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CONTENTS

M E D I C I N S K I P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad *Vase Stajića 9* *Srbija*

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THE QUALITY OF LIFE OF WOMEN WITH GENITAL WARTS

KVALITET ŽIVOTA ŽENA SA GENITALNIM BRADAVICAMA

Ljiljana MLADENOVIĆ SEGEDI and Artur BJELICA

Summary

Introduction. The aim of this study was to examine the quality of life and psychological state of women with genital warts. **Material and Methods.** Three questionnaires have been used to evaluate the quality of life: the generic questionnaire European quality of life index version 5D, disease-specific questionnaire for Condylomata Acuminata, and dermatology life quality index. **Results.** The average age of patients was 29.72 +/- 8.54 years. The most common location of genital warts was the vulva (33.8%) and then vulva and vagina (22.54%). The mean value of the visual analogue scale was 70.37 +/- 20.8. The mean value of the specific questionnaire for Condylomata Acuminata-10 questionnaire was 26.61 +/- 10.09. The patients with higher scores in specific questionnaire for Condylomata Acuminata-6 and -10 questionnaires evaluated their quality of life as significantly better (specific questionnaire for Condylomata Acuminata-10: $r = 0.251$; $p < 0.05$) (specific questionnaire for Condylomata Acuminata-6: $r = 0.263$; $p < 0.05$). The mean score of the dermatology life quality index questionnaire was 6.42 +/- 5.82. About 81.4% of women were concerned, anxious and depressed, 54.3% felt pain and discomfort in the genital region, and 57.7% suffered from itching and burning. Shame and insecurity was reported by 64.8% of patients. About 50.7% had a problem with genital warts that affected their relationship, while 62.1% had problems with sexual intercourse. **Conclusion.** Our examination showed that genital warts adversely affected the emotional and sexual aspects of the quality of life. There is a need for better education of the general population on human papillomavirus infection and its prevention.

Key words: Condylomata Acuminata; Quality of Life; Female; Surveys and Questionnaires; Stress, Psychological; Papillomavirus Infections; Signs and Symptoms

Sažetak

Uvod. Cilj našeg rada bio je da se ispita kvalitet života žena sa genitalnim bradavicama. **Materijal i metode.** Za merenje kvaliteta života korišćena su tri upitnika: generički upitnik – *the generic questionnaire European Quality of Life index version 5D*, specifičan za bolest upitnik – *disease-specific questionnaire for Condylomata Acuminata* i *Dermatology life quality index*. **Rezultati.** Prosečna starost ispitanica bila je 29,72 +/- 8,54 godine. Genitalne bradavice su najčešće bile lokalizovane na vulvi (33,8%), vulvi i vagini (22,54%). Srednja vrednost *visual analogue scale* iznosila je 0,37 +/- 20,8. Srednja vrednost *specific questionnaire for Condylomata Acuminata-10* iznosila je 26,61 +/- 10,09. Ispitanice sa višim skorovima u *specific questionnaire for Condylomata Acuminata-6 and -10* upitniku procenjivale su svoj kvalitet života kao bolji (*specific questionnaire for Condylomata Acuminata-10*: $r = 0{,}251$; $p < 0{,}05$) (*specific questionnaire for Condylomata Acuminata-6*: $r = 0{,}263$; $p < 0{,}05$). Srednja vrednost *Dermatology life quality index* upitnika iznosila je 6,42 +/- 5,82. Anksioznost, zabrinutost i depresivnost ispoljavalo je oko 81,4% ispitanica, dok je 64,8% ispitanica osećalo sram i nesigurnost. Oko 54,3% se žalilo na bol i nelagodnost u genitalnoj regiji, dok je 57,7% ispitanica osećalo svrab i peckanje. Kod oko 50,7% ispitanica prisustvo genitalnih bradavica je negativno uticalo na njihov odnos sa partnerom dok se kod 62,1% ispitanica javio problem u vezi sa seksulanim odnosima. **Zaključak.** Naše istraživanje je pokazalo da prisustvo genitalnih bradavica negativno utiče na emocionalnu i seksualnu dimenziju kvaliteta života. Neophodno je sprovođenje bolje edukacije stanovništva u vezi sa infekcijom humanim papiloma virusom i zaštitom od infekcije.

Ključne reči: genitalne bradavice; kvalitet života; žensko; ankete i upitnici; psihički stres; HPV infekcije; znaci i simptomi

Introduction

Human papillomavirus (HPV) infection is the most common viral sexually transmitted infection [1]. It is estimated that about 80% of sexually active women get a HPV infection by the age of 50 [2]. HPV infection is usually asymptomatic and in about 70 – 90% of cases is transient; it spontaneously resolves within two years, due to good immune response of the body [3, 4]. The HPV infection is most commonly associated with sexually active people under the age of 25 [4]. Genital warts represent a clinical manifestation of a persistent HPV infection caused by low-risk HPV types (most commonly HPV

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Abbreviations

EQ-5D – European quality of life index version 5D
CECA-10– Specific Questionnaire for Condylomata Acuminata
DLQI – Dermatology life quality index
HRQoL – health related quality of life
VAS – visual analogue scale
HPV – human papillomavirus

types 6 and 11), characterized by the appearance of single or multiple papules on the skin and mucous membranes in the anogenital region [1, 3, 5]. The prevalence of genital warts, according to several epidemiological studies, is becoming more frequent [6].

Although the symptoms are usually less pronounced (itching, burning, increased vaginal discharge and bleeding), the genital warts affect the patients' quality of life [2, 7].

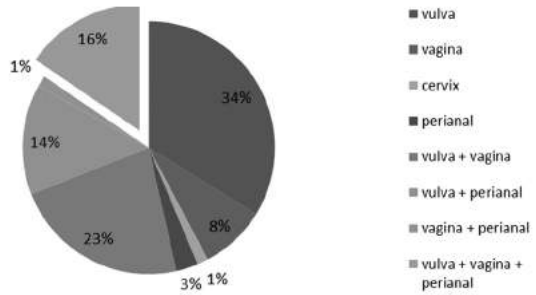
Studies have shown that genital warts have negative psychological effects. Women with genital warts suffer from stress and anxiety, as well as fear related to their social and sexual life being affected by the disease [7–10]. Patients with genital warts are overwhelmed with feelings of shame, embarrassment and guilt and feel less attractive, which adversely affects their sexual activity and enjoyment and may lead to issues with their partner [1, 7, 10]. Besides, there is a feeling of anger, guilt and concern for the future, as well as fear of getting cervical cancer [11].

The aim of this study was to examine the influence of genital warts on the quality of life of infected women and their psychological state by using standardized instruments for measuring the quality of life.

Material and Methods

This prospective study was conducted at the Clinic of Gynecology and Obstetrics of the Clinical Center of Vojvodina and it was approved by the Ethics Committee. Patients with genital warts were invited to participate in the study and after signing the Informed Consent, the following data were studied: age, education, marital status/current relationship, past sexual behavior, condom use, smoking, as well as the location of genital warts. Patients' knowledge about HPV infection was checked through non-standardized questionnaires designed for research purposes.

Three questionnaires were used to measure the patients' quality of life:



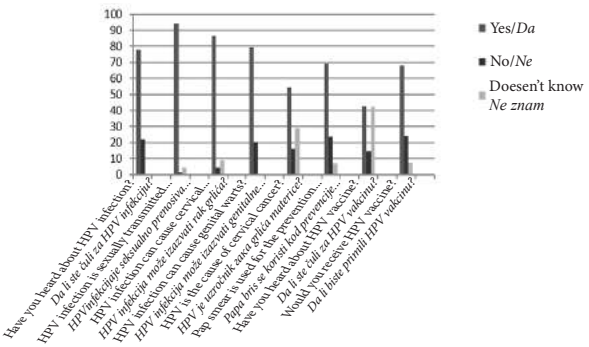
Graph 1. Location of genital warts
Grafikon 1. *Lokalizacija genitalnih bradavica*

1) The generic questionnaire European quality of life index version 5D (EQ-5D) measured the impact of the disease on the general health of patients. It consists of two parts. The first is the descriptive part in which patients describe their problems (three levels: I have no problem, I have a problem, I have a pronounced problem) in relation to five dimensions of health: mobility, self-care, performing usual activities, pain/discomfort and anxiety/depression. The second part of this questionnaire contains a visual-analogue scale (EQ-VAS) where the patients numerically assess their current health status (0 - the worst imaginable health state and 100 - the best imaginable health state) [9].

2) The disease-specific Specific questionnaire for Condylomata Acuminata-10 (CECA is a Spanish acronym for Specific Questionnaire for Condylomata Acuminata) was used to evaluate health related quality of life (HRQoL) in people with genital warts. The impact on the emotional dimension (the first 6 items (CECA-6)) and the sexual dimension (the last 4 items (CECA-4)) was evaluated using these 10 items. On a five-step scale, the respondents chose one answer (never = 5, rarely = 4, sometimes = 3, almost always = 2 and always = 1). The higher the score, the higher the quality of life. The total score of the questionnaire was 10 – 50, the total CECA-6 score ranged from 6 – 30 and CECA-4 from 4 – 20 [9, 10].

3) The general questionnaire for measuring the quality of life in dermatology - Dermatology life quality index (DLQI) consists of 10 multiple choice questions concerning to what extent the skin changes affect the quality of life (very much = 3, a lot = 2, a little = 1, not at all/not relevant = 0) in 10 areas. The total DLQI score ranged from 0 to 30. The higher the score, the greater the negative impact of skin changes on the quality of life: (0 – 1) - no effect, (2 – 5) - small effect, (6 – 10) - moderate effect, (11 – 20) – very large effect, (21 - 30) – extremely large effect [10, 12].

In the statistical data processing, the statistical package for social sciences (SPSS) version 20 was used. The numerical variables were calculated by the arithmetic mean and standard deviation, and categorical variables through percentages. To determine the existence of a difference and correlation between the parameters Student's t-test and F-test were used. The value $p < 0.05$



Graph 2. Patients' knowledge on HPV infection
Grafikon 2. *Znanje pacijentkinja o infekciji humanim papiloma virusom (HPV)*

Table 1. Socio-demographic characteristics of patients with genital warts
Tabela 1. *Socio-demografske karakteristike pacijentkinja sa genitalnim bradavicama*

Age (mean +/- SD)/*Godine (srednja vrednost +/- SD)*	29.72 +/- 8.54
Level of education (%)/*Stepen obrazovanja (%)*	
Primary school/*Osnovna škola*	7.1
Secondary school/*Srednja škola*	64.8
College/*Viša škola*	5.6
Faculty/*Fakultet*	22.5
Marital status (%)/*Bračno stanje (%)*	
Single/*Nije udata*	71.8
Divorced/*Razvedena*	8.5
Married/*Udata*	19.7
Currently has a partner (yes, %)/*Ima seksualnog partnera (da, %)*	69.01
Employment (%)/*Zaposlenost (%)*	
Student/*Student*	25.4
Employed/*Zaposlena*	53.5
Housewife/*Domaćica*	21.1
Smoking (yes, %)/*Pušač (da, %)*	49.3
Number of cigarettes per day (mean +/- SD)/*Broj cigareta dnevno (srednja vrednost +/- SD)*	13.46 +/- 7.97
Since what age (age; mean +/-SD)/*Početak pušenja (godine; srednja vrednost +/- SD)*	17.62 +/- 2.55
The first sexual intercourse (age +/- SD)/*Prvi polni odnos (godine +/- SD)*	18.33 +/- 2.33
Number of sexual partners (mean +/-SD)/*Broj seksualnih partnera (srednja vrednost +/- SD)*	4.32 +/- 2.49
Previous sexually transmitted infection (yes, %)/*Imala seksualno prenosivu infekciju (da, %)*	25.4
Delivery (yes, %)/*Porođaj (da, %)*	21.1
Abortion (yes, %)/*Prekid trudnoće (da, %)*	23.9
Uses condoms (yes, %) /*Koristi kondom (da, %)*	33.33

SD - standard deviation/*SD - standardna devijacija*

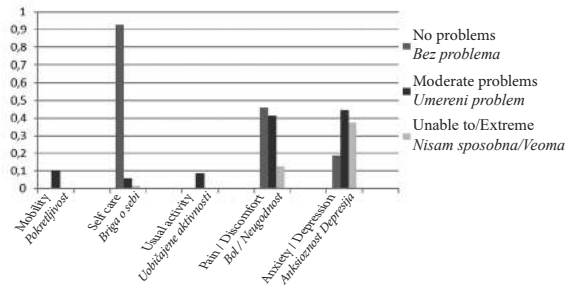
was considered statistically significant. The results are shown in tables and graphs.

Results

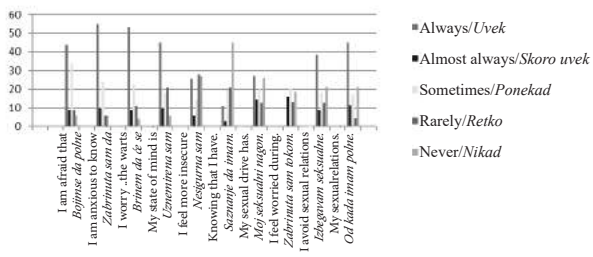
The general characteristics of the study group are presented in **Table 1**. On average, the patients were 29 years old (range 17 – 63 years), while 60.6% of them were aged 21 – 30 years. Of all the examinees, 64.8% were high school graduates and 53.5% of them were employed.

About 69% had a sexual partner, while only 19.7% of patients were married. The patients had their first sexual intercourse at the age of 18 years. On average, they had 4 sexual partners. About 79% of patients were nulliparous. Only 33.33% of patients used condoms, and 25.4% has already had a sexually transmitted infection. About 49.3% of patients were smokers. They smoked since the age of 17 years, on average 13 cigarettes per day.

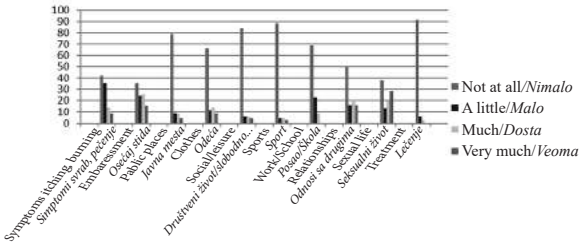
Graph 1 shows the location of genital warts. The genital warts most commonly showed up on the vulva (33.8%), vulva and vagina (22.54%), vul-



Graph 3. Effects of genital warts on 5 dimensions of health (EQ-5D)
Grafikon 3. *Uticaj genitalnoh bradavica na 5 dimenzija zdravlja European quality of life index version 5D*



Graph 4. Patients' answers to questions from the CECA-10 questionnaire (%)
Grafikon 4. *Odgovori pacijentkinja na pitanja iz Specific Questionnaire for Condylomata Acuminata -10 upitnika (%)*



Graph 5. Respondents' answers, to questions from the DLQI questionnaire (%)
Grafikon 5. *Odgovori pacijentkinja na pitanja iz Dermatology life quality index upitnika (%)*

va, vagina and perianal region (15.49%), as well as the vulva and perianal region (14.08%). Knowledge about HPV infection is shown in the **Graph 2**.

About 94 % of patients knew that HPV infection was a sexually transmitted disease, 79.4% knew that HPV infection could cause genital warts, and 86.6% of patients knew that HPV infection was associated with cervical cancer, but only 54.4% knew that HPV was the cause of cervical cancer. Fifty point eight percent of patients heard about HPV vaccine, while 42.9% of them knew that it was a vaccine for the prevention of HPV infection; 68.2% of patients would get vaccinated with this vaccine.

EQ-5D score

All patients completed the EQ-5D questionnaire. A very high percentage of them (81.4%) felt anxious and worried, 54.3% felt pain and discomfort, while significantly fewer patients had problems with mobility (10%), performing usual activities (8.6%) and self-care (7.1%) **(Graph 3)**.

The mean value of the Q-VAS visual scale was 70.37 +/- 20.8. Patients who had no difficulties with mobility ($F = 5.33$; $p < 0.05$), those without problems performing usual activities ($F = 9.46$; $p < 0.05$), as well as those who were not anxious/depressed ($F = 3.62$; $p < 0.05$) assessed their quality of life as higher.

CECA-10 questionnaire

Seventy respondents completed the CECA-6 questionnaire - emotional dimension, while 69 respondents completed the CECA-4 questionnaire (sexual dimension). The mean scoring for CECA-6 questionnaire was 15.63 +/- 5.99 while the mean scoring for CECA-4 questionnaire was 10.81 +/- 5.55. The mean scoring for CECA-10 questionnaire was 26.61 +/- 10.09. The patients with higher scores in CECA-6 and CECA-10 questionnaires had better quality of life (CECA-10: $r = 0.251$; $p < 0.05$) (CECA-6: $r = 0.263$; $p < 0.05$).

There was a statistically significant difference in CECA-6 and CECA-10 scores between housewives and students (CECA-6: $F = 5.11$; $p < 0.05$; CECA-10: $F = 3.56$; $p < 0.05$) as well as between patients without and with sexually transmitted infections, respectively (CECA-6: $F = 0.757$; $p < 0.05$; CECA-10: $F = 0.150$; $p < 0.05$) **(Graph 4)**.

Dermatology Life Quality Index

The dermatological questionnaire was completed by all patients. The average score of the questionnaire was 6.42 +/- 5.82. The patients who previously had sexually transmitted infections had more skin problems than those who had no sexually transmitted infections ($F = 0.856$, $p = 0.005$). The patients who had more skin problems estimated that they had a lower quality of life ($r = -0.274$, $p < 0.05$). The patients with higher DLQI scores also had lower scores for CECA-6 ($r = -0.554$; $p < 0.01$), CECA-4 ($r = -0.468$; $p < 0.01$), and CECA-10 questionnaires ($r = -0.598$; $p < 0.01$) **(Graph 5)**.

Discussion

The genital warts primarily cause stress among the infected women and may have a negative impact on their psychological state and social life. These women have expressed negative emotions such as shame, fear and anxiety, worry about reproductive health, the possibility of having children, as well as about the possibility of further spreading the infection. For that reason, they suffer from an elevated level of anxiety and distress [1–3]. They often blame their partners for getting the infection, which negatively affects their sexual life and the relationship. Similarly, the fear of disease progression negatively affects both their sexual life and health [1–3, 13].

In his study, Waller has proved that the level of knowledge about HPV infection is associated with an emotional reaction and feelings of stigma, shame and anxiety. The knowledge about HPV infection being sexually transmitted, and ignorance about the fact that it is highly prevalent in the population of sexually active women is associated with the highest level of feeling shame and stigma [13]. In our study, although a high percentage of patients heard about HPV infection and answered the questions correctly, generally speaking, the knowledge about HPV virus and the possible consequences of HPV infection is insufficient [6, 14]. We believe that the limitation of the questionnaire on the knowledge about HPV infection is that the questions are direct and suggestive. The real level of the respondents' knowledge on HPV infection would have been checked more successfully if they had to fill in the answers themselves [14].

In our study, a large number of patients (81.4%) were concerned, anxious or depressed due to having genital warts and they estimated that it negatively affected their quality of life. Ninety four point four percent of patients were worried that the warts would not disappear and that they would not completely recover from the infection. About 95.8% of patients worried that the warts would spread causing complications. Additionally, more than half of the patients (54.3%) felt pain and discomfort in the genital region which represented a relevant dimension that genital warts negatively affected their quality of life. Furthermore, 57.7% of women found itching and burning to be significant symptoms, while 64.8% of them felt shame and insecurity [3, 15–18].

The presence of genital warts has more negatively influenced the emotional dimension of the quality of life among housewives compared to students, as well as among women who have not previously had sexually transmitted infections compared to those who had. This may be the consequence of the fact that housewives and women who have not had any sexually transmitted infections have less knowledge about HPV infection, and hence the appearance of genital warts had a significantly higher impact on their quality of life and emotional health.

According to previous studies, HPV infection also has a significant negative impact on the sexuality of women because: a) the infection is associated with changes that occur on the genital organs which play a key role in the female eroticism; b) it may cause vulvodynia and dyspareunia [1]. Pain and discomfort in the genital region, feeling shame and embarrassment, knowledge that they have a viral sexually transmitted infection that can potentially lead to serious health issues affects the sexual life of women in a considerably negative manner [2, 3, 19].

In our study, genital warts showed to have a great impact on the sexual life of women. In about 74.3% of the patients, sexual drive decreased as well as the quality and frequency of sexual intercourse. About 62% of the patients had problems during sexual intercourse, while almost 40% avoided sexual intercourse. One patient (1.4%) even ceased having sexual intercourse after getting genital warts.

Conclusion

Our examination showed that genital warts adversely affected the emotional and sexual dimension of women's quality of life. Although it is a benign disease, the patients suffer from anxiety and depression. They are afraid that they will not recover from the infection and that warts will spread and cause complications. Better education of the population on human papillomavirus infection and its prevention is necessary.

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CONDITIONED PAIN MODULATION ASSESSMENT USING CONTACT HEAT AS CONDITIONING STIMULUS AND TWO DIFFERENT TEST STIMULI

PROCENA USLOVNE MODULACIJE BOLA IZAZVANE KORIŠĆENJEM KONTAKTNE TOPLOTE KAO USLOVLJAVAJUĆEG STIMULUSA I DVA RAZLIČITA TEST STIMULUSA

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Summary

Introduction. The objective of the study was to determine the potentials and reliability of conditioned pain modulation effect in healthy population by application of a conditioning contact heat stimulus, and heat and pressure applied to the low back region as a test stimulus. **Material and Methods.** The study included 33 healthy subjects (average age 25.73 ± 5.35 years). Pressure and heat pain thresholds were examined on the paravertebral musculature of the lower back as test stimuli. Contact heat was used on the contralateral forearm as a conditioning stimulus. Conditioned pain modulation was calculated as the difference between pain thresholds after and before conditioning stimulus application. To assess the reliability, identical testing was performed 14 ± 2 days later. **Results.** The pressure and heat pain thresholds, after the conditioning stimulus, were significantly higher compared to pain thresholds obtained before the conditioning stimulus (101,63 N/cm^2 ± 45,21N/cm^2 vs 82,15 N/cm^2 ± 36,15 N/cm^2, t = -7,528, p < 0,001 and 47,08° C ± 2,19° C vs 45,00 ± 3,05° C, t = -6,644, p < 0,001, respectively). The reliability of the same protocol, measured 14 ± 2 days after the previous testing, showed good reliability of the pressure pain threshold (intraclass correlation coefficient = 0,636, 95% confidence interval 0,240 - 0,825), and fair of the heat pain threshold (intraclass correlation coefficient = 0,435, 95% confidence interval - 0,070 - 0,713). **Conclusion.** Conditioned pain modulation was successfully induced by contact heat applied via a thermode, a conditioning stimulus. The reliability of this method of testing proved to be fair when it comes to the heat pain threshold and good when it comes to the pressure pain threshold.

Key words: Pain Measurement; Pain; Pain Threshold; Heating; Pressure; Conditioning (Psychology); Nociception; Analgesia; Back Muscles

Sažetak

Uvod. Cilj ove studije bio je da se utvrdi potencijal i pouzdanost izazivanja efekta uslovne modulacije bola u zdravoj populaciji korišćenjem lokalno aplikovane toplote kao uslovljavajućeg stimulusa, a toplote i pritiska aplikovane na donji deo leđa kao test-stimulusa. **Materijal i metode.** Istraživanje je obuhvatilo 33 zdrava ispitanika (prosečne starosti 25,73 ± 5,35 godina). Kao test-stimulusi ispitivani su pragovi za bol za pritisak i toplotu na paravertebralnoj muskulaturi donjeg dela leđa. Kontaktna toplota je primenjena na kontralateralnoj podlaktici kao uslovljavajući stimulus. Uslovna modulacija bola izračunata je kao razlika između pragova bola nakon i pre primene stimulusa. Kako bi se procenila pouzdanost protokola, identično ispitivanje je sprovedeno nakon 14 ± 2 dana. **Rezultati.** Prag bola za pritisak i toplotu koji su dobijeni nakon uslovljavajućeg stimulusa bili su značajno viši u poređenju sa pragovima bola dobijenim pre primene ovog stimulusa (101,63 N/cm^2 ± 45,21 N/cm^2 vs 82,15 N/cm^2 ± 36,15 N/cm^2, t = -7,528, p < 0,001 i 47,08° C ± 2,19° C vs 45 ± 3,05° C, t = -6,644, p < 0,001, respektivno). Procena pouzdanosti protokola, merena 14 ± 2 dana nakon inicijalnog ispitivanja pokazala je dobru pouzdanost za prag bola za pritisak (ICC = 0,636, 95% CI 0,240–0,825) i osrednju za prag bola za toplo (ICC = 0,435, 95% CI -0,070–0,713). **Zaključak.** Kontaktna toplota aplikovana preko termode pokazala se kao efikasan uslovljavajući stimulus jer je uspešno indukovala uslovnu modulaciju bola. Puzdanost ovog načina testiranja pokazala se kao osrednja kada je bio u pitanju prag bola za toplo, odnosno dobra kada je u pitanju bio prag bola za pritisak.

Ključne reči: merenje bola; bol; prag bola; zagrevanje; pritisak; uslovljavanje; nocicepcija; analgezija; leđni mišići

Introduction

The pain perception is regulated by the intrinsic pathways of pain modulation which can increase or reduce the harmful afferent stimulus. The inhibitory mechanism of modulation is known as endogenous analgesia [1]. It operates through several mechanisms mediated mainly by neurotransmitters - serotonin, dopamine, and noradrenaline [2]. In 1979, Le Bars et al. described the inhibitory mechanism, diffuse noxious inhibitory controls (DNIC). They proved a decrease of the rats' response to pain in the neurons of the posterior horn of the spinal cord after application of nociceptive stimulus distant from the excitatory peripheral receptive field [3]. Today, endogenous analgesia can be examined in humans in experimental

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Abbreviations

CPM – conditioned pain modulation
PPT – pressure pain threshold
HPT – heat pain threshold
ATS – advanced thermal stimulation
CS – conditioning stimulus
NPS – numerical pain scale
ICC – intraclass correlation coefficient
SEM – standard error of measurement
TS – test stimulus

conditions using a paradigm of conditioned pain modulation (CPM) [4]. The CPM involves lowering the intensity of pain or boosting the threshold of pain to a particular stimulus (test stimulus), during or after the application of another painful stimulation (conditioning stimulus) applied to a distant part of the body [4, 5]. Healthy people are generally able to successfully inhibit pain which is an indicator of the efficacy of CPM, while in patients with chronic pain CPM shows a lower efficiency [6]. The studies have shown a dysfunctional CPM in different chronic painful conditions, such as fibromyalgia [7], temporomandibular disorders [8], irritatable bowel syndrome, migraine, tension headache [9], back pain [10].

There are numerous CPM research protocols which differ by the type of test and conditioning stimulus of the subject tested, temporary characteristics (parallel and sequential simulation), and pain parameters on stimulus test (pain resistance, intensity, and temporal summation). The most commonly used type of conditioning stimulus is cold pressor arm wrap test. Another frequent method is hot water immersion. There are numerous modalities used as stimulus tests - thermal, mechanical, electrical, chemical, all of them applied to different parts of the body [5]. However, there is no gold standard when it comes to CPM research, nor any proof that a specific protocol is superior to another [11]. Even though there are many conditional pain modulation research protocols, only a few have examined this effect by applying the test stimulus to low back region [12–14]. In their study, Gerthard et al. claimed that it was more clinically relevant to apply stimulus test on the area which already exhibited pain [12].

The objective of the study was to determine the potentials and reliability of conditioned pain modulation effect in a healthy population by using the contact heat as conditioning stimulus, and heat and pressure applied to the low back region as a test stimulus. We have put forward the hypothesis that a significant effect of conditioned pain modulation can be induced with good reliability.

Material and Methods

The study included 33 healthy subjects, students of the Faculty of Medicine of the University of Novi Sad, with an average age of 25.73 ± 5.35 years. The research was approved by the Ethics Committee of the Faculty of Medicine, University of Novi Sad and all respondents signed an informed consent.

The research inclusion criterion was age above 18 years. The exclusion criteria were: chronic pain or any other current pain (headache, injury, etc.), diabetes, uncontrolled arterial hypertension, cardiovascular or pulmonary disease with complications, psychiatric disorders, use of analgesics, tranquilizers, antidepressants, anticonvulsants, not understanding the task.

All subjects were instructed not to take analgesics or alcohol for 24 hours before taking part in the study, to get enough sleep before the examination, not to have coffee/caffeine-containing products for 4 hours before testing, and not to get into any heavy physical activity.

After the respondents were informed and acquainted with the procedure and devices, the testing started. In the first part of the research, we investigated the pressure pain threshold (PPT) and the heat pain threshold (HPT). A digital algometer was used for PPT (Wagner Instruments, FDX-50), with rubber tip of 1cm^2, at the paravertebral musculature of the lumbar segment (2 cm lateral to the spinous process L3) [13]. The pressure was gradually increased at the rate of 5 N/s until respondents said to stop, after the feeling turned painful, the point when we recorded the N/cm^2 value. There were 3 measurements at each point with 10s intervals, and the average value was recorded as the final. Tests were randomized between left and right sides [12].

The HPT testing was performed using a device with advanced thermal stimulation (ATS) Thermode of 30 x 30 mm (Pathway Pain and Sensory Evaluation System, Medoc Ltd, Ramat Yishai, Israel) at 2 points: 1) paravertebral musculature of the lumbar segment (2 cm lateral from spinous process L1) and 2) on the proximal volar side of the opposite forearm (C8 dermatome) [13]. Tests were randomized between the left and right sides [12]. When testing HPT, the initial temperature of 32° C was increased at a rate of 0.7° C/s. The examinees pressed the stop button as soon as the feeling of warmth turned into a burning sensation, feeling of puncture or pain. At that moment, the temperature was lowered to the initial temperature at the speed of 7° C/s. Four heat stimulations were performed with 10s intervals, and the last three measurements were taken into account for the final HPT value. The pain thresholds for heat on the forearm were first tested while the subject was comfortably lying on the back. Then the examinees laid down on the stomach, and PPT was examined first, followed by HPT on the lumbar segment at previously defined places.

After examining the pain thresholds, a 15 minutes pause was made, after which testing of effects of CPM was performed. In order to induce and test CPM, stimulation of the conditioning stimulus (CS) - contact heat on the proximal volar side of the opposite forearm (C8 dermatome) with the ATS thermodynamic pathway device was performed while the subject was lying on the stomach. The intensity of CS was determined individually as the HPT value (determined by the original measurement on the same

forearm) plus + 1° C [13]. In order to guarantee safety of the subjects, as well as activate the nociceptors, adjustments have been made in the following order: 1. if the HPT + 1° C was higher than 47° C, CS temperature was lowered to 47° C, 2. in subjects where HPT + 1° C was below 40° C, CS was adjusted to 40° C [12]. The CS stimulation starting temperature was 32° C and kept increasing at the rate of 0,7° C/s until the predetermined temperature that lasted 120s (Ramp and Hold program) [12, 13]. During those 120s subjects have measured the pain in 30s intervals on a numerical pain scale (NPS) from 0 – 100, in which case 0 meant "no pain" while 100 meant "worst imaginable pain" [13]. In case a subject could not tolerate the assigned temperature or have measured the pain with > 90/100 temperate was lowered by 0.5° C although keeping pain intensity above 45/100 on NPS. This way we have guaranteed the safe CS that is intensive enough to cause CPM effect [13]. In case some of the subjects have declared pain less than 45/100 stimulation was interrupted and the temperature was increased by 1° C until the pain has reached at least 45/100 [13]. After 120s, the temperature went back to default level at the rate of 7° C and CS stimulation was finalized [13]. One minute after the end of CS (sequential paradigm), second test stimulus (TS2) in the lumbar area (PPT first then HPT) were tested again [11, 12]. Two types of stimuli have been used as test stimulus: PPT and HPT measured in a lumbar segment. For values of the first test stimulus (TS1) PPT and HPT values were taken during pain threshold on paravertebral musculature of the lumbar segment [11, 13, 15]. The difference between TS values after and before CS (TS2 - TS1) resembled the values of CPM. Positive value underscores the existence of CPM effect (elevation of pain threshold) while negative shows nonexistence of CPM effect (pain facilitation) [11].

In order to test the reliability of this protocol, a retest was made, and the same subjects were called for identical testing after 14 ± 2 days.

A subgroup of 18 subjects, underwent the same protocol 30 days after the retest, except they did not receive CS. In this way, we wanted to test the magnitude of the error and to investigate the difference between the results of the HPT and PPT, 15 minutes later.

The normality and distribution of the sample were evaluated for skewness and kurtosis. Paired-Samples T-test was used in order to investigate differences between TS threshold before and after CS. The same test was used to compare CS temperatures and pain intensities at the test and retest after two weeks. For reliability testing intraclass correlation coefficient (ICC) (two way mixed, absolute agreement) was used [16]. The ICC values < 0,4 were interpreted as poor, 0,4 - 0,59 as fair, 0,6 - 0,75 as good, and > 0,75 as excellent reliability [17–19]. P values ≤ 0,05 were considered statistically significant.

For the subgroup of 18 subjects who underwent testing without CS, the standard error of measurement (SEM) was calculated according to the formula SEM = SD* (√(1-ICC). This measure was used to estimate the range of measurements that might have occurred in subjects after repeated testing [20]. The value of the SEM was added to the HPT and PPT and was transformed into percentages to show the highest limit of SEM in repeated measurement. Data analyses were performed using the statistical package for the social sciences (SPSS) 23.

Results

The sample included 33 students and the majority (23) were female (69.7%). Their average body weight was 66,44 ± 16,05 kg, average height 173,03 ± 9,28 cm, and body mass index (BMI) 22,01 ± 3,93 kg/m^2.

The average conditioned heat stimuli temperatures and average pain intensities on the test and retest are shown in **Table 1**. An average temperature of the CS was significantly higher on the retest, although this was not accompanied by significantly higher pain intensity reported by subjects.

Significantly higher pain thresholds were achieved after CS for both pressure and heat at test and retest after 14 days **(Table 2)**. The PPT after CS was higher (13.79%) on the retest (23.71%) compared to the basic

Table 1. Pain intensity during conditioning stimulation
Tabela 1. *Intenzitet bola tokom stimulacije uslovljavajućim stimulusom*

	CS* (° C ± SD)	NPS** (Average ± SD)/*Nps*** *(Prosečna vrednost ± SD)*					
		0s	30s	60s	90s	120s	Average
Test/*Test* (n=33)	45,3±1,7	60,3±10,9	61,4±11,6	65,8±13,1	68,0±18,9	71,7±17,6	65,4±11,9
Retest/*Ponovljen test* (n=32)	46,0±1,3	63,4±12,9	64,8±12,5	67,5±14,6	71,1±14,6	73,2±15,1	68,0±11,6
t	-2,432	-1,824	-1,722	-0,956	-1,751	-0,913	0,368
p	0,021	0,078	0,095	0,347	0,090	-1,782	0,085
Intracalss correlation coefficient†	0,675	0,737	0,526	0,720	0,815	0,821	0,771
95% Confidence interval‡	0,336-0,841	0,469-0,871	0,062-0,765	0,429-0,863	0,623-0,910	0,636-0,912	0,536-0,888

Legend/*Legenda:* *Conditioning stimulus/*Uslovljavajući stimulus*; **Numerical rating pain scale/*Numerička skala bola*; †Intracalss correlation coefficient/*Intraklasni koeficijent korelacije*; ‡Confidence interval/*Interval pouzdanosti*

Table 2. Description of the stimulation scores before and after the conditioning stimulus
Tabela 2. *Rezultati merenja praga bola pre i posle uslovljavajućeg stimulusa*

	Test stimulus *Uslovljavajući stimulus*	Before CS* *Pre CS**	After CS* *Posle CS**	CPM** response (After CS* – Before CS*)/*CPM*** *odgovor (Pre CS* – Posle CS*)*	t	p
Test/*Test* (n=33)	PPT† (N/cm²±SD)	82,15±36,15	101,63±45,21	19,48±14,86	-7,528	<0,001
	HPT‡ (°C±SD)	45,00±3,05	47,08±2,19	2,08±1,80	-6,644	<0,001
Retest/*Ponovljen test* (n=32)	PPT† (N/cm²±SD)	84,36±28,15	96,00±33,32	11,63±14,15	-4,576	<0,001
	HPT‡ (°C±SD)	46,38±2,14	47,51±1,53	1,13±1,24	-5,146	<0,001

Legend/*Legenda:* *Conditioning stimulus/*Uslovljavajući stimulus*; **Conditioned pain modulation/*Uslovna modulacija bola*; †Pressure pain threshold/*Prag bola na pritisak*; ‡Heat pain threshold/*Prag bola na toplotu*

values. The PHT after CS was higher (2.43%) on the retest (4.62%) compared to the basic values.

The ICC was good for the PPT (ICC = 0.636), and fair for the PHT (ICC = 0.435). **Table 3** provides more detailed results.

The calculated ICC for the PPT was 0.98, while for the HPT it was 0.96. In the subgroup of 18 subjects, who underwent two consecutive testing without CS, the SEM was 4.09 N/cm² and 4.08 N/cm² for the PPT, in percentage 105.01% and 104.99% (after 15 minutes without CS). The SEM for the HPT was 0.5° C and 0.43° C and in percentage we got 101.08% and 100.93% (after 15 minutes without CS). The average SEM percentage for the PPT was 105.00% and 101.00% for the HPT. It was determined that any CPM value over 5% for the PPT and over 1% for the HPT indicated effect greater than the inherent error of measurement. The further calculation showed that a significant CPM for the PPT was obtained in 28 (84.8%) and 31 (93.9%) subjects for the HPT.

Discussion

Although there are many different protocols for testing CPM, the data are insufficient to support a specific CPM protocol [11]. There is also a rising need for more practical, reliable, clinically oriented protocol. Availability of equipment, need for a specific location for test stimulus application, an overly complicated procedure for patients (e.g., limb immersion into cold or hot water) are some of the reasons for adaptation of the existing protocols. Low back is probably one of the most frequent locations of chronic pain [21]. At the Medical Rehabilitation Clinic, we treat a substantial number of patients with low back pain, so we have modified some previous protocols in order to develop a new one, more practical for clinical use [5, 13, 15, 18, 22]. Our protocol produced highly significant CPM effect for both TS (HPT and PPT). Although the majority of papers suggest that PPT is the preferred way to test CPM [18, 23] our results support HPT as an eligible TS. There is a lack of reports on the percentage of participants not tolerating TS in the literature [23]. It is most likely that these short-lasting painful stimuli were well tolerated, which was the case in our study. Different modalities are used as CS in studies investigating CPM [18, 19, 24–26]. There are some recommendations that immersion in cold water (8 – 12° C) up to 2 minutes and immersion into hot water (46.5° C) up to 1 minute are sufficient to induce inhibitory mechanisms, and are well tolerated by examinees [23]. In this study, the temperature of the CS was "individualized" so it could provide minimal above threshold painful stimulus for induction of the descendent inhibitory mechanisms [13]. Duration of the CS of 2 minutes was sufficient, a similar protocol for CS was proposed by other researches using thermode [12, 13]. Subjects in our study tolerated both CS and TS well, and only a few of them have reported temporary redness in the region of the CS application which lasted

Table 3. Intraclass correlation coefficient and 95% confidence intervals in test-retest reliability
Tabela 3. *Intraklasni koeficijent korelacije i 95% interval pouzdanosti primenjenog provg testa i ponovljenog testa*

	Intracalss Correlation Coefficient *Intraklasni koeficijent korelacije*	95% Confidence Interval *95% Interval pouzdanosti*
PPT† test before CS*/*PPT† test pre CS**	0,838	0,666-0,921
PPT† test after CS*/*PPT† test posle CS**	0,901	0,794-0,952
HPT‡ test before CS*/*HPT‡ test pre CS**	0,709	0,296-0,870
HPT‡ test after CS*/*HPT‡ test posle CS**	0,829	0,647-0,917
CPM** response PPT†/*CPM** odgovor PPT†*	0,636	0,240-0,825
CPM** response HPT‡/*CPM** odgovor HPT‡*	0,435	-0,070-0,713

Legend/*Legenda:* *Conditioning stimulus/*Uslovljavajući stimulus*; **Conditioned pain modulation/*Uslovna modulacija bola*; †Pressure pain threshold/*Prag bola na pritisak*; ‡Heat pain threshold/*Prag bola na toplotu*

for several hours. There were no other adverse events reported, and all subjects successfully completed the planned protocol.

The issue regarding non-responders is very important [23]. Locke et al. found that a significant CPM effect was an increase in PPT values from baseline greater than 5.3% [27]. This percentage represents the calculated inherent error of measurement. In our study, we found that SEM was 5% for the PPT and 1% for the HPT, which was similar to results of Locke et al. [27]. Our results showed that CPM effect was reached in 85% of subject for the PPT that was by 10% lower than found by Locke et al. [27], while CPM effect for HPT was reached in 94% of subjects. Interestingly, CPM effect was present in several subjects for the HPT, but it was lacking for the PPT and vice versa.

In the majority of studies, immersion into the hot or cold water was a preferable option for the CS as it has shown better reliability [9, 18, 23]. However, usage of thermode as CS has its benefits, such as the ability to test pain thresholds in the specific region such as the low back region. Parallel paradigm is more frequently used compared to sequential paradigm [9, 23], although there is no clear recommendation which one is more reliable [23]. One can argue that the simultaneous application of two painful stimuli might confuse the examinees and make it harder to estimate the pain thresholds or pain intensities. Therefore, we used a sequential paradigm, where we divided CS and TS with one-minute pause in between. It is estimated that CPM effect probably lasts 10 – 15 minutes after CS [26]. During the research, we were able to finish all intended testing in a time frame of less than 5 minutes including a pause of one minute after CS. Time periods between test-retest vary in the literature from 2 days to 3 months [28–32]. We have decided to perform the retest after 14 ± 2 days.

Although thermode in our protocol was used on a relatively small area (9 cm^2), we obtained good ICC scores for CPM calculated via PPT as a TS and fair ICC scores for CPM calculated via HPT. These results are encouraging, having in mind that the previous studies showed poor reliability for contact heat as CS [18, 23]. It could be that the pain stimulus duration, as well as higher pain intensity of the CS in our study, was the reason why we obtained better reliability compared to the previous studies that used contact heat as CS. Some evidence suggest that pain intensity of the CS is an important factor for CPM magnitude [33].

In some of the previous studies, temperature used to provoke predetermined pain intensity was recorded, and the capacity of the CPM was calculated according to these values [18]. Other authors claim that pain threshold, which was used in our study, was a superior method for CPM testing [23].

The authors are searching for more practical and less time consuming protocols [18]. However, this must not decrease the reliability.

Conclusion

Conditioned pain modulation was successfully induced by contact heat applied via thermode as a conditioning stimulus. However, the reliability varied from good for pressure pain threshold as a test stimulus to fair for the heat pain threshold as a test stimulus. This protocol could be beneficial for testing different groups of patients, being a simple, quick and safe procedure. Therefore, we suggest further testing and recommend a comparison of the conditioned pain modulation phenomenon between genders and different age groups, as well as testing its reliability in larger samples.

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EPIDEMIOLOGICAL CHARACTERISTICS OF THE MOST COMMON ZOONOSES IN THE CENTRAL BANAT DISTRICT OF VOJVODINA FROM 2002 TO 2016

EPIDEMIOLOŠKE KARAKTERISTIKE VODEĆIH ZOONOZA U SREDNJOBANATSKOM OKRUGU VOJVODINE U PERIODU OD 2002. DO 2016. GODINE

Svetlana POPOV[1], Mirjana ŠTRBAC[2] and Mioljub RISTIĆ[2,3]

Summary

Introduction. The aim of this study was to determine the epidemiological characteristics of the most common zoonoses in the Central Banat District of Vojvodina. **Material and Methods.** A descriptive epidemiological study was conducted using data of the Centers for Disease Control and Prevention of the Institute of Public Health of Vojvodina and Public Health Institute Zrenjanin. The data for this study were collected from January 1, 2002 to December 31, 2016. **Results.** Apart from Q fever, which showed a stable incidence, the trend of incidence rates of other two zoonoses decreased between 2002 and 2016. Q fever was three times more common in males than females, while the incidence of males and females was similar among patients with Trichinellosis and Salmonellosis. Regarding the age distribution, the highest average incidence rate (12/100,000) of Trichinellosis was reported in patients aged 20–39 years; Salmonellosis predominated among patients aged 0–19 years (64.6/100,000), and Q fever in the 20–39 age group (9.2/100,000). Pork products were the most common source of outbreaks of Trichinellosis, while cakes and cookies were the most common source of outbreaks of Salmonelloses. Out of 92 interviewed patients with Q fever, 50 (54.3%) had a direct daily contact with their domestic animals during the maximum incubation period. **Conclusion.** In order to improve evaluation of epidemiological characteristics of the most common zoonoses and consequently their control in a timely manner, efficient exchange of information between health sectors for humans and animals is necessary, along with continuous education of food handlers and the general population of the Central Banat District of Vojvodina.

Key words: Zoonoses; Epidemiology; Disease Outbreaks; Q Fever; Trichinellosis; Salmonella Infections; Public Health Surveillance

Sažetak

Uvod. Cilj ove studije bio je da se odrede epidemiološke karakteristike vodećih zoonoza u Srednjobanatskom okrugu Vojvodine. **Materijal i metode**. Sprovedena je deskriptivna epidemiološku studija upotrebom podataka centara za kontrolu i prevenciju bolesti Instituta za javno zdravlje Vojvodine i Zavoda za javno zdravlje Zrenjanin. Podaci za ovu studiju prikupljeni su u periodu od 1. januara 2002. do 31. decembra 2016. godine. **Rezultati**. Osim *kju* groznice, koja je pokazala stabilan trend incidencije, trend incidencije za druge dve zoonoze u periodu od 2002. do 2016. godine bio je opadajući. *Kju* groznica je tri puta češće registrovana kod muškaraca u odnosu na žene, dok je učestalost obolevanja kod muškaraca i žena bila slična među obolelima od trihineloze ili salmoneloza. Što se tiče uzrasne distribucije, najviša prosečna stopa incidencije (12/100.000) trihineloze zabeležena je kod pacijenata uzrasta 20–39 godina, salmoneloza među pacijentima uzrasta 0–19 godina (64,6/100.000), a *kju* groznice u uzrastu 20–39 godina (9,2/100.000). Najčešće inkriminisane namirnice za trihinelozu bili su proizvodi od svinjskog mesa, dok su torte i kolači bili najčešći izvor infekcije za salmoneloze. Od 92 obolele osobe od *kju* groznice, ukupno 50 (54,3%) pacijenata je dalo podatak o svakodnevnom direktnom kontaktu sa domaćim životinjama tokom maksimalnog perioda inkubacije ovog oboljenja. **Zaključak**. Da bi se unapredila mogućnost procene epidemioloških karakteristika vodećih zoonoza, a time obezbedila i pravovremenost njihove kontrole, potrebna je brza razmena informacija između sektora za zdravstvenu zaštitu životinja i sektora za zaštitu zdravlja ljudi, uz kontinuiranu edukaciju osoba u prometu namirnicima i opšte populacije na teritoriji Srednjobanatskog okruga.

Ključne reči: zoonoze; epidemiologija; pojave bolesti; Q groznica; trihineloza; salmoneloza; nadzor javnog zdravlja

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Introduction

Zoonotic diseases (zoonoses) are infections that are spread between animals and people [1, 2]. Zoonoses have a significant public health impact worldwide. Due to the lack of control strategies and education, zoonoses have a higher incidence rate in developing than in developed countries [3].

It is estimated that zoonoses account for 58% to 61% of all communicable diseases causing illness in humans worldwide [4]. The estimates showed that 60.3% of the emerging infectious diseases were zoonoses [5].

According to the annual reports, the most common zoonoses in the Autonomous Province of Vo-

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Abbreviations

CBDV – Central Banat District of Vojvodina
APV – Autonomous Province of Vojvodina
EU/EEA – European Union/European Economic Area
US – United States

jvodina (APV) as well as in the Central Banat District of Vojvodina (CBDV) are Trichinellosis, Salmonellosis and Q fever [6].

Since 1966, when mandatory notification of Trichinellosis was introduced in the APV, this disease has been registered continuously with small or large outbreaks. The largest outbreak of Trichinellosis in the APV, with 907 human cases (the incidence rate of 45/100,000 inhabitants), was registered in 1985 [6].

A mandatory notification of Salmonellosis in the APV has been introduced since 1976. During the last 25 years of the XX century, there were several epidemics of human Salmonellosis that were registered mainly after eating improperly cooked contaminated foods [6, 7].

Q fever was the most common zoonotic disease in the APV until the 1990s, with the average annual incidence rate of 10.2/100.000 (incidence rate range: 3.8 - 20.4/100.000) [8]. After 1976, when 900 human Q fever cases were reported, which was the largest outbreak of the disease in Europe, Q fever remained an endemo-epidemic disease in the APV [9]. Until the 1990s, the disease showed a seasonal peak and it was registered mainly during the last winter months, as well as in the early spring months (during the lambing season) [10]. The CBDV was previously identified as a district with a potentially endemic area for Q fever [11].

The aim of this study was to determine the epidemiological characteristics of the most common zoonoses in the CBDV during 15 consecutive years (from 2002 to 2016).

Material and Methods

The study was conducted in the CBDV, one of seven administrative districts of APV, Serbia. It lies in the geographical region of Banat. According to the 2011 Census results, it has a population of 186.851 inhabitants. A retrospective, observational study was conducted during the period from January 1, 2002 to December 31, 2016. The data for this study were obtained from the communicable disease registries of the Centers for Disease Control and Prevention of the Institute of Public Health of Vojvodina and Public Health Institute Zrenjanin, which are responsible for the collection of passive surveillance data on the diseases for which notification is mandatory. Case inclusion criteria were based on the clinical signs specific for the analyzed zoonoses. The data on zoonoses in humans, collected as part of the routine system of infectious disease surveillance in the CBDV, included information on sociodemographic characteristics of patients, the date of disease onset, as well as the information related to the source and mode of transmission during the outbreak.

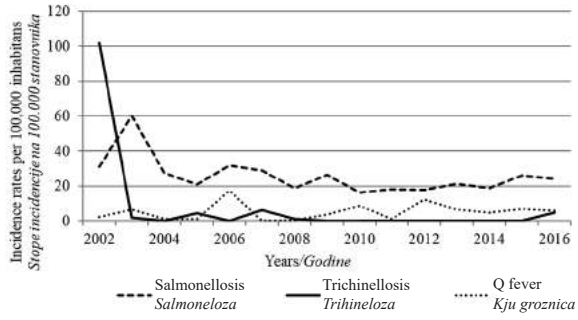
We used basic statistical indicators, general and specific incidence rates. Incidence rates were calculated using the annual number of registered human cases of zoonoses as the numerator and the number of inhabitants in the CBDV, according to the two Censuses in the Republic of Serbia (in 2002 and 2011) as the denominator, multiplied by 100.000 inhabitants per year.

Results

Structure and distribution of the most common zoonoses in the CBDV, 2002–2016

Throughout 2002–2016, the highest incidence rate (101.7/100.000) of Trichinellosis was registered during 2002 (212/249; 85.1%); Salmonellosis during 2003 (60/100.000), and Q fever during 2006 (17.3/100.000). Apart from Q fever, which showed a stable trend of incidence, the trend of incidence rate of other two zoonoses in the CBDV decreased between 2002 and 2016 (**Graph 1**).

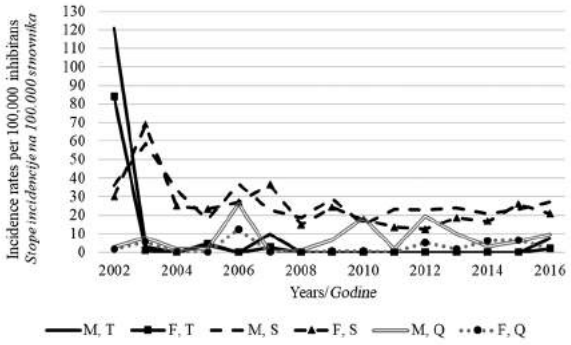
The average annual incidence rates of the three most common zoonoses with gender distribution are shown in **Graph 2**. The average annual incidence of Trichinellosis among males was 9.8/100.000, and 6.3/100.000 among females. The prevalence of Salmonellosis in males and females



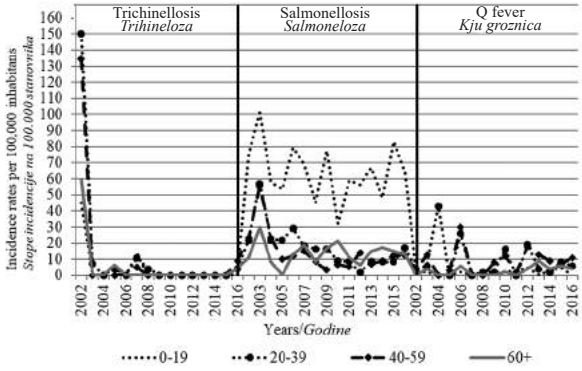
Graph 1. Incidence rates of the most common zoonoses in the Central Banat District, 2002–2016
Grafikon 1*. Učestalost vodećih zoonoza u Srednjobanatskom okrugu u periodu od 2002. do 2016. godine*

was nearly equal (51% vs. 49%), while Q fever was three times more frequently recorded in males than in females, with the average annual incidence rates of 7.9/100.000 and 2.9/100.000, respectively.

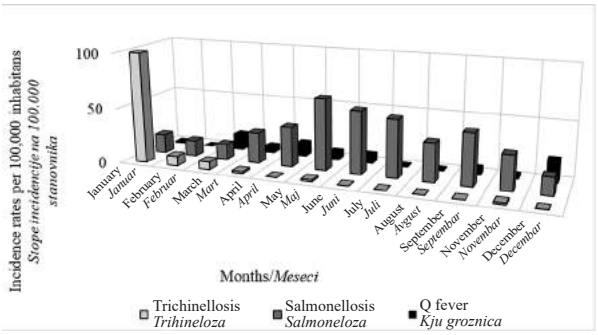
With regard to the age distribution, the highest average incidence rate (12/100.000) of Trichinellosis was reported in patients aged 20–39 years, while the lowest one was among patients aged 0–19 years (4.3/100.000). Human Salmonellosis predominated among patients aged 0–19 years (64.6/100.000), while the lowest average incidence rate was recorded among the oldest population (13.7/100.000). During 2002–2016, the majority of Q fever cases (81.2%) were reported in patients aged 20–59 years, with the highest average incidence rate (9.2/100.000) in the 20–39 age group (**Graph 3)**.



Graph 2. Gender-specific incidence rate of the most common zoonoses in the Central Banat District, 2002–2016
Grafikon 2*. Rodno specifična distribucija vodećih zoonoza u Srednjobanatskom okrugu u periodu od 2002. do 2016. godine*
Legend: M, T = males, *Trichinellosis*; F, T = females, *Trichinellosis*; M, S = males, *Salmonellosis*; F, M = females, *Salmonellosis*; M, Q = males, *Q fever*; F, Q = females, *Q fever*
***Legenda*:** M, T = muškarci, *trihineloza*; F, T = žene, *trihineloza*; M, S = muškarci, *salmoneloze*; F, M = žene, *salmoneloze*; M, Q = muškarci, *kju groznica*; F, Q = žene, *kju groznica*



Graph 3. Age–specific distribution of the most common zoonoses in the Central Banat District, 2002–2016
Grafikon 3*. Uzrasno specifična distribucija vodećih zoonoza u Srednjobanatskom okrugu u periodu od 2002. do 2016. godine*
Legend: Black vertical lines separate values of different zoonoses
Legenda: Crne vertikalne linije odvajaju vrednosti za različite zoonoze



Graph 4. Seasonal distribution of the most common zoonoses in the Central Banat District, 2002–2016
Grafikon 4*. Sezonska distribucija vodećih zoonoza u Srednjobanatskom okrugu u periodu od 2002. do 2016. godine*

Regarding the seasonal distribution, the majority of cases with Trichinellosis, Salmonellosis and Q fever were registered during January (84%); between June and August (42%), and during December, March and May (58%), respectively (**Graph 4**).

Outbreak occurrence, probable sources and modes of transmission according to available data after epidemiological investigation of the most common zoonoses in the CBDV, 2002–2016

From 2006 to 2016, there were 5, 28 and 6 outbreaks of Trichinellosis, Salmonellosis and Q fever in humans, accounting for 99%, 15% and 45% of the total number of human cases within the three observed zoonoses, respectively (**Table 1**).

According to the available data, pork products were the most common source of outbreaks of Trichinellosis (89%; 222/249), while cakes and cookies were the most common sources of outbreaks (36/114; 31.6%) of human Salmonella infections. Interestingly, two patients provided information that they had consumed quail eggs before the onset of Salmonellosis symptoms. Out of 92 interviewed patients with Q fever, 50 (54.3%) had a direct daily contact with their domestic animals during the maximum incubation period, while every fourth patient did not have any contact with animals (**Table 2**).

Table 1. Distribution of patients affected in epidemic outbreaks and sporadic cases of the most common zoonoses in the Central Banat District, 2002–2016
Tabela 1*. Distribucija pacijenata u epidemijama i distribucija sporadičnih slučajeva u tri vodeće zoonoze u Srednjobanatskom okrugu u periodu od 2002. do 2016. godine*

Zoonosis/*Zoonoza*	No of outbreaks *Broj epidemija*	Cases in epidemic *Broj obolelih u epidemijama*	Sporadic cases *Sporadični slučajevi*	Total *Ukupno*
		N %	N %	N %
Trichinellosis/*Trihineloza*	5	247	2	249
Percentage/*Procenat*		99.2%	0.8%	100%
Salmonellosis/*Salmoneloze*	28	114	666	780
Percentage/*Procenat*		14.6%	85.4%	100%
Q fever/*Kju groznica*	6	70	85	155
Percentage/*Procenat*		45.2%	54.8%	100%

Table 2. Probable sources and modes of transmission of the most common zoonoses in the Central Banat District, 2002–2016
Tabela 2*. Verovatni izvori i putevi prenošenja vodećih zoonoza u Srednjobanatskom okrugu u periodu od 2002. do 2016. godine*

Zoonosis *Zoonoza*	Probable source or mode of transmission *Verovatni izvor ili put prenošenja*	No of cases *Broj slučajeva*	% of total cases *% od ukupnog broja*
Trichinellosis *Trihineloza* N = 249	Pork products, bacon, sausages *Proizvodi od svinjskog mesa, slanina, kobasice*	222	89.1
	Grilled pork meat/*Svinjsko meso sa roštilja*	13	5.2
	Homemade smoked pork sausages *Domaće dimljene kobasice od svinjskog mesa*	10	4.1
	Unknown/*Nepoznato*	4	1.6
Salmonellosis *Salmoneloze* N = 114*	Cakes and cookies/*Torte i kolači*	36	31.6
	Ice cream/*Sladoled*	20	17.5
	Chicken eggs/*Kokošija jaja*	19	16.7
	White sausage/*Bela kobasica*	8	7.0
	Baked chicken wings/*Pečena pileća krilca*	3	2.6
	Mayonnaise/*Majonez*	3	2.6
	Quail eggs/*Prepeličja jaja*	2	1.8
	Unknown/*Nepoznato*	23	20.2
Q fever *Kju groznica* N = 92*	Contact with animals/*Kontakt sa životinjama*	50	54.3
	Livestock in the neighbourhood/*Uzgoj stoke u komšiluku*	19	20.7
	No contact with animals/*Bez kontakta sa životinjama*	23	25.0

*According to the available data after the epidemiological investigation/*Prema dostupnim podacima, nakon epidemiološkog ispitivanja*

Discussion

To our knowledge, this is the first study about the epidemiological characteristics of Trichinellosis, Salmonellosis and Q fever which are the three most common zoonoses in the CBDV.

Although these zoonoses are mainly clinically presented as mild diseases with minor complications, some of them can lead to development of severe complications with a potentially fatal outcome [3, 4].

Trichinellosis

Trichinellosis is a very common zoonotic disease across the world. Infection with Trichinella spp. has been documented in domestic animals as well as in wildlife worldwide. Globally, reporting of human Trichinellosis varies greatly. According to the analysis conducted between 1986 and 2009, a total of 56,912 cases of human Trichinellosis were registered in Europe. In addition, the highest ranges of the incidence rate per 100.000 inhabitants were registered in the following countries: Bosnia and Herzegovina, 4.1; Bulgaria, 2.4–2.9; Croatia, 1.7–4.8; Latvia, 1.1–1.3; Lithuania, 1.2–6.6; Romania, 2.9–8.5; and Serbia, 5.0 [12]. With regard to the annual epidemiological reports from the 29 European Union (EU)/ European Economic Area (EEA) countries, Bulgaria, Lithuania and Romania accounted for 63% of all confirmed cases in 2015 [13]. Our results showed that the average incidence of human Trichinellosis was 8/100,000, which is much higher than the average incidence registered in the APV during the same period [6], or the incidence registered in the EU/EEA countries during 2015, but it was close to the average incidence of the human Trichinellosis documented in Romania [12, 14]. Moreover, Romania was considered as one of the most affected countries in Southeastern Europe and in the world during 2004 [15]. With regard to the age and gender of patients, available data from clinical reports show that Trichinellosis is primarily a disease of adults, occurring almost equally in both genders. The results of a retrospectively reviewed Trichinellosis outbreaks, conducted worldwide during 1986–2009, showed the highest incidence among subjects aged 20–50 years in both genders [12]. Our findings showed that the highest incidence rate of Trichinellosis was detected in adults aged 20–39 years, which is consistent with the results obtained from the EU/EEA region [13]. A possible explanation for Trichinellosis predominance in adults may lie in food related behavior. Some previously published studies have reported that improperly cooked or prepared meat dishes may be more commonly eaten at adult–oriented events, particularly if alcohol is consumed [12, 15, 16]. Contrary to the age–specific distribution of human Trichinellosis obtained from several EU/EEA countries as well as from our findings during 2015, Lithuania and Romania were the countries in which almost all cases were reported among children and young teenagers (0−14 years of age) [13]. Similar to the results from the study conducted in Belgrade [17], and the previously published data in the APV [6], there was no significant difference regarding the gender of patients.

Multiple studies have reported a Trichinellosis outbreak in Europe among people who had consumed wild boar sausages [18]; bacon bought at the market [19]; homemade ground meat products after home slaughter [20]; infected pork salami [21]; raw pork meat and meat products produced by a meat processing plant and sold in shops [22]; ham produced from a pig slaughtered without veterinary inspection [23]; horse meat [24]; bear meat [25]; smoked wild boar ham [26]; grizzly bear meat [27]. We found evidence that almost all cases of registered human Trichinellosis were after eating some pork products. Hence, our results were supported by the findings from the latest described outbreak of human Trichinellosis in the CBDV where 309 cases were recorded which led to the highest incidence rate of Trichinellosis in 2002 (101.7/100.000) compared with other years. Smoked pork sausages produced by a slaughterhouse near Zrenjanin were the common source of this largest outbreak in the CBDV [28]. On the other hand, a total of 114 human cases were detected in the latest outbreak of human Trichinellosis in Serbia. This time wild boar (Sus scrofa) meat products were the source of the outbreak. Interestingly, this is the first case of Trichinella britovi confirmed in food samples linked to human Trichinellosis [29].

It is a known fact that some parts of APV have an endemic occurrence of human Trichinellosis. For comparison, in Serbia between 2005 and 2009, 62.8% of total human Trichinellosis cases were registered in the APV [30, 31]. Contrary to the fact that human Trichinellosis was permanently registered in the APV, outbreaks of this parasitic zoonosis in the CBDV occurred sporadically, with up to 24% of hospitalized cases [28]. In line with local customs, we found that most human cases of Trichinellosis were registered during January. This finding can be explained by increased pig slaughter for local consumption during Christmas and winter, and particularly due to consumption of pork sausages, salami and smoked meat [28]. Similar seasonal distribution of human Trichinellosis was described in Romania and Bulgaria, probably because of food behavior and customs similar to those in Serbia [13].

Salmonellosis

Salmonella is a significant public health concern worldwide and Salmonelloses in humans are a leading bacterial cause of acute gastroenteritis, both in children under 5 years of age and in the general population [32]. Previous estimates from four developed countries showed that the incidence of diarrheal disease ranged from 0.44 to 0.99 episodes per person–year, which possibly means that each year up to 2.8 billion cases of diarrheal diseases should be detected globally, and Salmonella infections account for around 3% of them [32, 33]. Global estimates of Salmonellosis are difficult to calculate because many countries, particularly the developing ones, have insufficient surveillance data [32]. However, the estimates for Salmonella infections in the 27 EU countries in the 2005–2009 period showed that about one out of every 80 inhabitants was affected by Salmonella spp. each year [34].

According to the data of the global population divided into 21 regions, the highest estimation of the Salmonella gastroenteritis per 100.000 person–years was detected in East Asia (3,980/100.000 person–years), and the lowest was detected in regions of Central Asia and Asia–Pacific (40/100.000 person–years). Interestingly, in the European region, the highest incidence rate of Salmonella gastroenteritis was registered in Central Europe (2.390/100.000), and the lowest in Western Europe (240/100.000) [32].

Similar to the results of recently published data for 37 European countries [35], we found a decreasing trend of Salmonellosis. In the rest part of the APV, there was a decreasing trend in the period between 1998 and 2008, but since 2009 the trend of Salmonellosis in the APV has been stable [6]. Although the reason for decrease of Salmonellosis in the CBDV remains unclear, experiences from the 37 European countries suggest that implementation of comprehensive veterinary control programs, particularly in poultry, may contribute to reduction in Salmonella infections in humans [35]. Similar to the reports from the European countries [36] and the United States (US) [37], there was no difference in the overall rates between males and females. The results of the reports mentioned above [36, 37] strongly suggested that most Salmonellosis cases were detected among children aged 0–4 years. This phenomenon can be explained by a higher proportion of symptomatic Salmonellosis in children and increased likelihood that parents will take their children to see a doctor who will then recognize and report the disease in this age group [36]. In support of the aforementioned facts, the highest incidence rate of Salmonellosis in the CBDV was registered in patients under 19 years of age. However, for a better evaluation of the age distribution of Salmonella infections in humans in a certain territory, a seroprevalence study should be implemented [38].

Regarding the seasonal pattern of Salmonellosis, we found that more than 40% of all cases were registered between June and August. Similar experiences were described in some European countries as well as in the US [36, 37], and this could be explained by particular or specific seasonal human behaviors as well as by variation in host susceptibility. Higher ambient temperature during summer months can itself be a risk factor for transmission because it drives bacterial survival and growth [39]. Other risk factors for Salmonellosis in humans include gastric hypoacidity, recent use of antibiotics, and different immunosuppressive conditions [40].

Homemade cakes and cookies consumed among family members were the most prevalent source of infection in the 28 outbreaks of human Salmonellosis in the CBDV. This is quite similar to the findings of the Belgrade study where 63.4% out of total outbreak cases of human Salmonellosis were registered after some family celebrations [41], as well as to the results obtained from other parts of APV [7].

Surprisingly, only 15% out of total human Salmonellosis cases were detected during the outbreak occurrence and the majority of Salmonellosis infections were classified as sporadic cases. Other authors have reported similar results [42]. Possible reasons for this might be a lack of consistency during the outbreak investigation or the fact that human Salmonellosis cases were registered mostly in families without a large outbreak appearance in the general population of CBDV. Additional reasons may be in the fact that a lot of patients with milder clinical presentation of disease symptoms did not consult a physician and/or specific etiology was not diagnosed. In accordance with the facts mentioned above, we believe that the number of cases reported during the study period represents only the "tip of the iceberg" of human Salmonellosis in the CBDV.

Most cases of Salmonellosis are caused by ingestion of contaminated food items such as eggs, dairy products, meat, even peanut butter [40]. It is noteworthy that previous investigations have established that a shell egg was the most important vehicle for the epidemic of human Salmonellosis in the US [43]. Although our results are consistent with the reports obtained from different European countries where either eggs or egg products were the most commonly identified sources of human Salmonella infections during summer months [34], findings of other authors also highlighted that barbeque and gardening were recognized as a specific source or mode of Salmonellosis transmission which was particularly noticed during June or July [39]. Similar to the findings in several European countries as well as the US [36, 37, 43], Salmonella Enteritidis was the most common serotype (96%) of Salmonella among the reported human Salmonellosis in the CBDV.

Q fever

With regard to Q fever, we provided evidence that the average incidence rate of Q fever was 5.2/100.000 which is higher than the average incidence rate of Q fever in the APV (1.4/100.000) in the 2008–2017 period. The analysis of all seven districts of APV during 2017, a higher incidence of Q fever was registered only in the Srem District (7.0/100.000) in comparison with the CBDV (3.7/100.000) [6]. Although only two large outbreaks of Q fever were registered in the CBDV during 2006 and 2010, a large number of sporadic human Q fever cases were registered in 2012, as well. The results of the study conducted by Ristić M. et al. [11] showed a strong positive correlation between the increase in Q fever cases and the increase in wind speed in the CBDV, which could potentially explain an endemo–epidemic character of human Q fever in this district.

Our results showed that human Q fever cases were more frequently registered among males than females, predominantly affecting the 20–59 age group. Furthermore, we found that the incidence of human Q fever was higher in December, March and May, and that more than one half of all patients had a direct daily contact with their domestic animals before the onset of the disease. If anything, most of the data presented above are in good agreement with the previously published data and their explanations in the study of Q fever conducted in the APV [11].

It is a known fact that the predominant mode of Q fever spreading is inhalation of aerosols generated from infected placenta, body fluids or contaminated dust resulting from contaminated manure and desiccation of infected placenta and body fluids [44]. In accordance with this, we believe that the aforementioned modes of transmission were presented in the majority of patients in our study. Findings of other authors across European countries also confirmed these observations [45].

In the latest largest community outbreak of Q fever ever reported in the literature in the Netherlands during 2007, 2008 and 2009, there were 168; 1,000 and 2,357 cases, respectively [46]. Findings of the aforementioned study highlighted that abortion waves in dairy goat farms were the primary source of infection in humans primarily affecting people living near these farms (within 5 km). It was noteworthy that one of the potential reasons for an obviously large number of human cases of Q fever was partly explained by an increased awareness of Q fever among general practitioners, specialists and medical microbiological laboratories, especially in the region where the 2007 outbreak firstly occurred. Similar to the results of the recently published systematic review in Galicia (north–west Spain) [47], we provided evidence that most human Q fever cases were sporadic, which additionally suggests that there was an endemic area of Q fever in Vojvodina. In accordance with the fact that Q fever in humans is often presented as an influenza–like illness and not correctly classified as Q fever, it can be assumed that the true number of persons with Q fever exceeds the number of reported cases [44]. In line with this, the implementation of a seroprevalence study in the CBDV should be considered.

Conclusion

The most common zoonoses in humans in the Central Banat District of Vojvodina are Trichinellosis, Salmonellosis and Q fever. In order to improve evaluation of epidemiological characteristics of these zoonoses and consequently the timely manner of their control, prompt exchange of information between health sectors for animals and humans, along with continuous education of food handlers and the general population in the Central Banat District of Vojvodina are needed.

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SIGNIFICANCE OF DELAYED SURGICAL TREATMENT OF SYMPTOMATIC NON-RUPTURED ABDOMINAL AORTIC ANEURYSM

ZNAČAJ ODLOŽENOG HIRURŠKOG LEČENJA SIMPTOMATSKE NERUPTURIRANE ANEURIZME ABDOMINALNE AORTE

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Summary

Introduction. An abdominal aortic aneurysm is a permanent focal dilation of the blood vessel wall to about 1.5 times larger than the normal diameter. Clinically, it may be divided into symptomatic and asymptomatic. It is still discussed whether patients with symptomatic non-ruptured abdominal aortic aneurysm benefit more from emergency or delayed surgical treatment. The aim of the study was to evaluate the results of the symptomatic non-ruptured aneurysms in regard to the diameter of ruptured and non-ruptured symptomatic aneurysms and the impact of the time elapsed from admission to surgery on its outcome. **Material and Methods.** The retrospective study included all 133 patients who underwent surgery due to symptomatic non-ruptured or ruptured abdominal aortic aneurysm at the Clinic of Vascular and Endovascular Surgery during the previous 3 years. **Results.** Out of a total of 133 patients, 75.19% underwent surgery in the first 24 hours after admission, while the rest 24.81% of patients were operated later. Intraoperative complications were recorded only in patients with ruptured aneurysms, 4% had cardiac arrest and 1.5% of patients had fatal outcome. The in-hospital mortality was 16.67% in patients with non-ruptured aneurysm of the abdominal aorta operated in the first 24 hours, and 9.91% in patients who were operated after 24 hours after admission. **Conclusion.** Early elective surgery is a method of choice in the treatment of symptomatic non-ruptured aneurysm of the abdominal aorta. However, surgical treatment in the first 24 hours is associated with a higher mortality rate than surgery after 24 hours after admission. Also, there is no statistically significant difference in the diameter of ruptured and non-ruptured symptomatic aneurysms, but the average size of the aneurysm diameter is higher in ruptured than in non-ruptured aneurysms, which confirms the fact that the increase in diameter increases the risk of aneurysm rupture.

Key words: Vascular Surgical Procedures; Aneurysm, Ruptured; Aortic Aneurysm, Abdominal; Risk Assessment; Signs and Symptoms; Comorbidity

Sažetak

Uvod. Aneurizma abdominalne aorte predstavlja trajno fokalno proširenje zida krvnog suda 1,5 puta veće od normalnog dijametra. Klinički može da se podeli na simptomatsku i asimptomatsku. Još uvek se diskutuje da li pacijenti sa simptomatskom nerupturiranom aneurizmom abdominalne aorte više profitiraju od hitnog hiruškog tretmana ili odloženog hirurškog pristupa. Cilj rada predstavlja detaljnu analizu rezultata simptomatske nerupturirane aneurizme prateći povezanost dijametra između rupturirane i nerupturirane simptomatske aneurizme, uz definisanje uticaja vremena proteklog od prijema pacijenta do operacije i njen ishod. **Materijal i metode.** Svih 133 pacijenta, koji su tokom prethodne tri godine podlegli hiruškom tretmanu zbog simptomatske nerupturirane i rupturirane aneurizme na Klinici za vaskularnu i transplantacionu hirurgiju, retrospektivno su analizirani. **Rezultati.** Od ukupno 133 pacijenta 75,19% je operisano u prvih 24 sata od prijema, dok je preostalih 24,81% operisano nakon 24 sata. Intraoperativne komplikacije su imali samo pacijenti sa rupturiranom aneurizmom i to 4% srčani zastoj i 1,5% smrtni ishod. Mortalitet u toku hospitalizacije kod pacijenata sa simptomatskom aneurizmom abdominalne aorte operisanih u prva 24 sata iznosi 16,67%, a kod pacijenata operisanih posle 24 sata iznosi 9,91%. **Zaključak.** Rana elektivna operacija je metoda izbora za tretman simptomatske nerupturirane aneurizme abdominalne aorte, uzimajući u obzir i činjenicu da je hirurški tretman u prvih 24 sata udružen sa većim stepenom smrtnosti nego nakon 24 sata od prijema pacijenta. Takođe, statistički nema značajne razlike u dijametru rupturiranih i nerupturiranih simptomatskih aneurizmi, ali prosečna vrednost dijametra aneurizme je veća kod rupturiranih aneurizmi, što nam potvrđuje tvrdnju da sa porastom dijametra raste i učestalost rupture.

Ključne reči: vaskularne hirurške procedure; ruptura aneurizme; aneurizme abdominalne aorte; procena rizika; znaci i simptomi; komorbiditet

Introduction

Aorta is the largest blood vessel that delivers oxygenated blood to all parts of the body. It belongs to a group of large, conducting, most elastic blood vessels in the body [1]. The aortic wall is composed of three concentric layers: tunica intima - the inner layer, tunica media - the middle layer, and tunica adventitia - the outer layer [2].

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Abbreviations

AAA	– abdominal aortic aneurysm
sAAA	– symptomatic abdominal aortic aneurysm
rAAA	– ruptured abdominal aortic aneurysm
MRA	– magnetic resonance angiography
US	– ultrasonography
MSCTA	– multi-slice computed tomography angiography
HTA	– hypertension
CMP	– cardiomyopathy
DM	– diabetes mellitus
COPD	– chronic obstructive pulmonary disease
AoFF bypass	– aortobifemoral bypass
AoII bypass	– aortoiliac bypass

In addition to the primary function of delivering blood to all parts of the body, the aorta and other elastic blood vessels play an important role in blood pressure control. This function is made possible due to the elasticity of the lamina which gets wider during the systole and thinner during the diastole. Owing to the tunica media, loaded with elastic fibers, the elastic wall of the aorta opposes pressure created after the contraction of the heart chamber, maintaining arterial pressure and blood flow even during relaxation of the chamber [1].

Aortic diseases are among the most important diseases of the vascular system. Apart from congenital malformations, aortic dissection and atherosclerotic changes, aneurysms are critical aortic diseases that may appear anywhere throughout the circulatory system [2].

Aneurysm is a permanent focal dilatation of the arterial wall to 1.5 times greater than its normal diameter. The real aneurysm of the aorta is defined as a dilation of all three layers (intima, media and adventitia) of the aortic wall, which differs from pseudo-aneurysms [7]. In addition to the thoracic part, aortic aneurysms are most commonly found in the infrarenal abdominal section [8] **(Figure 1)**.

According to the latest findings in the United States, about 150,000 new cases of abdominal aortic aneurysms (AAAs) are detected annually [4], while data in Western Europe show that over 700,000 people have AAAs [5]. The AAA is relatively frequent and sometimes fatal, which primarily affects the elderly, while the younger population is much less affected [7].

According to the clinical presentation, AAAs may be divided into two groups: asymptomatic AAA and symptomatic AAA (sAAA). Symptomatic aneurysms can be further divided into ruptured and non-ruptured aneurysms [9].

The pathophysiological basis of the AAA is multifactorial, while the degenerative process of the wall is most often found [10, 11]. The most common and most important etiological factor for aneurysm formation is atherosclerosis of the aortic wall, which is a disease of large and medium blood vessels. However, although much less often, AAA can also develop as a result of various infections including brucellosis, salmonellosis, and tuberculosis [12].

Other factors for AAA formation include a positive genetic predisposition, age, gender, smoking, hypertension (HTA), and chronic obstructive pulmonary disease (COPD). The significance of diabetes in the pathogenesis and course of AAA are still controversially discussed, often with contradictory results [13–15].

Based on information collected from numerous studies on the development of AAA, factors such as smoking, HTA and gender are listed as important. The process of AAA diameter enlargement mostly depends on the age, severity of heart disease and smoking. The worst potential outcomes, such as AAA ruptures, are associated with female gender, large initial AAA diameter, and elevated mean arterial pressure [16–22].

In addition to its unpredictability, AAA shows the so-called discontinuous growth pattern, unequal expansion of the aneurysm wall. In the natural course of untreated AAA, an aneurysm can be caused by wall stretching. Therefore, although the individual growth pattern cannot be accurately predicted, the average growth is about 3 to 4 mm per year [6]. In the past, the risk of AAA rupture has been overestimated, but recently two very significant studies have attempted to give a more precise estimate that is still in use [23, 24] **(Table 1)**.

Most commonly the AAAs rupture into the retroperitoneal area (83%) and much less likely into the intraperitoneal region (12%). In addition to the previously mentioned sites, aortocaval fistula (3 - 4%) and aortoduodenal fistula, although much less often (< 1%) are considered to be complications of ruptured AAA [23].

The aim of this study was to analyze demographic and medical history data, as well as the course and outcome of sAAA treatment. We also

Table 1. Annual risk of abdominal aortic aneurysm (AAA) rupture in relation to its size
Tabela 1. *Godišnji rizik od rupture aneurizme abdominalne aorte (AAA) u odnosu na njenu veličinu*

Description *Opis*	Diameter *Dijametar (cm)*	Estimated annual rupture risk *Procenjen godišnji rizik od rupture (%)*	Estimated five year rupture risk *Procenjen petogodišnji rizik od rupture (%)*
Normal aorta/*Normalna aorta*	2 - 3	0	0
Small AAA/*Mala AAA*	4 - 5	1	5 - 10
Medium AAA/*Srednja AAA*	5 - 6	2 - 5	30 - 40
Big AAA/*Velika AAA*	6 - 7	3 - 10	> 50
Large AAA/*Ogromna AAA*	> 7	>10	Approaching to 100/*Približava se 100*

Table 2. The comparison of the size of symptomatic abdominal aortic aneurysms (sAAA) and ruptured abdominal aortic aneurysms (rAAA)
Tabela 2. *Izmerena veličina aneurizmi i upoređena kod pacijenata sa simptomatskom aneurizmom abdominalne aorte (sAAA) i rupturirane aneurizme abdominalne aorte (rAAA)*

	sAAA (mm)	rAAA (mm)
Average value/*Prosečna vrednost*	77,24	83,08
Middle value/*Srednja vrednost*	76	76
Recorded minimum/*Zabeleženi minimum*	50	50
Recorded maximum/*Zabeleženi maksimum*	110	150

evaluated the results of the symptomatic non-ruptured aneurysms in regard to the diameter of ruptured and non-ruptured symptomatic aneurysms and the impact of the time elapsed from admission to surgery on the surgery outcome.

Material and Methods

The study was approved by the Ethics Committee of the Faculty of Medicine Novi Sad, and the authors strictly followed the principles and indications recommended and by the Declaration of Helsinki.

The retrospective study included 133 patients who underwent surgery for AAA in the period from January 2015 to the end of December 2017. Of these patients, 45 patients underwent open surgery for symptomatic non-ruptured abdominal aortic aneurysm, while 88 patients had emergency treatment of ruptured aneurysm of the abdominal aorta. Data were collected from the Medical Registry of the Clinic of Vascular and Endovascular Surgery of the Clinical Center of Vojvodina in Novi Sad and the Emergency Center Novi Sad (surgery protocols, medical histories, clinical, biochemical and radiological data, discharge letters, etc.).

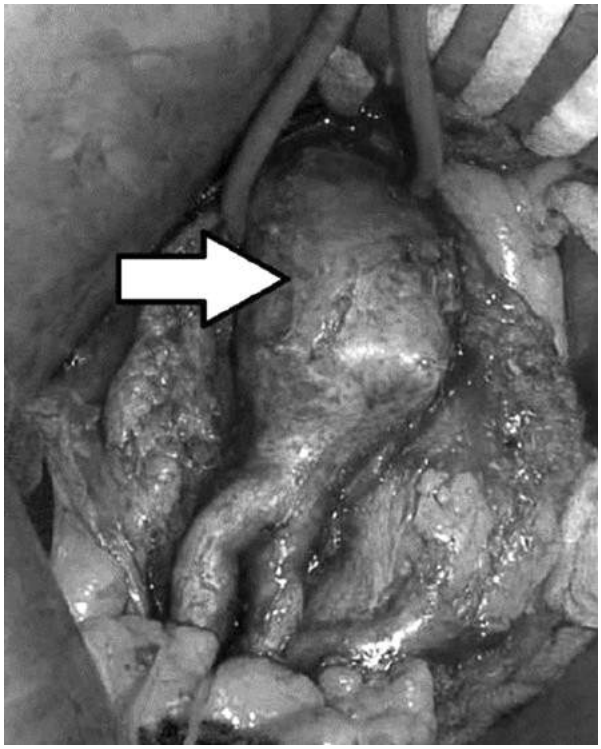


Figure 1. Intraoperative view of a non-ruptured infrarenal aneurysm (arrow) of the abdominal aorta
Slika 1. *Intraoperatvni pogled na nerupturiranu infrarenalnu aneurizmu (strelica) abdominalne aorte*

In addition to medical history and initial clinical examination, the precise diagnosis was set by multislice computed tomography angiography (MSCTA), and sometimes magnetic resonance angiography (MRA) and ultrasonography (US).

In order to analyze the results of previous AAA, the following parameters were preoperatively followed:

– Gender and age of the patients,

– Associated diseases (HTA, cardiomyopathy (CMP), diabetes mellitus (DM), chronic obstructive pulmonary disease (COPD), nicotinism),

– Diagnosis and clinical state of aneurysm (symptomatic non-ruptured/ruptured),

– Size of aneurysm.

The following parameters were monitored intraoperatively and postoperatively:

Applied surgical techniques (Dacron tube prosthesis, AoII-bypass (aortoiliac bypass), AoFF-bypass (aortobifemoral bypass)

– Blood products loss (ml),

– Recovery of blood (ml) from Blood Cell Saver,

– Intraoperative complications (cardiac arrest, lethal outcome),

– Treatment outcome,

– Length of survival after successfully performed surgery (intrahospital death/within the first 7 days after surgery/within the first 7 days after discharge).

All patients were operated in the operating rooms of the Clinical Center of Vojvodina and Emergency Center in Novi Sad. Interventions were carried out in collaboration with vascular surgeons and anesthesiologists, and all patients were operated under general endotracheal anesthesia. Postoperatively, patients were relocated to the Department of Vascular and Endovascular Surgery, and discharged after a recovery period with precise medication dosages and scheduled follow-ups.

Descriptive and comparative statistics were made between the groups with symptomatic non-ruptured and ruptured aneurysms. As part of the descriptive statistics, the following parameters were used: statistical mean and median, minimum and

maximum values, as well as standard deviation. The Pearson χ^2-test was used to compare the differences between the tested groups regarding the non-parametric characteristics.

Results

The study included 133 patients, of which 107/133 (80.5%) were male, while 26/133 (19.5%) were female. The average age of patients was 71.6 years and the median age was 69 years. The oldest patient was 93 years old and the youngest 55.

Based on the initial medical history data and a detailed clinical examination of all the patients, 45/133 (33.83%) patients had the diagnosis of non-ruptured sAAA. In this group, 10/45 (22.22%) patients were female, while the remaining 35/45 (77.77%) were male. The remaining 88/133 (66.15%) patients had the diagnosis of ruptured AAA (rAAA). In this group, 16/88 (18.18%) patients were female, while the remaining 72/88 (81.81%) were male.

Radiological diagnosis included preoperative assessment of the size of aneurysms. In the sAAA group **(Table 2),** the average size was 77.24 mm while the mean size was 76 mm. The recorded minimum and maximum was 50 mm and 110 mm. In the rAAA group, the average size was 83.08 mm while the mean size was 76 mm. The recorded minimum and maximum were 50 mm and 150 mm.

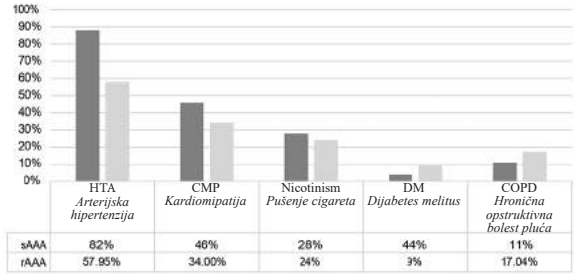
Of the observed preoperative comorbidities, the most common were: arterial hypertension (HTA) in 88/133 (66.16%) patients and CMP in 51/133 (38.34%) patients. Nicotinism was present in 34/133 (25.56%) patients, DM in 10/133 (7.52%), while COPD was found in 20/133 (15.4%) patients.

Of the observed comorbidities in the group with sAAA **(Graph 1)**, the most commonly present was HTA 82.22%, followed by CMP 46.67% of patients, and 4 from DM (4.44%). COPD was present in 11.11% of patients, while nicotinism was present in 28.89% of patients. Of the observed comorbidities in the group of patients with rAAA, the most commonly present were HTA 57.95%, then 34.09% of patients were suffering from CMP, and from DM 9.09% of patients. COPD was found in 17.04% of patients and nicotinism in 23.89%.

All patients underwent a surgical procedure, but at different time after admission. Of 133 patients, 100 (75.18%) underwent surgery in the first 24 hours after admission, and the remaining 33/133 (24.81%) were operated after 24 hours.

The operated patients underwent various procedures: Dacron tube interposition was performed in 98/133 (73.68%), aorto-biiliac-bypass (AoII-bypass) in 16/133 (12.03%), and aorto-bi-femoral bypass (AoFF-bypass) was performed in the remaining 19/133 (14.28%) patients.

The intraoperative procedure of patients with sAAA also varied; the Dacron tube interposition was performed in 36/133 (80%) patients, AoII-bypass in 4/133 (8.88%), and AoFF-bypass in the remaining 5/133 (11.11%). In patients with rAAA, the intraoperative procedures also varied: the Dacron tube interposition was performed in 62/133 (70.45%) patients, AoII-bypass in, 12/133 (13.63%), and AoFF-bypass was performed in the remaining 14/133 (15.9%) patients.



Graph 1. Comparative analysis of the comorbitities in patients with sAAA and rAAA

Grafikon 1. *Komparativna prezentacija komorbiditeta kod pacijenata sa simptomatskom i rupturiranom aneurizmom abdominalne aorte*

Intraoperative blood loss and blood replacement were also measured. In all the patients, the average blood loss was 2174 ml, while the median was 1500 ml. The minimum blood loss was 160 ml, and the maximum was 10 000 ml. The average blood products recovery by Cell Salvage was 786 ml and the mean was 570 ml. The minimum recorded value was 40 ml and the maximum was 6700 ml.

Intraoperative blood loss, blood replacement and cell salvage in the sAAA group were also precisely measured. The average loss of blood elements was 958.44 ml, while the median was 800 ml. The minimum loss was 160 ml, and the maximum was 2500 ml. The average value of blood product reimbursement was 342 ml and the mean value was 300 ml. The minimum value was 40 ml and a maximum 900 ml.

Intraoperative blood loss, blood replacement and cell salvage in the rAAA group were precisely measured too. The average loss of blood elements was 2796.64 ml, while the median loss was 2300 ml. The minimum loss was 250 ml, and the maximum was 10000 ml. The average value of blood products compensation was 1013 ml, and the median 820 ml. The minimum was 100 ml and the maximum 6700 ml.

In regard to complications, in the group of patients with sAAA, there were no cases of cardiac arrest, 0/45 (0%) or intraoperative lethal outcome, 0/45 (0%). In the group of patients with rAAA, the results of complication monitoring showed that intraoperative cardiac arrest was recorded in 6/88 (6.81%) patients, while intraoperative lethal outcome occurred in 2/88 (2.27%) cases.

As the last item, the surgical outcome was followed and of the total sample, 42/133 (31.57%) patients died in the hospital, while the remaining 91/133 (68.42%) patients were released from the hospital. In the group of patients with sAAA, 5/45

Table 3. Survival of patients with sAAA in regard to the admission and time of surgical procedure
Tabela 3. *Preživljavanje pacijenata sa simptomatskom aneurizmom abdominalne aorte u zavisnosti od pristizanja u bolnicu do početka operacije*

	Operated during first 24h *Operisan unutar prvih 24h*	Operated after 24h *Operisan posle 24h*
Died during hospitalization/*Preminuo u toku hospitalizacije*	16.67%	9.91%
Discharged from hospital/*Otpušten iz bolnice*	83.33%	90.09%

(11.11%) died in the hospital, while the remaining 40/45 (88.88%) patients were released home. In the group of patients with rAAA, 37/88 (42.04%) died in the hospital, while the remaining 51/88 (57.95%) patients were released home.

In the observed sample of patients with sAAA, 12/45 (26.67%) patients were operated in the first 24 hours after admission, and the remaining 33/45 (73.33%) patients were operated after 24 hours from the moment of admission to the hospital.

Out of the patients who underwent surgery in the first 24 hours **(Table 3)** after admission, 2/12 (16.67%) died in the hospital, while 10/12 (83.33%) patients were released from the hospital.

Out of the patients who were operated after the first 24 hours **(Table 3)** after admission, 3/33 (9.91%) patients died in the hospital, and the remaining 30/33 (90.09%) patients were discharged from the hospital.

Comparative statistics of comorbidity and other parameters in relation to the diagnosis are shown in **Table 4.**

Comparing two groups of symptomatic non-ruptured and ruptured AAA, hypertension was statistically significantly different ($p < 0.05$), while intrahospital survival and mortality during hospitalization showed a high statistically significant difference ($p < 0.001$) after successful surgery.

Discussion

The study includes 133 patients who underwent a surgical treatment of AAA at the Clinic of Vascular and Endovascular surgery in Novi Sad during the period from January 2015 to December 2017. Of the total number of patients (133), 107 (80.5%) were male and 26 (19.5%) were female. The male to female ratio among the investigated patients was 6 : 1, similar to the distribution found in the literature [9] of 7 : 1; this difference can be explained by the fact that the sample included almost three times more patients. Although the sample was much larger, in both studies the analyzed patients were most-

Table 4. Comparative statistics on comorbidity, intraoperative procedures, complications, intrahospital survival and mortality in relation to sAAA and rAAA
Tabela 4. *Komparativna statistika komorbiditeta, intraoperativnih procedura, komplikacija, intrahospitalnog preživljavanja kao i smrtnosti u odnosu na simptomatske (sAAA) i repturirane aneurizme abdominalne aorte (rAAA)*

Comorbidity/*Komorbiditeti*	sAAA	rAAA	p
HTA	37	51	0,009
CMP	21	30	0,221
Nicotinism	13	21	0,675
DM	2	8	0,539
COPD/*HOBP*	5	15	0,516
Intraoperative procedure/*Intraoperativna procedura*	sAAA	rAAA	p
Dacron tube/*Dakron tubus*	36	62	
Ao-II bypass	4	12	0,495
Ao-FF bypass	5	14	
Complications/*Komplikacije*	sAAA	rAAA	p
Exitus letalis/*Smrtni ishod*	0	2	0,790
Cardiac arrest/*Srčani zastoj*	0	6	0,177
Intrahospital survival/*Intrahospitalno preživljavanje*	sAAA	rAAA	p
Intrahospital lethality/*Intrahospitalno preminuli*	5	37	0,000
Mortality during hospitalization/*Smrtnost u toku hospitalizacije*	sAAA	rAAA	p
From 0 to 7 days/*Od 0 do 7 dana*	2	26	0,000

Legend: HTA - arterial hypertension; DM - diabetes mellitus; Nicotinism - smoking; COPD - chronic obstructive pulmonary disease; CMP - cardiomyopathy; Ao-II bypass - Aorto-biiliac baypas; Ao-FF bypass - Aorto-bifemoral bypass
Legenda: HTA – arterijska hipertenzija; DM – dijabetes melitus; Nicotinism – pušenje; HOBP – hronična opstruktivna bolest pluća; CMP – kardiomiopatija; Ao-II bypass – aortobiilijačni bajpas; Ao-FF bypass – aorto-bifemoralni bajpas

ly 60 years of age: in our case 87% while in the other study 79% [9].

The average age of our patients was 71.6 years; the oldest patient was 93 and the youngest 55 years of age. These data support the fact that aneurysm is a disease affecting the elderly population, mostly in the seventh decade, which was also confirmed by other available studies [9, 25].

A sAAA was diagnosed in 45 patients, while the remaining 88 had a rAAA, which is similar to the data available in the literature [9].

The question of the size of the aneurysm and its potential rupture is an abundantly discussed topic. Some studies, such as Ruby Lo et al. [26], have come to the conclusion that the risk of aneurysm rupture is directly associated with its size, but they have also proved that there was a correlation between the size of the aneurysm and the size of the patient's body. Due to the fact that data on the body size were generally not available to us, we could not check this hypothesis in the retrospective analysis of our patients.

In our patients with non-ruptured aneurysm, the mean size of the aneurysm was 77.24 mm while in the group of patients with ruptured aneurysm, the average size was 83.08 mm. Comparing the diameters of aneurysms in our study with those in the above mentioned study [26], where the average diameters of aneurysms were smaller than in ours, there were no statistically significant differences in diameters of ruptured aneurysms and symptomatic non-ruptured aneurysms of the abdominal aorta.

Of the preoperative comorbidities among our patients, the most common were HTA 66.16% and CMP 38.34%. DM was found in 28.12%, while 15.03% of patients had COPD. Nicotinism, although a very widespread comorbidity, was present only in 25.56% of patients. The results obtained are very similar to the results in the literature [9] with differences in response; DM 3% and nicotinism 67%, were much more prevalent in their patients. Thompson et al. [27] have come to the conclusion that HTA occurs more frequently in patients with rAAA versus sAAA in patients over 65 years of age, while in our sample there were more patients with HTA in those with sAAA.

In regard to the time when the surgical procedures were performed, we created two groups. The first group included patients who were operated in the first 24 hours after admission, while the second group included patients operated after 24 hours after admission. According to our data, 75.19% of patients were operated in the first 24 hours of admission, while the remaining 24.81% were operated after 24 hours.

During each operation, the amount of lost and recovered blood was monitored using the Cell-Saver apparatus. The average value of blood loss in our patients with symptomatic non-ruptured aneurysm was 958 ml, while patients with ruptured aneurysm lost an average of 2796 ml. The amount of blood returned to circulation via Cell-Saver in patients with sAAA was 342 ml, and 1013 ml in the group with rAAA. Other studies have also reported similar results [9].

Every surgery has risks and complications. In our sample, we followed the intraoperative lethal outcome and cardiac arrest in all operated patients, and found that only 4% of the patients experienced an intraoperative cardiac arrest, while lethal outcome occurred in 1.5%. It is very interesting that all intraoperative complications occurred in patients with rAAA, while patients with sAAA did not have any complications during the surgical procedure. Similar data are found in the literature [28].

Postoperatively, of the total sample, 68.42% of patients were released from the hospital after recovery, while the remaining 31.57% of patients died during the postoperative period. The highest number of deceased patients had rAAA 42.04% (37/88), and a much lower percentage of patients had sAAA 11.11% (5/45). Previous studies have shown that the postoperative mortality of patients with rAAA is estimated to be approximately 45.4%, which is very close to our 42.04% [28, 29].

A critical period for survival was also monitored; 42/133 (31.57%) patients died during the hospitalization, and the remaining 91/133 (68.42%) were released home. Patients who underwent surgery with sAAA were operated at different time after admission. Of the total sample of patients (45), 12/45 (26.67%) underwent surgery in the first 24 hours after admission, and the remaining 33/45 (73.33%) patients were operated after 24 hours after admission. In the group that was operated during the first 24 hours, 16.67% died, as well as 9.91% of the patients in the group operated after 24 hours, which is 1.6 times less compared to patients who were operated during the first 24 hours. Compared to two groups of symptomatic non-ruptured and ruptured AAA, HTA showed a statistically significant difference ($p < 0.05$), and our data for the period 2015 - 2017 largely coincide with those published for the period 2005 - 2007 [9]. One can only speculate that the cause of higher mortality of patients with HTA is the chronic change in the structure and elasticity of the blood vessels in general, as well as a greater prevalence of complications in the cardiovascular system such as cardiac insufficiency, chronic kidney disease, etc. [30].

We are aware of certain limitations of this study. Because of the retrospective nature of the study and the quality of the data collected from the registry, there are definitely some data that may have influenced the final results of the analysis, but were not recorded. It would also be more informative to quantify the status of comorbidity, but it was not possible to use the currently available data. Here, for example, we are thinking about nicotinism, for example concerning the period of smoking, the number of packs per day, as well as the duration of the abstinence period if it was present. We are also aware that the design of a prospective study in order to solve

this problem would be extremely difficult. Another clear limitation is that this analysis involved only patients with sAAA and rAAA who have been admitted to the tertiary vascular center of the Clinical Center Novi Sad. According to some information, it is estimated that one third to half of all patients with rAAA dies before reaching the hospital. There is a high possibility that patients, who died at home, or during transportation, were older and had more comorbidity. Despite the fact that our study is retrospective with a small number of patients, the volume of the data collected and the results obtained coincide and do not deviate to a large extent from the results of studies from other large centers.

Conclusion

This study shows that the surgery of symptomatic non-ruptured aneurysm of the abdominal aorta in the first 24 hours has a higher mortality rate than after 24 hours after admission. Early elective surgery is a method of choice in the treatment of symptomatic non-ruptured aneurysm of the abdominal aorta. There is no statistically significant difference in the diameter of ruptured and non-ruptured symptomatic aneurysms, but the average size of the aneurysm diameter is higher in ruptured, which confirms the fact that the increase in the diameter increases the risk of rupture as well.

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COSTS OF SEQUENTIAL MULTIPLE MYELOMA TREATMENT FOR ELDERLY TRANSPLANT-INELIGIBLE PATIENTS IN THE SERBIAN HEALTH CARE SYSTEM

TROŠKOVI TERAPIJSKIH SEKVENCI ZA LEČENJE MULTIPLOG MIJELOMA KOD STARIJIH PACIJENATA NEPODOBNIH ZA TRANSPLANTACIJU MATIČNIH ĆELIJA HEMATOPOEZE U ZDRAVSTVENOM SISTEMU SRBIJE

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Summary

Introduction. Multiple myeloma is an incurable plasma-cell proliferation mainly affecting the elderly population. The aim of this study was to analyze treatment patterns, utilization of health resources and treatment costs of multiple myeloma in the elderly patients ineligible for autologous hematopoietic stem cell transplantation in Serbia. **Material and Methods.** The analysis of the healthcare costs, from the perspective of the Serbian healthcare system, took into account the costs of medications, diagnostic procedures, inpatient and outpatient care, as well as the costs of drug administration and management of drug adverse effects. **Results.** Thalidomide based regimens were less costly than bortezomib-based regimens (average per-protocol costs 6,000 € vs. 64,700 €, respectively). The most expensive treatment regimen was lenalidomide-dexamethasone (average per-protocol costs 145,200 €). The sequential (four-line therapy) treatment costs varied from 85,800 €, starting with melphalan-prednisone-thalidomide to 153,800 €, starting with melphalan-prednisone-bortezomib. The estimated costs did not significantly differ during variation of the parameters in the sensitivity analysis. **Conclusion.** The costs of multiple myeloma treatment in the Republic of Serbia are mainly driven by the cost of anti-myeloma drugs. The most expensive treatment sequence was starting with melpha1an-prednisone-bortezomib treatment protocol.

Key words: Multiple Myeloma; Cost-Benefit Analysis; Economics, Pharmaceutical; Clinical Protocols; Aged; Serbia

Sažetak

Uvod. Multipli mijelom je neizlečiva maligna proliferacija plazmocita koja pogađa prvenstveno stariju populaciju. Cilj ovog rada je da se analiziraju protokoli lečenja, upotreba zdravstvenih resursa i troškovi tretmana multiplog mijeloma kod starijih pacijenta nepodobnih za autologu transplantaciju matične ćelije hematopoeze u Srbiji. **Materijal i metode.** Analiza troškova zdravstvene nege iz perspektive srpskog zdravstvenog sistema uzela je u obzir troškove lekova, dijagnostičkih procedura, hospitalizacije i vanbolničkog lečenja, kao i troškove administracije lekova i lečenja neželjenih efekata leka. **Rezultati.** Troškovi za protokole bazirane na talidomidu bili su manji od troškova za protokole bazirane na bortezomibu (prosečna cena 6,000 € tj. 64,700 € po protokolu). Tretman koji je iziskivao najviše troškova bio je lenalidomid-deksametazon (prosečna cena protokola 145,200 €). Troškovi sekvencionalnog lečenja (četiri terapijske linije) varirali su od 85,800 € za melfalan-prednizon-talidomid kao početni tretman do 153,800 € za melfalan-prednizon-bortezomib kao početni tretman. Procenjeni troškovi se nisu značajno menjali prilikom varijacije paramatara u analizi senzitivnosti. **Zaključak.** Troškovi tretmana multiplog mijeloma u Srbiji u najvećoj meri potiču od troškova antimijelomske terapije. Započinjanje terapije melfalan-prednizon-bortezomib protokolom rezultovalo je najvišim zdravstvenim troškovima.

Ključne reči: multipli mijelom; ekonomska evaluacija; farmakoekonomija; klinički protokoli; stari ljudi; Srbija

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Introduction

Multiple myeloma (MM) is an incurable malignant plasma-cell proliferation manifesting with bone pain, hypercalcemia, anemia, renal insufficiency and malaise [1]. It accounts for approximately 10% of all hematologic malignancies worldwide and it commonly affects the elderly population, and the median age at diagnosis is between 65 and 70 years [2]. The incidence of hematologic malignancies is remarkably lower in Eastern European coun-

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Abbreviations
MM – multiple myeloma
MPV – melphalan-prednisone-bortezomib
PFS – progression-free survival
NHIF – National Health Insurance Fund
MPT – melphalan-prednisone-thalidomide
CTD – cyclophosphamide-thalidomide-dexamethasone
VCD – bortezomib-cyclophosphamide-dexamethasone
VD – bortezomib-dexamethasone
RD – lenalidomide-dexamethasone

tries, although it is not completely clear whether the reason is a genuinely small number of cases, underdiagnosis or underreporting [3]. In 2014, 184 newly diagnosed cases were identified in Central Serbia [4]. The disease outcomes, expressed in terms of overall survival and progression-free survival (PFS), have been significantly improved after the introduction of novel immunomodulatory and proteasome inhibitor-based therapies [5]. The standard melphalan-prednisone treatment regimen for elderly transplant-ineligible MM patients has been ameliorated by incorporating the potent novel agents – thalidomide, bortezomib, and lenalidomide. Novel two- and three-drug combinations have been recommended for elderly transplant-ineligible MM patients in the United States of America (USA) [6], Europe [2, 7], as well as in Serbia [8] after assessing their effectiveness in clinical trials. However, the favorable health outcomes are associated with significantly higher costs, emphasizing the need for a pharmaco-economic evaluation. Only a third of cancer indications approved for treatment with targeted cancer therapies by the European Medicines Agency are reimbursed in Serbia [9]. There is a lack of published data on the reimbursement decisions and availability of novel MM treatment options in the Serbian setting. A previously published systematic literature review showed that there were no studies published on economic aspects of MM outside of the USA and Western Europe [10]. Due to constraints on the healthcare budget and an economic crisis spanning the last few decades, an assessment of the costs of alternative treatments in the Serbian setting is essential for a rational allocation of resources. The need for adopting pharmaco-economic aspects in healthcare policy decision making in Serbia is emphasized in case of MM, as a rare life-long treated disease with a spectrum of different medications some of which are available only at high costs. The economic burden of the MM is expected to steadily rise along with the aging of the Serbian population and also with prolonged patient survival due to novel drug generations. The aim of our study was to ascertain the treatment patterns available for elderly non-transplant eligible MM patients in Serbia, to analyze the total costs of different sequential treatment lines, and to guide efficient resource allocation by providing an insight into the costs of MM-related healthcare services.

Material and Methods

We have performed a micro-costing study from the perspective of the national health system of the Republic of Serbia. An Excel-based cost estimator was developed with the goal of assessing the total direct costs of sequential treatment options commonly used in the Serbian healthcare setting for elderly non-transplant eligible MM patients. Treatment options for the selected subgroup of patients were defined following the guidelines for diagnosis and treatment of MM in Serbia [8] and a clinical expert's opinion (coauthor A. Savić). In order to assess the lifetime per-patient costs, we considered three sequential treatment lines followed by palliative treatment **(Table 1)**. The principles of MM management in our analysis are based on the Serbian national clinical guidelines [8]. Further details, such as the frequency of diagnostic procedures throughout the treatment, duration of hospitalization for different treatment protocols, the necessity of physician examinations etc., were provided by nine clinical experts from Serbia in a three-round Delphi panel. The Delphi panel is a well-established method to achieve a consensus on clinical experts' opinion on a topic in their field of interest [11]. An overview of the drug protocols currently used in the Serbian clinical setting is presented in **Table 2** [12–29]. We assessed the costs of five different first-line treatment options and possible sequential treat-

Table 1. Assessed treatment sequences
Tabela 1. *Analizirane terapijske sekvence*

Analyzed Treatment Sequences/*Analizirane terapijske sekvence*						
First-line Tx/*Prva linija Tx*		Second-line Tx/*Druga linija Tx*		Third-line Tx/*Treća linija Tx*	Palliative Tx/*Palijativna Tx*	
MPT	→	VCD/VD/RD	→	RD/BTP/Chemo	→	CP
MPV	→	CTD/MPT/RD	→	RD/BTP/Chemo	→	CP
CTD	→	MPV/VD/RD	→	RD/BTP/Chemo	→	CP
VCD	→	MPT/ RD	→	RD/BTP/Chemo	→	CP
BP	→	RD	→	BTP/Chemo	→	CP

Legend/*Legenda*: B - bendamustine; C - cyclophosphamide; Chemo-standard chemotherapy/*standardna hemioterapija*; D - dexamethasone; M - melphalan; P -prednisone; R - lenalidomide; T - thalidomide; Tx – treatment/*terapija*; V - bortezomib

Table 2. Treatment protocols used in the clinical practice
Tabela 2. *Terapijski protokoli koji se primenjuju u kliničkoj praksi*

Protocol description *Opis protokola*	Recommended treatment duration and median treatment duration (MTD) reported in the trials *Preporučeno trajanje tretmana i srednje trajanje tretmana (MTD) prijavljeno u studijama*	Median progression-free survival (PFS) *Srednje vreme preživljavanja bez progresije bolesti (PFS)*	Source *Izvor*
BP, 1st-line/*prva linija* Bendamustine, 120-150 mg/m^2/d., i.v., on days/*na dan* 1, 2 Prednisone, 60 mg/m^2/d., p.o., on days/*na dan* 1 - 4	6 4-week cycles/*četvoronedeljnih ciklusa* MTD: 6,8 m.	PFS: 14 m.	Ponisch, 2006 [12]
CTD, 1st –line/*prva linija* Cyclophosphamide, 500 mg , i.v., on days/*na dan* 1, 8, 15 Thalidomide, 100-200 mg/d., p.o. Dexamethasone, 40 mg/d., i.v., on days/*na dan* 1 - 4 and 17 - 20 CTD, 2nd-line/*druga linija* Cyclophosphamide, 500 mg/m^2, i.v., on day/*na dan* 1 Thalidomide 100 mg/d., p.o. Dexamethasone 40 mg/d., i.v. on days/*na dan* 1 - 4 and *(i)* 17 - 20	6 4-week cycles/*četvoronedeljnih ciklusa* MTD, 1st line/*prva linija* : 9 m. MTD, 2nd line/*druga linija* : 6 m.	PFS, 1st line/*prva linija*: 13 m. PFS, 2nd line/*druga linija*: 10 m.	1st line/*prva linija*: Morgan, 2011 [13] 2nd line/*druga linija*: Dmoszynska, 2010 [14]
MPT, 1st- & 2nd-line/*prva i druga linija* Melphalan, 4 mg/m^2/d., p. o., 7 days/*dana* Prednisone, 40 mg/m^2/d., p.o., 7 days/*dana* Thalidomide, 100mg/d. p.o.	6 4-week cycles/*četvoronedeljnih ciklusa* MTD 1st line/*prva linija*: 9.6 m. MTD 2nd line/*druga linija*:4 m.	PFS, 1st line/*prva linija*: 21.8 m. PFS, 2nd line/*druga linija*: 9 m.	1st line/*prva linija*: Palumbo, 2008 [15] 2nd line/*druga linija*: Palumbo, 2006 [16]
MPV, 1st –line/*prva linija* a) Melphalan, 9 mg/m^2/d., p.o., on days *(na dan)* 1 - 4 Prednisone, 60 mg/m^2/d., p.o., on days/*na dan* 1 - 4 Bortezomib, 1.3 mg/m^2, i.v, on days/*na dan* 1, 4, 8, 11, 22, 25, 29, 32 b) Melphalan, 9 mg/m^2/d., p.o., on days/*na dan* 1 - 4 Prednisone, 60 mg/m^2/d., p.o., on days/*na dan* 1 - 4 Bortezomib, 1.3 mg/m^2, i.v., on days/*na dan* 1, 8, 15, 22 MPV, 2nd-line/*druga linija* Melphalan, 24 mg/d., p.o., 28 days *(dana)* Prednisone, 50 mg every other day/*svakog drugog dana*, p.o. Bortezomib, 1.3 mg/m^2, i.v., on days/*na dan* 1, 8, 15, 22	1st line/*prva linija* 4 6-week cycles/*četvoronedeljnih ciklusa* 5 4-week cycles/*četvoronedeljnih ciklusa* MTD, 1st line/*prva linija*: 11.5 m 2nd line/*druga linija* 9 4-week cycles/*četvoronedeljnih ciklusa* MTD, 2nd line/*druga linija*: ND	PFS, 1st line/*prva linija*: 24 m. PFS, 2nd line/*druga linija*: 18 m.	1st line/*prva linija*: San Miguel, 2008 [17] 2nd line/*druga linija*: Petrucci, 2013 [18]
Rd, 2nd and 3rd line/*druga i treća linija* Lenalidomide, 25 mg/d., p.o., on days/*na dan* 1 - 21 Dexamethasone 40 mg/d., i.v., on days/*na dan* 1 - 4, 9 - 12, 17 - 20	4-week cycles until progression/*četvoronedeljni ciklusi do progresije bolesti* MTD 2nd-line/*druga linija*: 12.5 m. MTD 3rd-line/*treća linija*: 9.2 m.	PFS 2nd-line/*druga linija*: 14.1 m. PFS 3rd-line/*treća linija*: 9.5 m.	Stadtmauer, 2009 [19]

VCD, 1st- & 2nd-line/*prva i druga linija* Cyclophosphamide, 500 mg i.v. on days/*na dan* 1, 8, 15 Bortezomib, 1.3 mg/m^2, i.v., on days/*na dan* 1, 4, 8, 11 Dexamethasone, 20 mg/d., i.v., days/*na dan* 1, 2, 4, 5, 8, 9, 11, 12	8 3-week cycles/*tronedeljnih ciklusa* MTD, 1st line/*prva linija*: 4.5 m. MTD, 2nd line/*druga linija*: 4.5 m.	PFS, 1st line/*prva linija*: 21 m. PFS, 2nd line/*druga linija*: 16 m.	1st line/*prva linija*: Kumar, 2012 [20] 2nd line/*druga linija*: Kropff, 2007 [21]
VD, 2nd-line/*druga linija* Bortezomib, 1.3 mg/m^2, i.v., on days/*na dan* 1, 4, 8, 11 Dexamethasone, 20 mg, i.v., on days/*na dan* 1, 2, 4, 5, 8, 9, 11, 12	8 3-week cycles/*tronedeljnih ciklusa* MTD: 3.5 m.	PFS: 7.4 m.	Hjorth, 2012 [22]
BPT, 3rd-line/*treća linija* Bendamustine, 60 mg/m^2, i.v., on days/*na dan* 1,8,15 Prednisone, 100 mg, p.o., on days/*na dan* 1,8,15,22 Thalidomide, 100 mg, p.o., on days/*na dan* 1 - 28	6 4-week cycles/*četvoronedeljnih ciklusa* MTD: 5.5 m.	PFS: 12 m.	Poenisch, 2008 [23]
Chemotherapy, 3rd-line/*treća linija* DCEP Dexamethasone, 40 mg/d., i.v., on days/*na dan* 1 - 4 Cyclophosphamide, 400 mg/m^2, i.v., on days/*na dan* 1 - 4 Etoposide, 40 mg/m^2, i.v., on days/*na dan* 1 - 4 Cisplatin, 10 mg/m^2, i.v., on days/*na dan* 1 - 4 DT-PACE Dexamethasone, 40 mg/d., i.v., on days/*na dan* 1 - 4 Thalidomide, 200 mg, p.o., on days/*na dan* 1 - 28 Cisplatin, 10 mg/m^2, i.v., on days/*na dan* 1 - 4 Doxorubicin, 10 mg/m^2, i.v., on days/*na dan* 1 - 4 Cyclophosphamide, 400 mg/m^2, i.v., on days/*na dan* 1 - 4 Etoposide, 40 mg/m^2, i.v., on days *(na dan)* 1 - 4	DCEP 6 3-week cycles/*tronedeljnih ciklusa* MTD: 1.5 m. DT-PACE 2 6-week cycles/*šestonedeljnih ciklusa* MTD: 3 m.	DCEP PFS: 3.7 m. DT-PACE PFS: 5.5 m.	DCEP Park,2014, obs. [24] Dadacaridou, 2007 [25] DT-PACE Gerrie, 2013, obs. [26] Lee, 2003 [27]
CP, palliative treatment/*palijativni tretman* Cyclophosphamide, 50 mg/II d., p.o. Prednisone, 30 mg/II d., p.o.	Continuously/*Kontinuirano* Median overall survival/*srednje vreme preživljavanja*: 16.4 m. MTD*: 9.2 m.	PFS: 11.6 m.	Weerdt, 2001, obs. [28] Zhou, 2010 [29]

Legend/*Legenda*: * MTD based on opinion of the clinicians from Serbia (Delphi panel)/*MTD na osnovu mišljenja srpskih kliničara*; B - bendamustine; C - cyclophosphamide; Chemo - chemotherapy/*hemioterapija*; d - day/*dan*; D - dexamethasone; i.v. - intravenous administration/*intravenska primena*; M - melphalan; m. - months/*meseci*; ND - not determined/*nije ustanovljeno*; obs. - observational studies/*obzervacione studije*; P - prednisone; p.o. - oral uptake/*oralni unos*; R - lenalidomide; T - thalidomide; Tx - treatment/*terapija*; V - bortezomib

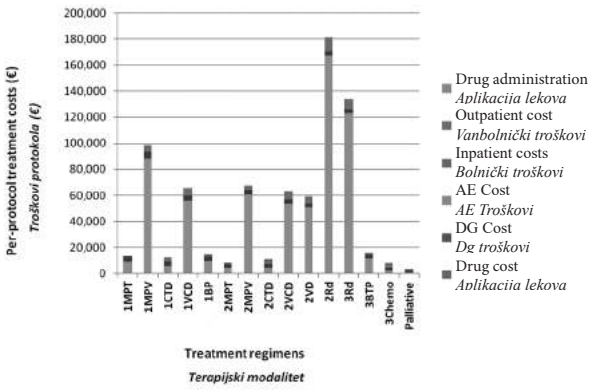
ment alternatives for a second- and third-line and palliative treatment. The total direct costs for MM management and treatment comprised the expenditures for drugs, diagnostic procedures, inpatient and outpatient care, administration of injectable drugs and management of adverse events for each treatment protocol. The healthcare service activities and the respective cost items are presented in **Scheme 1.** We followed the recommendations of the guidelines for disease-specific costing studies in low- and middle-income countries [30]. All costs were priced in 2016 and the values were converted to Euros (€)

Table 3. Sensitivity analysis; Varying the annual discount rate; Total costs and median sequence duration for the treatment strategies
Tabela 3. *Analiza osetljivosti. Menjanje godišnje diskontne stope. Ukupni troškovi i srednja vrednost trajanja terapijske sekvence*

Treatment Sequence *Terapijska sekvenca*	Base-Case (3% discount rate for costs and effectiveness outcomes) *Analiza referentnog slučaja (3%-na diskontna stopa na troškove i ishode)*		Recommendations of the Serbian guideline for farmacoeconomic evauations (3% discount rate for costs and 1.5% for effectiveness outcomes)/ *Preporuke vodiča za farmakoekonomke analize u Srbiji (3%-na diskontna stopa na troškove i 1,5%-na diskontna stopa na ishode)*		(5% discount rate for costs and effectiveness outcomes) *(5%-na diskontna stopa na troškove i ishode)*	
	Sequence Costs (€) *Troškovi sekvence (u evrima)*	Sequence Duration (months) *Trajanje sekvence (u mesecima)*	Sequence Costs (€) *Troškovi sekvence (u evrima)*	Sequence Duration (months) *Trajanje sekvence (u mesecima)*	Sequence Costs (€) *Troškovi sekvence (u evrima)*	Sequence Duration (months) *Trajanje sekvence (u mesecima)*
Starting with MPT *Započinjanje sa MPT*	85,800	49.3	85,800	50.9	82,100	47.3
Starting with CTD *Započinjanje sa CT*	96,400	42.8	96,400	44.0	93,200	41.3
Starting with MPV *Započinjanje sa MPV*	153,800	51.8	153,800	53.5	148,700	49.6
Starting with VCD *Započinjanje sa VCD*	118,600	48.4	118,600	49.9	114,800	46.4
Starting with BP *Započinjanje sa BP*	150,100	42.4	150,100	43.6	145,300	40.9

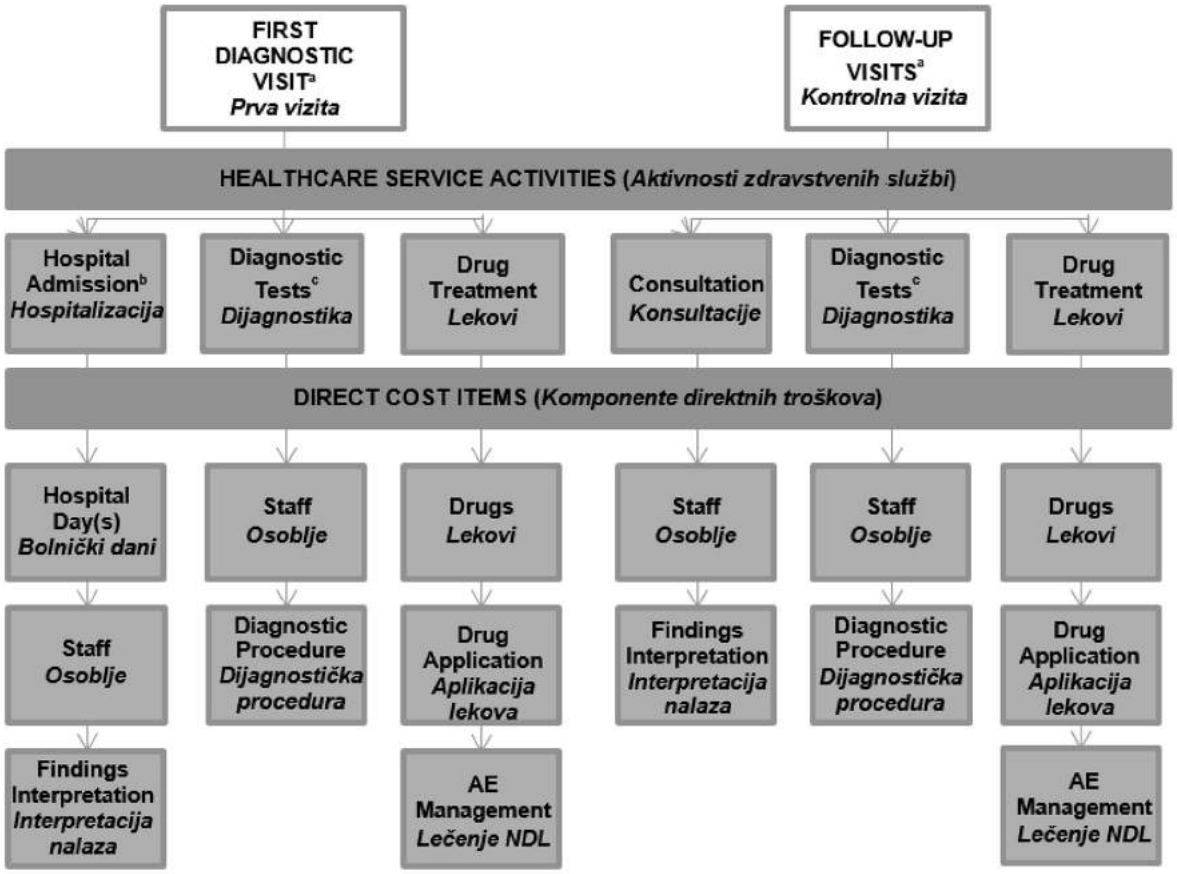
Legend/*Legenda*: BP – bendamustine-predinsone; CTD - cyclophosphamide, thalidomide, dexamethasone; MPT – melphalan, prednisone, thalidomide; MPV – melphalan, prednisone, bortezomib; VCD – bortezomib, cyclophosphamide, dexamethasone; € - Euro/*Evro*

according to the purchasing power parity rate in 2016 (i.e., the last reference year) [31]. Drug costs, costs of diagnostic procedures, inpatient and outpatient costs were provided by the National Health Insurance Fund (NHIF) of the Republic of Serbia



Graph 1. Direct per-protocol costs of the analyzed treatment regimens
Grafikon 1. *Direktni troškovi analiziranih terapijskih protokola*
Legend/*Legenda*: AE - adverse events/*neželjeni efekti*; B - bendamustine; C - cyclophosphamide; Chemo - chemotherapy/*hemioterapija*; D - dexamethasone; Dg - diagnosis/*dijagnostika*; M - melphalan; P - prednisone; R - lenalidomide; T - thalidomide; V - bortezomib. The numbers (1, 2, 3) indicate the treatment line/ *Brojevi (1, 2, 3) upućuju na liniju terapije*

[32]. The complete list of the analyzed drugs, their administration and the sources are shown in **Table 2**. The unit costs for staff, hospital day and injectable drug application were extracted from the NHIF's price list for health services for secondary and tertiary medical care [33]. The costs of prophylaxis and adverse events management were based on the frequency of their occurrence extracted from the respective clinical trials. The principles of management of these events are described in the guidelines for the management of adverse events in elderly MM patients [34] and adapted for the clinical setting by Serbian clinical experts. To estimate the total direct costs, we used the bottom-up microcosting methodology, since it results in the most precise cost estimates for health care [30]. Average patient costs were calculated by multiplying unit costs with the corresponding health resource consumption. We estimated per-protocol costs in each treatment line and the total direct per-patient cost of the life-long MM treatment for the alternative treatment sequences. The sequence costs were calculated based on the protocol costs, the survival probability and probability to switch to a particular subsequent treatment, discounted over the sequence duration. The sequence duration was calculated as a discounted sum of the reported median PFS for each of the treatment protocols in the sequence, taking into account the probability to switch to a particular subsequent treatment. Furthermore, we as-



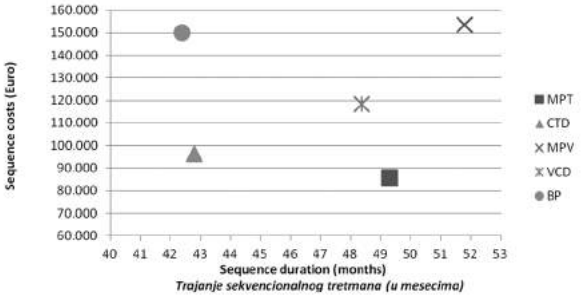
Scheme 1. Activities of healthcare services and cost item identification for MM treatment
Shema 1. *Aktivnosti zdravstvenih službi i identifikacija komponenata troškova MM tretmana*

Legend/*Legenda*: [a] Regular follow-up monthly visits during the first three months and then 3-month visits/ *Redovne mesečne kontrole tokom prva tri meseca terapije, a zatim na svaka tri meseca*; [b] Hospital admission for the first diagnostic visit and in case of severe adverse events/ *Hospitalizacija prilikom postavljanja dijagnoze i u slučaju ozbiljnih neželjenih reakcija*; [c] Diagnostic test: complete blood count, biochemical analyses of blood and urine samples, bone marrow puncture and aspiration, fluorescence in-situ hybridization of cytological specimens and imaging techniques (X-ray)/*Dijagnostički testovi: kompletna krvna slika, biohemijske analize krvi i urina, punkcija i aspiracija kostne srži, fluorescentna in situ hibridizacija citoloskih uzoraka i imidžing tehnike (RTG)*;. Inpatient costs: hospital admission, staff and interpretation of the findings during the hospital stay/*Bolnički troškovi: hospitalizacija, osoblje, interpretacija nalaza tokom hospitalizacije;* Outpatient costs: physician consultation during regular follow up visits and interpretation of diagnostic test findings/*Vanbolnički troškovi: troškovi redovnih kontrolnih poseta lekaru i interpretacija nalaza*; AE - adverse events; NDL - nežejena dejstva lekova.
Adopted from the guideline for disease-specific micro-costing studies [35]/*Prema vodiču za mikro-troškovne studije bolesti [35]*)

sessed the uncertainty of the cost estimates in several deterministic sensitivity analyses. We varied the discount rate, the protocol duration and the probabilities of switching to the second- and third-generation treatment alternatives.

Results

Per-protocol costs varied significantly among the treatment alternatives in all treatment lines **(Graph 1)**. Costs of thalidomide-based protocols in the first (melphalan-prednisone-thalidomide (MPT): 13,100 €, cyclophosphamide-thalidomide-dexamethasone (CTD): 12,500 €) and second line (MPT: 8,000 €, CTD: 10,900 €) were notably lower than the costs of bortezomib-based protocols (first line, melphalan-prednisone-bortezomib (MPV): 98,800 €, bortezomib-cyclophosphamide-dexamethasone (VCD): 65,800 €; second-line, melphalan-prednison-bortezomib (MPV): 67,500 €, bortezomib-cyclophosphamide-dexamethasone (VCD): 63,400 €, bortezomib-dexamethasone (VD): 59,200 €). The highest per-protocol costs were estimated for lenalidomide-dexamethasone (RD) protocols (second line: 181,200 €, third line: 133,700 €). Per-protocol costs were mainly driven by the drug acquisition costs, although the share of this cost component varied among the protocols (29 – 92%) **(Graph 1)**. The exception was pallia-



Graph 2. Total costs and median duration of the sequential treatment strategies

Grafikon 2. *Totalni troškovi i srednje vreme trajanja sekvencijalne terapije*

Legend/*Legenda*: BP – starting with bendamustine-prednisone/*Započinjanje terapije protokolom bendamustine-prednisone*; CTD - starting with cyclophosphamide, thalidomide, dexamethasone/*Započinjanje terapije protokolom cyclophosphamide, thalidomide, dexamethasone*; MPT – starting with melphalan, prednisone, thalidomide/*Započinjanje terapije protokolom melphalan, prednisone, thalidomide*; MPV – starting with melphalan, prednisone, bortezomib/*Započinjanje terapije protokolom melphalan, prednisone, bortezomib;* VCD – starting with bortezomib, cyclophosphamide, dexamethasone/*Započinjanje terapije protokolom bortezomib, cyclophosphamide, dexamethasone*; € - Euro/*Evro*

tive treatment, where the highest share of expenditures was allocated to the diagnostic procedures during the follow-up period (60%). In the remaining protocols, costs of diagnostic procedures accounted for 2% to 35% of the total per-protocol costs, and costs of inpatient care were the third largest component (3 - 21%), except in MPT protocols (less than 1%). Costs of administration of injectable drugs were on average estimated to be 2% of per-protocol costs. Outpatient care costs were smaller than 1% for all the protocols. The average per-patient cost of a life-long MM management and treatment for elderly patients who are not transplant candidates in Serbia is 120,100 €. The highest sequential treatment costs were associated with the treatment pathways starting with MPV (153,800 €) and bendamustine-prednisone (BP) (150,100 €). The most affordable sequential treatment was the one starting with MPT protocol (85,800 €), followed by the treatment sequence starting with CTD (96,400 €). The cost of the sequential treatment with the frontline VCD was 118,600 €. The total costs of the sequential treatment options in relation to the median duration of sequential treatment are shown in the **Graph 2**. The national guidelines for cost-effectiveness analysis in Serbia [35] recommend applying a 1.5% annual discount rate for effectiveness outcomes while keeping a 3% discount rate for costs. Furthermore, we analyzed the model results with a 5% discount rate applied for both costs and effectiveness outcomes. The estimated costs were robust to the discount rate variations - starting with MPV remained the most costly sequence. Starting with MPV was the sequence with the longest duration, followed by starting with MPT **(Table 3)**. The results of the Delphi panel we conducted suggested that, although recommended by the national clinical guidelines as an option for relapsed or refractory MM [8], lenalidomide is still not available in all the institutions in Serbia. In the sensitivity analysis, we analyzed the robustness of our results if we do not consider lenalidomide-based alternatives as an option in second- and third-line treatment. Furthermore, we analyzed the possibility that lenalidomide-based alternatives will be used more often in the clinical setting once they become available, so we increased the probability of switching to RD from the baseline value of 0.1 to 0.3 in the second-line, and from the baseline value 0.35 to 0.55 in the third-line setting, based on the results of the Delphi panel. The sensitivity analysis showed that incorporation of lenalidomide in the common treatment pathways increased the costs of the alternative sequential treatments by 30% on average, but also prolonged survival (treatment duration) on average by 3%. Meanwhile, the ranking of the strategies remained the same (results available on request). In the base-case analysis, we estimated treatment costs based on the guideline recommendations on the protocol treatment duration [8]. We assessed this assumption and analyzed the alteration in the cost estimates when assuming that the protocol duration equals the median treatment duration reported in the respective randomized controlled trials **(Table 2)**. The ranking of the strategies was different in this case. However, a substantial change in the sequence costs (> 10%) was noted only in case of the sequence starting with VCD (~120,000 €).

Discussion

Based on our analysis, the MM-related health care expenditures are mostly allocated to anticancer treatment. Costs of MM treatment and care vary substantially among the analyzed protocols. The most costly treatment protocol is RD, recommended for refractory or recurrent patients with MM. Among the routinely administered treatment options, the highest costs are estimated for treatment sequences starting with MPV. This can be explained by the fact that the first-line MPV costs are higher than the costs of bortezomib-based treatments administered as a second-line alternative to the patients who failed the upfront thalidomide-based regimens (85,200 € per protocol for the first-line MPV vs. 63,300 € per course for second-line bortezomib-based protocols on average).

In the base-case analysis, we estimated the costs of the sequential treatment alternatives under the assumption of the guideline-based treatment durations. Since this might not be the situation in the real-world setting, we tested this assumption by taking into the perspective the duration of the treatment reported in the respective studies. The resulting costs of the sequential treatments markedly differed only in case when starting with VCD. The VCD sequence costs were 33% lower due to the shortened treatment duration of both first-line VCD (from 6 to 4.5 months) and subsequent second-line MPT protocol (from 6 to 4 months).

Although there is a number of economic evaluations assessing the burden of MM [33, 36, 37], the conclu-

sions of these studies are not easily transferable to the Serbian healthcare system, due to variations in drug availability and costs and diverse patterns of treatment between the different healthcare systems. Our analysis resulted in a higher cost of bortezomib-based protocols in comparison to thalidomide- and bendamustine-based protocols for the first-line MM treatment. In the study of Garrison et al. [38], MPT and MPV protocols were compared from the perspective of the United States, where MPV protocol is shown to be less costly. However, the authors explained that the results of this comparison are strongly related to the price of thalidomide. Of note, in the United States, thalidomide is available only at a patent-protected price, while in Europe including Serbia, generic pricing is available. Thus, the cost analyses that adopted perspectives of the European Union countries [39– 41] resulted in higher costs of bortezomib-based protocols in comparison to thalidomide-based ones. Our analysis showed that the highest proportion of the healthcare expenditures for the treatment and management of MM in Serbia is allocated to drug acquisition. The proportion of total costs attributed to anti-cancer treatment in recurrent MM was 92% for lenalidomide-based regimens, 90% for bortezomib-based regimens and 54% for thalidomide-based protocols. The higher proportions of drug costs in comparison to other studies [39, 40) can be explained by the fact that the pricing of medications in Serbia is based on the European Union recommendations [42], while the unit costs of diagnostic procedures and inpatient care are significantly lower in Eastern Europe.

Our study has several limitations. In order to simplify the clinical reality, our analysis was based on several assumptions. We assumed that the effectiveness of the subsequent treatment lines is independent of the previous treatment. No dose modifications were assumed in the analysis. Clinical and methodological diversity between the studies was inevitable, considering a wide spectrum of analyzed treatment protocols. For example, variations in drug dosing and administration that exist even within a single regimen may affect the generalizability of our findings. Effectiveness estimates for chemotherapy and palliative treatment were based on the combination of evidence from clinical trials and observational studies since the data in the published randomized controlled trials were incomplete and unreliable; thus, we varied the estimates in a sensitivity analysis and the main conclusions remained the same. Data on treatment patterns and resource use in the Serbian settings are based on the national guideline recommendations, modified and precisely defined by the panel of clinical experts. Although the optimal size of the expert group in a Delphi panel is not defined [11], it would be interesting in future studies to re-analyze the estimates based on the data provided by a wider circle of clinical experts. Furthermore, validation of the findings with patient-level data would result in even more robust cost estimates. We consider the methodological approach and the results of our study generalizable to other hematologic institutions in Serbia and other similar publicly funded health care systems. However, the results of the Delphi panel that we conducted have shown that there might be individual practice variations among the oncologists of one institution, which could be even more evident across different hospital settings. Therefore, it is necessary to ensure that the recommendations of evidence-based national guidelines are followed in order to control costs, but also to provide the most beneficial patient outcomes. Based on our analysis, the most influential components of the total costs were costs of anti-cancer treatment, costs for diagnostic tests and inpatient care. Therefore, if the Serbian society that operates under the restricted healthcare budget tends to reduce the spending or reallocate the resources for management of elderly MM patients, the preferred approaches would be those reducing treatment costs and length of hospitalization. Costs of inpatient care could be reduced by redistribution of patients on injectable drugs to the less costly daily hospital or outpatient care setting. However, in elderly patients, not only costs of management of the disease but also the quality of life, travel distance to the institution and patient's wishes should also be considered and well balanced during the decision making [43].

Conclusion

In conclusion, the study has revealed that the sequential strategy, which provides the most durable life-long sequential treatment, meaning concurrently the most beneficial survival outcome, is the treatment sequence starting with **melphalan-prednison-bortezomib**, followed by the markedly less-costly sequence starting with **melphalan-prednisone-thalidomide**. The absolute difference in costs between these two strategies is 68,000 €, and the absolute difference in median sequential treatment duration is 2.5 months. A cost-effectiveness analysis, preferably employing carefully assessed quality of life for every multiple myeloma treatment protocol as an effectiveness measure, would result in more informative cost-effectiveness estimates, providing a reliable base for clinical decision making.

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INSTRUMENTAL AND INTEGRATIVE MOTIVATION IN TEACHING ENGLISH FOR MEDICAL PURPOSES

INSTRUMENTALNA I INTEGRATIVNA MOTIVACIJA U NASTAVI ENGLESKOG KAO JEZIKA MEDICINSKE STRUKE

Zoran MAROŠAN and Vuk MARKOVIĆ

Summary
Introduction. The aim of this paper is to determine the degree and type of motivation for learning English for specific purposes in the first year medical students of the Faculty of Medicine Novi Sad. The paper presents the results of the research on instrumental and integrative motivation carried out in a sample of 61 first year medical students of the the Faculty of Medicine in Novi Sad. **Material and Methods.** The paper is based on a survey carried out during the summer semester of 2017/18. An anonymous survey was conducted through a questionnaire that investigated instrumental and integrative motivation. In addition, the questionnaire included questions on the year of study, semester, number of years of learning English and regularity of attendance. **Results.** The results are presented in a table and the answers are expressed in percentages. The results were analyzed in two segments, based on the type of motivation that was tested. The research confirmed the hypothesis that medical students had high levels of instrumental motivation, mostly focused on their further advancement in the profession. The research also showed a strong integrative motivation of the respondents and their interest in the elements of culture and civilization of the target language. **Conclusion.** In conclusion, the possible impacts of this research on practical English teaching for medical purposes should be considered in order to provide guidance for further research.
Key Words: Students, Medical; Teaching; Motivation; Language; Education, Medical; Surveys and Questionnaires

Sažetak
Uvod. Cilj ovog rada je da se utvrdi stepen i vrsta motivacije u učenju engleskog jezika struke kod studenata prve godine medicine na Medicinskom fakultetu Novi Sad. U radu su predstavljeni rezultati istraživanja instrumentalne i integrativne motivacije sprovedenog na uzorku od 61 studenta prve godine medicine na Medicinskom fakultetu Novi Sad. **Materijal i metode.** Rad je zasnovan na anketi sprovedenoj tokom letnjeg semestra 2017/2018. školske godine. Anketa je bila anonimna, a istraživanje je izvedeno putem upitnika kojim se testira instrumentalna i integrativna motivacija. Pored toga, upitnik je sadržao i podatke o godini studija, polu, broju godina učenja engleskog jezika i redovnosti pohađanja nastave. **Rezultati.** Rezultati istraživanja prikazani su tabelarno i odgovori studenata izraženi su u procentima. Rezultati su analizirani u dva segmenta na osnovu vrste motivacije koja je testirana. Istraživanje je potvrdilo hipoteze o visokom stepenu instrumentalne motivacije kod studenata medicine, koja je u najvećoj meri usmerena ka njihovom daljem napretku u struci. Istraživanje je pokazalo i snažnu integrativnu motivaciju ispitanika i zainteresovanost za elemente kulture i civilizacije ciljnog jezika. **Zaključak.** U zaključku se razmatraju mogući uticaji ovog istraživanja na praktičnu nastavu engleskog kao jezika medicinske struke i daju smernice za dalja istraživanja.
Ključne reči: studenti medicine; predavanje; motivacija; jezik; medicinsko obrazovanje; ankete i upitnici

Introduction

Today, more than ever before, due to globalization, the Internet, politics and new trends in the world economy, as well as the need to keep up with the latest scientific achievements of utmost importance for all human activities, knowledge of English has become an imperative. That is why, apart from general English language courses, English for specific purposes (ESP) courses have gained in popularity and have become mandatory courses at most of the colleges and universities around the world. English, as a language of profession, encompasses almost all fields of science and humanities, such as business, law, economics, electrical and mechanical engineering, technology, mathematics, physics, medicine, and others. In each of these areas, emphasis is placed on professional terminology, its understanding and use. It should be noted that, in addition to this aspect, English language has to be taught and studied in everyday, social, cultural and historical contexts, as it contributes to a better understanding and contacts with people from these areas. One of the most important factors influencing success in foreign language acquisition is motivation, which, in addition to the attitudes that

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Abbreviations

ESP – English for Specific Purposes
ESL – English as a second language

students have towards learning a foreign language, both general as well as the language of the profession, has been a dynamic field of research in the last decades. Most of the studies were focused on the concept of motivation and attitude, obviously under the influence of the Gardner model, within the framework of which the attitude was considered a subcategory of the broader concept of motivation [1–3]. According to Dörnyei [4], motivation affects the basic dimensions of human behavior, its direction and magnitude (intensity). In addition to the attitudes, the concept of motivation is inextricably linked with the analysis of the needs of foreign language learners in the sense that if the program is more suited to the students, no matter whether it was a general or a language of the profession, the motivation and the final success in its accomplishment will be greater [5, 6].

The motivation of medical students to learn English as a language for specific purposes of medical profession has not been studied in our country. English as a language for medical purposes is largely different from other fields, primarily from humanities, but also natural sciences, since, on the vocabulary level, it has a much higher percentage of the words of Latin and Greek origin. Additionally, a large number of modern medical terms in Serbian are usually borrowed from English, often in a form that is not regular or systematized. This may at first glance lead to the conclusion that a greater percentage of the words of English origin are known or recognizable to the student of English for medical purposes, but may often result in the acquisition of incorrect forms and structures. At the same time, a large percentage of professional publications that are required for specialization are often available only in English, which further motivates students to improve both the general and medical English. The aim of this paper is to examine the degree of instrumental and integrative motivation of the first year medical students at Novi Sad University in learning English for medical purposes.

Motivation

Motivation is in the focus of a great deal of studies and research since it largely affects the acquisition of any foreign language. It is often mentioned by language teachers when they talk of success and failure to learn a foreign language [4]. Even though there are a lot of definitions of motivation, which is "not a simple construct" [7]. Dörnyei tried to define it as the "dynamically changing cumulative arousal in a person that initiates, directs, coordinates, amplifies, terminates, and evaluates the cognitive and motor processes whereby initial wishes and desires are selected, prioritized, operationalized and successfully or unsuccessfully acted out" [8].

Development of motivation research

Research on motivation in foreign language learning has been developing rapidly since the 1960s. The first significant results in this field were published by Gardner and Lambert in Canada in 1959. Within the Socio-psychological research period (1959 - 1990), the work of Gardner et al. had a decisive influence on the understanding of the role of motivation in learning a foreign language. They standardized techniques for measuring the broad range of motivation components based on socio-psychological approach and studied motivation as a complex system of different factors and attitudes. Probably the most important contribution they made is the identification of integrative and instrumental motivation [1]. The student is instrumentally motivated when learning the language to pass the exam, get a job, advance in his/her career, and use it when traveling abroad. Integrative motivation, on the other hand, encompasses his/her desire and motivation to identify or integrate into the community of the language he/she studies [9]. The main reasons for learning a foreign language are assimilation with the community and culture of the target language, with the possible powerful emotional interest in the speaker of that language. This dichotomy of motivation (instrumental and integrative) has also been expanded with the term orientation, taking into account whether the context of language learning is oriented towards academic learning and careers, or to the social and cultural model [10]. Gardner [7] also claimed that, when considering the roots of motivation for learning a foreign language in a school context, motivation must be considered not only at the educational level (as any other subject), but also in a cultural context, which is generally not relevant to the majority of other subjects of study. Most language teachers believe that motivation is a key factor in language acquisition. The importance of motivation in learning English cannot be disputed [10, 11]. The importance of motivation in the process of learning a foreign language is explained by Gardner [12] in the following way: "If a student is motivated, he or she has a reason (motive) to participate in relevant activities, to invest effort, to be persistent, dedicated to tasks, show the desire to achieve his/her goal, enjoying the activities, etc." In the analysis of 75 studies dealing with motivation, Masgoret and Gardner [13] concluded that "motivation is more associated with success in learning a different language than any other factor."

The research of motivation during the 1990s is characterized by work on cognitive theories in the psychology of education. During this period of learning a foreign language, motivation is called the cognitive period, and it brings a different view of the role of motivation, now from the cognitive aspect. The key aspects of motivation are viewed as two-fold, as the interaction of two trends: (a) the individual's estimate of one`s own abilities, poten-

Table 1. Results of a questionnaire on the instrumental and integrative motivation of the first year students of medicine at the Faculty of Medicine in Novi Sad
Tabela 1. *Rezultati upitnika za istraživanje instrumentalne i integrativne motivacije studenata prve godine Medicinskog fakulteta u Novom Sadu*

		1. I absolutely disagree *Apsolutno se ne slažem*	2. I disagree *Ne slažem se*	3. I mainly disagree *Uglavnom se ne slažem*	4. I mainly agree *Uglavnom se slažem*	5 I agree *Slažem se*	6. I absolutely agree *Apsolutno se ne slažem*
1.	Learning English is a pleasure for me, not an obligation. *Učenje engleskog jezika za mene predstavlja zadovoljstvo, a ne obavezu.*	3.2 %	4.9%		39.3%	29.5%	22.9%
2.	I feel that knowing English will be beneficial for my future career./*Smatram da ću tokom svoje buduće karijere imati koristi od poznavanja engleskog.*	3.2 %			6.5%	21.3%	68.8%
3.	The feeling of pleasure after successfully learning English language skills is enough to motivate me to learn./*Osećaj zadovoljstva posle uspešno savladanog gradiva iz engleskog jezika dovoljan mi je podsticaj za učenje.*		4.9%	3.2 %	39.3%	36%	16.4%
4.	I want to learn English well in order to achieve better overall results during my studies. *Želim da dobro naučim engleski jezik da bih postigao/la što bolji opšti uspeh u toku studija.*	3.2 %	3.2 %		11.4%	42.6%	39.3%
5.	While learning English I like to acquire new knowledge connected with my future profession *Kroz učenje engleskog jezika volim da stičem znanja u vezi sa svojom budućom profesijom.*	3.2 %	8.2%	6.5%	26.2%	22.9%	32.7%
6.	I want to learn English well in order to watch movies without subtitles./*Želim da dobro naučim engleski jezik da bih mogao/la da gledam filmove sa engleskog govornog područja bez titlova.*		4.9%	9.8%	26.2%	24.6%	34.4%
7.	I want to learn English in order to enroll at the master/PhD studies abroad./*Želim da dobro naučim engleski jezik da bih upisao/la master/doktorske studije u inostranstvu.*		1.6%	9.8%	22.9%	29.5%	36%
8.	I want to learn English because it is important for me to acquire wide general education./*Želim da dobro naučim engleski jezik jer mi je važno da steknem široko opšte obrazovanje.*		3.2 %	1.6%	19.6%	44.2%	31.1%
9.	I am learning English solely because it is a compulsory subject in the curriculum./*Učim engleski isključivo zato što mi je to obavezan predmet na studijama.*	41%	29.5%	19.6%	3.2 %	1.6%	4.9%

10.	I want to learn English well in order to read literature in the English language./*Želim da dobro naučim engleski jezik da bih pratio/la književnost engleskog govornog područja.*	26.2%	9.8%	26.2%	14.7%	6.5%	16.4%
11	I want to learn English in order to find a job abroad. *Želim da dobro naučim engleski jezik da bih pronašao/la posao u inostranstvu.*		4.9%	3.2 %	24.6%	39.3%	27.8%
12.	I want to learn English in order to find a well paid job. *Želim da dobro naučim engleski jezik da bih pronašao/la dobro plaćen posao.*		3.2 %		41%	24.6%	31.1%
13.	I am learning English as it will be beneficial during my studies./*Učim engleski jer će mi koristiti tokom studija.*		6.5%	8.2%	16.4%	36%	32.7%
14.	While learning English I like to learn about the way of life and the civilization of the English speaking cultures./*Kroz učenje engleskog jezika volim da učim i o načinu života i civilizaciji naroda sa engleskog govornog područja.*	9.8%	4.9%	16.4%	29.5%	24.6%	19.6%
15.	Getting a high grade in the exam is my primary motivation to learn English. *Postizanje dobre ocene na ispitu najvažniji mi je podsticaj za učenje engleskog.*	8.2%	16.4%	22.9%	22.9%	24.6%	4.9%
16.	I want to learn English in order to watch television shows in English without subtitles./*Želim da dobro naučim engleski jezik da bih mogao/la da pratim televizijske emisije na engleskom jeziku bez titlova.*		9.8%	9.8%	32.7%	14.7%	32.7%
17.	I want to learn English for further specialization within my profession abroad./*Želim da dobro naučim engleski jezik da bih se u okvirima posla stručno usavršavao/la u inostranstvu.*		1.6%	3.2 %	27.8%	32.7%	34.4%
18.	I want to learn English to be able to communicate with people from the English speaking countries./*Želim da dobro naučim engleski jezik kako bih mogao/la da razgovaram sa ljudima sa engleskog govornog područja.*			3.2 %	4.9%	42.6%	49.1%
19.	I want to learn English to be able to find my way when traveling abroad./*Želim da dobro naučim engleski jezik da bih mogao/la da se snađem kad putujem u inostranstvo.*		1.6%		3.2 %	32.7%	62.2%
20.	I want to learn English language terminology used in my profession./*Želim da dobro naučim izraze na engleskom jeziku koji se koriste u mojoj struci.*		1.6%		3.2 %	37.7%	57.3%

tials and limits, past achievements, as well as various aspects of task execution and maintenance of goals (values, beliefs, difficulties); and (b) the desire to narrow down the perspective of foreign language learning motivation, and to present concrete examples of functioning in language learning [14, 15].

The process-oriented period, which emerges after 2000, is focused on changes in motivation within the globalized world. When we analyze the motivation for foreign language learning today, it certainly cannot be the same as a few decades ago. In this sense, the motivation to learn English is largely different from that of earlier epochs, because English language in the process of globalization has earned a higher status and become more widespread. During this period, research by Dörnyei [16], Lamb and others highlights the need for reinterpretation of the classic Gardner term integrativeness. Also, a clear boundary between integrative and instrumental motivation has almost disappeared today. Lamb [17] points out that "integrative and instrumental motivation are difficult to distinguish as distinct concepts: contacts with Westerners, computer usage, understanding of pop poets, studying abroad, travel, career advancement – all of these pursuits are interrelated, and are also associated with English as an integral, central part of the process of globalization which transforms societies and has a crucial impact on the lives of people".

Related research

There are numerous studies aimed at determining the influence of motivation on the acquisition of a foreign language. Al-Tamimi and Shuib [18] published their research on the motivation and attitudes of students of the Petroleum Engineering studies towards learning English at Hadhramout University Of Sciences And Technology, Yemen. The result of their research, which included three motivational constructs (instrumental, integrative and personal), showed the dominant role of instrumental motivation for students in learning English, although personal motivation is also noticeable. On the other hand, Zanghar's research [19], conducted among university students in Libya, showed a somewhat stronger impact of integrative motivation, but did not establish the correlation between motivation and success in learning. Unlike this study, a study conducted in Spain [20] showed a positive correlation between motivation, motivational teacher strategies, and students' success in teaching foreign languages. The research conducted in Iran [21] has shown that motivation is a dynamic category, i.e. changing the nature and level over time. Similar conclusions have also been reached in Daftarifard and associates research [22], which also investigated the impact of motivation among university students in Iran. The research of Khansir and associates [23] shows a strong correlation between the socio-economic status of students and the motivation for learning a foreign language. Numerous studies on the role of motivation in foreign language learning have been conducted in Croatia [24, 25]. These authors studied individual differences regarding the affective characteristics of students, including motivation, attitudes and emotions towards learning English. The research included 2137 students of elementary and high schools, and showed a more positive attitude of high school students towards learning a foreign language. Martinović and Poljaković [26] conducted a research study at the University of Zadar among students studying (ESP), and the results showed the strongest impact of instrumental motivation, although integrative motivation was also highly ranked. A recent study by Pavičić, Takač and Berka [27] studied three components of motivation for learning English (pragmatic-communicative, affective and integrative) among students of secondary vocational schools and grammar schools, and the results showed that affective motivation is the strongest factor in their sample.

Earlier studies of motivation in English language teaching in our country [28–30] found that the motivation of students studying general English as a second language (ESL) is different from the motivation of those who study ESP. In teaching ESP, a significantly higher level of instrumental motivation was established compared to the integrative motivation. This difference is especially conspicuous among non-university students, whose language learning is closely related to the future needs of the profession. Similar conclusions have been drawn in recent studies of motivation among students of economics [31] and students of information technologies [32].

Material and Methods

This study was based on an anonymous survey conducted during the summer semester of 2017/18. The instrument of motivation research was a slightly adapted questionnaire, which was already used to test the motivation of students of natural sciences in teaching ESP [28]. The questionnaire contained 20 questions that tested instrumental and integrative motivation. In addition, it also contained questions about the year of studies, the number of years of English language learning before enrolling in the university, and whether the students attended classes regularly, occasionally or rarely. The questions in the survey dealing with different aspects of instrumental and integrative motivation were randomly ordered in the questionnaire. The answers were arranged on a scale of one (I absolutely disagree) to six (I absolutely agree), with three positive and three negative possible answers. The survey offered 12 questions which referred to instrumental and 8 to integrative motivation. The survey was conducted anonymously at the end of summer semester 2017/18 at the amphitheater of the Faculty of Medicine, the University of Novi Sad, in the presence of teachers, the authors of this paper. All 61 students that participated in the survey were in their first year of medical studies at the Faculty of Medicine in Novi Sad, attending English as a compulsory subject. All surveyed students regularly at-

tended English language classes, and their number of years of learning English as a foreign language before the university ranged from 6 to 15. Most of the students had studied English for 8 – 12 years, while a small number of them studied shorter or longer than that: two of them 6, and four of them 14 or 15 years. Due to the fact that students came from different secondary schools, different educational profiles and from different backgrounds, it was expected, given the great inconsistency in the number of years of learning English, and therefore the different level of knowledge, that the results would also show a certain degree of inconsistency in terms of instrumental and integrative motivation.

Results

The results of the research are shown in **Table 1**. The table lists all the 20 questions from the questionnaire in the order in which students responded to them. Their answers were scored on a scale of 1 to 6, and the results are shown in percentages.

Discussion

The following part of the paper analyzes the results obtained in our research based on the medical students' responses on the existence and degree of instrumental and integrative motivation for learning English.

Instrumental motivation

The research unambiguously showed an extremely high level of instrumental motivation. Namely, as much as 98.2% of the respondents expressed their desire to learn medical terminology in English (57.3% absolutely agreed, and 37.7% agreed with this statement). Very high results were also found in relation to statement No. 2 (students believed that they would benefit from knowing English during their future career) and statement number 17 (students expressed the desire to learn English in order to continue their professional training abroad). A slightly lower but still high percentage of students were motivated to learn English in order to find a well-paid job or a job abroad (statements 11 and 12). Motivation for learning in order to achieve better success in their studies was also high (statements 13 and 7), where it is noticeable that students recognized that their knowledge of English would be useful in their master`s and doctoral studies. It is extremely important to point out that less than 10% of students expressed motivation to learn English only because it was obligatory in the curriculum (statement No. 9), and that a relatively small number of students stated that they found getting a good grade at the exam the most important factor for learning English (statement 15).

Integrative motivation

By analyzing the results of the research on integrative motivation, its expected lower level than the previously analyzed instrumental motivation was confirmed. Most respondents expressed a motivation to learn English in order to communicate with people in English speaking countries (statement 18), and to make it easier for them to travel abroad (statement 19). A large number of students emphasized the feeling of satisfaction after successfully mastering the English language as a sufficient incentive for learning (statement 3), as well as the fact that learning English was a pleasure and not just a commitment (statement 1). A significant number of students were motivated to learn English in order to be able to watch movies (statement 6) and to watch television shows in English without subtitles (statement 16). A smaller number of students pointed out the desire to learn about the way of life and civilization of the people from English speaking countries (statement 14) as a factor of motivation, while most students have responded negatively to the statement that they wanted to learn English in order to read literature in English.

The results of the study confirmed its basic hypothesis, the dominant role of instrumental motivation in learning English as a language for medical purposes. Nevertheless, a very high degree of integrative motivation has been also determined, which is in line with the contemporary research in this field, showing that the boundaries within this classical division are getting thinner. It has been confirmed that the students are mostly motivated by the need to acquire professional terminology and to follow professional content in English in order to further improve in the field of medical science. Due to limited space, it was not possible to make a detailed statistical analysis of the results, including the correlation between factors such as gender and type of motivation, as well as the correlation between years of learning and motivation. All these topics could be the subject of further research and the analysis of the degree and type of motivation of medical students in English language teaching.

Conclusion

In this paper, we have confirmed the presence and degree of instrumental and integrative motivation for learning English among students of the first year of medicine at the Faculty of Medicine Novi Sad. The research showed an extremely high degree of not only instrumental but also integrative motivation, which confirmed our initial hypothesis. Such results should be taken into account when developing curricula for teaching English as a language for medical purposes and in designing materials and methods. Since a great deal of research points out motivation as one of the most important factors for successful foreign language learning, teaching materials and programs should be consistent with the degree and type of motivation of future learners in order to achieve optimal results in language learning.

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DRY EYE SYNDROME AND CATARACT SURGERY

SINDROM SUVOG OKA I OPERACIJA KATARAKTE

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Summary

Introduction. Dry eye syndrome has become a common problem after ocular surgeries with a significant impact on the quality of life. Many patients, who have undergone cataract surgery, postoperatively developed dry eye symptoms. Dry eye syndrome is one of the risk factors associated with cataract surgery. **Material and Methods.** The prospective study included 80 patients. We recorded the self-reported dry eye symptoms, the values of Schirmer test, tear breakup time, and best corrected visual acuity preoperatively, as well as 7 days and 1 month after the surgery. **Results.** A total of 80 patients were included in the study, 45 (56.2%) females and 35 (43.8%) males. The mean age of patients was 61.5 years (SD ± 6.2, range 57 - 70 years). The best corrected visual acuity at the time of surgery was 0.4 or less in 70 patients (87.5%). Most patients reported a significant improvement in visual acuity after surgery; 68 (85%) eyes achieved a best corrected visual acuity of 0.5 or higher (median 0.7; range 0.5 - 1.0). The mean tear breaking time in cataract patients before surgery was 12.4 sec, 7 days after the surgery it was 8.2 sec ($p < 0.05$) and 1 moth after the surgery 11.1 sec. The majority of patients had mild (47.5%) and moderate (33.75%) Schirmer test values. Dry eye with wetting < 5 mm after 5 minutes was found in 16.2% of patients before cataract surgery; 7 days after the surgery ($p < 0.05$) it was found in 23.75% of patients and one monht after surgery 11.1 sec. A foreign body sensation and watery eye were the most reported symptoms before cataract surgery. Seven days after the surgery foreign body sensation was present in 48.75% and watery eyes in 40% of patients. **Conclusion.** Significant increase in dry eye symptoms after cataract surgery was found with increasing age. Self reported dry eye problems are more common in patients with lower Schirmer test and best corrected visual acuity values before cataract surgery. Patients with concomitant dry eye disease require preoperative and postoperative treatment of dry eye to prevent aggravation of the existing symptoms that may affect the visual outcome after cataract surgery.

Key words: Cataract; Dry Eye Syndromes; Cataract Extraction; Risk Factors; Diagnostic Techniques, Ophthalmological; Tears; Signs and Symptoms; Age Factors

Sažetak

Uvod. Sindrom suvog oka je čest problem nakon oftalmoloških operacija sa signifikantnim uticajem na kvalitet života operisanih. Nakon operativnog lečenja katarakte, kod pacijenata su prisutni simptomi u vezi sa suvim okom. Sindrom suvog oka predstavlja jedan of faktora rizika nakonn operacije katarakte. **Materijal i metode.** Prospektivna studija je obuhvatila 80 pacijenata. Subjektivni simptomi u vezi sa suvim okom, Šrimerov (Schirmer) test, vreme prekida suznog filma i najbolja korigovana vidna oštrina evaluirani su preopravtivno, sedam dana i jedan mesec nakon operacije. **Rezultati.** Od 80 pacijenata 45 (56,2%) su žene, a prosečna starost 61,5 godina (SD ± 6,2, raspon 57−70 godina). Preopravtivna najbolja korigovana vidna oštrina iznosila je 0,4 ili manje kod 70 pacijenata (87,5%) a nakon operacije kod 68 (85%) postignuta je 0,5 ili veća (medijana 0,7; raspon 0,5−1). Prosečno vreme prekida suznog filma pre operacije bilo je 12,4 sec, nakon sedam dana 8,2 sec ($p < 0,05$), a mesec dana posle operacije 11,1 sec. Kod 47,5% pacijenata Šrimerov test je iznosio 10−14 i kod 33,75% 5−9 mm/5 min. Suve oči sa vrednostima < 5 mm preoperativno imalo je 16,2% pacijenata a 23,75% sedmog postoperativnog dana ($p < 0,05$). Sedmog postoperativnog dana 48,75% pacijenata imalo je osećaj prisustva stranog tela koji je u 40% praćen pojačanim suzenjem. **Zaključak.** Tokom starenja signifikantno se povećavaju simptomi suvog oka. Subjektivni simptomi su izraženiji kod pacijenata sa nižim preoperativnim vrednostima Šrimerovog testa i vrednostima vremena prekida suznog filma. U cilju sprečavanja pogoršanja osnovnog oboljenja koje može uticati na ishod operacije katarakte, potreban je adekvatan preoperativni i postoperativni tretman suvog oka.

Ključne reči: katarakta; sindroom suvog oka; ekstrakcija katarakte; faktori rizika; oftalmološke dijagnostičke procedure; suze; znaci i simptomi; faktori starosti

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Abbreviations
TBUT – tear breakup time
BCVA – best corrected visual acuity
IOL – intraocular lens
PHACO – phacoemulsification

Introduction

A healthy ocular surface and a stable tear film are essential to preserve the smooth optical surface of the cornea. Tears are made up of three layers: the lipid layer on the outside, the aqueous layer in the middle, and the inner mucus layer. The three layers together are known as the tear film. The main functions of the tear film are lubrication, nutrition and prevention of conjunctival and corneal infections.

Dry eye syndrome or keratoconjunctivis sicca is the most common ocular surface disease throughout the world [1]. This autoimmune disorder consists of qualitative and quantitative changes of the tear film. The cause of the tear film dysfunction is the alteration of tear aqueous, mucus and lipid component. It is estimated that 8 – 35% of the population has dry eye syndrome [1, 2].

Dry eye syndrome has a multifactorial origin and main risk factors are female gender, advanced age, and morphological changes in the lacrimal glands. Although the etiologies of dry eye are various, common to all ocular-surface disease is an underlying cytokine/receptor-mediated inflammatory process [3].

The Dry Eye Workshop, in 2007, defined dry eye as a multifactorial disease of tears and ocular surface that results in symptoms of discomfort, visual disturbance and tear film instability with potential damage to the ocular surface, which is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface [4]. The majority of dry eye symptoms (foreign body sensation, ocular fatigue, blurring, photophobia, red eye, feeling of heavy eyelids, epiphora etc.) are due to loss of tear film integrity.

Significant progress in the treatment of dry eye has been made in the last two decades; progressing from lubricating and hydrating the ocular surface with artificial tear to stimulating tear secretion; anti-inflammation and immune regulation [5].

Cataract surgery is a clinically effective and safe surgical procedure, and the risks of sight threatening complications are low. High rate of surgical success has created high expectations of patients regarding the vision outcome and improvement in vision related everyday activities [6, 7].

Dry eye has become a common problem after ophthalmic surgeries with a significant impact on the quality of life. Many patients who have undergone cataract surgery have complained of postoperative dry eye and symptoms of irritation [8, 9].

Dry eye is one of the risks associated with cataract surgery. Cataract surgery has been found to exacerbate pre-existing dry eye and to induce dry eye in patients with healthy corneas. This postoperative dry eye can negatively affect visual outcomes and visual recovery time [10]. Since the incidence of dry eye and cataract both increase with age, it is not a surprise that four out of five cataract patients may have moderate to severe dry eye symptoms [11].

The aim of this prospective study was to determine the incidence and natural course of dry eye, self reported dry eye symptoms, and to identify risk factors for development of dry eye after cataract surgery.

Material and Methods

This prospective study included 80 patients with senile cataract who underwent surgery at the Eye Clinic of the Clinical Center of Vojvodina in Novi Sad.

All patients underwent clinical and ophthalmological evaluation before cataract surgery with phacoemulsification (PHACO) and foldable intraocular lens (IOL) implantation (PHACO + IOL) and 7 days and 1 moth after the cataract surgery.

We recorded the self-reported dry eye symptoms (foreign body sensation, discomfort, dryness, redness of eye and watery eyes) preoperatively and after the surgery. Tear dynamics were assessed by Schirmer test. After placing a small strip of filter paper inside the inferior fornix, the eyes were closed for 5 minutes. The paper was then removed and the amount of moisture was measured. Both eyes were tested at the same time.

According to Schirmer test values, the patients were divided into 3 groups:

Group A. Mild (10 – 14 mm wetting of the paper after 5 minutes),

Group B. Moderate (5 – 9 mm wetting of the paper after 5 minutes),

Group C. Severe (< 5 mm wetting of the paper after 5 minutes).

To measure the tear breakup time (TBUT), 2% sodium fluorescein was instilled into the patient tear film, and all eyes were examined with a slit lamp under cobalt blue light. The TBUT was recorded as the number of seconds that elapsed between the last blink and the appearance of the first dry spot in the tear film. A TBUT under 10 seconds was considered abnormal.

The values of Schirmer test, TBUT, self-reported dry eye symptoms and visual acuity were evaluated before and after the cataract surgery. Patients with other general health problems (allergy, rheumatoid arthritis, stroke etc.) or any eye conditions accompanied with cataract (pterygium, cornea guttata, glaucoma, diabetic eye disease etc.) or any intraoperative and postoperative complications that may have contributed dry eye symptoms (corneal trauma, vitreous loss, corneal edema, Descemet folds, bullous keratopathy etc.) were not included in the study.

Results

A total of 80 patients were included in this study, 45 (56.2%) females and 35 (43.8%) males. The mean age of patients was 61.5 years (SD ± 6.2, range 57 – 70 years). According to maturity, an immature cataract was found in 47 (58.75%) eyes, intumescent

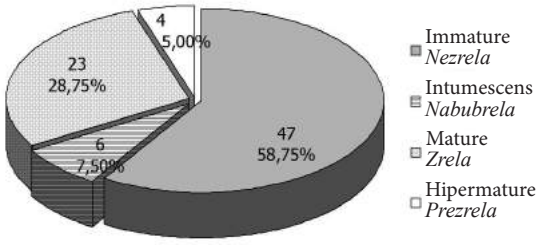

Figure 1. Maturity of cataracts
Slika 1. *Zrelost katarakte*

cataract in 6 (7.5%), mature cataract in 23 (28.75%), and hypermature in 4 (5%) eyes **(Figure 1)**.

At the time of surgery, best corrected visual actuivity (BCVA) was 0.4 or lower in 70 (87.5%) patients. A significantly better postoperative BCVA was established in most patients; 68 (85%) eyes achieved a BCVA of 0.5, or better (median 0.7, range 0.5 – 1.0). The remaining 12 (15%) eyes had a BCVA of 0.2, or better (median 0.3, range 0.2 – 0.4). The main preoperative and postoperative BCVA values are shown in **Figure 2**.

The mean TBUT in cataract patients before surgery was 12.4 seconds, 7 days after the surgery 8.2 seconds ($p<0.05$) and 1 month after the surgery 11.1 seconds. The mean values of TBUT are shown in **Figure 3.**

The majority of patients had mild (47.5%) and moderate (33.75%) Schirmer test values. Dry eye with wetting < 5 mm after 5 minutes were found in 16.2% before cataract surgery, and 23.75% 7 days ($p < 0.05$) and one month after the surgery 21.25%. Schirmer test values in A, B and C groups before and after the surgery are shown in **Table 1.**

Foreign body sensation and watery eye were the most commonly reported symptoms before the

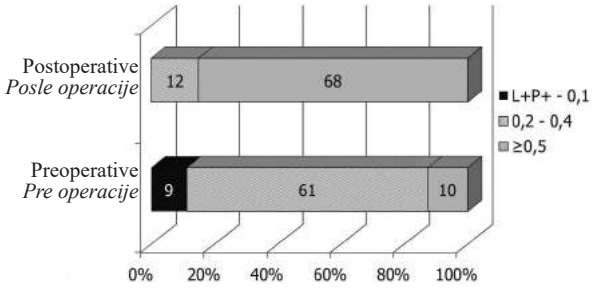

Figure 2. Preoperative and postoperative best corrected visual acuity
Slika 2. *Preoperativna i postoperativna najbolja korigovana vidna oštrina*

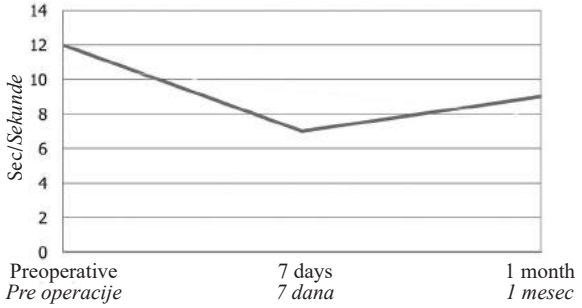

Figure 3. Mean values of the tear breakup time
Slika 3. *Srednje vreme prekida suznog filma*

cataract surgery. Seven days after the surgery, foreign body sensation was present in 48.75%, and watery eye in 40%. There were more self reported dry eye problems 7 days and 1 month after the surgery. Self reported dry eye symptoms in cataract patients are shown in **Table 2.**

Table 1. Schirmer test values (80 eyes)
Tabela 1. *Vrednosti Širmerovog testa (80 očiju)*

Schirmer test values *Vrednosti Širmerovog testa*	Before surgery *Pre operacije*	7 days after *7 dana posle operacije*	1 month after surgery *1 mesec posle operacije*
A (10 – 14 mm)	40	35	38
B (5 – 9 mm)	27	26	25
C (< 5 mm)	13	19	17

Table 2. Self reported dry eye symptoms
Tabela 2. *Lična procena simptoma suvog oka*

Self-reported symptoms *Lična procena simptoma suvog oka*	Before surgery *Pre operacije*	7 days after surgery *7 dana posle operacije*	1 month after surgery *1 mesec posle operacije*
Foreign body sensation *Osećaj stranog tela*	24	39 ($p < 0.05$)	27
Discomfort/*Neprijatnost*	12	24 ($p < 0.05$)	21
Dryness/*Suvoća*	14	21	19
Redness/*Crvenilo*	6	9	7
Watery eyes/*Suzenje oka*	20	32 ($p < 0.05$)	23

Discussion

Cataract surgery has become one of the most frequently performed and most successful surgical procedures with positive outcome [12]. Advances in new technology have led to the development of small incision PHACO surgery. These techniques have increased the efficacy of surgery with faster rehabilitation and reintegration in daily life activities.

High rate of surgical success has led to high expectations of patients regarding the vision outcome and improvement in vision related everyday activities [13]. In our study, visual acuity after cataract surgery was improved in the majority of the operated eyes. The largest group achieved good visual results after cataract surgery, 85.2% of operated patients with BCVA > 0.5.

Epitropoulos et al., in the research on the effect of tear osmolarity on repeatability of keratometry for cataract surgery planning, showed that taking care of dry eye syndrome before cataract surgery provides patients not only with more comfortable eyes but also better vision after surgery [14].

The prevalence and risk factors associated with dry eyes were evaluated in many studies. Lee et al., in their population based study, reported most dry eye symptoms in 40–49 year age group (37.6%), and a significant increase in dry eye symptoms was found with increasing age. The prevalence of dry eye was 1.4 times higher in men than in women [15].

In our study, 13/80 (16.2%) cataract patients had severe dry eye evaluated using Schirmer test with values 5 mm or less. The mean age of patients was 61.5 years (SD ± 6.2, range 57 – 70 years) and 45 (56.2%) were females.

Increased age and dry eye has also been reported in the study of Moss [16] and McCarty [17], although Schein et al. [18] found no age correlation et all.

In our study, the Schirmer test values showed significant changes 7 days after the surgery in the group with severe values before the cataract surgery (group C).

Barabino et al. conducted a study including forty subjects undergoing PHACO for cataract extraction and reported that Schirmer test values did not show any significant changes throughout the study, but statistically significant changes in symptoms, corneal fluorescein staining, lissamine green conjunctival staining, and TBUT occurred at day 1 and 7 after the surgery. This study indicates that cataract surgery may induce a clinical picture similar to dry eye, pointing out possible risks in patients with previous ocular surface diseases [19].

The mean TBUT of patients in our study was 12.4 seconds and 7 days after the surgery 8.2 seconds ($p < 0.05$). There were more self reported problems like foreign body sensation, discomfort, dryness and redness 7 days and 1 month after the surgery.

Self-reported dry eye (discomfort, dryness and watery eyes) up to 6 months after surgery was described to be among the most common complaints after cataract surgery in the study of Recchioni et al. [20].

Many reports of dry eye syndrome have focused on patients who had undergone PHACO and subsequently developed dry eye. Kasetsuwan et al. reported that the incidence of dry eye after PHACO was 9.8%. Symptoms and signs of dry eye occurred as early as seven days post-surgery and the severity pattern improved over time. According to the Schirmer test, 88.89% had a normal test [21].

In our study, 7 days after cataract surgery 26/80 (32.5%) patients had Schirmer test values 5– 9 mm (group B) and 19/80 (23.7%) < 5 mm (group C). There were no statistically significant changes in Schirmer test values 1 month after the surgery.

The study of Ishrat et al. concluded that the incidence of dry eye is higher in small incision cataract surgery (SICS) than PHACO due to tear film instability. Dry eyes were found in 42% ($p < 0.001$) of patients at 1 week follow-up. Fifteen percent of eyes were showing symptoms of dry eye at 1 month and 9% at 3 months after surgery. The majority of eyes in their study (27/42, 64.3%) had mild dryness. They also reported the significant differences in TBUT at 1 week, 1 month and 3 months postoperatively [22].

Optimization of the ocular surface before moving the patient to the operating room is essential for achieving the best surgical outcome. An impaired ocular surface has an impact on preoperative planning for cataract surgery [23]. Patients with cataract and concomitant dry eye disease require special consideration in order to prevent impairment of the existing symptoms, as well as being properly informed of the risks of dry eye associated with cataract surgery [24].

While several supportive measures have been developed and clinically applied, effective treatment and prophylaxis of dry eye has not yet been established. Further studies are necessary for a better comprehension of the consequence of cataract surgery on the ocular surface.

Conclusion

Concerning the results of many previous clinical studies and results of our study, cataract surgery with intraocular lens implantation in patients with dry eye syndrome is an effective and safe surgical procedure with good visual outcome. The risk of developing postoperative dry eye problems is related to preoperative qualitative and quantitative tear film status. Patients with concomitant dry eye disease require preoperative and postoperative treatment of dry eye to prevent impairment of symptoms that may affect the visual outcome after the cataract surgery.

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DETERMINATION OF DOPAMINE RECEPTOR D2 GENE RS1800497 POLYMORPHISM USING REAL-TIME POLYMERASE CHAIN REACTION

DETERMINACIJA POLIMORFIZMA GENA ZA DOPAMINSKI RECEPTOR D2 RS1800497 PRIMENOM REAL-TIME POLIMERAZNE LANČANE REAKCIJE

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Summary

Introduction. After analyzing a specific nucleotide sequence, located near the dopamine receptor D2 gene, rs1800497 polymorphism was detected, that affects dopamine D2 receptor expression, its density and affinity. This polymorphism is associated with many disorders such as: aggressive behavior, response to stress, sensation seeking, impulsivity, extraversion, anger, random memory, impaired cognitive function, brain glucose metabolism, obesity, pathologic gambling, substance addiction, depression, schizophrenia, that are related to modified gene expression, receptor density and affinity. Due to this association, a considerable interest in detecting a reliable dopamine receptor D2 gene allele was developed. The aim of this paper is optimization of real-time polymerase chain reaction in genotyping dopamine receptor D2 gene rs1800497 polymorphism. **Material and Methods.** Deoxyribonucleic acid was extracted from the blood of ten healthy individuals. Deoxyribonucleic acid samples were genotyped for dopamine receptor D2 gene rs1800497 (context sequence part containing targeted single nucleotide polymorphism CACAGCCATCCTCAAAGTGCTGGTC[A/G]AGGCAGGCGCCCAGCTGGACGTCCA) by polymerase chain reaction. The TaqMan assays were used to determine single-nucleotide polymorphisms. The products of amplification were analyzed using Applied Biosystems 7500 fast real-time polymerase chain reaction instrument. **Results.** After a successful polymerase chain reaction, two alleles that differed in one nucleotide were detected in our samples. **Conclusion.** Even though it is financially more demanding, real-time polymerase chain reaction method is recommended for dopamine receptor D2 gene genotyping in routine diagnostics because it is simple, accurate, and fast.

Key words: Receptors, Dopamine D2; Real-Time Polymerase Chain Reaction; Polymorphism, Genetic; Genotyping Techniques; Diagnosis

Sažetak

Uvod. Analizom specifične sekvence nukleotida u blizini gena za dopaminski receptor D2 otkriven je polimorfizam *rs*1800497 koji utiče na njegovu ekspresiju, broj receptora i afinitet. Ovaj polimorfizam je povezan sa: agresivnošću, odgovorom na stres, impulsivnošću, traženjem senzacija, radnom memorijom, kognicijom, metabolizmom glukoze u mozgu, gojaznošću, bolestima zavisnosti, depresijom i šizofrenijom za koje se pretpostavlja da su posledica izmenjene ekspresije gena za dopaminski receptor D2, broja D2 receptora i njegovog afiniteta. Zbog ove povezanosti, postoji značajan interes za efikasnom detekcijom alela gena za dopaminski receptor D2. Cilj rada jeste optimizacija *real-time polymerase chain reaction* metode u genotipizaciji polimorfizma rs1800497 gena za dopaminski receptor D2. **Materijal i metode.** Izolovani su uzorci dezoksiribonukleinske kiseline iz krvi zdravih osoba. Uzorci dezoksiribonukleinske kiseline uzeti od deset osoba, genotipizirani su za *single nucleotise polymorphism rs* 1800497 (sekvenca koja uključuje SNP:CACAGCCATCCTCAAAG TGCTGGTC[A/G]AGGCAGGCGCCCAGCTGGACGTCCA tokom *real-time polymerase chain reaction.* Upotrebljeni su *TaqMan* eseji za određivanje prisustva polimorfizama pojedinačnih nukleotidnih pozicija. Produkti amplifikacije su analizirani upotrebom 7500 *System SDS* programa na *real-time polymerase chain reaction* aparatu. **Rezultati.** Uspešnom *polymerase chain reaction*, u našim uzorcima detektovano je prisustvo dva alela koji se razlikuju u jednom nukleotidu. **Zaključak.** *Real-time polymerase chain reaction* metoda, iako finansijski zahtevnija, preporučuje se za genotipizaciju gena za dopaminski receptor D2 u rutinskoj dijagnostici zbog svoje jednostavnosti, brzine i uspešnosti.

Ključne reči: dopamin D2 receptori; polimeraza lančana reakcija u realnom vremenu; genetski polimorfizam; genotipizacija; dijagnoza

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Introduction

The dopamine receptor D2 (DRD2) is a transmembrane protein which belongs to the class of G-protein-coupled receptors (also known as the seven-transmembrane domain receptors) [1]. Its role is reflected in the dopamine binding and inhibiting the activity of adenylyl cyclase, an enzyme from the lyase group which is a part of the cyclic adenosine monophosphate (cAMP) signal pathway which, in addition to the phosphatidylinositol pathway, represents the way in which the G-protein coupled re-

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Abbreviations

DNA – deoxyribonucleic acid
DRD2 – dopamine receptor D2
SNP – single nucleotide polymorphism
cAMP – cyclic adenosine monophosphate
GDP – guanosine diphosphate
GTP – guanosine triphosphate
qPCR – real-time polymerase chain reaction
Ct – cycle threshold

ceptors transmit a signal. G-proteins consist of alpha, beta and gamma chains. Guanosine diphosphate (GDP) or guanosine triphosphate (GTP) is bound to the alpha chain, while the remaining two chains provide a connection to the cell membrane. At the moment when the ligand is bound to the receptor, the G-protein, which is connected to it, releases GDP in order to bind GTP. Then, the alpha chain to which GTP is bound, dissociates from the complex that it builds with other chains and goes to the inactivated adenylyl cyclase molecule and activates it. Adenylyl cyclase acts by converting adenosine triphosphate (ATP) into cAMP. The resulting cAMP further causes a cascade activation of the whole family of protein kinases that phosphorylate, thereby activating a large number of specific cell enzymes, which ultimately leads to the final intracellular effect of ligand. Accordingly, the indirect inhibitory effect of dopamine on the adenylate-cyclase prevents this cascade of events. A large number of hormones, such as adrenaline, noradrenaline, antidiuretic hormone, glucagon, and others, act through the cAMP signal pathway [2]. The DRD2 gene, localized on chromosome 11 (11q23.2), encodes the D2 dopamine receptor synthesis [3]. The aforementioned DRD2 gene is located within the genomic region that is significant because of the single-nucleotide polymorphism (SNP) rs1800497 (Taq1A). Earlier it was believed that the SNP rs1800497 was 10 kb away downstream from the DRD2 gene and was placed in the 3' non-coding region. However, it was proven that the SNP rs1800497 is a part of the exon 8 of ankyrin repeat and protein kinase domain-containing protein 1 (ANKK1) gene [4]. The function of this gene is reflected in the encoding of ANKK1 enzyme (commonly known as non-specific protein kinase - PKK1) [5]. The mentioned enzyme is a part of the Ser/Thr protein kinase family, whose function is to phosphorylate a hydroxyl (-OH) group of serine or threonine in proteins with the help of cAMP-dependent protein kinase which activates it through phosphorylation [6, 7]. Studies have shown that the SNP rs1800497 leads to the replacement of glutamic acid with lysine at position 713 (Glu713Lys) in protein [8], thereby affecting the expression of the DRD2 receptor by changing its density and affinity [9]. As a result, two alleles (genetic variants) of the DRD2 gene, A1-A and A2-G, occur and A is a mutated allele [10].

Studies conducted in vivo, using positron emission tomography (PET), showed significant individual variations in terms of the density of DRD2 receptors in human striatum. A higher incidence of A2-G allele was observed in schizophrenic patients, which are claimed to have an increased density of DRD2 receptors [8]. The correlation of the Taq1A rs1800497 polymorphism with the reduced density of DRD2 receptors was also proven in persons who abuse alcohol, cocaine and opiates compared to healthy individuals [11].

Dopamine receptors D2 are also present in the kidneys where they have an anti-inflammatory role and are involved in blood pressure control. Deficit of DRD2 receptors in the kidneys, caused by rs1800497 polymorphism disrupts their protective function, which increases the incidence of inflammatory kidney diseases. An example of such a disease is the inflammation of the cells of the proximal kidney tubules which occurs due to the consequent increased expression of the pro-inflammatory tumor necrosis factor alpha (TNFα). In addition to inducing inflammatory reactions, the tumor necrosis factor stimulates the production of the transforming growth factor β1 (TGFβ1), the main mediator in the kidney fibrosis development. Also, a reduced number of these receptors are associated with increased blood pressure. This is explained by the fact that DRD2 receptors participate in the inhibition of ion transport through proximal tubule cells in conditions when there is an optimal quantity of water (euvolemia) and a moderate increase in the volume of body fluids. A reduced number of DRD2 receptors can lead to increased transport of ions from proximal tubules to the interstitium, which is associated with the development of essential hypertension [12].

Certain studies demonstrated the correlation between the Taq1A rs1800497 polymorphism and obesity. Namely, as the Taq1A rs1800497 polymorphism affects the DRD2 receptor density, it is considered that the reduced density of the DRD2 receptor lies in the basis of the increased need for food. An analysis of the results obtained using the functional magnetic resonance imaging (fMRI) showed a reduced activation of dopamine pathways at the level of the orbitofrontal cortex and striatum of individuals who are carriers of the Taq1A rs1800497

Table 1. Results of genotyping
Tabela 1. *Rezultati genotipizacije*

Heterozygote/*Heterozigot*	Homozygote/*Homozigot*	
A1A2	A1A1	A2A2
6 samples/*6 uzoraka*	1 sample/*1 uzorak*	3 samples/*3 uzorka*

polymorphism. This contributes to development of an unhealthy diet pattern that is characterized by excessive eating, weight gain and obesity [13].

The research is based on the hypothesis that the application of the real-time polymerase chain reaction (qPCR) method represents an optimal method for detecting the rs1800497 polymorphism of the DRD2 gene.

The main goal of the research is optimization of the qPCR method for detection of the DRD2 gene rs1800497 polymorphism.

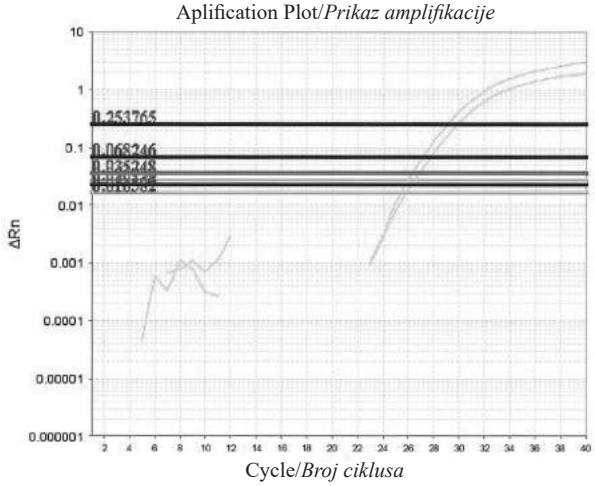
Material and Methods

In this prospective study, five venous whole blood samples of healthy voluntary blood donors were used, from which the deoxyribonucleic acid (DNA) was isolated in order to test the outcome of the qPCR method. A total of 2 ml of venous blood was collected from each person into glass test tubes with 3.2% sodium citrate. Signed informed consents were obtained from all participants, and the study was approved by the Institutional Ethics Committee. The research was performed according to the Declaration of Helsinki. The samples were kept at -20° C until analysis. The DNA was extracted from all samples using DNA Blood Prep Isolation Kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions. The genotyping of the DRD2 gene rs1800497 (context sequence part containing targeted SNP:CACAGCCATCCTCAAAGTGCTGGT C[A/G]AGGCAGGCGCCCAGCTGGACGTCCA) was carried out using qPCR and a set of reagents (assays) in order to determine the polymorphisms of a nucleotide with the corresponding fluorescent probes (TaqMan SNP, Applied Biosystems, Warrington, UK). The PCR was performed using 20 ng of genomic DNA together with 1µl TaqMan genotyping assay (TaqMan fluorescent probes with targeted specific primers) and 12.5 µl of TaqMan universal master mix supplied at a 2X concentration. The mix contained AmpliTaq Gold® DNA polymerase, Uracil-DNA glycosylase, dNTPs with dUTP, Passive Reference dye Rox and optimized buffer components. Genotyping was done in final 25µl reaction mix in separate PCR tubes using ABI 7500 Fast PCR platform (Applied Biosystems, Foster City, California, USA). Two TaqMan probes were marked with ''VIC®'' and ''FAM®'' fluorescent dyes with two target-specific primers obtained from the manufacturer's assay kit (TaqMan SNP, Applied Biosystems, Warrington, UK) emitting fluorescence of different wavelengths. If the device detected one dye it was a VIC homozygote (A1A1-AA); if it detected another dye, it was a homozygote FAM (A2A2-GG); the detection of both dyes signified a heterozygote (A1A2-AG) [14, 15].

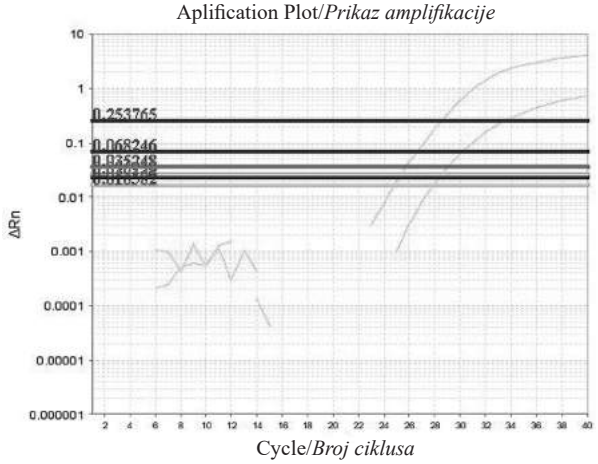
Results

The allele with the specific fluorescence curve was detected and analyzed using the 7500 System SDS program, integrated into the ABI 7500 Fast PCR platform. Ten samples gave the following results **(Table 1)**:

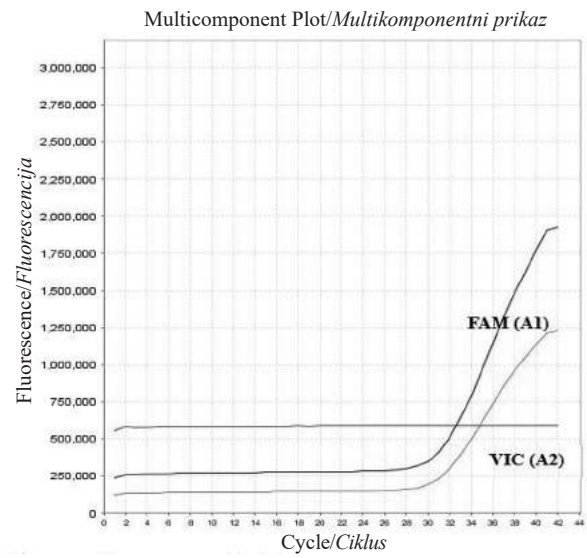
When interpreting the results, two values, determined by the program in the exponential phase of the amplification, are important. The threshold line determines the level of detection at which the reaction reaches the threshold (the intensity of the fluorescence above the background "noise"). The cycle threshold (Ct) is the PCR cycle in which the threshold value is reached. The greater the initial quantity of a gene in the sample, the fewer cycles are required in order for the fluorescence to reach the threshold value, therefore the Ct value is lower. For the purpose of determining polymorphism, in the amplification plot section, we analyzed whether the sample is homozygote or heterozygote based on the fluorescence of the curve of a particular color. It is important that, in the case of heterozygote A1A2, the curves are close to each other and that the threshold is reached



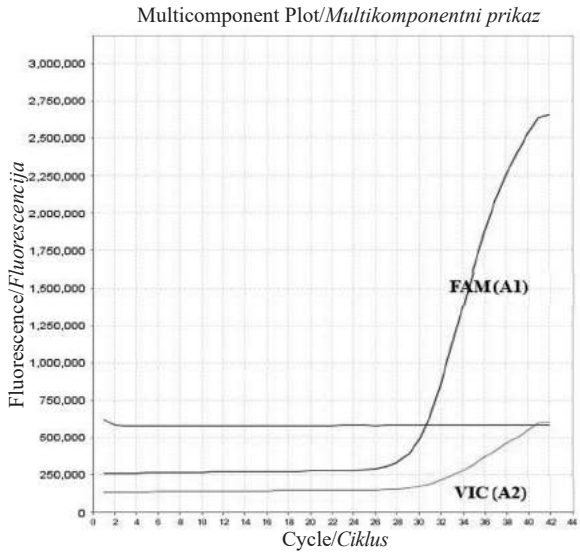
Graph 1. Heterozygote (A1A2)
Grafikon 1. *Heterozigot (A1A2)*



Graph 2. Homozygote (A1A1)
Grafikon 2. *Homozigot (A1A1)*



Graph 3. *Heterozygote (A1A2)*
Grafikon 3. Heterozigot (A1A2)



Graph 4. Homozygot (A1A1)
Grafikon 4. *Homozigot (A1A1)*

at about the same time, which confirms this genotype **(Graph 1)**. In case where the distance of two curves is greater, the second curve is delayed and reflects a non-specific binding, which confirms the presence of the homozygote **(Graph 2)**. The multicomponent plot section shows which alleles are present in the sample based on the dyes they are marked with **(Graph 3)** and **(Graph 4)**. A1 allele (with polymorphism) was marked with FAM® and A2 allele with VIC.

Discussion

By applying the PCR method, a successful detection of the DRD2 gene polymorphism rs1800497 was carried out. Through analyzing the results of the PCR method, the presence of genetic variants of the DRD2 gene were successfully observed, and A1 and A2 alleles were found. One of ten samples was a A1A1 homozygote for mutated allele, three of ten were A2A2 homozygotes for wild type allele and six were A1A2 heterozygotes for mutated allele.

In earlier works, long-range PCRs with capillary electrophoresis, Polymerase Chain Reaction - Restriction Fragment Length Polymorphism (PCR-RFLP), Polymerase Chain Reaction – Single-Strand Conformation Polymorphism (PCR-SSCP) and Southern blot technique were used to genotype the DRD2 gene rs1800497 polymorphism. These methods have proven to be successful in genotyping the specific polymorphism of DRD2 gene rs1800497. However, they are technically demanding, their performance is time consuming, the analysis of the results is more complex, and the reagent prices are higher; therefore, PCR method is more suitable in routine diagnostics.

Conclusion

The results of this research have shown that the application of the real-time polymerase chain reaction method is currently optimal for detecting the polymorphism. Although financially more demanding than other methods, due to its simplicity, speed, and reliability, it is recommended for detecting the rs180049 of the dopamine receptor D2 gene polymorphism in routine diagnostics.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
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DUODENAL DIVERTICULUM ASSOCIATED WITH PANCREATITIS – A CASE REPORT

DIVERTIKULUM DUODENUMA UDRUŽEN SA PANKREATITISOM – PRIKAZ SLUČAJA

Ivan BUSARČEVIĆ

Summary

Introduction. Acute pancreatitis is usually caused by biliary lithiasis and alcohol consumption. Pancreatitis in the elderly is a problem of increasing occurrence. Anatomic abnormalities may represent a less frequent, but important etiological factor. The duodenal diverticula rarely produce signs of inflammation, obstruction, hemorrhage or perforation. In some cases secondary biliary-pancreatic complications are found when a diverticulum originates from the papilla of Vater. **Case Report.** This case report describes a patient diagnosed with duodenal diverticulum who developed a framework of abdominal pain and laboratory findings compatible with acute pancreatitis which occurred two times in a short period of time. **Conclusion**. The association of duodenal diverticulum and acute pancreatitis has been reported, but it is important to point out that anatomic abnormalities may represent a less frequent but important etiological factor for acute pancreatitis.

Key words: Diverticulum; Duodenal Diseases; Pancreatitis; Ampulla of Vater; Digestive System Abnormalities; Signs and Symptoms; Risk Factors; Aged

Sažetak

Akutni pankreatitis je obično posledica dobro poznatih uzroka kao što su bilijarni konkrementi i konzumacija alkohola. Pankreatitis kod starijih osoba je sve zastupljeniji i to predstavlja problem. Anatomske promene predstavljaju ređi, ali važan, etiološki faktor. Duodenalni divertikulum retko uzrokuje znake poput inflamacije, opstrukcije, hemoragije ili perforacije. U nekim slučajevima sekundarne bilijarno-pankreatične komplikacije javljaju se ukoliko je divertikulum u neposrednoj blizini papile Vateri. **Prikaz slučaja.** Rad prikazuje slučaj pacijentkinje sa dijagnostikovanim duodenalnim divertikulumom kod koje se dva puta u kratkom vremenskom periodu javio bol u trbuhu a laboratorijski nalazi ukazivali su na akutni pankreatitis. **Zaključak**. Udruženost duodenalnog divertikuluma i akutnog pankreatitisa poznata je od ranije ali je važno istaći da anatomske promene predstavljaju ređi, ali podjednako važan, etiološki faktor akutnog pankreatitisa.

Ključne reči: divertikulum; oboljenja duodenuma; pankreatitis; Vaterova ampula; abnormalnosti digestivnog sistema; znaci i simptomi; faktori rizika; stari ljudi

Introduction

Duodenum is the second most common site of diverticulum formation after colon. The incidence of duodenal diverticula varies, from 1 – 6% in upper gastrointestinal contrast studies, 12 – 27% in endoscopic studies, and up to 22% in postmortem findings [1]. Duodenal diverticulum was first reported by a French pathologist, Chomel in 1710 [2] and diagnosed radiologically by Case [3] in 1913. Most of the duodenal diverticula occur in the periampullary region, within 2.0 cm of the ampulla of Vater [4]. Even though some studies report no gender predisposition [5–7], Grant Boileau [8] reported two diverticula in 11 female subjects, compared to 13 from 122 male subjects. In Case's [3] series of 85 cases of duodenal diverticula, 60% occurred in females. Mackenzie et al. [9] have also reported a female preponderance, female to male ratio of 1.6 to 1. Duodenal diverticula are asymptomatic in 90% of cases, less than 10% of patients develop nonspecific clinical symptoms like abdominal pain or discomfort [10]. The diagnosis of duodenal diverticula is incidental, found only during other diagnostic or therapeutic procedures. However, 6.5% of patients may develop complications [11] which include common bile duct obstruction, acute or chronic recurrent pancreatitis, partial duodenal obstruction, diverticulitis, ulceration, hemorrhage, enterolith formation, malignant degeneration, and perforation [12].

Case Report

An 85-year-old female was admitted to the Department of Gastroenterology and Hepatology with

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Abbreviations

GGT – gamma-glutamyl transferase
AST – aspartate aminotransferase
ALT – alanine aminotransferase
ALP – alkaline phosphatase
CT – computed tomography
JDD – juxtapapillary duodenal diverticulum

a two day history of dull epigastric pain radiating to the flanks and lower abdomen. The pain was associated with episodes of vomiting and chills. She had a history of similar painful episodes occurring infrequently during a few years, but the pain had never been so severe. She had never previously sought medical attention and treatment for such symptoms. Her past medical history was positive for hypertension and appendectomy at the age of 20. She was a non-smoker, and did not consume alcohol. The abdomen was soft, no masses were felt, while tenderness on deep palpation was evident on physical examination. The patient was given nil per mouth while workup for abdominal pain was started. Her pancreatic enzymes were found to be elevated: amylase level was 974 IU/L (reference value: less than 118 IU/L) and the lipase level was 720 U/L (reference value: less than 78U/L). Liver enzymes were mildly elevated: gamma-glutamyl transferase (GGT) 62 U/l (< 38), aspartate aminotransferase (AST) 68 (< 37), alanine aminotransferase (ALT) 138 U/l (< 48), alkaline phosphatase (ALP) 313 U/l (< 290), while bilirubin was within the reference range. Serum lipid values determined on the second day of hospitalization were in the reference ranges. Upper abdominal ultrasound showed no abnormalities, gallbladder was with no gallstones or other pathology. A computed tomography (CT) scan of the abdomen was performed on the fifth day of admission.

The common bile duct was dilated up to 15.32 mm without intraluminal content **(Figure 1)**. Pancreas was of normal size, clear contours, and of homogeneous structure **(Figure 2)**, without dilation of the main pancreatic duct. The CT also showed that common bile duct dilatation was the consequence of the compression of diverticulum near the site of papilla of Vater **(Figure 1)**. Upper gastroduodenal endoscopy revealed diverticular opening near the site of ampulla of Vater (approximately 1.2 cm in diameter and depth around 1 cm). The overlying mucosa appeared normal. While these examinations were being carried out, the patient underwent clinical treatment with parenteral hydration, third generation cephalosporin (ceftriaxone), H2 (histamine-2) blocker (ranitidine) and symptomatic therapy. The treatment provided suggestive regression of the acute-phase symptoms. The abdominal pain and vomiting resolved quickly after intravenous fluids were given, and within nine days the patient was discharged. The laboratory test results have improved as follows: amylase level was 90 IU/L (reference value: less than 118 IU/L) and the lipase level was 44 U/L (reference value: less than 78U/L), GGT 43 U/l (< 38), AST 21 (< 37), ALT 36 U/l (< 48), ALP 97 U/l (< 290). The patient was well for

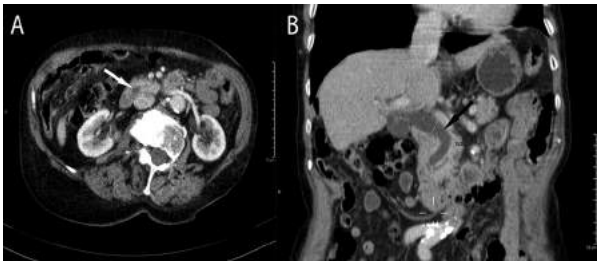

Figure 1. CT scan of the abdomen. A) Periampullary diverticulum (white arrow) and B) consequent extrahepatic bile ducts (common hepatic duct and common bile duct) dilatation (black arrow)

Slika 1. *Kompjuterizovana tomografija abdomena. A) Periampularni divertikulum (bela strelica), B) Posledična dilatacija ekstrahepatičnih žučnih kanala (zajednički jetreni kanal i zajedničku žučni kanal) (crna strelica)*

one week and then required readmission with a further bout of pancreatitis, amylase 1975 IU/l, GGT 210 U/l (< 38), AST 288 (<37), ALT 75 U/l (<48), ALP 202 U/l (< 115). The initial upper abdominal ultrasound showed hypoechoic body of pancreas, dilatation of the common bile duct up to 13 mm without intraluminal content and gallbladder with no gallstones or other pathology.

A control upper abdominal ultrasound confirmed dilatation of intra and extrahepatic biliary ducts, while a small amount of suspected intraluminal bile content was detected in the gallbladder. The values of oncomarkers (Ca 19-9 and carcinoembryonic antigen (CEA)) were in the normal range. The case was presented to an abdominal surgeon, a continuation of conservative therapy was proposed over the next ten days, after which decisions

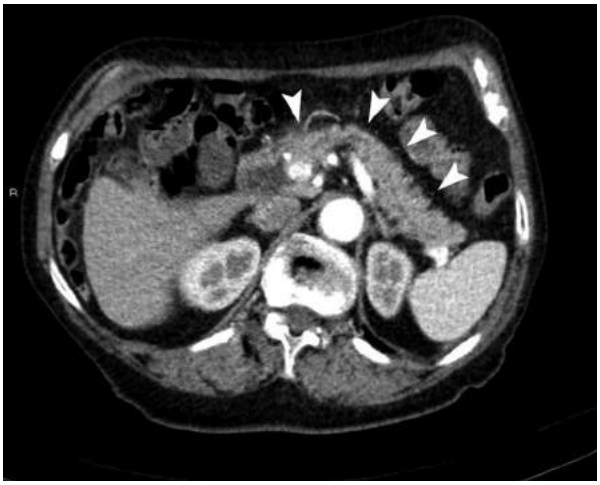

Figure 2. CT scan of the abdomen. Normal size, clear contours and homogenous structure of pancreas marked with arrows

Slika 1. *Kompjuterizovana tomografija abdomena. Normalna veličina, jasne konture i homogena struktura pankreasa (obeležena strelicama)*

on further treatment modalities were to be made. Following this period, a continuation of conservative treatment was proposed as well as further outpatient examinations by the gastroenterologist. The last gastroenterological control was four months after discharge from the hospital after which the patient continued receiving the previously recommended therapy: proton pump inhibitor and capsules of Ursofalk (ursodeoxycholic acid).

Discussion

We presented a case of an older woman with two episodes of pancreatitis in a short period of time caused by duodenal diverticulum near the site of papilla of Vater. Diverticula occur at weak spots in the duodenal wall such as the site of entry of the common bile duct, pancreatic duct and perivascular connective tissue sheath. The exact etiology is not clear; however, it might be the end result of disordered duodenal motility. Advancing age, progressive weakening of intestinal smooth muscles and increase in intraduodenal pressure may all encourage the outpouching of the duodenum [13]. Reported complications include hemorrhage from ulceration within diverticula [14] and cholangitis [15] or pancreatitis [16] which are thought to occur as a result of occlusion of the respective duct by an enlarging pouch [17]. The prevalence of juxtapapillary duodenal diverticula (JDD) in the general population is around 20%; they are often associated with biliary lithiasis. Patients with JDD have bile duct stones alone more often than patients without JDD (44% vs. 24%) [18]. In gallbladder of our patient, on the control abdominal ultrasonography during the second hospitalization, a small amount of suspected intraluminal bile content was detected. In various studies, upper gastrointestinal endoscopy has been shown to be a useful diagnostic tool; however, if the diverticula are located in the third or fourth part of the duodenum, then the sensitivity decreases [19]. It has been shown that there is an association between periampullary diverticula, which can lead to abscess formation, and biliary duct stones. Thus, in a patient suffering from pancreatitis with dilated bile ducts but no gallstones, the diagnosis of perivaterian abscess should be considered. Radiological diagnosis of these abscesses can be difficult [20].

The CT and magnetic resonance imaging scans are useful to distinguish between abscesses and neoplasms of the pancreas arising from the head of the pancreas by demonstrating characteristic air-fluid levels within these lesions [21, 22].

Surgical or endoscopic interventions should only be reserved for symptomatic diverticulum [23]. Furthermore, only 50% of patients treated with diverticulectomy were relieved of their symptoms [23, 24]. There is a consensus that elective surgical treatment of asymptomatic or minimally symptomatic diverticulum is not justified [13]. The existence of JDD influences bile duct diameter regardless of the presence of bile duct stones [18]. Classifying the severity of acute pancreatitis is important when comparing different institutional experiences, when talking with patients about prognosis, when planning therapy, and when comparing the new methods of management. New classification defines 3 degrees of severity: mild, moderately severe, and severe acute pancreatitis. Mild acute pancreatitis usually resolves within several days to a week, moderately severe acute pancreatitis resolves more slowly, may require interventions, and prolongs hospitalization; severe acute pancreatitis demands a longer hospital stay, usually some form of intervention, and may also be associated with multiple organ failure and death [25]. In order to establish the cause of the disease and prevent complications it is important to exclude anatomical abnormalities. We wanted to emphasize that application of different radiological diagnostic procedures could be very useful in establishing the proper diagnosis, as well as ruling out the existence of abscess collections and intraluminal collections in the dilated common bile duct. The abdominal CT revealed the presence of duodenal diverticulum, which was later-on confirmed by esophagogastroduodenoscopy. Care must be taken to diagnose the condition correctly by using appropriate radiological and endoscopic diagnostic procedures.

Conclusion

Anatomic abnormalities, such as duodenal diverticula, may represent a rare but important etiological factor of pancreatitis. We concluded that in our patient the duodenal diverticulum might have been a major contributing factor in repeated attacks of mild self-resolving pancreatitis.

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ECTHYMA GANGRENOSUM IN HEMATOLOGICAL PATIENTS – A REPORT OF TWO CASES

EKTIMA GANGRENOZUM KOD HEMATOLOŠKIH PACIJENATA – PRIKAZ DVA SLUČAJA

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Summary
Introduction. Ecthyma gangrenosum is a rare skin disorder which commonly affects immunocompromised patients. **Case Report.** We report two hematological patients suffering from ecthyma gangrenosum. Pseudomonas aeruginosa was isolated from skin lesions of both patients, but bacteremia was present only in the first case. These two cases had different pathogenic mechanisms and outcomes. **Conclusion.** Early diagnosis and prompt combination antibiotic therapy are of utmost importance in the treatment of this condition. On the other hand, the underlying disease has a great impact on the outcome as well.
Key words: Ecthyma; Hematologic Diseases; Pseudomonas aeruginosa; Bacterial Infections; Skin Ulcer; Gangrene; Early Diagnosis; Treatment Outcome

Sažetak
Uvod. *Ecthyma gangrenosum* je retka kožna bolest koja se obično javlja kod imunokompromitovanih bolesnika. **Prikaz slučaja.** Opisali smo dva slučaja hematoloških bolesnica sa gangrenoznim ektimom. U oba slučaja iz kožnih lezija izolovan je *Pseudomonas aeruginosa*, a u prvom slučaju bila je prisutna i bakterijemija. Ova dva stanja su imala različite patofiziološke mehanizme i ishode. **Zaključak.** Rana dijagnoza i rana antibiotska terapija najznačajniji su u lečenju. S druge strane, stanje osnovne bolesti ima veliki uticaj na ishod bolesti.
Ključne reči: ektima; hematološka oboljenja; Pseudomonas aeruginosa; bakterijske infekcije; kožni ulkus; gangrena; rana dijagnoza; ishod lečenja

Introduction

Ecthyma gangrenosum (EG) is a rare skin lesion usually found in neutropenic, immunocompromised patients with underlying hematological malignancies and patients after bone marrow transplantation [1, 2]. Mostly, EG appears secondary to Pseudomonas aeruginosa (PA) bacteremia [3, 4]. There are a few case reports of otherwise healthy individuals and patients without proven bacteremia where the skin lesions are considered to be primary [5]. Other pathogens, like Staphylococcus aureus, Klebsiella species, Escherichia coli, Neisseria meningitidis, Stenotrophomonas maltophilia, Aspergillus, Candida, Fusarium, and Rhizopus may also be causal agents [1]. Through a few stages, EG usually evolves from a macula or papula which progresses into a hemorrhagic bulla and a black necrotic eschar [1, 2, 5, 6].

Herein, we present two patients with hematologic malignancies and EG, with and without PA bacteremia. Alongside these cases, we presented a brief literature review on the essential issues associated with this condition.

Case Reports

Case 1

A 50-year-old female was admitted for the treatment of acute myeloblastic leukemia M2 French-American-British (FAB) classification, transformed from myelodysplastic syndrome. Bone marrow aplasia was induced after standard induction treatment with continuous intravenous cytarabine (7 days) and daunorubicin (3 days). On day 12 after the beginning of chemotherapy, the patient developed febrile neutropenia; the fever was 38.7° C, white blood cell count (WBC) was 2.0 x 10^9/L, and neutrophil granulocytes were 0.44 x 10^9/L. On day 14, hemorrhagic bullae appeared on her right hemiabdomen, right leg and vulva and in the course of a few days, they progressed into necrotic ulcers. Swabs were taken and sent for microbiological testing. In the meantime, empiric antibiotic treatment was initiated (ceftazidime and amikacin). When blood cultures, leg and abdominal ulcer swab results returned positive for PA, the treatment was continued according to the antibiogram. The patient was persistently febrile during the prolonged period of neutropenia (from day 14 to day 42). She was clinically at her worst on day 20, when she was hypotensive (blood

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Abbreviations
EG – ecthyma gangrenosum
PA – Pseudomonas aeruginosa
WBC – white blood cell

pressure was 85/50 mmHg) but responded well to volume challenge. Ulcer swabs were repeated and came back positive for PA. Intermittently, two separate strains of PA were present, alongside the development of antibiotic resistance, which resulted in frequent changes of medications **(Table 1)**. Additional ulcer treatment included surgical debridement and daily topical use of ethacridine lactate. After 118 days of hospitalization, the patient was discharged with an ulcer on her right foot measuring around 20 cm x 8 cm still positive for PA **(Figure 1)**. Four months later, she was readmitted for further ulcer treatment. Another necrosectomy was performed, and the patient received seven doses (one every other day) of autovaccine. However, the swab remained positive for PA, and the ulcer was still open. The underlying disease was treated with 500 mg hydroxyurea a day. In the following year, she was hospitalized several times due to high fever. The blood cultures were negative. The patient received parenteral antibiotic treatment together with a topical ulcer treatment. Due to the inability to continue high dose chemotherapy, the patient died as a result of infection and underlying disease progression.

Case 2

A 38-year-old male was admitted for autologous bone marrow transplantation. Two years earlier, he was diagnosed with nodular sclerosing Hodgkin's lymphoma, stage IV Bb. In the course of time, he received multiple lines of chemotherapy including doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD), bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone (BEACOPP) and dexamethasone, cisplatin, cytarabine (DHAP). Bone marrow transplantation was performed following the conditioning regimen, which included carmustine, etoposide, cytarabine, and melphalan (BCNU).

Figure 1. Ecthyma gangrenosum on the patient's right foot and foreleg
Slika 1. *Ecthyma gangrenosum kod pacijenta na desnom stopalu i potkolenici*

On day 3, the patient experienced a burning sensation and itching in the scrotal region, accompanied by an erythematous macular rash. He was afebrile with leukopenia (WBC 1.9 x 10^9/L). The patient was already on empirical antimicrobial prophylaxis, as part of the transplantation protocol (ciprofloxacin, acyclovir, and fluconazole). Due to the appearance of skin changes on day 3, the prophylactic treatment was switched to cefepime. The skin swab taken on day 3 cultivated a normal bacterial flora. After dermatological consultations, the erythematous macula was treated with topical corticosteroids and 3% solution of boric acid. Despite treatment, from day 5 to day 25, the lesion progressed into an ulcer typical for EG. Deterioration of the local finding was accompanied by a fever of 38° C starting from day 15. At that time, the patient presented with leukopenia (lowest WBC on day 16 was 0.4 x 10^9/L). He started receiving meropenem, vancomycin, and metronidazole, while blood cultures and swab results were pending. Blood cultures came back negative, but the swab from the scrotal ulcer taken on day 25 was positive for PA sensitive to piperacillin/tazobactam and cefepime. Piperacillin/tazobactam was

Table 1. Choice of antibiotics and the length of therapy
Tabela 1. *Spisak tipova antibiotika i dužine primene*

Antibiotic/*Antibiotik*	Days of application/*Dan primene*	Days in total/*Ukupno dana*
Ceftazidime/*Ceftazidim*	12 - 18, 44 - 51	15
Amikacin/*Amikacin*	12 - 18, 26 - 28, 31 - 35, 40 - 44	20
Piperacillin/Ttazobactam/*Piperacilin/Tazobactam*	20 - 21, 29 - 41	15
Vancomycin/*Vancomycin*	21 - 27, 29 - 40	19
Meropenem/*Meropenem*	21 - 26	6
Ciprofloxacin/*Ciprofloxacin*	19 - 20	2
Imipenem/Cilastatin/*Imipenem/Cilastatin*	28 - 30, 73 - 90	11
Cephalexin/*Cephalexin*	70-73	4
Gentamicin/*Gentamicin*	113 - 118	6

initiated on day 25 and continued for 12 days along with topical use of 3% solution of boric acid and silver sulfadiazine. The patient had a complete ulcer resolution. After that, the patient's stay was uneventful, and he was discharged.

Discussion

There are two possible pathogenic mechanisms involved in the development of EG; it either develops secondary to PA bacteremia, or it is a primary, localized skin infection without bacteremia [2]. Pseudomonas secretes enzymes such as elastase, exotoxin A, and protease, which lead to the development of necrotizing vasculitis, hemorrhage, and ulceration [1, 5]. In our cases, both mechanisms were augmented.

Most commonly, lesions develop in the anogenital and axillary regions, but any other body part, like the trunk and the extremities can be affected [1, 2, 4, 6]. Predisposition can be explained by the bacteria's affinity for moist and warm environments [1]. Unexpectedly, the worst lesion in the cases we presented was localized on the foot and foreleg. The rest followed a familiar pattern.

The treatment of EG is complicated for several reasons; development of multi-drug-resistant strains, presence of a severe underlying disease and usually intensive chemotherapy which further debilitates the host's weakened immune system [1, 4, 6]. These factors create a kind of vicious cycle. There are no standard guidelines for the treatment. Authors agree, and it is our opinion as well, that early diagnosis, regular sampling of tissue specimens, prompt initiation of combined antibiotic therapy, knowledge of local Pseudomonas' resistance profile, as well as individual approach to each patient are of great importance [1, 2, 5, 6].

In our first case, we tried additional therapy with autovaccination. These vaccines, which consist of inactivated microorganisms, can be used in the treatment of chronic or recurrent infections [7, 8]. Their utilization in humans is seldom, and they are proven to boost cytokine levels, but not a specific immune response towards a particular microorganism [7]. There were no clinical benefits of this treatment in our patient, since the infection persisted with the same intensity and the swab of the ulcer remained positive.

Our cases differed in courses and outcomes. As shown in previous studies, patients with Pseudomonas bacteremia have a higher mortality rate in comparison to patients without it [3, 4]. However, in our cases, the underlying diseases had a significant impact on the patients' outcome.

Conclusion

Early diagnosis and prompt combined antibiotic treatment are essential in the treatment of ecthyma gangrenosum. On the other hand, the underlying disease has a great impact on the outcome as well.

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SEDATION IN THE INTENSIVE CARE UNIT

SEDACIJA U JEDINICI INTENZIVNE NEGE

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Summary

Introduction. Sedation is the reduction of irritability or agitation by the use of certain drugs mostly to facilitate therapeutic or diagnostic procedures. **Scales for evaluation of the depth of sedation.** Riker Sedation-Agitation Scale and Richmond Agitation-Sedation Scale are the most commonly used scales. **Drugs.** Sedation is generally produced by using medications from the group of opioids, benzodiazepines, intravenous and inhalation general anesthetic agents, neuroleptics, phenothiazines, α-agonists and barbiturates**. Adverse effects of sedatives.** Sedation is often associated with hypotension, prolonged mechanical ventilation and longer time on respiratory support, higher frequency of delirium, immunosuppression, deep vein thrombosis, increased risk for development of nosocomial pneumonia, all of which leads to the prolonged recovery time. **Conclusion.** Sedatives currently used in intensive care units are widely used, but they have limitations. The goal is to get the desired level of sedation with as few side effects as possible.

Key words: Intensive Care Units; Hypnotics and Sedatives; Monitoring, Physiologic; Risk Assessment; Clinical Protocols

Sažetak

Uvod. Sedacija predstavlja stanje smanjene razdražljivosti ili uznemirenosti, koje nastaje upotrebom određenih lekova, a u svrhu terapijskih ili dijagnostičkih postupaka. **Skale za procenu dubine sedacije.** Najčešće se koriste Rikerova *Sedation-Agitation* skala i Ričmondova *Agitation-Sedation* skala. **Lekovi.** Za sedaciju se uglavnom koriste lekovi iz grupe opioida, benzodijazepina, intravenskih i inhalacionih anestetika, neuroleptika, fenotijazina, α-agonista i barbiturata. **Neželjeni efekti lekova koji se koriste za sedaciju.** Kao rezultat sedacije, često dolazi do hipotenzije, dužeg trajanja mehaničke ventilacije pluća i produženog odvajanja od respiratora, veće učestalosti delirijuma, imunosupresije, duboke venske tromboze, povećanog rizika za razvoj bolničke pneumonije, što sve dovodi do dužeg oporavka. **Zaključak.** Trenutno dostupni sedativi koji se koriste u jedinicama intenzivne nege su u širokoj upotrebi, ali imaju svoja ograničenja. Cilj je da se postigne željeni nivo sedacije, uz minimum neželjenih efekata.

Ključne reči: jedinice intenzivne nege; hipnotici i sedativi; monitoring; procena rizika; klinički protokol

Introduction

Sedation is the reduction of irritability or agitation by the use of certain drugs mostly to facilitate therapeutic or diagnostic procedures. The first drug which was used as a hypnotic was chloral hydrate. Although it was synthesized in 1832, it was not analyzed as a hypnotic until 1869 by the Berlin chemist Oscar Liebreich. Chloral hydrate substituted morphine very quickly, due to its simple and practical use. It has shown its effect without the application of injections, which is why it has been rather suitable for home use [1]. This drug still has its place in sedation of pediatric patients.

Nitrous oxide, "laughing gas", was discovered in 1844 by Horace Wells, an American dentist. He first tried the effect of this gas himself, with a help of a colleague while the extraction of wisdom tooth. Afterwards, Wells applied nitrous oxide on his patients, using an animal bladder and a wooden tube, which he put into the patient's mouth, while the nose was blocked. He performed successful operations during a period of one month. The first public demonstration was unfortunately unsuccessful, since the gas was not applied properly. Wells was declared a fraud and he gave up dentistry. In 1848, disappointed, he committed suicide using chloroform [2].

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Abbreviations
ICU – intensive care unit
GABA – gamma-aminobutyric acid
CNS – central nervous system
ETT – endotracheal tube

In the period between 1920s and mid-1950s, barbiturates were practically the only drugs used both as sedatives and hypnotics. Barbiturates were synthesized in 1864 by Adolf fon Bayern, although the synthetic process was developed and perfected by a French chemist Edouard Grimaux in 1879. He facilitated further development of barbiturate derivatives, which were widely applied [3].

As far as contemporary sedation is concerned, benzodiazepines (midazolam), dexmedetomidine opioids are most often used [4]. Morphine still occupies a significant place in the therapy, due to its analgesic and mildly euphoric effect and cost-effectiveness. Chloral hydrate is used for sedation in pediatrics, whereas in developed countries, nitrous oxide is the drug of choice, due to its practical use by a mask, leading to a mild euphoria and having an optimal analgesic effect. In dental practice, nitrous oxide is most often used in combination with oxygen [5, 6].

The development of intensive care units dates back to the times when artificial ventilation was established using rudimentary machines which did not have the ability to synchronize with patient's respiratory efforts. The consequence was deep sedation, up until the point when the patient was able to breathe without the help of a respirator. Apart from the use of microprocessor controlled ventilators in last decades, which are synchronized with patient's respiratory effort, new, short-acting sedatives and analgesics have significantly changed this approach. Today, intensive care is part of a multidisciplinary approach including a large team which participates in treating critically ill patients. As far as the sedation of patients is concerned, the choice of analgesics and sedatives is important, taking into consideration the potential allergy to drugs, organ dysfunction (especially liver and kidneys), the need for rapid start of action and/or cessation of the drug induced effect, the extended duration of therapy, as well as the primary response to the therapy. Analgesics and sedatives are used according to patients' needs, using the smallest effective dosage. The accumulation of drugs and their metabolites is being taken into consideration, as well as the adverse effects to which the application of these drugs may lead, particularly in critically ill patients. The manner of drugs administration is being planned, in the sense of continuous or intermittent administration [7].

Scales for evaluation of the depth of sedation

The assessment of the depth of sedation implies the use of various scales. Riker Sedation-Agitation Scale (SAS) **(Table 1)** and Richmond Agitation-Sedation Scale (RASS) **(Table 2)** are the most commonly used. These scales are also a part of the protocol for the assessment of the state of delirium in the intensive care unit (ICU), Confusion Assessment Method (CAM) for the ICU, as a part of Intensive Care Delirium Screening Check-list (ICDSC).

Scales for assessment of the sedation depth are used in order to achieve the optimal level of sedation. However, if that is not achieved, the patient is agitated, which leads to a poor synchronization between the patient and the ventilator and consequently to insufficient ventilation. A possibility of delirium must be considered, involuntary removal of

Table 1. Riker Sedation-Agitation Scale (SAS)
Tabela 1. *Rikerova Sedation-Agitation skala (SAS)*

Level *Vrednost*	Term *Termin*	Description *Opis*
7	Dangerous Agitation *Opasna agitiranost*	Pulling at endotracheal tube (ETT), trying to remove catheters, striking at staff, thrashing side-to-side./*Povlačenje endotrahealnog tubusa (ETT), pomeranje katetera, agresivnost prema osoblju, bacakanje iz strane u stranu.*
6	Very Agitated *Veoma agitiran*	Frequent and strict verbal reminding of limits is necessary, patient is biting ETT. *Potrebno strogo i stalno verbalno podsećanje na limite, pacijent grize ETT.*
5	Agitated *Agitiran*	Anxious and agitated, calms down to verbal instructions and commands. *Anksiozan i agitiran, miran na verbalne instrukcije i naređenja.*
4	Calm and Cooperative *Miran i kooperabilan*	Calm, awakens easily and follows commands. *Miran, lako se budi, sledi komande.*
3	Sedated *Sediran*	Difficult to arouse, awakens to verbal command and gentle shaking, follows simple commands, but drifts off again./*Teško se budi, ali na verbalne komande i blago drmanje, sledi jednostavne komande, ali ponovo „se isključi".*
2	Very Sedated *Veoma sediran*	Arouses to physical stimuli but does not communicate or follow commands, spontaneous movements are present./*Budi se na fizičke stimuluse, nije komunikativan i ne sledi komande, spontani pokreti prisutni.*
1	Unarousable/*Ne mogu da se probude*	Minimal or no response to stimuli, does not communicate or follow commands. *Minimalan odgovor, ili ga nema, na stimulus. Nije komunikativan niti sledi komande.*

Legend/*Legenda: ETT* – endoracheal tube/*endotrahealni tubus*

electrodes and catheters, as well as the development of the post-traumatic stress. On the other hand, the elevated level of sedation leads up to unnecessarily prolonged mechanical ventilation, which can be accompanied by complications such as ventilator associated pneumonia or other lung damage, neuromuscular dysfunction, diaphragm dysfunction and numerous other damages. Due to these reasons, it is very important to find the right balance and to establish the appropriate level of sedation in ICU [8].

Drugs

Numerous types of drugs are used for sedation. They include opioids, benzodiazepines, intravenous and inhalation general anesthetic agents, neuroleptics, phenothiazines, α-agonists and barbiturates. On one side these drugs are used in order to help the patient, while on the other they have potentially harmful and adverse effects. Therefore, doctors in the ICU have to be well acquainted with all characteristics of these medications, in order to provide the patient with the most adequate care [9].

Sedatives are drugs most often used in the ICU. However, there are no ideal sedatives. The properties of an ideal sedative include: sedative, analgesic and anxiolytic effects, minimal cardio-vascular and respiratory side-effects, rapid onset and offset of its effects, no adverse effects on kidney and liver functions, having inactive metabolites, no interactions with other drugs and being cost-effective. This is exactly why there is no ideal sedative agent, and a large number of drugs and their combinations are in use. There are no defined sedation regimes, so the choice of suitable drugs is being made according to individual needs of the patient, his characteristics and clinical symptoms [10].

Intravenous anesthetic agents

Propofol. Propofol is an intravenous anesthetic agent which has a sedative, hypnotic, anxiolytic and retrograde effect in subanesthetic doses, but has no analgesic effects. It has a wide range of advantages including anticonvulsant and antiemetic effects, and it decreases the intracranial pressure [11, 12]. The most important side-effect of propofol is that it leads to hypotension due to peripheral vasodilation and negative inotropic and chronotropic effects. It is a lipid emulsion; therefore its intravenous application is painful. Out of other side effects, a dose dependent respiratory depression and hyperlipidemia may occur. Propofol infusion syndrome is rare, but a very serious drug reaction. It is characterized by a progressive heart dysfunction, a severe metabolic acidosis, hyperkalemia, hyperlipidemia, acute renal insufficiency, and rhabdomyolysis. Hemodialysis or hemofiltration is recommended for elimination of propofol and its toxic metabolites [13].

Benzodiazepines

Benzodiazepines are most frequently used drugs for sedation of patients with severe illnesses or injuries. They lead to sedation, anxiolysis or hypnosis, depending on the number of receptors which are activated. Anxiolytic effect is manifested by binding to so called benzodiazepine receptors, which represent locations in the limbic system, after which occurs the activation of the inhibitory transmitter gamma-aminobutyric acid (GABA) affecting the nearby neurons (serotonin, dopamine, acetylcholine, noradrenaline and others) via GABA receptors. There are GABAa, GABAb and GABAc receptors. Benzodiazepines manifest their effect via GABAa receptors [14]. Generally, they lead to enhanced affinity of GABAa towards receptors, which consequentially makes easier for chloride channels to open up and lead to fast hyperpolarization. This is how their sedative and hypnotic effect is explained [15]. They do not lead to general anesthesia, but can induce respiratory and cardiovascular depression. They are bound with plasma proteins and are not eliminated by dialysis.

*Midazolam***.** Midazolam is the most commonly administered short-lasting, water soluble benzodiazepine which becomes liposoluble in the blood and rapidly crosses the hematoencephalic barrier and enters the central nervous system. Midazolam is suitable for sedation in ICU due to titration to a desired level of sedation, anterograde amnesia which does not change previously learned information, respiratory and cardiovascular stability and existence of a specific antagonist [16]. Anterograde amnesia is developing almost momentarily after the intravenous administration and it usually persists for 20 – 40 minutes after a single dose. It significantly influences the patients' stay in ICU, since they do not remember unpleasant experiences. The time of half-elimination varies greatly, and neutralization is unpredictable due to prolonged distribution. This is exactly why unpredicted awakening and extended extubation time can appear if it is administered for more than 72 hours. In comparison to propofol, midazolam induces a lower frequency of hypotension, but a greater time variation in the recovery after the cessation of drug administration [17]. The antagonist is Flumazenil (Anexate), which can neutralize the effect of benzodiazepine.

Lorazepam. Lorazepam is a long-acting benzodiazepine, relatively low as far as liposolubility is concerned and relatively slow acting. Due to these features, it is not a good choice for rapid agitation control. If administered through continuous intravenous infusion, it has a long time of half-elimination (10 - 30 hours). Because of that, it is cumulating and the sedation is prolonged. That is why it is more appropriate for bolus administration. Solutions which are used for preparation of lorazepam may lead to hyperosmolarity, lactic acidosis and renal tubular acidosis, if administered alongside the drug in extended or higher dose. If higher dose is taken orally, it may cause diarrhea [18, 19].

Table 2. Richmond Agitation-Sedation Scale
Tabela 2. *Ričmondova Agitation-Sedation skala*

Level/*Vrednost*	Term/*Termin*	Description/*Opis*
+4	Combative *Borben*	Overtly combative, violent, dangerous to staff. *Otvoreno borben, nasilan, neposredna opasnost po osoblje.*
+3	Very agitated *Veoma agitiran*	Aggressive, removes endotracheal tube, catheters. *Agresivan, uklanja endotrahealni tubus, katetere.*
+2	Agitated *Agitiran*	Frequent non-purposeful movements, "fights against ventilator". *Često nesvrsishodni pokreti,"bori se sa ventilatorom".*
+1	Restless *Uznemiren*	Anxious, but not aggressive. *Anksiozan, ali nije agresivan.*
0	Alert and calm *Oprezan i miran*	
-1	Drowsy *Pospan*	Not fully awaken, but is opening eyes to voice (longer than 10 seconds). *Nije potpuno budan, ali otvara oči na dozivanje (duže od 10 s)*
-2	Light sedation *Blaga sedacija*	Briefly awakens, eye contact to voice (less than 10 seconds). *Kratko se budi, kontakt očima na dozivanje (kraće od 10 s)*
-3	Moderate sedation *Umerena sedacija*	Movement or eye opening to voice, without eye contact. *Otvaranje ili pokreti očiju na dozivanje, ali bez kontakta očima.*
-4	Deep sedation *Duboka sedacija*	No response to voice, eye opening to physical (pain) stimulation. *Nema odgovora na dozivanje, oči otvara samo na fizičku (bolnu) stimulaciju.*
-5	Unarousable *Ne mogu da se probude*	No response to voice or pain stimulus. *Nema odgovora ni na poziv ni na stimulaciju.*

Other benzodiazepines. Diazepam is not commonly used for sedation of patients in ICU and it can be administered intravenously; however, continuous administration should be avoided due to a long half-time of elimination, from 30 - 60 hours. It may lead to renal dysfunction [20]. Diazepam is also used in pediatrics, especially when administered rectally.

Barbiturates

Thiopentone. Barbiturates are still occasionally used in ICU. Deep sedation with thiopentone can be used for burst suppression of status epilepticus, although today propofol is more often used. Also, by administering continuous infusion one can induce so called "barbiturate coma" with severe trauma of the central nervous system (CNS), with the aim of decreasing cerebral metabolism. Thiopentone has immunosuppressive effect in certain doses. Literature data indicate the influence on serum potassium level during thiopentone induced coma. It is necessary to monitor serum potassium level in these cases, in order to avoid additional complications [21].

Alpha2 agonists

Dexmedetomidine. Dexmedetomidine is an alpha2 agonist of newer generation, with sedative, sympatholytic and anxiolytic properties. It shows greater affinity towards alpha2 receptors compared to Clonidine, due to which it has more expressed sedative effects. Sedation by alpha2 agonists differs from sedation with other sedatives. Patients can be awaken readily and their cognitive performance on psychometric tests is usually preserved. This is exactly why patients are more communicative and they cooperate better compared to other types of sedation. Dexmedetomidine reduces the postoperative vomiting reflex and enables better tolerance of endotracheal tube, in comparison to other sedatives [22]. Bolus administration has an important influence on cardiovascular system. Initially it leads to peripheral vasoconstriction, which consequently induces hypertension and reflex bradycardia, and later leads to central effects which are shown in vasodilation, hypotension and bradycardia. Cases of arrhythmia and sinus arrest have also been recorded. Due to these reasons, bolus administration of dexmedetomidine is not recommended, that is caution and monitoring is necessary during administration. Dexmedetomidine provides the anesthesiologist to rapidly awake the patient, who tolerates the endotracheal tube well, without respiratory depression, which makes it an ideal sedative [23].

Clonidine. Clonidine is also from the group of alpha2 agonists which reduces blood pressure and lowers heart rate by reducing sympathetic stimulation. Although it was initially used as an antihypertensive drug, it has not found its adequate and expected application in the field of cardiology. Clonidine provides sedation with minimal respiratory depression and has analgesic properties in larger doses, with scarce opioid effects. It also decreases cerebral blood flow and cerebral oxygen consumption [24]. There are some data showing that sedative doses of Clonidine lead to reduced rapid eye movement sleep phase in healthy volun-

teers. It is often used as the second choice drug with good effects in controlling tachycardia and hypertension which occur as the consequence of sedation. The effects of the drug are significant in controlling delirium and abstinence syndromes when taking opioids, benzodiazepines, alcohol and nicotine. It is being excreted via kidneys, unchanged in 40 – 60%, and around 40% of the drug is metabolized into inactive metabolites [25, 26].

Opioids

Opioids such as morphine, fentanyl, sufentanil, alfentanil and remifentanil represent the basic pain therapy in ICU. They are agonists of μ receptors of the CNS, which lead to analgesia, sedation, but also to respiratory depression, nausea, constipation, urine retention and occasional confusion and bewilderment. The choice of opioid depends on the preferred commencement and duration of the effects of the drug. It is important to take care of their solubility in fatty tissue, since their continuous infusion may lead to accumulation and consequently prolonged duration of the drug effects. Doses are titrated according to patient's individual needs [7].

Morphine. Morphine is still considered a significantly strong and very frequently used opioid analgesic. It causes depression of the respiratory, vasomotor, and cough center, but on the other side, it stimulates the vomiting center. It causes a decrease of basal metabolism and circumstantially the decrease of the body temperature. It also causes bradycardia, miosis, and increased intraocular and intracranial pressure [27]. Morphine is metabolized in the liver into morphine-6-glucuronide, which is being eliminated a lot slower than the morphine itself and it crosses the brain barrier a lot slower, which, as an aftereffect, causes the prolongation of its impact. The analgesic effect is the most important characteristic of morphine. In a dosage-dependent manner it causes the increase of the pain barrier, and it also changes the emotional reaction to the pain and causes general sedation [15]. Euphoria occurs with approximately half of the patients, whereas with some of the patients dysphoria is possible as well [15]. Due to its positive characteristics, as well as its efficiency, morphine still represents ''the gold standard'' in the postoperative period.

Fentanyl. Fentanyl is a synthetic opioid, which is 100 times more potent than morphine. It has, above all, a wide application in the treatment of intraoperative pain. In case of prolonged infusion, its accumulation occurs, and this is the matter one should take care of [28].

Sufentanil. Sufentanil is an opioid with the most powerful analgesic effect. It is 500 – 1000 more potent than morphine. It is suitable for sedation, since if it is used in mild dosage, it does not compromise hemodynamic stability. It is metabolized in the liver, metabolites are inactive and they are being eliminated through the kidneys.

Alfentanil. Alfentanil is an analogoue to fentanyl with approximately 1/10 of the fentanyl potency, but it is a short-acting opioid used in a single dosage. It is frequently metabolized in the liver. It has a small volume of distribution. A smaller bit is being egested with no alterations, whereas the greater part is eliminated in the form of metabolites, through urine [7].

Remifentanil. Remifentanil is a popular opioid analgesic of newer generation and its metabolism does not depend on the liver function. Studies show a higher quality of sedation, good hypnotic effects and a shorter time for extubation. When using this medicine, it is very important to know its characteristics. Special bolus application is unnecessary and it is potentially hazardous due to bradycardia and hypotension [29].

Ketamine

Ketamine is an antagonist of N-methyl-D-aspartate receptors. It can be used for the introduction and maintenance of anesthesia, as well as a medication for sedation in the ICU. It causes a condition which is, due to is symptoms, known as ''dissociative anesthesia''. In some aspects, it might be an ideal sedative, since it has both sedative and analgesic impact. It is also significant that it causes cardiovascular stability and bronchodilation. However, due to its connection with hallucinations, its independent usage in the ICU is not recommended. Ketamine is useful for patient comfort in painful procedures within the scope of intensive care, especially in pediatrics (punctures, drainages) and with bending of burns. It is also useful with patients who endured trauma, for maintenance of the respiratory musculature tonus and reflexes and for preservation of hemodynamic stability. It is frequently used in prehospital conditions, as well as a supplement to the opioids in the check-up of the post-operative pain. Ketamine was traditionally contraindicated for the check-up of increased intracranial pressure. However, contemporary attitudes have changed, since if there is a risk of hemodynamic instability, ketamine might be a very useful medication. It is also used with very severe bronchospasm, although its bronchodilator effect is very small. Inhalation anesthetics and propofol are more efficient in this respect [30, 31].

Inhalation anesthetics

In sedation, of inhalation anesthetics, the following are most frequently used: isoflurane, sevoflurane and desflurane. According to some studies, isoflurane has presented efficient, safe sedation up to 96 hours, with quicker awakening in relation to midazolam, and similar awakening in relation to propofol, however with increased number of patients with delirium. Isoflurane is also a powerful bronchodilator and it has a significant role in therapy of status asthmaticus [32]. Desflurane has also shown faster awakening after a short-term postoperative sedation (< 12h), as well as quicker mental recovery in relation to propofol. There are special systems for application of inhalation anesthetics (sevoflurane) in the ICU.

Antipsychotics (tranquilizers)

Neuroleptics are used in the treatment of agitation caused by hyperactive delirium, with the option to include haloperidol and oral antipsychotics, such as chloropromazine, olanzapine, risperodine. Haloperidol is most frequently used since it can be applied intravenously, frequently as a preventive measure, taking care of adverse effects for the cardiovascular system. Patients should be followed for arrhythmia, such as torsade de pointes, and it should be applied with precaution in the patients whose QT interval is prolonged. Dosing of medicine is performed according to the individual needs of the patient. Contemporary manuals for the control of delirium recommend a short-time application of haloperidol or olanzapine, with the recommendation of dexmedetomidine for prevention [33].

Non-opioid analgesics

Nonsteroidal anti-inflammatory drugs (NSAID) are used as a supplement to opioids in the therapy of pain in certain patients in the ICU. They must be used with precaution since they might cause damage to kidneys and erosion of gizzard mucosa due to their impact on renal production of prostacycline. They also have a characteristic of higher risk of myocardial heart failure and a stroke [34].

Adverse effects of sedatives

Prolonged sedation is an intervention whose side effects are often underestimated. They cause hypotension and decrease in perfusion, prolong mechanical ventilation, and in the worst case, the need for tracheostomy. Apart from this, prolonged sedation causes postponed respiratory support, higher frequency of delirium, immunosuppression, deep vein thrombosis, increased risk for development of nosocomial pneumonia, all of it leading to prolonged recovery time [35]. On the other side, decreased sedation causes the condition of general discomfort, as well as hypertension, tachycardia, hypercatabolism, increased usage of oxygen, atelectasis, infection and psychological trauma [36]. Consequently, due to this very reason, doctors in ICU must be very familiar with the medicines applied in the therapy, in order to achieve desired effects. As it was stated beforehand, ideal sedative does not exist. Every medicine has certain side-effects, and it is the task of every doctor to estimate whether the application of medicine causes more benefit than harm to the patient.

Sedation and functions of the Central Nervous System

A great number of clinical studies have analyzed the relationship between the usage of benzodiazepines and deterioration of the CNS functions, especially in severely sick patients (critically ill, surgical patients, with trauma, burns) treated in ICU for a long period of time.

Data on the effects of opioids differ a great deal. Sedation with dexmedetomidine, in comparison with benzodiazepine, decreases the possibility of damage or duration of dysfunctions of the CNS. The ABCDE strategy, that stands for AB (Awakening and Breathing trials), C (Choice of sedation), D (Delirium monitoring and management), as well as E (Early Exercise), may decrease the incidence of acute and prolonged dysfunction of the CNS [37]. In the same manner, using bispectral index (BIS) for monitoring the depth of sedation makes it possible to establish the level of sedation. Monitoring the impact of sedatives on the CNS expressed numerically (above 60 - 80) may, correctly represent the level of consciousness i.e. the level of being awake [38].

Early mobilization

Contemporary studies show that early mobilization has a significant impact on the patients' functional outcomes, safety, and the length of stay in the ICU. Early physical therapy significantly reduces the incidence of delirium in the ICU. In the same manner, the protocol of early mobilization decreases the usage of sedatives and analgesics, and supports enhanced recovery after surgery. In patients on mechanical ventilation, every day without sedation, alongside the physical therapy, significantly improves the functional status and shortens the time in the ICU [39, 40].

Conclusion

Sedation is a very significant issue in the management of the critically ill patients. Consultations with doctors and the manner of sedation according to specific and individual characteristics of patients provide safe and adequate treatment. Currently accessible sedatives that are used in Intensive Care Units are acceptable and are widely used, but they also have limitations. Instead of searching for ideal sedatives for critically ill patients, their application should be based on the principles of pharmacology and pharmacokinetics of medicines. By establishing the aims of sedation, according to individual characteristics and current conditions of the patient, it is possible to provide a rational treatment strategy for each patient in the intensive care unit. In the same way, early mobilization of patients who are still in the intensive care unit, reduces the occurrence of intensive care unit delirium and consequently reduces the usage of sedatives and analgesics, thus contributing to enhanced recovery and shorter stay in the intensive care unit.

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Prihvaćen za štampu 22. III 2019.
BIBLID.0025-8105:(2019):LXXII:3-4:123-130.

UPUTSTVO ZA AUTORE

Časopis ***Medicinski pregled*** objavljuje radove koji prethodno nisu objavljeni niti poslati u drugi časopis. U Časopisu mogu biti objavljeni radovi iz različitih oblasti biomedicine, koji su namenjeni lekarima različitih specijalnosti.

Od 1. januara 2013. godine *Medicinski pregled* je počeo da koristi usluge *e-Ur* − Elektronskog uređivanja časopisa. Svi korisnici sistema − autori, recenzenti i urednici, moraju biti registrovani korisnici sa jednom elektronskom adresom.

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U postupku prijave neophodno je da se pošalje saglasnost i izjava autora i svih koautora da rad nije delimično ili u celini objavljen ili prihvaćen za štampu u drugom časopisu.

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Primaju se samo radovi koji su napisani na engleskom jeziku, uz sažetak rada i naslov rada koji treba da budu napisani na engleskom i srpskom jeziku.

Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

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

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

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

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

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

Priprema rukopisa

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

Propratno pismo:

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

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

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

Rukopis

Opšta uputstva

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

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

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

2. Sažetak

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

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

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

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

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

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

3. Tekst članka

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

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

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

Uvod

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

Materijal i metode

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

Rezultati

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

Diskusija

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

Zaključak

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

4. Literatura

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

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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

∗ Organizacija kao autor

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

∗ Bez autora

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

∗ Volumen sa suplementom

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

∗ Sveska sa suplementom

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

∗ Sažetak u časopisu

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

Knjige i druge monografije

∗ Jedan ili više autora

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

∗ Urednik (urednici) kao autor (autori)

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

∗ Poglavlje u knjizi

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

∗ Zbornik radova sa kongresa

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

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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

∗ Kompjuterska datoteka

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

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

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

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

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

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

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

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

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

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

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

6. Dodatne obaveze

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

INFORMATION FOR AUTHORS

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

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

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

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

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

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

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

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

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

Preparation of the manuscript

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

The covering letter:

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

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

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

The manuscript:

General instructions.

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

The manuscript should contain the following elements:

1. The title page.

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

2. Summary.

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

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

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

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

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

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

3. The text of the paper.

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

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

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

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

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

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

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

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

Articles in journals

∗ *A standard article*

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

∗ *An organization as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

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

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

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