ЗВАНИЧАН ЧАСОПИС СРПСКОГ ЛЕКАРСКОГ ДРУШТВА

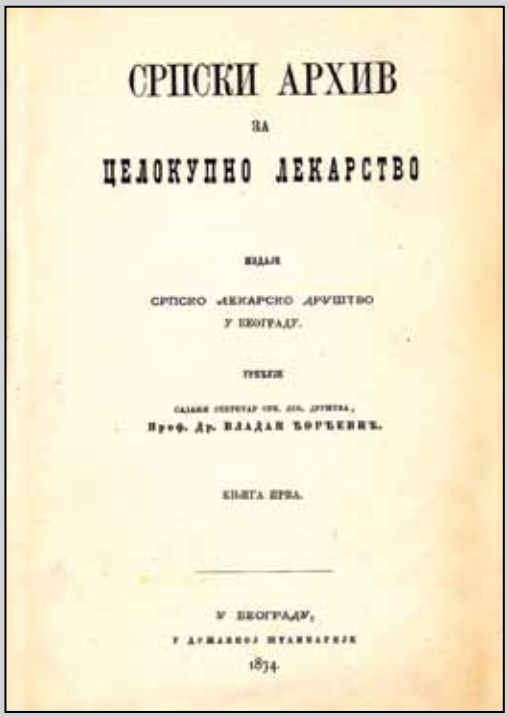
СРПСКИ АРХИВ

ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

ГОДИШТЕ / VOLUME 146 • МАЈ–ЈУН / MAY–JUNE 2018 • СВЕСКА / ISSUE 5–6

OFFICIAL JOURNAL *of* THE SERBIAN MEDICAL SOCIETY, Est.1872

SERBIAN ARCHIVES *of* MEDICINE



СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

ТОМ ПРВИ

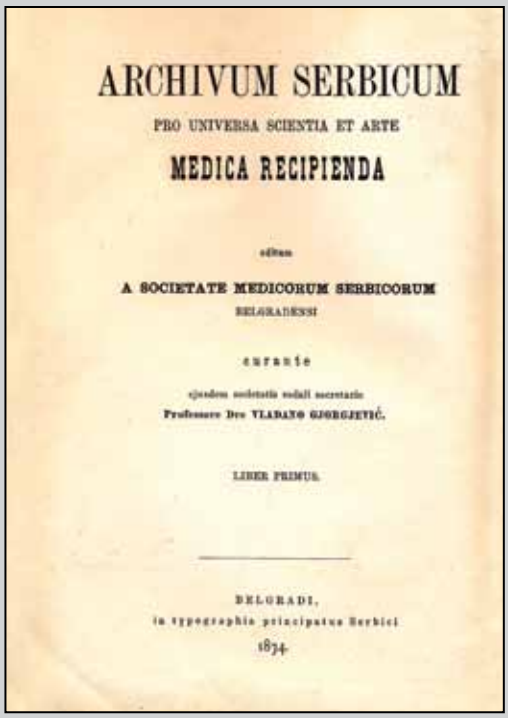
Прof. Др. ВЛАДАН ЂОРЂЕВИЋ.

КЊИГА ПРВА.

У БЕОГРАДУ,

1874.

Прва страна првог броја часописа на српском језику



ARCHIVUM SERBICUM

PRO UNIVERSA SCIENTIA ET ARTE

MEDICA RECIPIENDA

A SOCIETATE MEDICORUM SERBICORUM

BELGRADENSI

curante

Professore Dre VLADALO GJORGJEVIĆ.

LIBER PRIMUS.

BELGRADI,

1874.

The title page of the first journal volume in Latin

Корице/Cover
Председник Српског лекарског друштва
Ђорђе Клинковски (1827–1905)
Часопис се уређивао на састанцима Друштва (1895)
President of the Serbian Medical Society
Ђорђе Клинковски (1827–1905)
The editorial process of the journal used to take place at the Society meetings (1895)

Српски архив за целокупно лекарство је часопис Српског лекарског друштва основан 1872. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Часопис објављује: оригиналне радове, саопштења, приказе болесника, прегледе литературе, актуелне теме, радове из историје медицине, радове за праксу, радове који се односе на језик медицине, радове из медицинске етике (клиничка етика, етика публиковања, регулаторни стандарди у медицини), извештаје с конгреса и стручних састанака, стручне вести, приказе књига и дописе за рубрике Сећање, *In memoriam* и *Promemoria,* као и коментаре и писма Уредништву.

Сви рукописи који се разматрају за штампање у „Српском архиву за целокупно лекарство“ не могу да се поднесу или да буду разматрани за публиковање на другим местима. Радови не смеју да буду претходно штампани на другим местима (делимично или у потпуности).

Приспели рукопис Уређивачки одбор шаље рецензентима ради стручне процене. Уколико рецензенти предложе измене или допуне, копија рецензије се доставља аутору с молбом да унесе тражене измене у текст рада или да аргументовано образложи своје неслагање с примедбама рецензента. Коначну одлуку о прихватању рада за штампу доноси главни и одговорни уредник.

За објављене радове се не исплаћује хонорар, а ауторска права се преносе на издавача. Рукописи и прилози се не враћају. За репродукцију или поновно објављивање неког сегмента рада публикованог у „Српском архиву“ неопходна је сагласност издавача.

Радови се штампају на енглеском језику са кратким садржајем на енглеском и српском језику, односно на српском језику, ћирилицом, са кратким садржајем на српском и енглеском језику.

Аутори прихватају потпуну одговорност за тачност целокупног садржаја рукописа. Материјал публикације представља мишљење аутора и није нужно одраз мишљења Српског лекарског друштва. С обзиром на брз напредак медицинске научне области, корисници треба да независно процењују информацију пре него што је користе или се на њу ослањају. Српско лекарско друштво, уредник или Уређивачки одбор „Српског архива за целокупно лекарство“ не прихватају било какву одговорност за наводе у радовима. Рекламни материјал треба да буде у складу с етичким (медицинским) и правним стандардима. Рекламни материјал укључен у овај часопис не гарантује квалитет или вредност оглашеног производа, односно тврдње произвођача.

Поднесени рукопис подразумева да је његово публиковање одобрио одговорни ауторитет установе у којој је истраживање обављено. Издавач се неће сматрати правно одговорним у случају подношења било каквог захтева за компензацију. Треба да се наведу сви извори финансирања рада.

Serbian Archives of Medicine is the Journal of the Serbian Medical Society, founded in 1872, which publishes articles by the members of the Serbian Medical Society, subscribers, as well as members of other associations of medical and related fields. The Journal publishes: original articles, communications, case reports, review articles, current topics, articles of history of medicine, articles for practitioners, articles related to the language of medicine, articles on medical ethics (clinical ethics, publication ethics, regulatory standards in medicine), congress and scientific meeting reports, professional news, book reviews, texts for "In memory of...", i.e. In memoriam and Promemoria columns, as well as comments and letters to the Editorial Board.

All manuscripts under consideration in the Serbian Archives of Medicine may not be offered or be under consideration for publication elsewhere. Articles must not have been published elsewhere (in part or in full).

The submitted manuscripts are forwarded by the Editorial Board to reviewers for editing and evaluation. If the reviewers find that the manuscript needs to be modified or amended, the copy of the report is sent to the author(s), requiring of them to make necessary modifications or amendments of the text or to provide argumentative explanation of their disagreement with the suggested reviewer's remarks. The final decision on acceptance of the article for publication is made by the Editor-in-Chief.

The authors shall not be remunerated for the published articles, and they are required to assign copyright of their papers to the publisher. Manuscripts and enclosures shall not be returned to the authors. Reproduction or repeated publication of any section of the manuscript already published in the "Serbian Archives" requires the publisher's approval.

The articles are printed in the English language with an abstract both in English and Serbian, or in the Serbian language, Cyrillic alphabet, with an abstract in Serbian and English.

Authors accept full responsibility for the accuracy of all content within the manuscript. Material in the publication represents the opinions of the authors and does not necessarily reflect opinions of the Serbian Medical Society. Because of rapid advances in the medical sciences, users should independently evaluate information before using or relying on it. Serbian Medical Society, the Editor or Editorial Board of the Serbian Archives of Medicine does not accept any responsibility for the statements in the articles. Advertising material is expected to conform to ethical (medical) and legal standards. Inclusion of advertising material in this publication does not guarantee the quality or value of such product or claims made by its manufacturer.

Submission of the manuscript implies that its publication has been approved by the responsible authorities at the institution where the work has been carried out. The publisher will not be held legally responsible should be any claims for compensation. Details of all funding sources for the work should be given.

Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.11)
COBISS.SR-ID 3378434
Српски архив за целокупно лекарство
Званичан часопис Српског лекарског друштва
Излази шест пута годишње

Оснивач, власник и издавач
Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
Председник
Академик Радоје Чоловић
Интернет страна: http://www.sld.org.rs

Адреса уредништва
Српски архив
Краљице Наталије 1, 11000 Београд, Србија
Телефон: +381 (0)11 409 27 76
+381 (0)11 409 44 79
Е-пошта: office@srpskiarhiv.rs
Интернет страна: www.srpskiarhiv.rs

Претплата и експедиција
Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
Телефон: +381(0)11 3245-149
Текући рачуни: 205-8041-21 и
355-1009094-22

Чланци у целости доступни су на интернет страници: www.srpskiarhiv.rs

Цена претплате за календарску годину је 3.000,00 динара за појединце, 6.000,00 динара за установе и 100 евра за читаоце ван Србије. Цена појединачног примерка из текуће године је 600,00 динара, а свеске из претходних година 300,00 динара.

Штампање „Српског архива за целокупно лекарство" током 2018. године помогло је Министарство просвете, науке и технолошког развоја Републике Србије

ISSN 0370-8179; ISSN Suppl 0354-2793

eISSN 2406-0895
Отворен приступ

Штампано у Србији

ГОДИШТЕ 146. МАЈ–ЈУН 2018. СВЕСКА 5–6

Часопис „Српски архив за целокупно лекарство" је индексиран у базама: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Index Medicus (Medline, PubMed), Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

Редакција
Технички уредник: Јасмина Живковић
Лектор за српски језик: Дивна Продановић
Лектор за енглески језик: Мирко Рајић
Корице и лого: Златко Т. Урошевић

Штампа: ЈП „Службени гласник", Београд

Тираж: 700 примерака

СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

VOLUME 146 MAY–JUNE 2018 ISSUE 5–6

The journal "Srpski arhiv za celokupno lekarstvo" (Serbian Archives of Medicine) is indexed in: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Index Medicus (Medline, PubMed), Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

EDITORIAL OFFICE
Technical editor: Jasmina Živković
Serbian language editor: Divna Prodanović
English language editor: Mirko Rajić
Cover & Logo: Zlatko T. Urošević

Printed by: JP "Službeni glasnik", Belgrade

Circulation: 700 copies

Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.11)
COBISS.SR-ID 3378434
Serbian Archives of Medicine
Official Journal of the Serbian Medical Society
Published six times per year

FOUNDER, OWNER & PUBLISHER
Serbian Medical Society
President
Radoje Čolović, academician

PUBLISHER'S ADVISORY BOARD
Prof. Pavle Milenković, MD, PhD
Prof. Vladimir Bumbaširević, MD, PhD, MSASA
Prof. Ljiljana Vučković-Dekić, MD, PhD
Prof. Ljubica Đukanović, MD, PhD
Prof. Nebojša Lalić, MD, PhD, MSASA
Prof. Milica Čolović, MD, PhD

EDITORIAL OFFICE
Serbian Archives of Medicine
Kraljice Natalije 1, 11000 Belgrade, Serbia
Phone: +381 (0)11 409 27 76
+381 (0)11 409 44 79
E-mail: office@srpskiarhiv.rs
Website: www.srpskiarhiv.rs

SUBSCRIPTION AND DISTRIBUTION
Serbian Medical Society
Džordža Vašingtona 19, 11000 Belgrade
Serbia
Phone: +381(0)11 3245-149
Bank accounts: 205-8041-21 and
355-1009094-22

Full-text articles are available at website: www.srpskiarhiv.rs

Calendar year subscription prices are as follows: 3,000 dinars for individuals, 6,000 dinars for institutions, and 100 euros for readers outside Serbia. The price of a current year issue is 600 dinars, and of issues from previous years 300 dinars.

The publishing of the Serbian Archives of Medicine during 2018 is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

ISSN 0370-8179; ISSN Suppl 0354-2793

eISSN 2406-0895
Open Access

Printed in Serbia

САДРЖАЈ • CONTENTS

УВОДНИК • EDITORIAL

ORIGINAL ARTICLES • ОРИГИНАЛНИ РАДОВИ

CASE REPORTS • ПРИКАЗИ БОЛЕСНИКА

REVIEW ARTICLES • ПРЕГЛЕДИ ЛИТЕРАТУРЕ

CURRENT TOPIC • АКТУЕЛНА ТЕМА

ИСТОРИЈА МЕДИЦИНЕ • HISTORY OF MEDICINE

INVITED COMMENTARY • КОМЕНТАР ПО ПОЗИВУ

УВОДНИК / EDITORIAL

My Dear and Honorable Colleagues, Members of the Serbian Medical Society,

Thank you so much for granting me the most prestigious award a medical doctor born, raised, and educated in Serbia can receive: to be the editor-in-chief of the Serbian Archives of Medicine, the oldest journal of the kind in a country with over 800-year-long tradition of medicine. This is an immense honor, but at the same time an even greater responsibility. Established in 1872, the Serbian Archives of Medicine is a living testimonial of modern Serbian medicine growth and development over the last two centuries.

Today, we are at a historical turning point when it is of paramount importance to strengthen our ties nationally and internationally, in a wholehearted effort fueled by endless desire to provide our journal the position it once had both in the region and worldwide. The fact that over 30,000 medical doctors in the country support the Serbian Medical Society is both promising and encouraging. Although the restitution of our property – donated to the Society during the 19th and in the first half of the 20th century by our successful colleagues and noble countrymen – is still in process, we have faith in our government to find an adequate solution.

The medical education in our country has been always keeping pace with the times. When in 1920, although established in 1905, the Faculty of Medicine at the University of Belgrade enrolled its first generation of students, they were taught according to medical curriculum similar to the leading European ones. The same happened later, when medical schools were established at the universities of Novi Sad and Niš simultaneously in 1960, followed by Priština in 1969, and, last but not least, Kragujevac in 1977. The growing number of medical schools in the country as well as their collaboration and healthy competition are of great importance. Over time, we have been witnessing the increasing influence of American medicine both in Western Europe and in Serbia. The United States of America always welcomed our medical doctors for clinical training and post-doctoral studies in basic medical sciences. Thus, English became the second language of instruction at the Faculty of Medicine in Novi Sad, and later on at the Faculty of Medicine of the University of Belgrade, where Studies in English have been operating successfully and unceasingly since 1995.

From all said and mentioned, you can see that we have been keeping up the pace with the global academia rather successfully, irrelevant of the testing decades behind us. As a matter of fact, although we have a habit of saying it was done both in basic and clinical medicine, that division is actually artificial, for one cannot exist without the other and exclusive divisions bring good nowhere, least of all in medicine, where research endeavors are joint and aim to benefit all in any setting: bench or bedside.

I spent so many lines on medical education for I'm a firm believer that it is crucial for all of us practicing and teaching medicine, and particularly for those not in the vicinity of big medical centers. It is good training and skillful observation hand-in-hand that save lives in all four corners of the world and that can still help increase the knowledge of colleagues anywhere. Therefore, I urge you to share your work with us at the Serbian Archives of Medicine. Whether you are working at a small general hospital or you are affiliated with the leading university

hospital center, it is important to share the knowledge you acquire in different steps of your careers.

I am happy to inform you that members of the journal's Editorial Board, at our recent meeting, promised to contribute with their latest or best manuscripts, hoping to improve our journal's metrics, which shall continue to be a painstaking procedure.

While seeking new, modern, and better solutions for our journal, striving to increase its visibility and access, I will also do my very best to preserve our medical heritage. In the same spirit, I will practice a statement we all preach in the Hippocratic Oath by honoring one of my lifelong mentors who was a great supporter of the Serbian medicine, in an article dedicated to his memory that you will find in this issue.

Editor-in-Chief
Prof. Gordana Teofilovski-Parapid, M.D., Ph.D.
Honorary President, International Committee of
Symposia on Morphological Sciences
President, European Federation
for Experimental Morphology
Adviser to the Dean, Studies in English,
University of Belgrade, Faculty of Medicine
office@srpskiarhiv.rs

DOI: https://doi.org/10.2298/SARH170428184D
UDC: 615.468.6:616.31-089

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Microbial adherence affinity and clinical characteristics of polypropylene versus silk sutures in oral surgery

Miroslav Dragović[1], Marko Pejović[1], Jelena Stepić[1], Svetlana Dragović[2], Nađa Nikolić[2], Jovana Kuzmanović-Pfićer[3], Snježana Čolić[1], Jelena Milašin[2]

[1]University of Belgrade, School of Dental Medicine, Department of Oral Surgery, Belgrade, Serbia;
[2]University of Belgrade, School of Dental Medicine, Department of Human Genetics, Belgrade, Serbia;
[3]University of Belgrade, School of Dental Medicine, Department of Medical Statistics and Informatics, Belgrade, Serbia

SUMMARY

Introduction/Objective The purpose of this study was to compare polypropylene and silk suture materials in terms of bacterial adherence and clinical features including the impact on soft tissue healing.

Methods Ten healthy patients were included in this study. Unilateral upper and lower wisdom teeth were extracted at the same time and wounds were sutured with different threads (one monofilament – polypropylene – and one multifilament – silk suture). Stitches were removed seven days postoperatively. Real-time polymerase chain reaction was used to analyze bacterial adherence. Intraoperative handling and ease of removal were assessed with the help of Visual Analogue Scale. Landry healing index was used for evaluation of soft tissue healing.

Results Significantly more pronounced bacterial adherence was found on silk compared to polypropylene sutures ($p = 0.005$). Superior intraoperative handling properties were registered suturing with polypropylene compared to silk ($p = 0.005$). Soft tissue healing was significantly better around polypropylene sutures, both on the third and the seventh postoperative day ($p = 0.016$). Patient discomfort was slightly higher for polypropylene sutures, but without statistical significance.

Conclusion Polypropylene suture material showed significantly lower bacterial adherence and superior clinical features compared to silk, including better soft tissue healing.

Keywords: bacterial adherence; oral soft tissue healing; non-absorbable suture materials; oral surgery; real time-PCR

INTRODUCTION

It is widely accepted that *per primam* soft tissue healing, as well as the absence of infection during the postoperative period, is crucial for a successful outcome of every procedure in oral surgery. Primary healing is most frequently obtained by means of sutures, which serve as tissue support until enough tensile strength and integrity is regained [1, 2]. Although various suture materials are used for wound closure, one should always opt for the best thread in regard to biocompatibility and handling characteristics. According to their origin, suture materials can be natural or synthetic. Depending on the number of threads, monofilament sutures (made of a single strand or filament) and multifilament sutures (made of several braided/twisted strands or filaments) may be distinguished.

Nowadays, in oral surgery, silk is the only natural suture material that is still widely used. Ease of manipulation and low cost are the main reasons for that [3, 4]. However, many studies emphasized that tissue reaction is more pronounced around sutures of natural origin than around synthetic ones [5–10]. Technological advancements in the field of synthetic fibers have enabled the development of high quality threads, very stable in terms of physical configuration, showing high biocompatibility [11, 12, 13].

From a biological point of view, the ideal suture material should be as inert as possible and should not impede tissue regeneration. Due to anatomical and physiological complexity of the oral cavity, clinical and histological studies have suggested quite different oral tissue reactions to sutures in comparison with other parts of the human body [6, 14]. Oral cavity may be compared to a bioreactor, where in warm and damp environment bacteria are in constant interaction with present food detritus, enhancing the risk of superinfection [1]. It has been shown that in the presence of sutures, only 100 CFU of bacteria are sufficient to induce the onset of infection [15].

The aim of this study was to compare polypropylene and silk suture materials in terms of bacterial adherence and clinical features, including the influence on wound healing.

Received • Примљено: April 28, 2017
Revised • Ревизија: September 12, 2017
Accepted • Прихваћено: September 14, 2017
Online first: October 10, 2017

Correspondence to:
Jelena MILAŠIN
School of Dental Medicine, University of Belgrade
Dr Subotica 8, 11000 Belgrade, Serbia
jelena.milasin@stomf.bg.ac.rs

METHODS

Patients

Ten healthy female patients aged 21–27 years, undergoing surgical extraction of two impacted third molars, were included in the study. Using standard surgical protocol, unilateral upper and lower wisdom teeth were extracted at the same time and wounds were sutured with simple interrupted sutures. The envelope design for mucoperiosteal flap was used in mandible, with sulcular incision going from mesial part of the first molar, engaging second molar and extending buccally along the external oblique ridge. In the maxilla, standard triangular flap was performed with the vertical releasing incision made at the distal part of the interdental papilla between the first and the second molar. Each wound was sutured with different thread (one monofilament and one multifilament) taking care of equal distribution between jaws, i.e. both threads were used five times in the upper and five times in the lower jaw. The suture materials were black braided silk (Sofsilk®, Covidien LLC, USA) 4/0 gauge, with a 19 mm, 3/8 circle "reverse cutting" needle, and polypropylene (Surgipro®, Covidien LLC) 4/0 gauge, with a 19 mm, 3/8 circle "reverse cutting" needle. All sutures were placed and removed by the same surgeon in order to avoid inter-examiner variability. The sutures were removed seven days postoperatively. The study was approved by the institutional Ethics Committee and is in compliance with the Helsinki Declaration. Accordingly, all included patients signed a detailed informed consent.

Microorganisms' quantification

Knots of both sutures, obtained from each patient, were placed into sterile tubes (Eppendorf, Hamburg, Germany), transferred to the lab, and prepared for microbial analysis. In order to obtain consistent results, a portion of 4 mm in length of each sample was used for real-time polymerase chain reaction (PCR). Bacterial DNA was isolated using a KAPA Express Extract DNA Extraction Kit (Kapa Biosystems, Wilmington, MA, USA) according to the manufacturer's instructions. DNA extracts were stored at -20°C prior to PCR analysis. Total gene copy number determination was done as described by Brajović et al [16], using Maxima™ SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) and the following primers: Fw 5'-TCCTACGGGAGCACAGT'-3 and Rv (5'GGACTACCAGGGTATCTAATCCTGTT-3'. Real-time PCR analyses were performed on Line Gene-K Fluorescence Real-time PCR Detection System (Hangzhou Bioer Technology Co. Ltd., Hangzhou, China).

Clinical parameters

Control check-ups were performed on the first, third, and seventh day postoperatively. Soft tissue healing was judged by the operator with the help of healing index shown in Table 1 and presented numerically [17]. Using the Visual Analogue Scale (VAS), the operator rated threads with respect to the ease of intraoperative handling properties and the ease of removal. Patients, using the same scale, evaluated the suture discomfort and suture removal pain for each type of suture.

Table 1. Soft tissue healing index by Landry et al. [17]

Very poor (1) (has 2 or more of the following)	Tissue color: **≥ 50% of gingiva red** Response to palpation: **bleeding** Granulation tissue: **present** Incision margin: **not epithelialized, with loss of epithelium beyond incision margin** Suppuration: **present**
Poor (2)	Tissue color: **≥ 50% of gingiva red** Response to palpation: **bleeding** Granulation tissue: **present** Incision margin: **not epithelialized, with connective tissue exposed**
Good (3)	Tissue color: **≥ 25 and < 50% of gingiva red** Response to palpation: **no bleeding** Granulation tissue: **none** Incision margin: **no connective tissue exposed**
Very good (4)	Tissue color: **< 25% of gingiva red** Response to palpation: **no bleeding** Granulation tissue: **none** Incision margin: **no connective tissue exposed**
Excellent (5)	Tissue color: **all tissues pink** Response to palpation: **no bleeding** Granulation tissue: **none** Incision margin: **no connective tissue exposed**

Scanning electron microscopy

Samples of both suture materials used in this study were chosen randomly and analyzed by scanning electron microscopy (SEM). Specimens of silk and polypropylene were placed on specimen holders and coated with gold in a gold sputter at 18 mA for one minute. The specimens were analyzed descriptively and photographed in a VEGA TS 5133MM SEM high vacuum mode using the SE detector with accelerating voltage.

Statistical analysis

All statistical analyses were done using SPSS software package, version 18.0 (SPSS Inc., Chicago, IL, USA). Mean, median, SD, and range values were used for the description of numerical data. Descriptive data were expressed as percentage for discrete measures. Categorical variables were compared using the χ^2 test. Numerical data were analyzed using Friedman and Wilcoxon test. Spearman's correlation coefficient was done in order to assess the relationship between clinical parameters and microbial adherence. Differences were considered significant when the p-value was less than 0.05.

RESULTS

Microorganisms' quantification

A total of 20 suture samples were examined for microbial adherence and a statistically significant difference was found between the average gene copy number of bacteria

on silk sutures (2.33E + 10 ± 2.60E + 10 SD) and polypropylene (1.46 E + 8 ± 2.68E + 8 SD) (Figure 1). Not only the average number of bacteria on silk was higher than on polypropylene, but also all 10 silk samples, considered individually, had higher bacterial load than the corresponding polypropylene samples.

Clinical parameters

Postoperative period was uneventful in all patients. There were no postoperative complications such as wound dehiscence, immediate or delayed infection, dry socket etc. In the present study, a better regeneration was found around polypropylene sutures than around silk sutures, both on the third and on the seventh day postoperatively (Figure 2). No significant correlation was found between suture microbial adherence and soft tissue healing.

Superior intraoperative handling properties were registered for polypropylene sutures (mean VAS 96.40 mm ± 4.01 SD) compared to silk sutures (mean VAS 60 mm ± 17.15 SD; p = 0.005). Removal of both sutures was effortless and without significant difference between the two groups (Figure 3). In addition, mean values for suture removal pain data were higher for silk suture; however, it was not statistically significant (Figure 3).

The degree of discomfort due to suture presence on the first, third, and seventh postoperative day, as depicted in Figure 4, indicates that there was no significant difference between silk and polypropylene.

An important correlation was found between bacterial adherence and patient discomfort for silk (r_s = 0.84; p = 0.002), whilst such an association was not found for polypropylene (r_s = 0.44; p = 0.21).

Scanning electron microscopy

Representative micrographs of silk and polypropylene threads are given in Figure 5, depicting obvious differences related to debris accumulation.

DISCUSSION

Establishing primary wound closure without tension and avoiding postoperative infection are essential factors for optimal wound healing. Various suture materials are used in oral surgery for that purpose. One could find himself in a dilemma whether to use absorbable or non-absorbable, monofilament or multifilament, natural or synthetic materials. Non-absorbable sutures are widely used in oral surgery due to their satisfactory clinical properties. On the other hand, complex suturing techniques require utilization of absorbable sutures occasionally. Absorbable materials are often indispensable in pediatric surgery to protect children from additional trauma at the time of removal. In addition, for high-risk patients (HIV, HBV, etc.), it is preferable to use absorbable sutures in order to avoid unnecessary exposure of medical staff to pathogens [1].

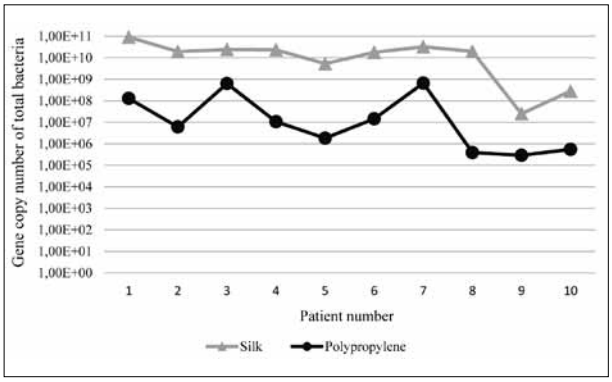


Figure 1. Individual values of total gene copy number of bacteria on silk and polypropylene sutures for each patient and type of suture (p = 0.005)

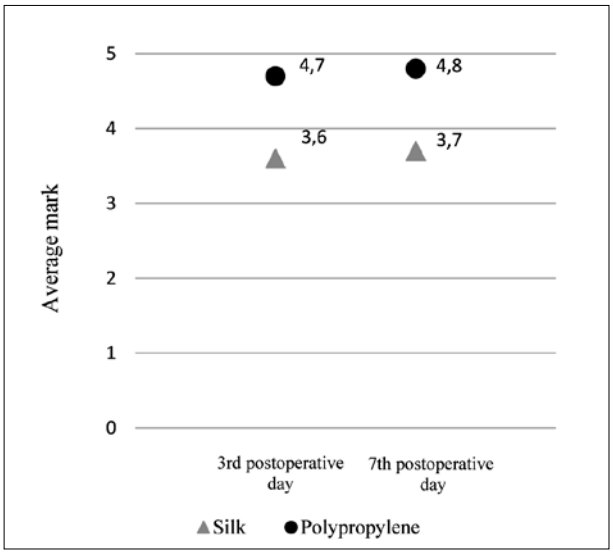


Figure 2. Average mark for each type of suture on the 3rd and 7th postoperative day according to soft tissue healing index by Landry et al. [17] (p = 0.016)

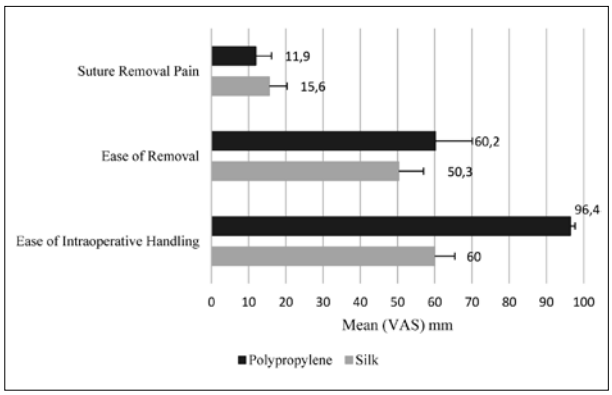


Figure 3. Visual Analogue Scale results (mean values) for clinical features related to suture materials

Silk is a non-absorbable multifilament suture of natural origin, well known as an easy-handling material, very pliable and strong enough to resist breaking during surgery. What is regarded as its negative feature is a significant tensile strength loss in early postoperative days in conjunction with swelling and fragmentation due to soaking

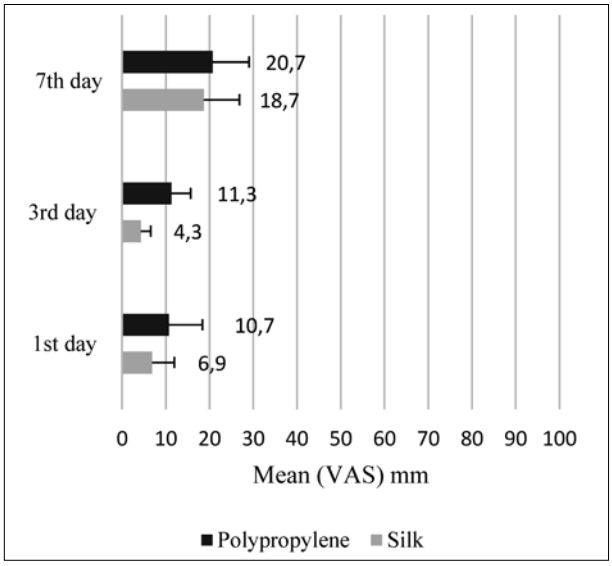


Figure 4. Visual Analogue Scale (VAS) results (mean values) for patient discomfort on the 1st, 3rd, and 7th postoperative day

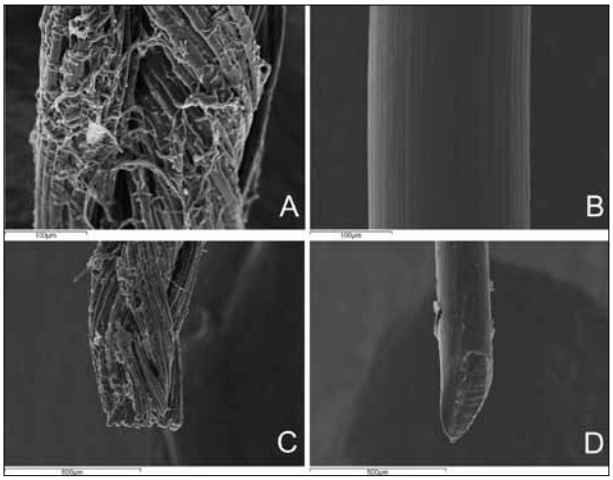


Figure 5. Scanning electron micrograph of (A) silk and (B) polypropylene sample, 1 mm from the free end; free end of (C) silk and (D) polypropylene

with saliva [7, 18, 19, 20]. Nevertheless, the necessity for longer tissue support inevitably imposes the use of non-absorbable synthetic materials, as they maintain tensile strength for a long time. In the case of polypropylene, it has been shown that tensile strength is modified very little immediately after knot tying [21]. Moreover, it has also been shown on animal models that polypropylene retained its tensile strength even after a period of two years [11]. Additionally, polypropylene as a monofilament synthetic suture elicits less pronounced tissue reaction then multifilament sutures [11, 12]. It has also been confirmed that silk induces remarkably greater tissue reaction in comparison with monofilament synthetic sutures [5–7, 22].

To the best of our knowledge, there are no studies on patients dealing with clinical implications of polypropylene versus silk use in oral surgery. It may be that the smooth surface and the absence of capillarity enable polypropylene thread to not only engage tissue with minimal friction and trauma but also to cause less tissue irritation during the healing period. The latter is of special importance, since strong tissue reaction around a suture could impede tissue regeneration and prolong healing. Despite some limitations, the present study confirmed significantly better soft tissue healing around polypropylene sutures as compared to silk ones, on both the third and the seventh postoperative day.

According to the literature, greater risk of bacterial colonization and migration along the suture is related to multifilament materials due to "wicking" phenomenon and interstices between twisted/braided threads [23, 24, 25]. Consequently, microorganisms might be transferred into deeper parts of the wound, where they may be harmful, causing an infection and delay of healing. However, our results showed no correlation between bacterial adherence and soft tissue healing. Quantification of bacteria by real-time PCR is reliable to a great extent, although the number of bacteria includes both viable and nonviable microbial species. The analysis of collected data in our study clearly indicates that silk is far more susceptible to bacterial adherence than polypropylene. These results are in accordance with findings of other authors [20, 23, 24, 26, 27, 28]. Despite different methods used for bacterial identification, data from all studies are consistent regarding the fact that monofilament sutures are less prone to microbial adherence than multifilament sutures. It is also widely accepted that physical configuration of threads, more than the material itself, contributes to different affinity of bacteria.

Concerning polypropylene features, its outstanding breaking strength, tying fluency, and knot security additionally recommend it as the material of choice for surgical sutures [21, 29]. Although sutures with low friction coefficient are at greater risk of being undone untimely, this may be successfully prevented by selecting adequate knots [1, 2]. In our study, polypropylene was estimated as highly preferable to silk due to easiness in intraoperative manipulation. Our study showed no significant difference between polypropylene and silk sutures in relation to the easiness of thread removal and accompanying removal pain (Figure 3). Higher mean value of suture removal pain for silk suture might be a consequence of inferior healing, as well as higher friction, as compared to polypropylene. Namely, when a thread with huge friction coefficient is glided through tissue with considerable speed, that friction is converted into heat, which ultimately may result in the onset of micro-burns along the line of the suture [1]. In our study, polypropylene was found to be slightly easier to remove, most likely due to its low friction rate, as well as an absence of fluid absorption.

As polypropylene is not widely used in oral surgery due to its rigidity, in particular caliber 3-0, almost all available information about this thread comes from other fields of surgery. Our study showed no significant difference between silk and polypropylene sutures regarding patient discomfort, albeit values for polypropylene were higher, especially on the first and the third postoperative day. Presumably, the main reason for patient annoyance is related to pricking, which could sometimes lead to the appear-

ance of decubitus in the postoperative period. In order to avoid that kind of complication, it is recommended to use threads with smaller diameter (4-0, 5-0, 6-0), as well as to cut them with scissors at a right angle, thereby evading the formation of a sharp free end. Likewise, leaving free ends at least 5–7 mm long may contribute to improved acceptance of these sutures by reducing inflexibility and pricking effect. It can be assumed that the lack of bacteria on polypropylene suture knots may compensate for their pricking effect. Hence, in our study, patient's subjective sensations of comfort/discomfort were quite similar for silk and polypropylene.

CONCLUSION

Polypropylene suture material showed significantly lower microbial adherence and superior clinical features compared to silk, including significantly better soft tissue healing.

ACKNOWLEDGMENT

This work was supported by a grant No. 175075 of the Ministry of Education, Science and Technological Development of the Republic of Serbia.

REFERENCES

1. Siervo S, Lorenzini L. Suturing Techniques in Oral Surgery. 2008. 223 p.
2. Silverstein LH, Kurtzman GM, Shatz PC. Suturing for optimal soft-tissue management. J Oral Implantol. 2009; 35(2):82–90.
3. Silverstein LH, Kurtzman GM. A review of dental suturing for optimal soft-tissue management. Compend Contin Educ Dent. 2005; 26(3):163–6, 169–70, 209.
4. Pons-Vicente O, López-Jiménez L, Sánchez-Garcés MA, Sala-Pérez S, Gay-Escoda C. A comparative study between two different suture materials in oral implantology. Clin Oral Implants Res. 2011; 22(3):282–8.
5. Abi Rached RS, de Toledo BE, Okamoto T, Marcantonio Júnior E, Sampaio JE, Orrico SR, et al. Reaction of the human gingival tissue to different suture materials used in periodontal surgery. Braz Dent J. 1992; 2(2):103–13.
6. Selvig KA, Biagiotti GR, Leknes KN, Wikesjö UME. Oral tissue reactions to suture materials. Int J Periodontics Restor Dent. 1998; 18(5):475–87.
7. Leknes KN, Røynstrand IT, Selvig KA. Human gingival tissue reactions to silk and expanded polytetrafluoroethylene sutures. J Periodontol. 2005; 76(1):34–42.
8. Yilmaz N, Inal S, Muğlali M, Güvenç T, Baş B. Effects of polyglecaprone 25, silk and catgut suture materials on oral mucosa wound healing in diabetic rats: an evaluation of nitric oxide dynamics. Med Oral Patol Oral Cir Bucal. 2010; 15(3):e526–30.
9. Kakoei S, Baghaei F, Dabiri S, Parirokh M, Kakooei S. A Comparative in vivo study of tissue reactions to four suturing materials. Iran Endod J. 2010; 5(2):69–73.
10. Yaltirik M, Dedeoglu K, Bilgic B, Koray M, Ersev H, Issever H, et al. Comparison of four different suture materials in soft tissues of rats. Oral Dis. 2003; 9(6):284–6.
11. Postlethwait RW. Long-term comparative study of nonabsorbable sutures. Ann Surg. 1970; 171(6):892–8.
12. Postlethwait RW. Five year study of tissue reaction to synthetic sutures. Ann Surg. 1979; 190(1):54–7.
13. Burkhardt R, Lang NP. Influence of suturing on wound healing. Periodontol. 2000. 2015; 68(1):270–81.
14. Wallace WR, Maxwell GR, Cavalaris CJ. Comparison of polyglycolic acid suture to black silk, chromic, and plain catgut in human oral tissues. J Oral Surg. 1970; 28(10):739–46.
15. Elek SD, Conen PE. The virulence of *Staphylococcus pyogenes* for man; a study of the problems of wound infection. Br J Exp Pathol. 1957; 38(6):573–86.
16. Brajović G, Popović B, Puletić M, Kostić M, Milašin J. Estimation of total bacteria by real-time PCR in patients with periodontal disease. Srp Arh Celok Lek. 2016; 144(1–2):10–4.
17. Landry RG, Turnbull RS, Howley T. Effectiveness of benzydamyne HCl in the treatment of periodontal post-surgical patients. Res Clin Forums. 1988; 10:105–18.
18. Zederfeldt B. Choice of suture materials for wound closure. Eur Surg Res. 1983; 15(2):57–8.
19. Meyer RD, Antonini CJ. A review of suture materials, Part II. Compendium. 1989; 10(6):360–8.
20. Parirokh M, Asgary S, Eghbal MJ, Stowe S, Kakoei S. A scanning electron microscope study of plaque accumulation on silk and PVDF suture materials in oral mucosa. Int Endod J. 2004; 37(11):776–81.
21. Von Fraunhofer J, Storey R, Masterson BJ. Tensile properties of suture materials. Biomaterials. 1988; 9(4):324–7.
22. Postlethwait RW, Willigan DA, Ulin AW. Human tissue reaction to sutures. Ann Surg. 1975; 181(2):144–50.
23. Katz S, Izhar M, Mirelman D. Bacterial adherence to surgical sutures. A possible factor in suture induced infection. Ann Surg. 1981; 194(1):35–41.
24. Merritt K, Hitchins VM, Neale AR. Tissue colonization from implantable biomaterials with low numbers of bacteria. J Biomed Mater Res. 1999; 44(3):261–5.
25. Masini BD, Stinner DJ, Waterman SM, Wenke JC. Bacterial adherence to suture materials. J Surg Educ. 2011; 68(2):101–4.
26. Otten JE, Wiedmann-Al-Ahmad M, Jahnke H, Pelz K. Bacterial colonization on different suture materials – A potential risk for intraoral dentoalveolar surgery. J Biomed Mater Res - Part B Appl Biomater. 2005; 74(1):627–35.
27. Banche G, Roana J, Mandras N, Amasio M, Gallesio C, Allizond V, et al. Microbial adherence on various intraoral suture materials in patients undergoing dental surgery. J Oral Maxillofac Surg. 2007; 65(8):1503–7.
28. Sala-Pérez S, López-Ramírez M, Quinteros-Borgarello M, Valmaseda-Castellón E, Gay-Escoda C. Antibacterial suture vs silk for the surgical removal of impacted lower third molars. A randomized clinical study. Med Oral Patol Oral Cir Bucal. 2016; 21(1):e95–102.
29. Capperauld I. Suture materials: A review. Clin Mater. 1989; 4(1):3–12.

Свилени и полипропиленски материјал за шавове у оралној хирургији – колонизација микроорганизмима и клиничке карактеристике

Мирослав Драговић[1], Марко Пејовић[1], Јелена Степић[1], Светлана Драговић[2], Нађа Николић[2], Јована Кузмановић-Пфифер[3], Сњежана Чолић[1], Јелена Милашин[2]

[1]Универзитет у Београду, Стоматолошки факултет, Клиника за оралну хирургију, Београд, Србија;
[2]Универзитет у Београду, Стоматолошки факултет, Одељење за хуману генетику, Београд, Србија;
[3]Универзитет у Београду, Стоматолошки факултет, Одељење за медицинску статистику и информатику, Београд, Србија

САЖЕТАК

Увод/Циљ Циљ ове студије био је поређење свиленог (СК) и полипропиленског конца (ППК) у погледу пријемчивости за бактерије и клиничких карактеристика, укључујући утицај на зарастање меког ткива у усној дупљи.

Методе У студију је укључено десет здравих испитаника код којих су хируршки извађени горњи и доњи умњак са једне стране истовремено, а ране су ушивене различитим концима (један монофиламентни – ППК и један полифиламентни – СК). Квантификација бактерија на узорцима конаца који су уклоњени седам дана после операције урађена је методом ланчане реакције полимеразе у реалном времену. Орални хирург је уз помоћ Визуелне аналогне скале оцењивао лакоћу интраоперативног руковања, као и лакоћу уклањања конаца. За процену квалитета зарастања меког ткива коришћен је индекс по Ландрију.

Резултати Статистички значајно више бактерија нађено је на свим узорцима СК у поређењу са ППК ($p = 0,005$). ППК се показао значајно лакшим за интраоперативно руковање у односу на СК ($p = 0,005$). Такође, зарастање меког ткива, 3. и 7. дана постоперативно, било је значајно успешније око ППК него око СК ($p = 0,016$). Непријатност због присуства конаца била је већа код примене ППК у односу на СК, али без статистички значајне разлике.

Закључак Полипропиленски конац је у односу на свилени конац показао значајно мању пријемчивост за бактерије и боље клиничке карактеристике, укључујући и боље зарастање меког ткива.

Кључне речи: бактеријска пријемчивост; зарастање меког ткива; нересорптивни хируршки конци; орална хирургија; *real time-PCR*

DOI: https://doi.org/10.2298/SARH170508191S
UDC: 616.24-002.5:613.84

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The impact of smoking on clinical characteristics and treatment outcome of patients with pulmonary tuberculosis

Sonja Smiljić
University of Priština, Faculty of Medicine, Department of Physiology, Kosovska Mitrovica, Serbia

SUMMARY
Intoroduction/Objective The objectives of our study were to determine the impact of smoking on clinical characteristics, the scope of radiological severity, and treatment outcome of patients with pulmonary tuberculosis (PTB).
Method This prospective study included patients suffering from PTB treated at the Pulmonology Department of the Clinical Hospital Center in Kosovska Mitrovica in the period between 2010 and 2016.
Results Among smokers suffering from PTB there were significantly more males ($p = 0.05$) between 30 and 49 years of age ($p < 0.001$). There was significantly more alcohol consumption present in smokers ($p < 0.001$), whose social factor for developing PTB ($p = 0.002$) was more expressed. A more severe form of PTB with cavitation was more common in smokers (38.8%), while a milder, parenchymatous, unilateral PTB was present in non-smokers (31.8%). Extensive X-ray changes were more common in smokers ($p = 0.002$). Relapse of the disease was more prevalent in smokers ($p = 0.05$). In multivariate logistic regression, the risks of being a smoker included years of age: 30–39 [odds ratio (OD) = 18.11], 40–49 (OR = 19.66), and 50–59 (OR = 9.06), and alcohol consumption habits (OR = 9.32).
Conclusion Smokers were more often afflicted with sputum-positive PTB, had extensive radiological changes, and the relapse of the disease was more common. Smokers were mostly middle-aged, with alcohol consumption habits, and constructed a group of patients whose habits were a critical factor for the eradication of tuberculosis.
Keywords: pulmonary tuberculosis; smoking; alcohol; X-ray abnormality

INTRODUCTION

Smoking and tuberculosis (TB) are the two biggest public health problems in the world. Smoking is one of the leading causes of premature death, causing six million deaths annually. Today, about 33% of the world's population smokes, mainly in countries with a high prevalence of TB. In 2015, pulmonary tuberculosis (PTB) affected 10.4 million people and one fifth of TB cases can be blamed on tobacco smoking [1]. The 2014 United States Surgeon General's Report implicates smoking as a cause of TB disease among those latently infected with *Mycobacterium tuberculosis* [2].

While the link between smoking and PTB was recognized almost a century ago, the impact of smoking on the development of TB has mostly been explained in the last few decades [3]. Active and passive exposures to tobacco smoke are independent risk factors for TB infection, the progression of TB infection to an active disease, severe clinical profile, and an increased risk of relapse and mortality. Thus, smokers are at a higher risk of developing TB than non-smokers. In smokers, the latent form of TB often turns into an active one, and the determining factor in this is their general health status. Smoking affects health and modifies the immune response, which favors the development of TB [4, 5, 6].

A recent study based on mathematical modeling estimated that, between 2010 and 2050, smoking can increase the number of TB patients in the world by 18 million, and cause a significant increase in mortality if the current trend in tobacco consumption continues unchecked [7]. Smoking prevalence among TB patients is higher than among the general population in many countries. However, the data are different, from 48% in Catalonia, 54.6% in China, to 81.5% in rural India [8, 9, 10]. Patients who are smokers often spread the disease to other family members [11]. The World Health Organisation estimates that about one third of people in the world are infected with *Mycobacterium tuberculosis* and that 90% of them exhibit no symptoms, making it a latent TB infection [1]. Smoking, alcohol consumption, and malnutrition can influence the transition from the latent to the active form of TB.

Possible mechanisms of the impact of smoking include reduced clearance of secretions on tracheobronchial mucosal surface, reduced phagocytic function of alveolar macrophages, reduced alveolar macrophage production of tumor necrosis factor (TNF), and increased macrophage hemochromatosis [3, 12]. Smoking reduces the effectiveness of the alveolar macrophages in developing effective immune response by altering the expression of cell

Received • Примљено: May 8, 2017
Revised • Ревизија: October 23, 2017
Accepted • Прихваћено: October 24, 2017
Online first: October 31, 2017

Correspondence to:
Sonja SMILJIĆ
Faculty of Medicine, University of Priština
Anri Dinana bb
38220 Kosovska Mitrovica
Serbia
sonja.smiljic@med.pr.ac.rs

proinflammatory cytokines [13]. In addition to nicotine, which modulates the activity of macrophages, about 5,000 more substances present in tobacco smoke modulate the activity of inflammatory cells. The correlation of TB and smoking can be a result of the inhibitory effect of nicotine in the production of TNF-alpha and anti-inflammatory cytokines CKSCL8 that make patients susceptible to the progression of the latent form of the infection into the active one. Smoking disrupts the phagocytic function of alveolar macrophages and induces apoptosis in macrophages. Chronic exposure to cigarette smoke reduces the expression of surface proteins related to antigen presentation by macrophages. Monocyte macrophage system in TB patients has a reduced phagocytic capacity, which is further reduced in patients with TB who smoke [3, 14].

Chronic alcohol use can reduce the response of macrophages and the immune system [15]. Body mass index value under 18.5 kg/m^2 is seen as a marker for malnutrition, and malnutrition can reduce immune response either through the interaction of monocyte-macrophages and T-lymphocytes and their cytokines or the secondary immune deficiency that increases the susceptibility of the host to infection [16]. Alcohol consumption significantly worsens the clinical manifestation of the disease and the outcome of the treatment, which may be the result of the adverse reactions to medication used in the treatment of PTB. People that abuse alcohol are 1.32 times more likely to exhibit an adverse reaction to tuberculostatics [10, 17]. The relation between the manifestation of adverse drug effects during treatment and unsuccessful treatment plays an important role in controlling the spread of TB.

The social and clinical reasons for the failure of antituberculosis therapy can be seen as a result of smoking and alcoholism. Smoking and alcohol are related to other pathological conditions that can be among the causes of treatment failure, and often of the premature discontinuation of therapy.

Unsuccessful treatment outcome is an adverse health condition for both the patients and for the public health, because it increases the duration of infectiousness; thus, individual and public health concerns should be considered together in planning effective control strategies. A large proportion of cases in which treatment will be unsuccessful could be predicted at entry through screening for age group, smoking, and alcoholism, and specially targeted measures could be taken in such cases.

The aim of our study was to determine the impact of smoking on clinical characteristics, the scope of radiological severity, and the treatment outcome of patients with PTB.

METHODS

The survey was conducted in accordance with the ethical principles and was approved by the Ethics Committee of the Priština Faculty of Medicine, temporarily seated in Kosovska Mitrovica.

This prospective study was conducted at the Department of Pulmonology of the Clinical-Health Center in Kosovska Mitrovica, the reference hospital for TB treatment. The study included patients suffering from TB, a total of 104 subjects treated during the period between 2010 and 2016.

The inclusion criteria for the selection of patients in this study were as follows: 1) older than 20 years of age; 2) typical symptoms of PTB (cough, sputum production, fever, night sweats, and weight loss); 3) typical fibrocavitary pulmonary infiltrates on chest radiographs standard; 4) at least one smear-positive sputum, with the subsequent positive culture of *Mycobacterium tuberculosis*, and 5) all study patients could already have been on the antituberculosis treatment (processed with all necessary radiological, microbiological, and laboratory and spirometric examinations, before starting the antituberculosis treatment).

On admission, the patients' data regarding demographics, age, sex, residence, marital status, education, occupation and possible contact with people suffering from TB were gathered. Special attention was paid to risk factors for developing PTB: smoking, alcohol consumption, drug use, and social status. In regard to comorbidities, we processed instances of diabetes mellitus.

All the patients were divided into two groups – smokers and non-smokers. The subjects were considered smokers if they reported that they smoked one or more cigarettes per day during the year preceding the diagnosis of TB, or non-smokers if they consumed less than 100 cigarettes in their life [8, 18]. Alcohol consumption was considered significant if male subjects consumed more than 280 g of alcohol per week, and female subjects over 168 g per week. None of our subjects used drugs (intravenous heroin and/or cocaine, or other).

We processed the following initial symptoms and signs in our patients: cough, sputum production, hemoptysis, chest pain, fever, night sweats, fatigue, and weight loss. In regard to the laboratory parameters, we processed the parameters for anemia (hemoglobin, hematocrit, hematological indices) and sedimentation rate. Sputum samples were taken from all the patients for a direct microscopy of the preparations stained according to the Ziehl–Neelsen method. Also, cultivation of the bacillus on the Lowenstein–Jansen medium was performed for all samples. The sputum was collected in the morning, before eating, after a spontaneous expectoration. Each sputum positive for direct microscopy was verified by the culture on the Lowenstein–Jansen medium. PTB was bacteriologically confirmed if the two sputum findings confirmed bacillus and/or in the case of positive sputum cultivation.

Chest X-ray results were categorized according to the scale of changes, their localization and their morphological structure. The interpretation of chest X-ray abnormalities was performed by a radiologist. Chest X-rays were focused on pulmonary parenchyma and caverns. The interpretation of abnormalities in the pulmonary parenchyma included unilateral or bilateral changes, the location of changes (in the lower, medium and upper fields) and the scale of changes (minimum/moderate and extensive).The final diagnosis of PTB was made based on the positive findings pertaining to acid-resistant bacilli in sputum and/or chest X-rays.

Statistical analysis

The data were analyzed by descriptive statistical methods and presented as frequencies and relative numbers. For the analysis of frequency differences between the groups, χ^2 test was used. Binary logistic regression was the technique used to analyze the dependencies between activities. The multiple logistic regression model included all the predictors that had statistical significance at 0.05 in a single logistic regression model. The criterion for statistical significance was $p < 0.05$.

For statistical data analysis, we used the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The study involved patients with PTB treated at the Department of Pulmonology in the period between 2010 and 2016. During these six years, we treated 104 patients with TB, who were predominantly male (67%). Out of the total number of patients with PTB, 60 were smokers (57.7%) and were 44 non-smokers (42.3%). Among smokers suffering from TB there were significantly more males ($p = 0.05$), between 30 and 49 years of age ($p < 0.001$). The majority of non-smokers who became ill (19) with TB are among women of the age between 20 and 29 years.

There was no significant difference in relation to the place of residence, family status, and education level between smokers and non-smokers. However, smokers suffering from TB were more likely to be manual laborers (40%). Smokers consumed alcohol significantly more often than non-smokers did ($p = 0.001$). The social determinant was significant in smokers with PTB ($p = 0.002$). A possible contact with the PTB affected people and diabetes mellitus were equally present in smokers and non-smokers (Table 1).

Respiratory symptoms were typical for both groups of patients, and did not differ significantly. Laboratory parameters for anemia and erythrocyte sedimentation rate did not differ significantly in smokers and non-smokers. A direct sputum bacilloscopy was more frequently positive in smokers than in non-smokers (Table 2).

There were some significant differences in radiographic severity. Smokers were often diagnosed with TB with cavitation, while in non-smokers the more common form was unilateral parenchymal PTB. Upper lung fields were significantly more affected by the changes in smokers (68.3%). The extent of radiological changes was significantly higher in smokers ($p = 0.002$). The incidence of relapse in smokers was 23.3% and 9.1% in non-smokers. The relapse of the disease was significantly more common in smokers ($p = 0.05$) (Table 3). Multiple logistic regression of the variables related to socio-demographic characteristics and risk factors associated with smoking included age [30–39 (OR = 11.18), 40–49 (OR = 19.66), and 50–59 (OR = 9.06)] and the habit of alcohol consumption (OR = 9.32) (Table 4).

Table 1. Sociodemographic characteristics and risk factors for pulmonary tuberculosis in smokers and non-smokers (n = 104)

Baseline patient characteristics	Population examined		p
	Smoker n (%)	Non-smoker n (%)	
	60 (57.7)	44 (42.3)	
Age, years			
20–29	3 (5)	17 (38.6)	< 0.001
30–39	11 (18.3)	3 (6.8)	
40–49	13 (21.7)	2 (4.5)	
50–59	21 (35.0)	10 (22.7)	
> 60	12 (20)	12 (27.3)	
Sex			
Male	45 (75)	25 (56.8)	0.050
Female	15 (25)	19 (43.2)	
Residence			
Rural	45 (75.0)	32 (72.7)	0.794
Urban	15 (25.0)	12 (27.3)	
Marital status			
Single	25 (41.7)	25 (56.8)	0.127
Married	35 (58.3)	19 (43.2)	
Education			
Primary	36 (60)	23 (52.3)	0.432
Secondary	22 (36.7)	21 (47.7)	
High	2 (3.3)	0 (0)	
Employment status			
Unemployed	13 (21.7)	11 (25)	0.185
Manual laborer	24 (40)	9 (20.5)	
Office worker	9 (15)	8 (18.2)	
Pensioner	14 (23.3)	16 (36.4)	
Alcohol use			
Yes	19 (31.7)	1 (2.3)	
No	41 (68.3)	43 (97.7)	< 0.001
Social determinants			
Yes	30 (50)	9 (20.5)	
No	30 (50)	35 (79.5)	0.002
Tuberculosis contact			
Yes	6 (10)	4 (9.1)	
No	54 (90)	40 (90.9)	0.877
Tuberculosis history			
Yes	9 (15)	7 (16.3)	
No	51 (85)	37 (83.7)	0.860
Diabetes mellitus			
Yes	9 (15)	5 (11.4)	
No	51 (85)	39 (88.6)	0.591

DISCUSSION

The World Health Organisation estimates that, during 2015, 9.4 million people were affected by TB, with a fatal outcome in 1.4 million of the treated patients. The main reasons for maintaining this high number of patients in the past two decades are the large number of HIV-infected patients and multidrug-resistant TB [19, 20]. Another very important risk factor whose effects on morbidity related to TB have been explained in the past several years is smoking [21]. In European countries, 16% of fatalities among adults older than 30 years were caused by tobacco consumption [21]. Other risk factors for developing TB include alcohol, drugs and associated diseases, especially diabetes, contact

Table 2. Symptoms and clinical signs between smokers and non-smokers with pulmonary tuberculosis (n = 104)

Symptoms	Population examined		p
	Smokers n (%)	Non-smokers n (%)	
	60 (57.7)	44 (42.3)	
Cough			
Yes	53 (88.3)	34 (77.3)	0.132
No	13 (11.7)	11 (22.7)	
Productive cough			
Yes	36 (60)	21 (47.7)	0.214
No	24 (40)	23 (52.3)	
Hemoptysis			
Yes	5 (8.3)	7 (15.9)	0.232
No	55 (91.7)	37 (84.1)	
Fever			
Yes	41 (68.3)	24 (54.5)	0.151
No	19 (31.7)	20 (45.5)	
Night sweats			
Yes	34 (58.6)	25 (56.8)	0.935
No	26 (41.4)	19 (43.2)	
Asthenia			
Yes	37 (61.7)	30 (68.2)	0.493
No	23 (38.3)	14 (31.8)	
Weight loss			
Yes	27 (45)	25 (56.8)	0.234
No	33 (55)	19 (43.2)	
Anemia			
Yes	19 (31.7)	11 (25)	0.458
No	41 (68.3)	33 (75)	
Sedimentation rate			
Yes	42 (70)	29 (65.9)	0.658
No	18 (30)	15 (34.1)	
Sputum			
Negative	17 (28.3)	20 (45.5)	0.072
Positive	43 (71.7)	24 (54.5)	

Table 3. Comparison of severity of pulmonary tuberculosis and treatment outcome between smokers and non-smokers

Clinical characteristics	Population examined		p
	Smokers n (%)	Non-smokers n (%)	
	60 (57.7)	44 (42.3)	
Case type			
New	46 (76.7)	40 (90)	0.050
Retreatment	14 (23.3)	4 (10)	
Diagnosis			
PTB unilateral	14 (23.3)	14 (31.8)	0.273
PTB bilateral	18 (30)	17 (38.6)	
PTB with multiple cavities	23 (38.3)	9 (20.5)	
Pleural effusion	5 (8.3)	4 (9.1)	
Location of CXR abnormality			
Upper field	41 (68.3)	27 (61.4)	0.390
Medium field	11 (18.3)	7 (15.9)	
Lower field	8 (13.4)	10 (22.7)	
Radiological severity			
Initial	21 (48.1)	29 (65.9)	0.002
Advanced tuberculosis	39 (51.9)	15 (34.1)	
Outcomes			
Cure	46 (44.2)	39 (97.5)	0.475
Relapse	11 (10.6)	4 (3.8)	
Death	3 (2.9)	1 (1)	
Intra hospital therapy (days)			
< 30	11 (18)	9 (20.5)	0.786
> 30	49 (81.7)	35 (79.5)	

CXR – chest X-ray; PTB – pulmonary tuberculosis

Table 4. Multivariate logistic analysis of the association of demographic characteristics and risk factors with smoking

Factors	OR (CI 95%)	p
Age (years)		
20–29 (reference)	-	-
30–39	11.18 (1.75–71.52)	0.011
40–49	19.66 (2.67–144.94)	0.003
50–59	9.06 (2.10–39.01)	0.003
> 60	5.29 (1.21–23.04)	0.027
Sex	0.8 (0.28–2.25)	0.615
Alcohol use	9.32 (1.12–77.67)	0.039
Social determinants	2.01 (0.72–5.66)	0.172

OR – odds ratio; CI – confidence interval

with infected persons, as well as poor living conditions such as overcrowding and poor ventilation. There are no detailed data on the global prevalence of risk factors, so it is assumed that the prevalence of risk factors is the same in all segments of the adult population.

Smokers suffering from TB had a more severe clinical and radiological presentation of the disease, a more common sputum-positive TB in the beginning, as well as two months after the treatment, a lower therapy success rate, and a higher risk of relapse [13]. Patients treated for TB who were included in our study were mostly smokers (57.7%). This is a higher percentage of smokers than in studies conducted in different parts of the world, including countries with high PTB prevalence [9, 10, 15]. A similar number of smokers suffering from TB were recorded in Russia (49%) and Spain (48%). Also, the number of smokers among our patients suffering from PTB was higher than in the general population, where it amounted to 41.2% in people between the ages of 18 and 64 years [8, 22].

Socio-demographic characteristics of smokers and non-smokers suffering from PTB included in our study did not notably differ. A significant difference was detected in relation to patients' sex. Smokers suffering from TB were mostly middle-aged men, between 30 and 49 years of age, who were manual laborers [23]. Among the non-smokers, ill with TB were mainly females between 20 and 29 years of age, from the rural environment. The traditional way of behavior among unemployed women from the countryside continues to be present in this region, so that women are less likely to be smokers. Place of residence, family status, and education level did not differ significantly, which is in contrast with data from other regions, where there the number of affected smokers in the rural areas was greater [24]. The results of the 2013 Health Survey in Serbia show that the percentage of smokers in the cities is statistically significantly higher compared to inhabitants of rural areas. It also showed that there are fewer females among smokers.

Risk factors considered important in the occurrence of PTB were a possible contact with an affected person and a positive family history. However, these were equally present in non-smokers and smokers. We obtained the same results concerning comorbidities. Our study was not able to confirm the existing evidence that the association of smoking and diabetes increases the risk of developing TB, probably because among our patients, smokers suffering from TB were mostly middle-aged persons who did not suffer from diabetes mellitus [23, 25]. Older patients suffering from TB who were treated for diabetes mellitus were rarely smokers.

The risk factors are more common in men than in women, which was concluded in the study that covered 14 countries with the highest rate of TB incidence. In addition to cigarette smoking, alcohol consumption and the social determinant were the most important predictors for developing PTB among our subjects. Men who drank alcohol and smoked cigarettes were more susceptible to TB. Among the general population, 4.7% were daily alcohol consumers, while the percentage was several times higher among our TB patients (31.7%). There were a significant number of smokers who were also alcohol consumers [18]. Among the affected women, there were fewer smokers and they rarely drank alcohol [19]. Fewer smokers and alcohol consumers among our female patients treated for PTB can be explained by the fact that these habits are not socially acceptable in their social settings, where men drink six times more than women.

Important symptoms in the diagnosis of TB are the following: a cough present for at least two weeks, sputum, fever, night sweats, weight loss, asthenia, and hemoptysis [18]. A single symptom can be present, or there can be a combination of several TB-sensitive symptoms. In patients involved in our study, the symptoms of the disease occurred earlier in smokers than in non-smokers, but there were no significant differences present in the manifestations of symptoms of the disease, which was not in line with other studies. We confirmed that the sputum-positive PTB was more common in smokers. Smokers who suffer from TB are likely to have a greater ability to spread germs and a greater risk to infect their family members. It is possible to protect the family by changing their habits and quitting smoking. In patients with PTB, we detected laboratory abnormalities such as anemia and accelerated erythrocyte sedimentation rate, which did not significantly differentiate between smokers and non-smokers. Chronic infections, including TB, cause anemia, which is explained by the suppression of erythropoiesis of inflammatory mediators. On the other hand, the disruption of iron homeostasis develops with an increased absorption and retention of iron in the reticuloendothelial system during a chronic infection, such as TB [26].

Cigarette smoking is associated with an increased risk of advanced and more severe forms of the disease, such as cavitation, a positive sputum culture, and subsequent conversion of sputum culture after starting the treatment. Smoking has adverse effects on the completion of the treatment and relapse [27, 28]. In our study, smokers with PTB were more likely to experience bilateral changes on lung parenchyma or caverns. An Indian study showed that among non-smokers there were more people with minimal changes, while extensive changes were more common among smokers. Cavitation is more common in smokers [29].

In our patients, in addition to severe clinical manifestations of the disease, poorer treatment outcomes and relapse were significantly more frequent. This is consistent with other studies, where relapse of TB was recorded in 10.4% of patients [30]. For example, in a survey conducted in Georgia, smokers had a 70% poorer outcome than non-smokers [22]. Patients experiencing extensive changes on the lung parenchyma often suffer from relapses and are at increased risk of mortality [29, 30]. Among our patients, there were more death outcomes in smokers suffering from TB. We could not statistically confirm this fact due to a relatively small sample and because the treatment of PTB in the Northern Kosovo region is mostly successful. The effect of smoking on clinical parameters (lung cavitation and positive sputum culture) and a slower sputum conversion rate after the start of treatment has a serious impact on the prevention of disease transmission. Even in patients who are sensitive to treatment with tuberculostatics, the success rate of the treatment is lower than the desired 85%, which is the objective set by the World Health Organisation.

Several lifestyle factors are associated with an increased risk of PTB, including smoking and alcohol abuse [15, 16]. Smoking and excessive alcohol consumption are major health risks globally and are targets for interventions to reduce the global burden of disease. Ensuring that patients make appropriate lifestyle changes would help reduce the overall burden of PTB.

The impact on the social determinants that are significant predictors for developing PTB must take place through a number of actions at the social level in order to minimize poverty and promote better education on prevention measures. Integrated public health programs are needed, which can help reduce the number of patients with diabetes mellitus, smoking, and excessive alcohol use [31].

CONCLUSION

Smokers suffering from TB were more often middle-aged males that consumed alcohol and lived in poor social conditions. They had more severe clinical manifestations of TB with extensive X-ray changes in the lungs, often with caverns. Smokers who suffered from TB had a higher risk of relapse. The risk of death was higher in smokers than in non-smokers.

A detailed understanding of the diffusion of smoking in our environment, as well as the socio-demographic and clinical factors associated with smoking among patients with PTB, is the first step towards the formation of effective strategies for early diagnosis, control, and monitoring, in order to reduce the number of patients and improve treatment outcomes. Smoking is a risk factor for more frequent incidence of PTB with severe clinical forms and poorer treatment outcome. A part of the strategy for eradicating TB needs to be directed towards the campaign against smoking.

REFERENCES

1. World Health Organization. Global tuberculosis report 2016. Geneva: World Health Organization. 2016 (WHO/HTM/TB/2016).
2. ASPA. The Health Consequences of Smoking–50 Years of Progress: A Report of the Surgeon General, 2014. Available from: http://www.surgeongeneral.gov/library/reports/50- years-of progress/ index.html
3. Aryanpur M, Mortaz E, Masjedi MR, Tabarsi P, Garssen J, Adcock IM, et al. Reduced Phagocytic Capacity of Blood Monocyte/ Macrophages in Tuberculosis Patients Is Further Reduced by Smoking. Iran J Allergy Asthma Immunol. 2016; 15(3):174–82.
4. Aryanpur M, Masjedi MR, Hosseini M, Mortaz E, Tabarsi P, Soori H, et al. Cigarette smoking in patients newly diagnosed with pulmonary tuberculosis in Iran. Int J Tuberc Lung Dis. 2016; 20(5):679–84.
5. Altet-Gômez MN, Alcaide J, Godoy P, Romero MA, Hernández del Rey I. Clinical and epidemiological aspects of smoking and tuberculosis: a study of 13038 cases. Int J Tuberc Lung Dis. 2005; 9(4):430–6.
6. Zellweger JP, Cattamanchi A, Sotgiu G. Tobacco and tuberculosis: could we improve tuberculosis outcome by helping patients to stop smoking? Eur Respir J. 2015; 45(3):583–5.
7. Basu S, Stuckler D, Bitton A. Projected effects of tobacco smoking on worldwide tuberculosis control: mathematical modelling analysis. BMJ. 2011; 4(343):2–11.
8. Hernáiz CR, Giardín JM, Clotet CN, Lebrato CJ. Tuberculosis and immigration in an area of southwest Madrid. Int J Tuberc Lung Dis. 2016; 20(4):530–5.
9. Wang J, Shen H. Review of cigarette smoking and tuberculosis in China: intervention in needed for smoking cessation among tuberculosis patients. BMC Pub Health. 2009; 9:292.
10. Gajalakshmi V, Peto R. Smoking, drinking and incident tuberculosis in rural India: population-based case–control study. Int J Epidemiol. 2009; 38(4):1018–145.
11. Huang CC, Tchetgen E, Becerra MC, Cohen T, Galea J, Calderon R, et al. Cigarette smoking among tuberculosis patients increases risk of transmission to child contacts. Int J Tuberc Lung Dis. 2014; 18(11):1285–91.
12. van Zyl-Smit RN, Binder A, Meldau R, Semple PL, Evans A, Smith P, et al. Cigarette smoke impairs cytokine responses and BCG containment in alveolar macrophages. Thorax. 2014; 69(4):363–70.
13. Gegia M, Magee MJ, Kempker RR, Kalandadze I, Chakhaia T, Golub JE, et al. Tobacco smoking and tuberculosis treatment outcomes: a prospective cohort study in Georgia. Bull World Health Organ. 2015; 93(6):390–9.
14. Feng Y, Kong Y, Barnes PF, Huang FF, Klucar P, Wang X, et al. Exposure to cigarette smoke inhibits the pulmonary T-cell response to influenza virus and *Mycobacterium tuberculosis*. Infect Immun. 2011; 79(1):229–37.
15. Davis A, Terlikbayeva A, Aifah A, Hermosilla S, Zhumadilov Z, Berikova E, et al. Risks for tuberculosis in Kazakhstan: implications for prevention. Int J Tuberc Lung Dis. 2017; 21(1):86–92.
16. Patra J, Jha P, Rehm J, Suraweera W. Tobacco smoking, alcohol drinking, diabetes, low body mass index and the risk of self-reported symptoms of active tuberculosis: individual participant data (IPD) meta-analyses of 72,684 individuals in 14 high tuberculosis burden countries. PLoS One. 2014; 9(5):e96433.
17. Lin HH, Ezzati M, Murray M. Tobacco smoke, indoor air pollution and tuberculosis: a systematic review and meta-analysis. PLoS Med. 2007; 4(1):e20.
18. Jiménez-Fuentes MA, RodrigoT, Altet MN, Jiménez-Ruiz AC, Casals M, Antón Penas A. Factors associated with smoking among tuberculosis patients in Spain. BMC Infect Dis. 2016; 16:486.
19. Glaziou P, Falzon D, Floyd K, Raviqilione M. Global epidemiology of tuberculosis. Semin Respir Crit Care Med. 2013; 34(1):3–16.
20. Ferrara G, Murray M, Winthrop K, Centis R, Sotgiu G, Migliori GB, et al. Risk factors associated with pulmonary tuberculosis: smoking, diabetes and anti-TNFα drugs. Curr Opin Pulm Med. 2012; 18(3):233–40.
21. WHO global report: mortality attributable to tobacco. Geneva: World Health Organization 2012; (Accessed 9 Septmber 2014). Available from: http://www.who.int/tobacco/publications/ surveillance/ rep_mortality_attributable/en/
22. WHO Report on the Global Tobacco Epidemic 2013. World health organization. Available from: http://www.who.int/gho/countries/en/
23. Bai KJ, Lee JJ, Chien ST, Suk CW, Chiang CY. The influence of smoking on pulmonary tuberculosis in diabetic and non-diabetic patients. PLoS One. 2016; 11(6):e0156677.
24. Kolappan C, Gopi PG. Tobacco smoking and pulmonary tuberculosis. Thorax. 2002; 57(11):964–6.
25. Reed GW, Choi H, Lee SY, Lee M, Kim Y, Park H, et al. Impact of diabetes and smoking on mortality in tuberculosis. PLoS One. 2013; 8(2):e58044.
26. Pednekar MS, Hakama M, Gupta PC. Tobacco use or body mass – do they predict tuberculosis mortality in Mumbai, India? Results from a population-based cohort study. Plos One. 2012; 7:e39443.
27. Leung CC, Yew WW, Chan CK, Chan KC, Law WS, Lee SN, et al. Smoking adversely affects treatment response, outcome and relapse in tuberculosis. Eur Respir J. 2015; 45(3):738–45.
28. Lin HH, Murray M, Cohen T, Colijn C, Ezzati M. Effects of smoking and solid-fuel use on COPD, lung cancer, and tuberculosis in China: a time-based, multiple risk factor, modelling study. Lancet. 2008; 372(9648):1473–83.
29. Waitt CJ, Squire SB. A systematic review of risk factors for death in adults during and after tuberculosis treatment. Int J Tuberc Lung Dis. 2011; 15(7):871–5.
30. Mahishale V, Patil b, LollyM, Eti A, Khan S. Prevalence of smoking and its impact on treatment outcomes in newly diagnosed pulmonary tuberculosis patients: a hospital-based prospective study. Chonnam Med J. 2015; 51(2):86–90.
31. Lönnroth K, Castro KG, Chakaya JM, Chauhan LS, Floyd K, Glaziou P, et al. Tuberculosis control and elimination 2010–50: cure, care, and social development. Lancet. 2010; 375(9728):1814–29.

Утицај пушења на клиничке карактеристике, радиолошке промене и исход лечења оболелих од туберкулозе плућа

Соња Смиљић

Универзитет у Приштини, Медицински факултет, Институт за физиологију, Косовска Митровица, Србија

САЖЕТАК

Увод/Циљ Циљ нашег истраживања био је да утврдимо утицај пушења на клиничке карактеристике, обим радиолошких промена и исход лечења оболелих од туберкулозе плућа.

Методологија Проспективном студијом обухватили смо све оболеле од туберкулозе плућа (ТБП) лечене на Одељењу пулмологије Клиничко-болничког центра у Косовској Митровици у периоду од 2010. до 2016. године.

Резултати Међу пушачима оболелим од ТБП било је значајно више особа мушког пола ($p = 0,05$), старости између 30 и 49 година ($p < 0,001$). Пушачи су значајно више конзумирали алкохол ($p < 0,001$) и имали су изражен социјални фактор за обољевање од ТБП ($p = 0,002$). Тежа клиничка форма ТБП са кавернама била је чешћа код пушача (38,8%), а код непушача лакша, паренхиматозна, једнострана плућна туберкулоза (31,8%). Обимне радиолошке промене су биле израженије код пушача ($p = 0,002$). Рецидив болести се чешће јављао код пушача ($p = 0,05$). У мултиваријантној логистичкој регресији ризик да неко буде пушач јесу године живота 30–39 ($OR = 11,18$), 40–49 ($OR = 19,66$) и 50–59 ($OR = 9,06$) и навика конзумације алкохола ($OR = 9,32$).

Закључак Пушачи су чешће боловали од спутум позитивне туберкулозе плућа, имали су обимније радилошке промене и рецидив болести је био чешћи. У највећем броју били су средњих година живота са навиком конзумирања алкохола и чинили су групу оболелих чије навике представљају главни разлог за искорењивање туберкулозе.

Кључне речи: туберкулоза плућа; пушење; алкохол; РТГ абнормалности

DOI: https://doi.org/10.2298/SARH1806271M
UDC: 616.31-006.6; 616.98:578.827

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Prevalence of human papillomavirus in oropharyngeal squamous cell carcinoma in Serbia

Jovica Milovanović[1,2], Ana Jotić[1,2], Dragoslava Andrejić[3], Aleksandar Trivić[1,2], Bojan Pavlović[1,2], Katarina Savić-Vujović[4], Ana Banko[5], Anđela Milovanović[6], Vojko Đukić[1,2]

[1]Clinical Center of Serbia, Clinic for Otorhinolaryngology and Maxillofacial Surgery, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Department of Otorhinolaryngology and Maxillofacial Surgery, Belgrade, Serbia;
[3]Dr Simo Milošević Primary Healthcare Center, Belgrade, Serbia;
[4]University of Belgrade, Faculty of Medicine, Department of Pharmacology, Clinical Pharmacology and Toxicology, Belgrade, Serbia;
[5]University of Belgrade, Institute of Microbiology and Immunology, Faculty of Medicine, Serbia;
[6]Clinical Center of Serbia, Clinic for Physical Medicine and Rehabilitation, Belgrade, Serbia

SUMMARY

Introduction/Objective Oropharyngeal carcinoma makes up to 3% of all newly diagnosed carcinomas in the world. In Serbia, oropharyngeal carcinoma constitutes 1.8% of all malignancies. Studies have shown a growing role of infections with human papilloma viruses (HPV) in oropharyngeal cancer etiology. HPV-positive patients have a more favorable prognosis and significantly higher rate of overall survival. The purpose of this paper was to establish how HPV status influenced Serbian patients' overall survival and the disease-free survival according to known risk factors (tobacco and alcohol consummation), clinical TNM stage of the disease, and modality of treatment.

Methods The study included 87 patients treated for oropharyngeal carcinoma in a one-year period with a five-year follow-up. Treatment modalities included surgery with or without postoperative radio- or chemoradiotherapy, only radiotherapy or chemoradiotherapy. Sex, common risk factors, TNM stage, and treatment method were considered, as well as the influence of HPV status on the overall survival and the disease-specific survival depending on the presence of risk factors.

Results HPV-positive patients with oropharyngeal carcinoma were more frequently men, smokers, and alcohol consumers. Considering clinical T, N, and M stage of the disease, the overall survival and the disease-specific survival rates were better in HPV-positive patients, who had better survival if they were treated with primary surgical therapy rather than primary radiotherapy.

Conclusion HPV status significantly influenced survival and locoregional control in Serbian patients with oropharyngeal carcinoma. This implies possible modifications of treatment strategies for these patients in order to further improve their prognosis and treatment outcomes.

Keywords: oropharyngeal carcinoma; human papillomavirus; overall survival; disease-specific survival

INTRODUCTION

Oropharyngeal squamous cell carcinoma (OPSCC) makes up to 3% of all newly diagnosed carcinomas in the world, with majority occurring in developing countries [1]. In Serbia, oropharyngeal carcinoma constitutes 1.8% of all malignancies. According to the latest available data from 2013, the ascertained incidence of diagnosed oropharyngeal squamous cell carcinoma in the general population of Serbia was 5.3/100,000 [2].

There have been changes in demographic characteristics, risk factors, and subsite of the OPSCC over the last few decades. Though they are still more frequent in men than in women, incidence was higher in younger white males, for human papilloma virus (HPV)-related cancers [3]. Subsites, like lingual and palatine tonsils, became more frequently involved, followed by the base of the tongue [4]. Studies in European countries and the United States have shown a growing role of infections with HPV in increasing incidence of OPSCC [5]. In the United States, approximately 40–80% of oropharyngeal carcinomas are considered to be caused by HPV [6]. However, these patients have a more favorable prognosis and significantly higher rate of overall survival, with reported 50% improvement in the overall survival compared with HPV-negative patients [7, 8]. Studies that compare HPV status and outcomes of treatment in patients with OPSCC are trending in the literature, and this is the first study of that kind done on patients with oropharyngeal carcinoma in Serbia.

The purpose of this paper was to establish the demographic characteristics and risk factors in patients treated for OPSCC in Serbia, and to contemplate how HPV status influenced the patients' overall survival and disease-free survival taking into consideration clinical TNM stage of the disease and the modality of treatment.

Received • Примљено:
February 28, 2017
Revised • Ревизија:
October 23, 2017
Accepted • Прихваћено:
October 24, 2017

Correspondence to:
Ana JOTIĆ
Clinic for Otorhinolaryngology and Maxillofacial Surgery
Clinical Centre of Serbia
Pasterova 2
11000 Belgrade, Serbia
anajotic@yahoo.com

METHODS

The study included 87 patients treated for OPSCC at the Clinic for Otorhinolaryngology and Maxillofacial Surgery of the Clinical Centre of Serbia in Belgrade in a one-year period (from January 2009 to January 2010). This study was approved by the Institutional Ethics Committee (440/IX-3/09), and all the patients signed the informed consent form prior to their inclusion in the study. The patients were divided into age groups according to the International Cancer Survival Standard using the Five Default Age Groups (15–44, 45–54, 55–64, 65–74, 75+) [9]. Risk factors such as continuous tobacco use and alcohol consumption during the follow-up period were also noted. The patients were treated after undergoing necessary diagnostic procedures (clinical examination, tumor biopsy, and histopathology verification, radiological diagnostics). Patients with previous treatment or relapse of the OPSCC were not included in the study. HPV positivity was estimated by positive p16 immunohistochemical staining of the tissue samples [10, 11]. The modality of treatment for every patient was decided on the Oncological Board (consisting of a radiotherapist, head and neck surgeons, an oncologist, and a histopathologist). The choice of primary and adjuvant treatment was decided based on the National Comprehensive Cancer Network and the American Society of Radiation Oncology guidelines, which are recommended and used at the Clinic for Otorhinolaryngology and Maxillofacial Surgery and the Institute for Oncology and Radiology of Serbia in Belgrade [12, 13]. Surgical therapy involved resection of the tumor with some form of neck dissection in case of cervical lymphadenopathy. Radiotherapy consisted of external radiotherapy with a total dose of 60–70 Gy in 30–35 fractions for six to seven weeks. The patients received chemotherapy concurrently with radiotherapy; three courses of cisplatin intravenously, on the first, fourth, and seventh week during radiotherapy. The follow-up period was five years. The patients were examined every month postoperatively during the first year, every three months during the second and third year, and every six months during the fourth and fifth year. Lethal outcome and relapse of the disease was noted in the follow-up period.

Overall survival and disease-free survival in HPV-positive and HPV-negative patients were analyzed depending on demographic data of the patients, such as age and sex, common risk factors, tobacco and alcohol use, T, N, M stage of the disease, and the modality of treatment. Overall survival and the disease-specific survival were assessed at one, three, and five years after the treatment.

IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) was used for the data analysis. To determine demographic characteristics of the patients, descriptive statistics were used. The χ^2 test was used to compare monitored parameters between groups. Overall survival and the disease-specific survival were calculated according to the Kaplan–Meier method; the logrank test was used to compare survival parameters between patient groups. P-values lower than 0.05 were considered statistically significant.

RESULTS

The study comprised 69 (79.3%) males and 18 (20.7%) females of an average age of 59.64 years (SD ± 8.46 years). HPV-positive patients were of an average age of 54.5, and HPV-negative of 64 years. The youngest patient was 35, and the oldest 82 years old. Continuous tobacco use during the follow-up period was noted in 53 (60.9%) of the patients, and 34 (39,1%) were non-smokers. Continuous consumption of alcohol during the follow-up period was noted in 47 (54%) of the patients, while 40 (46%) were non-drinkers. Fourty-eight (55.2%) patients were HPV-positive. The patients were in different stages of the disease, without evident majority of the patients in a certain stage. Most of the patients were treated with surgery, followed by postoperative radiotherapy or chemoradiotherapy and concomitant chemoradiotherapy (Table 1).

Three-year and five-year overall survival was better for all age groups in HPV-positive patients, but the difference wasn't statistically significant. Overall survival was better for male patients who were HPV-positive after one-, three-, and five-year periods, and for women who were

Table 1. Characteristics of the patients included in the study

Parameter	HPV-negative n (%)	HPV-positive n (%)	Total n (%)
Age (years)			
45	2 (2.3)	3 (3.4)	5 (5.8)
45–54	3 (3.4)	15 (17.2)	18 (20.7)
55–64	23 (26.4)	17 (19.6)	40 (46)
65–74	16 (18.4)	5 (5.8)	21 (24.1)
≥ 75	3 (3.4)	0 (0)	3 (3.4)
Sex			
male	34 (39.1)	35 (40.2)	69 (79.3)
female	13 (14.9)	5 (5.8)	18 (20.7)
Smoking			
Smokers	27 (31)	26 (29.9)	53 (60.9)
Non-smokers	20 (23)	14 (16.1)	34 (39.1)
Alcohol			
Consumers	23 (26.4)	24 (27.6)	47 (54)
Non-consumers	24 (27.6)	16 (18.4)	40 (46)
T stage			
T1	8 (9.2)	6 (6.9)	14 (16.1)
T2	9 (10.3)	13 (14.9)	22 (25.2)
T3	12 (13.8)	15 (17.2)	27 (31)
T4	17 (19.6)	7 (8)	24 (27.6)
N stage			
N0	19 (21.9)	11 (12.6)	30 (34.5)
N1–3	28 (32.2)	29 (33.3)	57 (29.9)
M stage			
M0	44 (50.7)	37 (42.5)	81 (93.2)
M1	3 (3.4)	3 (3.4)	6 (6.8)
Treatment			
OP	7 (8)	2 (2.3)	9 (10.3)
RT	5 (5.8)	3 (3.4)	8 (9.2)
OP + RT or OP + RT/CH	24 (27.6)	19 (21.9)	43 (49.5)
RT/CH	12 (13.8)	15 (17.2)	27 (31)

RT – radiotherapy; RT/CH – chemoradiotherapy; OP – surgery

Table 2. Overall survival for HPV-negative and HPV-positive patients depending on their age, sex, tobacco use, alcohol consumption, TNM stage, and treatment modality

Parameter	1-year overall survival (%)		3-year overall survival		5-year overall survival		p
	HPV-negative	HPV-positive	HPV-negative	HPV-positive	HPV-negative	HPV-positive	
Age (years)							
45	50	100	50	50	50	50	0.038
45–54	66.7	92.3	66.7	46.2	33.3	38.5	
55–64	73.9	100	56.5	76.9	56.5	76.9	
65–74	75	100	62.5	80	56.3	60	
≥ 75	100	/	66.7	/	66.7	/	
Sex							
Male	62.9	96.8	45.7	64.2	45.7	56.7	0.042
Female	83.3	100	83.3	80	83.3	60	
Smoking							
Smokers	55.6	100	40.7	59.4	40.7	37.8	0.001*
Non-smokers	90	92.9	75	85.1	75	85.1	
Alcohol							
Consumers	76.2	95.7	61.9	66.2	61.9	56	0.558
Non-consumers	65.4	100	50	66.7	50	58.3	
T stage							
T1	100	100	100	83.3	85.7	66.7	0.002*
T2	92.3	100	84.6	87.5	84.6	62.5	
T3	69.2	92.3	46.2	67.1	46.2	67.1	
T4	35.7	100	21.4	57.1	21.4	28.6	
N stage							
N negative	78.9	100	68.4	100	68.4	85.7	0.018*
N positive	64.3	96.2	46.4	52.2	46.4	48.2	
M stage							
M0	68.9	96.9	55.6	65.4	55.6	54.5	0.531
M1	100	100	50	75	50	75	
Treatment							
OP	100	100	60	100	60	100	0.006*
RT	100	100	60	100	20	100	
OP+RT or OP+RT/HT	82.6	100	73.9	64.4	73.9	58	
RT+HT	33.3	90	13.3	64.3	13.3	64.3	

OP – surgery; RT – radiotherapy; RT/CH – chemoradiotherapy;
*p < 0.05

HPV-positive after a one-year period. However, this difference was not statistically significant (logrank test, p = 0.042) (Table 2). In addition, overall survival was better for HPV-positive non-smokers one, three, and five years after the treatment (logrank test, p = 0.001). Alcohol consumers and non-consumers who were HPV-positive had better overall survival rates after periods of one year and three years, but it was not statistically significant (logrank test, p = 0.558) (Figure 1).

Overall survival for patients who were HPV-positive was better compared to those who were HPV-negative for T1, T3, and T4 stage after one-, three-, and five-year periods (logrank test, p = 0.002) (Table 2). Overall survival was better in both groups of patients for those who had metastatic lymph nodes (96.2 vs. 64.3 for the period of one year, 52.2 vs. 46.4 for three years, and 48.2 vs. 46.4 for the period of five years), and in those without metastatic lymph nodes who were HPV-positive (100 vs. 78.9 for for the period of one year; 100 vs. 68.4 for three years, and 85.7 vs. 68.4 for the five-year period), compared to HPV-negative patients (logrank test, p = 0.018) (Figure 2). Significantly better overall survival was detected in HPV-positive patients treated with radiotherapy (logrank test, p = 0.006). HPV-positive patients had better overall survival one, three, and five years after the treatment if the primary treatment was surgery and chemoradiotherapy (Figure 2).

Disease-free survival was better for almost all age groups in HPV-positive patients, except in patients younger than 45 years, but the difference between the HPV-positive and HPV-negative groups was not statistically significant. Disease-free survival in HPV-positive male patients was better, compared to HPV-negative patients (96.8 vs. 80.3 for the period of one year, 76.7 vs. 58.4 for three years, and 72.2 vs. 58.4 for the five-year period, p = 0.153). Considering continuous smoking during the follow-up, HPV-positive patients had significantly better disease-free survival rates compared to HPV-negative ones (logrank test, p = 0.04) Alcohol consumption did not significantly influence the overall survival in our patients, although survival was higher in HPV-positive patients one year (95.7 vs. 88.9) and three years after the treatment (78.3 vs. 72.2) (Figure 3).

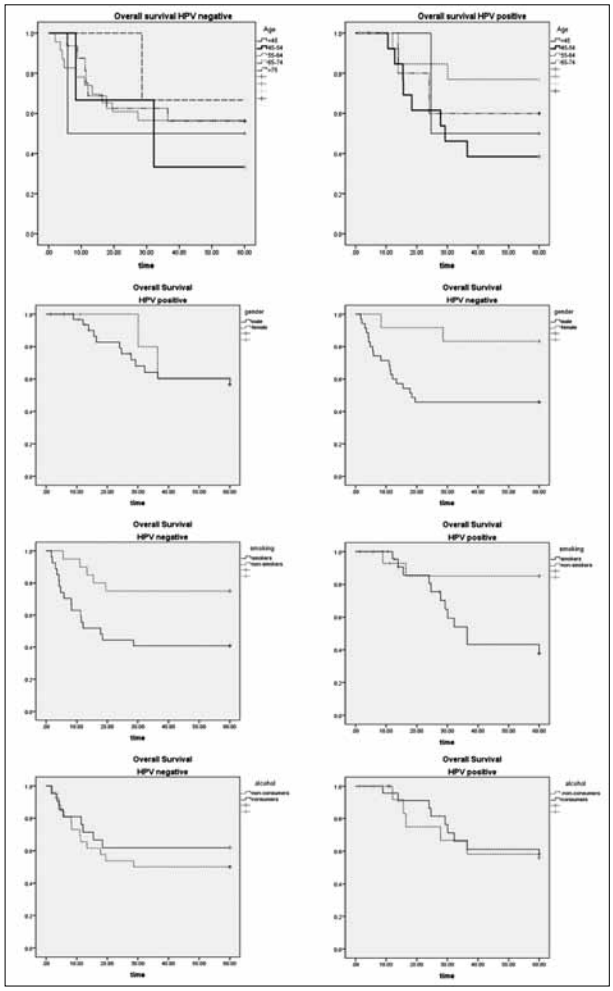


Figure 1. Kaplan–Meier curves for overall survival for HPV-negative and HPV-positive patients depending on age, sex, tobacco use, and alcohol consumption

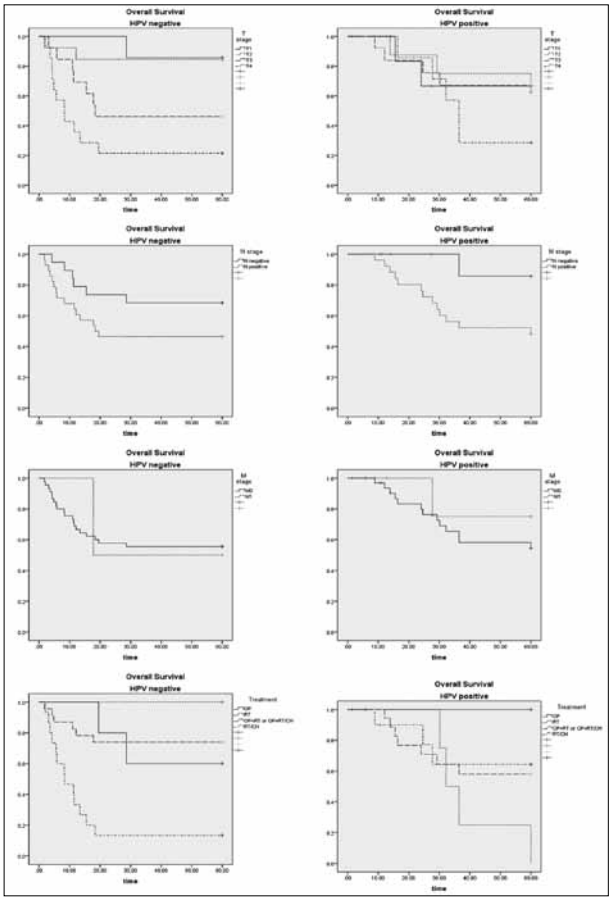


Figure 2. Kaplan–Meier curves for overall survival for HPV-negative and HPV-positive patients depending on the TNM stage and treatment modality

Disease-free survival for patients who were HPV-positive was better than in those who were HPV-negative for T1, T2, and T4 stage after one-, three-, and five-year periods (Table 3). Disease-free survival was better for HPV-positive patients with metastatic lymph nodes (96.2 vs. 64.3 for the one-year period, 52.2 vs. 46.4 for the three-year period, and 48.2 vs. 46.4 for the five-year period), compared to HPV-negative patients. Disease-free survival was worse in HPV-positive patients without metastatic lymph nodes than in those who were HPV-negative five years after the treatment (logrank test, p = 0.002). Regarding the M stage, better disease-free survival was again detected in the HPV-positive group of patients (Figure 4). Significantly better disease-free survival was detected in HPV-positive patients treated with radiotherapy after three- and five-year periods, compared to HPV-negative patients (100 vs. 60 for the three-year period and 100 vs. 20 for the five-year period (log rank test, p = 0.006). HPV-positive patients had better overall survival one year, three years, and five years after treatment if the primary treatment was surgery and chemoradiotherapy (Figure 4).

DISCUSSION

The correlation between outcomes and survival in patients with OPSCC with HPV positivity has been demonstrated in many studies [14, 15, 16]. No research was done in Serbia concerning this matter until the present study.

There were no significant differences between demographic characteristics in our study, comparing HPV-positive and HPV-negative patients. HPV-positive patients treated for OPSCC were more likely to be men (40.2%), smokers (26.4), and alcohol consumers (27.6%). In the HPV-positive group, women were less frequent (5.8% vs. 14.9%). Others studies documented the same findings, though it is noticed in the literature that the incidence of women diagnosed with OPSCC is rising [16, 17]. This trend is expected to demonstrate itself in the Serbian population as well.

Earlier research showed overall worse prognosis for active smokers with head and neck carcinoma, regardless of their HPV status [18, 19]. In our study, overall survival and the disease-free survival were better in HPV-positive patients who were not smokers. On the other hand, after a five-year follow-up period, HPV-negative smokers had better disease-free survival than non-smokers did, but no sig-

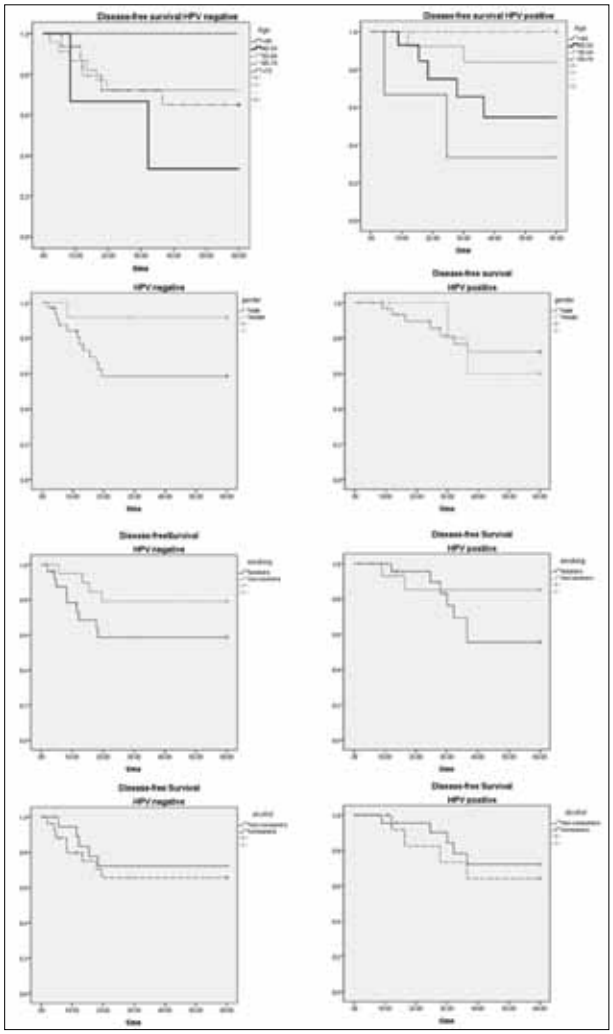


Figure 3. Kaplan–Meier curves for disease-free survival for HPV-negative and HPV-positive patients depending on age, sex, tobacco use, and alcohol consumption

nificant effect of smoking on the disease-free survival was detected. Similar results were published by Liskamp et al. [20]. Poorer results in smokers were attributed to hypoxia, microvascular damage, the immune suppressive effect of tobacco, and smoking-induced genetic alteration. Benson et al. [21] accounted age and tobacco for only 9% variation in overall survival by HPV status. The degree of cellular deregulations and response to therapy were proven to have bigger influence on improved prognosis in HPV-positive compared to HPV-negative patients. Alcohol consumption was not proven significant in overall and disease-free survival in our study, although some researches indicate that HPV positivity in non-drinking patients is a negative predicting factor [22].

Considering clinical T, N, and M stage of the disease, many studies to date have stressed better overall, disease-specific, and progression-free survival rates in HPV-positive OPSCC patients than in HPV-negative patients [14, 16, 23, 24]. This was observed in our study as well, except for some discrepancies in the three-year disease-free survival for T3 stage and in the five-year overall survival in T1 and T2 stages. These finding in our patients could be explained by lethal outcome caused by diseases other than cancer. Some publications identified advanced T stage (3 or 4) as a significant risk factor for overall survival, especially in HPV-negative patients [5, 14, 23]. On the other hand, N stage did not prove to be a statistically significant risk factor for overall survival, but rather extranodal spreading of the disease [14, 23]. In this study, disease-free survival was significantly longer in HPV-positive patients in advanced stages of the disease with positive nodal disease, compared to HPV-negative patients.

In our study, HPV-positive patients treated primarily with radiotherapy had better overall and disease-free survival compared to HPV-negative patients. A systematic

Table 3. Disease-free survival for HPV-negative and HPV-positive patients depending on their age, sex, tobacco use, alcohol consumption, TNM stage and treatment modality

Parameter	1-year disease-free survival (%)		3-year disease-free survival		5-year disease-free survival		p
	HPV-negative	HPV-positive	HPV-negative	HPV-positive	HPV-negative	HPV-positive	
Age (years)							
45	100	66.7	33.3	33.3	33.3	33.3	0.074
45–54	66.7	92.9	33.3	65.7	33.3	54.7	
55–64	86.7	100	72.3	83.9	72.3	83.9	
65–74	86.5	100	72.1	100	64.9	100	
≥ 75	100	/	100	/	100	/	
Sex							
Male	80.3	96.8	58.4	76.7	58.4	72.2	0.153
Female	91.7	100	91.7	80	91.7	60	
Smoking							
Smokers	73.3	100	58.7	69.2.	58.7	55.4	0.040*
Non-smokers	90.7	94.1	81.1	74.8	81.1	74.8	
Alcohol							
Consumers	88.9	95.7	72.2	78.3	72.2	72.3	0.442
Non-consumers	95	92.9	79.2	85.1	79.2	85.1	

T stage							
T1	100	100	100	100	100	100	0.000*
T2	100	100	91.7	100	91.7	100	
T3	100	92.3	88.9	67.1	66.7	67.1	
T4	42.2	100	25.3	57.1	25.3	28.6	
N stage							
N0	94.1	100	94.1	100	94.1	88.9	0.002*
N1–N3	76.5	95.8	51	65.7	51	60.2	
M stage							
M0	82.7	96.9	69.4	77.3	69.4	68.7	0.976
M1	100	100	50	75	50	75	
Treatment							
OP	100	100	80	100	80	100	0.006*
RT	100	100	60	100	20	100	
OP + RT or OP + RT/HT	90.9	100	81.3	87.7	81.3	78.9	
RT + HT	55	90	22	64.3	22	64.3	

OP – surgery; RT – radiotherapy; RT/CH – chemoradiotherapy;
*$p < 0.05$;

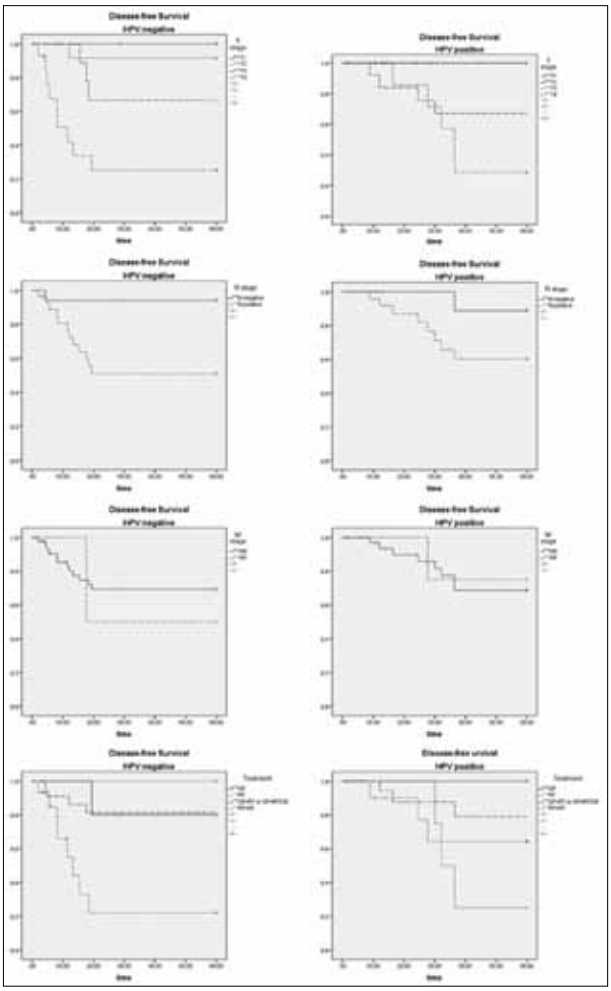


Figure 4. Kaplan–Meier curves for disease-free survival for HPV-negative and HPV-positive patients depending on the TNM stage and treatment modality

review, done in 2015, claimed better response rates to radiotherapy in HPV-positive patients, even in T3–4 tumors [25]. Also, for HPV-positive OPSCC patients, overall and disease-free survival was better if their primary treatment was surgical therapy rather than radiotherapy, which was confirmed by results. It was suggested that HPV-negative patients have poorer response to multimodality treatment than HPV-positive patients [26]. In addition, some studies indicated that HPV-positive patients after being treated with chemoradiotherapy had significantly longer time to distant metastatic failure compared to HPV-negative patients [27]. Considering patients treated with surgery and adjuvant radiotherapy or surgery with adjuvant chemoradiotherapy in our study, no significant differences were noted between the HPV-positive and HPV-negative groups. The management of locoregionally advanced oropharyngeal cancer still focuses on definitive chemoradiotherapy. In addition to organ preservation, concomitant chemoradiotherapy can offer significant improvement in the five-year overall survival in poor-prognosis tumors and survival could be prolonged up to 16% [28].

Given all the advances in understanding the role of HPV infection in OPSCC pathogenesis, treatment, and outcomes, new strategies are needed to achieve better results. Some recent studies suggested deintensification of therapy for low-risk patients (HPV-positive patients who smoke less than 10 pack years, and have low volume tumors). Proposed methods of deintensification include decreasing doses of radiation (de-escalation) or replacing cisplatin radio-sensitization to targeted therapy with cetuximab [29]. Minimally invasive surgical techniques are extensively used for the management of early-stage tumors, and they involve transoral robotic surgery and transoral laser microsurgery. Tumors previously resected through external and extensive surgical approaches are now being treated by the transoral approach with less morbidity and complications, achieving satisfactory locoregional control [30].

CONCLUSION

In our study, no significant differences between demographic characteristics were found in comparing HPV-positive and HPV-negative patients' overall survival and

disease-free survival. HPV-positive non-smokers had better overall survival and the disease-free survival. They also had better overall survival and disease-free survival depending on the clinical TNM stage of the disease. Due to significant influence of HPV positivity on survival and locoregional control of the disease, introduction of better diagnostic and therapeutic strategies in Serbia is needed. Routine detection of HPV should be done in all patients diagnosed with OPSCC before undergoing a specific oncology treatment. In addition to satisfactory oncological results, this would result in less morbidity, improved quality of life, and benefits to the patient.

REFERENCES

1. GLOBOCAN (2012). http://globocan.iarc.fr/old/summary_table_site_prev.asp?selection=13010&selection=21030&title=Lip%2C+oral+cavity%2C+Other+pharynx&sex=0&africa=1&america=2&asia=3&europe=4&oceania=5&build=6&window=1-&sort=0&submit=%C2%A0 Accessed 09 June 2016.
2. Institute of Public Health of Serbia Dr Milan Jovanovic- Batut, Center for prevention and control of noncommunicable diseases. Cancer incidence and mortality in central Serbia 2013. Cancer registry of Central Serbia 2013. 2015; 15
3. Brown LM, Check DP, Devesa SS. Oral cavity and pharynx cancer incidence trends by subsite in the United States: changing gender patterns. J Oncol. 2012; 2012:649498.
4. Weatherspoon DJ, Chattopadhyay A, Boroumand S, Garcia I. Oral cavity and oropharyngeal cancer incidence trends and disparities in the United States: 2000-2010. Cancer Epidemiol. 2015; 39(4):497–504.
5. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010; 363(1):24–35.
6. Mehanna H, Beech T, Nicholson T, El-Hariry I, McConkey C, Paleri V, et al. Prevalence of human papilloma virus in oropharyngeal and nonoropharyngeal head and neck cancer – systematic review and meta-analysis of trends by time and region. Head Neck. 2013; 35(5):747–55.
7. Ragin CC, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papilloma virus infection: review and meta-analysis. Int J Cancer. 2007; 121(8):1813–20.
8. O'Rorke MA, Ellison MV, Murray LJ, Moran M, James J, Anderson LA. Human papillomavirus related head and neck cancer survival: a systematic review and meta-analysis. Oral Oncol. 2012; 48(12):1191–201.
9. Corazziari I, Quinn M, Capocaccia R. Standard cancer patient population for age standardising survival ratios. Eur J Cancer. 2004; 40(15):2307–16.
10. Singhi AD, Westra WH. Comparison of human papilloma virus in situ hybridization and p16 immunohistochemistry in the detection of human papilloma virus-associated head and neck cancer based on a prospective clinical experience. Cancer. 2010; 116(9):2166–73.
11. Cai C, Chernock RD, Pittman ME, El-Mofty SK, Thorstad WL, Lewis Jr JS. Keratinizing-type squamous cell carcinoma of the oropharynx: P16 over expression is associated with positive high-risk HPV status and improved survival. Am J Surg Pathol. 2014; 38(6):809–15.
12. National Comprehensive Cancer Network. Head and neck cancers (Version 2.2016). http://www.nccn.org/professionals/physician_gls/pdf/head-and-neck.pdf. Accessed December 2016
13. Sher DJ, Adelstein DJ, Bajaj GK, Brizel DM, Cohen EEW, Halthore A, et al .Radiation therapy for oropharyngeal squamous cell carcinoma: Executive summary of an ASTRO Evidence-Based Clinical Practice Guideline. Pract Radiat Oncol. 2017; 7(4):246–53.
14. Amini A, Jasem J, Jones BL, Robin TP, McDermott JD, Bhatia S, et al. Predictors of overall survival in human papilloma virus-associated oropharyngeal cancer using the National Cancer Data Base. Oral Oncol. 2016; 56:1–7.
15. Sedghizadeh PP, Billington WD, Paxton D, Ebeed R, Mahabady S, Clark GT, et al. Is p16-positive oropharyngeal squamous cell carcinoma associated with favorable prognosis? A systematic review and meta-analysis. Oral Oncol. 2016; 54:15–27.
16. Lim MY, Dahlstrom KR, Sturgis EM, Li G. Human papilloma virus integration pattern and demographic, clinical, and survival characteristics of patients with oropharyngeal squamous cell carcinoma. Head Neck. 2016; 38(8):1139–44.
17. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005; 55(2):74–108.
18. Chen AM, Chen LM, Vaughan A, Sreeraman R, Farwell DG, Luu Q, et al. Tobacco smoking during radiation therapy for head-and-neck cancer is associated with unfavorable outcome. Int J Radiat Oncol Biol Phys. 2011; 79:414–9.
19. Hoff CM, Grau C, Overgaard J. Effect of smoking on oxygen delivery and outcome in patients treated with radiotherapy for head and neck squamous cell carcinoma – a prospective study. Radiother Oncol. 2012; 103(1):38–44.
20. Liskamp CP, Janssens GO, Bussink J, Melchers WJ, Kaanders JH, Verhoef CG. Adverse effect of smoking on prognosis in human papillomavirus-associated oropharyngeal carcinoma. Head Neck. 2016; 38(12):1780–7.
21. Benson E, Li R, Eisele D, Fakhry C. The clinical impact of HPV tumor status upon head and neck squamous cell carcinomas. Oral Oncol. 2014; 50(6):565–74.
22. Dediol E, Sabol I, Virag M, Grce M, Muller D, Manojlović S. HPV prevalence and p16INKa overexpression in non-smoking non-drinking oral cavity cancer patients. Oral Dis. 2016; 22(6):517–22.
23. Kumar B, Cipolla MJ, Old MO, Brown NV, Kang SY, Dziegielewski PT, et al. Surgical management of oropharyngeal squamous cell carcinoma: Survival and functional outcomes. Head Neck. 2016; 38(1):1794–802.
24. Goodman MT, Saraiya M, Thompson TD, Steinau M, Hernandez BY, Lynch CF, et al. Human papillomavirus genotype and oropharynx cancer survival in the United States of America. Eur J Cancer. 2015; 51(18):2759–6
25. Wang MB, Liu IY, Gornbein JA, Nguyen CT. HPV-positive oropharyngeal carcinoma: a systematic review of treatment and prognosis. Otolaryngol Head Neck Surg. 2015; 153(5):758–69.
26. Masterson L, Moualed D, Liu ZW, Howard JE, Dwivedi RC, Tysome JR, et al. De-escalation treatment protocols for human papilloma virus-associated oropharyngeal squamous cell carcinoma: a systematic review and meta-analysis of current clinical trials. Eur J Cancer. 2014; 50(15):2636–48.
27. Trosman SJ, Koyfman SA, Ward MC, Al-Khudari S, Nwizu T, Greskovich JF, et al. Effect of human papillomavirus on patterns of distant metastatic failure in oropharyngeal squamous cell carcinoma treated with chemoradiotherapy. JAMA Otolaryngol Head Neck Surg. 2015; 141(5):457–62.
28. Furness S, Glenny AM, Worthington HV, Pavitt S, Oliver R, Clarkson JE, et al. Interventions for the treatment of oral cavity and oropharyngeal cancer: chemotherapy. Cochrane Database Syst Rev. 2011; 13(4):CD006386.
29. O'Sullivan B, Huang SH, Siu LL, Waldron J, Zhao H, Perez-Donez B, et al. Deintensification candidate subgroups in human papillomavirus-related oropharyngeal cancer according to minimal risk of distant metastasis. J Clin Oncol, 2013; 31(5):543–50.
30. 3Hinni ML, Nagel T, Howard B. Oropharyngeal cancer treatment: the role of transoral surgery. Curr Opin Otolaryngol Head Neck Surg. 2015; 23(2):132–8.

Учесталост хуманог папилома вируса у орофарингеалном планоцелуларном карциному у Србији

Јовица Миловановић[1,2], Ана Јотић[1,2], Драгослава Андрејић[3], Александар Тривић[1,2], Бојан Павловић[1,2], Катарина Савић-Вујовић[4], Ана Банко[5], Анђела Миловановић[6], Војко Ђукић[1,2]

[1]Клинички центар Србије, Клиника за оториноларингологију и максилофацијалну хирургију, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Катедра за оториноларингологију са максилофацијалном хирургијом, Београд, Србија;
[3]Дом здравља „Др Симо Милошевић", Београд, Србија;
[4]Универзитет у Београду, Медицински факултет, Институт за фармакологију, клиничку фармакологију и токсикологију, Београд, Србија;
[5]Универзитет у Београду, Институт за микробиологију и имунологију, Медицински факултет, Београд, Србија;
[6]Клинички центар Србије, Клиника за физикалну медицину и рехабилитацију, Београд, Србија

САЖЕТАК

Увод/Циљ Орофарингеални карцином чини око 3% новодијагностикованих карцинома на свету. У Србији 1,8% болесника са малигнитетом болује од орофарингеалног карцинома. Студије указују на све већу улогу инфекције хуманим папилома вирусом (ХПВ). У литератури је показано да болесници који су позитивни на инфекцију ХПВ-ом имају бољу прогнозу и значајно веће укупно преживљавање.

Циљ овог рада је био да установи како ХПВ статус утиче на укупно преживљавање и преживљавање без знакова болести болесника са орофарингеалним карциномом у Србији, у зависности од познатих фактора ризика (конзумирање алкохола и цигарета), клиничког ТНМ стадијума и начина лечења.

Методе У студију је укључено 87 болесника којима је дијагностикован орофарингеални карцином током периода од једне године, са петогодишњим периодом праћења. Терапија је укључила хируршко лечење са постоперативном радиотерапијом или хемиорадиотерапијом, само радиотерапију или само хемиорадиотерапију. Испитивани су пол, фактори ризика, клиничка ТНМ класификација, као и утицај ХПВ статуса на укупно преживљавање и преживљавање без знакова болести у зависности од присуства фактора ризика.

Резултати Болесници са орофарингеалним карциномом позитивни на ХПВ најчешће су били мушког пола, пушачи и конзументи алкохола. Што се тиче клиничког ТНМ стадијума, преживљавање је било боље код ХПВ позитивних болесника. Боље преживљавање су имали ХПВ позитивни болесници лечени примарно хируршки у односу на оне примарно лечене радиотерапијом.

Закључак ХПВ статус значајно утиче на преживљавање и локорегионалну контролу код болесника са орофарингеалним карциномом у Србији. Ова чињеница указује на потребу модификовања терапијских стратегија за ове болеснике, у циљу даљег побољшавања прогнозе њихове болести и исхода терапије.

Кључне речи: орофарингеални карцином; хумани папилома вирус; укупно преживљавање; преживљавање без знакова болести

DOI: https://doi.org/10.2298/SARH170509173F
UDC: 616.24-002.5:613.84; 616.441-006.6

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Lymphocytic infiltration as a prognostic factor in papillary thyroid carcinoma

Aleksandar Filipović[1], Ljiljana Vučković[2]

[1]University of Montenegro, Faculty of Medicine, Clinical Centre of Montenegro, Department of Endocrine Surgery, Podgorica, Montenegro;
[2]University of Montenegro, Faculty of Medicine, Clinical Centre of Montenegro, Department of Pathology, Podgorica, Montenegro

SUMMARY

Introduction/Objective We examine the prognostic significance and association between lymphocytic infiltration and papillary thyroid carcinoma. This manuscript aims to establish whether the presence of lymphocytic infiltration in the classical type of papillary thyroid carcinoma is a favorable prognostic factor for survival.

Methods This is a retrospective study of patients treated for papillary thyroid carcinoma at the Clinical Centre of Montenegro over a period of seven years (2010–2017). A total of 105 patients aged 12 to 84 years were included in the study, of which 74% showed concomitant histological evidence of lymphocytic infiltration. The patients were divided into two groups – one with lymphocytic infiltration and the other without it. Anti-CD3 and anti-CD20 antibodies were used to identify T and B lymphocytes. The prognostic outcome was assessed using the Kaplan–Meier survival plots.

Results The cohort with lymphocytic infiltration revealed a lower frequency of extrathyroidal invasion ($p < 0.0001$), nodal metastases ($p < 0.0001$), and the absence of distant metastases, compared with those without lymphocytic infiltration. Chronic lymphocytic thyroiditis is a favorable prognostic factor for survival in our examined group ($p < 0.0001$).

Conclusion The present study shows that immune reaction involving lymphocytic infiltration plays a role in extrathyroidal tumor growth and development of nodal and distant metastases in patients with papillary thyroid cancer. The presence of lymphocytic infiltration is a favorable factor for survival in the classical form of papillary thyroid carcinoma.

Keywords: papillary thyroid carcinoma; lymphocytic infiltration; prognostic factors

INTRODUCTION

Lymphocytic infiltrates (LI) may be found in the context of or surrounding a variety of malignancies and are commonly believed to represent the host's immune response to the tumor [1]. A more favorable clinical evolution has been reported in various malignancies when associated with LI [2]. Heterogeneous immune infiltrates have been shown in diverse tissue types and may portend an improved prognosis [3, 4]. Different types of tumor-associated lymphocytes have been identified in thyroid cancer [5]. These consist of cells of the immune system, macrophages, mast cells, and neutrophils, and are associated with an adaptive immune response (T and B cells) [6]. Several studies have shown that patients whose tumors are not infiltrated by lymphocytes present a high recurrence rate, suggesting that the presence of lymphocytes in the thyroid tumor microenvironment indicate a favorable prognosis [7]. The results from a meta-analysis investigating the correlation between papillary thyroid carcinoma and Hashimoto's thyroiditis revealed that thyroiditis was more frequently observed in papillary thyroid cancer than in benign thyroid diseases, and cancer patients with thyroiditis had a longer duration of recurrence-free survival [8]. According to the current classifications of the World Health Organization, papillary carcinoma is the most frequent type of differentiated thyroid carcinoma. Differentiated thyroid cancer is a suitable model to study tumor-associated macrophages, as they are associated with signs of immune reaction, including LI. Surgery represents primary and basic treatment for papillary thyroid cancer. The occurrence of LI in patients with papillary carcinoma has been reported to predict fewer recurrences and improved survival [9].

The aim of our study is to determine the statistical relationship between groups with and without lymphocytic infiltration, from a retrospective review of our series of 105 patients with the classical type of papillary thyroid carcinoma, treated at the Clinical Centre of Montenegro from 2010 to 2017, and to establish whether the presence of lymphocytic infiltration in the classical type of papillary thyroid carcinoma is a favorable prognostic factor for survival.

Received • Примљено: May 9, 2017
Revised • Ревизија: September 4, 2017
Accepted • Прихваћено: September 6, 2017
Online first: September 15, 2017

Correspondence to:
Aleksandar FILIPOVIĆ
Department of Endocrine Surgery
Clinical Centre of Montenegro
Faculty of Medicine
University of Montenegro
Ljubljanska 1, 20000 Podgorica
Montenegro
a.filipovic@t-com.me

METHODS

This study was done at the Surgery Clinic of the Clinical Centre of Montenegro, and all data are from the history of diseases. This is a retrospective cohort study of patients treated for papillary thyroid carcinoma at the Clinical Centre of Montenegro over a period of seven years (2010–2017). A total of 105 patients aged 12 to 84 years were included in the study. Including factors are all patients with the classical type of papillary thyroid carcinoma. Excluding factors are all subtypes of papillary carcinoma – follicular, medullar, and anaplastic carcinomas. Based on insight into the medical documentation and medical history, the official pathohistological logbook of the Clinic of Pathology of the Clinical Centre of Montenegro, and the electronic database of the patients, we have collected all data regarding the following: age, sex, tumor size, presence of the lymph or distant metastases, histopathologic characteristics of the tumor, surgical treatment, tumor invasion, multicentric tumors, and clinical feature classification of tumors (tumor, node, metastasis). This study was carried out in archival, paraffin-embedded tissues from 105 patients with the classical form of papillary carcinoma. All the patients underwent a total thyroidectomy and central lymph node dissection, adjuvant radioactive iodine-131 therapy, and postoperative L-thyroxine TSH-suppressive therapy. After surgery, distant metastases were diagnosed, where, in addition to high serum thyroglobulin levels, the metastatic tissue was localized by total body scanning and other imaging tests (X-rays, computed tomography scan). A diagnosis of regional lymph node recurrence was made when ultrasound evidence of suspicious lymph nodes was confirmed by either radioiodine uptake or a cytological finding of neoplastic epithelial cells in a lymph node.

Patients were classified on the extent of disease at presentation as class I – patients with intrathyroid disease, class II – patients with positive cervical lymph nodes, class III – patients with extrathyroid tumor invasion, and class IV – patients with distant metastases. The patients were divided into two groups – those with or without lymphocytic infiltration. Both groups were mutually compared for their prognostic factors.

The group of patients with the presence of lymphocytic infiltration was divided into four groups of various extent of lymphocytic infiltration. The follow-up period was between six and 84 months.

The diagnosis of differentiated thyroid carcinoma was made according to the WHO criteria [10]. At the Institute of Pathology of the Clinical Centre of Montenegro, the samples obtained by surgical resection were fixed in 10% buffered formalin, then molded into paraffin blocks from which semi-serial cuts 3 μm thick were obtained. Based on hematoxylin and eosin processing of the sample, one molded extract from each processed case was obtained of the bordering area of non-necrotic tumor tissue and thyroid gland tissue.

The occurrence of peritumoral LI or Hashimoto's thyroiditis was recorded. The group of patients with the presence of lymphocytic infiltration was divided into four groups by various extent of lymphocytic infiltration. The degree of lymphocytic infiltration varied from a small number of lymphocytes (grade I), islands of lymphocytic infiltration in or around the tumor (grade II), thyroid tissue surrounding groups of a large number of lymphocytes (grade III), to Hashimoto's thyroiditis (grade IV) (Figure 1), characterized by the presence of Hürthle cells and a varying degree of acini atrophy. Identification of lymphocytes was done on hematoxylin and eosin-stained slides and confirmed using anti-CD3 and anti-CD20 antibodies for identification of T and B lymphocytes. All antibodies were obtained from Dako (Copenhagen, Denmark).

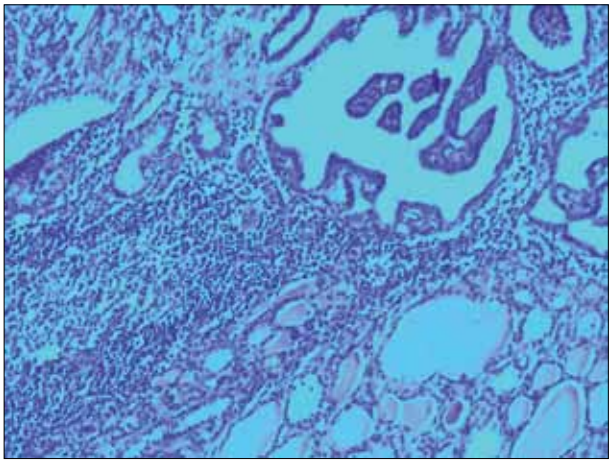


Figure 1. Lymphocytic infiltration – grade IV (H&E; ×10)

Student's t-test was used for comparison of clinical and pathological parameters between the groups. The survival curve (Kaplan–Meier) was used for comparing time-dependent variables (survival, death). Prognostic outcome was assessed using the Kaplan–Meier survival plots. The quantitative variables were expressed as mean ± standard deviation, while the categorical ones were presented as percentages. A univariate Cox regression analysis was performed in order to determine which variables were significantly associated with survival. Statistical analyses were performed using SPSS for Windows, Version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Histological evidence of lymphocytic infiltration was found in 74% of the patients evaluated. The mean or median age at diagnosis did not differ between the cohorts. Our series showed that lymphocytic infiltration could be identified in the thyroid tissue of patients of all ages, with the highest prevalence noted in the fifth decade of life.

Factors with a significant impact on survival rate include age, sex, size of tumor, invasiveness, and lymphogenic and hematogenic metastases, as shown in Table 2. The median tumor size was 20 mm in the group with lymphocytic infiltration, with a range of 3–60 mm. In the group without lymphocytic infiltration, median tumor size was 35 mm, with a range of 4–140 mm.

Table 1. Clinical characteristics and clinical stages of disease in patients with papillary thyroid carcinoma associated with lymphocytic infiltration (LI) or without it

Variable	LI present 78 (74%)	LI absent 27 (26%)	p
Age (years)	12–84 (48 ± 36)	25–72 (49 ± 23)	No significance (NS)
Sex (male:female)	1:7	1:4	< 0.0001
Tumor size (mm)	3–60 (20)	4–140 (35)	< 0.0001
Lymph metastases	21 (27%)	5 (18%)	NS
Distant metastases	0	4 (15%)	< 0.0001
Multicentric	11(14%)	4 (15%)	NS
Tumor invasion	8 (10%)	9 (33%)	< 0.0001
Stage I	37 (48%)	10 (37%)	NS
Stage II	25 (32%)	4 (15%)	NS
Stage III	7 (9%)	6 (22%)	< 0.0001
Stage IV	9 (11%)	7 (26%)	< 0.0001

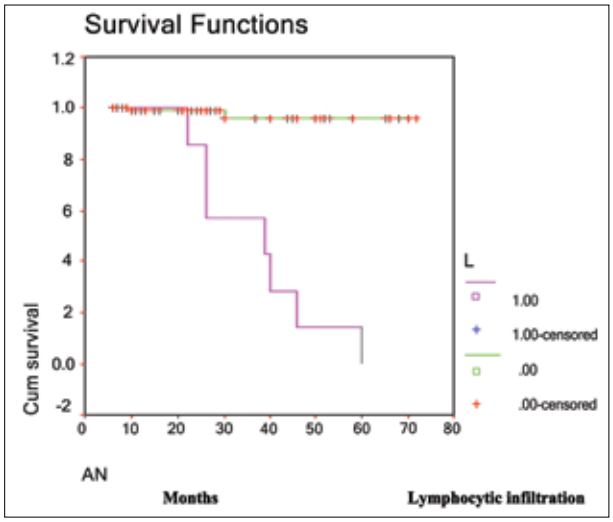


Figure 2. Data on disease-free survival and cancer-specific survival for the respective cohorts, with and without lymphocytic infiltration

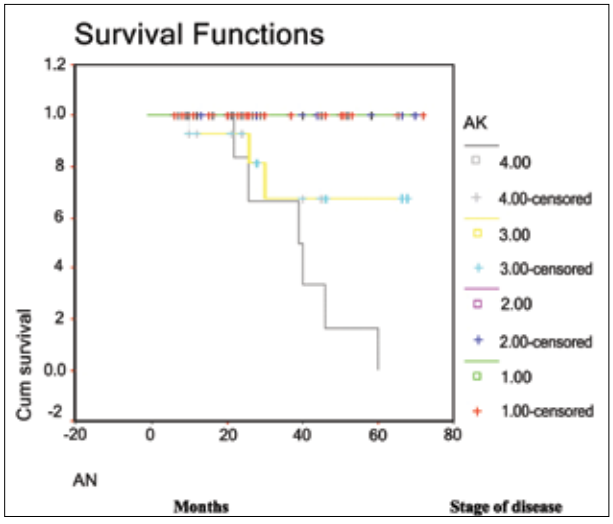


Figure 3. Comparison of disease-free survival of patients in different stages of the disease, showing a significant difference in the fourth stage

By means of univariate analysis, we found that factors with a significant impact on survival rate and shorter survival rate include age ($p < 0.0001$), tumor size ($p < 0.018$), regional tumor infiltration ($p < 0.0001$), and hematogenic metastases ($p < 0.049$).

In our study of age > 45 years, male, tumors up to 30 mm in size are a worse significant prognostic factor for survival ($p < 0.0001$).

A greater female preponderance was noted in the group with lymphocytic infiltration. At diagnosis, patients with lymphocytic infiltration tend to have more limited disease, with a significantly lower frequency of extrathyroidal invasion (10% vs. 33%), distant metastases (0% vs. 15%), and better survival compared to those without lymphocytic infiltration (Figure 2). The average overall survival was 97.3%. The average cancer-specific survival in the group with lymphocytic infiltration was 95%. The average cancer-specific survival in the group without lymphocytic infiltration was 72%.

There was no distant metastases occurrence in the group with lymphocytic infiltration.

The cohort without lymphocytic infiltration had a higher proportion of patients in the fourth stage of the disease ($p < 0.0001$), compared to those with lymphocytic infiltration (Figure 3).

DISCUSSION

The etiological ratio between thyroid carcinoma and lymphocytic infiltration remains a cause for concern. The presence of lymphocytic infiltration was noted in four fifths of our series of patients with papillary carcinoma.

The prognosis of thyroid cancer with Hashimoto's thyroiditis is better than that of patients with thyroid cancer alone [11]. About 75% of patients with papillary thyroid cancer had lymphocytic infiltration of the thyroid gland, and this group with infiltration had a high frequency of positive anti-thyroid antigen antibody, mild extent of the tumor at diagnosis, and better prognosis of non-recurrence of the tumor [12]. A character of infiltrated lymphocytes is cytotoxic T cells with natural or lymphokine-associated killer activity acting as carcinoma cell killers and secreting cytokines, such as interleukin-1, which inhibit thyroid carcinoma cell growth.

In the 10-year follow-up of patients with differentiated thyroid carcinoma, Matsubayashi et al. [2] found a significantly smaller incidence of recurrence in the group of patients with lymphocytic infiltration, compared to the group of patients without lymphocytic infiltration.

However, the presence of lymphocytic infiltration as part of the immune response has a significant impact on tumor variables, such as size of tumor, local invasiveness, incidence of lymphogenic and hematogenic metastases. The absence of lymphocytic infiltration is a poor prognostic factor in patients with invasive tumors. In addition, the absence of lymphocytic infiltration is a poor prognostic factor for the incidence of lymphogenic and hematogenic metastases, as well as for a histologic type of tumor [13, 14].

Most patients are older than 45 years and represent two thirds of examined patients. Older age as a poor prognostic factor was described earlier. Using univariate analysis of 234 patients, Bellantone et al. [15] have reported that survival is significantly affected by age ($p = 0.0001$), tumor size ($p = 0.018$), extrathyroid expansion ($p = 0.000001$), lymphogenic metastases ($p = 0.03$), and distant metastases ($p = 0.0149$). A study by Mazzaferri and Kloss [16] confirmed that the main factor affecting the disease prognosis is the age, which is confirmed by our study.

In our study, papillary carcinoma occurs in females in 83.8% of cases. Cancer was most commonly reported in women in the fifth decade of life. In men, it occurs more frequently in the sixth decade of life. In groups with present or absent lymphocytic infiltration, the results of age and sex show that females are dominant in both groups, except that in the group with LI the female sex is more prevalent.

The male sex aged over 60 years, tumors over 4 cm in size, a poorly differentiated histological type of tumor, and the presence of hematogenic metastases in a multivariate analysis are important factors of poor prognosis for mortality in differentiated thyroid carcinoma [15]. Similar results were obtained in our study. The male sex, according to a study by Cunningham et al. [17], has a worse prognosis in differentiated thyroid carcinoma ($p = 0.003$), but does not affect the recurrence.

In our study, cervical lymphogenic metastases were verified in 25.7% of patients, and hematogenic metastases in 8% of patients, which correlates with the results of other authors. The incidence of relapse in the form of lymphogenic metastases (level III) was recorded in 16% of patients, which also corresponds to the results of similar studies. Similar results are found in a large study by Gilliland et al. [18], where, with the association of lymphogenic metastases with relapse, they reported greater tumor-specific mortality. The study by Mazzaferri and Kloss [16] from 2001 found that bilateral jugular metastases and mediastinal metastases are poor prognostic factors. In our study, the presence of lymphogenic metastases does not affect survival, while the presence of hematogenic metastases significantly increases mortality in the studied group of patients.

Our study showed that invasive tumors that show infiltrative growth, compared to healthy thyroid tissue as well as in relation to the gland capsule and prethyroid muscles, lymph, and blood vessels or trachea, represent a poor prognostic factor. In our series, 28 patients had infiltrative tumors. Infiltration of the gland capsule appeared in 14 patients; infiltration of prethyroid muscles was confirmed in 13 patients, and infiltration of all layers in front of the gland and even on the skin appeared in one patient.

In the group without hematogenic metastases, cancer-specific mortality was significantly dependent on the age of over 40 years, on the tumor size of more than 1.5 cm with invasive tumors, with lymphogenic metastases present. In our study, mortality was significantly higher in patients with invasive extrathyroid tumors, which corresponds to the results of other authors [17].

Rare forms of papillary carcinoma were recorded in 12% of patients – as a follicular variant of papillary carcinoma. Shaha [10] published the last TNM classification of cancers of the thyroid gland and confirmed that the clinical stages of the disease to assess risk factors are more useful than histopathological grouping of patients, because histological stages overestimate the biological characteristics of many N1b tumors.

Given that all patients underwent total thyroidectomy, multifocality as a poor prognosis factor is reduced to a minimum. In our study, multifocality of the tumor did not affect survival.

In our study, tumors T1 and T2 represent a good prognostic factor, while larger tumors with extrathyroid expansion are a poor prognostic factor. Most studies that examine the importance of the size of the primary thyroid cancer suggest that T1 tumors up to 10 mm in size are a favorable prognostic factor, which proved right in our study [19].

Clinical staging of the disease was more useful than TNM pathological staging of the disease as a predictor of prognosis in a study of 5,768 patients with papillary thyroid carcinoma, done by Ito et al. [20]. Cancer-specific survival was significantly lower in groups T4a and T4b with tumors with extrathyroid invasion. The largest number of thyroid cancers is in the first and the second stage of the disease, and it is expected that the number of high-risk patients is reduced, which fits the results obtained in our study [21].

In our study, the absence of LI is a poor prognostic factor for the occurrence of hematogenic metastases, as well as recurrence.

Chronic lymphocytic thyroiditis represents a favorable prognostic factor in our study group ($p < 0.0001$), while local invasiveness and extrathyroid expansion were significantly lower in the group of patients with lymphocytic thyroiditis present ($p < 0.0001$). In our group, we also had significantly lower survival in patients who have no LI.

The etiological relationship between thyroid cancer and lymphocytic infiltration remains ambiguous, as it is not clear whether lymphocytic thyroiditis is induced by neoplasm or the presence of lymphocytic thyroiditis induces the neoplastic process. A second series of patients showed that patients with chronic thyroiditis have a lower level of TNM at diagnosis. It was demonstrated that cytotoxic T lymphocytes are most frequent, and can secrete interleukin-1, which inhibits tumor growth [22]. During 11 years of follow-up, Kimura et al. [23] reported a lower incidence of recurrence and cancer-specific mortality in patients with present lymphocytic thyroiditis. In a study of 1,533 patients, Kashima et al. [24] reported a 5% cancer-specific mortality and an 85% 10-year period without relapse in the group of patients without lymphocytic thyroiditis.

In our study of a group of patients without LI, there was a significantly higher incidence of recurrent disease in the form of hematogenic metastases. In the group with the presence of LI, relapses were exclusively lymphogenic metastases. Since lymphogenic metastases do not affect survival, and hematogenic metastases significantly reduce survival, we can say that the absence of LI is a poor prognostic

factor for cancer-specific survival. Other studies suggest that cervical metastases are found in 50–80% of cases of papillary carcinoma, most commonly in the central section of the neck (level VI), followed by middle jugular (level III), supraclavicular (level IV), and subdigastric node (level I) [25]. In a study by Mazzaferri [26], it was found that bilateral jugular metastases and mediastinal metastases are poor prognostic factors and influence recurrence and survival.

CONCLUSION

Lymphocytic infiltration surrounding or inside the tumor might be useful in establishing a good prognosis.

This research has shown that lymphocytes in tissue as part of the immune response have a good effect on factors related to the tumor characteristics such as size, invasiveness, extrathyroid tumor growth, and incidence of hematogenic metastases.

REFERENCES

1. Li Volsi VA. The pathology of autoimmune thyroid disease. Thyroid. 1994; 4(3):333–9.
2. Matsubayashi S, Kawai K, Matsumoto Y, Mukuta T, Morita T, Hirai K, et al. The correlation between papillary thyroid carcinoma and lymphocytic infiltration in the thyroid gland. J Clin Endocrinol Metab. 1995; 80(12):3421–4.
3. Iglesia MD, Parker JS, Hoadley KA, Serody JS, Perou CM, Vincent BG. Genomic analysis of immune cell infiltrates across 11 tumor types. J Natl Cancer Inst. 2016; 108(11).
4. Kuo CY, Liu TP, Yang PS, Cheng SP. Characteristics of lymphocyte-infiltrating papillary thyroid cancer. J Cancer Res Pract. 2017; 4(3):95–9.
5. Pusztaszeri MP, Faquin WC, Sadow PM. Tumor-associated inflammatory cells in thyroid carcinomas. Surg Pathol Clin. 2014; 7(4):501–14.
6. Li B, Severson E, Pignon JC, Zhao H, Li T, Novak J, et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. Genome Biol. 2016; 17(1):174.
7. Cunha LL, Marcelio MA, Ward LS. The role of the inflammatory microenvironment in thyroid carcinogenesis. Endocr Relat Cancer. 2014; 21(3):85–103.
8. Lee JH, Kim Y, Choi JW, Kim YS. The association between papillary thyroid carcinoma and histologically proven Hashimoto thyroiditis: a meta-analysis. Eur J Endocrinol. 2013; 168(3):343–9.
9. Loh KC, Greenspan FS, Dong F, Miller RT, Yeo PP. Influence of lymphocytic thyroiditis on the prognostic outcome of patients with papillary thyroid carcinoma. J Clin Endocrinol Metab. 1999; 84(2):458–63.
10. Shaha AR. TNM classification of thyroid carcinoma. World J Surg. 2007; 31(5):879–87.
11. Liu LH, Bakhos R, Wojcik EM. Concomitant papillary thyroid carcinoma and Hashimoto thyroiditis. Semin Diagn Pathol. 2001; 18(2):99–103.
12. Ito Y, Miyauchi A, Jikuzono T, Higashiyama T, Takamura Y, Miya A, et al. Risk factors contributing to a poor prognosis of papillary thyroid carcinoma: validity of UICC/AJCC TNM classification and stage grouping. World J Surg. 2007; 31(4):838–48.
13. Bagnasco M, Venuti D, Paolieri F, Torre G, Ferrini S, Canonica GW. Phenotypic and functional analysis at the clonal level of infiltrating T lymphocytes in papillary carcinoma of the thyroid: prevalence of cytotoxic T cells with natural killer-like or lymphokine-activated killer activity. J Clin Endocrinol Metab. 1989; 69(4):832–6.
14. Van Savell H Jr, Hughes SM, Bower C, Parham DM. Lymphocytic infiltration in pediatric thyroid carcinomas. Pediatr Dev Pathol. 2004; 7(5):487–92.
15. Bellantone R, Lombardi CP, Boscherini M, Ferrante A, Raffaeli M, Rubino F, et al. Prognostic factors in differentiated thyroid carcinoma: a multivariate analysis of 234 consecutive patients. J Surg Oncol. 1998; 68(4):237–41.
16. Mazzaferri EL, Kloss RT. Clinical review 128: Current propositions to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab. 2001; 86(4):1447–63.
17. Cunningham MP, Duda RB, Recant W, Chmiel JS, Sylvester JA, Fremgen A. Survival discriminates for differentiated thyroid cancer. Am J Surg. 1990; 160(4):344–7.
18. Gilliland FD, Hunt WC, Morris DM, Key CR. Survival discriminates for differentiated thyroid cancer. A population-based study of 15,698 cases from the Surveillance, Epidemiology and End Results (SEER) program 1973–1991. Cancer. 1997; 79(3):564–73.
19. Ito Y, Kudo T, Takamura Y, Kobayashi K, Miya A, Miyauchi A. Lymph node recurrence in patients with N1b papillary thyroid carcinoma who underwent unilateral therapeutic modified radical neck dissection. World J Surg. 2012; 36(3):593–7.
20. Ito Y, Kudo T, Kobayashi K, Miya A, Ichihara K, Miyauchi A. Prognostic factors for recurrence of papillary thyroid carcinoma in the lymph nodes, lung, and bone: analysis of 5,768 patients with average 10-year follow-up. World J Surg. 2012; 36(6):1274–8.
21. Roh JL, Kim JM, Park CI. Central lymph node metastasis of unilateral papillary thyroid carcinoma: patterns and factors predictive of nodal metastasis, morbidity, and recurrence. Ann Surg Oncol. 2011; 18(8):2245–50.
22. Wu MH, Shen WT, Gosnell J, Duh QY. Prognostic significance of extranodal extension of regional lymph node metastasis in papillary thyroid cancer. Head Neck. 2015; 37(9):1336–43.
23. Kimura H, Yamashita S, Namba H, Tominaga T, Tsuruta M, Yokoyama N, et al. Interleukin-1 inhibits human thyroid carcinoma cell growth. J Clin Endocrinol Metab. 1992; 75(2):592–602.
24. Kashima K, Yokoyama S, Noguchi S, Murakami N, Yamashita H, Watanabe S, et al. Chronic thyroiditis as favorable prognostic factor in papillary thyroid carcinoma. Thyroid. 1998; 8(3):197–202.
25. Lee YC, Shin SY, Kwon KH, Eun YG. Incidence and clinical characteristics of prelaryngeal lymph node metastasis in papillary thyroid cancer. Eur Arch Otorhinolaryngol. 2013; 270(9):2547–50.
26. Mazzaferri EL. Management of low risk differentiated thyroid cancer. Endocr Pract. 2007; 13(5):4987–512.

Лимфоцитна инфилтрација као прогностички фактор папиларног карцинома штитасте жлезде

Александар Филиповић[1], Љиљана Вучковић[2]

[1]Универзитет Црне Горе, Медицински факултет, Клинички центар Црне Горе, Одељење ендокрине хирургије, Подгорица, Црна Гора;
[2]Универзитет Црне Горе, Медицински факултет, Клинички центар Црне Горе, Клиника за патологију, Подгорица, Црна Гора

САЖЕТАК

Увод/Циљ Циљ рада је био да утврди да ли присуство лимфоцитне инфилтрације представља повољан прогностички фактор за преживљавање код класичне форме папиларног карцинома штитасте жлезде.

Методе У ретроспективну студију укључено је 105 болесника лечених због папиларног карцинома штитасте жлезде у периоду од седам година (2010–2017). Болесници старости 12–84 година су подељени у две групе: са лимфоцитном инфилтрацијом (74% болесника) и без ње. За идентификацију Т и Б лимфоцита коришћена су анти-ЦД3 и анти-ЦД20 антитела. Каплан–Мејерова крива је коришћена за поређење временски зависних варијабли.

Резултати Одсуство лимфоцитних инфилтрата је лош прогностички фактор за преживљавање код болесника са инвазивним екстратироидним туморима ($p < 0,0001$) и у односу на појаву лимфогених и хематогених метастаза ($p < 0,0001$). Хронични лимфоцитарни тироидитис представља повољан прогностички фактор за преживљавање у нашој испитиваној групи ($p < 0,0001$).

Закључак Лимфоцити у ткиву као део имуног одговора имају позитиван утицај на екстратироидни раст тумора и мању учесталост лимфогених и хематогених метастаза. Присуство лимфоцитне инфилтрације представља повољан прогностички фактор за преживљавање код класичне форме папиларног карцинома штитасте жлезде.

Кључне речи: папиларни карцином тиреоидеје; лимфоцитна инфилтрација; прогностички фактори

DOI: https://doi.org/10.2298/SARH170124200R
UDC: 616.379-008.64-074:577.112

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Serum resistin and adiponectin relationships with glucometabolic control in patients with type 2 diabetes mellitus

Khalid A. Al-Regaiey[1], Syed S. Habib[1], Laila Al Dokhi[1], Anwar A. Jammah[2], Mohammad M. Subhan[3]

[1]King Saud University, College of Medicine, Department of Physiology, Riyadh, Saudi Arabia;
[2]King Saud University, College of Medicine, Department of Medicine, Riyadh, Saudi Arabia;
[3]Plymouth University, School of Biomedical and Healthcare Sciences, United Kingdom

SUMMARY

Introduction/Objective Adiponectin and resistin are important adipokines that play an important role in the regulation of blood sugar, beta-oxidation in muscles, and insulin resistance. This study aimed to assess and compare the relationships of resistin and adiponectin concentrations with glucometabolic control in patients with type 2 diabetes mellitus (T2DM).

Methods A total of 191 subjects were studied. The final selection included 107 patients with T2DM (67 males and 40 females) and 84 healthy control subjects (45 males and 39 females). Fasting venous blood samples were analyzed for glucose (FBG), glycosylated hemoglobin (HbA1c), insulin, lipids, adiponectin and resistin levels. Body composition was evaluated in all subjects by the body mass index (BMI) and waist–hip ratio (WHR).

Results BMI, WHR, FBG, HbA1c, homeostatic model assessment of insulin resistance (HOMA-IR), total cholesterol, and triglycerides were significantly higher in individuals with T2DM compared to healthy volunteers. Serum resistin levels were significantly higher ($p = 0.0259$) and serum adiponectin levels were significantly lower ($p = 0.0001$) in T2DM patients than in control subjects. Adiponectin levels were significantly lower ($p = 0.0411$) in diabetes patients with poor glycemic control, compared to those with good glycemic control, while the difference was non-significant for resistin ($p = 0.8899$). Serum adiponectin levels were discordant with HbA1c ($r = -0.274$, $p = 0.004$). Linear-by-linear association showed significant trend of better glycemic control at increasing quartiles of adiponectin levels ($p = 0.042$), while the trend was not significant for resistin levels ($p = 0.904$). Multiple regression analysis revealed FBG, insulin, HOMA-IR, and HbA1c as significant predictors of adiponectin.

Conclusions T2DM patients have significantly higher resistin and lower adiponectin levels compared to healthy controls. Adiponectin levels were significantly lower in patients with poor glycemic control.

Keywords: adiponectin; resistin; dyslipidemia; type 2 diabetes mellitus

INTRODUCTION

It is well known that obesity increases the risk of developing type 2 diabetes mellitus (T2DM) [1]. Adipose tissue is a complex endocrine organ with potential implications on insulin resistance, obesity and diabetes. Many researches have resulted in identification of a large group of adipocyte-specific proteins, such as adiponectin, acylation-stimulating protein, resistin, leptin, which are involved in regulating glucose and lipid metabolism and insulin resistance in obesity and diabetes [1, 2, 3]. Therefore, high visceral fat and insulin resistance have been reported to be independently associated with prediabetes and T2DM [4]. Adipose tissue dysfunction is characterized by ectopic fat deposition in the abdominal viscera and liver, inflammatory and adipokine dysregulation, and insulin resistance, which may be a more important mediator of diabetes development than total fat mass as such [5, 6].

Adipocyte-specific proteins, such as adiponectin, acylation-stimulating protein, resistin and leptin, have recently been identified [7]. Resistin and adiponectin are important adipokines that regulate insulin sensitivity. Adiponectin, synthesized in the adipose tissue, appears to play an important role in inflammatory mechanisms, glycemic and lipid control, which cluster together to markedly increase the atherosclerotic risk in diabetes subjects. Plasma adiponectin concentrations are reported to be decreased in patients with obesity, T2DM, insulin resistance syndromes, dyslipidemia, and coronary artery disease [8–12]. Resistin is secreted by adipocytes and leads to insulin resistance in vivo and in vitro and is considered to be an important link between obesity and diabetes [13].

Lower levels of adiponectin in obese subjects are associated with higher levels of resistin and are considered to contribute to insulin resistance and accelerated atherogenesis [14]. Plasma adiponectin levels correlate negatively with adiposity, and serum adiponectin levels and waist–hip ratio (WHR) are independent predictors of high-sensitivity C-reactive protein levels in normoglycemic subjects [15, 16, 17]. Hence, both adiponectin and resistin have important biological activity on glucose and lipid

Received • Примљено: January 24, 2017
Revised • Ревизија: November 2, 2017
Accepted • Прихваћено: November 7, 2017
Online first: November 17, 2017

Correspondence to:
Syed Shahid HABIB
Department of Physiology (29)
College of Medicine
PO Box 2925
King Saud University
Riyadh 11461
Kingdom of Saudi Arabia
shahidhabib44@hotmail.com

metabolism. However, the comparison of these adipokines on glucometabolic control needs further investigation. Therefore, the aim of this study was to assess and compare the relationships of resistin and adiponectin concentrations with glucometabolic control in patients with T2DM.

METHODS

This case–control study was carried out at the Department of Physiology and Medicine, College of Medicine, and King Khalid University Hospital, King Saud University, Riyadh. The study was approved by the Institutional Review Board of the College of Medicine. A total of 191 subjects were selected for the study. Final analysis included 107 patients with T2DM (67 males and 40 females). The control group included 84 healthy subjects (45 males and 39 females) matched for age, sex, and weight, recruited from staff members and the patients' companions. All the patients were diagnosed with T2DM based on American Diabetes Association criteria and were in stable metabolic condition with at least one year of duration of T2DM [18]. Patients with acute diabetes states, acute or chronic renal problems, thyroid diseases, acute and chronic infections, stroke, taking oral contraceptives or steroids, were excluded. Clinical and demographic data from all the participants was recorded on a predesigned form, which included weight, height, the body mass index (BMI), WHR measurements, and exercise habits. The patients were divided into a good and a poor glycemic control group based on a cut-off glycosylated hemoglobin (HbA1c) value of 7.5% [18]. After 10–12 hours of overnight fasting, venous blood samples were analyzed for total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), fasting blood glucose (FBG), HbA1c, basal insulin, adiponectin, and resistin levels. Human insulin, adiponectin, and resistin immunoassays were carried out by quantitative standard sandwich enzyme-linked immunosorbent assay (ELISA) technique using a monoclonal antibody specific for resistin with kits supplied by R&D Systems, (Abingdon, United Kingdom). Insulin resistance was calculated by homeostasis model assessment of insulin resistance (HOMA-IR) using the formula HOMA-IR = (FPI (mU/L) × FPG (mmol/L)) / 22.5 [19].

Bioelectrical impendence analysis was used to measure body composition with InBody 3.0 (Biospace Inc., Seoul, Korea) body analyzer according to the manufacturer's instructions. All assessments were made with the respondents being in the early morning fasting state, wearing light clothes, and after emptying of the urinary bladder. The machine calculated the amount of each tissue with the difference in electrical impedance [20].

Statistical analysis

The data was analyzed by IBM SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA). Descriptive characteristics of the study patients were calculated as mean ± standard deviation. The tests applied for statistical analysis were Student's t-test for normally distributed data and Mann–Whitney U-test for skewed data. We applied and used the linear-by-linear association p-value for significant difference at different quartiles of adiponectin and resistin levels in diabetes patients. Spearman's correlations and multiple regression analysis were performed to show the predictors of adiponectin and resistin levels. A p-value of < 0.05 was taken as statistically significant.

RESULTS

This study reveals relationships between adiponectin and resistin concentrations with glycemic and lipid control in patients with T2DM. Table 1 shows comparison of descriptive characteristics and biochemical profile between control and diabetes patients. BMI, WHR, FBG, HbA1c, HOMA-IR, TC, and TG were significantly higher in individuals with diabetes compared to healthy volunteers. Exercise prevalence in each group was also compared and it was non-significant (Table1). T2DM patients were divided into good and poor glycemic control group based on a cut-off HbA1c value of 7.5%. Table 2 expresses the comparison of descriptive characteristics and biochemical profile between good and poor glycemic control in T2DM patients. BMI ($p = 0.0257$), HOMA-IR ($p = 0.0002$), and TG ($p = 0.0006$) were significantly higher in the poor glycemic control group than in the good glycemic control group.

Box plot represents serum adiponectin and resistin levels in controls, all subjects with diabetes, and in those with good and poor glycemic control (Figures 1 and 2). Serum resistin levels were significantly higher ($p = 0.0259$) (Figure 1) and serum adiponectin levels significantly lower ($p = 0.0001$) (Figure 2) in T2DM patients than in healthy

Table 1. Comparison of descriptive characteristics and biochemical profile between controls and T2DM patients (mean ± SD)

Characteristics	Controls n = 84	T2DM n = 107	p
Male/Female	45/39	67/40	/
Age (years)	50.16 ± 12.58	52.20 ± 11.07	0.2735
Height (cm)	166.80 ± 8.52	165.71 ± 13.93	0.2641
Weight (kg)	77.66 ± 14.78	84.23 ± 20.60	0.0515
WHR	0.94 ± 0.12	1.11 ± 0.09	0.0001
BMI (kg/m^2)	28.13 ± 4.80	29.72 ± 5.27	0.0227
FBG (mmol/dl)	5.06 ± 0.99	8.88 ± 3.29	0.0001
HbA1c (%)	5.01 ± 0.56	7.67± 1.53	0.0001
Insulin (μIU/ml)	22.69 ± 6.12	24.81 ± 9.19	0.0708
HOMA-IR	5.20 ± 2.46	9.73 ± 5.08	0.0001
TC (mmol/L)	4.25 ± 0.96	4.48 ± 1.12	0.0515
TG (mmol/L)	1.41 ± 1.19	2.04 ± 1.44	0.0463
LDL (mmol/L)	2.68 ± 0.86	2.62 ± 0.91	0.1264
HDL (mmol/L)	1.17 ± 0.23	1.04 ± 0.31	0.1020
TC (mmol/L)	4.25 ± 0.96	4.48 ± 1.12	0.0515
Exercise n (%) No Yes	 45 (42.66) 39 (41.34)	 52 (54.34) 55 (52.66)	0.4949

BMI – body mass index; WHR – waist–hip ratio; FBG – fasting blood glucose; HbA1c – glycosylated hemoglobin ; TC – total cholesterol; TG – triglycerides; LDL – low-density lipoprotein; HDL – high-density lipoprotein

Table 2. Comparison of descriptive characteristics and biochemical profile between good and poor glycemic control in T2DM patients (mean ± SD)

Characteristics	HbA1c < 7.5 n = 50	HbA1c ≥ 7.5 n = 57	p
Male/Female	28/22	30/27	
Age (years)	53.90 ± 10.51	51.32 ± 11.15	0.2283
Height (cm)	167.63 ± 6.01	164.48 ± 17.53	0.2474
Weight (kg)	79.94 ± 15.10	84.78 ± 17.17	0.1342
WHR	0.99 ± 0.07	1.01 ± 0.09	0.1613
BMI (kg/m^2)	28.46 ± 4.97	30.77 ± 5.26	0.0257
FBG (mmol/dl)	7.10 ± 1.64	10.20 ± 3.61	0.0001
HbA1c (%)	6.58 ± 0.44	9.66 ± 2.58	0.0001
Insulin (μIU/ml)	23.91 ± 7.51	25.24 ± 10.68	0.4705
HOMA-IR	7.66 ± 3.47	11.19 ± 5.49	0.0002
TC (mmol/L)	4.19 ± 0.84	4.58 ± 1.26	0.1279
TG (mmol/L)	1.47 ± 0.69	2.36 ± 1.30	0.0006
LDL (mmol/L)	2.54 ± 0.85	2.75 ± 0.93	0.3307
HDL (mmol/L)	1.00 ± 0.24	1.08 ± 0.49	0.3783

BMI – body mass index; WHR – waist–hip ratio; FBS – fasting blood glucose; HbA1c – glycosylated hemoglobin; TC – total cholesterol; TG – triglycerides; LDL – low-density lipoprotein; HDL – high-density lipoprotein

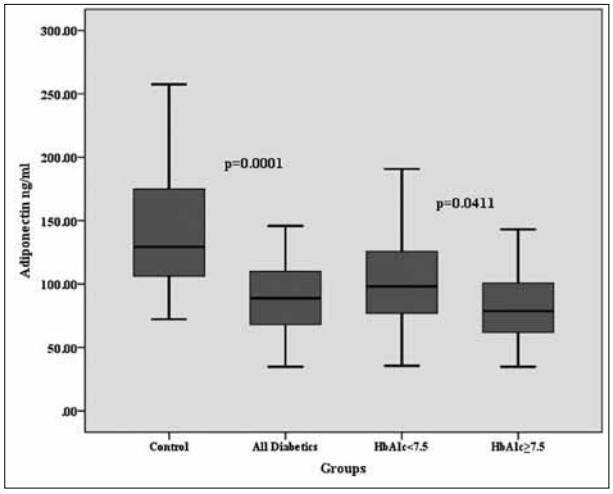


Figure 1. Comparison of adiponectin levels between control subjects, all patients with type 2 diabetes mellitus, and those with good (HbA1c < 7.5) and poor glycemic control (HbA1c ≥ 7.5)

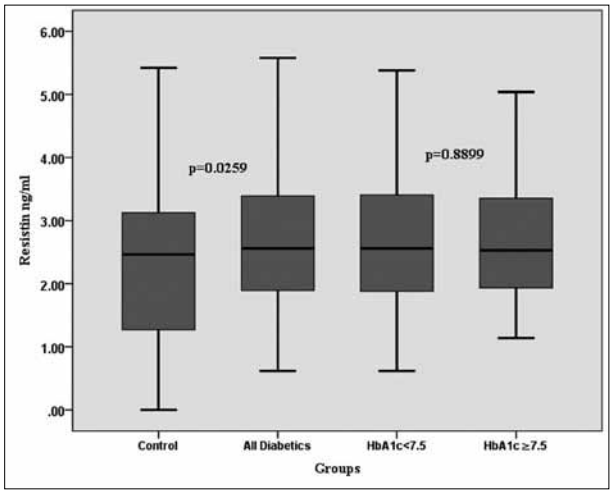


Figure 2. Comparison of resistin levels between controls, all patients with type 2 diabetes mellitus, and those with good (HbA1c < 7.5) and poor glycemic control (HbA1c ≥ 7.5)

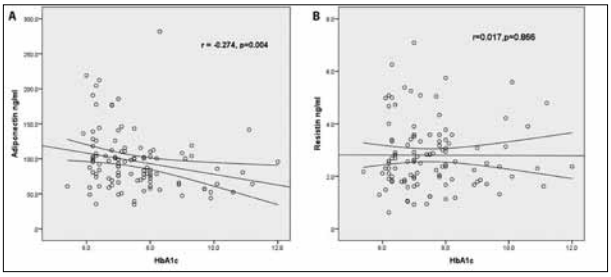


Figure 3. Scatter plot showing the association of circulating levels of adiponectin (a) and resistin (b) with glycosylated hemoglobin (HbA1c)

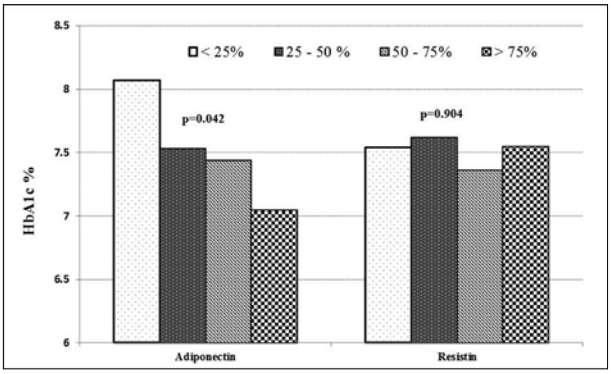


Figure 4. Linear-by-linear association of HbA1c% at different quartiles of adiponectin and resistin levels in diabetes patients; there is a significant trend of better glycemic control at increasing levels of adiponectin levels (p = 0.042), while the trend was not significant for resistin levels (p = 0.904)

subjects. We observed that serum adiponectin levels were significantly lower (p = 0.0411) in diabetes patients with poor glycemic control, compared to those with good glycemic control, but the difference was non-significant for resistin (p = 0.8899). Scatter plot in Figure 3 shows the relationship of adiponectin (a) and resistin (b) with glycemic control. Serum adiponectin levels were discordant with HbA1c (r = -0.274, p = 0.004). No relationship between HbA1c and resistin levels was observed (r = 0.017, p = 0.866). Linear-by-linear association of HbA1c% at different quartiles of adiponectin and resistin levels in diabetes patients was also determined. There was a significant trend of better glycemic control at increasing levels of adiponectin levels (p = 0.042), while the trend was not significant for resistin levels (p = 0.904), which was depicted by linear-by-linear association of HbA1c% at different quartiles of adiponectin and resistin levels in diabetes patients as shown in Figure 4.

Multiple regression analysis was performed keeping adiponectin and resistin as dependent variables to determine their predictive factors (Table 3). Significant predictors of adiponectin levels were FBS, insulin, HOMA-IR, and HbA1c. For resistin, none of the variables was significant. Table 4 expresses the proportion of patients using medications for diabetes and comorbidities in patients with T2DM.

DISCUSSION

The present study aims to assess and compare the relationships of resistin and adiponectin concentrations with

Table 3. Multiple regression analysis for prediction of adiponectin and resistin levels in T2DM patients

Parameter	Adiponectin (ng/ml) Standardized Beta Coefficients	p	Resistin (ng/ml) Standardized Beta Coefficients	p
FBS (mmol/L)	1.498	0.015	0.481	0.507
Insulin (μIU/ml)	1.524	0.018	0.518	0.493
HOMA-IR	-2.317	0.016	-0.855	0.450
HbA1c (%)	-0.237	0.014	0.284	0.182
TG (mmol/L)	-0.150	0.315	-0.201	0.268
TC (mmol/L)	-0.260	0.122	0.153	0.448
HDL (mmol/L)	0.074	0.618	-0.089	0.620
LDL (mmol/L)	0.133	0.472	-0.401	0.079
Duration (years)	0.268	0.063	-0.186	0.283

BMI – body mass index; WHR – waist–hip ratio; FBS – fasting blood glucose; HbA1c – glycosylated hemoglobin; TC – total cholesterol; TG – triglycerides; LDL – low-density lipoprotein; HDL – high-density lipoprotein

Table 4. Use of medications for diabetes and comorbidities in patients with T2DM

Medicines for diabetes	n (%)
Biguanides	14 (13.1)
Sulphonylureas	25 (23.4)
Glinides	11 (10.3)
Alpha-glucosidase inhibitors	21 (11.2)
Thiazolidinedione	23 (21.5)
Lipid-lowering medicines	25 (23.4)

glycemic and lipid control in patients with T2DM. We observed that T2DM patients have significantly higher resistin and lower adiponectin levels. The effect of glycemic control on resistin levels was not significant. However, adiponectin was significantly lower in patients with poor glycemic control, compared to those with good glycemic control. Similar to a report by Schulze et al. [21], our study supports the hypothesis that increased adiponectin levels might be associated with better lipid and glycemic control with reduced inflammation in patients with T2DM. Measures that could increase adiponectin levels might be valuable targets for decreasing the higher coronary artery disease risk in diabetes.

In another similar study, adiponectin was found to be significantly decreased in T2DM patients as compared to normal control subjects. Adiponectin levels were negatively associated with high-sensitivity C-reactive protein, LDL-C, HbA1c, TG, TC, and positively with HDL-C. HbA1c had a negative correlation with serum adiponectin. This shows that adiponectin may play an important role in the pathogenesis of diabetes, and may emerge as an independent predictor of the development of T2DM [22]. Nayak et al. [23] showed that adiponectin decreases with increasing adiposity and insulin resistance regardless of diabetes status. Among non-obese subjects, adiponectin correlated negatively with TG, interleukin-6, and HOMA-IR, and correlated positively with HDL. Diabetes status, tumor necrosis factor-α (TNF-α), and BMI were identified as independent predictors of adiponectin. Glucose and adiponectin were useful indicators of T2DM. Moreover, insulin-mediated glucose turnover was significantly affected by adiponectin and TNF-α [23]. Adiponectin negatively correlated with BMI after adjusting for age, sex, and diabetes status [24]. In an interesting study, Lau et al. [25] proposed a novel adiponectin–resistin (AR) index by taking into account both adiponectin and resistin levels to provide a better indicator of the metabolic homeostasis and metabolic disorders. A novel insulin resistance (IRAR) index was derived to provide an improved diagnostic biomarker of insulin sensitivity.

Adipocytokines that have been implicated in the pathogenesis of metabolic syndrome include TNF-α, interleukin-6, angiotensinogen, leptin, plasminogen activator inhibitor-1, and resistin [26]. The present study supports the evidence that resistin plays an important role in the pathogenesis of obesity and insulin resistance. We reported previously that higher resistin levels in T2DM have a significant positive correlation with body fat mass [27]. However, in the present study, although T2DM patients had higher resistin levels, the effect of glycemic control on resistin levels was not significant. In a study on Chinese T2DM patients, 16 weeks of liraglutide administration led to increased adiponectin and decreased resistin levels compared to glimepiride-treated subjects, while inducing similar glycemic changes [28]. Adiponectin, leptin, and resistin levels are affected by the use of anti-diabetes drugs among which glimepiride shows more effect on adiponectin and resistin levels, while leptin gets affected more by metformin. It shows that the adipokine levels are not affected by diabetes only, suggesting that their alterations in T2DM may be due to obesity. Therefore, there might be important links between adiposity and insulin resistance [29].

The limitation of the present work is its cross-sectional design and small sample size. We recommend further large-scale prospective studies to additionally explore the true homeostatic roles of adiponectin and resistin in patients with T2DM. Since they are related to glucose and lipid metabolism, it would be worth studying them as an integrated approach in relation to different pharmacological interventions and physical fitness programs. They may prove to be useful integrated biomarkers to predict metabolic dysregulation and cardiovascular risk in T2DM.

CONCLUSION

T2DM patients have significantly higher resistin and lower adiponectin levels when compared to healthy controls. Adiponectin levels were significantly lower in patients with poor glycemic control. However, the effect of glycemic control on resistin levels was not significant.

ACKNOWLEDGMENT

The authors are thankful to Dr. Shaikh Mujeeb Ahmed and Mr. James Chu for collection of data and blood assays. We are thankful to King Abdul Aziz City for Science and Technology for supporting this project (Project No. 26-60).

REFERENCES

1. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. J Clin Endocrinol Metab. 2008; 93(11 Suppl 1):S57–63.
2. Ahima RS. Central actions of adipocyte hormones. Trends Endocrinol Metab 2005; 16(7): 307–13.
3. Faraj M, Lu HL, Cianflone K. Diabetes, lipids, and adipocyte secretagogues. Biochem Cell Biol. 2004; 82(1):170–90.
4. Neeland IJ, Turer AT, Ayers CR, Powell-Wiley TM, Vega GL, Farzaneh-Far R, et al. Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. JAMA. 2012; 308(11):1150–9.
5. McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. J Clin Endocrinol Metab. 2011; 96(11):E1756–60.
6. Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasan RS, Irlbeck T, et al. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham Heart Study. Obesity (Silver Spring). 2010; 18(11):2191–8.
7. Faraj M, Lu HL, Cianflone K. Diabetes, lipids, and adipocyte secretagogues. Biochem Cell Biol. 2004; 82(1):170–90.
8. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. 1999. Biochem Biophys Res Commun. 2012; 425(3):560–4.
9. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, et al. Plasma concentrations of a novel, adiposespecific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 2000; 20(6):1595–9.
10. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Platley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001; 86(5):1930–5.
11. Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. J Clin Endocrinol Metab. 2002; 87(6):2764–9.
12. Nanayakkara G, Kariharan T, Wang L, Zhong J, Amin R. The cardio-protective signaling and mechanisms of adiponectin. Am J Cardiovasc Dis. 2012; 2(4):253–66.
13. Flier JS. Diabetes. The missing link with obesity? Nature. 2001; 409(6818):292–3.
14. Beltowski J. Adiponectin and resistin – new hormones of white adipose tissue. Med Sci Monit. 2003; 9(2):RA55–61.
15. Habib SS, Al Regaiey KA, Al Dokhi L. Assessment of adipokines relationships with cardiovascular risk Markers in relation to body indices in normoglycemic males. Pak J Med Sci. 2013; 29(1):21–6.
16. Asayama K, Hayashibe H, Dobashi K, Uchida N, Nakane T, Kodera K, et al. Decrease in serum adiponectin level due to obesity and visceral fat accumulation in children. Obes Res. 2003;11(9):1072–9.
17. Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S. The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? J Endocrinol Invest. 2007; 30(3):210–4.
18. American Diabetes Association. Standards of medical care in diabetes – 2010. Diabetes Care. 2010; 33 Suppl 1:S11–61.
19. Singh B, Saxena A. Surrogate markers of insulin resistance: A review. World J Diabetes. 2010; 15; 1(2):36–47.
20. Boneva-Asiova Z, Boyanov MA. Body composition analysis by leg-to-leg bioelectrical impedance and dual-energy X-ray absorptiometry in non-obese and obese individuals. Diabetes Obes Metabol. 2008; 10(11):1012–8.
21. Schulze MB, Rimm EB, Shai I, Rifai N, Hu FB. Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. Diabetes Care. 2004; 27(7):1680–7.
22. Goodarzi MT, Babaahmadi-Rezaei H, Kadkhodaei-Eliaderani M, Haddadinezhad S. Relationship of serum adiponectin with blood lipids, HbA(1)c, and hs-CRP in type II diabetic postmenopausal women. J Clin Lab Anal. 2007; 21(3):197–200.
23. Nayak BS, Ramsingh D, Gooding S, Legall G, Bissram S, Mohammed A, et al. Plasma adiponectin levels are related to obesity, inflammation, blood lipids and insulin in type 2 diabetic and non-diabetic Trinidadians. Prim Care Diabetes. 2010; 4(3):187–92.
24. Nayak S, Soon SQ, Kunjal R, Ramadoo R, Baptiste O, Persad J, et al. Relationship between adiponectin, inflammatory markers and obesity in type 2 diabetic and non-diabetic Trinidadians. Arch Physiol Biochem. 2009; 115(1):28–33.
25. Lau CH, Muniandy S. Novel adiponectin-resistin (AR) and insulin resistance (IRAR) indexes are useful integrated diagnostic biomarkers for insulin resistance, type 2 diabetes and metabolic syndrome: a case control study. Cardiovasc Diabetol. 2011; 10(1):8.
26. Kusminski CM, McTernan PG, Kumar S. Role of resistin in obesity, insulin resistance and Type II diabetes. Clin Sci (Lond). 2005; 109(3):243–56.
27. Habib SS. Serum resistin levels in patients with type 2 diabetes mellitus and its relationship with body composition. Saudi Med J. 2012; 33(5):495–9.
28. Li D, Xu X, Zhang Y, Zhu J, Ye L, Lee KO, et al. Liraglutide treatment causes upregulation of adiponectin and downregulation of resistin in Chinese type 2 diabetes. Diabetes Res Clin Pract. 2015; 110(2):224–8.
29. Farooq R, Amin S, Hayat Bhat M, Malik R, Wani HA, Majid S. Type 2 diabetes and metabolic syndrome - adipokine levels and effect of drugs. Gynecol Endocrinol. 2017; 33(1):75–8.

Однос серумског резистина и адипонектина и глукометаболичка контрола код болесника са дијабетесом мелитусом типа 2

Халид А. ел Регаиеј[1], Сајед Ш. Хабиб[1], Лејла ел Дохи[1], Анвар А. Џамах[2], Мохамед М. Субхан[3]

[1]Универзитет краља Сауда, Медицински колеџ, Катедра за физиологију, Ријад, Саудијска Арабија;
[2]Универзитет краља Сауда, Медицински колеџ, Катедра за медицину, Ријад, Саудијска Арабија;
[3]Универзитет у Плимуту, Факултет биомедицинских и здравствених наука, Плимут, Велика Британија

САЖЕТАК

Увод/Циљ Адипонектин и резистин су адипокини који играју важну улогу у регулисању шећера у крви, бета-оксидацији у мишићима и инсулинској резистенцији.

Циљ ове студије је био да процени и упореди односе концентрација резистина и адипонектина са глукометаболичком контролом код болесника са шећерном болешћу типа 2 (ШБТ2).

Методе Испитан је укупно 191 испитаник. Коначна селекција обухватила је 107 болесника са ШБТ2 (67 мушкараца и 40 жена) и 84 здравих, контролних особа (45 мушкараца и 39 жена). Анализирани су узорци венске крви за глукозу (ВГ), гликозиловани хемоглобин (*HbA1c*), инсулин, липиди (укупни холестерол – УХ, триглицериди – ТГ), адипонектин и резистин. Грађа тела оцењена је код свих и то индексом телесне масе (ИТМ) и односом струк–кукови (ОСК).

Резултати: ИТМ, ОСК, ВГ, *HbA1c*, инсулинска резистенција (ИР), УХ и ТГ били су значајно већи код особа с дијабетесом у поређењу са здравим добровољцима. Ниво резистина у серуму био је значајно виши ($p = 0,0259$), а ниво серумског адипонектина значајно нижи ($p = 0,0001$) код ШБТ2 него код контролних субјеката. Нивои адипонектина били су значајно нижи ($p = 0,0411$) код болесника са лошом контролом гликемије у поређењу са онима са добром гликемијском контролом, док је разлика нивоа резистина била безначајна ($p = 0,8899$). Нивои адипонектина у серуму нису у корелацији са *HbA1c* ($p = -0,274$, $p = 0,004$). Линеарна корелација показала је значајан тренд боље контроле гликемије код повећања нивоа адипонектина ($p = 0,042$), док тренд није био значајан за нивое резистина ($p = 0,904$). Мултипла регресиона анализа открила је ВГ, инсулин, ИР и *HbA1c* као значајне предикторе адипонектина.

Закључак: Болесници са ШБТ2 имају знатно повишен резистен и снижен адипонектин у поређењу са здравим особама. Нивои адипонектина су знатно нижи код болесника са слабом контролом гликемије.

Кључне речи: адипонектин; резистин; дислипидемија; шећерна болест тип 2

DOI: https://doi.org/10.2298/SARH171227028R
UDC: 616.24-002.5:613.84; 616.921.8-053.2

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Pertussis in children under the age of 10

Mioljub Ristić[1,2], Biljana Radosavljević[1], Vladimir Petrović[1,2]

[1]Institute of Public Health of Vojvodina, Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia

SUMMARY

Introduction/Objective Pertussis is a vaccine-preventable disease that causes a large number of cases and hospitalizations worldwide.

The aim of this study was to determine predictors of hospitalization in cases of pertussis among children under 10 years of age in the South Bačka District of Vojvodina Province, Serbia.

Methods Data for this observational study were obtained from inpatient and outpatient healthcare facilities in the South Bačka District from January 1, 2013 to December 31, 2016. We evaluated predictors of hospitalization among the patients who fulfilled the criteria of case definitions of pertussis proposed by the Global Pertussis Initiative. Pertussis was confirmed by DNA polymerase chain reaction or ELISA serology tests.

Results Out of 122 laboratory-confirmed pertussis cases, 43 (35.2%) were hospitalized. Apnea and pneumonia were associated with hospitalization, and all six hospitalized patients aged 0–3 months had cyanosis. Apnea was a good predictor of hospitalization among children with any duration of cough ($p < 0.05$). Among children with a cough that lasted longer than 14 days, post-tussive emesis or pneumonia or contact with a person who had a prolonged cough were associated with hospitalization ($p = 0.035$, $p = 0.042$, and $p = 0.046$, respectively). There were fewer hospitalizations in properly vaccinated cases than in partly or non-vaccinated cases between two months and four years of age ($p < 0.008$).

Conclusions Among the pertussis cases under 10 years of age, apnea, pneumonia, and cyanosis were factors associated with hospitalization. Immunization against pertussis corresponding to age reduces the disease severity and hospitalizations in children from two months to four years of age.

Keywords: pertussis; hospitalization; surveillance; epidemiology

INTRODUCTION

Pertussis (whooping cough) as a vaccine-preventable disease is a prevalent cause of acute cough in both children and adults occurring in outpatient and inpatient health care facilities [1]. Despite high immunization coverage, pertussis is still present around the world [2–5]. In 2016, more than 139,000 pertussis cases were reported worldwide [6]. The majority (approximately 95%) of infections occurred in developing countries; with most deaths occurring in young infants who were either unvaccinated or incompletely vaccinated [3, 4, 5]. Even in countries with high vaccination coverage, pertussis causes a high number of cases and hospitalizations [2, 5]. A dramatic resurgence of pertussis worldwide, with large outbreaks and deaths mainly in infants, has drawn the attention of healthcare providers [5].

Protection against pertussis was achieved only after completed three-dose primary vaccination series at approximately six months of age [2, 3, 7]. According to the annual reports in the South Bačka District of Vojvodina Province, Serbia, between 2013 and 2016, the average immunization coverage of pertussis was 95% for the primary series (at two, four, and six months), and 90% for one booster dose (one year after the third dose of the vaccine) [8].

The main goal of this study was to determine predictors of hospitalization in cases of pertussis among children under 10 years of age in the South Bačka District.

METHODS

Study design

The design and methods of improved surveillance of pertussis have been described previously [9, 10]. Surveillance of pertussis from inpatient and outpatient healthcare facilities in the South Bačka District was conducted for four consecutive years, in the period from January 1, 2013 to December 31, 2016. We included all children under 10 years of age, regardless of the duration of cough. Additionally, when children fulfilled one or more criteria of pertussis proposed by the Global Pertussis Initiative (GPI), they were enrolled after admission to healthcare facilities for a period of one week [9]. During the study period, we included children who were hospitalized at the Department of Pulmonology of the Institute for Child and Youth Health Care of Vojvodina (an inpatient facility), and at 11 health centers (the primary healthcare level) of the South Bačka District.

Eligible were children under 10 years of age who met one or more criteria of clinical case definitions of pertussis proposed by the GPI for two age groups (0–3 months old, and four months to nine years old) (Table 1).

Received • Примљено: December 27, 2017
Revised • Ревизија: March 16, 2018
Accepted • Прихваћено: March 19, 2018
Online first: March 27, 2018

Correspondence to:
Mioljub RISTIĆ
Institute of Public Health of Vojvodina
Futoška 121
21000 Novi Sad
Serbia
mioljub.ristic@mf.uns.ac.rs

Table 1. Clinical case definitions of pertussis and diagnostic tests proposed by the Global Pertussis Initiative[a] for patients under 10 years of age

Age groups	0–3 months	4 months – 9 years
Signs/symptoms/contact	Cough and coryza with no or minimal fever plus: – whoop or – apnea or – post-tussive emesis or – cyanosis or one of the following: – seizure – pneumonia – close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness	Paroxysmal cough with no or minimal fever plus: – whoop or – apnea or one of the following: – post-tussive emesis – seizure – worsening of symptoms at night – pneumonia – close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness
Diagnostic method – cough illness in a person with no or minimal fever plus cough duration[b]	PCR for all children aged 0–3 months	PCR or serology (IgG-PT), if ≥ 1 year post-pertussis vaccination

PCR – polymerase chain reaction; IgG – immunoglobulin G; PT – pertussis toxin;
[a]Adapted from the Global Pertussis Initiative;
[b]For patients aged four months to nine years: PCR if cough duration is ≤ 3 weeks, and serology if cough duration is > 3 weeks

We excluded children who did not fulfil the GPI clinical case definitions of pertussis proposed for the aforementioned age groups.

This research was conducted as a part of the daily clinical routine practice. The training of all included physicians and nurses was conducted before starting our research. Verbal informed consent was obtained from parents or guardians of children at the moment of swab taking in accordance with national regulations. All data about the children were anonymized and de-identified.

Participants

We obtained children's demographic and clinical data as well as the data about vaccination against pertussis in a structured questionnaire to parents or guardians. At the primary healthcare level, vaccination status was obtained from the participants' vaccination records. Vaccination status at inpatient facilities was determined from a parental report of the child's vaccination record, and therefore it was checked from vaccination records at the primary healthcare level. The child's physician determined clinical management, including hospitalization and laboratory procedures. Depending on the clinical course of the disease, all clinical and laboratory data were obtained at inpatient or outpatient healthcare facilities.

Posterior nasopharyngeal swabs and whole blood samples (single-serum) from the patients were collected by trained physicians and nurses at inpatient and outpatient medical facilities, as well as at the Institute of Public Health of Vojvodina, Novi Sad. All samples were analysed at the Centre for Microbiology of the Institute of Public Health of Vojvodina. According to the GPI case definitions of pertussis, the type of laboratory method (real-time polymerase chain reaction or serology tests) depends on the duration of cough and on the age of the suspected patient (Table 1) [9].

As we previously described in detail, nasopharyngeal specimens were defined as positive if *Bordetella pertussis* was detected by the real-time polymerase chain reaction [10]. Additionally, ELISA antibody test from whole blood samples was considered positive if cut-off values were above 100 IU/mL. Because of potentially false positive results, we excluded all participants between four months and nine years of age who had been vaccinated within one year before the collection of whole blood samples [9].

Statistical analysis

We examined an association between potential predictors of hospitalization regarding certain signs/symptoms, sex, the duration of cough, residence, asthma, prescribed antibiotics, diagnostic methods, and vaccination status. In accordance with the vaccination status, we divided participants into two groups: 1) properly vaccinated (children who received the number of vaccine doses corresponding to their age), and 2) partly or non-vaccinated participants (partly vaccinated children were the ones who received some but not all vaccines, while the non-vaccinated patients were those who did not receive any dose of pertussis vaccine). For the analysis of the association between hospitalization and vaccination status, we excluded patients under two months of age because they were below the vaccination age.

The two-tailed Fisher's exact test or χ^2 were used for associations between categorical variables, with the Yate's correction for continuity used for the analysis of dichotomous variables, and the Mann–Whitney U-test for continuous variables. We calculated the difference between the laboratory-confirmed pertussis in inpatient and outpatient healthcare facilities using univariate and multivariate logistic regression models by the odds ratio with 95% confidence interval regarding certain signs/symptoms.

The results were considered statistically significant when the p-value of all applied models was < 0.05. The data were analyzed using the IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) and MedCalc for Windows, Version 12.3.0 (MedCalc Software, Mariakerke, Belgium).

RESULTS

General characteristics of children with laboratory-confirmed pertussis

During 2013–2016, 122 laboratory-confirmed pertussis cases under 10 years of age were reported. Of these, 43 (35.2%) were inpatients and 79 (64.7%) were outpatients.

Table 2. Characteristics of laboratory-confirmed pertussis patients under 10 years of age in the South Bačka District, Vojvodina, 2013–2016

Variable	Total cases No. / total No. (%)	Hospitalized No ./ total No. (%)	Outpatients No. / total No. (%)	p[a]
Sex				
Female	59/122 (48.4)	23/43 (53.5)	36/79 (45.6)	0.518
Male	63/122 (51.6)	20/43 (46.5)	43/79 (54.4)	
Age				
0–3 months	8/122 (6.6)	6/43 (14.0)	2/79 (2.5)	**< 0.001[b]**
4–12 months	11/122 (9.0)	9/43 (20.9)	2/79 (2.5)	
2–5 years	22/122 (18.0)	7/43 (16.3)	15/79 (19.0)	
6–9 years	81/122 (66.4)	21/43 (48.8)	60/79 (76.0)	
Duration of cough in days (Mean ± SD)	25.5 ± 17.3	26.5 ± 19.3	25.0 ± 16.1	0.920[c]
Residence				
Urban area	87/122 (71.3)	29/43 (67.4)	58/79 (73.4)	0.626
Rural area	35/122 (28.7)	14/43 (32.6)	21/79 (26.6)	
Asthma or bronchitis or laryngitis				
Yes	34/122 (27.9)	15/43 (34.9)	19/79 (24.1)	0.288
No	88/122 (72.1)	28/43 (65.1)	60/79 (75.9)	
Diagnostic method				
PCR positive	37/122 (30.3)	17/43 (39.5)	20/79 (25.3)	0.154
Serology (IgG-PT) positive	85/122 (69.7)	26/43 (60.5)	59/79 (74.7)	
Vaccination status[d]				
Properly vaccinated according to age	100/116 (86.2)	28/38 (73.7)	72/78 (92.3)	**0.015**
Partly vaccinated or non-vaccinated	16/116 (13.8)	10/38 (26.3)	6/78 (7.7)	
Antibiotic treatment before sampling				
Yes	25/122 (20.5)	14/43 (32.6)	11/79 (13.9)	**0.028**
No	97/122 (79.5)	29/43 (67.4)	68/79 (86.1)	

SD – standard deviation; PCR – polymerase chain reaction;
IgG – immunoglobulin G; PT – pertussis toxin;
[a]χ^2 test;
[b]Two-tailed Fisher's exact test;
[c]Mann–Whitney test;
[d]Only for patients aged two months to nine years;
Values that differ significantly ($p < 0.05$) between hospitalized and outpatient laboratory-confirmed cases are marked in bold

Patients aged 0–3 months and 4–12 months had a higher risk of hospitalization in comparison with the other two age groups ($p < 0.001$). Among all laboratory-confirmed cases, inpatients were significantly more partly or non-vaccinated against pertussis, and had antibiotic treatment prior to inclusion in the study, in comparison with outpatients ($p = 0.015$ and $p = 0.028$, respectively) (Table 2).

Risk factors for hospitalization

To assess the effects of certain signs/symptoms of clinical case definitions of pertussis for the two age groups and the vaccination status of participants, we compared the results of pertussis-positive children who were hospitalized with those who were not (Table 3, 4, and 5).

Taking into account the required signs/symptoms (RSS) in children aged 0–3 months and four months to nine years (Table 1), Table 3 shows the signs/symptoms in hospitalized and outpatient cases. The most frequent clinical sign/symptom among inpatients and outpatients was whoop (58.1% and 48.1%, respectively). In patients four months to nine years of age, the prevalence of worsening of symptoms at night was 72.7% in outpatients and 67.6% among inpatients. All six hospitalized patients aged 0–3 months with laboratory-confirmed pertussis had cyanosis. According to univariate and multivariate logistic regression analysis, we revealed that RSS in combination with apnea or pneumonia was associated with hospitalization ($p < 0.05$). Although the combinations of RSS and whoop or post-tussive emesis were not significantly associated with hospitalization, the association of these variables increased after adjustment for the confounding effect of the vaccination status.

The RSS along with apnea were a good predictor of hospitalization among children with any duration of cough ($p < 0.05$). In children who had a cough for more than 14 days, the RSS combined with post-tussive emesis or pneumonia or with information of close exposure to a person with a prolonged cough were associated with hospitalization ($p = 0.035$, $p = 0.042$, and $p = 0.046$, respectively) (Table 4).

We analyzed the association between hospitalization and vaccination in properly vaccinated cases and among those who were partly or non-vaccinated against pertussis. There were 116 pertussis cases two months to nine years of age. Of these, 38 (32.8%) were hospitalized. There were fewer hospitalizations in properly vaccinated cases than in partly or non-vaccinated ones between two months and four years of age ($p < 0.008$). However, there was no significant difference between hospitalization in properly and partly or non-vaccinated children against pertussis in patients 5–9 years old ($p = 0.570$) (Table 5).

DISCUSSION

This is the first study to evaluate predictors of hospitalization among laboratory-confirmed pertussis cases in our country. Our findings provide a comprehensive view of

Table 3. Predictive signs/symptoms and contact in hospitalized and outpatients under 10 years of age in the South Bačka District, Vojvodina, 2013–2016

Signs/symptoms/contact	Hospitalized No. / total No. (%)	Outpatients No. / total No. (%)	crude OR (95% CI)	p	adjusted OR[a] (95% CI)	p
Whoop	25/43 (58.1)	38/79 (48.1)	1.50 (0.71–3.17)	0.290	1.63 (0.69–3.89)	0.268
Apnea	18/43 (41.9)	11/79 (13.9)	4.45 (1.85–10.72)	**0.001**	3.05 (1.11–8.39)	**0.031**
Post-tussive emesis	24/43 (55.8)	31/79 (39.2)	1.96 (0.92–4.15)	0.081	2.26 (0.94–5.44)	0.068
Cyanosis[b]	6/6 (100)	1/2 (50)	NA	ND	-	-
Seizure	5/43 (11.6)	0 (-)	NA	ND	-	-
Worsening of symptoms at night[c]	25/37 (67.6)	56/77 (72.7)	0.78 (0.33–1.83)	0.570	0.91 (0.28–2.03)	0.585
Pneumonia	9/43 (20.9)	1/79 (1.3)	20.65 (2.52–169.43)	**0.005**	15.21 (1.60–144.71)	**0.018**
Contact[d]	16/43 (37.2)	21/79 (26.6)	1.64 (0.74–3.62)	0.224	1.14 (0.43–2.99)	0.794

OR – odds ratio; CI – confidence interval; NA – not applicable; ND – not determined;
[a]Adjusted for the following variables: symptoms, sex, duration of cough, residence, asthma, antibiotic prescribed, diagnostic method, and vaccination status;
[b]Only for patients aged 0–3 months;
[c]Only for patients aged ≥ 4 months;
[d]Close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness;
Values that differ significantly ($p < 0.05$) between hospitalized and outpatient laboratory-confirmed cases are marked in bold

Table 4. Predictive signs/symptoms and contact in hospitalized and outpatients under 10 years of age in accordance with the cough duration before sampling in the South Bačka District, Vojvodina, 2013–2016

Signs/symptoms/contact	Cough duration of ≤ 14 days (n = 39)			Cough duration of > 14 days (n = 83)		
	Hospitalized No. / total No. (%)	Outpatients No. / total No. (%)	p[a]	Hospitalized No./total No. (%)	Outpatients No. / total No. (%)	p[a]
Whoop	8/15 (53.3)	14/24 (58.3)	NS	17/28 (60.7)	22/55 (40)	NS
Apnea	6/15 (40)	1/24 (4.2)	0.008	12/28 (42.9)	8/55 (14.5)	0.007
Post-tussive emesis	7/15 (46.7)	10/24 (41.7)	NS	17/28 (60.7)	19/55 (34.5)	0.035
Worsening of symptoms at night[b]	10/12 (83.3)	18/23 (78.3)	NS	15/25 (60)	31/54 (57.4)	NS
Pneumonia	5/15 (33.3)	0 (-)	NA	4/28 (14.3)	1/55 (1.8)	0.042
Contact[c]	6/15 (40)	9/24 (37.5)	NS	10/28 (35.7)	8/55 (14.5)	0.046

NS – not significant; NA – not applicable;
[a]Two-tailed Fisher's exact test;
[b]Only for patients aged ≥ 4 months;
[c]Close exposure to an adolescent or adult (usually a family member) with a prolonged afebrile cough illness

Table 5. Association between vaccination and hospitalization for pertussis among children aged two months two nine years in the South Bačka District, Vojvodina, 2013–2016

Age group	Vaccination status	Hospitalized No. / total No. (%)	Outpatients No. / total No. (%)	p[a]
2 months – 4 years n = 26	Properly vaccinated	4/14 (28.6)	10/12 (83.3)	0.008
	Partly vaccinated or non-vaccinated	10/14 (71.4)	2/12 (16.7)	
5–9 years n = 90	Properly vaccinated	24/24 (100)	62/66 (93.9)	0.570
	Partly vaccinated or non-vaccinated	0 (-)	4/66 (6.1)	

[a]Two-tailed Fisher's exact test

the pertussis burden among children during the first nine years of life.

We revealed that 35.2% of laboratory-confirmed cases under 10 years of age were hospitalized because of pertussis. Furthermore, out of the total number of pertussis patients younger than 12 months of age, about 80% were hospitalized. The study conducted by Crespo et al. [7] found that more than 90% of hospitalized patients with pertussis were younger than 12 months. A probable explanation for the obvious high prevalence of hospitalized cases lies in the fact that the authors of the mentioned study included not only the primary cases, but also all secondary cases of pertussis (contacts with primary cases).

Our results show that RSS in combination with apnea increased the probability of hospitalization by about four times and the combination of RSS accompanied with pneumonia by more than 15 times. In addition, among patients aged 0–3 months, a cough and coryza with no or minimal fever, as the RSS, combined with cyanosis was a good predictor of hospitalization. Considering a lot of research conducted with heterogeneous inclusion/exclusion criteria in varying clinical settings, with different types of diagnostic pertussis tests, as well as various immunization schedules, multiple studies reported different results regarding predictors of pertussis hospitalizations. The results of the aforementioned study highlighted that whoop, apnea, and cyanosis were more frequent in hospitalized than in outpatient cases, and pneumonia was not associated with an increasing risk of hospitalization [7]. The limitation of the mentioned study was the duration of the study period (only two years).

The results of another study, which was conducted among children with pertussis in a hospital setting, showed that children who were readmitted had more cyanotic episodes per day, with a greater number of hospital

days [11]. Furthermore, the results of a recently published meta-analysis indicated that apnea and cyanosis are helpful for detection of pertussis in infants younger than 12 months of age [1].

Many of the implemented case definitions of pertussis predicted the cough duration of ≥ 2 weeks for patients of all ages. Due to the implementation of the new GPI case definitions of pertussis, which predicted inclusion of patients under 10 years of age, regardless of the cough duration, we found that as many as 32% (39/122) of the total number of cases had a cough duration of less than 14 days [9]. Our results are very important if we know that early diagnosis of pertussis in infants allows targeted antibiotic therapy, which could reduce the severity of the disease, the duration of cough and could play an important role in reducing pertussis transmission to close contacts. It is noteworthy that both vaccination and early treatment strategies are equally important for improving outcomes [12–16].

Observing the vaccination status among children from two months to four years of age, we clearly demonstrated an increasing risk of hospitalization among partly or non-vaccinated children in comparison with those who were fully immunized against pertussis. Multiple studies have reported similar results [7, 15–19]. Similar to the results of our research, a study conducted among infants (aged < 12 months) found that properly vaccinated children were protected against hospitalization [20]. In addition, probably because only participants aged < 12 months were included, the authors of the stated study revealed that protection against hospitalization was the same after immunization with whole-cell or acellular pertussis vaccines [20].

Our study findings suggest that the risk of hospitalization was the same regardless of vaccine doses among children aged 5–9 years. We believe that the reasons for this lie in the fact that vaccine-induced immunity waned over time, which consequently led to a decrease of the protective role of vaccination regarding the hospitalization. According to the recently published review data, the estimated duration of protection obtained from the whole-cell pertussis vaccine is 5–14 years, and the one from the acellular vaccine is 4–7 years [21, 22]. One of the recently mentioned explanations for the resurgence of pertussis worldwide, both in schoolchildren and adolescents is connected with changes in the antigens in circulating *Bordetella pertussis* in comparison with the vaccine strains [23].

In regard to the signs/symptoms, the results of the study among fully immunized children with the median age of nine years and the median cough duration of 14 days showed that only 21% of the patients had paroxysmal cough, 13% had post-tussive emesis, 7% apnea, and 6% had classic whoop [24]. Results of another study which was conducted on hospitalized children from one month to 15 years of age with prolonged cough (duration ≥ 14 days) who were previously vaccinated with four doses of vaccine against pertussis, demonstrated that the prevalence of paroxysmal cough was 84.4%, but post-tussive emesis and whoop were rare (31.3% and 28.2%, respectively) [25]. The mentioned differences can be interpreted as the result of various inclusion criteria of the study population. In our research, there were 74% of properly immunized hospitalized children from two months to nine years of age. Probably because of the implementation of active surveillance combined with training of the staff included in our research and low vaccination coverage among hospitalized children, there were 58.1% patients with whoop, 55.8% with post-tussive emesis, and as many as 41.9% children with apnea.

Due to the quality and comparability of the results of our study, we are convinced that this research has the potential to be a standard model in the preparation of more comprehensive hospital surveillance among children with pertussis infection throughout the Republic of Serbia.

CONCLUSION

We revealed that apnea, pneumonia, and cyanosis were good predictors of hospitalization in pertussis cases. In addition, apnoea was a good predictor for hospitalization among children, regardless of the duration of cough. On the other hand, post-tussive emesis, pneumonia, and contact with a person with the prolonged afebrile cough illness were associated with hospitalization among children with the cough duration of > 14 days. Immunization against pertussis corresponding to age reduces the disease severity and hospitalization in children from two months to four years of age.

ACKNOWLEDGMENT

We thank all the parents of the children for accepting to participate in this study. We would also like to thank all health professionals who took part in the surveillance of the pertussis system in the South Bačka District of Vojvodina, Serbia, during 2013–2016. Special thanks go to Mr. Milan Đilas (Institute of Public Health of Vojvodina, Novi Sad, Serbia) and Ms. Svetlana Milovančev for their invaluable contributions to this study.

The study was financially supported by the Institute of Public Health of Vojvodina.

REFERENCES

1. Moore A, Ashdown HF, Shinkins B, Roberts NW, Grant CC, Lasserson DS, et al. Clinical characteristics of pertussis-associated cough in adults and children: a diagnostic systematic review and meta-analysis. Chest. 2017; 152(2):353–67.
2. Edwards K, Decker MD. Whooping cough vaccine. In: Plotkin SA, Orenstein WA, Offit PA, editors. Vaccines. 6th ed. Philadelphia: Elsevier; 2013. p. 447–92.
3. World Health Organization. Pertussis vaccines: WHO position paper, August 2015 – Recommendations. Vaccine. 2016; 34(12):1423–5.
4. Ghanaie RM, Karimi A, Sadeghi H, Esteghamti A, Falah F, Armin S, et al. Sensitivity and specificity of the World Health Organization pertussis clinical case definition. Int J Infect Dis. 2010; 14(12):e1072–5.
5. Hartzell JD, Blaylock JM. Whooping cough in 2014 and beyond: an update and review. Chest. 2014; 146(1):205–14.
6. World Health Organization, 2014. Immunization, vaccines and biologics (pertussis). World Health Organization, Geneva, Switzerland. [accessed 2017 December 5]. Available from: http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/passive/pertussis/en/.
7. Crespo I, Toledo D, Soldevila N, Jordán I, Solano R, Castilla J, et al. Characteristics of hospitalized cases of pertussis in Catalonia and Navarra, two regions in the north of Spain. PLoS One. 2015; 10:e0139993.
8. Institute of Public Health of Vojvodina. Communicable diseases in South Bačka District, 2013–2016. Annual report. Novi Sad: Institute of Public Health of Vojvodina; 2017. (Serbian)
9. Cherry JD, Tan T, Wirsing von König CH, Forsyth KD, Thisyakorn U, Greenberg D, et al. Clinical definitions of pertussis: summary of a Global Pertussis Initiative roundtable meeting, February 2011. Clin Infect Dis. 2012; 54(12):1756–64.
10. Petrović V, Šeguljev Z, Ristić M, Radosavljević B, Đilas M, Heininger U. Pertussis incidence rates in Novi Sad (Serbia) before and during improved surveillance. Srp Arh Celok Lek. 2017; 145(3-4):165–72.
11. Lurie G, Reed PW, Grant CC. When to discharge children hospitalized with pertussis? Acad Pediatr. 2009; 9(2):118–22.
12. Lasserre A, Laurent E, Turbelin C, Hanslik T, Blanchon T, Guiso N. Pertussis incidence among adolescents and adults surveyed in general practices in the Paris area, France, May 2008 to March 2009. Euro Surveill. 2011; 16(5).
13. Muloiwa R, Dube FS, Nicol MP, Zar HJ, Hussey GD. Incidence and diagnosis of pertussis in South African Children hospitalized with lower respiratory tract infection. Pediatr Infect Dis J. 2016; 35(6):611–6.
14. Tiwari T, Murphy TV, Moran J. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC Guidelines. MMWR Recomm Rep. 2005; 54(RR-14):1–16.
15. Cortese MM, Baughman AL, Zhang R, Srivastava PU, Wallace GS. Pertussis hospitalizations among infants in the United States, 1993 to 2004. Pediatrics. 2008; 121(3):484–92.
16. Barlow RS, Reynolds LE, Cieslak PR, Sullivan AD. Vaccinated children and adolescents with pertussis infections experience reduced illness severity and duration, Oregon, 2010–2012. Clin Infect Dis. 2014; 58(11):1523–9.
17. Wymann MN, Richard JL, Vidondo B, Heininger U. Prospective pertussis surveillance in Switzerland, 1991-2006. Vaccine. 2011; 29(11):2058–65.
18. Juretzko P, von Kries R, Hermann M, Wirsing von König CH, Weil J, Giani G. Effectiveness of acellular pertussis vaccine assessed by hospital-based active surveillance in Germany. Clin Infect Dis. 2002; 35(2):162–7.
19. Briand V, Bonmarin I, Lévy-Bruhl D. Study of the risk factors for severe childhood pertussis based on hospital surveillance data. Vaccine. 2007; 25(41):7224–32.
20. Crespo Fernández I, Soldevila N, Carmona G, Sala MR, Godoy P, Domínguez A. Surveillance of hospitalized and outpatient cases of pertussis in Catalonia from 2003 to 2009. Hum Vaccin Immunother. 2013; 9(3):667–70.
21. Kilgore PE, Salim AM, Zervos MJ, Schmitt HJ. Pertussis: microbiology, disease, treatment, and prevention. Clin Microbiol Rev. 2016; 29(3):449–86.
22. Burdin N, Handy LK, Plotkin SA. What is wrong with pertussis vaccine immunity? The problem of waning effectiveness of pertussis vaccines. Cold Spring Harb Perspect Biol. 2017; 9(12).
23. Plotkin SA. The pertussis problem. Clin Infect Dis. 2014; 58(6):830–3.
24. Yaari E, Yafe-Zimerman Y, Schwartz SB, Slater PE, Shvartzman P, Andoren N, et al. Clinical manifestations of *Bordetella pertussis* infection in immunized children and young adults. Chest. 1999; 115(5):1254–8.
25. Narkeviciute I, Kavaliunaite E, Bernatoniene G, Eidukevicius R. Clinical presentation of pertussis in fully immunized children in Lithuania. BMC Infect Dis. 2005; 5:40.

Велики кашаљ код деце млађе од десет година

Миољуб Ристић[1,2], Биљана Радосављевић[1], Владимир Петровић[1,2]

[1]Институт за јавно здравље Војводине, Нови Сад, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија

САЖЕТАК

Увод/Циљ Велики кашаљ је вакцинама спречива заразна болест која је узрок великог броја оболелих и хоспитализованих широм света.

Циљ рада је био да се одреде предиспонирајући фактори за хоспитализацију оболелих од великог кашља у узрасту млађих од десет година у Јужнобачком округу (Војводина).

Методе рада Подаци за ову опсервациону студију добијени су из болничких и ванболничких здравствених установа Јужнобачког округа, у периоду од 1. јануара 2013. до 31. децембра 2016. године. Предиспонирајући фактори за хоспитализацију оболелих процењивани су на основу клиничких критеријума Глобалне пертусисне иницијативе за дефиницију великог кашља. Велики кашаљ је доказиван употребом *PCR* метода или серолошким (*ELISA*) тестовима.

Резултати Од 122 потврђена случаја великог кашља, хоспитализовано је 43 (35,2%). Апнеа и пнеумонија су корелирали са хоспитализацијом, а свих шест хоспитализованих болесника млађих од три месеца имали су цијанозу. Апнеа је била добар прогностички знак хоспитализације за оболеле, без обзира на дужину трајања кашља ($p < 0,05$). Код оболелих чији је кашаљ трајао дуже од 14 дана прогностички знаци за хоспитализацију су били повраћање после кашља, пнеумонија и контакт са особом која је имала дуготрајни кашаљ ($p = 0,035$, $p = 0,042$ и $p = 0,046$). Потпуно имунизована деца узраста од два месеца до четири године била су ређе хоспитализована у односу на непотпуно имунизовану и невакцинисану децу истог узраста ($p < 0,008$).

Закључак Код деце млађе од десет година, апнеа, пнеумонија и цијаноза показали су се као добри прогностички знаци за хоспитализацију оболелих од великог кашља. Вакцинација против пертусиса у складу са узрастом доприноси појави блажих клиничких форми обољења и смањењу броја хоспитализација код деце узраста од два месеца до четири године.

Кључне речи: велики кашаљ; хоспитализација; надзор; епидемиологија

DOI: https://doi.org/10.2298/SARH170811175S
UDC: 616.72-002-085-053.2; 616.71-007.234-053.2; 606:616.72-002.77

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Bone mineral density in children with juvenile idiopathic arthritis after one year of treatment with etanercept

Gordana Sušić[1], Marija Atanasković[1], Roksanda Stojanović[1,2], Goran Radunović[1,2]

[1]Institute of Rheumatology, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction/Objective Juvenile idiopathic arthritis (JIA) is the most frequent chronic inflammatory, rheumatic disease of childhood, associated with disturbance of bone mineral metabolism, which develops gradually and progressively, and if untreated eventually leads to osteoporosis in adulthood.
The aim of our study was to evaluate bone mineral density (BMD) in patients with JIA treated with etanercept over a period of one year.

Methods The prospective cohort study included 94 JIA patients (66 female, 28 male), their median age being 14.77 years. BMD was measured by dual-energy X-ray absorptiometry on the lumbar spine. Disease activity was assessed using the American College of Rheumatology Pedi 50 criteria.

Results After one year of treatment with etanercept, we found a statistically significant increment in all osteodensitometry variables ($p < 0.001$). Annual enhancement for the whole group was as follows: bone mineral content 15.8%, BMD 7.2%, BMD_{vol} 4.2%. Z-score improved from -0.86 to -0.58 SD at the last visit, but decreased in rheumatoid factor-positive polyarthritis patients. Patients with systemic JIA had the lowest Z-score. Z-score correlated with functional disability level. BMD was lower in the group treated with glucocorticoids.

Conclusion Our results showed significant improvement of bone mineral density in children with JIA after one year of treatment with etanercept. Rheumatoid factor-positive and systemic JIA subtypes and treatment with glucocorticoids are the risk factors for impairing bone mineral metabolism.

Keywords: juvenile idiopathic arthritis; bone mineral density; anti-TNF

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most frequent autoimmune, rheumatic disease in childhood. Inflammatory process affects primarily the synovial joints and cartilage, leading to excessive production of proinflammatory cytokines. Longstanding inflammation beginning in childhood, when the development of the skeletal system and growth are not yet completed, may cause many complications such as growth retardation, disturbance of bone metabolism leading to osteopenia and osteoporosis, which become apparent when fractures occur [1, 2, 3].

Disturbance of bone metabolism in JIA develops gradually and progressively and it is the result of interaction of many factors. The most important is disease activity and severity, genetic predisposition, duration of the disease, number of affected joints, poor nutrition, medications, especially glucocorticoids (GC), delayed puberty, reduced physical activity, lack of exposure to the sun, and others [4].

There is evidence that JIA is associated with low bone mineral density (BMD), as a result of impairment of bone mineral acquisition during adolescent growth spurt and the inability of achieving optimal peak bone mass. Forty-one percent of adolescents with early-onset JIA had low bone mass > 11 years after disease onset. The development of low total-body bone mineral content (BMC) correlated with the duration of active disease, disease severity, measures of bone resorption, weight, and height [5]. It is a reasonable approach in improving bone health and preventing fractures to suppress disease activity using more efficient therapeutic options. Etanercept (ETN), as a tumor necrosis factor (TNF) blocker, has shown its effectiveness inducing prompt and sustained suppression of disease activity [6, 7]. In addition, there is growing evidence that ETN could have a protective role in preventing structural bone damage by increasing osteoblastic and decreasing osteoclastic activity [8].

There are several modalities of assessing pediatric skeleton, but dual-energy X-ray absorptiometry (DXA) remains the preferred method because of its high precision, accuracy, reproducibility, speed, and especially low radiation exposure and availability of reference data [9].

The aim of our study was to examine bone mineral status in patients with JIA after one year of treatment with ETN.

Received • Примљено: August 11, 2017
Accepted • Прихваћено: September 11, 2017
Online first: September 22, 2017

Correspondence to:
Gordana SUŠIĆ
Institute of Rheumatology
Resavska 69, 11000 Belgrade
Serbia
susic.gordana@gmail.com

METHODS

We undertook a prospective study that included 94 consecutive children with established diagnosis of JIA, referred to the Institute of Rheumatology, the main tertiary care referral hospital in the country, between January 2010 and December 2016. The main inclusion criterion was that those failed to achieve inactive disease according to Wallace et al. [10] criteria despite previously treatment with GC and/or methotrexate (MTX), or had intolerance to MTX have been started on ETN 0.4 mg/kg of body weight twice weekly. The biologic therapy was commenced according to the local criteria for reimbursement covered by health insurance.

According to the International League of Associations for Rheumatology classification, 10 (10.6%) patients had systemic onset (sJIA), 28 (29.8%) patients had polyarthritis rheumatoid factor (pJIA RF) negative, 15 (16%) had polyarthritis RF positive, 20 (21.3%) patients had extended oligoarthritis, 19 (20.2%) had enthesitis and arthritis, and two patients (2.1%) had psoriatic arthritis, who were included in the polyarticular seronegative JIA group for futher analysis, because their clinical presentation was arthritis of peripheral joints [11].

Physical examination, laboratory investigation, and functional ability assessment were done at baseline and 12 months after introducing ETN.

The impact of arthritis on physical ability was estimated using the Serbian version of the Childhood Health Assessment Questionnaire (CHAQ) [12]. This questionnaire, which contains 69 questions regarding usual daily living activities, was completed by a parent or a child if he/she was older than 12 years, and refers to physical ability during the week prior to the clinic visit. CHAQ disability index (CHAQ DI) ranges from 0 (best) to 3 (worst). CHAQ DI represents the average sum of the entire eight areas covered by CHAQ and is divided into four categories: 0 = no disability, 0.1–0.5 = mild, 0.6–1.5 = moderate, and > 1.5 = severe disability [13].

Disease activity was assessed according to the criteria by Giannini et al. [13], which include physician's global assessment of disease activity (PGA) on a 100-mm visual analogue scale (VAS), parent's or patient's assessment of overall well-being on VAS (ranging from 0 mm, being the best, to 100 mm, being the worst), functional ability (CHAQ), number of joints with active arthritis and number of joints with limited range of motion (LOM) as clinical variables, erythrocyte sedimentation rate (ESR) as a laboratory sign of inflammation. Active joint was defined if joint swelling or any two of the following signs were present: LOM, joint pain/tenderness, or joint warmth. The patients were divided into two groups according to the American College of Rheumatology Pediatric 50 definition of improvement [10]. If the patients demonstrated at least 50% improvement from the baseline in at least three of any six core set variables with no more than one indicator worsening by more than 30%, they were considered as responders.

The treatment was analyzed recording the number of patients receiving GC, MTX administered at a dose of 10–15 mg/m^2 of body surface area per week.

Written informed consent form was obtained from parents or from patients if they were older than 18 years. The study was approved by the Ethics Committee of the Institute of Rheumatology of Belgrade, Serbia.

Osteodensitometry examination was performed using LUNAR DPX-L pediatric software DXA absorptiometry device. Measurements of BMD were performed on the lumbar spine (anterior–posterior scan), and the value from the L2–L4 segment was taken for analysis at the beginning of treatment with ETN and 12 months later. During the examination, the patients were in the supine position with flexed hips and knees at 90°, in order to correct the physiological lordosis of the lumbar spine.

BMD area expressed in g/cm^2, bone mineral content (BMC) in g/cm, and Z-score expressed in standard deviation (SD) were taken for analysis. Z-score signifies a patient's BMD mean – BMD from an age- and sex-matched reference group, divided by SD for the reference group; the manufacture's database of Italian population of children was used. According to the Z-score, the patients were divided into two groups: group I with Z-score < -1 SD, and group II with Z-score ≥ -1 SD.

In order to eliminate the effect of the length of the bone on BMD, we used the formula $BMD_{vol} = BMD \times [4 / \pi \times$ L2–L4 region width in cm], representing volumetric bone mineral density (BMD_{vol}) expressed in g/cm^3 [14].

Statistical analysis

Data were evaluated by descriptive statistics and analytical models using SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA). To describe the groups, descriptive statistical methods were used: the grouping and graphical representation; calculating measures of central tendency (arithmetic mean – $\bar{x}$, the median – Med.); calculating measures of variability (standard deviation – SD, standard score variations – SSD), and calculating the relative numbers.

From analytical statistical methods, Student's t-test and analysis of variance were used to test for differences between groups of respondents for parametric data, and Mann–Whitney U-test and χ^2 test were used for nonparametric data. To test the normality of distribution of data, Kolmogorov–Smirnov test was used. To assess correlation, the data were applied to a single linear correlation and Spearman's rank correlation.

RESULTS

The median baseline age of 94 patients with JIA was 14.77 years (range of 5–20 years), the median disease duration was 4.42 years (0.72–19). Almost three quarters of patients were female. Demographic and clinical characteristics of the patients are shown in Table 1.

At the entry of study, 12 patients (12.8%) were of preschool age, 20 (21.3%) were 7–12 years old, and 62 (66%) were teenagers. The patients were nine years old (median) at the time the first symptoms of JIA appeared, and 33

Table 1. Baseline demographic and clinical characteristics of the patients

Characteristics	Value (n = 94)
Female/male ratio	66/28 (70.2/29.8)
Age, median (min.–max.), years	14.77 (5.0–20.0)
Age at the onset of the disease, median (min.–max.), years	9.0 (1.08–16.0)
Disease duration, median (min.–max.), years	4.42 (0.72–19)
JIA subtypes	
Systemic	10 (10.6)
Polyarticular, RF negative	28 (29.8)
Polyarticular, RF positive	15 (16.0)
Oligoarticular, extended	20 (21.3)
Enthesitis-related arthritis	19 (20.2)
Psoriatic arthritis	2 (2.1)
No patients on methotrexate	79 (84.0)
No patients on glucocorticoids	22 (23.4)

Table 2. Disease activity core set variables at baseline and after 12 months

Variables	Baseline	After 12 months	% of improvement	p
PGA (mm)	46.4	9.2	81.8	< 0.001
Parent/patient assessment of well-being (mm)	37.5	12.4	47.9	< 0.01
Number of joints with LOM	10.1	4.7	69.9	< 0.001
Number of active joints	9.7	0.2	81.1	< 0.001
CHAQ	0.7	0	66.8	< 0.001
ESR (mm/h)	34	17	39.8	< 0.001

All values are median;
LOM – limited range of motion; PGA – physician global assessment; CHAQ – childhood health assessment questionnaire; ESR – erythrocyte sedimentation rate

Table 3. Osteodensitometry evaluation of the patients at baseline and after 12 months

Variables	Baseline (mean ± SD)	After 12 months (mean ± SD)	p
BMD	0.90 ± 0.25	0.95 ± 0.240	< 0.001
BMC	32.75 ± 16.65	36.19 ± 16.18	< 0.001
Z-score	-0.86 ± 1.23	-0.58 ± 1.21	< 0.001
BMD_{vol}	0.31 ± 0.05	0.32 ± 0.55	< 0.001

BMD – bone mineral density; BMC – bone mineral content; BMD_{vol} – bone mineral density volumetric

Table 4. Z-score according to the arthritis subtype

Arthritis subtype	Baseline*	After 12 months**	p
Systemic	-2.14	-1.87	0.085
Polyarticular, RF negative	-0.66	-0.36	0.005
Polyarticular, RF positive	-0.63	-0.69	0.377
Oligoarticular, extended	-0.85	-0.66	0.041
Enthesitis-related arthritis	-0.76	-0.31	0.010

Intergroup (Kruskal–Wallis)
*p = 0.052
**p = 0.033

(35.1%) were of preschool age. More than half of the patients (57.4%) had disease duration of less than two years, and 11 patients (11.7%) suffered from arthritis more than 10 years.

All the patients had polyarthritis, were non-responsive or had adverse reactions to previous MTX treatment. It was the first condition for prescribing biologic therapy covered by health insurance system in our country. The most frequent was polyarticular seronegative arthritis group, comprising one third of the patients.

At baseline, 79 patients (84%) were treated with MTX, and 22 patients (23.4%) received oral GC.

Disease activity core set criteria according to Giannini et al. [13] at baseline and at the last visit after 12 months are presented in Table 2.

We found significant improvement in all six core set variables representing disease activity, a year after introducing ETN ($p < 0.001$); the most pronounced improvement was observed in PGA and clinical manifestations of the disease (81%). At baseline, 56 (59.5%) patients had moderate and severe functional disability (DI 0.5–1.5 and above 1.5, respectively), but at the end of the follow-up period, 77 (81.9%) patients had no limitations or had mild limitations in performing everyday activities ($p < 0.001$).

At the last visit, 82 (87.2%) patients met the American College of Rheumatology Pedi 50 criteria and were assigned to the group of responders, while 12 (12.8%) patients did not show satisfied therapeutic effect and were assigned to the group of non-responders. Among non-responders, there were three patients with sJIA, five with pJIA RF negative, and three patients with RF positive polyarthritis. Non-responders, with the exception of one female patient, were slightly older and were younger at the onset of the disease; however, this was not statistically significant except in relation to GC treatment and disease duration, which was longer in non-responders. In the non-responder group at baseline, 50% of the patients continued GC treatment, and 33.3% of patients were continuing the treatment at the last visit. In the responder group, only two patients used GC at the end.

At baseline, the two groups did not differ with regard to osteodensitometry variables (BMD, BMC, Z-score, and BMD_{vol}). Bone mineral status of patients at baseline and at the last evaluation is presented in Table 3.

After one year of treatment with ETN, statistically significant increment in all osteodensitometry variables was present ($p < 0.001$). Mean annual enhancement for BMC was 15.8%, 7.2% for BMD, and 4.2% for BMD_{vol} for the entire group of patients. The Z-score also improved from -0.86 to -0.58 SD after one year of treatment.

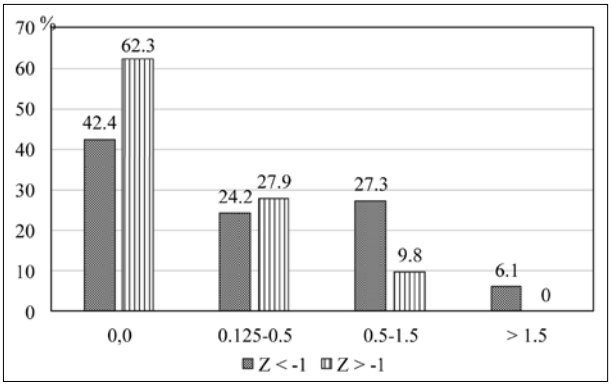
According to the type of arthritis, there was an intergroup difference in the Z-score at baseline ($p = 0.052$) and after one year ($p = 0.033$). Patients with sJIA had the lowest Z-score (-2.14 SD, -1.87 SD, respectively); all were on GC.

Analyzing the groups separately according to the arthritis subtype, after 12 months of treatment, all osteodensitometry values significantly improved for the seronegative polyarticular onset ($p < 0.001$), and enthesitis-related arthritis patients (for BMD and BMD_{vol}, $p < 0.001$, for Z-score and BMC $p < 0.01$). In contrast, in pJIA RF-positive patients, Z-score decreased from -0.63 SD to -0.69 SD. In this group, the only statistically significant improvement was found with regard to BMC ($p = 0.041$).

The results are presented in Table 4.

At the first osteodensitometry measurement, 44 patients (46.8%) had a Z-score below -1 SD; one year after, 11 out of 44 patients improved their Z-score ($p < 0.01$). During the study period, we observed significant height and weight increase in the patients (data not shown).

Disability index calculated from the CHAQ negatively correlated with the Z-score at baseline, as well one year after ($p = 0.017$ vs. $p = 0.002$). One third of the patients with moderate and severe functional limitations had a Z-score below -1 SD from the reference value. Seventy percent of patients with very low BMD (< -2 SD) had moderate or severe disability at baseline, and 50% at the last examination. The values are presented in Figure 1.



p = 0.021

Figure 1. Correlation of Z-score with disability level on the last evaluation

BMD, BMC, and BMD_{vol} were lower in the GC group ($p < 0.01$). At the end of the study, GC were stopped in 12/22 patients (54.4%); the dosage of GC was decreased in 6/22 patients (27.3%), and the dosage of GC was not changed in four patients (18.3%); all were nonresponders.

DISCUSSION

Over the last 15 years, the outcome of children with JIA dramatically improved with introducing biologic therapy as a regular treatment option. ETN has been the first anti TNF-blocker licensed for use in JIA. Remarkably, rapid and sustained efficacy of ETN in controlling inflammation, inhibiting progression of joint destruction, and acceptable safety profile was confirmed [6, 7].

We performed a prospective study to investigate bone mineral status in patients with JIA treated with ETN over a period of one year. Our results confirmed excellent ETN efficacy in suppressing disease activity, which reflected on PGA (the improvement was as much as 82%), on parents' or patients' assessment from the clinical point of view (number of joints with active arthritis decreased from 9.7 to 0.2), as well on laboratory signs of inflammation. Rapid decrement of C-reactive protein and thrombocytes and enhancement of hemoglobin were also recorded, but the data are not shown.

There are many risk factors that contribute to bone fragility: high disease activity, poor nutrition, reduced physical activity, growth impairment, puberty delay and inability to reach adequate peak bone mineral accretion, treatment with GC especially, and others. According to Markula-Patjas et al. [15], compressive fractures, mainly thoracic, were associated with a high level of disease activity, high body mass index, and exposure to a high dose of GC, but not with disease duration, nor with BMD.

Assessment of pediatric skeletons can be performed by many methods: X-ray, quantitative computed tomography, quantitative ultrasonography, magnetic resonance imaging, but DXA remains a preferred method for clinical measurement of bone mineral density in children because of many advantages previously mentioned. Paediatric Position Development Conference of the International Society of Clinical Densitometry put JIA on the list of secondary diseases that may affect the skeleton and gave recommendations for interpretation of DXA results in pediatric population. The terms "osteopenia" and "osteoporosis" should be avoided at pediatric age. BMC and BMD Z-score of ≥ 2 SD below what is expected should be labelled "low for age." Diagnosis of osteoporosis in children can be made when both low bone mass and bone fracture history are present [16].

In our previous study, we confirmed decreased BMD in JIA patients, compared with healthy peers (Z-score -1.02 vs. -0.09 SD, $p < 0.001$). Systemic onset, polyarthritis, longer treatment, and higher cumulative GC dosage, higher damage (functional status and radiologic stage) were risk factors for low BMD. Some of these patients participated in this study but were subsequently not treated with biologics [17]. This study did not include a control group, but the patients were prospectively followed during one year and results at baseline served for further statistical analysis.

Lien et al. [18] explored predictors of bone mass in children with early arthritis (mean disease duration 19.3 months) compared with healthy children. A value between -1 SD and -2 SD was defined as low BMD and BMC, and very low if it was less than -2 SD of the reference value of the healthy population. During a two-year follow-up period, it was found that 24% of patients and 12% of controls had low and very low total BMC. Bone formation and resorption were reduced in the patient group. The results of that study point out that bone metabolism disturbance begins in the early stages of the disease. Patients with polyarthritis had significantly lower BMC compared to the children with oligoarthritis.

In our study, 28.7% of the patients had low BMD, and 18.1% had very low BMD at baseline. This group comprised 17 patients, their median disease duration being 6.3 years, mean number of active joints 12, with moderate functional disability (CHAQ DI 0.88). After one year of treatment, number of patients with low BMD decreased to 19.2%, and 15.9% of the patients had a Z-score below -2 SD. Only one girl with sJIA had a compressive vertebral fracure. Her Z-score at baseline was -3.9, which decreased by the end of study to -4.4 SD; she was a non-responder with a seven-year-long GC treatment.

French et al. [5] found that the average age of 40% of patients with JIA is 35years, that their disease duration is

27 years, and that they had osteopenia at the spine and femoral neck. Risk factors for developing osteopenia were functional class II and higher during adolescence, inadequate participation in organized sports and other forms of physical activity during adolescence, smoking, insufficient dietary calcium intake during adolescence. According to the results, the majority of adult patients reached normal peak of bone mass, but it was significantly lower compared to the healthy population.

Unquestionably, the role of bone remodeling belongs to proinflammatory cytokines such as the TNF, interleukin-1, interleukin-6, interleukin-17, as well as matrix metalloproteinases produced in synovial membrane, which cause the destruction of joint bone and cartilage. Their presence in affected joints can cause excessive osteoclastogenesis, bone resorption, and suppression of osteoclastogenesis [19].

The first study addressing bone mineral status on JIA patients treated with ETN published by Simonini et al. [20] included 20 patients. The patients were younger and disease duration was shorter, and functional disability was higher than in our study. Bone status was determined by broadband ultrasound attenuation at the calcaneus; however, they agreed that DXA remains the gold standard for measuring BMD. After one year of ETN treatment, responders showed higher broadband ultrasound attenuation and Z-score than non-responders.

In the responder group, we found important improvements in all densitometry variables compared to the baseline; in the nonresponder group, statistically significant improvement was only for BMC, which could be explain by the increased linear growth, which was observed, but the data are not presented.

Patients with sJIA on both evaluations had the lowest Z-score and there was no increasing during treatment with anti-TNF blocker. Stagi et al. [21] presented similar results from a large cohort group of 245 patients, with a wider range of age (9–28 years) than that in our study. Patients with sJIA had significant reduction in cortical and trabecular BMD as well, compared to the control group. In our group of patients, the Z-score decreased in RF pJIA patients during one year. The patients were all female, 6/15 were treated with GC, CHAQ 0.725 corresponded to moderate functional disability. In sJIA patients, the Z-score did not significantly increase, confirming that both JIA subtypes have unfavorable outcome, resulting in joint destruction and higher disability in adulthood.

Deleterious effect of GC on inhibition of bone formation caused by a decrease in the number of osteoblasts is well known. It eventually leads to decreasing bone remodeling and increases the tendency towards fractures. Patients treated with GC had lower BMD compared to non-GC patients in our study. At the last observation, BMD_{vol} and Z-score did not improve significantly. We did not analyze in more details the GC group (duration, cumulative dosage, etc.). Thornton et al. [22] examined bone health in adults with a history of JIA; oral GC was associated with lower BMD at both the spine and the hip. Similar results were revealed in a study by Tang et al. [23] – main predictors of low spine BMD were the JIA subtype, disease activity, BMD, and GC exposure.

Doubtlessly, reduced bone mass and density in JIA develop as a result not only of impairment of bone turnover, but also due to lower muscle strength, poorer physical health, and high level of functional disability.

We are aware of some limitation of our study. We had no control group of healthy children and did not include biochemical markers of bone turnover. One year of follow-up period is too short for understanding all aspects of influence of anti-TNF blockers on bone mineral metabolism.

CONCLUSION

Our results confirmed a significant improvement in BMD during one year of treatment with ETN, as well as its efficacy on disease activity. Longitudinal studies and larger cohorts could give better understanding of the long-term outcome and safety of anti-TNF blockers.

In the meantime, our task as physicians is to carefully monitor our patients, apply the best therapeutic options for better control of disease activity, and advise them to make some lifestyle changes, such as reducing or discontinuing smoking and excessive alcohol intake, participation in weight-bearing exercises and sports activities, consumption of dietary calcium and vitamin D supplementation in order to prevent long-term consequences.

ACKNOWLEDGEMENT

We wish to thank Dr. Dejan Stevanović for helpful comments on the manuscript.

REFERENCES

1. Roth J, Bechtold S, Borte G, Dressler F, Girschick HJ, Borte M. Osteoporosis in juvenile idiopathic arthritis: a practical approach to diagnosis and therapy. Eur J Pediatr. 2007; 166(8):775–84.
2. Burnham JM. Inflammatory diseases and bone health in children. Curr Opin Rheumatol. 2012; 24(5):548–53
3. Brabnikova Maresova K. Secondary osteoporosis in patients with juvenile idiopathic arthritis. J Osteoporosis. 2011; 569417.
4. Lien G, Flatø B, Haugen M, Vinje O, Sørskaar D, Dale K, et al. Frequency of osteopenia in adolescents with early-onset juvenile idiopathic arthritis: a long-term outcome study of one hundred five patients. Arthritis Rheum. 2003; 48(8):2214–23.
5. French AR, Mason T, Nelson AM, Crowson CS, O'Fallon MW, Khosla S, et al. Osteopenia in adults with history of juvenile rheumatoid arthritis. A population based study. J Rheumatol. 2002; 29(5):1065–70.
6. Lovell DJ, Giannini EH, Reiff A, Cawkwell GD, Silverman ED, Nocton JJ, et al. Etanercept in children with polyarticular juvenile rheumatoid arthritis. N Engl J Med. 2000; 342(11):763–9.
7. Horneff G, Burgos-Vargas R, Constantin T, Foeldvari I, Vojinovic J, Chasnyk VG, et al. Efficacy and safety of open label etanercept on extended oligoarthticular juvenile idiopathic arthritis, enthesitis

related arthritis and psoriatic arthritis: part 1 (week 12) of CLIPPER study. Ann Rheum Dis. 2014; 73(6):1114–22.
8. Seriolo B, Paolino S, Sulli A, Cutolo M. Are there any positive effect of TNF-alfa blockers on bone metabolism? Reumatismo. 2006; 58(3):199–205.
9. Bacharach L, Sills I. Clinical report – bone densitometry in children and adolescents. Pediatrics. 2011; 127(1):189–94.
10. Wallace CA, Ruperto N, Giannini EH, for the Childhood Arthritis and Rheumatology Research Alliance (CARRA), the Pediatric Rheumatology International Trials Organization (PRINTO) & Pediatric Rheumatology Collaborative Study Group (PRCSG). Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis (JIA). J Rheumatol. 2004; 31(11):2290–94.
11. Petty RE, Southwood TR, Manners P, Baum J, Glass DN, Goldenberg J, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. J Rheumatol. 2004; 31(2):390–2.
12. Sušić G, Ruperto N, Stojanović R, Gacić D, Pilipović N, Pašić S, et al. The Serbian version of the Childhood Health Assessment Questionnaire (CHAQ) and the Child Health Questionnaire (CHQ). Ciln Exp Rheum. 2001; 19 (suppl. 23):S168–72.
13. Giannini EH, Ruperto N, Ravelli A, Lovell DJ, Felson DT, Martini A. Preliminary definition of improvement in juvenile arthritis. Arthritis Rheum. 1997; 40(7):1202–9.
14. Kröger H, Kotaniemi A, Kröger L, Alhava E. Development of bone mass and bone density of the spine and female neck – a prospective study of 65 children and adolescent. Bone Miner. 1993; 23(3):171–82.
15. Markula-Patjas KP, Valta HL, Kerttula LI, Soini IH, Honkanen VE, Toiviainen-Salo SM, et al. Prevalence of vertebral compression fracture and associated factors in children and adolescents with severe juvenile idiopathic arthritis. J Rheumatol. 2012; 39(2):365–73.
16. Rauch F, Plotkin H, DiMeglio L, Engelbert RH, Henderson RC, Munns C, et al. Fracture prediction and the definition of osteoporosis in children and adolescents: the ISCD 2007 Pediatric Official Positions. J Clin Densitom. 2008; 11(1):22–8.
17. Susic G, Pilipovic N, Stojanovic R. Bone mineral density in patients with juvenile idiopathic arthritis. Srp Arh Celok Lek. 2009; 137 (7-8):396–401.
18. Lien G, Selvaag AM, Flatø B, Haugen M, Vinje O, Sørskaar D, et al. A two-year prospective controlled study of bone mass and bone turnover in children with early juvenile idiopathic arthritis. Arthritis Rheum. 2005; 52(3):833–40.
19. Viswanathan A, Sylvester FA. Chronic paediatric inflammatory diseases: effects on bone. Rev Endocr Metab Disord. 2008; 9(2):107–22.
20. Simonini G, Giani T, Stagi S, de Martino M, Falcini F. Bone mineral status over 1 year of etanercept treatment in juvenile idiopathic arthritis. Rheumatology. 2005; 44(6):777–80.
21. Stagi S, Cavalli L, Signorini C, Bertini F, Cerinic M, Brandi ML, et al. Bone mass and quality in patients with juvenile idiopathic arthritis: longitudinal evaluation of bone-mass determinants by using dual-energy x-ray absorptiometry, peripheral quantitative computed tomography and quantitative ultrasonography. Arthritis Res Ther. 2014; 16(2):R83.
22. Thornton J, Pye SR, O'Neill TW, Rawlings D, Francis RM, Symmons DP, et al. Bone health in adult men and women with a history of juvenile idiopathic arthritis. J Rheumatol. 2011; 38(8):1689–93.
23. Tang T, Tang Xu, Hu L, Huang Y, Li Q. Evaluation of bone mass in children and young adults with juvenile idiopathic arthritis. Clin Exp Rheumatol. 2015; 33(5):758–64.

Густина кости код деце са јувенилним идиопатским артритисом после годину дана лечења етанерцептом

Гордана Сушић[1], Марија Атанасковић[1], Роксанда Стојановић[1,2], Горан Радуновић[1,2]

[1]Институт за реуматологију, Београд, Србија
[2]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод/Циљ Јувенилни идиопатски артритис (ЈИА) јесте најчешће хронично, запаљенско реуматско обољење у детињству, удружено са поремећајем минералног костног метаболизма, који се развија постепено и прогресивно и доводи до остеопорозе у одраслом добу.

Циљ наше студије је био да се испита костни минерални статус код болесника са ЈИА после годину дана лечења етанерцептом.

Метод Проспективна кохортна студија је укључила 94 болесника са ЈИА (66 девојчица, 28 дечака) просечног узраста 14,77 година. Минерална густина кости (МГК) мерена је двоструком апсорпциометријом *X* зрака на лумбалној кичми. Степен активности болести је процењиван *ACR*50 критеријумима.

Резултати После годину дана лечења етанерцептом установили смо статистички значајно побољшање у свим остеодензитометријским показатељима ($p < 0,001$). Годишње повећање за целу групу било је: укупно минерала у костима 5,8%, МГК 7,2%, $МГК_{вол}$ 4,2%. З-скор се поправио са -0,86 до -0,58 СД на крају истраживања, али је у групи болесника са РФ позитивним полиартритисом дошло до смањења З-скора. Најнижи З-скор су имали болесници са системским ЈИА. З-скор је корелисао са степеном функцијске неспособности. Болесници лечени гликокортикоидима имали су значајно нижу минералну густину.

Закључак Наши резултати су показали значајно побољшање минералне густине кости код деце са ЈИА после годину дана лечења етанерцептом. Болесници са РФ позитивним полиартритисом и системским артритисом, као и они који примењују гликокортикоиде, имају већи ризик за поремећај костног метаболизма.

Кључне речи: јувенилни идиопатски артритис; минерална густина кости; анти-ТНФ

DOI: https://doi.org/10.2298/SARH180222038M
UDC: 616.14-005.6-07

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Sensitivity and specificity of D-dimer tests compared to ultrasound examination of deep vein thrombosis

Dragan Marković[1,2], Dragan Vasić[1,2], Jelena Bašić[3], Slobodan Tanasković[1,4], Slobodan Cvetković[1,2], Zoran Rančić[5,6]

[1]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[2]Clinical Centre of Serbia, Clinic for Vascular and Endovascular surgery, Belgrade, Serbia;
[3]Rudolfstiftung, Department of Cardiology, Vienna, Austria;
[4]Dedinje Cardiovascular Institute, Vascular Surgery Clinic, Belgrade, Serbia;
[5]University Hospital Zurich, Department of Cardiovascular Surgery, Zurich, Switzerland;
[6]University of Zurich, Faculty of Medicine, Zurich, Switzerland

SUMMARY

Introduction/Objective Untreated deep vein thrombosis (DVT) is associated with a high risk of pulmonary embolism (PE), and false diagnosis of DVT results in unnecessary anticoagulant therapy, with a risk of bleeding. Accurate diagnosis of DVT and prompt therapy are essential to reduce the risk of thromboembolic complications.

The aim of our study was to evaluate the sensitivity and specificity of three D-dimer tests (DD PLUS, HemosIL, and VIDAS) comparing to compression ultrasonography (CUS) examination.

Methods We observed 350 patients, some with different risk factors. The patients underwent the same protocol (evaluation of the patient's history, physical examination, and D-dimer testing), and CUS was used as a reference for all the patients. According to Wells score, the patients were divided into groups with low, moderate, and high pretest probability (PTP).

Results Most of the examined patients were with moderate PTP. The CUS showed that there was the highest number of examined patients without DVT. Most of the examined patients with a positive CUS finding had proximal iliac and femoral DVT.

VIDAS test was positive in the highest percentage in the group of patients with CUS-documented thrombosis.

Conclusion All three D-dimer tests used in our study had similar sensitivity and specificity. However, VIDAS test had higher levels of positive and negative predictive values comparing to the others. The comparison of three D-dimer tests by an ROC curve showed that VIDAS test has the highest overall statistical accuracy of all three D-dimer tests.

Keywords: D-dimer test; compression ultrasonography; deep vein thrombosis

INTRODUCTION

Venous thromboembolism (VTE) is a common disorder associated with significant morbidity and mortality, with annual incidence in developed countries of 1 in 1,000 [1].

That might be a problem, because untreated deep vein thrombosis (DVT) is associated with a high risk of pulmonary embolism (PE), and false diagnosis of DVT results in unnecessary anticoagulant therapy, with a risk of bleeding. Accurate diagnosis of DVT and prompt therapy are essential to reduce the risk of thromboembolic complications. DVT also predisposes patients to post-thrombotic or post-phlebitic syndrome in 40–75% of cases. Between 90% and 95% of PEs arises from lower extremity DVT [2, 3].

In the past, contrast venography has been the gold standard for the diagnosis of DVT, but nowadays it has been replaced in most centers by color duplex ultrasonography. Venography is invasive and is associated with a small but significant risk of complications [4].

The recommended protocol for the diagnosis of DVT consists of the following: 1) Wells score used for diagnosing DVT [5]; 2) D-dimer assay for DVT; and 3) compression ultrasonography (CUS).

Careful history has to be taken considering risk factors. Physical examination is inadequate for establishing the diagnosis of VTE. In recent years, use of D-dimer tests has been increased since the testing is quick and non-invasive [6, 7].

The fragments of the disintegrating fibrin in the clot are fibrin degradation products. One of the fibrin degradation products produced is D-dimer, which consists of variously sized pieces of cross-linked fibrin. D-dimer levels in the blood are normally very low and concentrations are raised by thrombolysis. D-dimer tests generally have a high negative predictive value and should not be used in isolation as screening tests. Therefore, they are often used in conjunction with clinical probability scoring or CUS to reduce the need for further imaging.

There are four types of D-dimer assays commercially available: enzyme-linked im-

Received • Примљено: February 22, 2018
Revised • Ревизија: April 16, 2018
Accepted • Прихваћено: April 18, 2018
Online first: April 25, 2018

Correspondence to:
Dragan VASIĆ
Clinic for Vascular and endovascular Surgery
Clinical Centre of Serbia
Koste Todorovića 8
11000 Belgrade, Serbia
dr_dragan_vasic@yahoo.com

munosorbent assay (ELISA), latex agglutination assay, whole-blood agglutination assay (SimpliRED) and immunochromatographic test (Simplify). Many quantitative latex agglutination and ELISA tests are available and the conventional ELISA is considered the gold standard for determination of D-dimer concentration.

CUS, due to its high sensitivity, specificity, and reproducibility, has replaced venography as the most widely used test in the evaluation of this disease. In symptomatic patients, CUS has shown to be highly specific and sensitive for both proximal and distal DVT. The sensitivity has ranged 90–100% for the diagnosis of symptomatic DVT. The specificity has ranged 95–100%. In high-risk asymptomatic patients, its sensitivity ranges 50–80% and specificity ranges 95–100%. The safety, availability, and well-documented accuracy of this technique justify its widespread use [8, 9].

D-dimer tests should not be used as stand-alone tests, nor are they useful in situations of concurrent anticoagulant use, malignancies, post-surgery, pregnancy, or severe infections. Problems can also occur due to the fact that 30% of patients with PE will have normal D-dimer.

The aim of our study was to evaluate the sensitivity and specificity of three D-dimer tests (DD PLUS, HemosIL, and VIDAS), comparing to CUS examination.

METHODS

This study has been performed over the June 2016 – October 2017 period at the Clinic for vascular and endovascular surgery, Clinical Centre of Serbia.

All the patients underwent the same protocol consisting of patient's history evaluation and physical examination, as well as D-dimer testing as a second step. Finally, CUS of the symptomatic leg was used as a reference test in all the patients.

Physicians at the Vascular Department filled in a questionnaire (modified Wells score) comprising details of history (risk factors) and physical examination (clinical signs). Pretest probability score models for predicting the probability of DVT, based on history and examination, were used in order to help clinicians improve the accuracy of diagnosis of DVT (Table 1).

According to Wells score, all the patients were divided into three groups: patients with a score of 0 or less had low pretest probability, patients with a score of 1 or 2 were considered moderate, and patients with a score that of 3 or more were with high pretest probability [5].

Three D-dimer assays were used: DD PLUS – a latex-enhanced immunoturbidimetric assay (Dade-Behring, Marburg, Germany) on the BCT analyzer, HemosIL – a latex-enhanced immunoassay (Instrumentation Laboratory, Milan, Italy) on the ACLTM 7000 analyzer, and VIDAS (ELISA) DD Exclusion (DD2) (bioMérieux, Marcy L'Etoile, France) on the VIDAS analyzer. D-dimer tests were performed within one hour of admission to the vascular ambulance.

Table 1. Pretest probability scale for deep vein thrombosis used in this study

Risk factors	Score
Active cancer: curative or palliative treatment initiated within 6 months	2
Prior history of idiopathic VTE or known primary thrombophilia	2
Paralysis, paresis, plaster immobilization within 12 weeks	1
Bedridden ≥ 3 days or major surgery within 12 weeks	1
Clinical signs	**Score**
Entire symptomatic leg swollen (the asymptomatic leg is not swollen)	2
Calf swelling > 3 cm compared to the asymptomatic leg	1
Pitting edema, greater in the symptomatic leg	1
Alternative diagnosis (usually muscle pain or venous insufficiency)	-2
- Tenderness or Homan's sign is nonspecific and receives no points - High probability ≥ 3, Moderate probability 1–2, Low probability ≤0	

A D-dimer test was considered positive if the values were > 149–196 μg/L for DD PLUS, > 268 μg/L for HemosIL, and > 650–676 μg/L for VIDAS test.

CUS of the veins of the symptomatic leg was used as the reference test in all the patients. All examinations were performed on a single Acuson Antares ultrasound machine (Siemens, Munich, Germany), using a linear array 7 MHz scan head (7540) with standardized image settings, including resolution mode, depth of field, gain, and transmit focus. CUS examinations were made according to a standardized protocol and report form, performed within three hours of admission to the vascular ambulance. The patients were classified as DVT-positive if they had DVT confirmed with CUS, or as DVT-negative if they had no CUS-confirmed DVT. Patients with unclear CUS findings were excluded from the data analysis. The results of the D-dimer assay were unknown to the ultrasonographer.

Data analysis was assessed using statistical evaluation in addition to various descriptive and analytic statistical methods (t-test, χ^2 test, McNemar's test, and others).

RESULTS

We observed 350 patients, 168 of whom were male and 182 female. Their average age was 62.5 ± 8.4, the youngest being 18 and the oldest one 85 years old.

Several risk factors were present in our patients with different frequency. Malignant diseases were previously diagnosed in 24 patients (6.8%) included in our study (active cancer, either previously surgically treated, on chemo- or radio-therapy). There were six female patients with gynecologic cancers (cervical, ovarian, uterine, vaginal, and vulvar), five patients with cancer of the gastrointestinal tract and liver, four patients with leukemias and lymphomas, and two female patients with breast carcinoma.

Previous episodes of VTE had 26 (7.4%) patients, and seven patients (2%) were with known and documented primary thrombophilia [three patients with activated protein C resistance (factor V Leiden), three patients with

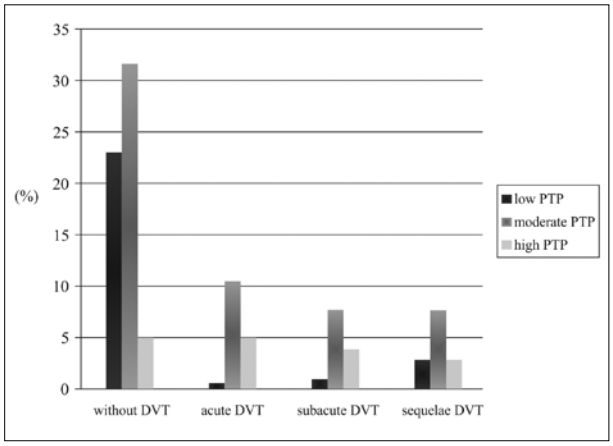


Figure 1. Cumulative compression ultrasonography results for low, moderate, and high PTP groups
PTP – pretest probability; DVT – deep vein thrombosis

protein C and protein S deficiency, and one patient with prothrombin gene mutation].

DVT was present in 13 patients (3.7%) with lower-extremity plaster immobilization at the moment the diagnosis was established. Lower-extremity paresis and paralysis were present in nine patients (2.6%) – either as a result of spinal cord trauma (three patients), cerebrovascular insult (three patients), progressive myelitis (one patient), or cerebral tumor (two patients).

15 patients (4.3%) with CUS-documented DVT were bedridden (seven patients in the end-stage of malignant diseases, two patients in the end-stage of renal failure, two patients with AIDS, and four patients with sequelae of cerebrovascular disease).

Major surgery procedures were performed in 23 patients (6.6%) (orthopedic, vascular/cardiac, abdominal, gynecological, or neurosurgical procedures) two days to 12 weeks before the CUS examination.

The patients in our study had the following clinical sign distribution: entire leg swelling was present in 59 (16.9%) and calf swelling was present in 48 (13.7%) patients, 36 (10.3%) patients had unilateral pitting edema, and 52 (14.9%) patients had alternative clinical signs (i.e. muscle pain, chronic venous insufficiency, isolated joint pain, cellulitis, etc.).

Most of the examined patients (56.8%) were with moderate PTP according to the modified Wells score used.

CUS examination results for all three PTP groups are presented in Figure 1. The highest number of examined patients in all PTP groups was without DVT (59.2%).

Proximal DVT localization (iliac and femoral DVT) was found in 60.5% and distal DVT localization (popliteal and crural DVT) in 39.5% of patients with DVT.

The comparison of D-dimer test results and CUS examination is presented in Table 2. The results show that VIDAS test was positive in the highest percentage in the group of patients with CUS-documented thrombosis. In the group without CUS-documented thrombosis, HemosIL test was negative in the highest percentage.

Important statistical parameters of D-dimer tests compared in our study are presented in Table 3. VIDAS had the highest sensitivity, while HemosIL had the highest specificity. Comparing to the other tests, VIDAS had the highest levels of both positive and negative predictive values.

Table 2. Compression ultrasonography and D-dimer test results comparison

Tests	Without thrombosis		With thrombosis		Whole group	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)
DD PLUS	40.3	59.7	93	7	73.3	26.7
HemosIL	33.8	66.2	88.4	11.6	56.1	43.8
VIDAS	42.6	57.4	95.3	4.7	62.8	37.2

Table 3. Statistical parameters of DD PLUS, HemosIL, and VIDAS test

D-dimer test	Sn (%)	Sp (%)	PPV (%)	NPV (%)
DD PLUS	93	40	51	89
HemosIL	84	66	62	89
VIDAS	95	59	64	94

Sn – sensitivity; Sp – specificity; PPV – positive predictive value; NPV – negative predictive value

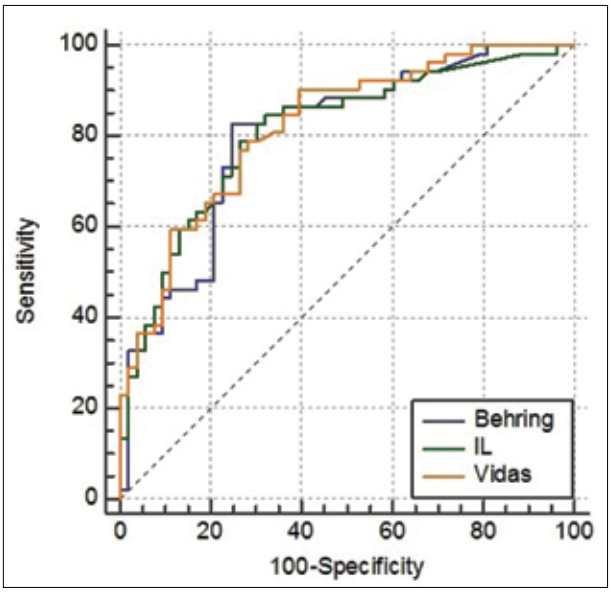


Figure 2. D-dimer tests comparison by sensitivity and specificity (ROC curve)

The comparison of three D-dimer tests by receiver operating characteristic (ROC) curve is represented in Figure 2. In this curve, the sensitivity is plotted in function of the specificity for different cut-off points, where each point represents a pair corresponding to a particular decision threshold, and test with perfect discrimination has a plot that passes through the upper left corner. Therefore, the closer the ROC plot is to the upper left corner – the higher the overall accuracy of the test. In our study, it was the case with VIDAS test.

DISCUSSION

Patients with acute VTE require clinical assessment and objective testing to be accurately diagnosed. Almost all patients with acute VTE have an elevated D-dimer level. An elevated D-dimer is associated with many illnesses and,

therefore, is not specific to VTE. D-dimer tests can have a high sensitivity, which is useful because a normal test excludes the diagnosis of VTE. D-dimer testing is most appropriate in the assessment of outpatients because the prevalence of disease and the likelihood of comorbidity are lower than in inpatient populations, making a test of exclusion particularly valuable [10, 11].

The role of the pretest clinical probability score and/or the D-dimer concentration in the diagnostic management of DVT has been the objective of many studies.

While reviewing management outcome studies, Carrier et al. [12] have found that the three-month PTE risk in patients left untreated on the basis of a low/intermediate or unlikely PTP and a negative D-dimer test was very low and that the combination of a negative VIDAS D-dimer result and a non-high PTP effectively and safely excludes PE.

The results of a study by Van der Graaf et al. [13] suggest that VIDAS and Tinaquant D-dimer assays have the highest sensitivity for the exclusion of DVT in outpatients. In outpatients that have a low or moderate pretest probability for DVT, these tests may be used in management studies where anticoagulation is withheld on the basis of D-dimer testing alone.

Vermeer et al. [14] tested samples from 274 consecutive symptomatic patients with suspected PE, DVT, or suspected for both complications, with DD PLUS assay. The conclusion of their study shows that this appears to be safe when implemented in an algorithm based on clinical assessment, D-dimer concentration, and radiological diagnostic techniques to stratify the risk for PE or DVT.

The objective of a study by Legnani et al. [15] was to evaluate possible advantages of using quantitative D-dimer assays (VIDAS, Innovance, HemosIL, and STA Liatest) performed in plasma aliquots sampled after cessation of vitamin K-antagonism in 321 patients enrolled in the PROLONG study. Their conclusion was that quantitative D-dimer assays may provide information useful for evaluating the individual risk of recurrent VTE and they seem particularly advantageous since they allow the selection of different cut-off levels according to the age and other patients' characteristics.

Djurabi et al. [16] studied the VTE failure rate of 2,206 consecutive patients with an unlikely clinical probability where VIDAS or Tinaquant D-dimer tests were performed. Their conclusion was that both tests perform equally well in combination with an unlikely clinical probability in excluding PE, but the VIDAS test was shown to be more efficient.

Gardiner et al. [17] evaluated the performance of eight D-dimer assays, including VIDAS, DD PLUS, and HemosIL, both as stand-alone tests and in combination with pretest probability. Their conclusion was that the highly variable diagnostic performance of these D-dimer assays means that some assays can be unsuitable for certain diagnostic strategies, but the combination of sensitive D-dimer assays with an assessment of PTP may be used to exclude the diagnosis of DVT.

Bogavac-Stanojević et al. [18] analyzed the total cost of three D-dimer assays (VIDAS, DD Plus, and HemosIL). The total cost of the diagnostic procedure was calculated based on the consumed resources for diagnostic tests, laboratory time, and consumables). Their study group consisted of 96 outpatients with clinically suspected DVT. In the selection of patients for CUS, they used one diagnostic algorithm for the entire patient group and another for patients selected for CUS according to clinical PTP. The conclusion was that a diagnostic algorithm using PTP assessment, DD assay, and CUS could effectively diagnose DVT and reduce CUS utilization and costs per patient.

Many authors emphasize the advantages of other non-invasive diagnostic procedures in establishing the diagnosis of DVT. In combination with CUS, they can estimate the diagnostic accuracy, clinical and cost effectiveness.

CUS, due to its high sensitivity, specificity, and reproducibility has replaced venography as the most widely used test in the evaluation of this disease. The safety, availability, and well-documented accuracy of this technique justify its widespread use.In symptomatic patients, CUS has shown to be highly specific and sensitive for both proximal and distal DVT.

Michiels et al. [19] found that pulmonary angiography could be the gold standard for segmental PE and that normal pulmonary ventilation/perfusion scan and normal rapid ELISA VIDAS D-dimer test safely exclude PE. The combination of clinical assessment and a rapid ELISA VIDAS D-dimer, followed by CUS, will reduce the need for helical spiral CT by 40–50%.

Le Gal et al. [20] showed that the presence of a clot – even an asymptomatic one – in the proximal lower limb veins of a patient with clinically suspected PE, confirmed by CUS, provides evidence for VTE and indicates anticoagulant therapy in such patients. Their experience is that invasive tests are often unavailable and their use is therefore limited to selected patients and non-invasive management (clinical probability, D-dimer, and multislice CT) is feasible in most patients with suspected PE.

Goodacre et al. [21] searched through electronic medical databases and additional data from article bibliographies. Their conclusion was that old techniques as plethysmography and rheography have modest sensitivity for proximal DVT, poor sensitivity for distal DVT, and modest specificity. Ultrasound has 94% sensitivity for proximal DVT, 64% sensitivity for distal DVT, and 94% specificity. Computed tomography scanning has 95% sensitivity for all DVT (proximal and distal combined) and 97% specificity. Magnetic resonance imaging has 92% sensitivity and 95% specificity [21].

Diagnostic algorithms based on a combination of Wells score, D-dimer, and ultrasound (with repeat if negative) are feasible at most worldwide hospitals and are among the most cost-effective diagnostic methods. Pretest probability and D-dimer tests can decrease the need for CUS in young and healthy patients suspected of DVT. D-dimer tests should not be used as a stand-alone test or in situations such as the use of anticoagulants, presence of malignant diseases, post-surgical procedures, during pregnancy, in patients with severe infections, etc.

CONCLUSION

All three D-dimer tests used in our study were with similar sensitivity and specificity. However, the VIDAS test had higher levels of positive and negative predictive values compared to the DD plus and HemosIL tests. A comparison of the three D-dimer tests by the ROC curve showed that the VIDAS test has the highest overall statistical accuracy.

REFERENCES

1. Heit JA. The epidemiology of venous thromboembolism in the community: implications for prevention and management. J Thromb Thrombolysis. 2006; 21(1):23–9.
2. Meissner MH, Strandness JR. Pathophysiology and natural history of acute deep venous thrombosis. In: Rutherford RB Vascular surgery. 6th ed. Philadelphia: Elsevier Saunders; 2005. p. 2124–57.
3. Sakuma M, Takahashi T. Epidemiology and therapy of acute pulmonary thromboembolism. In: Shirato K. Venous thromboembolism, prevention and treatment. Tokyo: Springer-Verlag; 2005. p. 3–47.
4. Bounameaux H. Integrated diagnostic approach to venous thromboembolism. In: Goldhaber SZ, Ridker PM. Thrombosis and thromboembolism. New York: Marcel Dekker; 2002. p. 225–34.
5. Wells PS, Anderson DR, Rodger M, Forgie M, Kearon C, Dreyer J, et al. Evaluation of D-dimer in the diagnosis of suspected deep-vein thrombosis. New Engl J Med. 2003; 349(13):1227–35.
6. Neale D, Tovey C, Vali A, Davies S, Myers K, Obiako M, et al. Evolution of the Simplify D-dimer assay as a screening test for the diagnosis of the deep vein thrombosis in an emergency department. Emerg Med J. 2004; 21(6):663–6.
7. Janes S, Ashford N. Use of a simplified clinical scoring system and D-dimer testing can reduce the requirement for radiology in the exclusion of deep vein thrombosis by over 20%. Br J Haematol. 2001; 112(4):1079–82.
8. Zwiebel WJ. Venous thrombosis. In: Introduction to vascular ultrasonography, 4th Ed. Zwiebel WJ. Elsevier Saunders: Philadelphia, 2000; 23:340.
9. Schäberle W. Peripheral veins. Deep vein thrombosis of the leg. In: Ultrasonography in Vascular Diagnosis: A Therapy-Oriented Textbook and Atlas. New York: Springer-Verlag; 2005.
10. Kearon C, Ginsberg JS, Douketis J, Crowther M, Brill-Edwards P, Weitz JI, et al. Management of suspected deep venous thrombosis in outpatients by using clinical assessment and D-dimer testing. Ann Inter Med. 2001; 135:108–11.
11. Huisman MV, Klok FA. Diagnostic management of clinically suspected acute pulmonary embolism. J Thromb Haemost. 2009; 7(Suppl 1):312–7.
12. Carrier M, Righini M, Djurabi RK, Huisman MV, Perrier A, Wells PS, et al. VIDAS D-dimer in combination with clinical pre-test probability to rule out pulmonary embolism - A systematic review of management outcome studies. Thromb Haemost. 2009; 101(5):886–92.
13. van der Graaf F, van den Borne H, van der Kolk M, de Wild PJ, Janssen GW, van Uum SH. Exclusion of deep venous thrombosis with D-dimer testing – comparison of 13 D-dimer methods in 99 outpatients suspected of deep venous thrombosis using venography as reference standard. Thromb Haemost. 2000; 83(2):191–8.
14. Vermeer HJ, Ypma P, van Strijen MJ, Muradin AA, Hudig F, Jansen RW, et al. Exclusion of venous thromboembolism: evaluation of D-Dimer PLUS for the quantitative determination of D-dimer. Thromb Res. 2005; 115(5):381–6.
15. Legnani C, Palareti G, Cosmi B, Cini M, Tosetto A, Tripodi A. Different cut-off values of quantitative D-dimer methods to predict the risk of venous thromboembolism recurrence: a post-hoc analysis of the PROLONG study. Haematologica. 2008; 93(6):900–7.
16. Djurabi RK, Klok FA, Nijkeuter M, Kaasjager K, Kamphuisen PW, Kramer MH, et al. Comparison of the clinical usefulness of two quantitative D-Dimer tests in patients with a low clinical probability of pulmonary embolism. Thromb Res. 2009; 123(5):771–4.
17. Gardiner C, Pennaneac'h C, Walford C, Machin SJ, Mackie IJ. An evaluation of rapid D-dimer assays for the exclusion of deep vein thrombosis. Br J Haematol. 2005; 128(6):842–8.
18. Bogavac-Stanojević N, Dopsaj V, Jelić-Ivanović Z, Lakić D, Vasić D, Petrova G. Estimation of the total cost per patient with clinically suspected deep vein thrombosis in two diagnostic strategies. Clin Chem Lab Med. 2009; 47(Issue S1):S1–S409.
19. Michiels JJ, Gadisseur A, van der Planken M, Schroyens W, De Maeseneer M, Hermsen JT, et al. Screening for deep vein thrombosis and pulmonary embolism in outpatients with suspected DVT or PE by the sequential use of clinical score: a sensitive quantitative D-dimer test and noninvasive diagnostic tools. Semin Vasc Med. 2005; 5(4):351–64.
20. Le Gal G, Righini M, Sanchez O, Roy PM, Baba-Ahmed M, Perrier A, et al. A positive compression ultrasonography of the lower limb veins is highly predictive of pulmonary embolism on computed tomography in suspected patients. Thromb Haemost. 2006; 95(6):963–6.
21. Goodacre S, Sampson F, Stevenson M, Wailoo A, Sutton A, Thomas S, et al. Measurement of the clinical and cost-effectiveness of non-invasive diagnostic testing strategies for deep vein thrombosis. Health Technol Assess. 2006; 10(15):1–168.

Компарација Д-димер теста са ултразвучним прегледом код дијагностике дубоке венске тромбозе

Драган Марковић[1,2], Драган Васић[1,2], Јелена Башић[3], Слободан Танасковић[1,4], Слободан Цветковић[1,2], Зоран Ранчић[5,6]

[1]Универзитет у Београду, Медицински факултет, Београд, Србија;
[2]Клинички центар Србије, Клиника за васкуларну и ендоваскуларну хирургију, Београд, Србија;
[3]Болница „Рудолфштифтунг", Клиника за кардиологију, Беч, Аустрија;
[4]Институт за кардиоваскуларне болести „Дедиње", Клиника за васкуларну хирургију, Београд, Србија;
[5]Универзитетска болница у Цириху, Клинка за кардиоваскуларну хирургију, Цирих, Швајцарска;
[6]Универзитет у Цириху, Медицински факултет, Цирих, Швајцарска

САЖЕТАК

Увод/Циљ Нелечена дубока венска тромбоза (ДВТ) повезана је са увећаним ризиком за плућну емболију, а погрешно дијагностикована ДВТ доводи до сувишне антикоагулационе терапије и тиме до повећаног ризика крварења. Тачна дијагностика и брза терапија ДВТ су важне за редукцију ризика од тромбоемболичких компликација.

Циљ наше студије је да процени сензитивност и специфичност три Д-димер теста (*DD plus, HemosIL i VIDAS*) у поређењу са ултразвучним испитивањем.

Методе У студију је укључено 350 болесника за различитим факторима ризика. Болесници су подвргнути истом протоколу (евалуација историје болесника, физички преглед и контрола Д-димера), а ултразвучни (УЗ) преглед коришћен је као акредитив за све болеснике.

Резултати Болесници су по Велсовој скали подељени у групе са ниском, средњом и високом предтест вероватноћом. Већина прегледаних болесника је била у групи са средњом предтест вероватноћом. На УЗ је показано да највећи број болесника није имао ДВТ. Већина болесника са позитивним УЗ прегледом је имала проксималну – илијачну или феморалну ДВТ. *VIDAS* тест је у највећем проценту био позитиван у групи болесника са доказаном ДВТ на УЗ. Поређење сва три Д-димер теста на *ROC* кривој је показало највишу статистичку тачност *VIDAS* теста.

Закључак Сва три Д-димер теста коришћена у нашој студији имала су сличне вредности сензитивности и специфичности, с тим што је *VIDAS* тест имао виши ниво позитивне и негативне предиктивне вредности него тестови *DD plus* и *HemosIL*.

Кључне речи: Д-димер тест; компресивна ултрасонографија; дубока венска тромбоза

DOI: https://doi.org/10.2298/SARH170503198T
UDC: 616.61-089.843

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

En bloc paired cadaveric renal transplantation from an 18-month-old infant as a donor to an adult recipient – case report and literature review

Aleksandar Tomić, Ivan Marjanović, Saša Micković

University of Defence, Military Medical Academy, Clinic for Vascular and Endovascular Surgery, Belgrade, Serbia

SUMMARY

Introduction Even in the modern era of kidney transplantation, the use of small grafts from pediatric cadaver donors remains controversial. This kind of transplantation is rare and, to date, limited data are presented. Major problems with infant kidney transplantation are difficulties in performing vascular anastomosis, vasospasm, renal vein thrombosis, and small infant kidneys with poor venous runoff. However, en bloc infant kidney transplantation could resolve this problems.

Case outline We report on transplantation of en bloc cadaver kidneys from an 18-month-old infant. The transplant recipient was a 32-year-old male, with a body weight of 65 kg. Abdominal ultrasonography showed kidneys growing, and no hydronephrosis, perirenal, or retroperitoneal collections were seen.

Conclusion Transplantation of infantile kidneys en bloc in our adult recipient provided good results. The follow-up will show the final effect.

Keywords: kidney transplantation, methods; dual kidney transplant; pediatric kidney donor; patient selection

INTRODUCTION

Single infant kidney transplantation is technically more demanding; complications, as well as graft loss, are present more often, either due to injury at the beginning or later due to hyperfiltration [1, 2]. Transplantation of a pair of kidneys in the block constitutes a solution, and in the era of ever-increasing organ needs, it halves the number of potential recipients. Even in the modern era of kidney transplantation, the use of small grafts from pediatric cadaver donors remains controversial. This kind of transplantation is rare and, to date, limited data are presented. This was the first case of infant kidney cadaver transplantation to an adult recipient performed at our institution. We report a case of kidney transplantation from an 18-month-old infant to an adult man.

CASE REPORT

An 18-month-old male infant was diagnosed with brain death as a consequence of extreme dehydration. After parents' approval, organ procurement was planned. En bloc kidney procurement was performed, including both kidneys with segments of the aorta, inferior vena cava, and both ureters (Figure 1). Kidney perfusion was performed in situ with Euro-Collins solution (1,000 mL). Cold ischemia time was 13 hours. Kidney measurements were 5.7 × 4.2 × 1.7 cm and aortic and inferior vena cava diameter was about 10 mm.

A 32-year-old male (65 kg, 175 cm) with end-stage renal disease was chosen as the recipient, as no child with terminal kidney disease was compatible. The donor was A-positive, with 3/6 human leukocyte antigen compatibility and negative "cross-match" using complement dependent cytotoxicity. The patient was on hemodialysis for the previous four years (three times per week), with no diuresis, and his serum creatinine level was 1,052 mmol/L. Preoperative examination included abdominal ultrasonography, multislice computed tomography with pelvic angiography and intravenous urography. The operation was performed through the right Gibson incision. The kidneys were placed at both sides of the right iliac blood vessels: the left kidney in the right iliac fossa, and the right kidney below aortic bifurcation (Figure 2). End-to-side anastomosis of the infrarenal infant aorta and inferior vena cava and external iliac vessels with continuous GORE-TEX CV7 sutures (W. L. Gore & Associates, Inc., Newark, DE, USA) were performed. Suprarenal portions of the aorta and inferior vena cava were oversewn with continuous 5-0 nonabsorbable monofilament sutures. Separate anti-reflux uretero-cysto-neostomies with two J-J stents were made (Figure 3). Diuresis started after 15 minutes with good perfusion of both kidneys. In the postoperative period, diuresis was about 9 L/24 hours, and creatinine level fell to 200 mmol/L.

Received • Примљено: May 3, 2017
Revised • Ревизија: November 3, 2017
Accepted • Прихваћено: November 9, 2017
Online first: November 17, 2017

Correspondence to:
Aleksandar TOMIĆ
Clinic for Vascular and Endovascular Surgery
Military Medical Academy
Crnotravska 17, 11000 Belgrade
Serbia
tomicdoc@gmail.com

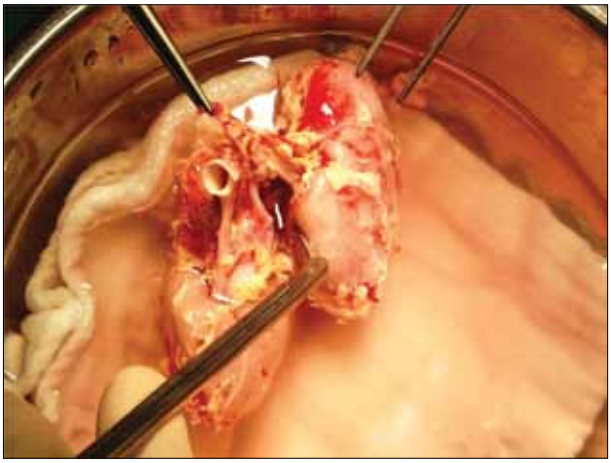


Figure 1. Preparation of the kidneys

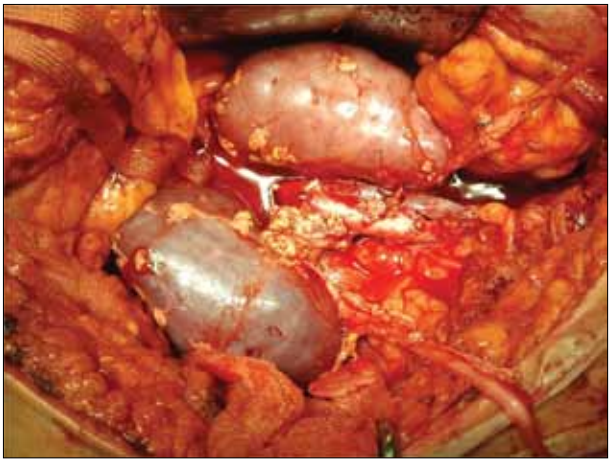


Figure 2. Sutured proximal end of the infant's aorta and the inferior vena cava; end-to-side anastomosis of the infant's inferior vena cava to the recipient's external iliac vein; end-to-side anastomosis of the infant's aorta to the recipient's external iliac artery

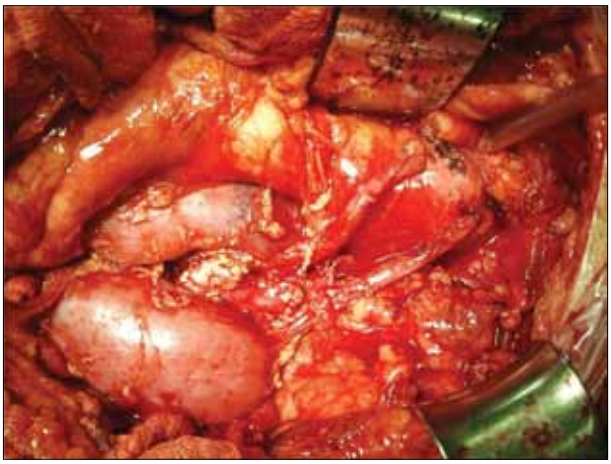


Figure 3. Separate uretero-cysto-neostomies for both ureters

Abdominal ultrasonography showed kidneys' enlargement (8 × 4 × 2 cm). Moreover, no signs of hydronephrosis, perirenal, or retroperitoneal collections were recorded. Good flow through arterial and venous anastomosis, and good perfusion of both kidneys were recorded by a color Doppler examination. Anti-thymocyte globulin, methylprednisolone, mycophenolate mofetil, and tacrolimus were used as immunosuppressive therapy. There were no surgical complications, postoperative recovery was uneventful, and the patient was discharged on the 15th postoperative day.

DISCUSSION

Modern transplant surgery is challenged with a lack of available organs needed for transplantation. This is the main reason why marginal donors are used more frequently for organ procurement. The main characteristics of these donors are increased transplantation risks, deficiency of functional nephrons, diabetes, hypertension, and the age of over 60 years. In recent years, more infant organ donors have been accepted for kidney procurement. In 1969, a report was submitted on the first successful transplantation of a pair of kidneys from a pediatric kidney donor to an adult [3]. Different techniques are being developed; Salehipour et al. [4] explain this in their paper. Small vascular and urethral anastomoses present a technical challenge for the surgeon. Small and immature kidney vascular vessels and large and mature vessels of the recipient make vascular anastomosis technically more difficult. This problem is overcome with end-to-side anastomoses of the infrarenal donor vena cava and aorta to the recipient vessels [5]. The other method is interposition grafting of the aorta and vena cava to the recipient vessels [6]. Moreover, tiny and underdeveloped ureters and strong adult urinary bladder detrusor make uretero-cysto-neostomy anastomosis very difficult. Venous thrombosis, the major complication and cause of kidney rejection was reported by García Buitrón et al. [7]. They noted that two of four en bloc renal transplants from pediatric donors aged less than one year were lost due to venous thrombosis.

Kayler et al. [8] had good results with the opposite technique, where distal parts of the infant aorta and inferior vena cava were oversewn and anastomoses were done with proximal parts of the mentioned vessels. The UCLA Renal Transplant Registry reported that a one-year graft survival rate after kidney transplantations obtained from 276 donors younger than three years was less than 54% [9]. Small infant kidneys with poor venous runoff make transplantation of one kidney to an adult insufficient. In 1967, Kelly et al. [10] showed that only one in five individual single kidneys transplanted to an adult recipient has an adequate function. Uemura et al. [11] found that kidneys from pediatric donors > 6 cm in size can be successfully transplanted as single kidneys. Sureshkumar et al. [12] suggested that pediatric donors weighing more than 10 kg were suitable for single-kidney transplantations. In some retrospective studies, it was noted that hyperfiltration remains the main problem with single infant kidney transplantations [13, 14]. Vascular hyperinfiltration injury can be reduced by dividing the blood flow into both small graft kidneys. In addition, the overall survival rate is significantly higher from paired kidneys compared to a single transplanted kidney [15]. Therefore, both kidneys were transplanted to

one recipient as en bloc transplantation. Enlargement of both kidneys after transplantation has also been noticed by other authors [14]. Another challenge for small organs is the creation of ureterocystostomy, so Lippman et al. [16] believe that poor surgical technique is a curve for urinary leakage, occurring secondary to necrotic ureter caused by inadequate blood supply [16]. However, successful transplantation of a pair of cadaveric renal kidneys aged six months with 4.75 cm in length performed by Huang et al. [13] once again demonstrates that it is possible to extend the marginal donor limit when it relates to the size of the organs and the donor's age.

CONCLUSION

Transplantation of infantile kidneys en bloc in our adult recipient provided good results. This kind of transplantation is a good option, for it extends the number of marginal donors. The follow-up will show the final effect.

REFERENCES

1. Sharma A, Fisher RA, Cotterell AH, King AL, Maluf DG, Posner MP. En bloc kidney transplantation from pediatric donors: comparable outcomes with living donor kidney transplantation. Transplantation 2011; 92(5):564–9.
2. Beltran S, Kanter J, Plaza A, Pastor T, Gavela E, Avila A, et al. One-year follow-up of en bloc renal transplants from pediatric donors in adult recipients. Transplant Proc. 2010; 42(8):2841–4.
3. Martin LW, Gonzalez LL, West CD, Swartz RA, Sutorius DJ. Homotransplantation of both kidneys from an anencephalic monster to a 17 pound boy with Eagle-Barret syndrome. Surgery. 1969; 66(3):603–7.
4. Salehipour M, Bahador A, Nikeghbalian S, Kazemi K, Shamsaeifar AR, Ghaffaripour S, et al. En-bloc transplantation: an eligible technique for unilateral dual kidney transplantation. Int J Organ Transplant Med. 2012; 3(3):111–4.
5. Nghiem DD. En bloc transplantation of kidneys from donors weighing less than 15 kg into adult recipients. J Urol. 1991; 145(1):14–6.
6. Amante AJ, Kahan BD. En bloc transplantation of kidneys from pediatric donors. J Urol. 1996; 155:852.
7. García Buitrón J, Rodríguez-Rivera García J, Chantada Abal V, González Martín M.. [Kidney transplant with pediatric grafts from donors under a year old. The implantation technics]. Arch Esp Urol. 1993; 46(9):793–7.
8. Kayler L, Blisard D, Basu A, Tan HP, McCauley J, Wu C, et al. Transplantation of En Bloc Pediatric Kidneys When the Proximal Vascular Cuff Is Too Short. Transplantation. 2007; 83(1):104–5.
9. Bretan PN, Koyloe M, Singh K, Barba L, Ward H, Sender M, et al. Improved survival of en bloc allografts from pediatric donors. J Urol. 1997; 157(5):1592–5.
10. Kelly WD, Lillehi RC, Aust JB. Kidney transplantation: experience at the University of Minnesota Hospital. Surgery. 1967; 62:704.
11. Uemura T, Liang J, Khan A, Kwon O, Ghahramani N, Wang Li, et al. Outcomes of transplantation of single pediatric renal allografts equal to or more than 6 cm in length. Transplantation. 2010; 89(6):710–3.
12. Sureshkumar KK, Patel AA, Arora S, Marcus RJ. When is it reasonable to split pediatric en bloc kidneys for transplantation into two adults? Transplant Proc. 2010; 42(9):3521–3.
13. Huang CP, Ho HC, Su CK, Ou YC, Cheng CL, Yang CR. Successful Kidney Transplantation Using Paired Cadaver Kidneys Procured From a 6-month-old Brain Death Infant. JTUA. 2005; 16(3):120–3.
14. Modi P, Rizvi SJ, Trivedi HL. Succesful en bloc transplantation of pediatric deceased donor kidneys with grade 1 injury. Indian J Nephrol. 2009; 19(4):167–9.
15. Gruessner RWG, Matas, AJ, Lloveras G, Fryd DS, Dunn DL, Payne, WD, et al: A comparison of single and double pediatric cadaver donor kidneys for transplantation. Clin Transplant. 1989; 3(4):209–14.
16. Lippman H, Jacoby K, McFarlin L, Nicastro C, Aaberg RJ, Banowsky L. Surgical complications in 50 adult renal transplant recipients of single kidneys from cadaveric donors aged 11 to 48 months. Clin Transplant. 1992; 6:350–6.

En bloc кадаверична трансплантација два бубрега са детета старости 18 месеци као даваоца на одраслог примаоца – приказ случаја и преглед литературе

Александар Томић, Иван Марјановић, Саша Мицковић

Универзитет одбране, Војномедицинска академија, Клиника за васкуларну и ендоваскуларну хирургију, Београд, Србија

САЖЕТАК

Увод И у модерној ери трансплантације бубрега, употреба малих органа са кадаверичних педијатријских давалаца остаје контроверзна. Оваква трансплантација је ретка и са мало објављених података. Главни проблеми код трансплантација са једним дечјим бубрегом су тешкоћа у креирању васкуларних анастомоза, спазам крвних судова, тромбоза реналне вене и мала величина дечјег бубрега са недовољним венским протоком. Трансплантација два дечја бубрега „у блоку" могла би да реши ове потенцијалне проблеме.

Приказ болесника Приказујемо „у блоку" кадаверичну трансплантацију бубрега са 18 месеци старог, мождано мртвог дечјег даваоца. Прималац је био одрасли мушкарац старости 32 године и телесне тежине 65 *kg*. Контролни ултразвук трбуха је потврдио увећање оба бубрега, без присутне хидронефрозе, перирепаналне и ретроперитонеалне течности.

Закључак Трансплантација парних дечјих бубрега „у блоку" на одраслог примаоца у нашем случају је дала добре резултате. Праћење ће показати коначни резултат.

Кључне речи: трансплантација бубрега, методе; трансплантација два бубрега, дете давалац бубрега; селекција болесника

DOI: https://doi.org/10.2298/SARH161213162L
UDC: 616-006.3-02; 615.27.06

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Iatrogenic Kaposi's sarcoma following immunosuppressive treatment of the recurrent longitudinally extensive transverse myelitis

Jovan Lalošević[1], Dusan Škiljević[1,2], Irena Dujmović[3,2], Jelena Drulović[3,2], Ljiljana Medenica[1,2]

[1]Clinical Centre of Serbia, Clinic of Dermatovenereology, Belgrade Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Clinical Centre of Serbia, Clinic of Neurology, Belgrade, Serbia

SUMMARY

Introduction Iatrogenic Kaposi's sarcoma (KS) represents a multifocal, angioproliferative tumor that develops in patients undergoing immunosuppressive treatment and is considered to be induced by activation of latent human herpes virus type 8 (HHV8) infection.
The aim of this report is to present a patient with iatrogenic KS due to immunosuppressive treatment.

Case outline We present a 69-year-old male non-HIV patient, previously treated for anti-aquaporin-4 antibody negative recurrent longitudinal extensive transverse myelitis with prednisolone and azathioprine for one year. The patient developed bluish and violet plaques and nodules on his face, trunk, and extremities. Skin biopsy findings (histopathology and immunohistochemical detection of CD31 expression and anti-HHV8 antibodies in the spindle cells) confirmed the diagnosis of KS. The reduction of immunosuppression and topical treatment with imiquimod resulted in a partial but significant regression of skin lesions, but the patient had another relapse of myelitis following the cessation of azathioprine and a reduction in the dose of prednisolone.

Conclusion To the best of our knowledge, this is the first case of an inflammatory and demyelinating central nervous system disease treated with corticosteroids and azathioprine that was associated with iatrogenic KS. The efficient treatment of both conditions is highly challenging and can be troublesome in specific cases.

Keywords: Kaposi's sarcoma; longitudinally extensive transverse myelitis, immunosuppression; human herpes virus 8; imiquimod

INTRODUCTION

Kaposi's sarcoma (KS) represents a multifocal, angioproliferative tumor that is currently recognized in four different clinical types: classical, endemic in Africa, epidemic associated with Acquired Immune Deficiency Syndrome, and iatrogenic. Iatrogenic KS develops in patients undergoing immunosuppressive treatment and is considered to be induced by activating the latent human herpes virus type 8 (HHV8) infection [1, 2].

The aim of this report is to present a patient with iatrogenic KS due to immunosuppressive treatment.

Received • Примљено: December 13, 2016
Accepted • Прихваћено: August 8, 2017
Online first: August 11, 2017

Correspondence to:
Dušan ŠKILJEVIĆ
Pasterova 2, Belgrade 11000
Serbia
dusanskiljevic@yahoo.com

CASE REPORT

We describe a 69-year-old man who developed multiple KSs on his face, trunk, and extremities following long-term immunosuppressive therapy for recurrent longitudinally extensive transverse myelitis (LETM).

The patient was admitted to our dermatology department with multiple violaceus plaques and nodules, some of which were ulcerated. The lesions were present on his face, neck, and trunk (Figure 1 A and B). During the previous one-year period, the patient was treated by a neurologist for a recurrent, anti-aquaporin-4 antibody negative LETM, affecting six vertebral segments on the thoracic spinal cord, with no clinical or subclinical signs of optic nerve or brain involvement. As part of the neurological work-up, neurosarcoidosis, systemic autoimmunity, and paraneoplastic conditions were considered, but could not have been confirmed. The patient was also tested negative for syphilis, *Borrelia burgdorferi*, hepatitis B virus, hepatitis C virus, and human immunodeficiency virus (HIV). Cerebrospinal fluid tests for herpes simplex virus type I and II, varicella zoster virus and cytomegalovirus were also negative, while tests for human T-lymphotropic virus type I or II could not have been performed due to technical limitations. Prior to the skin lesion eruption, the patient had had five attacks of myelitis over a period of three years. At his first myelitis attack, the patient was treated with high-dose methylprednisolone (1,000 mg/day) for five days, intravenously, followed by oral steroid taper. However, a reduction in the maintenance dose of prednisolone below 30 mg/day was always associated with a relapse of myelitis and therefore azathioprine was introduced as

Figure 1. A) Violaceus plaques present on the patient's face, trunk, and extremities; B) close-up of the lesions, infiltrated violaceus plaques with telangiectasia on the periphery of the lesions; C) almost complete resolution of the lesions during the immunosuppression-free period

steroid-sparing agent. Prior to the first skin eruption, the patient had been treated with azathioprine in addition to corticosteroids for one year. At the time of skin lesion eruption, the patient received oral prednisolone (50 mg/day) in addition to azathioprine (2.5 mg/kg/day).

Digital dermoscopy revealed a vascular lesion consisting of homogenous whitish and reddish formations and multiple telangiectasia. Histopathological analysis of the skin biopsy showed multiplied and dilated vascular formations comprised of spindle cells with numerous extravagated erythrocytes and siderophages, admixed with lymphocytes and eosinophils. Additional immunohistochemistry analysis revealed high intensity expression of CD31 and HHV8 nuclear antigen in the spindle cells (Figure 2). The tests for HIV infection were repeated (both anti-HIV antibodies and tests for HIV antigens) and were consistently negative.

Considering the diagnosis of widespread KS, the neurologist ceased azathioprine administration and tapered the dose of prednisolone to a daily dose of 30 mg. The patient was additionally treated with topical 5% imiquimod cream for five months and partial regression of skin lesions occurred.

However, the reduction in immunosuppression resulted in another severe relapse of myelitis and high-dose methylprednisolone treatment (1,000 mg/day over five days) was reintroduced, followed by oral prednisolone in a daily dose of 1 mg/kg. Unfortunately, this treatment was complicated by *Salmonella enteritidis* sepsis and disseminated intravascular coagulation (DIC) syndrome. During the treatment of sepsis and DIC as life-threatening conditions, the steroid treatment was temporarily stopped for one month, and after the resolution of both sepsis and DIC, it was again introduced in the dose of 20 mg / alternate days of prednisolone. During the immunosuppression-free period, KS lesions resolved almost completely (Figure 1C), but his neurological condition further worsened to the level of spastic paraplegia.

DISCUSSION

To the best of our knowledge, this is the first case of an inflammatory and demyelinating disease of the central nervous system associated with iatrogenic KS treated with corticosteroids and azathioprine.

To date, the majority of cases of iatrogenic KS were described as developing after one year of immunosuppressive therapy with steroids, cyclosporine or azathioprine [3]. There are only two cases of iatrogenic KS induced by immunosuppression in a neurological disease [4, 5]. In both of these cases, the immunosuppressant agent was suspended, with partial regression of KS lesions in one case [5], and a total regression after an additional local radiotherapy in the other [4]. The main therapy protocol in iatrogenic KS is the discontinuation of the culprit immunosuppressive agent since it has been demonstrated that a re-activation of the latent HHV8 infection, which can be induced by immunosuppression, is essential for the onset of all KS forms [6]. In our patient, KS lesions partially resolved following the reduction in immunosuppressive therapy and almost completely resolved following a temporary discontinuation of steroids during the severe life-threatening infection.

Kotter et al. [7] reported a promising result of treating KS with interferon-alpha (IFN-α) in a patient with Behçet's disease who developed this malignancy (skin, mucosa, and pulmonary lesions) after a long-term triple immunosuppressive therapy with prednisolone, cyclosporine A, and

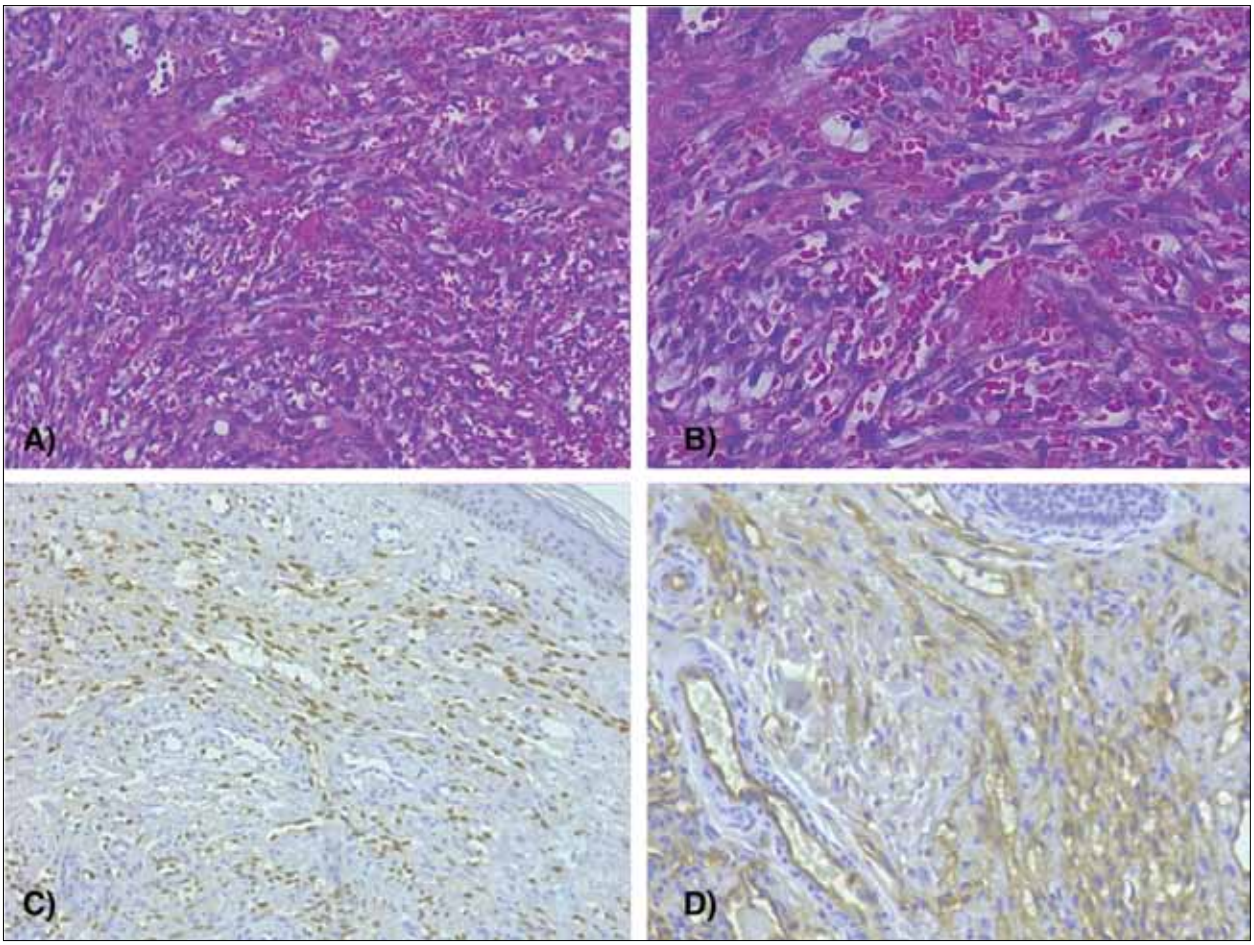


Figure 2. A) and B) Multiplied and dilated vascular formations comprised of spindle cells with numerous extravagated erythrocytes and siderophages, admixed with lymphocytes and eosinophils; H&E, original magnification A) ×100, B) ×200; C) IHH HHV8 positive cells; original magnification ×100; D) IHH CD31 positive cells; original magnification ×100

azathioprine. Although we also considered treatment with IFN-α alpha in our patient, we did not introduce such a therapy since off-label treatment with IFN-α was not available in our country. Additionally, type-I interferons might potentially induce worsening of neuromyelitis optica (NMO) patients [8]. Although our patient was anti-aquaporin-4 antibody negative, and since we have not identified any other neurological condition that might be associated with recurrent LETM, we initially considered this patient to have neuromyelitis optica spectrum disease according to the definition of Wingerchuk et al. [9]. However, the clinical presentation of the recurrent LETM would not meet the latest neuromyelitis optica spectrum disease diagnostic criteria [10]. Another treatment option in our patient was local radiotherapy, which had to be disregarded considering the large number and dissemination of KS lesions.

To the best of our knowledge, we here present the first case of an inflammatory and demyelinating disease of the central nervous system associated with iatrogenic KS presenting with typical course followed by the resolution after the complete withdrawal of immunosuppressive drugs. The efficient treatment of both conditions is highly challenging and can be troublesome in specific cases. Since there are no unequivocal treatment guidelines for cases when a disease needed to be treated with immunosuppressants is associated with a disorder caused by immunosuppressants, there is always a necessity to balance the seesaw in order to offer the best possible treatment.

ACKNOWLEDGEMENT

This report was partially supported by a grant from the Ministry of Education, Science and Technological Development of the Republic of Serbia, projects No. 175031 (Dr. Dujmović and Dr. Drulović), No. 175065 (Dr. Škiljević), and No. 175038 (Dr. Medenica).

REFERENCES

1. Tornesello ML, Biryahwaho B, Downing R, Hatzakis A, Alessi E, Cusini M, et al. Human herpesvirus type 8 variants circulating in Europe, Africa and North America in classic, endemic and epidemic Kaposi's sarcoma lesions during pre-AIDS and AIDS era. Virology. 2010; 398(2):280–9.
2. Moore AY. American Academy of Dermatology 1999 Awards for Young Investigators in Dermatology. Active transcription of human herpesvirus 8 cellular homologue genes and HIV-1 tat in various forms of Kaposi's sarcoma. J Am Acad Dermatol. 1999; 41(3 Pt 1):458–9.
3. Rady PL, Hodak E, Yen A, Memar O, Trattner A, Feinmesser M, et al. Detection of human herpesvirus-8 DNA in Kaposi's sarcomas from iatrogenically immunosuppressed patients. J Am Acad Dermatol. 1998; 38(3):429–37.
4. Tully T, Barkley A, Silber E. Kaposi sarcoma in a patient with relapsing-remitting multiple sclerosis receiving fingolimod. Neurology. 2015; 84(19):1999–2001.
5. Celik Y, Turgut N, Turgut B, Pamuk GE, Demir M. Chronic idiopathic demyelinating polyneuropathy (CIDP) associated with Kaposi's sarcoma. J Neurooncol. 2006; 79(3):323–4.
6. Buonaguro FM, Tornesello ML, Buonaguro L, Satriano RA, Ruocco E, Castello G, et al. Kaposi's sarcoma: aetiopathogenesis, histology and clinical features. Journal of the European Academy of Dermatology and Venereology: JEADV. 2003; 17(2):138–54.
7. Kotter I, Aepinus C, Graepler F, Gartner V, Eckstein AK, Stubiger N, et al. HHV8 associated Kaposi's sarcoma during triple immunosuppressive treatment with cyclosporin A, azathioprine, and prednisolone for ocular Behcet's disease and complete remission of both disorders with interferon alpha. Ann Rheum Dis. 2001; 60(1):83–6.
8. Feng X, Reder NP, Yanamandala M, Hill A, Franek BS, Niewold TB, et al. Type I interferon signature is high in lupus and neuromyelitis optica but low in multiple sclerosis. J Neurol Sci. 2012; 313(1-2):48–53.
9. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG. The spectrum of neuromyelitis optica. Lancet Neurol 2007; 6(9):805–15.
10. Wingerchuk DM, Banwell B, Bennett JL, Cabre P, Carroll W, Chitnis T, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology. 2015; 85(2):177–89.

Јатрогени Капошијев сарком као последица имонусупресивне терапије рекурентног лонгитудиналног екстензивног трансверзалног мијелитиса

Јован Лалошевић[1], Душан Шкиљевић[1,2], Ирена Дујмовић[3,2], Јелена Друловић[3,2], Љиљана Меденица[1,2]

[1]Клинички центар Србије, Клиника за дерматовенерологију, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Клинички центар Србије, Клиника за неурологију, Београд, Србија

САЖЕТАК

Увод Јатрогени Капошијев сарком (КС) представља мултифокални, антипролиферативни тумор који се најчешће јавља код болесника који примају неки вид имуносупресивне терапије и сматра се да је последица реактивације латентне инфекције хуманим херпес вирусом 8 (ХХВ8).

Циљ овог рада је био да прикаже болесника са јатрогеним КС услед имуносупресивног лечења.

Приказ болесника Приказујемо ХИВ негативног мушкарца, старог 69 година, који је годину дана лечен преднизолоном и азатиоприном, због антиаквапорин-4 негативног рекурентног лонгитудиналног екстензивног трансверзалног мијелитиса. Код болесника је дошло до појаве ливидних папула и нодулуса на носу, лицу, трупу и екстремитетима. Хистопатолошки и имунохистохемијски налази биоптата коже потврдили су дијагнозу КС (позитивност ћелија на *CD*31, а такође и на ХХВ8 антитела). Редукција имуносупресије и локална терапија имиквимодом довели су до парцијалне регресије кожних промена, али се развио релапс мијелитиса због искључивања азатиоприна и смањења дозе преднизолона.

Закључак На основу доступне литературе, ово је први описани случај јатрогеног КС удруженог са инфламаторним и демијелинизационим обољењем централног нервног система. Ефикасно лечење оба стања је изузетно комплексно и тешко у одређеним случајевима, као што је и наш.

Кључне речи: Капошијев сарком; лонгитудинални екстензивни трансверзални мијелитис; имуносупресија; хумани херпес вирус 8; имиквимод

DOI: https://doi.org/10.2298/SARH161228138D
UDC: 616.61-78; 616.13/.14-089.843

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Two-stage forearm brachio-basilic loop arteriovenous graft for hemodialysis

Branislav S. Donfrid[1], Olivera B. Lozanče[2], Zvezdan B. Stefanović[1,3], Aleksandar N. Janković[4], Nada B. Dimković[4,5]

[1]University of Belgrade, Faculty of Dentistry, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Veterinary Medicine, Department of Anatomy, Belgrade, Serbia;
[3]Zvezdara University Medical Center, Clinic for Surgery, Belgrade, Serbia;
[4]Zvezdara University Medical Center, Clinical Department for Nephrology, Belgrade, Serbia;
[5]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction The autologous radio-cephalic arteriovenous fistula (AVF) is the best vascular access for patients on chronic hemodialysis. In some patients with inadequate blood vessels, it is necessary to create proximal AVF, or arteriovenous grafts. High percentage of primary graft failure is noted in cases where diameters of the brachial artery and the basilic vein are insufficient.

The aim of this work was to introduce a new surgical technique for arteriovenous creation in patients with inadequate blood diameter.

Case outline The authors have proposed implantation of brachio-basilic polytetrafluoroethylene AV forearm loop graft in two acts. In the first act, the native brachio-basilic AVF was created in the distal region of the arm by side-to-end anastomosis. Three to four weeks after the first act, significant dilatation of brachial artery and basilic vein was noted (confirmed by the use of color duplex sonography technique). During the second act, polytetrafluourethylene graft was implanted by end-to-end anastomosis on the dilated basilica vein.

Conclusion AV graft that was created in two acts has sufficient blood flow without early or late complications. Primary patency was 30 months and secondary patency was 50 months. As an original method in the current literature, we recommend it in different clinical settings when there are no better alternatives for vascular access.

Keywords: arterio-venous fistula; arterio-venous graft; hemodialysis; vascular access

INTRODUCTION

Adequate and functional long-term vascular access is crucial for efficient hemodialysis (HD) [1]. The new era of hemodialysis has begun thanks to the design of the native arteriovenous fistula (AVF) by Brescia et al. [2]. During the 60 years of application, this vascular access has showed the best results and now it is considered the "gold standard." However, in some patients, native AVF cannot be done due to inadequate anatomical and functional characteristics of blood vessels, damage of the blood vessels due to frequent puncturing, exhaustion of blood vessels due to repeated surgical procedures, aging of the dialysis population, high prevalence of diabetics, mineral metabolism disturbances, pronounced vascular calcifications, etc. [3]. In these patients, the options are proximal AVF, arteriovenous grafts, and tunneled catheters [4].

Good understanding of the anatomy and topography of the vascular system, primarily of the brachial artery (a. brachialis) and the basilic vein (v. basilica) is very important for a good estimation of the possibilities for creating a vascular access for dialysis. In 80% of patients, brachial artery continues to the axillary artery, follows the medial nerve, and in the cubital area gives two terminal branches – the radial and ulnar arteries [5]. Anatomical variations or deviations of the brachial artery refer to the phenomenon of double brachial arteries (superficial and deep), which occurs in 2–12% of cases [6]. Branching of the superficial brachial artery from the main brachial tree can occur at different levels. In the cubital part, this artery usually extends as radial, while the brachial artery continues to the ulnar artery [7]. At the level of the elbow, basilic vein is located in front of the medial humeral epicondyle, continues along the medial side of the arm, in the initial part just below the skin, and in the proximal part penetrating the deep fascia and stretching along the brachial artery until its confluence. In a situation when native AVF cannot be created, possible solutions are vascular graft and tunneled catheter [8]. Vascular graft has the advantage over tunneled catheter, particularly in the region of the forearm (brachio-basilic „loop graft") [8].

CASE REPORT

We present a male patient, 23 years old, who was on hemodialysis for nine years due to severe congenital malformations of the urogenital

Received • Примљено: December 28, 2016
Revised • Ревизија: May 29, 2017
Accepted • Прихваћено: May 30, 2017
Online first: July 7, 2017

Correspondence to:
Branislav DONFRID
KBC Zvezdara, D. Tucovića 161
11000 Belgrade, Serbia
branislav.donfrid@gmail.com

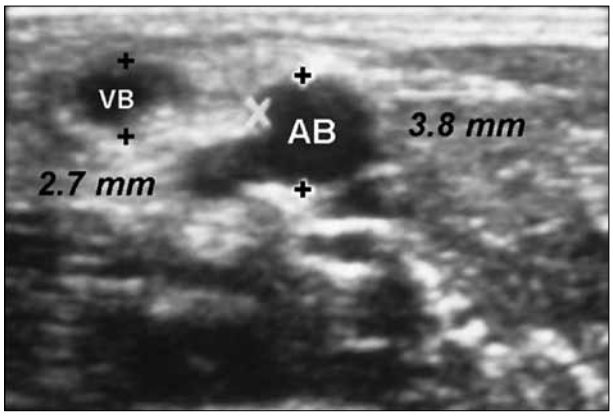


Figure 1. Initial color duplex sonography examination of the diameter of the brachial artery (3.8 mm) and the basilic vein (2.7 mm) in the region of the distal left arm

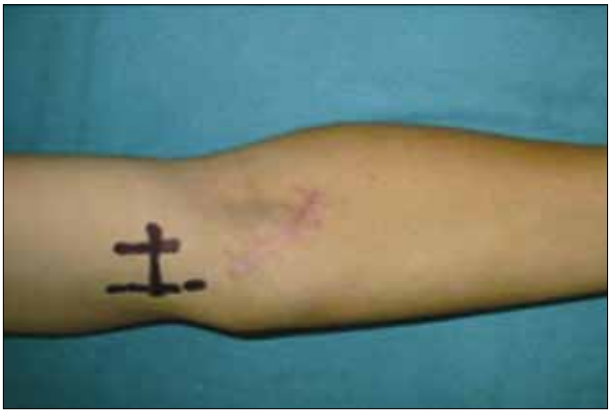


Figure 2. Mapping of the brachial artery and the basilic vein in the distal part of the left arm

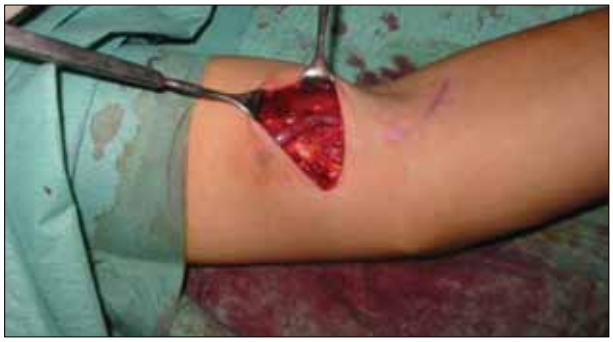


Figure 3. The first act shows latero-terminal anastomosis between the brachial artery and the basilic vein

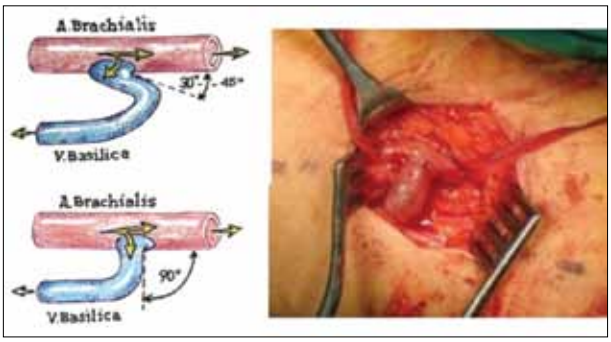


Figure 4. Brachio-basilic anastomosis; the angle of the vein to the artery has to be between 90° and 120°

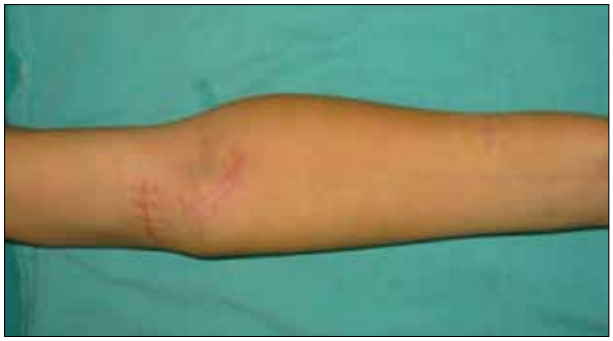


Figure 5. The left arm and forearm four weeks after the surgery – the first act

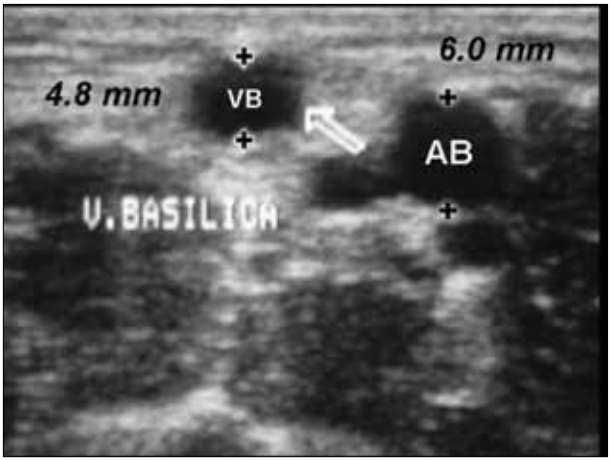


Figure 6. Color duplex examination after four weeks: both the basilic vein and the brachial artery were dilated to 4.8 mm and to 6 mm, respectively

tract. Native blood vessels (forearm cephalic vein, cubital vein, arm cephalic vein and radial artery, distal and proximal) on both arms were seriously damaged by repeated attempts to form an AVF. Over the last several months the patient was dialyzed through a central venous catheter in various positions. Color duplex examination was used to measure the diameters of the brachial artery (3.6 mm) and the basilic vein (2.7 mm) in the distal part of the left arm (Figures 1 and 2). In the middle third of the arm, basilic vein joins the deep venous system making it unfeasible for transposition and creation of native brachial-basilic AVF. Due to all of the above, implantation of the polytetrafluorethylene (PTFE) graft in two acts was attempted.

The first act of the procedure was performed under regional anesthesia (axillary block). After cross-section of the distal third of the arm, side-to-end anastomosis of the brachial artery and the basilic vein was made (Figure 3). During the formation of the anastomosis, it is very important to adjust the angle of the vein to the artery, which has to be between 90° and 120° (Figure 4). Four weeks later, color duplex scan showed that the basilic vein was arterialized (4.8 mm in diameter) and the brachial artery was dilated to 6.0 mm (Figures 5 and 6). These diameters of the blood vessels allowed for the safe graft implantation.

The second act of the procedure was also performed in regional anesthesia, through the scar tissue of the first

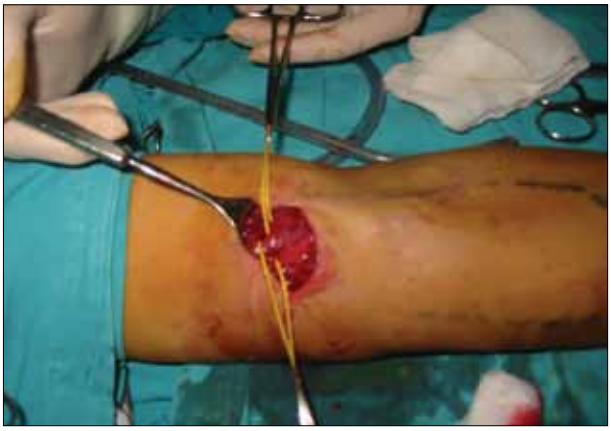


Figure 7. Through the previous scar, the basilic vein was prepared close to the brachial artery without attacks on the brachial artery (four weeks after the first act)

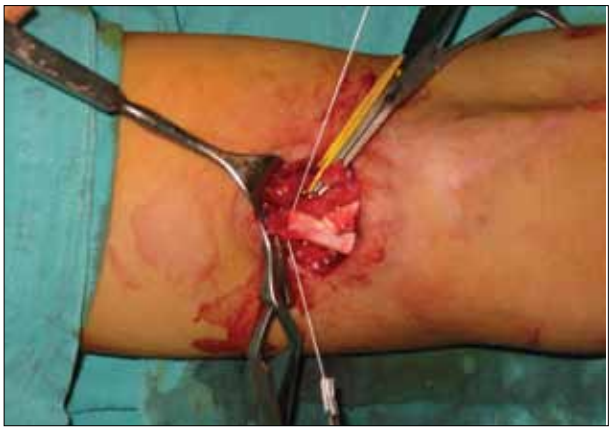


Figure 8. Implanted PTFE 5 mm graft in the loop position in the left forearm with two T–T anastomoses; the suture of the back wall of the basilica vein and the graft

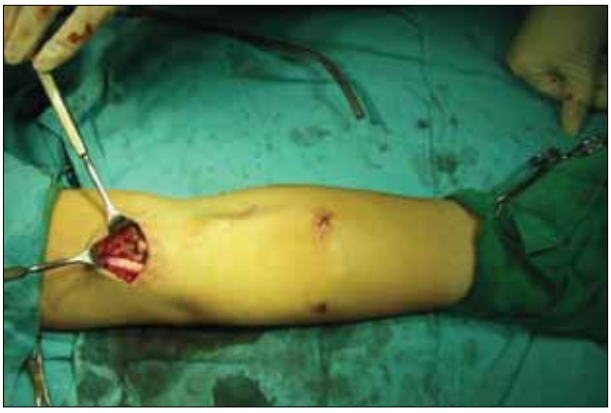


Figure 9. Implanted PTFE 5 mm graft in the loop position in the left forearm

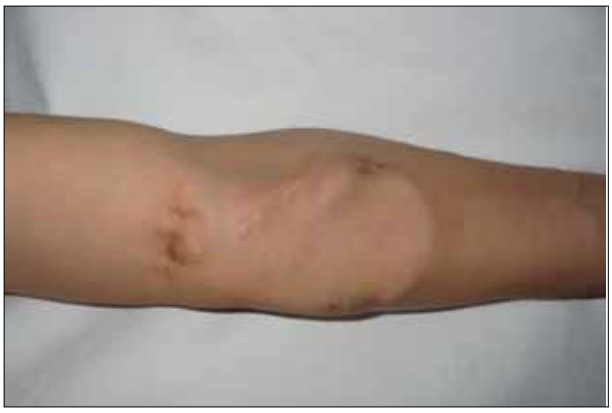


Figure 10. Forearm appearance at the 30th week after the implantation of the graft, which is in good function

surgery. The basilic vein near AVF anastomosis was prepared and a 5 mm e-PTFE vascular graft was placed subcutaneously in the position of the "loop" graft. Using vascular clamps, a cross-cut of the basilic vein was made 2 cm away from the anastomosis with the brachial artery. After instillation of heparin in both ends, the second act was completed by creation of end-to-end anastomosis between vein and graft using Gore-Tex (W. L. Gore & Associates, Inc., Newark, DE, USA) 6-0 suture (Figures 7, 8, 9). From the functional point, arterial anastomosis remained latero-terminal. The graft was ready to be used for hemodialysis after four weeks. Leaving about 2 cm of the basilic vein between the arterial end of the graft and the brachial artery significantly simplifies the process in the second act since the artery remains intact. This is particularly important in case of need for extirpation of the graft due to infection. In that case, the remaining part of the basilic vein is ligated by suture ligature and the infected graft is easily completely removed.

The patient was successfully treated by hemodialysis using the implanted graft over a period of 30 months (primary patency) (Figure 10). The partial replacement of the graft due to pseudoaneurysms at the puncturing places was done and the graft was used for additional 20 months (secondary patency 50 months).

DISCUSSION

Referral to the surgeon and waiting time for vascular access creation are important determinants of the type of vascular access and its usability [9]. According to the Dialysis Outcomes and Practice Patterns Study (DOPPS) V, in most of the DOPPS countries, the frequency of native AVFs is usually less than 80%, ranging from 49% in Canada up to 92% in Russia [10]. By multivariate logistic regression analysis, vein diameter was the sole independent predictor of functional fistula maturation [11].

Transposition of the basilic vein for arteriovenous fistula is the last possibility for the creation of vascular access native vessels in the upper extremity. It is important to have the basilic vein of sufficient length in order to obtain a sufficient conduit for the two-needle butting and to avoid recirculation.

In 66% of cases, the basilic vein can be used for the formation of native AVF after vein transposition and superficialization. However, in 34% of cases the basilic vein is short and cannot be used for native AVF [12]. In that case, superficialization on a deep brachial vein is possible, but the primary and secondary flow of such vascular access is insufficient. In addition, an aneurysm at the puncture site for hemodialysis, axillary vein thrombosis, "steal syndrome," and other complications are frequent [13].

It is well known that implantation of the vascular graft requires appropriate diameter and quality of arterial and venous blood vessels, which may be a major obstacle for this type of surgery [14]. Literature data about graft implantation vary from center to center and it is not surprising that average primary and secondary patency after six months were found to be 58% and 76%, respectively [15].

In cases of inadequate blood vessels when there is no possibility of creating vascular access native vessels, we suggest arteriovenous graft implantation in two acts. Such operation should take place before the decision to create a vascular access of the lower extremities. Primary and secondary patency of this graft is excellent.

Instead of conclusion, this case report demonstrates the successful creation of a vascular graft in a two time-separated acts in patients where it was not possible to create other vascular accesses (native AVF, standard graft). This technique has excellent early results, the same as the primary implanted grafts on good blood vessels. We recommend it for patients with previously exhausted vascular accesses, as well as for patients with pronounced and long-lasting hypotension (regardless of the quality of the blood vessels), diabetics, and the elderly who have advanced atherosclerosis. Since this method is not described in the available literature, we consider it an original contribution named "forearm brachio-basilic arteriovenous grafts in two acts – Zvezdara method."

REFERENCES

1. Ng YY, Wu SC, Hung YN, Ko PJ. Effect of demographic characteristics and timing of vascular access maturation on patency in Chinese incident haemodialysis patients. Nephrol Dial Transplant. 2009; 24(11):3447–53.
2. Brescia MJ, Cimino JE, Appel K, Hurwich BJ. Chronic hemodialysis using venepuncture and a surgically created arteriovenous fistula. N Engl J Med. 1966; 275:1089–92.
3. Hammes M. Hemodynamic and biologic determinates of arteriovenous fistula outcomes in renal failure patients. Biomed Res Int. 2015; 2015:171674.
4. Vascular Access 2006 Work Group. Clinical practice guidelines for vascular access. Am J Kidney Dis. 2006; 48(Suppl 1):176–247.
5. Shenoy S. Surgical anatomy of upper arm: what is needed for AVF planning. J Vasc Access. 2009; 10(4):223–32.
6. Krstonosic B, Srdic B, Maric D, Gudovic R, Mijatov S, Babovic S. An anatomical study of double brachial arteries – a case report. International Journal of Anatomical Variations. 2010; 3:6–8.
7. Anaya-Ayala JE, Younes HK, Kaiser CL, Syed O, Ismail N, Naoum JJ, et al. Prevalence of variant brachial-basilic vein anatomy and implications for vascular access planning. J Vasc Surg. 2011; 53(3):720–4.
8. Tordoir J, van Loon MM, ter Meer M, van Laanen J, Bode AS, Weijmer MC, et al. Hemodialysis vascular access management in the Netherlands. J Vasc Acess. 2015; 16(Suppl. 9):S11–5.
9. Schild AF, Gillaspie E, Perez E. When an AVF isn't possible for HD, AV grafts are the preferred access. J Vasc Access. 2007; 8(3):140–1.
10. Pisoni RL, Zepel L, Port FK, Robinson BM. Trends in US vascular access use, patient preferences, and related practices: An update from the US DOPPS Practice Monitor with international comparisons. Am J Kidney Dis. 2015; 65(6):905–15.
11. Lazarides MK, Georgiadis GS, Antoniou GA, Staramos DN. A meta-analysis of dialysis access outcome in elderly patients. J Vasc Surg. 2007; 45(2):420–6.
12. Pach AR. A two-staged technique for basilic vein transposition. J Vasc Access. 2007; 8(4):225–7.
13. Al Shakarchi J, Stolba J, Houston JG, Inston N. Surgical techniques for haemodialysis access-induced distal ischaemia. J Vasc Access. 2016; 17(1):40–6.
14. Lauvao LS, Ihnat DM, Goshima KR, Gruessner AC, Mills JL Sr. Vein diameter is the major predictor of fistula maturation. J Vasc Surg. 2009; 49(6):1449–504.
15. Goldin ShD, Verstandig I, Berelowitz D, Zaghal I, Olsha O. Upper limb grafts for hemodialysis access. J Vasc Access. 2015; 16(Suppl. 9):S34–9.

Подлакатни брахио-базилични артериовенски графт за хемодијализу у два акта

Бранислав С. Донфрид[1], Оливера Б. Лозанче[2], Звездан Б. Стефановић[1,3], Александар Н. Јанковић[4], Нада Б. Димковић[4,5]

[1]Универзитет у Београду, Стоматолошки факултет, Београд, Србија;
[2]Универзитет у Београду, Факултет ветеринарске медицине, Катедра за анатомију, Београд, Србија;
[3]Клиничко-болнички центар „Звездара", Хируршка клиника, Београд, Србија;
[4]Клиничко-болнички центар „Звездара", Клиничко одељење за нефрологију и метаболичке поремећаје са центром за дијализе „Професор др Василије Јовановић", Београд, Србија;
[5]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Аутологна радиоцефалична артериовенска фистула је најбољи васкуларни приступ за болеснике на хемодијализи. Код неких болесника са неадекватним крвним судовима потребно је креирати проксималну артериовенску фистулу или имплантирати артериовенски графт. Високи проценат раног затајивања графта најчешће настаје услед малог пречника базиличне вене или брахијалне артерије.

Циљ овога рада је био да прикаже нову хируршку технику за креирање артериовенског графта код болесника са неодговарајућим дијаметром крвних судова.

Приказ болесника Урађена је имплантација *PTFE* графта у виду петље на подлактици у два акта. У првом акту је у дисталном делу надлактице креирана брахио-базилична латеро-терминална артериовенска фистула под углом од 90 степени. После четири недеље, контролном доплер-сонографијом је констатовано знатно повећање пречника базиличне вене и брахијалне артерије. У другом акту је базилична вена попречно пресечена и са две темино-терминалне анастомозе у виду петље уметнут *PTFE* графт.

Закључак Овако креиран артериовенски графт у два акта давао је задовољавајући проток крви без компликација. Примарна функционалност је износила 30 месеци, а секундарна 50 месеци. У доступној литератури овакав поступак није објављен, а препоручујемо га код болесника без других могућности за креирање васкуларног приступа.

Кључне речи: артериовенска фистула; артериовенски графт; васкуларни приступ; хемодијализа

DOI: https://doi.org/10.2298/SARH160629207O
UDC: 616.411-007.43; 616:98:578.825

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Spontaneous splenic rupture in infectious mononucleosis

Božidar Odalović[1,2], Milan Jovanović[3], Radojica Stolić[4], Branislav Belić[4], Simon Nikolić[1], Predrag Mandić[1]

[1]University of Priština, Faculty of Medicine, Kosovska Mitrovica, Serbia;
[2]Clinical Center of Serbia, Emergency Center, Belgrade, Serbia;
[3]Clinical Center of Serbia, Center for Endocrine Surgery, Belgrade, Serbia;
[4]University of Kragujevac, Faculty of Medicine, Kragujevac, Serbia

SUMMARY

Introduction Spontaneous splenic rupture is a rare but potentially fatal complication of infectious mononucleosis (IM). It occurs in only 0.1–0.5% of cases of this disease.
The aim of this paper was to present a case with spontaneous splenic rupture after IM.
Case outline A 22-year-old female patient was feeling better one month after she was treated for infectious mononucleosis, and started training volleyball. Two weeks after starting the training, she felt severe abdominal pain. The diagnosis of rupture was confirmed with computer tomography. Splenectomy was successfully performed. The postoperative course was uneventful and the patient recovered with no need for blood transfusion.
Conclusion Timely diagnosis and setting indications for surgical treatment are crucial in healing. Patients should wait to start with sport activities at least two months if the size of the spleen is within normal range.
Keywords: infectious mononucleosis, complications; rupture, spontaneous; splenic rupture, etiology, surgery; splenectomy

INTRODUCTION

Infectious mononucleosis is a common viral illness caused by an infection with the Epstein–Barr virus and it is manifested with fever, sore throat, fatigue, and lymphadenopathy [1]. Complications are rare including splenic rupture with an incidence between 0.06–0.5% [2]. Splenic rupture is considered as the most dangerous complication that may lead to fatal outcome [1]. Symptoms of splenic rupture include abdominal pain, syncope, and rapid drop in blood pressure, while the diagnosis is mostly established with ultrasonography or computer tomography of the abdomen [3]. Recommended treatment for splenic rupture is splenectomy in order to avoid sudden death [4].

CASE REPORT

A 22-year-old female patient previously diagnosed with infectious mononucleosis presented herself to the Emergency Department of the Clinical Center of Serbia, as an emergency case due to severe abdominal pain. Six weeks previously she had gone to the hospital, where she had been diagnosed with infectious mononucleosis. The diagnosis was reached based on medical history, clinical examination, and elevated levels of immunoglobulin M and immunoglobulin G antibodies against Epstein–Barr virus. Abdominal ultrasound revealed enlarged liver and spleen; axial diameter of the spleen was 14.2 cm. After a month of treatment, the patient was feeling better and started to train volleyball. Two weeks after she started to train, the patient felt severe abdominal pain and presented herself to the Emergency Department of the Clinical Center of Serbia. The pain onset was sudden, occurred while the patient was playing volleyball, and was accompanied by malaise, dizziness, and general weakness. The patient's skin and visible mucous membranes were pale; the skin was covered with cold sweat. The patient was alert and oriented but hemodynamically unstable with a heart rate of 122 beats per minute and low blood pressure (90/50 mmHg). Blood test results showed a low hemoglobin level (93 g/L), leukocytosis (17×10^9/L) and a low level of red blood cells (3.28×10^{12}/L). On palpation, the patient's abdomen was firm, very sensitive, and painful, especially in the left upper quadrant. The diagnosis was confirmed with computer tomography (CT) scan of the abdomen. The CT scan showed spleen enlargement and fluid (14×7 cm in size) (Figure 1). The presence of free fluid was noticed intraintestinally and in the left paracolic gutter. CT morphology of the liver, kidneys, and pancreas was normal. As intensive reanimation therapy did not help, as the heart rate was still accelerated in spite of the reanimation therapy, it was decided that the patient should undergo surgery. After opening the abdominal cavity and evacuating 800 ml of

Received • Примљено: June 29, 2016
Revised • Ревизија: December 18, 2017
Accepted • Прихваћено: December 20, 2017
Online first: December 29, 2017

Correspondence to:
Milan D. JOVANOVIĆ
Center for Endocrine Surgery
Clinical Center of Serbia
8 Dr Koste Todorovića Street
Belgrade 11000, Serbia
milanfenix@yahoo.com

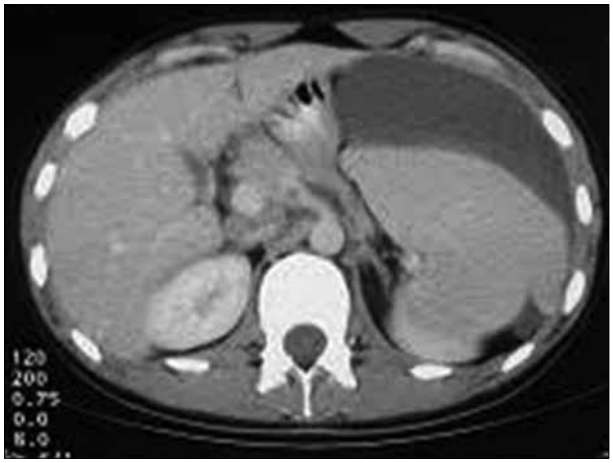


Figure 1. Computerized tomography of the enlarged spleen and free abdominal fluid

Figure 2. Intraoperative finding of a cleft on the upper splenic pole

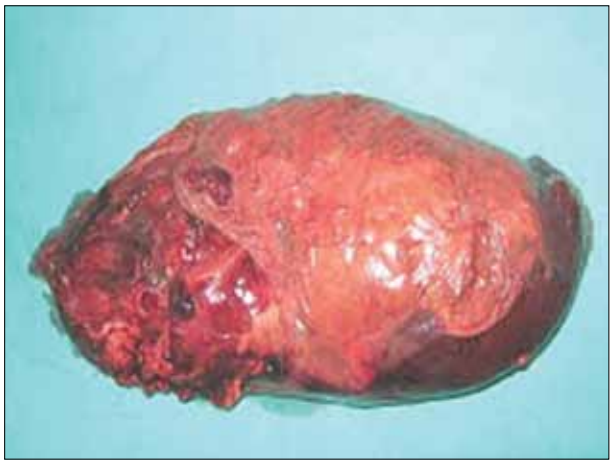


Figure 3. Macroscopic view of the removed enlarged spleen

hemoperitoneum, splenectomy was performed since the cleft on the upper pole of the spleen could not be surgically repaired. After splenectomy and revising the abdominal cavity for hemostasis, abundant lavage was performed and drains were placed in the left subphrenic space, prior to the closure of the abdominal wall. The postoperative was uneventful and the patient was recovering without any need for blood transfusion. The drains were removed at the optimum time and on the day 6 the patient received vaccination against *Pneumococcus*, *Meningococcus* and *Haemophilus influenzae*. On day 7, the patient was discharged in good general condition with written information about post-splenectomy risks and an up-to-date vaccination card.

DISCUSSION

Complications of infectious mononucleosis could be serious and fatal and splenic rupture is considered the most frequent cause of death in infectious mononucleosis [4]. Unfortunately, mortality rate is relatively high when rupture occurs (approximately 30%) [5]. Detailed mechanism of splenic rupture remains unclear. Some authors consider the increase in portal venous pressure and sudden compression of the enlarged spleen due to diaphragm contraction the most frequent factor that may cause spontaneous splenic rupture [6], while Patel et al. [7] consider the expanding of subcapsular hematoma the most important factor that causes splenic rupture in infectious mononucleosis. As our patient started feeling severe pain while she was playing volleyball, the most likely cause of the splenic rupture is sudden compression of the enlarged spleen. Splenic rupture, especially when a patient is hemodynamically unstable, should be treated by splenectomy, while some authors recommend transcatheter arterial embolization [1, 8]. We have treated our patient by splenectomy after a surgical consultation, in order to prevent sudden death. Repair was considered, but it was not possible to perform, due to spleen enlargement and high risk of bleeding. The patient was vaccinated against *Pneumococcus*, *Meningococcus,* and *Haemophilus influenzae*, as vaccination against these pathogens should be conducted after splenectomy [1]. Survival rate for patients who undergo splenectomy is high and is close to 100%. Therefore, the survival benefit from splenectomy outweighs post-splenectomy risks, since mortality rate in vaccinated patients is very low [9]. While this case concludes with the etiology of splenic rupture remaining unclear in regard to infectious mononucleosis, this report has important implications for clinicians of emergency, intensive care, general surgery, hematology, as well as the infectious disease medicine. The spleen is most vulnerable to rupture in the second and third week after the onset of infectious mononucleosis [4]. This report illustrates that splenic rupture may develop six weeks after the onset of infectious mononucleosis, which has been rarely described in medical literature to date. Also, the report shows that we need better monitoring of patients with infectious mononucleosis and, according to that, attending physicians may have to improve surveillance and treatment plans. It is necessary to warn patients to wait before undertaking sports activities for a long time after treating infectious mononucleosis, considering that the risk of spleen rupture obviously exists a couple of week after treating the disease. Patients should wait to start with sport activities at least two months if the size of the spleen is within normal range.

REFERENCES

1. Dunmire SK, Hogquist KA, Balfour HH. Infectious Mononucleosis. Curr Top Microbiol Immunol. 2015; 390:211–40.
2. Bartlett A, Williams R, Hilton M. Splenic rupture in infectious mononucleosis: A systematic review of published case reports. Injury. 2016; 47(3):531–8.
3. Putukian M, O'Connor FG, Stricker P, McGrew C, Hosey RG, Gordon SM, et al. Mononucleosis and athletic participation: an evidence-based subject review. Clin J Sport Med. 2008; 18(4):309–15.
4. Rinderknecht AS, Pomerantz WJ. Spontaneous splenic rupture in infectious mononucleosis: case report and review of the literature. Pediatr Emerg Care. 2012; 28(12):1377–9.
5. Aubrey-Bassler FK, Sowers N. 613 cases of splenic rupture without risk factors or previously diagnosed disease: a systematic review. BMC Emerg Med. 2012; 12:11.
6. Koebrugge B, Geertsema D, de Jong M, Jager G, Bosscha K. Spontaneous splenic rupture in infectious mononucleosis. JBR-BTR. 2013; 96(4):234–5.
7. Patel JM, Rizzolo E, Hinshaw JR. Spontaneous subcapsular splenic hematomas as the only clinical manifestation of infection mononucleosis. JAMA. 1982; 247(23):3243–4.
8. Jenni F, Lienhardt B, Fahrni G, Yuen B. Nonsurgical management of complicated splenic rupture in infectious mononucleosis. Am J Emerg Med. 2013; 31(7):1152.e5–6.
9. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. 2011; 378(9785):86–97.

Спонтана руптура слезине после инфективне мононуклеозе

Божидар Одаловић[1,2], Милан Јовановић[3], Радојица Столић[4], Бранислав Белић[4], Симон Николић[1], Предраг Мандић[1]

[1]Универзитет у Приштини, Медицински факултет, Косовска Митровица, Србија;
[2]Клинички центар Србије, Ургентни центар, Београд, Србија;
[3]Клинички центар Србије, Центар за ендокрину хирургију, Београд, Србија;
[4]Универзитет у Крагујевцу, Медицински факултет, Крагујевац, Србија

САЖЕТАК

Увод Спонтана руптура слезине је ретка али потенцијално фатална компликација инфективне мононуклеозе (ИМ). Јавља се само у 0,1–0,5% случајева ове болести.
Циљ овог рада је био да прикаже болесницу са спонтаном руптуром слезине после ИМ.

Приказ болесника Болесница стара 22 године осећала се добро месец дана после лечења ИМ и почела је да тренира одбојку. Две недеље после почетка тренирања, у току тренинга осетила је јаке болове у трбуху. Дијагноза спонтане руптуре слезине потврђена је компјутеризованом томографијом. Урађена је спленектомија, а постоперативни ток је био повољан и она се опоравила без трансфузија.

Закључак Правовремена дијагноза и постављање индикације за оперативним лечењем од пресудног су значаја за излечење. Са спортским активностима се може отпочети два месеца после лечења ИМ уколико је величина слезине нормална.

Кључне речи: инфективна мононуклеоза, компликације; спонтана руптура; руптура слезине; етиологија, хирургија; спленектомија

DOI: https://doi.org/10.2298/SARH170308179A
UDC: 615.816/.817:616-073

REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Application of ultrasound diagnostics in cardiopulmonary resuscitation

Slađana Anđelić[1], Aleksandar Pavlović[2], Slađana Trpković[2], Ana Šijački[3], Aleksandra Janićijević[4], Biljana Putniković[4]

[1]Municipal Institute for Emergency Medical Care, Belgrade, Serbia;
[2]University of Priština, Faculty of Medicine, Kosovska Mitrovica, Serbia;
[3]University of Belgrade, Faculty of Medicine, Clinical Center of Serbia, Clinic for Emergency Surgery, Belgrade, Serbia;
[4]University of Belgrade, Faculty of Medicine, Zemun Clinical Hospital Centre, Department of Cardiology, Belgrade, Serbia

SUMMARY
Ultrasound is becoming increasingly available and incorporated into emergency medicine. Focused echocardiographic evaluation in resuscitation (FEER) is a training program available to emergency doctors in order to ensure adequate application of echocardiography in the cardiac arrest setting. The FEER protocol provides an algorithm, whereby a "quick view" can be provided in 10 seconds during minimal interruptions in chest compressions. Performing ultrasound in the cardiac arrest setting is challenging for emergency doctors. The International Liaison Committee on Resuscitation recommend the 'quick look' echocardiography view can be obtained during the 10-second pulse check, minimizing the disruption to cardiopulmonary resuscitation.
Keywords: cardiopulmonary resuscitation; critical care; advanced cardiac life support; ultrasound

INTRODUCTION

Over the last several decades, a rapidly growing trend of the application of ultrasonography (USG) in out-of-hospital settings has become evident [1]. Along with the technological development, ultrasound (US) apparatuses have been improved, while the trend of minimization leads to the creation of small, manually transportable equipment of good resolution and accessible prices [2]. Easy to employ, it finds application at various locations where cardiac arrest (CA) might occur (in patients' homes, outside, at working places, etc.) as well as in the ambulance vehicle, under different conditions of external circumstances (light, temperature).

USG utilization in emergency medicine (EM) is presented through the focused cardiac ultrasound where the EM physician who is not an imaging specialist but has undergone a corresponding education within the field of echocardiography (echo) performs a focused USG examination. The advantages of prehospital application of a portable US apparatus are in its non-invasive method, in the fact that it can be done in a short amount of time beside the patient's bed, and in the possibility of multiple repetitions, while sensitivity in the detection of free liquid is equal to the computed tomography examination [3]. Insufficiencies are that US findings depend on the physician's education; specific injuries cannot be identified, the visualization of retroperitoneal injuries is low, and obesity and subcutaneous emphysema can substantially interfere with the examination. Although tempting for EM physicians, the US apparatus has its limitations, such as technical possibilities (small monitor, lower resolution), length of the examination, and the patient's condition and the possibility of positioning the patient into a certain position.

After adequate anamnesis/heteroanamnesis and a complete physical examination, USG represents the unavoidable differential-diagnostic method in the early detection of emergency conditions at the prehospital level [4]. A detailed description of US findings is not expected from the EM physician, but only a statement whether a certain clinical pathology is present or not.

US DURING CARDIOPULMONARY RESUSCITATION

A novelty within the field of US diagnostics during the application of the Advanced Life Support is that an educated EMS physician can use portable (handheld) devices with transthoracic echocardiogram [5]. According to the current guidelines by the American Heart Association and the European Resuscitation Council, and on the basis of the consensus of the International Liaison Committee on Resuscitation (ILCOR), a possible role of USG during CPR is recognized (Figure 1) [6, 7, 8].

Ultrasound assessment is addressed above to identify and treat reversible (hypovolemia,

Received • Примљено: March 8, 2017
Accepted • Прихваћено: September 25, 2017
Online first: October 3, 2017

Correspondence to:
Slađana ANĐELIĆ
Municipal EMC Institute of Belgrade
Franše d`Eperea 5
11000 Belgrade, Serbia
novizivot94@gmail.com

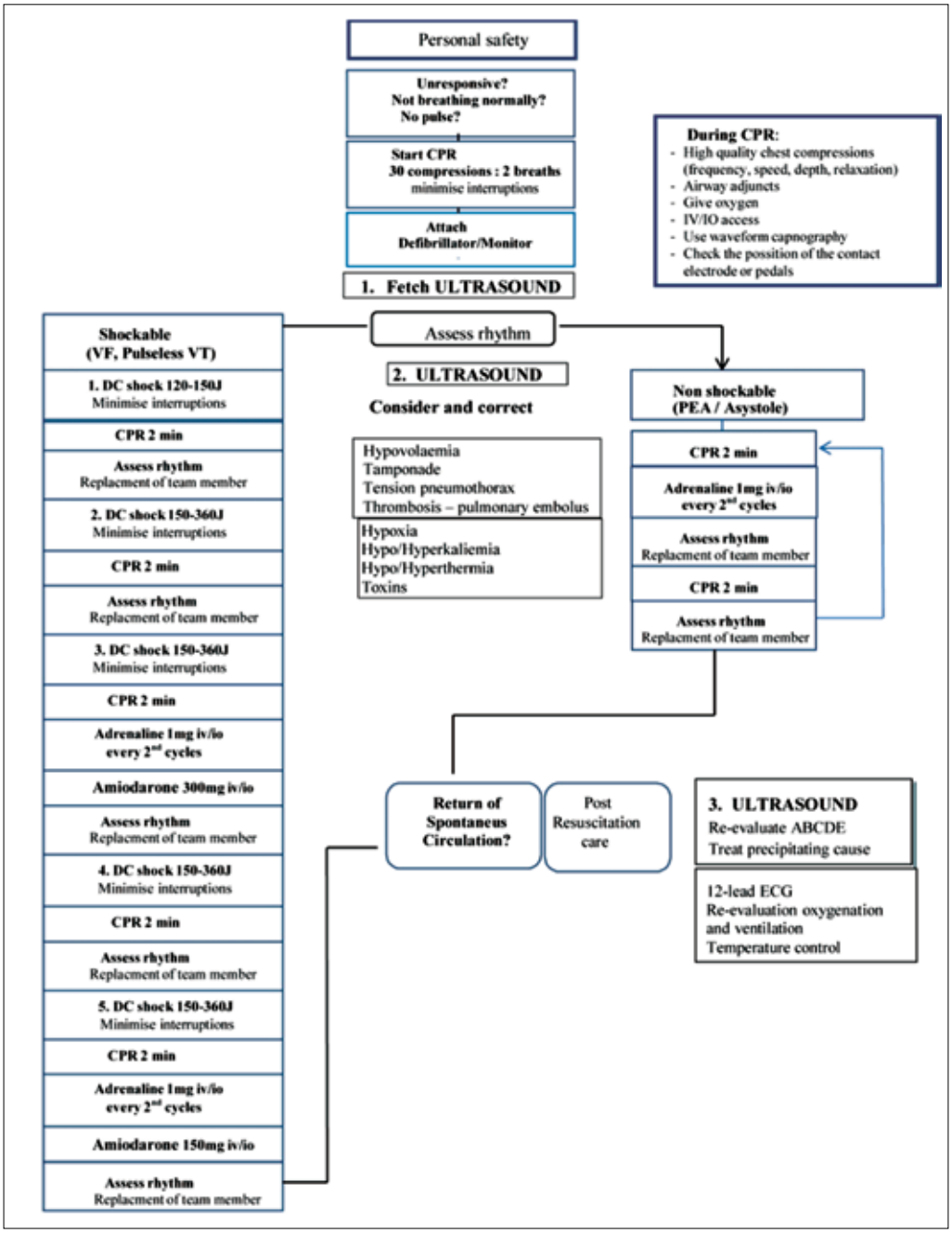


Figure 1. Ultrasonography during Advanced Life Support

cardiac tamponade, tension pneumothorax, pulmonary thromboembolism, aortic dissection, ischemia-region wall motion abnormality) causes of CA and to identify low cardiac output states ('pseudo-PEA'). Several studies have investigated the application of USG during CPR with the aim of detection of reversible causes [9, 10].

Focused Echo Evaluation in Resuscitation involves the application of emergency procedures in 10 steps with CPR measures applied at the same time (Table 1) [11]. It is recommended to perform USG examination during the pause in chest compressions of about 10 seconds, for carotid pulse check-up. Indications for immediate echosonography are presented in Table 2.

The Echo-Guided Life Support algorithm contains the "Airway–Breathing–Circulation" sequence of five questions [12]: 1) Is it a pneumothorax? 2) Is there cardiac tamponade? 3) Is the patient hypovolemic? 4) If poor function of the left ventricle is noted, is this the main cause of shock? 5) Are there signs of right ventricle failure?

USG as a diagnostic method can also answer the following questions: Is the tracheal tube correctly positioned? Are there signs of return of spontaneous circulation (ROSC)? Is there cardiac rhythmic motion in pulseless electrical activity (PEA)?

Table 1. Focused Echo Evaluation in Resuscitation [11]

Phases	Steps
High quality CPR, preparation and information of prehospital team members	1. Begin CPR (5 sets of 30 chest compressions: 2 artificial ventilations) 2. Inform other team members: "Preparing the echo" 3. Prepare and test the US apparatus 4. Adapt to the situation (assume the best position in relation to the patient and other team members)
Application of US	5. Inform team members that US examination will be performed no longer than 10 seconds in the periods of minimal interruptions of chest compressions planned so that another team member can check the carotid pulse 6. Give the command: "Stop the compressions" 7. Place the US probe above the patient's subxiphoid region during the last (30th) chest compression 8. Perform US examination of the subcoastal region as soon as possible; if the echogram is not done after 3 seconds, attempt US examination of the parasternal zone during the next 5 seconds
Continue CPR	9. After 9 seconds give the command: "Continue with CPR"
Interpretation of findings and planned emergency interventions	10. Communicate with team members (inform them about US findings) and apply appropriate emergency therapeutic intervention (pericardiocentesis, thoracocentesis, thrombolytic drugs, etc.)

CPR – cardiopulmonary resuscitation; US – ultrasound

Table 2. Indications for immediate echosonography [11]

Before reanimation	Unconscious person Acute myocardial infarction Atypical chest pain: suspected aortic dissection, suspected aneurysm Penetrating trauma, blunt trauma Hypotension, shock of unknown cause Acute severe dyspnea Syncope in a young person Venous thrombosis of thoracic or abdominal aorta Iatrogenic complications
During CPR	PEA Suspected cardiac tamponade Early detection of ROSC Bradycardia – asystole, pacemaker – ECG Performing CPR Efficient chest compression
Post-resuscitation care	Hypotension, adaptation to vasopressors

PEA – pulseless electrical activity; ROSC – return of spontaneous circulation CPR – cardiopulmonary resuscitation

TENSION PNEUMOTHORAX

Dramatic clinical condition can be exclusively caused by a mistake during the mechanical ventilation enabling the breach of the fresh air into the pleural space. Thoracic needle decompression is lifesaving in tension pneumothorax (TP). In the TP diagnostics, USG was used for the first time in 1987 in a study by Wernecke et al. [13]. Since then, a great number of investigations have proved a high sensitivity and specificity of USG in the diagnostics of TP, which have overcome the classical radiography of the thorax and are close in sensibility and specificity to multidetector computed tomography [14]. Owing to the technological progress, US diagnostics has gained its position in the TP diagnostics in emergent conditions when reaching diagnosis is of vital significance. Therefore, Focused Assessment with Sonography in Trauma (FAST) protocol has been supplemented with a chest examination (lungs), so that now E-FAST (Extended-FAST) protocol is used, which also contains a standardized lung examination [15].

On examination, the patient lies on the back, while the physician is mostly positioned on his/her right side. Initially, the insertion site of the catheter is located in the second intercostal space on the mid-clavicular line in the sagittal plane (with positioning indicator situated via cranium) [16, 17]. At the beginning of the examination, anatomic structures should be identified so that two ribs and the belonging intercostal space are within the visual field. The ribs can be visualized as glowing hyperechogenic reflective areas with posterior acoustic shadow, while in the intercostal area a hyperechogenic line can be seen that corresponds to the pleural leaves ("the bat sign").

By the normal US finding, the most significant sign is the sliding of the visceral towards the parietal pleura ("pleural sliding," "lung sliding sign") during respiratory movements [18]. The M-mode cursor is positioned over the hyperechogenic pleural line, while two different pictures appear on the screen. Immobile parts of the chest wall above the pleural line form a picture of horizontal lines ("waves"), while pleural leaves create a granular form ("sand") below the pleural line that gives a characteristic "seashore sign" [18].

During the examination in B-mode, in a normal finding we can notice B-lines or "comet-tail artifacts (sign)" [19], which is the consequence of reverberation artifacts that present as vertical hyperechogenic lines extending from the pleural line to the pleural parenchyma.

The US signs of pneumothorax are described by Zhang et al. [20]**:** the absence of the "lung sliding sign" in TP is the consequence of air presence among the leaves of the parietal and visceral pleura; the air separates pleural leaves, thus enabling the visceralization of the visceral pleura and leads to the absence of pleural sliding in B-mode. By using M-mode, the absence of pleural sliding is presented as a uniform picture of horizontal lines above and below the pleural line ("barcode sign" or "stratosphere sign"). The existence of this phenomenon presents the picture

of TP in this space. The absence of B-lines ("comet-tail sign") in TP is the result of air accumulation in the pleural space, which disturbs the propagation of ultrasound waves and eliminates the gradients of acoustic impedance. The negative predictive value of B-lines is high and amounts to 98–100%, so that the visualization of only one B-line excludes the TP diagnosis.

A-lines are present in patients with TP, while, due to the absence of B-lines, they are clearly seen. "Lung-point sign" represents the TP border, which develops on the periphery and defines the real size of TP. In this sign, in M-mode, the "seashore sign" and the "stratosphere sign" are exchanged over time. This sign has a sensitivity of 79% and a specificity of 100%.

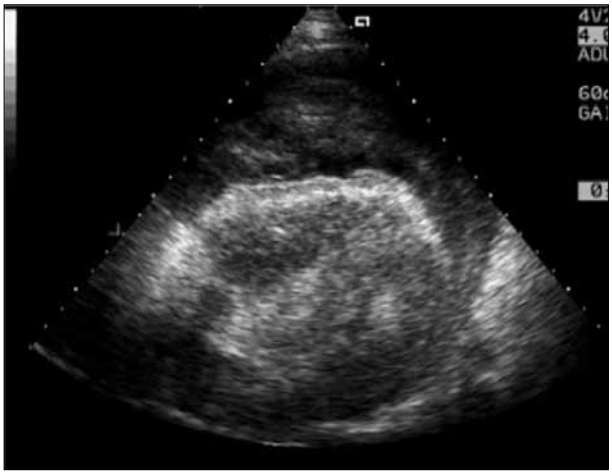


Figure 2. Cardiac tamponade by Dr. Ivan Stanković, Zemun Clinical Hospital Centre, Department of Internal Medicine, Belgrade, Serbia

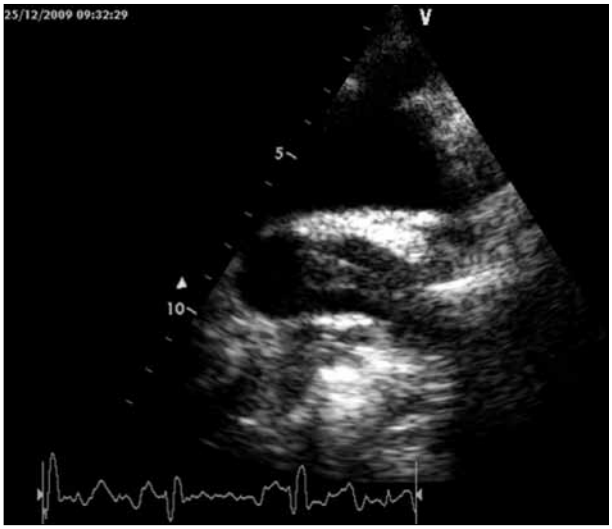


Figure 3. Thrombus in the pulmonary artery right branch – suprasternal cross-section (source: Putniković B. Echocardiography in pulmonary thromboembolism; Medical Days of Sokobanja 2013, Sokobanja, Serbia, October 4–6, 2013)

CARDIAC TAMPONADE

Clinical syndrome is a life-threatening condition caused by the increased pericardial pressure developed due to the accumulation of the fluid into the pericardial space [21]. With each cardiac contraction, this condition deteriorates resulting in PEA. US heart examination is the diagnostic method of choice in the diagnostics of cardiac tamponade, which should be done without delay. The most frequent finding is the feature of pericardial discharge, i.e. the separation of pericardial leaves during the entire cycle [21]. There are several echo signs of TP danger, so that the 2D mode can accordingly register the increase of the right ventricle (RV) in expirium and collapse of the right atrium (RA) in inspirium (Figure 2), RA collapse in systole in the duration longer than 1/3 of the systole duration of over 1/3, paradoxical movements of the interventricular septum, and RV collapse in diastole. FAST examination includes the subxiphoid window and/or parasternal "long axis" approach [22]. In penetration wounds, bedside US has 100% sensitivity and 97% specificity in detecting pericardial effusion and the need for emergent pericardiocentesis [23]. The level of suspected cardiac tamponade must be increased to "present until proven differently" when the injury is rectangular (so-called cardiac box), so that it forms horizontal lines along the clavicle over the nipples to the rib edges, and a lower horizontal line that joins the vertical lines at the site of connection with rib edges. Echocardiographically, separation of epicardial layers around the heart can be discovered when the quantity of fluid around the heart is over 15–35 ml. The speed of fluid accumulation defines the clinical course. Therapeutic care assumes emergent pericardiocentesis performed under US guidance at the site where "the deepest pocket" of fluid is perceived. Resuscitation with needle pericardiocentesis can be of help in carefully selected patients.

PULMONARY THROMBOEMBOLISM

Pulmonary thromboembolism (PE) is one of frequent immediate causes of CA in prehospital conditions [24, 25]. Therefore, it seems a logical and appealing test for many patients with acute chest pain and/or heavy breathing where PE is one of potential disorders. US examination in patients with suspected PE offers numerous data on the morphology and function of RV, RA, pulmonary artery visualization, and indirect assessment of pulmonary hypertension. In addition, we may exclude with certainty other hemodynamically unstable diseases that can imitate PE, such as extensive myocardial infarction, cardiac tamponade, or aortic dissection. Echocardiographically, signs of acute overload of RV by pressure (dilatation and hypokinesis of RV, tricuspid regurgitation, increased systolic pressure in RV < 60 mmHg, dilated non-collapsible right hollow vein) that indirectly indicate PE (Figure 3).

The most direct sign of PE is a direct visualization of thrombosis in the pulmonary artery. In PE, it has a sensitivity of 80% and a specificity of 97%, and it is increased with RV dysfunction. Ribeiro et al. [26] concluded that RV dysfunction confirms the diagnosis of PE and that it is associated with mortality.

There are several echo signs of PE.

RV dilatation – due to pulmonary hypertension caused by PE, increased pressure in the right heart, and increased relative separation of RV that is normally about 2/3 the cross-section of the LV (left ventricle). These changes are best detected in the apical projection of four cavities [27, 28]. RV dilatation in the apical projection is considered the increased relation of the RV end-diastolic dimension in comparison with LV larger than 1 mm. In the assessment of RV dilatation, the long parasternal axis is also of great use, in which the infundibular portion can be best visualized. According to different authors, RC dilatation is considered the end-diastolic dimension of the RV larger than 27, i.e. 30 mm. If the RV is not evidently larger than the LV, PE can be excluded as the cause of CA with high probability.

Right ventricular hypokinesis – McConnell's sign (Figure 4) (RV dilatation with decreased size of the LV (ratio RV/LV > 0.7); in addition, the association between hypokinesis of the RV free wall and preserved top contractility (McConnell sign). Specificity of this sign in the diagnostics of PE is about 94%, while sensitivity is about 20% [29].

Flattening of interventricular septum [30] – remodeling of the RV due to overburden by pressure during PE; echocardiographically, it is viewed by its dilatation, regional disorder of free wall kinetics, as well as by the pathological mobility of the interventricular septum. Having in mind that the compensatory mechanism of dilatation is limited, acute pressure increase in the RV can additionally result in the interventricular septal shift from the right toward the left that can be seen during systole and diastole. By flattening of the interventricular septum, LV acquires the letter D shape. Such change is best perceived in the short parasternal projection, but can be also visualized in M-mode in the long parasternal projection or double-dimensionally in the abovementioned short parasternal and apical projection of four cardiac chambers. The interventricular septum shift, particularly if it occurs during diastole, can compromise the LV diastolic filling and cause the patient's additional hemodynamic deterioration. Therefore, US detection of interventricular septal shift is of high importance in the decision to administer fluid therapy with the aim to prevent additional LV dilatation and obstruction of inflow into the right heart.

Tricuspid regurgitation (TR) – maximal speed of TR is the most useful method for the stratification of patients according to the level of systolic pressure in the RV, i.e. pulmonary artery. In support of PE speaks the speed of TR outflow that is between 2.8–3.8 cm/s, as well as the pressure gradient which is not over 60 mmHg. The speeds above the stated ones indicate primary PE or hypertension of some other etiology.

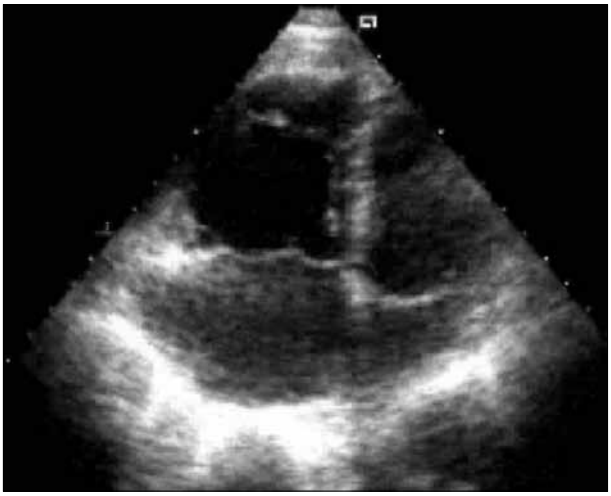


Figure 4. Right ventricle dilatation and hypokinesis – McConnell's sign, by Dr. Ivan Stanković, Zemun Clinical Hospital Centre, Department of Internal Medicine, Belgrade, Serbia

The 60/60 sign – dicrotic notch in the profile of the RV [acceleration time (AcT)] outflow tract. It represents the AcT in the RV outflow tract < 60 ms in the presence of systolic gradient TR < 60 mmHg gradient [30].

Other indirect morphological characteristic of the RV burdened by pressure include the following: dilated (> 20 mm) inferior vena cava (IVC), non-collapsible in inspirium and dilated RA (IVC ≥ 2 cm and IVC collapse < 40% is a significant sign of elevated RA pressure).

HYPOVOLEMIA

Inadequate volume of the circulating blood is the most frequent cause of PEA form of CA [6]. A hypovolemic patient has insufficient filling (<1 cm) or dynamic IVC. In patients with spontaneous breathing, the size of IVC is increased with each inspirium, while in patients with CA, the opposite occurs if applying artificial ventilation. In patients on mechanical ventilation, the increase of IVC size > 18% is in harmony with preload dependence. Each change in size is an indication for a rapid application of fluid during CPR. Echo finding (Table 3, Figure 5) includes a significantly reduced end-diastolic chamber size and "kissing" ventricular walls during systole [10]. If hypovo-

Table 3. Echocardiographic signs of hypovolemia [10]

Parameter	Static/dynamic	Finding suggestive of hypovolemia	Pitfalls
LV cavity size and function	Dynamic	Small, hyperkinetic with end-systolic cavity obliteration	Inotropic support, severe valvular regurgitation, left ventricular hypertrophy
LV end-diastolic area	Static	5.5 cm^2/m^2 of BSA	As above
IVC size and inspiratory collapse	Dynamic	> 10 mm collapse on inspiration	Spontaneously breathing patients in sinus rhythm only
IVC size and expiratory airway collapse	Dynamic	Variable	Intubated and mechanically ventilated, in sinus rhythm only

BSA – body surface area; IVC – inferior vena cava; LV – left ventricle

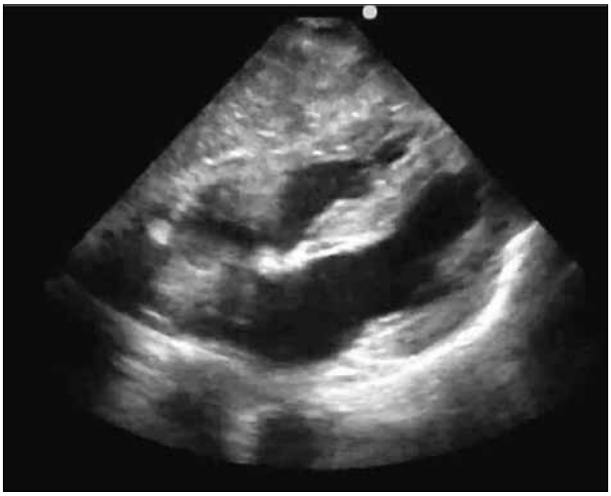


Figure 5. Echocardiographic signs of hypovolemia, by Dr. Ivan Stanković, Zemun Clinical Hospital Centre, Department of Internal Medicine, Belgrade, Serbia

lemia is indicated, possible causes should be searched for using abdominal US examination by implementing the FAST protocol and aortal check-up (aneurysm or rupture of abdominal aorta). Echo finding can confirm the presence of internal bleeding.

POSITION OF THE ENDOTRACHEAL TUBUS

If the endotracheal tube is in its correct position during US examination, only one air-filled structure is visible on the neck [31]. The "double tract" sign indicates esophageal intubation. US in CA can be a diagnostic method, a prognostic method (ROSC/non-ROSC), and an ethical method (making decision on emergency transport or cessation of CPR in the field). However, the decision on the cessation of resuscitation efforts cannot be passed on US findings alone.

If the patient is in ventricular fibrillation or pulseless ventricular tachycardia, US finding is of limited value. In patients in PEA or asystole, US has significantly higher diagnostic significance. According to the new CPR algorithms (2015), if the initial rhythm of CA is PEA, the application of portable US can enable the differentiation between true PEA from pseudo-PEA [32]. In addition to the aforementioned, US findings can indicate reduced LV ejection fraction, free fluid in the peritoneal cavity, the size of abdominal aortic aneurysm, the presence of deep vein thrombosis, etc.

In an interesting letter to the editor, its authors have presented the analysis to evaluate the possibility of checking the efficacy of heart compressions using US and of guiding the hands' position in order to improve cardiac contractility. Preliminary observations indicate that changing the position of hands guided by USG could improve the quality of chest compressions and important hospital information could be obtained without stopping CPR [33].

CONCLUSION

Ultrasound during CPR should be performed during the rhythm check, preferably the first rhythm check and each subsequent check, which is recommended to be no longer than 10 seconds, to prevent a fall in coronary perfusion pressure. It is therefore advisable to get the ultrasound machine to the patient's bedside as early as possible. The time required to position the machine, to turn it on, to ensure that the correct probe is attached and in position, and that screen settings are optimized, can be significant. Hence, the second action in the Extracorporeal Life Support algorithm – 'attach defibrillator/monitor' – should be replaced with 'attach defibrillator/monitor and fetch ultrasound.' A priority during CPR is to minimize interruptions in CPR, which is associated with a drop in coronary blood flow and outcome – 'plan before interrupt compressions.' This would include planning to perform USG during the pulse check, i.e. positioning the probe on the patient and readying the US machine.

REFERENCES

1. Moore CL, Copel JA. Point-of-care ultrasonography. N Engl J Med. 2011; 364(8):749–57.
2. Putniković B, Stanković I, Miličević P, Marjanović M, Nešković AN. Hand-held echo is not so handy in everyone's hands: misdiagnosing congenital septal defects in patients with heart murmurs. Srp Arh Celok Lek. 2015; 143(5-6):322–5.
3. Lichtenstein DA, Menu Y. A bedside ultrasound sign ruling out pneumothorax in the critically iii: lung sliding. Chest. 1995; 108(5):1345–45.
4. Stankovic I, Marcun R, Janicijevic A, Farkas J, Kadivec S, Ilic I, et al. Echocardiographic predictors of outcome in patients with chronic obstructive pulmonary disease. J Clin Ultrasound. 2017; 45(4):211–21.
5. Testa A, Cibinel GA, Portale G, Forte P, Giannuzzi R, Pignataro G, et al. The proposal of an integrated ultrasonographic approach into the ALS algorithm for cardiac arrest: the PEA protocol. Eur Review Med Pharmacol Sci. 2010; 14(2):77–88.
6. Pavlović A, Trpković S, Anđelić S, Marinković O. Kardiopulmonalna reanimacija – nove preporuke 2015–2020. NČ urgent medic HALO 194. 2015; 21(3):181–98.
7. Anđelić S. Najvažnije promene u preporukama za vanbolničku kardiopulmonalnu reanimaciju odraslih. ABC časopis urgentne medicine. 2014; 14(1):7–14.
8. Trpković S, Pavlović A, Anđelić S, Sekulić A. Nove preporuke za kardiopulmonalnu reanimaciju u posebnim stanjima. NČ urgent medic HALO 194. 2015; 21(3):199–211.
9. Bernardin G, Mazerolles M. Les critères hémodynamiques statiques prédictifs de l' efficacitéd' un remplissage vasculaire. Réanimation. 2004; 13:288–98.
10. Zafiropoulos A, Asrress K, Redwood S, Gillon S, Walker D. Critical care echo rounds: echo in cardiac arrest. Echo Res Pract. 2014; 1(2):D15–21.
11. Breitkreutz R, Walcher F, Seeger FH. Focused echocardiographic evaluation in resuscitation management: concept of an advanced life support-conformed algorithm. Crit Care Med. 2007; 35(5 Suppl):S150–61.
12. Lancto^t JF, Valois M, Beaulieu Y. EGLS: Echo-guided life support. Crit Ultrasound J. 2011; 3:123–9.
13. Wernecke K, Galanski M, Peters PE, Hansen J. Pneumothorax: Evaluation by ultrasound-preliminary results. J Thorac Imaging. 1987; 2(2):76–8.

14. Mišović M, Kosanović T. Ultrasound in diagnostics of pneumothorax. MD-Medical Data. 2015; 7(4):311–4.
15. Kirkpatrick AW, Sirois M, Laupland KB, Liu D, Rowan K, Ball CG, et al. Hand-held thoracic sonography for detecting post-traumatic pneumothoraces: the extended focused assessment with sonography for trauma (EFAST). J Trauma. 2004; 57(2):288–95.
16. Coats TJ, Wilson AW, Xeropotamous N. Pre-hospital management of patients with severe thoracic injury. Injury. 1995; 26(9):581–5.
17. Ball CG, Kirkpatrick AW, Laupland KB, Fox DL, Litvinchuk S, Dyer DM, et al. Factors related to the failure of radiographic recognition of occult posttraumatic pneumothoraces. Am J Surg. 2005; 189(5):541–6.
18. Cunningham J, Kirkpatrick AW, Nicolaou S, Liu D, Hamilton DR, Lawless B, et al. Enhanced recognition of "lung sliding" with power Doppler imaging in the diagnosis of pneumothorax. J Trauma. 2002; 52(4):769–71.
19. Lichtenstein D, Meziere G, Biderman P, Gepner A. The "'comet-tail artifact"': an ultrasound sign ruling out pneumothorax. Intensive Care Med. 1999; 25(4):383–8.
20. Zhang M, Liu ZH, Yang JX, Gan JX, Xu SW, You XD, et al. Rapid detection of pneumothorax by ultrasonography in patients with multiple trauma. Crit Care. 2006; 10(4):R112.
21. Sakač D, Kovačević DV, Koraćević G. Pericarditis and cardiac tamponade: urgent condition not only in cardiology. Med Pregl. 2011; 64(3-4):194–7.
22. Tsang TS, Enriquez-Sarano M, Freeman WK, Barnes ME, Sinak LJ, Gersh BJ, et al. Consecutive 1127 therapeutic echocardiographically guided pericardiocenteses: clinical profile, practice patterns, and outcomes spanning 21 years. Mayo Clin Proc. 2002; 77(5):429–36.
23. Ghafouri HB, Zare M, Bazrafshan A, Modirian E, Farahmand S, Abazarian N. Diagnostic accuracy of emergency performed focused assessment with sonography for trauma (FAST) in blunt abdominal trauma. Electron Physician. 2016; 8(9):2950–3.
24. Tamburkovski V, Andjelic S. Wells' score for early prehospital screening of pulmonary embolism. Signa vitae. 2016; 12(1):131–3.
25. Andjelic S, Panic G, Sijacki A. Emergency response time after out-of-hospital cardiac arrest. Eur J Intern Med. 2011; 22(4):386–93.
26. Ribeiro A, Lindmarker P, Juhlin-Dannfelt A, Johnsson H, Jorfeldt L. Echocardiography Doppler in pulmonary embolism: right ventricular dysfunction as a predictor of mortality rate. Am Heart J. 1997; 134(3):479–87.
27. Torbicki A. Echocardiographic diagnosis of pulmonary embolism: a rise and fall of McConnell sign? Eur J Echocardiogr. 2005; 6(1):2–3.
28. Heintzen MP, Strauer BE. Acute cor pulmonale associated with pulmonary embolism. Internist. 1999; 40:710–21.
29. Unluer EE, Senturk GO, Karagoz A, Uyar Y, Bayata S. Red flag in bedside echocardiography for acute pulmonary embolism: Remembering Mc Connell's sign. Am J Emerg Med. 2013; 31:719–21.
30. Movahed MR, Hepner A, Lizotte P, Milne N. Flattening of the interventricular septum (D-shaped left ventricle) in addition to high right ventricular tracer uptake and increased right ventricular volume found on gated SPECT studies strongly correlates with right ventricular overload. J Nucl Cardiol. 2005; 12(4):428–34.
31. Chou HC, Tseng WP, Wang CH, Ma MHM, Wang HP, Huang PC, et al. Tracheal rapid ultrasound exam (T.R.U.E.) for confirming endotracheal tube placement during emergency intubation. Resuscitation. 2011; 82(10):1279–84.
32. Soar J, Nolan JP, Böttiger BW, Perkins GD, Lott C, Carli P, et al. European Resuscitation Council Guidelines for Resuscitation 2015. Section 3. Adult advanced life support. Resuscitation. 2015; 95:100–47.
33. Zanatta M, Benato P, Cianci V. Corrigendum to "Letter to the editor: Ultrasound guided chest compressions during cardiopulmonary resuscitation". Resuscitation. 2015; 87:e13–4.

Примена ултразвучне дијагностике у кардиопулмоналној реанимацији

Слађана Анђелић[1], Александар Павловић[2], Слађана Трпковић[2], Ана Шијачки[3], Александра Јанићијевић[4], Биљана Путниковић[4]

[1]Градски завод за хитну медицинску помоћ, Београд, Србија;
[2]Универзитет у Приштини, Медицински факултет, Косовска Митровица, Србија;
[3]Универзитет у Београду, Медицински факултет, Клинички центар Србије, Клиника за ургентну хирургију, Београд, Србија;
[4]Универзитет у Београду, Медицински факултет, Клиничко-болнички центар Земун, Одељење интерне медицине, Београд, Србија

САЖЕТАК

Ултразвук се све више користи у ургентној медицини. Фокусирана ехокардиографска процена у реанимацији (ФЕПР) програм је обуке намењен лекарима хитне помоћи који желе да се оспособе за примену ехокардиографије на локацији срчаног застоја. ФЕПР протокол садржи алгоритам у којем се „брзи поглед" може обезбедити у року од 10 секунди, током минималних прекида у грудним компресијама. Примена ултразвука у срчаном застоју представља велики изазов за лекаре хитне медицинске помоћи. Међународни комитет за реанимацију препоручује ехокардиографију по принципу „брзог погледа" који се може урадити током десетосекундне провере пулса уз минимално ометање кардиопулмоналне реанимације.

Кључне речи: кардиопулмонална реанимација; интензивна нега; ФЕПР протокол; ултразвук

DOI: https://doi.org/10.2298/SARH160822016I
UDC: 616.12-008.331.1-085.22; 615.22

REVIEW ARTICLE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Fixed combination of bisoprolol and low-dose hydrochlorothiazide in arterial hypertension

Branislava Ivanović[1], Milan Pavlović[2], Arsen Ristić[1], Dragan Kovačević[3]

[1]Clinical Center of Serbia, Clinic for Cardiology, Belgrade, Serbia;
[2]Niš Clinical Center, Clinic for Cardiovascular Diseases, Niš, Serbia;
[3]Institute for Cardiovascular Diseases of Vojvodina, Sremska Kamenica, Serbia

SUMMARY
Beta-blockers showed better results in lowering elevated blood pressure in the younger age group of patients with higher renin plasma levels. Actual recommendations from the European Society of Cardiology for treatment of arterial hypertension from 2013 insist that heart rate should always be measured along with blood pressure. These recommendations point out the significance of resting heart rate as an independent predictor of cardiovascular morbidity and mortality in patients with arterial hypertension. Beta-blockers have a compelling indication for treatment of arterial hypertension in patients with coexistence of coronary artery disease, especially post myocardial infarction, as well as in patients with systolic heart failure. Bisoprolol, a highly selective beta-blocker with a long half-life and a prolonged antihypertensive effect, has shown consistent blood pressure control over a period of 24 hours.
It has been demonstrated in placebo-controlled studies that administration of thiazide diuretics, in addition to lowering blood pressure levels, had also been associated with a reduction of cardiovascular morbidity and mortality rates. It is evident that effectiveness of thiazide diuretics is dose-dependent; however, undesirable effects of drugs are also dose-dependent. Depending on the dose, they aggravate glucose intolerance, increase lipid levels, cause hypokalemia, hyponatremia, and hypomagnesemia, and increase levels of uric acid. Administration of very low doses of a thiazide diuretic is acceptable in combination with other antihypertensive drugs, because it potentiates the action of other drugs without causing undesirable metabolic effects. The effectiveness and safety of the combination of bisoprolol (in various doses) and a thiazide diuretic in a small dose has been proven in clinical trials.
Keywords: hypertension, drug therapy; antihypertensive agents; administration and dosage; beta-blockers; bisoprolol; thiazides

INTRODUCTION

The main treatment goal in patients with arterial hypertension is the normalization of blood pressure levels, prevention, stopping, or regression of the target organ damage, with the ultimate goal to decrease cardiovascular morbidity and mortality rate. Under normalization of blood pressure levels we consider a decrease to less than 140/90 mmHg, and to less than 140/85 mmHg in patients with diabetes mellitus. However, this goal is very difficult to achieve for most patients. According to results from Blood Pressure Control Rate and Cardiovascular Risk Profile study, in which 7,860 patients from Central and Eastern Europe treated for arterial hypertension were included, satisfactory control of blood pressure was achieved in only 27.1%. According to that study, target blood pressure levels were reached in 23.3% of patients in Serbia [1]. Possible reasons for treatment failure are physicians' inertia on the one hand, and patients' low awareness of the problem severity and significance of adherence to advised treatment on the other. Therapeutic inertia is reflected in satisfaction with achieved reduction without normalization of blood pressure values, and in insisting on monotherapy.

Administration of more than one antihypertensive agent is advised in international guidelines, for patients with systolic blood pressure that is 20 mmHg higher, and diastolic one that is 10 mmHg higher than border levels, as well as in patients with high level risk due to associated risk factors and subclinical damage to target organs, diabetes mellitus, associated cardiovascular or kidney disease. The best evidence in favor of benefits of combined therapy are the results of a meta-analysis in which 42 studies (10,968 patients) were included, and which showed that a combination of two antihypertensive agents lowered arterial blood pressure five times better than the maximal dose of a single drug [2]. It is possible to achieve the desired effectiveness by combining medicaments with different mechanisms of action due to their synergistic action. Fixed combinations of diuretics and renin–angiotensin–aldosterone system inhibitors or beta-blockers are useful, as they ensure numerous benefits, starting with better blood pressure control, a simplified dosage regime, and improvement of compliance with reduction of dose-dependent undesirable effects.

The objective of this paper is to summarize advantages of administering a fixed combination of bisoprolol and a low-dose thiazide diuretic.

Received • Примљено: August 22, 2016
Revised • Ревизија: February 19, 2018
Accepted • Прихваћено: February 22, 2018
Online first: February 27, 2018

Correspondence to:
Milan PAVLOVIĆ
Branka Miljkovića 51
18000 Niš, Serbia
milanpa@open.telekom.rs

BETA-BLOCKERS IN THERAPY OF ARTERIAL HYPERTENSION

A 10-year follow-up of 3,195 healthy persons with an average age of 48.5 years in the Framingham Heart Study has shown that overweight younger men, especially if they continue to further gain weight, predominantly develop diastolic arterial hypertension [3]. Isolated systolic hypertension in older patients was found more often in women, and usually occurred in persons with history of normal or high–normal blood pressure. It has been found that heart rate, as well as minute volume, and peripheral vascular resistance as well, were increased in proportion with body mass index and waist circumference [4]. Sympathetic neural activity in skeletal muscles, the index of sympathetic nervous system activation, increases in proportion with waist circumference and abdominal fat tissue mass [5]. Abdominal adipocytes cause sympathetic nervous system activation trough leptin production. An increased sympathetic tone leads to an increase in renin and angiotensin II production, which in turn additionally activate sympathetic nervous system through feedback. Abdominal adipocytes stimulate an increase in interleukin 6 and tumor necrosis factor alpha trough adipokines, and cause the development of increased insulin resistance. An increase in insulin level occurs with additional feedback activation of sympathetic nervous system. In this manner, central obesity and an increased leptin production increase catecholamines plasma levels with unfavorable effects on the heart and the blood vessels.

The increased sympathetic nervous system activation leads to an increase in heart rate and an increased strength of the myocardial contraction, and causes an increase in ventricular stroke volume and an increase in minute volume (minute volume is equal to the product of heart rate and stroke volume). The elevated adipokines', interleukin-6, and tumor necrosis factor alpha levels intensify oxidative stress and cause the development of endothelial dysfunction and inflammation [6]. The disorder of endothelial function is the underlying cause of inadequate vasodilatation of the arterioles in response to metabolic stimuli and an increased peripheral vascular resistance. In addition to the functional component, an anatomical substrate of an increased peripheral vascular resistance develops. Hypertrophy of small artery and arteriole media develops at the expense of lumen reduction; this is an anatomical change, which is the underlying cause of the development and progression of diastolic arterial hypertension and is referred to as eutrophic remodeling of small resistant arteries. The activation of the sympathetic nervous system causes the development and the progression of arterial hypertension trough an increment of minute volume and peripheral vascular resistance. It has been demonstrated that in the treatment of arterial hypertension, beta-blockers showed better results in lowering elevated blood pressure in the younger-age group of patients with higher renin plasma levels [7]. Older patients with lower renin levels show a better therapeutic response to calcium antagonists [8]. It has been found that highly selective beta-blocker bisoprolol, in a group of male patients, age between 35 and 60 years, and with moderate hypertension, showed better results after four weeks of treatment, compared to drugs from other groups of antihypertensive agents [9]. In these patients, monotherapy with bisoprolol with a daily dose of 5 mg showed a better efficacy in lowering both systolic and diastolic blood pressure compared to treatment with losartan 50 mg, amlodipine 5 mg, or hydrochlorothiazide 25 mg once a day. The strategy for arterial hypertension treatment in earlier guidelines was based on assessed sympathetic nervous system activity degree [10, 11, 12]. In younger patients with arterial hypertension and increased sympathetic tone and higher renin plasma levels, drugs with antiadrenergic effects were recommended for initiation of hypertension treatment: beta-blockers and angiotensin-converting-enzyme (ACE) inhibitors, or angiotensin receptor blockers. Diuretics and calcium antagonists (ABCD treatment strategy for arterial hypertension) were recommended as the initial therapy for elderly patients with lower rennin levels.

While comparing antihypertensive effects of bisoprolol and atenolol in a single daily dose by using the method of ambulatory blood pressure monitoring, bisoprolol showed a better blood pressure control in 659 patients with mild to moderate hypertension during the entire period of twenty-four hours' follow-up. A greater efficacy of bisoprolol was exhibited especially in the last hours of the twenty-four-hour interval, in the morning of the following day, before the next scheduled dose of the drug [13]. For consistent blood pressure control during the period of twenty-four hours, it is necessary to use a medicament with longer elimination half-life and higher minimal-to-maximal effect ratio as a monotherapy. As opposed to atenolol, which has an average elimination half-life of six to seven hours and minimal-to-maximal effect ratio of 31%, the highly selective beta-blocker bisoprolol has an elimination half-life of 10–12 hours and minimal-to-maximal effect ratio of 78%. Under the current US Food and Drug Administration criteria for approval of a single daily dose drug for the treatment of arterial hypertension, minimal-to-maximal effect ratio over the period of 24 hours must be higher than 50%. Blood pressure values in the early morning hours, prior to taking the next dose for the following day, is of particular importance. At that time of the day, a pronounced increase in the sympathetic nervous system activity and an increased catecholamine level occur with awakening and beginning of daily activities. At that time, a sudden increase in heart rate and blood pressure is recorded, and increased frequency of ischemic episodes, both symptomatic and asymptomatic ones, is also registered in patients with coronary disease at that time. In the early morning hours, after awakening, a higher frequency of sudden cardiac death, as well as ischemic stroke, is recorded. Bisoprolol, a beta-blocker with a long half-life and a prolonged antihypertensive effect, and a consistent blood pressure control over the period of twenty-four hours, has shown a reduction of ischemic episodes in patients with coronary artery disease in the early morning hours [14].

HEART RATE AND CARDIOVASCULAR MORTALITY

In a study with 2,037 men with untreated arterial hypertension, and a 36-year-long follow-up, it has been found that elevated resting heart rate at baseline increased the risk of mortality [15, 16]. It has been demonstrated that elevated resting heart rate increased the risk of mortality from coronary artery disease, the risk of cardiovascular mortality, but also the risk of overall mortality. It has been reported that resting heart rate higher than 84 beats per minute at baseline had been associated with an increased mortality, compared to lower heart rate levels. It has been confirmed in a study with elderly patients with arterial hypertension that elevated resting heart rate at baseline increased the risk of mortality. This has been demonstrated in both men and women [17]. Patients with arterial hypertension and resting heart rate over 79 beats per minute have a risk of mortality increased by 89%, in comparison to patients with heart rate under 79 beats per minute. It has been found, trough a follow-up of 5,713 asymptomatic men over the period of 23 years, that elevated resting heart rate at baseline increased the risk of sudden cardiac death and the risk of mortality from myocardial infraction [18].

An analysis of risk factors has shown that in addition to classic risk factors for atherosclerosis, resting heart rate values in men had an independent significance as a predictor of mortality risk [19]. Heart rate higher than 80 beats per minute, along with classic risk factors – systolic and diastolic blood pressure, diabetes, smoking and age – indicate an increased risk of mortality. Actual recommendations from the European Society of Cardiology for treatment of arterial hypertension from 2013 insist that heart rate should be always measured along with blood pressure [20]. These recommendations point out the significance of resting heart rate as an independent predictor of cardiovascular morbidity and mortality in patients with arterial hypertension. [21].

An elevated heart rate increases the myocardial oxygen demand, and may cause occurrence of myocardial ischemic episodes in patients with coronary disease. It is less known that an elevated heart rate accelerates the progression of atherosclerosis, predisposes occurrence of a vulnerable atheroma rupture and the development of acute coronary syndrome. An increased activation of the sympathetic nervous system has unfavorable effects on the heart and stimulates hypertrophy of the left ventricle in patients with arterial hypertension. A sudden increase in the catecholamine plasma level may even lead to a direct toxic myocardial lesion, with an increase in markers of myocardial necrosis level. An excessive sympathetic stimulation may have initiation of apoptosis of myocardial cells as a result. An elevated sympathetic nervous system tone increases the frequency of disorders of heart rhythm and lowers the threshold for occurrence of malignant arrhythmias.

Secondary prevention with beta-blockers reduces cardiac mortality rate in patients with a history of myocardial infarction. A meta-analysis of 12 studies has shown that each 10-beat per minute reduction in heart rate reduce the cardiac mortality rate by 26% on an annual basis [22]. It has been also demonstrated that an excessive neurohumoral stimulation had an unfavorable effect in patients with heart failure. An increased sympathetic activation accelerates the dilatation of the left ventricle, intensifies the remodeling of the ventricle, and aggravates heart failure. A meta-analysis of a larger number of studies with patients with heart failure has shown an unfavorable effect of elevated resting heart rate on an increased patient mortality rate [23]. Guidelines for treating systolic heart failure propose beta-blockers bisoprolol, carvedilol, and metoprolol succinate as chronic treatment. It has been demonstrated that these drugs exhibit a prognostic effect and reduce the mortality of patients with heart failure. It has been found that the risk from cardiovascular mortality in such patients had been decreased in proportion with reduction in resting heart rate [23].

ACE inhibitors, angiotensin receptor blockers, and calcium antagonists in therapy of arterial hypertension have shown a better effect on reduction of cardiovascular risk in a larger number of studies. In studies, the most common comparator from the beta-blocker group was atenolol, a moderately selective beta-blocker. Atenolol, with its shorter elimination half-life, in a single daily dose, could not maintain a consistent antihypertensive effect during the period of 24 hours. As opposed to highly selective beta-blockers and vasodilating beta-blockers, atenolol has shown a weaker effect and has had a lower efficacy in reduction of central arterial pressure in the population of advanced age patients. Because of this, some more recent guidelines from national and international cardiology associations have diminished the role of beta-blockers in initiation of treatment of uncomplicated arterial hypertension. The new American JNC 8 guidelines for the treatment of arterial hypertension emphasize that in making recommendations only the most recent clinical studies have been included [24]. Nowadays, widely used beta-blockers, with expired patents' protection, have not been studied in new megatrials of arterial hypertension treatment, and it is required to limit treatment concerns to earlier comparators from the beta-blocker group, which were studied in the older trials. This is also the case with the current European Society of Cardiology guidelines for the treatment of arterial hypertension from the year 2013. In these recommendations, from the beta-blocker group, vasodilating beta-blockers and highly selective beta-blockers with a better blood pressure regulation profile, better metabolic effects, and more effective reduction of the cardiovascular risk, stand out [25].

THE ADVANTAGES OF A LOW-DOSE THIAZIDE DIURETIC ADMINISTRATION

The use of thiazide diuretics started in the late 1950s. Since then, they have remained one of the most significant groups of drugs for lowering blood pressure due to their effectiveness and low cost. Their primary mechanism of action is the inhibition of Na-Cl cotransporter in the renal distal convoluted tubules and adjacent nephron segments. This inhibition initially leads to a diuretic effect,

which causes a reduction of plasma volume and decreased blood pressure. After the continuation of therapy, a partial restitution of blood volume occurs, and the vasodilating mechanism based on opening the ATP-sensitive potassium channels is responsible for the continuation of antihypertensive action.

The effectiveness of thiazide diuretics in blood pressure regulation is shown in comparison with placebo and other antihypertensive agents. It has been demonstrated in placebo-controlled studies that administration of thiazide diuretics, besides lowering blood pressure levels, had also been associated with a reduction of cardiovascular morbidity and mortality rates. A meta-analysis of these studies has proven that a low dose of a diuretic reduced the overall mortality rate by 10%, the stroke risk by 29%, decreased heart failure risk by 49%, and cardiovascular disease risk by 24% [26]. At the same time, it has been demonstrated that neither group of antihypertensive agents, with which they had been compared (beta-blockers, ACE inhibitors, potassium channel blockers, alpha-blockers, and AT1 receptor blockers), had not been significantly better than thiazide diuretics administered in low doses. Numerous randomized studies have determined that thiazide diuretics, in addition to efficient blood pressure regulation, also reduced the risk from coronary events, strokes, and heart failure in the elderly [27, 28]. The ALLHAT study has shown that a thiazide diuretic was equally efficient as an ACE inhibitor and a calcium channel blocker in coronary and cerebrovascular events prevention, and that it was more efficient than a calcium channel blocker in heart failure prevention, and more efficient than an ACE inhibitor in stroke prevention [29].

There are disagreements concerning the existence of differences in effectiveness of individual thiazide diuretics. In the MRFIT study, patients were treated with chlorthalidone or hydrochlorothiazide (HCTZ), depending on the clinical centers' experiences. After seven years, the Advisory Committee recommended chlorthalidone for use in all patients treated with a thiazide diuretic, based on an unfavorable mortality trend in patients treated with HCTZ in nine centers, in comparison to a favorable trend registered in patients treated with chlorthalidone in six centers [30]. A meta-analysis and a mini-study conducted in one centre, in which effectiveness of administered diuretics was studied during 24-hour ambulatory monitoring, confirmed the advantage of chlorthalidone in comparison with HCTZ [31, 32]. More complete data on the effectiveness of individual thiazide diuretics (bendrofluazide, cyclopenthiazide, indapamide, hydrochlorothiazide, chlorthalidone, metolazone) were obtained in the Cochrane analysis [33]. Sixty double-blind placebo-controlled studies published until February 2014 were included in the stated analysis. The analysis comprised 11,282 patients with an average age of 55 years, out of which 53% were male. The mean blood pressure in the subjects was 158/99 mmHg. A similar effectiveness of studied thiazide diuretics was found. In general, systolic blood pressure was reduced by 9 mmHg, and diastolic by 4 mmHg in comparison with the placebo group. The most information about HCTZ was obtained from 35 randomized studies that included 6,725 patients. HCTZ was administered in daily doses of 6.25 mg, 12.5 mg and 25 mg. The dose of 6.25 mg showed a statistically significant better reduction of blood pressure levels in comparison with the placebo; however, in lowering systolic blood pressure, a clear dose-dependent response was registered. HCTZ dose of 12.5 mg lowered systolic pressure by 2.2 mmHg more than the dose of 6.25 mg, and the dose of 25 mg lowered systolic pressure by 2.7 mmHg more than the dose of 12.5 mg. The dose of 12.5 mg lowered diastolic pressure by 1.1 mmHg more than the dose of 6.25 mg, and the difference in effectiveness was only 1 mmHg for doses of 12.5 and 25 mg. The more efficient dose-dependent reduction of systolic, but not diastolic, blood pressure was also responsible for dose-dependent reduction in pulse pressure, which was up to 4–6 mmHg at maximal doses. Other groups of antihypertensive agents, such as ACE inhibitors and AT1 receptor blockers, lower pulse pressure independently of administered dose by 3 mmHg, and non-selective beta-blockers by 2 mmHg. It is obvious that the reduction in pulse pressure achieved by HCTZ administration in the lowest dose (6.25 mg) was bigger than the one achieved by other antihypertensive agents.

It is evident that effectiveness of thiazide diuretics is dose-dependent; however, undesirable effects of a drug are also dose-dependent. Depending on the dose, they aggravate glucose intolerance, increase lipid levels, cause hypokalemia, hyponatremia, and hypomagnesemia, and increase the levels of uric acid.

A meta-analysis of 22 studies has shown that the use of thiazide diuretics, in comparison with placebo and other antihypertensive agents, was associated with an increased risk of new-onset diabetes mellitus [34]. The mechanism of development of glucose intolerance and reduction in insulin sensitivity is not completely clear. According to the generally accepted assumption, thiazide-induced decreased level of potassium is responsible for the reduction in insulin secretion and the reduction in insulin-mediated glucose uptake in skeletal muscles with consequential reduction in insulin sensitivity, which leads to impaired glucose tolerance and hyperglycemia. This assumption is based on results from smaller studies, and it was reinforced in a post hoc analysis of the SHEP study, in which it was found that the risk of the new-onset diabetes mellitus was increased with each 0.5 mEq/L decrease in K level [35, 36]. Zillich et al. [37] have found a significant negative correlation between the potassium level and the glucose level ($r = -0.28$, 95% confidence interval -0.47 to -0.07, $p < 0.01$). They have concluded that maintaining potassium levels above 4 mEq/L may reduce the risk of hyperglycemia development caused by treatment with thiazide diuretics. Obesity has been reported as a contributing factor to the development of this unfavorable effect of thiazide diuretics, as well as treatment duration of more than nine years, which may contribute to the development of diabetes mellitus [38].

In addition to individual risk assessment for each patient (existing disorder of glucose metabolism, obesity), as a preventive measure for diabetes mellitus development, administration of a thiazide diuretic in a low dose is reported.

A dose-dependent increase in total cholesterol, low-density lipoprotein and triglyceride levels may occur even after short-term administration of thiazide diuretics [39, 40]. These changes are more frequent in patients with diabetes mellitus. The mechanism of development of these changes is not clear, but it is associated with a decrease in insulin sensitivity and/or reflex activation of the sympathetic nervous system and renin–angiotensin–aldosterone system in diuretic-induced volume depletion. This increase is a reversible one – lipid levels return to the range prior to therapy initiation, a year after cessation of diuretics' administration [41]. Considering that a thiazide diuretic in a low dose does not induce a significant change in volume, it is expected that it will not have an impact on disorders of lipid status.

Hypokalemia (potassium level < 3.5 mEq/L) is often an adverse effect of thiazide diuretics [42]. In the first few days of therapy, thiazide diuretics cause the average of 0.6 mEq/L dose-dependent decrease in the potassium level (which is more than the decrease of 0.3 mEq/L caused by administering loop diuretics). An increased sodium intake, a decrease in chloride levels in the distal tubules, metabolic alkalosis, and secondary hyperaldosteronism contribute to the increase in flow-dependent potassium secretion.

Hypokalemia is, in addition to the aforementioned metabolic disorders, responsible for other disorders as well. In the MRFIT study, a significant inverse relationship between potassium concentrations and ventricular extrasystoles was found [30]. The risk of thiazide-induced hypokalemia and rhythm disorders is more significant in patients with existing hypertrophy of myocardium, heart failure, and ischemia; therefore, in such patients, greater caution should be exercised and the dosage of a thiazide diuretic should be lowered.

Hyponatremia is a rare but serious complication of diuretic therapy, with the remark that thiazide diuretics are more likely to cause it than loop diuretics. Possible hyponatremia has very rarely a degree that requires a correction [43]. Metabolic alkalosis is also a very rare complication of administering thiazide diuretics. Its occurrence is also dose-dependent, as are all listed adverse effects.

Thiazide diuretics, as well as loop diuretics, increase excretion of magnesium trough urine, which may lead to a decrease in magnesium levels by 5–10%. Hypomagnesemia is found more often in the elderly and in those on high doses of diuretic therapy, and often coexists with hypokalemia, hyponatremia, and hypocalcemia. Hypomagnesemia is suspected when electrocardiography signs develop (prolonged QT and/or PR interval, a wide QRS complex, ST depression and low amplitude T wave along with supraventricular and ventricular arrhythmias), neurological changes (the change in mental status, and/or neuromuscular irritability such as tetany, tremor, muscle spasms). Parenteral supplementation of magnesium required due to the administration of thiazide diuretics is very rare, but oral supplementation should be considered when any of the listed symptoms occur [43].

A high dose of thiazide diuretics causes the increase in urates concentration by more than 35% due to their clearance reduction. This reduction in clearance may be a consequence of an increased tubular reabsorption caused by diuretic-induced reduction of volume and/or competitive secretion of urates and thiazides since they have the same anion transporter. The occurrence of gout attacks requires temporary therapy cessation, and, if not acceptable, a drug should be administered in the lowest possible dose along with allopurinol [44].

The deleterious effect of thiazide and thiazide-like diuretics on male sexual function is not negligible. According to various studies, decreased libido, erectile dysfunction, and ejaculation problems are seen in 3–32% of patients [30].

In spite of all listed adverse effects of thiazide diuretics, the following Kaplan's [45] statement is undoubtedly acceptable: "... Appropriate use of diuretics can still be a safe and effective way to treat hypertension..." It is necessary to adapt the premise "appropriate use" to our midst, since only HCTZ and indapamide (which we have classified as thiazide-like diuretics) are registered. Having in mind the wide use of HCTZ, it is necessary to define the "low" dose that would be both effective in blood pressure control and have least adverse effects. It has been shown in the aforementioned Cochrane analysis that HCTZ administered in the dose of 6.25 mg achieves an antihypertensive effect. The administration of this very low dose of the thiazide diuretic is indeed acceptable in combination with other antihypertensive drugs, because it potentiates the action of other drugs without causing undesirable metabolic effects.

THE IMPORTANCE OF A FIXED COMBINATION OF BISOPROLOL AND LOW-DOSE HIDROCHLORTHIAZIDE

The effectiveness and safety of a combination of bisoprolol (in various doses) and a thiazide diuretic in a small dose (6.25 mg) has been proven in experimental and clinical trials. It has been demonstrated in an animal model that this combination caused a reduction in blood pressure and heart rate, that it caused a lower renin activation compared to a thiazide diuretic alone, and that it caused a reduction of myocardial hypertrophy [46].

It has been demonstrated on the sample of 106 patients that an eight-week treatment with a combination of bisoprolol and a low-dose thiazide diuretic caused a successful reduction of systolic blood pressure from 157.4 mmHg to 137.3 mmHg, and of diastolic blood pressure from 98.8 mmHg to 87.4 mmHg [47]. The desired therapeutic response was achieved in 61% of the patients – normalization of blood pressure levels, with adverse effects occurring in 18.9%, out of which headache and fatigue were the most common ones. Disorders of glucose and lipid metabolism, potassium and uric acid levels were not recorded.

Even better results of blood pressure control in patients with mild to moderate hypertension have been obtained in a multicentric, randomized, double-blind, placebo-controlled study. A combination of bisoprolol and a thiazide diuretic reduced systolic blood pressure levels by

15.8 mmHg, and diastolic blood pressure by 12.6 mmHg, which was significantly more, compared to bisoprolol or a thiazide diuretic as monotherapy [48]. Normalization of blood pressure levels was achieved in 71% of the patients. A similar result has been obtained in another randomized, double-blind, placebo-controlled study. Using a combination of a low-dose bisoprolol (2.5 mg) and a thiazide diuretic (6.25 mg), a reduction of diastolic blood pressure was achieved in 61% of the patients with safety profile comparable to placebo [49]. In a randomized, double-blind, parallel study, the effectiveness of a combination of bisoprolol and a low-dose thiazide diuretic was compared to enalapril or amlodipine monotherapy [50, 51]. The desired therapeutic response was achieved in 71% of the patients treated with the combination, and in 69% and 45% of the patients treated with amlodipine and enalapril, respectively. Another similar randomized, double-blind, parallel study has shown that a low-dose combination of bisoprolol and a thiazide diuretic and amlodipine were equipotent, but it was more efficient compared to enalapril [52]. The same authors have shown, using efficacy analysis depending on race, that a combination of bisoprolol and a thiazide diuretic in non-black population led to more significant lowering of diastolic pressure compared to amlodipine, enalapril, or placebo [53]. The incidence of adverse effects has been similar in all treatment modalities, but treatment cessation was rarer when using the combination of bisoprolol and a thiazide diuretic.

Papadopoulos and Papademetriou [54] have concluded in their review paper on effectiveness of a fixed low-dose combination of bisoprolol and a thiazide diuretic that the period in which a satisfactory response to therapy can be expected is about four weeks; after this period, in case of failure, a combination with a higher bisoprolol dose should be administered. A combination of bisoprolol and a low-dose of a diuretic showed both effectiveness and safety in treating isolated hypertension in elderly persons [55]. This combination has found its place in treatment of arterial hypertension in children [54].

CONCLUSION

Bisoprolol, a highly selective beta-blocker with long half-life and a prolonged antihypertensive effect, has shown a consistent blood pressure control over a period of 24 hours. The administration of a very low dose of a thiazide diuretic is acceptable in combination with beta-blockers, because it potentiates antihypertensive action without causing undesirable metabolic effects. The effectiveness and safety of a combination of bisoprolol (in various doses) and a thiazide diuretic in a small dose has been proven in clinical trials.

REFERENCES

1. Grassi G, Cifkova R, Laurent S, Narkiewicz K, Redon J, Farsang C, et al. Blood pressure control and cardiovascular risk profile in hypertensive patients from central and eastern European countries: results of the BP-CARE study. Eur Heart J. 2011; 32(2):218–25.
2. Wald DS, LawM, Morris JK, Bestwick JP, Wald NJ. Combination therapy versus monotherapy in reducing blood pressure: meta-analysis on 11,000 participants from 42 trials. Am J Med. 2009; 122(3):290–300.
3. Franklin SS, Pio JR, Wong ND, Larson MG, Leip EP, Vasan RS, et al. Predictors of new-onset diastolic and systolic hypertension: the Framingham Heart Study. Circulation. 2005; 111(9):1121–7.
4. Drukteinis J, Roman MJ, Fabsitz RR, Lee ET, Best LG, Russell M, et al. Cardiac and systemic hemodynamic characteristics of hypertension and prehypertension in adolescents and young adults: the strong heart study. Circulation. 2007; 115(2):221–7.
5. Joyner M, Charkoudian N, Wallin G. Sympathetic nervous system and blood pressure in humans: individualized patterns of regulation and their implications. Hypertension. 2010; 56(1):10–6.
6. Cruickshank J. Are we misunderstanding beta-blockers. Int J Cardiol. 2007; 120(1):10–27.
7. Bühler FR. Age and pathophysiology-oriented antihypertensive response to calcium antagonists. J Cardiovasc Pharmacol. 1988; 12(Suppl 8):S156–62.
8. Tadic M, Ivanovic B, Celic V, Kocabay G. The impact of metabolic syndrome, recently diagnosed diabetes and hypertension on right ventricular remodeling. Is there difference between risk factors? Clin Exp Hypertens. 2014; 36(5):295–301.
9. Hiltunen TP, Suonsyrjä T, Hannila-Handelberg T, Paavonen KJ, Miettinen HE, Strandberg T, et al. Predictors of antihypertensive drug responses: initial data from a placebo-controlled, randomized, cross-over study with four antihypertensive drugs (the GENRES study). Am J Hypertens. 2007; 20(3):311–8.
10. Cruickshank JM. The modern role of beta-blockers in cardiovascular medicine. Shelton: PMPH-USA; 2011.
11. Divac N, Naumović R, Ristić A, Milinković M, Brković V, Jovičić Pavlović S, et al. Patterns of antihypertensive medication use in kidney transplant recipients. Herz. 2017; 42(1):67–74.
12. Vujin B, Kovačević D, Petrović M, Ivanov I, Panić G. Takotsubo cardiomyopathy in pregnancy. Cent Eur J Med. 2014; 9(1):49–53.
13. Neutel JM, Smith DH, Ram CV, Kaplan NM, Papademetriou V, Fagan TC, et al. Application of ambulatory blood pressure monitoring in differentiating between antihypertensive agents. Am J Med. 1993; 94(2):181–7.
14. Arnim T. Medical treatment to reduce total ischemic burden: Total ischemic burden bisoprolol study (TIBBS), a multicenter trial comparing bisoprolol and nifedipine. J Am Coll Cardiol. 1995; 25(1):231–8.
15. Gillman MW, Kannel WB, Belanger A, D'Agostino RB. Influence of heart rate on mortality among persons with hypertension: the Framingham study. Am Heart J. 1993; 125(4):1148–54.
16. Ivanovic B, Dincic D, Tadic M, Simic D. Arterial hypertension in the elderly. Vojnosanit Pregl. 2011; 69(9):779–85.
17. Palatini P, Thijs L, Staessen JA, Fagard RH, Bulpitt CJ, Clement DL, et al. Predictive Value of clinic and ambulatory heart rate for mortality in elderly subjects with systolic hypertension. Arch Intern Med. 2002; 162(20):2313–21.
18. Jouven X, Empana JP, Schwartz PJ, Desnos M, Courbon D. Heart-rate profile during exercise as a predictor of sudden death. N Engl J Med. 2005; 352(19):1951–8.
19. Janssen I, Katzmarzyk PT, Church TS, Blair SN. The Cooper Clinic Mortality Risk Index: clinical score sheet for men. Am J Prev Med. 2005; 29(3):194–203.
20. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension. Eur Heart J. 2013; 34(28):2159–219.
21. Ivanović B, Tadić M, Dinčić D. Heart rate – predictor of cardiovascular risk. Vojnosanit Pregl. 2012; 69(9):799–802.
22. Cucherat M. Quantitative relationship between resting heart rate reduction and magnitude of clinical benefits in post-myocardial infarction: a meta-regression of randomized clinical trials. Eur Heart J. 2007; 28(24):3012–9.
23. Dobre D, Borer JS, Fox K, Swedberg K, Adams KF, Cleland JG, et al. Heart rate as therapeutic target in heart failure. Eur Heart J. 1999; 1:H64–9.

24. James P, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. 2014 Evidence-based guideline for the management of high blood pressure in adults report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA. 2014; 311(5):507–20.
25. Koraćević G, Sakač D, Pavlović M, Ilić D, Tomašević M, Kostić T. Should we prescribe "vasodilating" beta-blockers in Marfan syndrome to prevent aortic aneurysm and dissection? Vojnosanit Pregl. 2012; 69(2):195-200 [DOI: 10.2298/VSP1202195K]
26. Psaty BM, Lumley T, Furberg CD, Schellenbaum G, Pahor M, Alderman MH, et al. Health outcomes associated with various antihypertensive therapies used as first-line agents: a network meta-analysis. JAMA. 2003; 289(19):2534–44.
27. Ivanović B, Tadić M. When does low normal blood pressure become too low? The J-curve Phenomenon. Acta Cardiol. 2014; 69(2):121–9.
28. Aronow WS, Fleg JL, Pepine CJ, Artinian NT, Bakris G, Brown AS, et al. ACCF/AHA 2011 expert consensus document on hypertension in the elderly: A report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents developed in collaboration with the American Academy of Neurology, American Geriatrics Society, American Society for Preventive Cardiology, American Society of Hypertension, American Society of Nephrology, Association of Black Cardiologists, and European Society of Hypertension. J Am Soc Hypertens. 2011; 5(4):259–352.
29. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. Major outcomes in high-risk hypertensive patients randomized to angiotensin converting enzyme inhibitor or calcium channel blocker vs. diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). JAMA. 2002; 288 (23):2981–97.
30. Multiple Risk Factor Intervention Trial Research Group. Mortality after 10½ years for hypertensive participants in the Multiple Risk Factor Intervention Trial. Circulation. 1990; 82(5):1616–28.
31. Roush GC, Holford TR, Guddati AK. Chlorthalidone compared with hydrochlorothiazide in reducing cardiovascular events: systematic review and network meta-analyses. Hypertension. 2012; 59(6):1110–7.
32. Ernst ME, Carter BL, Goerdt CJ, Steffensmeier JJ, Phillips BB, Zimmerman MB, et al. Comparative antihypertensive effects of hydrochlorothiazide and chlorthalidone on ambulatory and office BP. Hypertension. 2006; 47(3):352–8.
33. Musini VM, Nazer M, Bassett K, Wright JM. Blood pressure-lowering efficacy of monotherapy with thiazide diuretics for primary hypertension. Cochrane Database Syst Rev. 2014; (5):CD003824.
34. Grossman E, Verdecchia P, Shamiss A, Angeli F, Reboldi G. Diuretic treatment of hypertension. Diabetes Care. 2011; 34(Suppl 2):S313–9.
35. Shafi T, Appel LJ, Miller ER, Klag MJ, Parekh RS. Changes in serum potassium mediate thiazide-induced diabetes. Hypertension. 2008; 52(6):1022–9.
36. Savage PJ, Pressel SL, Curb JD, Schron EB, Applegate WB, Black HR, et al. SHEP Cooperative Research Group. Influence of long-term, low-dose, diuretic-based, antihypertensive therapy on glucose, lipid, uric acid, and potassium levels in older men and women with isolated systolic hypertension. Arch Intern Med. 1998; 158(7):741–51.
37. Zillich AJ, Garg J, Basu S, Bakris GL, Carter BL. Thiazide diuretics, potassium, and the development of diabetes: a quantitative review. Hypertension. 2006; 48(2):219–24.
38. Mariosa LS, Ribeiro-Filho FF, Batista MC, Hirota AH, Borges RL, Ribeiro AB, et al. Abdominal obesity is associated with potassium depletion and changes in glucose homeostasis during diuretic therapy. J Clin Hypertens. 2008; 10(6):443–9.
39. Mantel-Teeuwisse AK, Kloosterman JM, Maitland-van der Zee AH, Klungel OH, Porsius AJ, de Boer A. Drug-induced lipid changes: a review of the unintended effects of some commonly used drugs on serum lipid levels. Drug Saf. 2001; 24(6):443–56.
40. Lakshman MR, Reda DJ, Materson BJ, Cushman WC, Freis ED. Diuretics and beta-blockers do not have adverse effects at 1 year on plasma lipid and lipoprotein profiles in men with hypertension. Arch Intern Med. 1999; 159(6):551–8.
41. Ernst ME, Carter BL, Zheng S, Grimm Jr RH. Meta-analysis of dose-response characteristics of hydrochlorothiazide and chlorthalidone: effects on systolic blood pressure and potassium. Am J Hypertens. 2010; 23(4):440–6.
42. Clayton JA, Rodgers S, Blakey J, Avery A, Hall IP. Thiazide diuretic prescription and electrolyte abnormalities in primary care. Br J Clin Pharmacol. 2005; 61(1):87–95.
43. Rob PM, Dick K, Bley N, Seyfert T, Brinckmann C, Höllriegel V, et al. Can one really measure magnesium deficiency using the short-term magnesium loading test? J Intern Med. 1999; 246(4):373–8.
44. Gurwitz JH, Kalish SC, Bohn RL, Glynn RJ, Monane M, Mogun H, et al. Thiazide diuretics and the initiation of anti-gout therapy. J Clin Epidemiol. 1997; 50(8):953–9.
45. Kaplan NM. The choice of thiazide diuretics. Why chlorthalidone may replace hydrochlorothiazide. Hypertension. 2009; 54(5):951–3.
46. Mougenot N, Mediani O, Lechat P. Bisoprolol and hydrochlorothiazide effects on cardiovascular remodeling in spontaneously hypertensive rats. Pharmacol Res. 2005; 51(4):359–65.
47. Luna RL, Oigman W, Ramirez JA, Mion D, Batlouni M, da Rocha JC, et al. Efficacy and tolerance of the bisoprolol/hydrochlorothiazide combination in arterial hypertension. Arq Bras Cardiol. 1998; 71(4):601–8.
48. Frishman WH, Bryzinski BS, Coulson LR, DeQuattro VL, Vlachakis ND, Mroczek WJ, et al. A multi- factorial trial design to assess combination therapy in hypertension. Treatment with bisoprolol and hydrochlorothiazide. Arch Intern Med. 1994; 154(13): 1461–8.
49. Frishman WH, Burris JF, Mroczek WJ, Weir MR, Alemayehu D, Simon JS, et al. First-line therapy option with low-dose bisoprolol fumarate and low-dose hydrochlorothiazide in patients with stage I and stage II systemic hypertension. J Clin Pharmacol. 1995; 35(2):182–8.
50. Tadic M, Ivanovic B. Why is functional capacity decreased in hypertensive patients? From mechanisms to clinical studies. J Cardiovasc Med (Hagerstown). 2014; 15(6):447–55.
51. Prisant LM, Weir MR, Papademetriou V, Weber MA, Adegbile IA, Alemayehu D, et al. Low-dose drug combination therapy: an alternative first-line approach to hypertension treatment. Am Heart J. 1995; 130(2):359–66.
52. Papademetriou V, Prisant LM, Neutel JM, Weir MR. Efficacy of low-dose combination of bisoprolol/hydrochlorothiazide compared with amlodipine and enalapril in men and women with essential hypertension. Am J Cardiol. 1998; 81(11):1363–5.
53. Prisant LM, Neutel JM, Ferdinand K, Papademetriou V, DeQuattro V, Hall WD, et al. Low-dose combination therapy as first-line hypertension treatment for blacks and nonblacks. J Natl Med Assoc. 1999; 91(1):40–8.
54. Papadopoulos DP, Papademetriou V. Low-dose fixed combination of bisoprolol/hydrochlorothiazide as first line for hypertension: a review of the rationale and clinical evidence. Angiology. 2009; 60(5):601–7.
55. Benetos A, Consoli S, Safavian A, Dubanchet A, Safar M. Efficacy, safety, and effects on quality of life of bisoprolol/hydrochlorothiazide versus amlodipine in elderly patients with systolic hypertension. Am Heart J. 2000; 140:623–9.

Фиксна комбинација бисопролола и хидрохлортиазида у малој дози у лечењу артеријске хипертензије

Бранислава Ивановић[1], Милан Павловић[2], Арсен Ристић[1], Драган Ковачевић[3]

[1]Клинички центар Србије, Клиника за кардиологију, Београд, Србија;
[2]Клинички центар Ниш, Клиника за кардиоваскуларне болести, Ниш, Србија;
[3]Институт за кардиоваскуларне болести Војводине, Сремска Каменица, Србија

САЖЕТАК

Бета-блокатори имају бољи учинак у смањењу повећаног крвног притиска код млађих болесника са вишим нивоом ренина у плазми. Актуелне препоруке Европског удружења кардиолога за лечење артеријске хипертензије из 2013. године предлажу да се увек уз мерење крвног притиска одређује и срчана фреквенција. Ове препоруке истичу значај срчане фреквенције у мировању, као независног предиктора кардиоваскуларног морбидитета и морталитета болесника са артеријском хипертензијом. Посебан разлог за увођење бета-блокатора у терапију артеријске хипертензије представља постојање придружене коронарне болести, као и систолне срчане инсуфицијенције. Бисопролол је високоселективни бета-блокатор са дугим полуживотом у плазми и продуженим антихипертензивним учинком, који показује сталну контролу крвног притиска у току двадесет четири сата.

У плацебо контролисаним студијама показано је да је примена тиазидних диуретика, поред редукције висине артеријског притиска, повезана са смањењем кардиоваскуларног морбидитета и морталитета. Очито је ефикасност тиазидних диуретика дозно зависна, међутим дозно зависни су и нежељени ефекти лека. Они дозно-зависно погоршавају интолеранцију глукозе, повећавају ниво липида, узрокују хипокалемију, хипонатремију и хипомагнезиемију и повећавају ниво мокраћне киселине. Примена веома ниске дозе тиазидног диуретика је прихватљива у комбинацији са другим антихипертензивима зато што она потенцира деловање других лекова без изазивања нежељених метаболичких ефеката. Ефикасност и сигурност комбинације бисопролола (у различитим дозама) и тиазидног диуретика у малој дози доказани су у клиничким студијама.

Кључне речи: хипертензија, лечење; антихипертензивни лекови, ординирање; бета-блокатори; бисопролол; тиазиди

DOI: https://doi.org/10.2298/SARH170609142P
UDC: 614.25:37

CURRENT TOPIC / АКТУЕЛНА ТЕМА

The application of simulation in medical education – our experiences "from improvisation to simulation"

Aleksandar Pavlović[1], Nevena Kalezić[2,3], Slađana Trpković[1], Nebojša Videnović[1], Ljiljana Šulović[1]

[1]University of Priština temporarily seated in Kosovska Mitrovica, Faculty of Medical Sciences, Kosovska Mitrovica, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Clinical Center of Serbia, Center for Anaesthesiology and Resuscitation, Belgrade, Serbia

SUMMARY
While the use of simulation in medical education has a long history, it has seen its greatest strides in the past 15-odd years. It may be defined as imitation, artificial while at the same time faithful, of various clinical situations through well-crafted medical "scenarios" where, instead of actual people, we use standardized patients: plant, animal, or synthetic models, computerized interactive manikins – simulators, with audiovisuals, as well as medical equipment used in everyday clinical practice. The fundamental goal of using simulation in medical education is an optimal balance between professional education on the one hand and complete safety and protection of patients on the other. Depending on the available finances and the level of advancement of the healthcare and education systems, medical simulation can take various forms – from simple improvisation to the creation of a high-fidelity simulation in centers for medical simulation. Our example shows that, even with modest financial means, enthusiasm, creativity, and good ideas make it possible to establish a center for medical simulation. A separate section of the paper is devoted to the staging of a simulation scenario based on the authors' experiences.
Keywords: education; medical; teaching; manikins

INTRODUCTION

Medical simulation (MS) is artificial and faithful imitation of various clinical situations using well-devised medical "scenarios" featuring, instead of actual patients, either standardized patients (patient actors), plant, animal, or synthetic models for practice, or computerized interactive manikins which include audiovisual equipment, in addition to the medical equipment employed in everyday clinical practice [1, 2]. The purpose of MS is to ensure the optimal proportion of educating healthcare workers on the one hand, and patient safety (including respecting patients' ethical and legal rights) on the other.

HISTORY

Archeological discoveries of sculptures, figurines, and carved models reveal that techniques of simulation had been part of education even in times of antiquity [3, 4]. In ancient China, teaching acupuncture involved life-size bronze human figures filled with liquid, which had wax-covered perforations for the insertion of acupuncture needles [3, 5]. As many as 2,500 years ago, a comprehensive text on surgery titled Sushruta Samhita emerged in India. This volume, considered the precursor to Arabian and European medicine, describes in great detail methods of medical education using plant and animal models [3]. Under the Qing dynasty in China (1644–1912), only men were permitted to enter the medical profession. Prohibited from touching female patients during examinations, they performed examinations indirectly, using miniature models of naked female figures made of ivory [6, 7]. Specialized workshops named La Specola appeared in Italy in the 18th century and made out of wax anatomically accurate models for student education, today preserved in the eponymous museum in Florence [8]. Louis XV, concerned over an increase in the mortality of newborns in rural France, ordered the construction of a childbirth simulator dubbed the Machine for the purpose of better educating midwives and obstetricians. The Machine was shaped like the lower half of a female torso and included uterus and genitalia, fetuses of various ages, and even contained a sponge that released colorless and red fluid (to simulate the amniotic fluid and blood) [9]. The first simulation-executed training programs in medical education were organized in the late 1960s, when researchers at the University of Miami made the Harvey Cardiology Simulator, which could provide realistic simulations of many cardiological conditions. The first realistic manikin, the Sim One simulator, was created in 1966, long before the introduction of computer technology in medicine [10, 11, 12].

MS is not an original method of teaching; rather, it evolved from simulation in other disciplines, where any error could have equally

Received • Примљено: June 9, 2017
Accepted • Прихваћено: July 7, 2017
Online first: July 11, 2017

Correspondence to:
Aleksandar PAVLOVIĆ
Faculty of Medical Sciences
University of Priština
Anri Dinana bb
38220 Kosovska Mitrovica
Serbia
sasaaleksandarpavlovic@gmail.com

disastrous consequences – primarily based on experiences in aviation, where simulation began to be used after World War I [11, 12, 13].

ADVANTAGES OF SIMULATION IN MEDICAL EDUCATION

MS is becoming an increasingly important link in the so-called education chain, where students move from the acquisition of theoretical to that of practical knowledge through the use of simple simulators, going on to learn in high-fidelity simulations and simulation scenarios, and completing their education at the end of the chain with real patients in real circumstances.

The application of simulation in medical education changes the motto of the old, traditional method of learning, "See one, do one, teach one," into the new, more contemporary and successful, "See one, practice many, do one" [1]. Simulation-based learning allows a move from the traditional to the much more integrative approach of using cognitive (perception, thinking, memory, learning), psychomotor (practical, manual skills and techniques, the execution of risky and complex procedures, managing new technology), executive (independent decision-making, building professional opinion), and interpersonal (interview, communication, teamwork) functions [2, 12].

MS erases the compromise inherent in balancing education and patient safety in a real clinical environment [1]. When a simulation is performed, the focus is on teaching and the student, while, in real conditions, the patients – their treatment and protection from medical error – are always in the forefront [1, 11, 14]. In this way, the fundamental medical ethics principle of "first, do no harm" is fully actualized [12].

MS permits the creation of scenarios that can rarely be encountered in typical student exercise classes, like emergencies, life-threatening or rare situations. Also, MS can be used in the design and testing of new clinical equipment [2, 11, 14].

Conversely, when working with actual patients, education time is limited and access to the patient for the purpose of education sporadic [14].

The greatest advantage of simulation is the possibility of repeating procedures that have not been learned to a satisfactory degree for the purpose of education, as the manikins and computer software absorb all mistakes made in the course of learning. This makes it possible to institute a very efficient educational principle – learning from mistakes [11, 14]. In a clinical setting, mistakes must be prevented or corrected immediately to protect the patient. In addition, errors made in simulation may be discussed by the doctor in training and evaluated without constraint, worry, guilt, ethical responsibility, or legal liability. The basic differences between MS and contact with real patients are shown in Table 1.

MS has applications both in undergraduate and postgraduate medical studies, in the continued education of medical professionals, as well as in the acquisition of the knowledge needed to resolve critical situations in emergency situations during natural disasters and armed conflicts [1, 11].

Table 1. Basic differences between medical simulation and learning with actual patients

Medical simulation	Actual contact with patients
Education and students are the priority	Priorities are treatment, patient safety, and respect for the patient's autonomy
Unlimited duration of education	Duration limited by the patient's condition and needs
Practicing skills while learning from mistakes	Limitless repetition and mistakes not permitted
Students actively participate in solving emergency, life-threatening situations	Medical professionals manage emergency situations without active student participation
Open discussion of omissions in debriefing, with no ethical, moral, or legal responsibility attached	Fear of liability and guilt, which are limiting factors for an objective critical review

The integration of simulation methods into educational curricula for healthcare workers at the national level has become standard practice in many developed countries and the day is not far when the formation of simulation centers will be a requirement in accrediting medical education facilities [2, 14]. One of the first obstacles is convincing healthcare institutions' financial sector of the economic justification and usefulness of applying simulation in the education of medical professionals. Examples from practice indicate indirect benefits in the long term in the form of increased patient safety, reduced likelihood of medical error and complications, and hence reduced cost of treatment [1].

However, learning in a simulated environment must on no account be allowed to become a goal unto itself. Simulation cannot replace clinical experience; but it can be a useful model for preparing the doctor for practice [2]. It is not realistic to expect students to exhibit complete mastery in the clinical world after simulation learning, but the training can be an ideal transition stage from theoretical knowledge to the practical experience of working with actual patients. Also, students must not be permitted to acquire a sense of comfort and ease through learning in simulation that may carry over into the real clinical setting, as this may have repercussions on patient safety [2].

TYPES OF SIMULATION AND THEIR BASIC CHARACTERISTICS

Based on the available literature and the authors' personal experience, we have divided all simulation into six basic groups according to fidelity [1, 2, 11, 12, 14]. Each has its advantages and drawbacks, as shown in Table 2.

Over the past 15 years, it has become almost unthinkable to educate medical students and healthcare workers without some of the abovementioned simulation techniques and methods. With regard to space, all high-fidelity simulations can be performed in three ways in virtual real-

Table 2. Types of simulators and simulations – basic characteristics

No.	Simulators by fidelity	Basic characteristics	Example of simulation
1.	Screen-based text simulators; clinical problems are solved using printed or electronically displayed material: presentation of the patient, laboratory test results, treatment charts, vital sign charts, photographs, X-ray and CT scans	• Low-fidelity simulators • Passive approach • Development of cognitive function • Non-realistic approach, no interactivity or teamwork • Low education cost • A large number of students may be educated simultaneously	ECG learning, interpretation of blood gas or laboratory test results
2.	Part task trainers: parts of plants, animal organs, static life-size manikins, models or human body parts	• Low-fidelity simulators • Used for hands-on practice • Unrealistic approach, no interactivity or teamwork • Low education cost • A large number of students may be educated simultaneously	Air management heads, central line placement torsos, cardiopulmonary resuscitation, store-bought items such as pigs' feet or banana peel (suturing)
3.	Screen-based computer simulators; simulation using computerized audiovisual and graphic animations: anatomic animations, monitoring physiological functions, pharmacokinetic and dynamic processes associated with drug administration, effects of applied treatment, watching surgical techniques, images, and animations	• Medium-fidelity simulators • Basic computer equipment and software necessary • Develop cognitive functions • Insufficiently realistic degree of interactivity • Good grasp of computer program use necessary • Independent learning and reception of feedback possible • Low education cost • A large number of students may be educated simultaneously	May be used in all pre-clinical and clinical branches of medicine; e.g.: simulation of general anesthesia, auscultation (heart sounds and murmurs and lung sounds)
4.	Standardized patients (patient actors) are educated and rehearsed actors (medical professionals) who simulate various clinical conditions and diseases and provide guidance toward a diagnosis in direct communication with students through information about patient history and partial physical examination	• Realistic approach • Partly interactive approach • Permits acquisition of cognitive, psychomotor and interpersonal knowledge, development of communication skills • Invasive procedures cannot be performed • Clinical signs cannot be acted out, only symptoms can • Low education cost • Education in small groups of students assembled around one "patient"	Simulated clinical situations
5.	Complex task trainers for performing certain diagnostic or treatment procedures; various physiological parameters may be automatically controlled by a physiological and pharmacological model incorporated within the software or may respond to instructor inventions in response to actions of the trainee	• Medium-fidelity simulators • Realistic approach, simulating various emergency medical conditions and diseases • Develops cognitive, psychomotor, and interpersonal knowledge • Interactive approach • High cost (includes manikins and software that can simulate the interaction between the student and the teacher) • Work in small groups	Ultrasound simulator, videolaryngoscopy simulator, advanced life support cardiopulmonary resuscitation protocol
6.	Integrated simulators with realistic patient simulators and environments; virtual reality trainers offer a transition from the two-dimensional world of the textbook to the three-dimensional world of simulated patients	• High-fidelity simulations • May only be performed in simulation centers or as simulation in situ with appropriate computer equipment, software, audio and visual equipment connected to manikins and medical equipment • Develops cognitive, psychomotor and interpersonal knowledge and teamwork • Multi-professional training possible • Evaluation after completed scenario • Sensitive and expensive equipment that presents difficulties in transportation	Creation of clinical scenarios of various medical conditions and diseases – diagnostics and treatment; example scenarios: anaphylactic shock treatment, tension pneumothorax treatment, treatment of venous air embolism, etc.

ity in medicine: in centers for MS, using in situ simulation, and in remote-facilitated simulations.

A center for MS is a spatial, technical, organizational, and personnel unit operating within medical schools or healthcare institutions. In addition to manikins of varying degrees of fidelity, computer and audiovisual equipment and accompanying software, adequate space is needed too. We shall discuss the establishment and operation of a simulation center further in the text based on our experience.

In situ simulation is a model of mobile training within the working environment of medical professionals, with the equipment they use daily, but employing standardized

patients or manikins. Its advantages, other than the economic factor, are a more humane and relaxed approach for the medical personnel, who are being educated in their own familiar and current clinical surroundings, in the same place where they treat their patients, together with colleagues by whose side they work every day, at the same time testing their own equipment. Simulation sessions can be performed in a similar way in pre-hospital conditions, like ambulances or helicopters [15].

Remote-facilitated simulations entail the existence of a centralized command center for simulation which runs simulations that can be held in distant areas hundreds of kilometers away. This, of course, requires sophisticated equipment, computers, software, audio and video equipment, but also a high-speed internet connection. Computer equipment in the command center is connected to high-fidelity simulators in the simulation unit, while instructors in the command center monitor students' work and communicate with them using cameras and microphones. Remote simulation is a combination of in situ simulation and a simulation center. This centralized approach provides a consistent system of education for a large number of healthcare workers [16].

There are over 20 associations of simulation medicine in Europe and worldwide: the Society in Europe for Simulation Applied to Medicine (SESAM), the Society for Simulation in Healthcare (SSH), the Association of Standardized Patient Educators (ASPE), the International Pediatric Simulation Society (IPSS), and there are also similar organizations in Canada, Australia, Latin America, Russia, Spain, Poland, India, and Japan. In addition, several medical publications in Europe and America publish papers solely on simulation medicine (The Journal of the Society for Simulation in Healthcare, BMJ Simulation & Technology Enhanced Learning, Advances in Simulation, The Internet Journal of Medical Simulation, etc.).

ESTABLISHMENT OF THE FIRST CENTER FOR MEDICAL SIMULATION IN SERBIA: "FROM IMPROVISATION TO SIMULATION"

"A journey of a thousand miles begins with a single step" – Laozi, ancient Chinese philosopher (6th–5th century BC).

The establishment of the Center for Medical Simulation at the Faculty of Medical Sciences in Kosovska Mitrovica had several stages. We named the first the "improvisation stage," due to the lack of adequate equipment and manikins for student education, especially in cardiopulmonary resuscitation, which cannot be learned from reference textbooks alone [17, 18]. Without any MS knowledge, our students assumed the roles of standardized patients and improvised various clinical conditions. After this came the manikin acquisition stage. With modest financial means, we purchased basic manikins for the basic life support measures of cardiopulmonary resuscitation (CPR), airway manikins (endotracheal intubation, placement of laryngeal mask and combitube, oropharyngeal, and nasopharyngeal tube), manikins for the parenteral administering of medication, oxygen therapy, advanced life support CPR measures, including the use of automated external defibrillator (AED) and manual defibrillators, and pediatric manikins. The introduction of mandatory continued medical education into the Serbian healthcare system ushered in the third, medical professional education stage, which included fundraising to establish an economic foundation for founding the Center for Medical Simulation [19–22].

From improvisation to the manikin room, we wanted to take a step further and enter the unknown world of simulation medicine.

The Center for Medical Simulation was established at our school in 2012. It occupies around 100 m^2 in the immediate vicinity of the Dean's Office of the Faculty of Medical Sciences in a space that was previously a disused warehouse. It consists of a room for introductory lectures, which seats 30 and has computer and audio and video equipment, an office for administrative work and preparation by instructors, two manikin rooms, a simulation room, a command room, two water lines, and a storage area for equipment. In addition to preparing participants in simulation scenarios, the introductory lecture room can be turned into a training center for first aid training, where students practice basic life support CPR measures on mats. The earlier mentioned manikins and equipment are set up in the manikin rooms, with an anesthesia machine in a separate area, where students gain basic anesthesiology skills with appropriate preparation of the manikins and equipment. Before entering the simulation room, all students go through training on static manikins, acquiring the manual skills and techniques necessary while discharging simulation scenarios [20, 21, 22]. The central portion of the Center is occupied by the simulation room, separated from the command room by a transparent (glass) partition. The schematic and real representation of the simulation and command rooms is provided in Figures 1 and 2.

The simulation room was turned into an intensive care unit with a manikin in the bed, connected to a vital sign monitor, controlled from the command room using the appropriate software to display a simulation of continuous ECG monitoring, systolic and diastolic blood pressure measurements, heart pulse, pulse oximetry and capnometry. A central venous catheter (attached to equipment measuring central venous pressure) is placed in the manikin, which can be submitted to intravenous cannulation, the administration of infusion solutions and blood transfusions (using imitation blood, a red-dyed fluid). A urinary catheter is also inserted in the manikin and there is a urine collection bag (with imitation urine – a yellow-dyed fluid).

One of our "inventions" is the installation of audio equipment into the torsos of static manikins, which is then connected to software in the command room, allowing students a more realistic approach to studying the auscultation of heart sounds and murmurs and lung sounds (Figure 3) [21, 22].

An "info" monitor in the simulation room briefs students on the parts of the scenario they cannot take in via audio equipment [voice recordings of instructor and/or manikin patient, files of heart sounds and murmurs, or

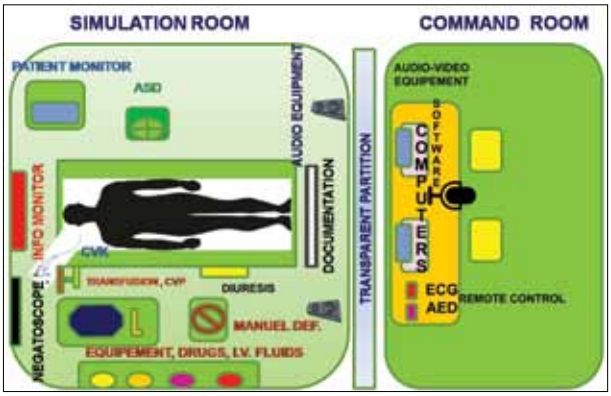


Figure 1. Schematic of the simulation room and the command room in our Simulation Center

Figure 2. Actual view of our Simulation Center and work done in it

Figure 3. Speakers installed in the manikin's torso connected to the computers in the command room to emit heart sounds and murmurs, as well as lung sounds

printed materials (anamnesis, disease history, laboratory test results, therapy chart, and list of vital signs]. There are also a lightbox, manual defibrillator, and AED (remote controlled from the command room) in the simulation room. A separate part of the room is used to keep medication and equipment for airway management, orotracheal aspiration, and thoracic drainage. Depending on the type of scenario, the simulation room can be turned into an emergency room, a pediatric intensive care unit, a or patient room.

The command room has computers (connected through the appropriate software with the vital sign monitor and info monitor), an AED remote control, an ECG simulator, and microphone and speakers for interactive contact with students. The Center for Medical Simulation has a high-speed internet connection and each simulation scenario includes, in addition to the equipment, electronic and printed material deployed as needed. We have downloaded the vital sign monitor software from free download sites online. In cooperation with the Faculty of Technical Sciences, we are in negotiations about creating computer programs tailor-made for our simulation equipment [19, 22].

PLANS FOR THE FUTURE

- Positive experiences to date in the application of this model of education have opened new vistas to us and paved the way toward plans for the future, including the following Acquiring modern manikins, equipment and software;
  - Organizing seminars and conferences on MS;
  - Connecting with other medical schools and healthcare institutions in relation to MS in Serbia and the region;
  - Publishing papers on MS in publications of reference;
  - Connecting with developed MS centers in Europe, instructor education, cooperation with other schools – an interdisciplinary approach;
  - Becoming involved in international projects and foundations;
  - Forming a national Center for Medical Simulation in the Republic of Serbia.

HOW TO MAKE A SIMULATION SCENARIO

A well-thought-out simulation scenario can be compared to a play that can only be devised by someone with vast clinical knowledge and experience in the area of medicine that the scenario treats. The creativity and innovation of the instructor are brought fully to light here.

In simulation scenarios, we used the experiences of other authors who permit free access to a Template for Simulation Patient Design, based on which we designed a template appropriate to our conditions [23].

The selection of each scenario requires defining the pedagogic goals and target trainees, the issues considered in the simulation, designing the simulation model, providing didactic materials, devices, instruments, manikins, equipment, consumable materials, and the video and audio files needed for executing the simulation. Before launching the scenario, the instructor prepares and double-checks the computer and audio equipment and rehearses the prepared simulation scenario. Each scenario entails a detailed preparation of the necessary data, which will show up on info screens and the patient monitor, controlled from the command room, audio and visual information, and heart sound and breathing sound files.

If the simulation involves an actor, the scenario must be well rehearsed to present situations where diagnostic or treatment decisions by trainees are expected as realistically as possible. The instructor's task is to simulate phases of the scenario on the computer through the info and patient screens and audio equipment.

The students get information about the patient from a text presented as disease history which they read prior to the scenario, by reading the vital signs on the patient monitor, obtaining information on vital symptoms in direct contact with the patient (voiced over by the instructor or an audio file), from the info monitor, reading data from a treatment chart, vital sign chart, laboratory test results and available diagnostic tests, and the course of the disease in the patient documentation. Each scenario is divided into "mini scenarios" or "conditions," which permit the scenario to unfold. Moving from one condition to the next is caused by triggers, which include time, the administered medication, or the manual technique performed by the trainee [13, 23].

During the simulation exercise, students are tasked with acquainting themselves with the clinical problem, making the diagnosis and responding in a timely fashion: with manual skills and techniques using the available equipment, administering medication (personally or with the help of a demonstrator, another participant in the simulation), and by responding to questions and interacting with the instructor through audio equipment.

In the course of the simulation, the instructor assesses the trainees in the following: interest and activity during the introductory interactive lectures and discussions; interpretation of vital signs and symptoms, medical history, and other documentation (therapeutic lists, laboratory findings, chest X-ray and ECG; diagnosis and differential diagnosis; emergency treatment (manual skills, administration of therapy; handling equipment, interpersonal and communications skills; teamwork) [13, 23].

Each simulation is recorded with a video camera. The final, vital part of MS is feedback, done through a reconstruction and detailed report of the simulation, with a critical review of the team members' actions, including comments and conclusions on their responses. In an interactive approach, the video and audio recording of the simulation is analyzed and unclear or poorly executed situations are explained. At the end, all participants make an evaluation by filling out a survey form rating the selection of the simulation topic, organization, duration, and pace of the training, manner and methods of working, and the spatial and technical conditions at the Center for Simulation. They also suggest new subjects, teachers, and methods for later training sessions and provide special comments and suggestions [3, 13].

We have designed and rehearsed some 10 scenarios, notably basic life support and advanced life support CPR measures in adults and children, anaphylactic shock treatment, treatment of hemorrhagic shock, venous air embolism, using an Early Warning Score, auscultation (heart sounds and murmurs, lung sounds), tension pneumothorax treatment, etc.

A video presentation of our Center for Medical Simulation is available at the following web address: https://www.youtube.com/watch?v=85K-AwR_ENQ [22].

CONCLUSION

The use of simulation in medical education has a long history. Over the past 15 years, it has become almost unthinkable to provide education to students and medical professionals without using some form of simulation. The idea of forming a simulation center is associated in the minds of many with costly, sophisticated equipment, which is hence unattainable when finances are scant. Our example proves that, even with modest financial means, when one has enthusiasm, creativity and good ideas, satisfactory results in simulation medicine are possible, allowing higher quality education for medical professionals on the one hand and the protection of patients on the other. We are aware that our results are modest and cannot compare to contemporary European and international MS centers. Yet we have taken the first steps and are trying to share them with you in this article.

REFERENCES

1. Rashmi D, Upadhyay KK, Jaideep CN. Simulation and its role in medical education. Med J Armed Forces India. 2012; 68(2):167–72.
2. Ker J, Bradley P. Simulation in Medical Education. In: Swanwick T, editor. Understanding Medical Education: Evidence, Theory and Practice. Hoboken, New Jersey: Wiley-Blackwell, 2010. p. 164–80.
3. Owen H. Early use of simulation in medical education. Simulation in healthcare: Journal of the Society for Simulation in Healthcare. 2012; 7(2):102–16.
4. Morriss-Kay GM. The evolution of human artistic creativity. J Anat. 2010; 216(2):158–76.
5. Schnorrenberger CC. Anatomical roots of Chinese medicine and acupuncture. J Chin Med. 2008; 19:35–63.
6. Russell KF. Ivory anatomical manikins. Med Hist. 1972; 16(2):131–42.
7. Bause S. Antique Chinese diagnostic dolls. Anesthesiology. 2010; 112(3):513.
8. De Ceglia FP. The rotten, the disembowelled woman, the skinned man: Body images from Eighteenth century Florentine wax modelling. JCOM. 2005; 4:1–7.
9. Carty E. Educating midwives with the world's first simulator: Madame du Coudray's eighteenth century mannequin. Can J Midwifery Res Pract. 2010; 9:35–45.
10. Cooper JB, Taqueti VR. A brief history of the development of mannequin simulators for clinical education and training. Qual Saf Health Care. 2004; 13 (Suppl 1):11–8.
11. Bradley P. The history of simulation in medical education and possible future direction. Med Educ. 2006; 40(3):254–62.
12. Jones F, Passos-Neto CE, Braghiroli OFM. Simulation in medical education: Brief history and methodology. PPCR. 2015; 1(2):56–63.
13. Rosen KR. The history of medical simulation. J Crit Care. 2008; 23(2):157–66.
14. Weller MJ, Nestel D, Marshall DS, Brooks MP, Conn JJ. Simulation in clinical teaching and learning. Med J Aust. 2012; 196(9):594.

15. Hssain I, Alinier G, Souaiby N. In-situ simulation: A different approach to patient safety through immersive training. Mediterranean Journal of Emergency Medicine. 2013; 15:17–28.
16. Ikeyama T, Shimizu N, Ohta K. Low-cost and ready-to-go remote-facilitated simulation-based learning. Simul Healthc. 2012; 7(1):35–9.
17. Pavlović PA. Kardiopulmonalno cerebralna reanimacija. 3rd ed. Beograd: Obeležja; 2011.
18. Pavlović PA, Trpković VS, Anđelić LJS, Marinković MO. Kardiopulmonalna reanimacija – nove preporuke 2015–2020. Naučni časopis urgentne medicine HALO 194. 2015; 21(3):181–98.
19. Pavlović PA, Trpković VS, Videnović DN, Šulović SL. Primena simulacije u mediicnskoj edukaciji. SEE Journal of Emergency Medicine [Internet]. 2015; 1(1):22–9. [Cited 2016 May 29]; Available from: http://www.seejournal.rs/pdf_see_izdanja/SSEJournal_2015_1.pdf
20. Pavlovic A, Trpkovic S, Videnovic N. Development of simulation medicine in Serbia. Proceedings of the 1st Belgrade Anaesthesia Forum; 2016 Apr 1–2; Belgrade, Serbia. Lajkovac: La-pressing; 2016. p. 25–39.
21. Pavlovic A, Trpkovic S, Kalezic N. Edukacija zdravstvenih radnika u zbrinjavanju urgentnih stanja u medicini - simulaciona medicina. In: Kalezic N, ed. Inicijalni tretman urgentnih stanja u medicini. Beograd: Medicinski fakultet Beograd: 2016. p. 939–67.
22. Pavlovic A. From improvisation to simulation [Internet]. 2015 Jun 18. Kampus Medicinski Fakultet Pristina - Kosovska Mitrovica. [Cited 2016 May 29]; Available from: https://www.youtube.com/watch?v=85K-AwR_ENQ
23. Jeffrey M, Taekman MD. (Human Simulation and Patient Safety Center, Duke University Medical Center). Template for Simulation Patient Design. Available from: http://simcenter.duke.edu/support.html

Примена симулација у медицинској едукацији – наша искуства „од импровизације до симулације“

Александар Павловић[1], Невена Калезић[2,3], Слађана Трпковић[1], Небојша Виденовић[1], Љиљана Шуловић[1]

[1]Универзитет у Приштини са привременим седиштем у Косовској Митровици, Медицински факултет, Косовска Митровица, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Клинички центар Србије, Центар за анестезиологију и реаниматологију, Београд, Србија

САЖЕТАК

Примена симулације у медицинској едукацији има дугу историју, али највећи напредак доживљава последњих петнаестак година. Она се може дефинисати као верна имитација различитих клиничких ситуација преко добро направљених медицинских „сценарија“ где се уместо реалних пацијената користе „стандардизовани пацијенти“, биљни, животињски или синтетички модели, компјутеризоване интерактивне лутке – симулатори, уз употребу аудио и видео опреме, као и медицинске опреме која се користи у свакодневној клиничкој пракси. Основни циљ коришћења симулације у едукацији је да се постигне оптималан баланс између стручне едукације, с једне стране, и заштите пацијената, с друге стране. У зависности од финансијских могућности, као и развијености здравственог и образовног система, медицинска симулација се може изводити на разне начине – од једноставне импровизације до стварања високо „верне“ симулације у центрима за медицинску симулацију. Наш пример показује да је и у условима скромних финансијских средстава, уз ентузијазам, креативност и добре идеје, могуће формирати центар за медицинску симулацију. Посебно је приказан симулациони сценарио на основу досадашњих искустава аутора.

Кључне речи: медицинска едукација; учење; лутке за едукацију

DOI: https://doi.org/10.2298/SARH170704168P
UDC: 61:929 Инглис Е.; 355.415.6(497.11)"1914/1915"

ИСТОРИЈА МЕДИЦИНЕ / HISTORY OF MEDICINE

Елси Инглис (1864–1917) и Болнице шкотских жена у Србији у Великом рату – 2. део

Славица Поповић-Филиповић

Секција за историју медицине Српског лекарског друштва, Београд, Србија

САЖЕТАК

Вест о великим победама херојске а мале Србије у Првом светском рату проширила се надалеко. Праћена апелом српских посланстава и српског Црвеног крста, помоћ је пристизала са свих страна. Прве медицинске мисије и санитетска и друга помоћ дошле су из Русије. Следиле су медицинске мисије из Велике Британије, Француске, Грчке, Холандије, Данске, Швајцарске, Америке... Материјална помоћ и појединци стигли су из Пољске, Канаде, Аустралије, Новог Зеланда, Ирске, Норвешке, Индије, Јапана, Египта, Јужне Америке и других земаља. Искрени пријатељи српског народа су формирали разне фондове под окриљем Црвеног крста и других удружења. Септембра 1914. формиран је Српски потпорни фонд у Лондону, а новембра исте године у Шкотској је основана прва јединица Болнице шкотских жена за службу у иностранству.

Циљ овог рада је био да вратимо сећање на Болнице шкотских жена у Србији и са Србима у току Првог светског рата. Оне у историји српског народа заузимају посебно место. Настале су иницијативом др Елси Мод Инглис (1864–1917), лекарке, хирурга, борца за женска права, а подршком Шкотске федерације сифражетских друштава. Искључиво у саставу жена, Болнице шкотских жена су својим учешћем у рату великим делом допринеле родној и професионалној равноправности, посебно у области медицине. Многи ставови садашњице произишли су захваљујући генерацији првих лекарки, које су се изборила за равноправност при избору и обављању професије лекара, како у миру тако и у рату.

Кључне речи: Први светски рат; Болнице шкотских жена; Шкотска; Србија; Инглис Е.

ПЕТА ЈЕДИНИЦА БОЛНИЦЕ ШКОТСКИХ ЖЕНА – ЈЕДИНИЦА „ГИРТОН И ЊУНАМ“, ФРАНЦУСКА, ЂЕВЂЕЛИЈА, СОЛУН

Пета јединица Болнице шкотских жена, Јединица „Гиртон и Њунам“, радила је у Француској (*Troyes*) од оснивања, маја 1915. Одлуком француског војног санитета, новембра 1915, пресељена је у Ђевђелију, а доласком окупације пребачена је на Солунски фронт (Слика 1). Јединица је била под руководством др Ен Луизе Макилрој (*Dr. Anne Louise McIlroy*, 1874–1968), која је остала на челу до краја деловања болнице, септембра 1919.

Слика 1. Болнице шкотских жена, Јединица „Гиртон и Њунам“ на Солунском фронту
Figure 1. Scottish Women's Hospitals, Girton and Newnham Unit, the Salonika Front

Received • Примљено: July 4, 2017
Revised • Ревизија: August 25, 2017
Accepted • Прихваћено: August 31, 2017
Online first: September 5, 2017

Correspondence to:
Slavica POPOVIĆ-FILIPOVIĆ
Ul. Koste Jovanovića 69/2
11040 Belgrade, Serbia
bobfilip@yahoo.com

Слика 2. Др Ен Луиза Макилрој, руководилац Јединице „Гиртон и Њунам", Болнице шкотских жена, Солунски фронт
Figure 2. Dr. Anne Louise McIlroy, CMO of the Girton and Newnham Unit, the Scottish Women's Hospitals, the Salonika Front

Слика 3. Болнице шкотских жена, Јединица „Корзика", Ајачо, Корзика
Figure 3. The Scottish Women's Hospitals, Corsica Unit, Ajaccio, Corsica

Лекарску екипу су чиниле др Луиза Макилрој (*Dr. Anne Louise McIlroy*), др Изабел Емсли (*Dr. Isabel Emslie*), др Онориа Кир (*Honoria Somerville Keer*), др Мабел Харди (*Dr. Mabel Hardie*), др Елиза Грејг (*Dr. Eliza Stephenson Greig*), др Џин Патрон Гордон (*Dr. Jean Patron Gordon*), др Рут Иден Конвеј (*Dr. Ruth Eden Conway*), др Каролин Лоу (*Dr. Caroline Victoria Lowe*), др Л. Доротеа Тејлор (*Dr. L. Dorothea Taylor*), др Гледис Ворд (*Dr. Gladys Ward*), др Мери Макнил (*Dr. Mary Lauchline McNeill*), др Етел Прајс (*Dr. Ethel Jane Mildred Pryce*), др Мери Александер (*Dr. Mary Alexander*) и др Барбара Макгрегор (*Dr. Barbara McGregor*). Јединица је имала зубну станицу, у којој су радиле зубарке Кејт Латарч (*Kate Latarche*) и Мери Џејн Рипли (*Mary Jane Ripley*). Поред хирургије, болница је примала оболеле од маларије и других инфективних обољења.

Др Луиза Макилрој, Иркиња, била је прва жена лекар са дипломом медицине и патологије у Глазгову. После рата постала је прва жена професор на Медицинском факултету у Лондону. Др Макилрој је, поред бројних српских и британских одликовања, била проглашена за даму Велике Британије (Слика 2).

У јединици је била радиолог госпођица Едит Стони (*Miss Edith Anne Stoney*, 1869–1938), ирског порекла, која је дипломирала математику на Кембрицу и Тринити колецу. У то време радиологија је била ретка у медицинској пракси. Зато је врло значајно што је радиолог Стони формирала рендгенску службу у неколико јединица Болница шкотских жена.

Јединица „Гиртон и Њунам" у Солуну била је у саставу француског санитета, са наменом појачања српског војног санитета на Солунском фронту. Само у периоду јул–август 1916. болница је примила 1000 пацијената, а најчешће са дијагнозом маларије и дизентерије. Након Горничевске и Кајмакчаланске битке, велику улогу у хируршком збрињавању имале су јединице Болница шкотских жена у Солуну и Острову. Јединица „Гиртон и Њунам" се бавила рехабилитацијом и ортопедском протетиком бројних инвалида. Постаће позната по оснивању Ортопедског центра „Калкута" (*Calcutta Orthopaedic Centre*), за лечење великог броја српских рањеника и инвалида. По ослобођењу, др Макилрој је организовала ортопедски центар у Београду, испод Авале.

ШЕСТА ЈЕДИНИЦА БОЛНИЦЕ ШКОТСКИХ ЖЕНА – ЈЕДИНИЦА „КОРЗИКА", НА КОРЗИЦИ

Шеста јединица Болнице шкотских жена, Јединица „Корзика" (*the Corsica Unit*), организовала је лечење и рехабилитацију српских војника и српске избегле колоније на Корзици. Главна болница је била смештена у једној вили, у Ајачију, изолована болница у Лазарету, док су амбуланте и диспанзери били у селима (Слика 3).

Јединица „Корзика" (*Serbian Refugee Hospital, Corsica*) имала је, поред Српског потпорног фонда, и материјалну помоћ и подршку града Манчестера и Уније уједињених синдиката. Истовремено, француска влада је подржала живот српске колоније, не само на Корзици већ и широм Француске. Јединица „Корзика" предвођена са др Мери Блер имала је тежак задатак да „излечи и поврати у нормалан живот део изнемогле српске нације... Било је то поновно стварање нације сломљене и расуте по свету – опоравити и вратити у живот људе, жене, децу која умиру"[1, 2].

Јединица је започела своје деловање на челу са др Мери Алис Блер (*Dr. Mary Alice Blair*, 1880–1962), лекарком са Новог Зеланда. Руководство су наставиле др Мери Елизабет Филипс (*Dr. Mary Elizabeth Phillips*), Велшанка, потом др Елизабет Кортланд (*Dr. Elizabeth Courtland*), завршила медицину у Брислу, др Матилда Макфејл (*Dr. Alexandrina Matilda MacPhail*), Шкотланђанка, др Една Гест (*Dr. Edna Mary Guest*), Канађанка и др Онориа Кир (*Dr. Honoria Somerville Keer*), Канађанка. Лекарску екипу су чиниле др Кетрин Ан-

Слика 4. Болнице шкотских жена, Јединица „Америка", у Острову, на Солунском фронту
Figure 4. The Scottish Women's Hospitals, America Unit, Ostrovo, Salonika Front

Слика 5. Др Агнес Бенет (1872–1960) године 1916.
Figure 5. Dr. Agnes Bennett (1872–1960), in 1916

дерсон (*Dr. Catherine Emslie Anderson*), др Мери Фергусон (*Dr. Mary Mitchelina Grant Ferguson*), др Едит Холвеј (*Dr. Edith Blake Hollway*), др Софи Џексон (*Dr. Sophie Bangham Jackson*) и др Хелена Џонс (*Dr. Helena G. Jones*).

Болница шкотских жена испловила је из Солунског залива 25. децембра 1915, а радила је до априла 1919. У време свог деловања, Јединица „Корзика" је примила 1704 болничких пацијената и 15.515 амбулантних пацијената. Др Елси Инглис, оснивач и руководилац Болнице шкотских жена, посетила је болницу на Корзици априла 1916. Она је била много задовољна радом и топлом атмосфером која је владала међу члановима ове велике заједнице, придошле са различитих страна света.

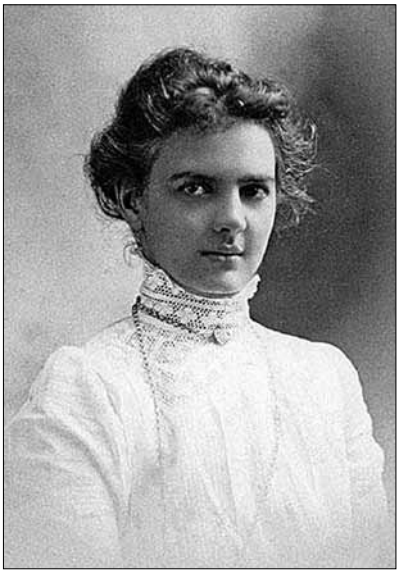
Др Мери Филипс је руководила Јединицом „Корзика" у Ајачију на Корзици, од 1. маја 1916. до 4. јануара 1917. У време када је др Филипс била руководилац болнице формирано је мушко, женско одељење и породилиште, где је рођено 79 беба. Популарност др Филипс потврђује и испраћај, када су у њену част младићи из Лазарета приказали српску верзију Молијера. Одласком није престала њена наклоност према Србима. Др Филипс је, по ослобођењу, покренула обуку српских девојака за медицинске сестре, која је постигла лепе резултате.

СЕДМА ЈЕДИНИЦА БОЛНИЦЕ ШКОТСКИХ ЖЕНА – ЈЕДИНИЦА „АМЕРИКА" У ОСТРОВУ, НА СОЛУНСКОМ ФРОНТУ

Седма јединица Болнице шкотских жена у Острову, на Солунском фронту (Слика 4), деловала је под управом др Агнес Бенет (*Dr. Agnes Bennett*, 1872–1960), аустралијске лекарке, која је дошла са Новог Зеланда. Болница је пратила деловање Треће српске армије и била је позната као Трећа пољска хируршка болница. Била је појачана Српским хируршким завојиштем, под руководством др Чедомира Ђурђевића (1866–1940), хирурга, који је завршио медицину у Бечу. Лекарски помоћник био је медицинар Урош Ружичић (1891–1966), који је поред болничких дужности имао улогу преводиоца. У болници је било око 60 српских болничара и помоћних радника.

У комплетно опремљеној пољској болници на обали језера Острово радиле су лекарке, медицинске сестре и болничарке, највећим делом из Аустралије и са Новог Зеланда. Болница је настала залагањем материјалне помоћи бројних америчких филантропа, па отуда и назив Јединица „Америка" (*the America Unit*). У оснивању ове болнице велики допринос дала је Кетлин Бурк, промотер Болнице шкотских жена у Америци и Канади.

Поред др Агнес Бенет (Слика 5) [3], лекарску екипу чиниле су: др Лилијан Купер (Dr. Lilian Violet Cooper), прва жена хирург у држави Квинсленд (Аустралија), Мери Џозефина Бедфорд (*Miss Mary Josephine Bedford*), филантроп из Бризбејна, др Џеси Скот (*Dr. Jessie Ann Scott*), са Новог Зеланда, чланица Болнице шкотских жена у Крагујевцу и Крушевцу. Фебруара 1917. придружила им се још једна лекарка из Аустралије, др Мери де Гарис (*Dr. Mary Clementina De Garis*) (Слика 6), из Викторије. У лекарској екипи су биле три Шкотланђанке: др Сибил Луис (Dr. Sybil Lonie Lewis), лекарка Болнице шкотских жена у Ваљеву, др Ана Лејла Мункастер (*Dr. Anna Leila Muncaster*), лекарка у јединици Српског потпорног фонда у Крагујевцу и др Изабел Емсли (*Dr. Isabel Emslie*) (Слика 7), која је пребацила болницу у ослобођену Србију. У екипи су такође биле др Мери Гордон (*Dr. Mary Louisa Gordon*), др Грејс Паилторп (*Dr. Grace Winifred Pailthorpe*), др Хелен Лили (*Dr. Hellen Lillie*), др Џоан Кенеди Роуз (*Dr. Joan Kennedy Rose*), др Мира Макензи (Dr. Myra



Слика 6. Др Мери Клементина де Гарис (1881–1963)
Figure 6. Dr. Mary Clementina De Garis (1881–1963)

Слика 7. Др Изабел Емсли, руководилац Болница шкотских жена у Врању
Figure 7. Dr. Isabel Emslie, CMO of the Scottish Women's Hospitals in Vranje

Слика 8. Др Елси Мод Инглис (1864–1917) године 1916.
Figure 8. Dr. Elsie Maud Inglis (1864–1917), in 1916

Mackenzie), др Мабел Блејк (*Dr. Mabel Nellie Blake*) и др Мери Фергусон (*Dr. Mary Mitchelina Grant Ferguson*).

Особље од 60 чланица чиниле су пет жена хирурга, 20 обучених медицинских сестара, 10 болничарки, главна медицинска сестра Мод Тејт (*Miss Maud Ellen Tate*), администратор Флоренс Џек (*Miss Florence Jack*). Болница шкотских жена у Острову почела је да ради 13. августа 1916. По ослобођењу пресељена је у Врање, где је радила годину дана. Болница у Острову је била праћена Транспортном јединицом, која се посебно истакла у време Горничевске и Кајмакчаланске битке и у пробоју Солунског фронта. Руководиоци Транспортне јединице су биле Кетрин Харли (*Mrs. Katherine Harley*), Мери Бедфорд (*Miss Mary Bedford*) и Кетлин Дилон (*Kathleen Dillon*).

Др Агнес Бенет сведочи: „Нема дилеме да су то Срби извели, просто невероватно... Како су они освојили Кајмакчалан право је чудо, пењући се све време уз те литице... без сумње, Срби су постигли диван напредак под командом генерала Васића... Све док има рањених на брдима наша кола ће одлазити по њих, ако је то људски могуће постићи".

Болница шкотских жена у Острову „била је једна од ретких болница која је имала 'привилегију' и 'војничку част' да буде на првој линији фронта... Сазнање да смо дошле овде добровољно, да им помогнемо, а нас није послало никакво војно министарство по наређењу донетом на некој конференцији, мења потпуно ситуацију", рекла је др Бенет [4].

Педесет две чланице Јединице „Америка" Болнице шкотских жена у Острову, дванаест лекара и четрдесет медицинских сестара и болничарки одликовано је Орденом српског Црвеног крста. Орден Светог Саве добиле су др Агнес Бенет, др Лилијан Купер, др Мери де Гарис, др Изабел Емсли, др Џеси Скот, др Сибил Луис, др Лилиан Мункастер и Мери Џозефин Бедфорд.

ОСМА ЈЕДИНИЦА БОЛНИЦЕ ШКОТСКИХ ЖЕНА – ЈЕДИНИЦА „ЛОНДОН", НА РУСКОМ ФРОНТУ И ДОБРУЏИ

Болница шкотских жена, Јединица „Лондон" (*London Unit*), под руководством др Елси Инглис, била је у пратњи Прве српске добровољачке дивизије, која је формирана у Русији. Др Елси Инглис (Слика 8) са 75 чланица Болице шкотских жена стигла је у Одесу августа 1916. Болница је имала пратњу Транспортне јединице, са 16 аутомобила за евакуацију рањеника. Руководилац Транспортне јединице била је баронеса Евелина Хаверфилд. Лекарску екипу су чиниле др Елси Инглис, руководилац и хирург, др Лилијан Мери Чесни (*Dr. Lilian Mary Chesney*), хирург, др Кетрин Корбет (*Catherine Louisa Corbett*), хирург, др Лиза Мери Потер (*Lisa Mary Potter*), др Џенет Лерд (*Dr. Janet Stewart Laird*) и др Гледис Ворд (Dr. Gladys Ward), медицинарке Агнес Марфи (*Agnes M. Murphy*) и Елинор Рендал (*Elinor Frances Rendel*). Главне медицинске сестре биле су Марион Фокс (*Marion Fox*) и Мери Визард (*Mary Vizard*).

Санитет при Првој српској добровољачкој дивизији био је организован у два лазарета. Руководилац првог лазарета био је др Драгослав Поповић, док је други лазарет предводио др Владимир Станојевић. У јесен 1916. године у Добруџи се већ борила Прва српска добровољачка дивизија, заједно са руским и румунским дивизијама. У борбама на фронтовима у Трансилванији и Добруџи, Српска дивизија је имала велике губитке, са још већим бројем рањеника. Болница шкотских жена је заједно са српским санитетом пратила страховите борбе на Руском фронту и у Румунији са великим бројем рањеника. Деловала је у Русији и Румунији од августа 1916. до новембра 1917, када се повукла из Меџидије [5].

Др Инглис је писала са фронта: „Руска револуција, која се догодила 1917, угрозила је безбедност Прве српске дивизије на Румунском фронту и рад шкотских жена...“ Уследила је наредба британске владе да се комплетно особље врати у домовину, али др Инглис је одбила речима да ће се вратити само са српском војском, али никако без ње. [6].

Др Инглис, болесна и исцрпљена, вратила се са Руског фронта у Шкотску, а војници Прве српске добровољачке дивизије напустили су Русију, да би се после дугог путовања придружили српској војсци на Солунском фронту. Мада с крајњим напором, др Инглис је извршила смотру српских војника у Глазгову, потом се повукла. Преминула је у Глазгову, 26. новембра 1917. Уз химне савезничких земаља, последњу пошту одали су јој британски, француски и српски војници. Др Елси Инглис је сахрањена у Катедрали Светог Џајлса, у Единбургу.

ДЕВЕТА ЈЕДИНИЦА БОЛНИЦЕ ШКОТСКИХ ЖЕНА – ЈЕДИНИЦА „ДР ЕЛСИ ИНГЛИС“, НА СОЛУНСКОМ ФРОНТУ, У ДРАГОМАНЦИМА

Девета јединица Болнице шкотских жена, Јединица „Др Елси Инглис“ (*Dr. Elsie Inglis Unit*) формирана је у знак сећања на др Елси Инглис, оснивача и лидера Болница шкотских жена. Оснивање јединице је уследило после повратка са Руског фронта, па је тако велики број чланица Лондонске јединице ушао у састав новоформиране болнице. Ова јединица је била под покровитељством Војног министарства и имала је част да пре поласка има смотру у присуству краља и краљице.

Јединица „Др Елси Инглис“, пољска болница на Солунском фронту, била је постављена на једној падини између Драгоманца и Вертекопа, у непосредној близини железничке пруге. Имала је капацитет од 100 постеља, а особље од 25 чланица. Болница, праћена Транспортном јединицом, на челу са Џералдин Хеџиз (*Miss Geraldine Hedges*) и Франсис Е. Робинсон (*Francis E. Robinson*), имала је 32 возила, од тога 25 кола. Од почетка априла 1918. болница је имала дужност првог завојишта на самом фронту. Пацијенти су били Срби, Французи, Италијани, Грци, Руси и други.

Јединица је формирана као појачање Болници шкотских жена у Острову. Септембра 1918. болница је, под руководством др Лилијан Чесни (*Dr. Lilian Chesney*), пребачена у Доњи Пожар, а октобра у Скопље. Болница је своје деловање завршила је у Сарајеву, где је радила до априла 1919.

Лекарску екипу су чиниле др Анет Матилда Бенсон (*Dr. Annette Matilda Benson*), др Лилијан Чесни (*Dr. Lillian Chesney*), др Гледис Ворд (*Dr. Gladys Ward*), др Елинор Рендел (*Dr. Elinor F. Rendel*) и медицинар Агнес М. Марфи (*Agnes M. Murphy*).

Др Анет Бенсон (*Dr. Annette Benson, M.D.*), хирург са дугогодишњим искуством из Индије, постављена је за руководиоца Јединице „Др Елси Инглис“. Др Бенсон је била међу првим женама које су стекле звање лекарке на Медицинском факултету за жене при Краљевској болници, у Лондону. Носилац је Ордена Светог Саве и других високих одликовања.

ДЕСЕТА ЈЕДИНИЦА БОЛНИЦЕ ШКОТСКИХ ЖЕНА У САЛАНШЕУ У ФРАНЦУСКОЈ

Десета јединица Болнице шкотских жена била је Меморијална болница (*Tuberculosis Sanatorium for Serbs at Sallanches, Haute Savoie*) са санаторијумом, намењена српским студентима и избеглицама оболелим од туберкулозе. Болница је била лоцирана у Француској, у Саланшеу (*Sallanches*), испод Монт Бланка, у близини швајцарске границе.

Руководиоци болнице су биле др Матилда Макфејл (*Dr. Matilda Alexandrina Macphail*, 1860–1946) и др Мариан Булок (*Dr. Marian Theresa Bullock*, 1877–1956). Лекарску екипу су чиниле др Матилда Макфејл као управница болнице, др Маријан Тереза Булок (*Dr. Marian Theresa Bullock*) и др Елизабет Нил (*Dr. Elisabeth Niel*). Главне медицинске сестре су биле Етел Мод (*Miss Ethel Maude*) и Мери Доналдсон (*Miss Mary Steuart Donaldson*), апотекарица Ела Моли Ертон (*Miss Ella Molloy Ayrton*) и домаћица, госпођа Мери Милн (*Mrs. Mary Milne*).

Болница шкотских жена у Саланшеу је радила од марта 1918. до јуна 1919. Све време болница је деловала као санаторијум, углавном намењена рековалесцентима. Због све веће потребе, капацитет болнице се повећао, од 100 на 150 постеља.

Др Матилда Макфејл, Шкотланђанка, студирала је медицину на Лондонском медицинском факултету за жене, где је дипломирала 1887. После студија, др Макфејл је преузела службу лекарке у медицинској мисији у Индији, где је основала диспанзер за сиромашне жене и децу у Мадрасу. Пре доласка у Саланше, др Макфејл је била руководилац Јединице „Корзика“, на Корзици.

ЕПИЛОГ

Из обимне литературе посвећене Болницама шкотских жена, завршавамо речима др Инглис: „Видите, ми се налазимо у врло тужној земљи, али то је најодважнија земља у Европи, без изузетка. Овде њихови најбољи људи страдају на све стране, али никад се не жале и никад не помисле ни за тренутак да се предају, иако Аустријанци још увек могу да нагрну. Аустрија их је страшно лоше третирала, али Срби нису огорчени на њих. Срби се односе према непријатељским затвореницима на потпуно исти начин као према својима, мада немају много шта да им понуде. Србија је јако поносна земља па отуда не воли да је други народи жале. Она је дивна мала земља, и ми смо је већ заволели, али је скроз опустошена са три последња рата. Тако ми имамо војнике у нашој болници који су скоро

само дечаци, али који поносно показују своје ожиљке из три рата" [6].

Др Елси Инглис је носилац Ордена Светог Саве и прва жена којој је уручен Орден Белог орла. Меморијална чесма у Младеновцу је најлепши споменик посвећен др Елси Инглис и Болници шкотских жена, јединственој медицинској мисији у историји медицине и историји Великог рата.

После Првог светског рата комитети Болница шкотских жена помогли су изградњу Меморијалне болнице за мајку и дете „Др Елси Инглис" на Дедињу, у Београду. На Физиолошком институту Медицинског факултета у Београду стоји спомен-плоча у част др Елси Инглис. У обележавању стогодишњице од Првог светског рата, а поводом Међународног дана жена, Британска резиденција у Београду именовала је Дом „Елси Инглис" по др Елси Инглис, лекарки, борцу за друштвена и политичка права жена. Меморијална болница за мајку и дете „Др Елси Инглис" налази се у Единбургу. Биста др Елси Инглис, поклон српског народа, а дело Ивана Мештровића, изложена је у Националној галерији Шкотске, у Единбургу.

Меморијална чесма у Младеновцу, спомен-плоче у Лазаревцу и Крушевцу, гроб Евелине Хаверфилд у Бајиној Башти, Савезничко гробље у Нишу, споменик чланицама Болнице шкотских жена и леди Хатон и Медицинска школа „Др Изабел Емсли Хатон" у Врању најбољи су доказ да се успомена на Болнице шкотских жена и др Елси Инглис чува и негује у Србији.

Речи Винстона Черчила су се обистиниле: „Ниједна женска организација није постигла већу репутацију. Записи о њиховом раду, осветљени славом др Елси Инглис, сијаће кроз историју".

После сто прохујалих година, историјска баштина српског народа из Великог рата, дуго занемарена и потиснута доласком савремене историје, данас добија на свом значају и вредновању у правом светлу. Ови редови су посвећени великом добротвору српског народа др Елси Инглис, шкотској лекарки, хирургу и оснивачу Болнице шкотских жена. Да судбине чланица Болнице шкотских жена нису остале потпуно анонимне и заборављење, потврђују савремена дела из пера писаца, историчара, социолога и лекара, са свих меридијана.

ЛИТЕРАТУРА

1. McLaren ES. A History of the Scottish Women's Hospitals. London: 1919. p. 88, 229, 234.
2. Powell A. Women in the War Zone. Hospital Service in the First World War. Stroud Gloucestershire: The History Press, 2009.
3. Popović-Filipović S. Dr Agnes Benet na čelu Bolnice škotskih žena na Solunskom frontu. Zbornik radova Drugog naučnog skupa 800 godina srpske medicine, Beograd: Infinitas i SLD; 2011.
4. Popović-Filipović S. Za hrabrost i humanost, Bolnice škotskih žena u Srbiji i sa Srbima za vreme Prvog svetskog rata 1914–1918. godine. Beograd: Signature; 2007.
5. Lazarević J. Engleskinje u srpskom narodu. Beograd: Beogradsko žensko društvo; 1929. p. 123.
6. Cahill AF, ed. Between the Lines. Letters and Diaries from Elsie Inglis's Russian Unit. Edinburgh, Cambridge, Durham: The Pentland Press Limited; 1999.

Elsie Inglis (1864–1917) and the Scottish Women's Hospitals in Serbia in the Great War – Part 2

Slavica Popović-Filipović

Serbian Medical Society, Section for History of Medicine, Belgrade, Serbia

SUMMARY

The news about the great victories of the Gallant Little Serbia in the Great War spread far and wide. Following on the appeals from the Serbian legations and the Serbian Red Cross, assistance was arriving from all over the world. First medical missions and medical and other help arrived from Russia. It was followed by the medical missions from Great Britain, France, Greece, the Netherlands, Denmark, Switzerland, America, etc. Material help and individual volunteers arrived from Poland, Canada, Australia, New Zealand, Ireland, Norway, India, Japan, Egypt, South America, and elsewhere. The true friends of Serbia formed various funds under the auspices of the Red Cross Society, and other associations. In September 1914, the Serbian Relief Fund was established in London, while in Scotland the first units of the Scottish Women's Hospitals for Foreign Service were formed in November of the same year.

The aim of this work was to keep the memory of the Scottish Women's Hospitals in Serbia and with the Serbs in the Great War. In the history of the Serbian nation during the Great War, a special place was held by the Scottish Women's Hospitals – a unique humanitarian medical mission. It was the initiative of Dr. Elsie Maud Inglis (1864–1917), a physician, surgeon, promoter of equal rights for women, and with the support of the Scottish Federation of Woman's Suffrage Societies. The Scottish Women's Hospitals, which were completely staffed by women, by their participation in the Great War, also contributed to gender and professional equality, especially in medicine. Many of today's achievements came about thanks to the first generations of women doctors, who fought for equality in choosing to study medicine, and working in the medical field, in time of war and peacetime.

Keywords: World War I; Scottish Women's Hospitals; Scotland; Serbia; Inglis E.

DOI: https://doi.org/10.2298/SARH180614044T
UDC: 61:929 Дидио Л.Ц.А.

ИСТОРИЈА МЕДИЦИНЕ / HISTORY OF MEDICINE

Professor Liberato J. A. DiDio – a great anatomist of the 20th century and an advocate of medicine without borders

Gordana Teofilovski-Parapid[1], Maria A. Miglino[2]

[1]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[2]University of Sao Paulo, School of Veterinary Medicine and Animal Science, Sao Paulo, Brazil

SUMMARY
Liberato J. A. DiDio (1920–2004) was one of the most prominent figures of anatomy belonging to the 20th century's second half and an open-minded man. In 1984, during the era of communism in Yugoslavia, he opened the doors of the Medical College of Ohio (MCO) in Toledo, OH, USA, to a Serbian doctor. During the troubled times for people and anatomists in Serbia in 1994, he saved their association from being expelled from the International Federation of Anatomical Associations. In 1999, only a few months after the bombing of Yugoslavia, he helped them to get the organization of the XVIII International Symposium on Morphological Sciences in 2005, the meeting of the leaders in the field. Serbian anatomists and clinicians proved that he was right when considering them on a par with their peers in the international anatomical and medical community.
Professor DiDio first showed talent with Gold Medal – top graduate at his high school, and La Royale Award (Graduation Golden Ring) – top graduate MD. He was trained in Brazil, Italy, and the US. He was the Founding Chairman – Department of Topographical Anatomy, Faculdade de Ciências Medicas, Belo Horizonte, Minas Gerais, Brazil; Head of Gross and Surgical Anatomy, Northwestern University Medical, Dental, and Graduate Schools; Founding Chairman, Department of Anatomy, MCO; Professor Emeritus at the age of 70 (1990), Assistant to the President of the MCO, Consultant to the President and the Emeritus Dean (1992–2004). He was a member of editorial boards of 34 journals, academic adviser in 92 M.S. and Ph.D. theses.
Keywords: Professor Liberato J. A. DiDio; anatomy; morphology; XVIII International Symposium on Morphological Sciences – Belgrade 2005

Liberato John Alphonse DiDio (1920–2004) was one of the most prominent figures of anatomy belonging to the second half of the 20th century. He was a great scientist and an open-minded man who, in 1984, during the communist era in Yugoslavia, generously opened the doors of the Medical College of Ohio in Toledo, OH, USA, to a Serbian anatomist. And these doors remained open for collaboration and support [1–5]. Moreover, during the tumultuous and troubled times for anatomists in Serbia in 1994, he saved their association from being expelled from the International Federation of Anatomical Associations (IFAA). However, this was not the end of his generosity. In 1999, only a few months after the bombing of the Federal Republic of Yugoslavia, during the Congress of the IFAA in Rome, he helped them and the Faculty of Medicine of the University of Belgrade to get the organization of the XVIII International Symposium on Morphological Sciences (ISMS) in 2005, the meeting of the leaders in the field, which was an honor and a privilege never before bestowed on anatomists of the entire former Yugoslavia (Figure 1). That scientific and moral giant had an extremely rich and fruitful history.

Liberato J. A. DiDio was born in 1920 to an Italian family living in Brazil. Early in his life, it became obvious he was exceptional as he got the Gold Medal as the top graduate of his high school (Instituto Pratola, Sao Paulo, Brasil, 1931), followed by the Certificate of Honor, as the top student at the end of his gymnasium education (Dante Alighieri College, Sao Paulo, 1936). Over the six-year medical school course (1940–1945), he was the annual laureate of the Montenegro Award as the leading student, and, finally, he was awarded the La Royale Award (Graduation Golden Ring) at the graduation ceremony as the best M.D. (Faculdade de Medicina, University of Sao Paulo, 1945). Even at that young age, his talent and hard work were internationally recognized and rewarded by the Rockefeller Foundation Award as the top student in basic medical sciences (1945). The aforementioned awards are but a few from a long list of 144 awards he received during his long and fruitful life.

Professor DiDio had a fascinating and rich educational background and training. First, in Brazil at the Faculty of Medicine of the University of Sao Paulo, from which he obtained the M.D. degree (1945) and the D.Sc. degree (1949), and had the postdoctoral training in embryology, postdoctoral course in labor medicine and forensic/medico-legal aspects, Ph.D.

Received • Примљено: June 14, 2018
Accepted • Прихваћено: June 20, 2018
Online first: June 22, 2018

Correspondence to:
Gordana TEOFILOVSKI-PARAPID
University of Belgrade
Faculty of Medicine
Dr. Subotića starijeg 8
11000 Belgrade, Serbia
gordana.teofilovski-parapid@med.bg.ac.rs

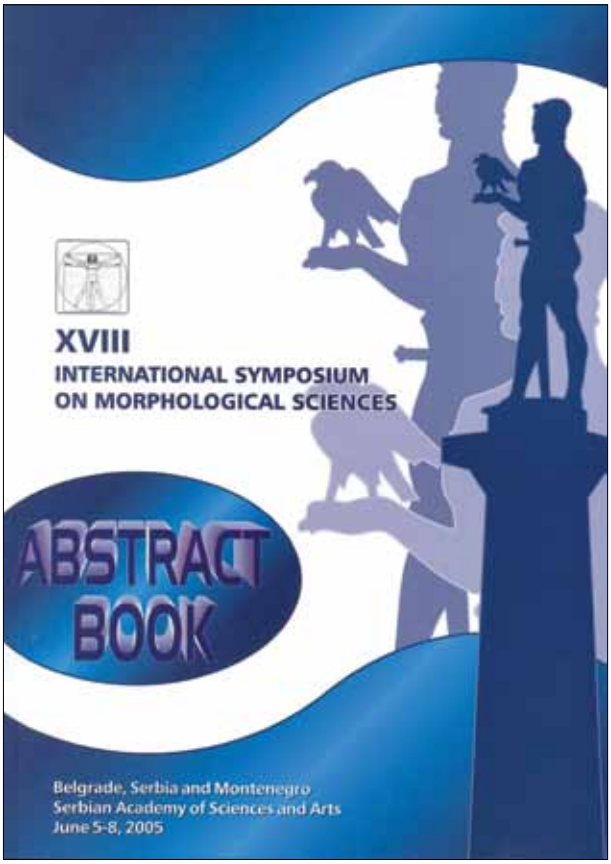


Figure 1. Abstract Book of the XVIII International Symposium on Morphological Sciences held in the Belgrade in the Serbian Academy of Sciences and Arts, June 5–8, 2005

in anatomy (*Summa cum laude*). At the University Hospital of the same school, he accomplished his internship in tropical medicine, and residency in surgery. Finally, he got the chairmanship in Anatomy and Embryology, by competitive examinations (*Summa cum laude*) at the Faculty of Medicine, Federal University of Minas Gerais, Belo Horizonte, Brazil. The need to broaden his scientific horizons and improve his skills took him out of Brazil. Hence, in 1955, he pursued his postdoctoral training in caryometry and histophotometry at the Messina University, Italy, and postdoctoral training in lymphology at the Parma University, Italy, in 1958. The crown of his education was the two-year fellowship (1961–1962) in the US with postdoctoral training in electron microscopy, polarization microscopy, and study of medical education in the following institutions: Department of Anatomy at the School of Medicine of the University of Washington, Seattle, WA; Department of Cytology at the Rockefeller University, New York City, NY; and at the Department of Anatomy of the Harvard University Medical School, Boston, MA.

The long list of his appointments begins with the position of the Research Assistant in Physiology at the Faculty of Medicine of the University of Sao Paulo (1942–1944), followed by the Research Assistant and Instructor (monitor) in Anatomy I (1943–1946). He left the School in 1954, as Associate Professor of Anatomy, to become Professor and Founding Chairman at the Department of Topographical Anatomy of the Faculdade de Ciências Medicas in Belo Horizonte, Minas Gerais, Brazil, where he stayed as Professor and Chairman until 1963. While at the University of Minas Gerais in Belo Horizonte, he also worked

Figure 2. The last meeting of professor and his alumna – Professor Liberato J. A. DiDio with professors (left to right) Gordana Teofilovski-Parapid, Carlos Baptista, and Angelica Miglino (State University of Rio de Janeiro, July 2002)

as Professor and Chairman at the Department of Veterinary Anatomy (1955–1956) and Director of the Institute of Morphology (Anatomy, Histology, Embryology, Electron Microscopy, Neuroanatomy) (1962–1963).

At the age of 43, he definitely moved to the US, where he continued adding prestigious titles and positions to his academic career: Professor, Head of Gross Anatomy and Surgical Anatomy, Department of Anatomy, Northwestern University Medical, Dental, and Graduate Schools, Chicago, IL (1963–1966); Professor and Founding Chairman, Department of Anatomy, Medical College of Ohio (MCO) in Toledo, OH (first founding faculty member) (1966–1988); Founding Dean of the Graduate School (1972–1986). Although recognized as Professor Emeritus at the age of 70 (1990), this didn't stop him from working at the MCO as the Assistant to the President (1988–1991), Interim Director of the Mulford Library (1990–1991), Consultant to the President and the Emeritus Dean (1992– 2004). He kept his international scientific activities as Visiting Professor at the Institute of Human Normal Anatomy of the La Sapienza University in Rome, Italy, as well as at the Department of Morphology of the Escola Paulista de Medicina, Federal University of Sao Paulo, Brazil, and as President of the Committee on Ethics in Scientific Research at the University of Santo Amaro, Sao Paulo, Brazil (Figure 2).

Dr. DiDio died in Sao Paolo (2004) as the Emeritus Dean of the MCO. At the time, he was performing several additional duties in Brazil: he was the Advisor for Graduate Studies at the Department of Surgery and Anatomy of Domestic Animals of the Faculty of Veterinary Medicine and Zootechnology, University of Sao Paulo, Vice-President of the Organizacao Santamarense de Educacao e Cultura, as well as Professor of Surgical Anatomy at the Department of Surgery, and of Scientific Methodology at the Department of Public Health (Faculty of Medicine), Professor of Veterinary Anatomy at the Faculty of Veterinary Medicine of the University of Santo Amaro, Sao Paulo, Brazil.

He left behind a legacy of 11 books as either the only or the first author, another 20 with chapters written by him, 379 scientific papers, and over 177 papers reported at morphological meetings worldwide. He reviewed 15 international books and translated another 11, which was possible due to his knowledge of English, Italian, Portuguese, French, and Spanish. He was an academic adviser in 92 M.S. and Ph.D. theses.

It is hard to find a system of the human body to whose anatomy data Professor DiDio has not contributed [1–24]. In his huge scientific body of work, he dealt with coronary circulation describing cardiac segments and subsegments

Figure 3. Professor David Brynmor Thomas (President of the International Federation of Anatomical Associations, IFAA, at the time) with professors (left to right): Beverly Kramer (current IFAA President), G. Strkalj (South Africa), Lev Kolesnikov (President of the All-Russian Scientific Society of Anatomists, Histologists and Embryologists), Mauricio Moscovici (Honorary Vice-President, International Committee of Symposia on Morphological Sciences) with wife Fella Moscovici (Belgrade City Hall, Opening Ceremony of the XVIII International Symposium on Morphological Sciences, June 5, 2005)

in humans and their importance for cardiac surgery, electron microscopic investigations on the myocardium under normal, experimental, and pathologic conditions, as well as cardiac valve bioprostheses in humans [3, 5–10]. The research comprises the anatomical and experimental investigations on anatomicosurgical segmentectomy in dogs and its application in human cardiac surgery. Furthermore, he conducted the research on anatomicosurgical segments of organs other than the heart, in both humans and other mammals [11, 12, 13]. His most recent research was related to several projects, such as aging, subcellular structure of the normal and neoplastic prostate, placenta of mammals, pineal gland, subcellular alterations induced by amiodarone, and innervations of muscles of the arm and their relationship with rehabilitation chronology [14–24].

Dr. DiDio was a member of editorial boards of 34 prestigious scientific journals in the field at the time, such as Acta Anatomica (Switzerland), Anatomia Clinica – now Surgical and Radiological Anatomy (France and Germany), Anatomischer Anzeiger – now Annals of Anatomy (Germany), Archivio Italiano di Anatomia ed Embriologia (Italy), Bulletin de l'Association des Anatomistes (France), Excerpta Medica (Anatomy, Histology, Embryology, Anthropology) (the Netherlands), Morphology Journal (Russia), Ohio Journal of Science (USA), Brazilian Journal of Morphological Sciences (Brazil), Revista Chilena de Anatomia (Chile), etc.

Throughout the world, scientific and professional associations were eager to have Dr. DiDio as a member and 55 succeeded in that effort. He was a prominent member of the American Association of Anatomists, American Association of University Professors, American Association of Veterinary Anatomists, American Cancer Society, American College of Legal Medicine, American Heart Association, International College of Surgeons (Fellow), Anatomical Society of Great Britain and Ireland, Anatomische Gesellschaft, Associacao Brasileira de Editores Cientificos, Associacao Medica do Parana, Portuguese Anatomical Society, Societe d'Anthropologie de Paris, etc. He was a founder of the American Association for Cell Biology, Ibero-American Society of Anatomy, Italian Society of Histochemistry, and Pan American Association of Anatomy. In the International Federation of Associations of Anatomists, Dr. DiDio served as the President (1986–1989), and afterwards as the Honorary President. He died as the President of the International Committee on Symposia on Morphological Sciences, generously helping in organizing the XVIII ISMS, to be held in Belgrade, Serbia, on June 5–8, 2005.

Dr. DiDio will be remembered as a man with immense knowledge that made him define the importance of the concept of "dynamic morphology" as an integration of structure and function. He will be remembered as a medical doctor and a scientist of great moral integrity, a demanding advisor, but nonetheless a supportive professor and a devoted friend – all at the same time (Figure 2). The 21st century anatomy – without him – will never be the same, for, among other things, Serbian anatomists and the Serbian Medical Society lost a selfless supporter and a faithful friend in the international medical science community. However, by successfully organizing the XVIII ISMS held in Belgrade, Serbia, on June 5–8, 2005, Serbian anatomists and clinicians side by side – strongly supported by the Faculty of Medicine of the University of Belgrade, the Board for Cardiovascular Pathology of the Serbian Academy of Sciences and Arts (SASA) and it's president Academician Vladimir Kanjuh, and the authorities of the Republic of Serbia – all united, lived up to Professor DiDio's expectations, proving he was right when considering them on a par with their international peers in the world of anatomical and medical society [25]. That meeting, after 10 years of sanctions, brought to the SASA for the first time over 100 foreign scientist, among whom Professor David Brynmor Thomas (Figure 3) and Professor Pierre Sprumont, presidents of the IFAA and the European Federation For Experimental Morphology (EFEM), respectively. They both held their associations' council meetings in Belgrade during the XVIII ISMS. Even nowadays, you will find "Belgrade 2005" in the EFEM Statute [26]. Our leading clinicians at the Faculty of Medicine of the University of Belgrade and affiliated hospitals adopted the concept of dynamic morphology and helped anatomists in creating an exceptional program. Today, many of them are members of the SASA, like professors Nebojša Radunović, Miodrag Ostojić, Đorđe Radak, Nebojša Lalić, Petar Seferović, Marko Bumbaširević, and other equally prominent ones, like professors Biljana Obrenović-Kirćanski, Nadežda Čovičković-Šternić, Miodrag Rakić, Mirko Teofilovski, and Mladen Kočica. The Serbian Medical Society showed its best, and our international colleagues have been fascinated.

ACKNOWLEDGEMENT

This text was partly reported at the XVIII International Symposium on Morphological Sciences in Belgrade, June 2–5, 2005.

Authors are indebted to the Ministry of Science and Environment Protection of the Republic of Serbia and to the Malpighi Foundation (Italy) for the financial support of the XVIII ISMS, and especially to Professor Guido Macchiarelli for his personal contribution. This work has been supported by the Ministry of Education and Sciences of the Republic of Serbia, grant No. III41022.

REFERENCES

1. Baptista CA, DiDio LJ, Teofilovski-Parapid G. Variation in length and termination of the ramus circumflexus of the human left coronary artery. Anat Anz. 1990; 171(4):247–53.
2. DiDio LJ, Baptista CA, Teofilovski-Parapid G. Anatomical variations of the abducent nerve in humans. Arch Ital Anat Embriol. 1990; 95(3-4):167–72.
3. Teofilovski-Parapid G, Baptista CA, DiDio LJ, Vaughan C. The membranous portion of the interventricular septum and its relationship with the aortic valve in humans. Surg Radiol Anat. 1991; 13(1):23–8.
4. Baptista CA, Didio LJ, Teofilovski-Parapid G. Variations of the blood supply of the human conus arteriosus. Bull Assoc Anat (Nancy). 1992; 76(232):9–18.
5. Teofilovski G, Filipovic B, Bogdanovic D, Trpinac D, Rankovic A, Stankovic G, et al. Myocardial bridges over coronary arteries in Cercopithecus. Ann Anat. 1992; 174(5):435–9.
6. Piras C, Rodrigues H, Lopes AC, DiDio LJ. The relationship between the papillary muscles and the anatomicosurgical segments of the left ventricle of the human heart. Rev Assoc Med Bras (1992). 1993; 39(3):135–40.
7. DiDio LJ. Electron microscopic and x-ray microanalytic evaluation of cardiac valve bioprostheses. Tokai J Exp Clin Med. 1983; 8(4):301–6.
8. Fentie IH, Allen DJ, Schenck MH, Didio LJ. Comparative electron microscopic study of bovine, porcine and human parietal pericardium, as materials for cardiac valve bioprostheses. J Submicrosc Cytol. 1986; 18(1):53–65.
9. Macchiarelli G, DiDio LJ, Allen DJ, Stolf NG, Pego-Fernandes P, Motta PM. Scanning electron microscopy study of endocardial regeneration in bovine pericardial patch-grafts implanted in the canine heart. Cardioscience. 1994; 5(1):43–9.
10. Didio LJ, Franco-Saenz R, Morse DE. Endocrine cardiomyocytes. J Submicrosc Cytol. 1987; 19(4):683–94.
11. Busnardo AC, DiDio LJ, Thomford NR. Anatomicosurgical segments of the human pancreas. Surg Radiol Anat. 1988; 10(1):77–82.
12. Christo MC, DiDio LJ. Anatomical and surgical aspects of splenic segmentectomies. Ann Anat. 1997; 179(5):461–74.
13. DiDio LJ, Lopes AC. Clinical and surgical importance of anatomosurgical segments and subsegments of the organs of the human body. Rev Assoc Med Bras (1992). 1995; 41(3):167–77.
14. Lopes AC, DiDio LJ. Positive aspects of geriatrics and gerontology. Rev Assoc Med Bras (1992). 1996; 42(1):1.
15. Timms BG, Mohs TJ, Didio LJ. Ductal budding and branching patterns in the developing prostate. J Urol. 1994; 151(5):1427–32.
16. Gross SA, Didio LJ. Comparative morphology of the prostate in adult male and female Praomys (Mastomys) Natalensis studied with electron microscopy. J Submicrosc Cytol. 1987; 19(1):77–84.
17. Gross SA, Didio LJ. The prostate in pregnant and non-pregnant Praomys (Mastomys) Natalensis at the subcellular level. J Submicrosc Cytol Pathol. 1988; 20(1):101–7.
18. Miglino MA, DiDio LJ. Vasculature of bovine placentas studied by scanning electron microscopy of corrosion casts. Ital J Anat Embryol. 1992; 97(1):23–35.
19. Hamlett WC, Miglino MA, Didio LJ. Subcellular organization of the placenta in the Atlantic sharpnose shark, Rhizoprionodon terraenovae. J Submicrosc Cytol Pathol. 1993; 25(4):535–45.
20. Lopes AC, Mora O, Sasso WS, DiDio LJ. Propranolol-like action of amiodarone. An electronmicroscopic study in rats under cold stress. J Submicrosc Cytol Pathol. 1997; 29(2):253–6.
21. Cricenti SV, Deangelis MA, Didio LJ, Ebraheim NA, Rupp RE, Didio AS. Innervation of the extensor carpi radialis brevis and supinator muscles: Levels of origin and penetration of these muscular branches from the posterior interosseous nerve. J Shoulder Elbow Surg. 1994; 3(6):390–4.
22. Sirigu P, Gross SA, DiDio LJ, Lantini MS. Histochemical localization of prostaglandin-synthetase in the salivary glands of Praomys (Mastomys) natalensis. Basic Appl Histochem. 1988; 32(3):321–5.
23. Cramer GD, Allen DJ, DiDio LJ, Potvin W, Brinker R. Comparison of computerized tomography with magnetic resonance imaging (MRI) in the evaluation of encephalic ventricular volume. Surg Radiol Anat. 1990; 12(2):135–41.
24. Manso JC, DiDio LJ. Anatomical variations of the human suprarenal arteries. Ann Anat. 2000; 182(5):483–8.
25. XVIII International Symposium on Morphological Sciences, Belgrade June 5–7, 2005. Abstract Book, p. 1–266.
26. European Federation for Experimental Morphology (EFEM), *http://www.efem.eu/statutes.php.*

Професор Либерато Џ. А. Дидио – велики анатом двадесетог века и заговорник медицине без граница

Гордана Теофиловски-Парапид[1], Марија А. Миглино[2]

[1]Универзитет у Београду, Медицински факултет, Београд, Србија;
[2]Универзитет у Сао Паулу, Ветеринарски факултет, Сао Пауло, Бразил

САЖЕТАК

Либерато Џ. А. Дидио (1920–2004) био је један од најеминентнијих анатома друге половине XX века и слободоуман човек. Он је 1984. године, у време комунистичког режима у Југославији, отворио врата Медицинског факултета Охаја (МФО) у Толеду српском доктору и анатому. У тешким временима за народ и анатоме у Србији, он је 1994. спасио њихову асоцијацију од избацивања из Интернационалне федерације анатомских асоцијација (ИФАА) и 1999, само неколико месеци после бомбардовања Србије, за време ИФАА конгреса у Риму, он је помагао њима и Медицинском факултету Универзитета у Београду да добију организацију XVIII Интернационалног симпозијума морфолошких наука 2005. године, састанак светских лидера у мофологији који ни анатоми у бившој Југославији нису добили. Српски анатоми и клиничари су искористили пружену прилику и доказали да је био у праву када их је сматрао равноправним у светској анатомији и медицини.

Професор Дидио је рано показао таленат, почевши Златном медаљом за завршену гимназију и краљевском наградом Дипломски златан прстен за најбоље дипломираног доктора. Изузетно се образовао у родном Бразилу, а затим у Италији и САД. Био је оснивач и директор Одељења топографске анатомије на Факултету медицинских наука у Бело Хоризонтеу (Минас Жерас, Бразил), руководилац анатомије и хируршке анатомије Одељења анатомије на Нортвестерн универзитету Медицинског, Стоматолошког и Постдипломског факултета; оснивач и директор Одељења за анатомију МФО у Толеду; оснивач и декан Дипломске школе; професор емеритус у 70. години (1990). После пензионисања ради као асистент председника МФО, саветник председника и декан емеритус (1992–2004). Био је члан издавачког савета 34 научна часописа, саветник у 92 магистеријума и доктората.

Кључне речи: професор Либерато Џ. А. Дидио; анатомија; морфологија; XVIII Интернационални симпозијум морфолошких наука – Београд 2005

DOI: https://doi.org/10.2298/SARH170908180R
UDC: 615.816/.817:616-073

INVITED COMMENTARY / КОМЕНТАР ПО ПОЗИВУ

Application of ultrasound diagnostics in cardiopulmonary resuscitation – invited commentary

Predrag Romić
Serbian Medical Society, Section for Anaesthesiology, Intensive Care and Pain Therapy, Belgrade, Serbia

The paper titled "Application of ultrasound diagnostics in cardiopulmonary resuscitation" [1] represents one of review articles which analyze the possibility of ultrasound (US) application during cardiopulmonary resuscitation (CPR). Having performed resuscitation for several decades, I believe that this represents a minor CPR topic since it can be applied only while verifying consciousness during CPR (which is to be avoided) since there are less than 10 seconds available (what can be achieved in such a short time frame?). In addition, the real indications are present only in the posttraumatic heart arrest (tension pneumothorax and hypovolemia, but these conditions are easily recognized and countered by anesthesiologists).

Pulmonary thromboembolism (PTE) is confirmed by indirect US findings ("dilatation and hypokinesis of RV, tricuspid regurgitation, increased systolic pressure in RV < 60 mmHg, dilated non-collapsible right hollow vein") – however, this can be useful only when the heart is functioning, and we are talking about heart arrest provoked by PTE. Hence, if the heart is beating, we cannot see the dilatation and hypokinesis of the right ventricle, tricuspid regurgitation, etc.

Consequently, the recommendation to the authors and everyone else who is planning on introducing urgent US during CPR is that this is only possible in intra-hospital CPR, when the patient is already admitted to intensive treatment. In fact, this is stated in the recommendations of the European Reanimation Council, which emphasize the application of urgent US during advanced cardiac life support, most frequently used in the hospital setting [2].

The insistence on dividing CPR into extra-hospital CPR and intra-hospital CPR is quite significant and has been put to practice ever since the time of projects researching extra-hospital heart arrest, and especially since a large multicentric study of the World Health Organization titled WHO MONICA Project (Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) – the study conducted between 1980 and 1990 in 21 countries unequivocally showed that cardiac arrests which occurred in extra-hospital settings were generally caused by coronary disease and developed according to the ventricular fibrillation type, while intra-hospital cardiac arrests developed according to asystole and electric activities without a pulse (PEA – pulseless electric activity). One study has demonstrated cardiogenic ethology in 91.5% of 10,861 extra-hospital cardiac arrest cases taken care by emergency medical teams [3, 4].

Since then, resuscitators have been aware that these basic measures of CPR (external heart massage and artificial respiration) in an extra-hospital cardiac arrest only "buy some time," and causal therapy, such as defibrillation, i.e. a countershock by electric current which interrupts ventricular fibrillation, is needed as soon as possible. At that time, automatic external defibrillators (AEDs) appeared. These devices independently analyze heart rhythm and decide when and if to perform defibrillation with the accuracy of > 98%, so that they can be used even by resuscitators who are not medical doctors. This represents the basis for the PAD (Public Access Defibrillation) program development as the objective of contemporary CPR, which also comprises a network of amateur-resuscitators equipped with AEDs at locations where a large number of people are likely to be [5, 6].

All of the aforementioned has been stated with the ultimate goal to emphasize the wish and willingness in contemporary CPR for the earliest possible application of defibrillation in extra-hospital cardiac arrests, which makes the practicality of US use in these conditions negligible. The only possibility is to apply this method intra-hospitally, but even then only during rare periods of consciousness verification in patients suffering from cardiac arrest.

Received • Примљено: September 8, 2017
Accepted • Прихваћено: September 27, 2017
Online first: October 3, 2017

REFERENCES

1. Anđelić S, Pavlović A, Trpković S, Šijački A, Janićijević A, Putniković B. Application of ultrasound diagnostics in cardiopulmonary resuscitation. Srp Arh Celok Lek. 2018; 146(5-6):323–9.
2. Neumar RW, Shuster M, Callaway CW, Gent LM, Atkins DL, Bhanji F, et al. Executive Summary 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2015; 132:S315–67.
3. Luepker RV. WHO MONICA project: what have we learned and where to go from here? Public Health Reviews. 2012; 33:373–96.
4. Rea TD, Cook AJ, Stiell IG, Powell J, Bigham B, Callaway CW, et al. Predicting survival after out-of-hospital cardiac arrest: Role of the utstein data elements. Ann Emerg Med. 2010; 55(3):249–57.
5. Romić P, Jovanović K, Jovićević K. Značaj rane defibrilacije u kardiopulmonalnoj reanimaciji. Anaesth Iugoslav. 2002; 23(3-4):11–6.
6. Romić P, Jovanović K, Simeunović S, Milojković A. Program masovne defibrilacije u slučajevima netraumatskog srčanog zastoja (PAD Program). ABC – Časopis Urgentne medicine, 2005; 1(4):5–14.

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

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

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

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

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

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

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

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

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало

би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

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

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

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

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом.

Уколико је рад у целости на српском језику (нпр. рад из историје медицине, језика медицине и др.), потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик. Сажетке и радове који су у целости на српском језику аутори из Србије треба да пишу ћирилицом.

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

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

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

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

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

ОБИМ РАДОВА. Целокупни рукопис рада – који чине насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, претходно и кратко саопштење, рад

из историје медицине и преглед литературе до 5.000 речи, а за приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи. Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

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

ТАБЕЛЕ. Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле.

Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

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

Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

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

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

Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

СХЕМЕ (ЦРТЕЖИ). Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме.

Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

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

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

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, а у метаанализи до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да

буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

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

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

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

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) за годину у којој се рад предаје Уредништву. Сви домаћи аутори такође морају бити претплаћени на часопис или измирити накнаду за обраду чланака (*article processing charge*) за годину у којој се рад предаје Уредништву, у износу од 3.000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*article processing charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

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

Додатне информације о чланарини и претплати могу се добити путем имејла (*office@srpskiarhiv.rs*) и на интернет-страници часописа *http://srpskiarhiv.rs/en/subscription/).*

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад могу се доставити имејлом (*office@srpskiarhiv.rs*), електронски преко система за пријављивање на интернет-страници часописа (*http://www.srpskiarhiv.rs*), препорученом пошиљком или лично, доласком у Уредништво. Уколико се рад шаље поштом или доноси у Уредништво, рукопис се доставља одштампан у три примерка и нарезан на *CD* (снимљени материјал треба да је истоветан оном на папиру).

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

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

АДРЕСА:
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
Ул. краљице Наталије 1
11000 Београд
Србија

ТЕЛЕФОН: + 381 11 409-2776
+ 381 11 409-4479
E-MAIL: *office@srpskiarhiv.rs*
ИНТЕРНЕТ АДРЕСА: *http://www.srpskiarhiv.rs*

ISSN 0370-8179 ISSN Online 2406-0895

Before submitting their paper to the Editorial Office of the *Serbian Archives of Medicine*, authors should read the Instructions for Authors, where they will find all the necessary information on writing their manuscript in accordance with the journal's standards. It is essential that authors prepare their manuscript according to established specifications, as failure to do so will result in paper being delayed or rejected. *Serbian Archives of Medicine* provides no fee for published articles. By submitting a paper for publishing consideration, authors of a paper accepted for publication in the *Serbian Archives of Medicine* grant and assign all copyrights to the publisher – the Serbian Medical Society.

GENERAL INSTRUCTIONS. *Serbian Archives of Medicine* publishes papers that have not been, either in their entirety or partially, previously published, and that have not been accepted for publication elsewhere. *Serbian Archives of Medicine* publishes papers in English and Serbian. For better availability and citation, authors are encouraged to submit articles of all types in English. The journal publishes the following article types: editorials, original papers, metaanalyses, review articles, preliminary and short communications, case reports, images in clinical medicine, video-articles, articles for practitioners, current topics, history of medicine articles, language of medicine articles, opinion essays, personal view articles, invited commentaries, letters to the editor, book reviews and other articles. Original papers, preliminary and short communications and case reports are published in English only, while other article types may be published in Serbian if the Editorial Office reaches such decision. The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., ^{99}Tc, IL-6, O_2, B_{12}, CD8). If something is commonly written in italics, such as genes (e.g. *BRCA1*), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

CONFLICT OF INTEREST STATEMENT. The manuscript must be accompanied by a disclosure statement from all authors (contained within the Submission Letter) declaring any potential interest or stating that the authors have no conflict of interest. For additional information on different types of conflict of interest, please see World Association of Medical Editors (WAME, *www.wame.org*) policy statement on conflict of interest.

AUTHORSHIP. All individuals listed as authors should be qualified for authorship. Every author should have participated sufficiently in writing the article in order to take responsibility for the whole article and results presented in the text. Authorship is based only on: crucial contribution to the article conception, obtaining of results or analysis and interpretation of results; design of manuscript or its critical review of significant intellectual value; final revision of the manuscript being prepared for publication.
The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institu-

tion in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, meta-analysis, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, preliminary communications, and meta-analyses, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

KEYWORDS. Below the summary, 3 to 6 keywords or phrases should be typed. The keywords need not repeat words in the title and should be relevant or descriptive. *Medical Subject Headings – MeSH* (*http://www.nlm.nih.gov/mesh*) are to be used for selection of the keywords.

TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine", etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

Summaries and articles written in Serbian by authors from Serbia need to be written in the Serbian Cyrillic alphabet.

STRUCTURE OF THE MANUSCRIPT. All section headings should be in capital letters using boldface. Original articles, meta-analyses and preliminary and short communications should have the following section headings: Introduction (objective is to be stated in the final paragraph of the Introduction), Methods, Results, Discussion, Conclusion, References. A review article includes: Introduction, corresponding section headings, Conclusion, References. The firstly named author of a meta-analysis or a review article should cite at least five auto-citations (as the author or co-author of the paper) of papers published in peer-reviewed journals. Co-authors, if any, should cite at least one auto-citation of papers also published in peer-reviewed journals. A case report should consist of: Introduction (objective is to be stated in the final paragraph of the Introduction), Case Report, Discussion, References. No names of patients, initials or numbers of medical records, particularly in illustrations, should be mentioned. Case reports cannot have more than five authors. Letters to the editor need to refer to papers published in the *Serbian Archives of Medicine* within previous six months; their form is to be comment, critique, or stating own experiences. Publication of articles unrelated to previously published papers will be permitted only when the journal's Editorial Office finds it beneficial.

All enclosures (tables, graphs, photographs, etc.) should be placed at the end of the manuscript, while in the body of the text a particular enclosure should only be mentioned and its preferred place indicated. The final arrangement (position) of the enclosures will depend on page layout.

ABBREVIATIONS. To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

DECIMAL NUMBERS. In papers written in English, including text of the manuscript and all enclosures, a decimal point should be used in decimal numbers (e.g. 12.5 ± 3.8), while in Serbian papers a decimal comma should be used (e.g. 12,5 ± 3,8). Wherever applicable, a number should be rounded up to one decimal place.

UNITS OF MEASURE. Length, height, weight and volume should be expressed in metric units (meter – m, kilogram – kg, gram – g, liter – l) or subunits. Temperature should be in Celsius degrees (°C), quantity of substance in moles (mol), and blood pressure in millimeters of mercury column (mm Hg). All results of hematological, clinical and biochemical measurements should be expressed in the metric system according to the International System of Units (SI units).

LENGTH OF PAPER. The entire text of the manuscript – title page, summary, the whole text, list of references, all enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian – must not exceed 5,000 words for original articles, preliminary and short communications, review articles and articles on history of medicine, and 3,000 words for case reports, articles for practitioners, educational articles and articles for "Language of medicine"; for any other section maximum is 1,500 words.

Video-articles are to last 5–7 minutes and need to be submitted in the *flv* video format. The first shot of the video must contain the following: title of the journal in the heading (*Serbian Archives of Medicine*), title of the work, last names and initials of first and middle names of the paper's authors (not those of the creators of the video), year of creation. The second shot must show summary of the paper, up to 350 words long. The final shot of the video may list technical staff (director, cameraman, lighting, sound, photography, etc.). Video-articles need to be submitted along with a separate summary (up to 350 words), a single still/photograph as an illustration of the video, and a statement signed by the technical staff renouncing copyrights in favor of the paper's authors.

To check the required number of words in the manuscript, please use the menu *Tools–Word Count*, or *File–Properties–Statistics*.

ARTICLE ENCLOSURES are tables, figures (photographs, schemes, sketches, graphs) and video-enclosures.

TABLES. Each table, with its legend, should be self-explanatory. The title should be typed above the table and any explanatory information under the table. Tables should be numbered in Arabic numerals in order of citation in the text. Use *MS Word*, the menu *Table–Insert–Table*, inserting the adequate number of rows and columns. By the right click of the mouse, use the options *Merge Cells* and *Split Cells*. Use *Times New Roman*, font size 12 pt, with single line spacing and no indent to draw tables. Abbreviations used in tables should be explained in the legend below each respective table.

If the manuscript is entirely in the Serbian language, tables and corresponding legend should be both in Serbian and English. Also, the table cells should contain text in both languages (do not create two separate tables with a single language!).

FIGURES. Figures are all types of visual enclosures, and photographs, schemes, sketches and graphs are published as 'figures' in the *Serbian Archives of Medicine*. Figures should be numbered in Arabic numerals in order of citation in the text. Only original digital photographs (blackand-white or color), of minimum 300 dpi, and *jpg* or *tiff* format, are acceptable (small, blurry and photographs of poor quality will not be accepted for publishing!). If authors do not possess or are not able to provide digital photographs, then the original photos should be scanned in 300 dpi, and saved in original size. If a paper needs to be illustrated with a considerable number of figures, several figures will be published within the paper, and the rest will be available in the electronic version of the paper as a PowerPoint presentation (every figure needs to be numbered and be accompanied by legend). Video-enclosures (illustrations of a paper) can last 1–3 minutes and are submitted in the *flv* format. Along with the video, a still/photograph representative of the video is also needed, as it will be used as a placeholder in the electronic version of the paper, and as an illustration in the printed version.

If the manuscript is entirely in the Serbian language, photographs and corresponding legend should be both in Serbian and English.

Photographs may be printed and published in color, but possible additional expenses are to be covered by the authors.

GRAPHS. Graphs should be plotted in *Excel* in order to see the respective values distributed in the cells. The same graphs should be copied and pasted to the *Word* document, numbered in Arabic numerals by order of citation in the text. The text in the graphs should be typed in *Times New Roman*. Abbreviations used in graphs should be explained in the legend below the respective graph. In the printed versions of papers, graphs are generally published in black-and-white; therefore, it is suggested to avoid the use of colors in graphs, or to utilize colors of significant difference in brightness.

If the manuscript is entirely in the Serbian language, graphs and corresponding legend should be both in Serbian and English.

SCHEMES (SKETCHES). Schemes and sketches are to be submitted in *jpg* or *tiff* format. Schemes should be drawn in *CorelDraw* or *Adobe Illustrator* (programs for drawing vectors, curves, etc.). The text in the schemes should be typed in *Times New Roman*, font size 10 pt. Abbreviations used in schemes should be explained in the legend below the respective scheme.

If the manuscript is entirely in the Serbian language, schemes and corresponding legend should be both in Serbian and English.

ACKNOWLEDGMENT. List all those individuals having contributed to preparation of the article but having not met the criteria of authorship, such as individuals providing technical assistance, assistance in writing the paper or running the department securing general support. Financial aid and all other support in the form of sponsorship, grants, donations of equipment and medications, etc., should be mentioned too.

REFERENCES. The reference list is the responsibility of the authors. Cited articles should be readily accessible to the journals readership. Therefore, following each reference, its DOI number and PMID number (if the article is indexed for MEDLINE/PubMed) should be typed.

References should be numbered in Arabic numerals in order of citation in the text. The overall number of references should not exceed 30, except in review articles, where maximum of 50 is acceptable, and in meta-analysis, where up to 100 referenceas are allowed. The number of citations of original articles must be at least 80% of the total number of references, and the number of citations of books, chapters and literature reviews less than 20%. If monographs and articles written by Serbian authors could be included

in the reference list, the authors are obliged to cite them. The majority of the cited articles should not be older than five years. Use of abstracts as references is not allowed. If it is important to comment on results published solely in the form of an abstract, it is necessary to do so within the text of the article. The references of articles accepted for publication should be designated as *in press* with the enclosed proof of approval for publication.

The references are cited according to the Vancouver style (*Uniformed Requirements for Manuscripts Submitted to Biomedical Journals*), rules and formats established by the International Committee of Medical Journal Editors (*http://www.icmje.org*), used by the *U.S. National Library of Medicine* and scientific publications databases. Examples of citing publications (journal articles, books and other monographs, electronic, unpublished and other published material) can be found on the web site *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. In citation of references the defined standards should be strictly followed, because it is one of the essential factors of indexing for classification of scientific journals.

SUBMISSION LETTER. The manuscript must be accompanied by the Submission Letter, which is signed by all authors and includes the following: 1) statement that the paper has never been published and concurrently submitted for publication to any other journal; 2) statement that the manuscript has been read and approved by all authors who have met the criteria of authorship; and 3) contact information of all authors of the article (address, email, telephone number, etc.). Blank Submission Letter form can be downloaded from the journal's web site (*http://srpskiarhiv.rs/global/pdf/SubmissionletterformFINAL.pdf*).

Additionally, the authors should submit the following copies of all permits for: reproduction of formerly published material, use of illustrations and publication of information on known people or disclosure of the names of people having contributed to the work.

MEMBERSHIP FEE, SUBSCRIPTION RATES AND ARTICLE PROCESSING CHARGE. In order to publish their article in the *Serbian Archives of Medicine*, all domestic authors and co-authors, medical doctors and doctors of dental medicine, must be members of the Serbian Medical Society (according to the Article #6 of the Statute of the SMS) for the year in which the manuscript is being submitted. All domestic authors and co-authors must also be subscribed to the journal for the year in which the manuscript is being submitted, or pay an article processing charge for the coverage of all editing and publishing expenses (3,000 RSD). Authors and co-authors from abroad need only pay the article processing charge (€35) – the fee is required for substantive editing, fact and reference validations, copy editing, and publishing online and in print. An author who had already paid the fee can have more articles submitted for publishing consideration in the year the fee was paid. All authors who pay this fee may, if they desire so, receive the printed version of the journal in the year when the fee is payed.

Please note that the payment of this charge does not guarantee acceptance of the manuscript for publication and does not influence the outcome of the review procedure, in accordance with good publishing practice.

The journal accepts donations from sponsors to create a sum for payment reductions or waivers for authors unable to cover the article processing charge (a justification of the inability to pay should be provided in such cases).

The requirement for paying the article processing charge does not apply to students or to journal subscribers. Institutions (legal entities) cannot by their subscription cover this condition on behalf of the authors (natural persons). Copies of deposit slips for membership and article processing charge should be enclosed with the manuscript. Foreign authors are under no obligation to be members of the Serbian Medical Society. All the relevant information can be obtained via email address of the Editorial Office (*office@srpskiarhiv.rs*) and on the journal's web site (*http://srpskiarhiv.rs/en/subscription/*).

SUBMISSION OF THE MANUSCRIPT. Manuscript and all enclosures can be sent by email (*office@srpskiarhiv.rs*) or via the journal's web site (*http://www.srpskiarhiv.rs*). If sent by registered mail or delivered in person at the Editorial Office in Belgrade, it should contain two printed copies and a CD with the version identical to that on paper, as well as all the necessary accompanying documentation.

NOTE. The papers not complying with these instructions will not be reviewed and will be returned to the authors for revision. Observing the instructions for preparation of papers for *the Serbian Archives of Medicine* will shorten the time of the entire process of publication and will have a positive effect on the quality and timely release of the journal's issues.

For further information, please contact us via the following address:

ADDRESS:
Srpsko lekarsko društvo
Uredništvo časopisa „Srpski arhiv za celokupno lekarstvo"
Ul. kraljice Natalije 1
11000 Beograd
Serbia

PHONE: +381 11 409 27 76
+381 11 409 44 79
E-MAIL: *office@srpskiarhiv.rs*
WEB SITE: *www.srpskiarhiv.rs*

ISSN 0370-8179
ISSN Online 2406-0895

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

61(497.11)

СРПСКИ архив за целокупно лекарство : званичан часопис Српског лекарског друштва = Serbian Archives of Medicine : official journal of the Serbian Medical Society / главни и одговорни уредник Гордана Теофиловски-Парапид. - Књ. 1 (1874)-књ. 2 (1875) ; књ. 3 (1879)- књ. 8 (1881) ; књ. 9 (1887)-књ. 10 (1888) ; књ. 11 (1894)-књ. 12 (1895) ; год. 1, бр. 1/2 (1895)- . - Београд : Српско лекарско друштво, 1874-1875; 1879-1881; 1887-1888; 1894-1895; 1895- (Београд : Службени гласник). - 29 cm

Двомесечно. - Текст на енгл. језику. - Има суплемент или прилог: Српски архив за целокупно лекарство. Суплемент = ISSN 0354-2793. - Друго издање на другом медијуму: Српски архив за целокупно лекарство (Online) = ISSN 2406-0895
ISSN 0370-8179 = Српски архив за целокупно лекарство
COBISS.SR-ID 3378434

Srp Arh Celok Lek ISSN 0370-8179 COBISS.SR-ID 3378434 UDC 61(497.11)

СРПСКО ЛЕКАРСКО ДРУШТВО БЕОГРАД

www.srpskiarhiv.rs