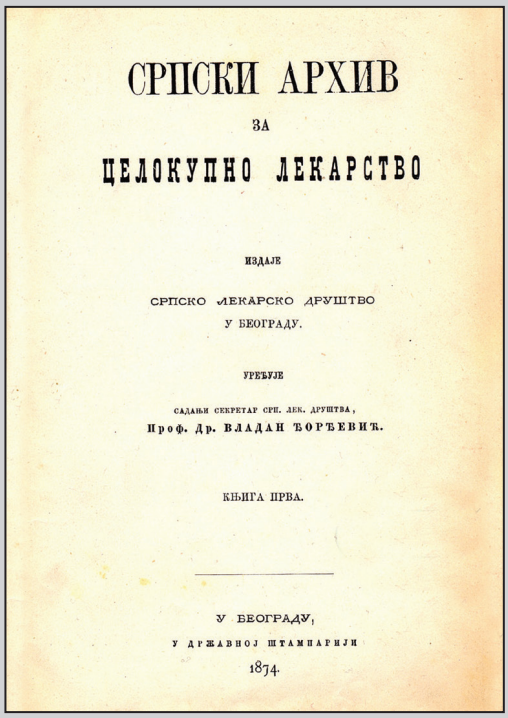

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ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

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У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

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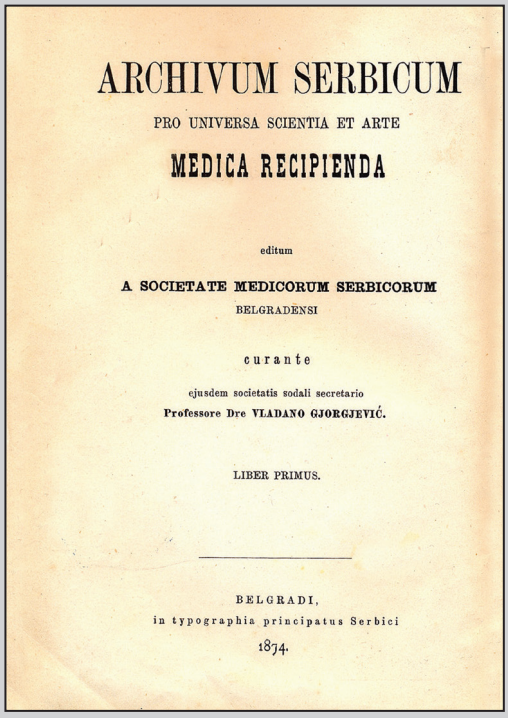

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effects of the fixed orthodontic therapy on biochemical and microbiological parameters of saliva

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SUMMARY

Introduction/Objective Malocclusions are one of the most frequent disorders in dentistry, and pose a risk for the onset of caries and periodontal diseases. Fixed orthodontic treatment solves the problem of malocclusions; however, it requires simultaneous cooperation of the patients, parents, and dentists involved.

The objective of this study is to examine the effects of fixed orthodontic therapy on the *Streptococcus mutans* and *Lactobacillus spp.* bacteria in saliva, the pH value, and buffering capacity of saliva.

Methods The research was carried out at the Faculty of Medicine in Foča, Department of Dentistry. The study included 100 respondents, aged 13 to 17 years. The respondents were divided into two groups: the study group (respondents wearing fixed braces) and the control group (respondents not subjected to fixed braces therapy). Saliva samples were taken from the respondents four, 12, and 18 weeks after the start of the orthodontic therapy. The study used the bacteria caries risk test (CRT) and CRT buffer (Ivoclar Vivadent).

Results The study showed an increased number of bacteria in saliva of the respondents during all three follow-up periods (χ^2 test, $p = 0.001$). The largest numbers of the *Streptococcus mutans* and *Lactobacillus spp.* bacteria were found in week 12 of the therapy. Saliva pH value and buffering capacity of saliva increased statistically significantly in week 12 of the therapy (χ^2 test, $p = 0.001$).

Conclusion Oral conditions in patients changed during the fixed orthodontic therapy: the number of bacteria increased, the pH value and buffering capacity of saliva changed. It was necessary to use preventive measures in order to avoid complications during the fixed orthodontic appliances therapy.

Keywords: malocclusion; *Streptococcus mutans*; bacteria

INTRODUCTION

Saliva is the main defense mechanism in the oral cavity and is a major factor for preserving and maintaining the health of oral tissue. Chemical properties of saliva are affected by local factors in the oral environment and by the general health of the individual. The physico-chemical properties of saliva determine the progress of orthodontic treatment and its adverse effects in an orthodontic patient.

Malocclusion is one of the most frequent dental disorders and increases the risk of the onset of caries and periodontal diseases [1]. The placement of orthodontic brackets and bands may compromise oral hygiene, because new retentive places are formed resulting in increased accumulation of dental plaque leading to gingival inflammation [2]. Orthodontic treatment may solve the problem of malocclusion, but it increases the risk of caries onset and the severity of this lesion may range from a white spot on a tooth or demineralization to the loss of integrity of the enamel surface and the onset of a cavity [3]. Some studies report that the prevalence of white spot lesions during orthodontic treatments ranges 30–70% [3]. The changes in salivary parameters such as the decline in the pH level and buffering capacity of saliva may contribute to the demineralization of enamel and increase the susceptibility of teeth to the onset of caries [4]. Amongst various microflora in the mouth, the *Streptococcus mutans* bacterium is the main culprit for the onset of caries. The presence of these microorganisms at high levels indicates an increased risk of caries. Caries is an undesirable side effect of treatment with fixed orthodontic appliances [5, 6]. Patients undergoing orthodontic therapy experience oral ecological changes that lead to an increased number of mutans streptococci in the saliva and dental plaque [7]. At the same time, the presence of the *Lactobacillus spp.* bacteria increases the severity and incidence of caries [8]. *Lactobacillus spp.* is a secondary invasive bacterium and is responsible for the progression of the caries lesions.

The side effects of the fixed orthodontic therapy on oral health are described in numerous studies [9, 10, 11]. The studies report that caries as a complication of fixed orthodontic

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therapy is present in 2–96% of patients [12, 13]. Chang et al. [14] were amongst the first who had examined the impact of the fixed orthodontic therapy on the salivary microbiological parameters, pH value, and buffering capacity of saliva. Other authors also examined the impact of the fixed orthodontic therapy on the salivary microbiological parameters, pH value, and buffering capacity of saliva, which is the objective of the present study as well [15, 16].

METHODS

The present study was designed as a prospective cohort one. The research included 100 respondents who reported to the Faculty of Medicine in Foča, the study program in Dentistry during the years 2015 and 2016, who then underwent dental check-ups and were diagnosed with malocclusions. The respondents were divided into two groups. The exposure group (the study group) consisted of 50 respondents who underwent the treatment for malocclusion. The non-exposed group (the control group) consisted of 50 respondents, who were also diagnosed with malocclusion, but did not receive treatment (financial means, durability of treatment, patients satisfied with the current positions of teeth). The criteria for the inclusion in the study were the following: the presence of permanent dentition with other permanent molars in place, good overall health condition of the patient, patients indicated as requiring the upper and lower fixed orthodontic appliances, without dental caries. The criteria for the exclusion from the study were the following: periodontal diseases, patients with clefts, syndromes, and orofacial diseases, and patients who suffer from a chronic disease and receive a form of therapy (diabetes mellitus, autoimmune diseases, epilepsy).

The samples of saliva in the control group were collected several days after the dental check-up (T0). The samples of saliva in the study group were taken four, 12, and 18 weeks (T1, T2, T3) after the attachment of the fixed orthodontic appliances. The saliva pH level, buffering capacity, and quantitative presence of the *Streptococcus mutans* and *Lactobacillus spp.* bacteria were determined for each patient. The respondents were instructed not to consume any food and drinks for at least one hour before giving a sample of saliva and to brush their teeth once, in the morning, on the day of giving saliva samples. The respondents were sitting in a dental chair leaning slightly forward and were chewing bilaterally a paraffin ball for five minutes to stimulate salivation. Saliva was collected in sterile plastic cups for each patient. A bacteria caries risk test (CRT) (Ivoclar Vivadent, Schaan, Liechtenstein) was used to determine the quantitative presence of bacteria. The agar carrier of the mentioned test was removed from the test vial and $NaHCO_3$ tablet (for the purpose of ensuring anaerobic conditions) was placed at the bottom of the vial. The nutrient base was moistened with a thin layer of saliva using a pipette, to the amount of 1 ml. The agar carrier (the base) was carefully returned to the vial, which was then firmly closed. The vials with the seeded base were incubated at 37°C over 48 hours. Thereafter the presence of the grown colonies on both seeded bases (colony-forming unit – per ml of saliva) was measured by way of comparison with the standardized scheme specified by the manufacturer. The buffering capacity of the saliva was determined using a CRT buffer (Ivoclar Vivadent). The saliva pH value was determined using a digital pH meter (4440 pH Type; Funke-Dr.N.Gerber Labortechnik GmbH, Berlin, Germany).

The research complies with the Helsinki Declaration. For the purpose of this study, Approval No. 01-1142 was obtained from the Ethics Committee of the Faculty of Medicine in Foča.

The data were analyzed using descriptive and analytical statistics methods; χ^2 test and Fisher's exact test, for qualitative variables, and Student's t-test, for quantitative variables, were used to determine the statistical significance between the two groups of respondents.

RESULTS

The study included 100 respondents, aged 13–17 years. The average age was 14.88 ± 1.35. The age group of 13–14 years made up 40% of the respondents, 24% of the respondents were 15 years old, while the remaining 36% of the respondents were in the age group of 16–17 years. Male respondents made up 48%, while female respondents made up 52% of the total number of respondents.

The numbers of the *Streptococcus mutans* were statistically significantly increased during all three periods of research when compared to the control group of respondents. The largest numbers of the *Streptococcus mutans* bacterium were found after 12 weeks of therapy (p = 0.001). The number of bacteria was reduced after 18 weeks when compared to week 12 of the treatment; however, a statistically significant difference still existed when compared to the control group of the respondents (Table 1). The numbers of the *Lactobacillus spp.* bacteria were statistically significantly increased during all three periods of research when compared to the control group of the respondents. The largest number of the *Lactobacillus spp.* bacteria was found after 12 weeks of therapy (p = 0.001). The number of the bacteria was reduced after 18 weeks when compared to week 12 of the treatment; however, a statistically significant difference still existed when compared to the control group of the respondents (Table 2).

Table 1. Data obtained by saliva analysis on the amount of *Streptococcus mutans* bacterium in subjects undergoing orthodontic treatment after four, 12, and 18 weeks and in the control group

Time (weeks)	Level of bacteria	Group of respondents number (%)		Total number (%)	χ^2; F	p
		Study group	Control group			
4	Low (< 10^5) High (≥ 10^5)	6 (12) 44 (88)	22 (44) 28 (56)	28 (28) 72 (72)	12.698	0.001
12	Low (< 10^5) High (≥ 10^5)	5 (10) 45 (90)	22 (40) 28 (56)	27 (27) 73 (73)	14.663	0.001
18	Low (< 10^5) High (≥ 10^5)	10 (20) 40 (80)	22 (44) 28 (56)	32 (32) 68 (68)	6.618	0.010

χ^2 – Chi-squared test; F – Fisher's exact test

Table 2. Data obtained by saliva analysis on the amount of *Lactobacillus spp*. bacterium in subjects undergoing orthodontic treatment after four, 12, and 18 weeks and in the control group of subjects

Time (weeks)	Level of Bacteria	Group of respondents number (%)		Total number (%)	χ^2; F	p
		Study group	Control group			
4	Low (< 10^5) High (≥ 10^5)	23 (46) 27 (54)	38 (76) 12 (24)	61 (61) 39 (39)	9.458	0.002
12	Low (< 10^5) High (≥ 10^5)	15 (30) 35 (70)	38 (76) 12 (24)	53 (53) 47 (47)	21.236	0.001
18	Low (< 10^5) High (≥ 10^5)	18 (36) 32 (64)	38 (76) 12 (24)	56 (56) 44 (44)	16.234	0.001

χ^2 – Chi-squared test; F – Fisher's exact test

Table 3. Buffer capacity of the control and study group

Buffer capacity in saliva samples	Level of buffer capacity	Respondents number (%)		Total number (%)	χ^2; F	p
		Study group	Control group			
Study group after 4 weeks and control group	Low Medium High	2 (2) 23 (23) 25 (25)	4 (4) 26 (26) 20 (20)	6 (6) 49 (49) 45 (45)	1.406	0.495
Study group after 12 weeks and control group	Low Medium High	2 (2) 6 (6) 42 (42)	4 (4) 26 (26) 20 (20)	6 (6) 32 (32) 62 (62)	20.973	0.001
Study group after 18 weeks and control group	Low Medium High	6 (6) 23 (23) 21 (21)	4 (4) 26 (26) 20 (20)	10 (10) 49 (49) 41 (41)	0.608	0.738

χ^2 – Chi-squared test; F – Fisher's exact test

Table 4. Saliva pH values of the study and the control group

pH value in saliva samples	Respondents AM (SD)		t	p
	Study group	Control group		
Study group after 4 weeks and control group	6.76 (0.40)	6.66 (0.26)	1.482	0.141
Study group after 12 weeks and control group	6.89 (0.21)	6.66 (0.26)	4.881	0.001
Study group after 18 weeks and control group	6.84 (0.47)	6.66 (0.26)	2.345	0.210

AM – arithmetic mean; SD – standard deviation, t – Student's t-test

The pH value and buffering capacity of saliva statistically significantly increased 12 weeks after the therapy when compared to the control group of the respondents (p = 0.001). Four and 18 weeks after the orthodontic treatment no statistically significant difference was found in comparison to the control group of the respondents (Tables 3 and 4).

DISCUSSION

This research showed that a statistically significant increase in the number of *Streptoccocus mutans* bacterium occurred in the patients' saliva after all three study periods (four, 12, and 18 weeks after the orthodontic therapy). The study results show that the adverse effects of the fixed orthodontic therapy occurred as early as four weeks after the treatment. Chang et al. [14] presented similar results, according to which they also found a statistically significant increase in the number of bacteria four weeks and three months after wearing the braces. The authors detected the peak in the number of microorganisms in week four of the therapy, in contrast to our research. This study shows that in week 18 of the therapy the number of bacteria starts to decline, which is a very significant piece of information when it comes to planning preventive and prophylactic measures. The increased number of the *Streptococcus mutans* bacteria during the therapy may be explained by the increased retention locations following the attachment of the appliance, which is conducive to the accumulation of plaque, which in turn increases the number of aciduric and acidogenic bacteria, which prefer hard and uneven surfaces for their growth [17]. The results of this study are consistent with the results of other authors, who also found the increased numbers of bacteria during all three follow-up periods, whereas they followed up on patients six, 12, and 18 weeks of the therapy [8, 16]. In addition, all these studies found the largest number of bacteria in week 12 of the orthodontic therapy. When compared to our study, the difference was the sample size. Numerous authors suggest that white spots on teeth, as the start of a caries lesion, are detected as early as four weeks after the orthodontic treatment; therefore, we are of the opinion that it is justified to start monitoring the changes in saliva after four weeks [18, 19, 20]. White spot lesions on teeth, if left untreated, may progress into a lesion on a tooth and this entire process progresses rapidly in orthodontic patients [21].

The study showed a statistically increased number of the *Lactobacilus spp.* bacterium in saliva in all three follow-up periods (four, 12, and 18 weeks after the orthodontic treatment). The greatest value of the number of the bacteria were found in week 12 of the therapy, while the decline in the number of the bacteria was found in week 18, which is a significant piece of information when planning preventive measures. The study showed no statistically significant difference in regard to the sex and age of patients. These results are consistent with the results of other authors, who also found an increase in the number of the *Lactobacillus spp*. bacteria in all three follow-up periods, with the highest number found in week 12 of the therapy [8, 16]. Chang et al. [14] studied the numbers of this microorganism four weeks and three months following the therapy and they found the greatest number of the bacterium in the third month (week 12) of the therapy, which matches the results of our study, as well as of other studies [16]. Other authors also found statistically significant increase of the number of this microorganism in week 12 of the therapy [22]. Eighteen weeks after the treatment, the results showed that the number of this microorganism was declining when compared to week 12; however, the number was still statistically significantly increased when compared to the control group of the respondents. Week 12 may be regarded as the period of the most intense growth of the *Lactobacillus spp*.

bacteria in the respondents' saliva. However, some authors found the statistically increased number of the *Lactobacillus spp*. bacterium in saliva six months after wearing the braces, while no statistically significant difference in the number of this microorganism was detected four and 12 weeks after the therapy when compared to the values before the attachment of the braces [23].

The study results show that statistically significant difference occurred between the study and control groups of respondents ($p = 0.001$) in week 12 of the therapy, in regard to the saliva pH values (Table 4), which is consistent with results of some other studies [14, 16]. Some studies found no changes in the saliva pH values during all three follow-up periods of patients with fixed orthodontic appliances [24].

The study results showed a statistically significant difference regarding the buffering capacity of saliva 12 weeks after the therapy ($p = 0.001$) (Table 3). Some studies reported the decline in the buffering capacity of saliva with the increase in the pH value, which is not consistent with the results of this study and compels one to consider other ions of the stimulated saliva which have the buffering effect apart from the bicarbonate ones [16]. Arab et al. [8] found a statistically significant decline in the saliva pH value as early as week six of the therapy, which continued in weeks 12 and 18 of the orthodontic treatment, which is in contrast to our research. These results support the notion of great risk for the onset of caries, given the weak defensive ability of the organism, while the number of bacteria is significantly increased. Lara-Carrillo et al. [25] found a statistically significant increase in the pH value and buffering capacity of saliva of the respondents four weeks after the orthodontic treatment.

CONCLUSION

The research showed the changes in biochemical and microbiological parameters during the fixed orthodontic appliances therapy. The patients wearing fixed orthodontic appliances require regular dental check-ups to be performed by orthodontists and other dental medicine specialists, in order to detect in due time the risk factors for the onset of oral and dental diseases.

Conflict of interest: None declared.

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Утицај фиксне ортодонтске терапије на биохемијске и микробиолошке параметре пљувачке

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САЖЕТАК

Увод/Циљ Малоклузије представљају један од најчешћих поремећаја у стоматологији, а уједно представљају и ризик за настанак каријеса и пародонталних обољења. Фиксна ортодонтска терапија решава проблем малоклузије, али истовремено захтева сарадњу како пацијента, тако и родитеља и стоматолога.

Циљ овог рада био је да се испита утицај фиксне ортодонтске терапије на количину бактерија *Streptococcus mutans* и *Lactobacillus spp.* у пљувачки, *pH* и пуферски капацитет пљувачке.

Методе Истраживање је рађено на Медицинском факултету у Фочи, на одсеку за стоматологију. У студији је учествовало 100 испитаника, узраста 13–17 година. Испитаници су подељени на студијску групу (испитаници који носе фиксни ортодонтски апарат) и контролну групу (испитаници без фиксне терапије). Испитаницима су узимани узорци пљувачке после четири, 12 и 18 недеља од почетка ортодонтске терапије. У студији су коришћени *CRT* тест на бактерије и *CRT* пуфер (*Ivoclar Vivadent*).

Резултати Студија је показала повећану количину бактерија у пљувачки испитаника у сва три пратећа периода (χ^2, p = 0,001). Највећа количина бактерија *Streptococcus mutans* и *Lactobacillus spp.* нађена је у 12. недељи терапије; *pH* вредност пљувачке и пуферски капацитет пљувачке су статистички значајно повећани у 12. недељи терапије (χ^2, p = 0,001).

Закључак Током фиксне ортодонтске терапије мењају се услови у устима пацијента: повећана је количина бактерија, долази до промене *pH* вредности и пуферског капацитета пљувачке. Неопходно је користити превентивне мере како би се спречиле компликације током терапије фиксним ортодонтским апаратима.

Кључне речи: малоклузија; *Streptococcus mutans*; бактерија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Applicability of the instruments for measuring pain intensity in persons with masticatory myofascial pain

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SUMMARY

Introduction/Objective The most frequent clinical presentation of myofascial pain (MFP) includes the presence of a deep local muscle pain, limited level of movements, heterotopic pain of trigger points in the referent zones, and the loss of the major symptoms by anesthetizing these points. The only manner to objectively and comprehensively evaluate pain, as a multidimensional experience, is by applying multiple methods in its diagnostics.

The objective of this paper was to corelate diagnostic possibilities of different quantification instruments for the assessment of pain intensity in persons with masticatory MFP.

Methods The study involved 60 subjects, divided into two groups stratified according to their sex and age. The Research Diagnostic Criteria for Temporomandibular Disorders diagnostic protocol was applied, within which the numeric scale of pain, digital palpation, graded chronic pain scale, the Visual Analogue Scale (VAS), and algometry were used.

Results The cardiac power index values are statistically significant and in negative correlation with the algometric measurements (from -0.48 to -0.59) and in positive, statistically significant, correlation with the VAS values (0.71).

Conclusion The results of studies we obtained lead us to the conclusion that there is an interdependence of these instruments for the measurement of pain intensity in persons with masticatory MFP and that the VAS and algometry are more objective and precise methods than the manual palpation.

Keywords: myofascial pain; diagnostics instruments; VAS; algometer; manual palpation

INTRODUCTION

The problems related to the etiology, occurrence, and particularly the diagnostics of the myofascial pain dysfunction syndrome (MPDS) in the head and neck region, due to a multitude of unknowns, have been the subject of a number of scientific discussions. The existing theories on the mechanisms of pain occurrence include local muscle hypoxia, centrally indicated sensitization, and neurogenically stimulated secretion of substances that cause the occurrence of pain in sensitive places [1].

The most frequent clinical presentation myofascial pain (MFP) includes the presence of deep local muscle pain and suffering, limited level of movements, heterotopic pain from the so-called trigger points in the referent zones, and the loss of major symptoms by anesthetizing these points [2]. It is a known fact that the diagnostic possibilities of quantification and characterization of the chronic MFP are definitely hardly feasible. That is why the precise diagnostic of these painful conditions is not always straightforward.

The only manner to objectively and comprehensively evaluate pain, as a multidimensional experience, is by applying multiple methods in its diagnostics. It is, therefore, not surprising that there is a multitude of studies that deal with this topic and confirm the positive correlation and interdependence of different instruments for the measurement of pain intensity [3–6].

In clinical practice, the manual muscle palpation is established as the "gold standard" and is still the most frequently applied method for examination of muscle sensitivity [7, 8]**.** However, the attitude that it is exclusively sufficient for diagnosing the masticatory MFP can mostly be found in older papers and, nowadays, it is considered outdated. Some of the main issues with this method are certainly the impossibility to sufficiently standardize the procedure, as well as different interpretations of a patient's reactions during its performance. That is exactly why other instruments for measuring pain intensity have been introduced. The Visual Analogue Scale (VAS) is one of the most frequently used unidimensional scales for the assessment of the pain threshold [9]. Algometry is a more objective, precise, standardized, repeatable, and valid method [10, 11]. The measurement of pain intensity and the documentation of its values are the basis of the proper and efficient treatment.

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The objective of this paper was to correlate diagnostic possibilities of different quantification instruments for the assessment of pain intensity in persons with masticatory MFP.

METHODS

The study, conducted at the Clinic for Dentistry of Vojvodina, Faculty of Medicine in Novi Sad, Serbia, in accord with standards of the institutional committee on ethics, involved 60 subjects divided into two groups stratified by their sex and age. The study group comprised 30 subjects with the diagnosis of MFP (16 men and 14 women) with the average age of 42.77 ± 11.57 years, while the control group comprised 30 healthy subjects without any signs and symptoms of MFP.

In addition, control group subjects were excluded if they had masticatory MFP, temporomandibular joint arthralgia, degenerative joint disease, and/or disc displacement without reduction, as well as if they complained of frequent and/or persistent pain in any bodily part, fibromyalgia syndrome, self-reported psychogenic illness, and the female subjects were not pregnant.

The basic criterion for patients to be involved in the study was the occurrence of pain in *m. masseter* and/or *m. temporalis* of longer than three months duration. The subjects did not experience neurological disorders, atypical pain, infections of the surrounding structures, acute pain caused by dental disorders, neuropathies, chronic immune-deficiency, neoplasms, and the female subjects were not pregnant.

The diagnosis was established using the detailed history, with a particular emphasis on the pain anamnesis, as well as clinical examination, performed by standardized procedures of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) protocol [12].

The MFP diagnosis was obtained on the basis of data processing conducted in the first and the second part of the clinical protocol, primarily from the positive results of the palpatory pain in three or more points, with or without functional limitation in the opening of the mouth [13]. Monitoring of the pain intensity was also performed by applying different instruments: the Graded Chronic Pain Scale (GCPS), the numeric pain rating scale 0–3 during palpation, VAS, and algometry.

The patients provided answers to palpation and graded the feeling of pain in the numeric rating scale (0 – no pain, 1 – mild pain, 2 – moderate pain, 3 – severe pain). The grades were added, and the overall sensitivity sum was obtained. The GCPS is a scale within the second part of the RDC/TMD protocol and its result is the value that is called the Characteristic Pain Intensity (CPI) [14]. The VAS was applied as a unidimensional scale for measuring intensity of the subjective feeling of pain prior to examination of the pressure pain threshold. The scale consists of one straight 10-cm-long line, the beginning of which is marked by 0, which is a numerically expressed value for the patient signifying the absence of pain, all the way to the end of the scale marked by 10, signifying that the pain is unbearable. The subject needed to express the point that correlates best with the intensity of experienced pain sensation. It is expressed in millimeters [1].

The algometric measurement was conducted with a digital algometer (FPIX 10, 2007, dimensions 23/4" WX" HX 1 1/4" d; Wagner Instruments, Riverside, CT, USA). The measuring locations were the precisely determined points on the masseter and temporal muscles on both sides [15]. The measuring was performed by applying a rubber probe with a certain intensity on the surface of 1 cm^2. The device has the capacity to render the calibration values into kgf/cm^2 – N, Ibf, and Ozf. The testing was conducted in the identical space and time conditions. The subjects were requested to inform us when they start experiencing pain (pressure pain treshold – PPT) and when that pain becomes unbearable (pain tolerance threshold – PTT). That moment was registered at the algometer display. The testing was repeated three times with rest phases in the duration of five minutes.

Before the beginning of the research, all the subjects had been familiarized with the experimental procedures and they gave their voluntary compliance with the signed consent for participating. The research was approved by the Ethics Committee of the Faculty of Medicine in Novi Sad.

The basic measurements and statistical analyses were used for establishing basic conclusions. The distributions of normal values were tested, the average and mean values of measurements, as well as the standard deviation were analyzed, while more thorough testing was checked with the t-test. Moreover, the Spearman's rank correlation coefficient for different variables was used for the detection of the connection of diagnostic methods. The level of relevance was considered significant if $p < 0.05$.

RESULTS

During palpation of the anterior fibers of the temporal muscle, as much as 40% of the study group subjects experienced severe pain on palpation of anterior fibers at the right side and 23.33% on the left side, while 10% of the subjects felt no pain on either side. Generally, patients experienced less pain on palpation of middle and posterior fibers of the temporal muscles, regardless of the side (Table 1). During palpation of the masseter muscle, patients experienced the worse (severe) pain in the lower and middle portions of the muscle on the right side, and in the upper portions on the left side (Table 1).

The values of algometric measurements for both muscles (masseter and temporal), in both groups of subjects, are shown in Tables 2 and 3. There were statistically significant differences in mean values of algometric measurements in both muscles between the two groups of subjects (Student's t-test). Statistically significant differences were noted between the values of the pain threshold and the pain tolerance threshold for both muscles (Tables 2 and 3).

The values of measuring the pain intensity using the VAS scale with the study group subjects are shown in Table 4.

Table 1. The numeric rating scale of pain during the application of manual pressure on masseter and temporal muscles

Muscle		Position	No pain	Mild pain	Moderate pain	Severe pain
			n (%)	n (%)	n (%)	n (%)
Temporal muscle	Right	Posterior	13 (43.33)	13 (43.33)	4 (13.33)	0
		Middle	10 (33.33)	2 (6.67)	11 (36.67)	7 (23.33)
		Anterior	3 (10)	8 (26.67)	7 (23.33)	12 (40)
	Left	Posterior	19 (63.33)	8 (26.67)	3 (10)	0
		Middle	10 (33.33)	7 (23.33)	10 (33.33)	3 (10)
		Anterior	3 (10)	9 (30)	11 (36.67)	7 (23.33)
Masseter muscle	Right	Upper portion	3 (10)	10 (33.33)	14 (46.67)	3 (10)
		Mid-belly	4 (13.33)	9 (30)	7 (23.33)	10 (33.33)
		Lower portion	6 (20)	7 (23.33)	7 (23.33)	10 (33.33)
	Left	Upper portion	2 (6.67)	8 (26.67)	9 (30)	11 (36.67)
		Mid-belly	3 (10)	7 (23.33)	14 (46.67)	6 (20)
		Lower portion	7 (23.33)	7 (23.33)	10 (33.33)	6 (20)

Table 2. The differences in the t-test values of algometric measurements of *m. masseter* and *m. temporalis* on both sides between the two groups of subjects

Muscle	Control group	Study group	t	p
	$\bar{x} \pm SD$	$\bar{x} \pm SD$		
Right masseter muscle PPT	4.87 ± 0.92	2.02 ± 0.69	13.54*	0.000000*
Right masseter muscle PTT	6.63 ± 0.77	2.95 ± 0.69	19.47*	0.000000*
Left masseter muscle PPT	4.94 ± 0.97	2.09 ± 0.58	13.86*	0.000000*
Left masseter muscle PTT	6.55 ± 0.89	2.97 ± 0.65	17.82*	0.000000*
Right temporal muscle PPT	5.24 ± 1.14	2.36 ± 0.68	11.88*	0.000000*
Right temporal muscle PTT	6.59 ± 1.16	3.33 ± 0.79	12.76*	0.000000*
Left temporal muscle PPT	5.17 ± 1.24	2.55 ± 0.58	10.48*	0.000000*
Left temporal muscle PTT	6.38 ± 1.23	3.47 ± 0.61	11.61*	0.000000*

PPT – pressure pain threshold; PTT – pain tolerance threshold

Table 3. The differences in the t-test values of algometric measurements (pressure pain threshold and pain tolerance threshold) of *m. masseter* and *m. temporalis* between the two groups of subjects

Muscle	Control group	Study group	t	p
	$\bar{x} \pm SD$	$\bar{x} \pm SD$		
Masseter muscle PPT	4.91 ± 0.91	2.05 ± 0.60	14.29*	0.000000*
Masseter muscle PTT	6.59 ± 0.76	2.96 ± 0.61	20.36*	0.000000*
Temporal muscle PPT	5.21 ± 1.15	2.46 ± 0.51	11.97*	0.000000*
Temporal muscle PTT	6.48 ± 1.14	3.40 ± 0.59	13.15*	0.000000*

PPT – pressure pain threshold; PTT – pain tolerance threshold

Table 4. Descriptive statistics for the visual analogue scale (VAS) in the study group

Study group	n	$\bar{x} \pm SD$	95% CI	Min.	Max.
VAS	30	7.24 ± 1.40	6.719–7.767	4.8	9.5

Table 5. The values of the characteristic pain intensity (CPI), measured with the Graded Chronic Pain Scale in the study group

Study group	$\bar{x} \pm SD$	96% CI	Min.	Max.
CPI	60.78 ± 15.80	54.88–66.68	30	90

The subjects considered that their pain had the average value on the scale of 7.24 cm.

The values of the graded chronic pain scale are shown in Table 5. The mean values of the characteristic pain intensity (CPI) were between 54.88 and 66.68 in the 96% confidence interval.

The obtained algometric values (the PPT and the PTT) for both muscles and the values of the VAS measurements were in a reverse correlation concerning statistical significance (the correlation coefficient for the masseter muscle was -0.50 and -0.64, and for the temporal muscle -0.42 and -0.38, respectively). The correlation coefficient was negative, considerable, and strong. The manual pressure values were in negative correlation with the values of algometric measurements and in positive correlation with the values measured with the VAS, but with no statistical significance. The CPI values were statistically significant and in negative correlation with algometric measurements (from -0.48 to -0.59) and positive, statistically significant correlation with the VAS values (0.71). The correlation of the palpation values of the masseter muscle and CPI was statistically significant (0.50), while for the temporal muscle it was not (0.18). It was established that in subjects with a higher level of disability, lower pressure induced by the algometer caused pain, i.e. they were in negative, statistically significant correlation with the values of algometric measurements on both muscles (Table 6).

DISCUSSION

Chronic MFP is often non-recognized in clinical practice. This is the reason why it is important to have precise and complete observation of the pain characteristics and intensity for its diagnosis, which should be founded on the manifold methodological approaches and use of different instruments for measuring pain intensity.

Table 6. Spearman's rank correlation coefficient for different variables

Variables	Manual palpation		Masseter muscle		Temporal muscle		VAS	CPI
	Temporal muscle	Masseter muscle	PPT	PTT	PPT	PTT		
Palpation of temporal muscle	1.00	0.05	-0.35	-0.35	-0.2	-0.32	0.12	0.18
Palpation of masseter muscle	0.05	1	-0.28	-0.35	-0.22	-0.28	0.34	0.50*
Masseter muscle PPT	-0.35	-0.28	1	0.95*	0.53*	0.62*	-0.54*	-0.50*
Masseter muscle PTT	-0.35	-0.35	0.95*	1	0.49*	0.61*	-0.60*	-0.59*
Temporal muscle PPT	-0.20	-0.22	0.53*	0.49*	1	0.91*	-0.42*	-0.48*
Temporal muscle PTT	-0.32	-0.28	0.62*	0.61*	0.91*	1	-0.38*	-0.49*
VAS	0.12	0.34	-0.54*	-0.60*	-0.42*	-0.38*	1	0.71*
CPI	0.18	0.50*	-0.50*	-0.59*	-0.48*	-0.49*	0.71*	1

PPT – pressure pain threshold; PTT – pain tolerance threshold; CPI – characteristic pain intensity; VAS – Visual Analogue Scale

The possibility of comparing the results of MPDS studies is additionally complicated due to insufficient standardization, inadequate controllability, application of different clinical and diagnostic criteria, incomplete observation, and diverse interpretation of diagnostic results.

It is clear that comprehensive and completely objective assessment and measurement of pain, as a multidimensional and multifactorial subjective phenomenon, does not exist. That is precisely the reason why the self-assessment of pain intensity is, *inter alia*, a foundation for pain management. The subjects with MFP usually and most often experience pain, i.e. sensitivity, of the masseter and/or temporal muscles. These two muscles are most frequently used in the studies measuring the orofacial muscle pain threshold. The highest number of neuromuscular filaments, conductivity, and physiological and anatomic domination make these two muscles completely representative for testing and diagnosing these disorders [1, 16]. Some authors obtained the values according to which they distinguished the anterior bundle of *m. temporalis* as the most certain and representative for the MFP measurement. According to them, there was a linearly proportional correlation between the applied pressure on trigger points and the caused pain. With healthy muscles, this correlation was not linearly proportional, which we also noticed in our study [17].

The MFP patients exhibit greater muscle sensitivity than the healthy subjects in the control group [15]. We have emphasized that a sensitivity to various types of pressure (manual and algometric) in the region of masseter muscles is one of the most significant features of the MFP and is applied as a criterion to distinguish it from other forms of painful conditions in the head and neck region [18, 19, 20]. The relation of trigger points and referent zones where the pain occurs is constant and significant for their detection and diagnostics [21].

Frequently, the feeling of pain is increased during palpation, as well as during masticatory function. Typically, the MPDS is a localized, unilateral, painful syndrome, in which bilateral symptoms occur only when combined with generalized disorders, such as fibromyalgia [22]. The values of pain intensity on the VAS vary during the day, usually 3–5 cm, to as much as 10 cm [1]. According to pain quality, it is usually deep, penetrating pain that varies from sensitivity to severe, devastating pain [23]. In our research, the pain had lasted two years on average, it was unilateral, mostly periodical, and its intensity measured with the VAS was averagely 7.24 cm. It is of great importance to conduct different, manifold measurements of muscle pain. Even though the VAS is the most frequently used instrument for the assessment of pain quantity, in our research, as well as in many others, it was applied in combination with the examination of muscle sensitivity to palpation, and algometry [9, 24].

The algometer was applied in the examination of painful sensitivity and a multitude of studies present it as a reliable instrument in the assessment of the MFP intensity [11, 15]. It is easy to use and the validity and reproducibility of algometric measurements for clinical practice are evaluated through different parameters as good to excellent [25]. The application of a modern digital algometer of this performance ensured additional precision in measurement in comparison to other types of manual algometers. In respect to the differences in the pain threshold between the MFP patients and healthy persons, many studies have shown that the pain threshold is essentially lower in MFP patients than in healthy subjects in the control group [7]. Accordingly, our research also discovered significant differences in the PPT and MPT values in all measurements (on individual points, combined values for each muscle individually on both sides and as a whole) between the experimental and the control group of subjects with 95% confidence.

A frequent approach in the relevant literature concerning MFP diagnostics was application of the quantitative algometric pain measurement, with comparative VAS measurements [8, 17]. The algometry is warmly recommended as an examination method in different scientific studies. It is easy, simple to apply, and reliable in long-term studies [26]. A properly calibrated algometer, in combination with other instruments for pain assessment, is an absolutely appropriate and necessary choice in the so-called auxiliary diagnostics of MFP. Based on our research, we concluded that there is a connection between the VAS and algometry, and that they are more objective and precise methods than the manual palpation. Algometry was in the statistically significant, negative correlation with the VAS values in a high ranking.

In this respect, future research should be aimed at completing and developing uniform, generally accepted diagnostic protocols for these orofacial region disturbances.

Conflict of interest: None declared.

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Применљивост инструмената за мерење интензитета бола код особа са мастикаторним миофасцијалним болом

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САЖЕТАК

Увод/Циљ Најчешћа клиничка презентација миофасцијалног бола (МФБ) укључује присуство дубоке мишићне боли, ограничен ниво покрета, хетеротопни бол из *trigger* тачака у референтне зоне и губитак водећих симптома њиховим анестезирањем. Дијагностичке могућности квантификације и карактеризације хроничног МФБ често су тешко изводљиве. Једини начин да се бол, као мултидимензионално искуство, објективно и свеобухватно процени је примена вишеструке методологије у његовој дијагностици.
Циљ овога рада био је корелирати дијагностичке могућности различитих квантификационих инструмената за процену интензитета бола код особа са мастикаторним МФБ.

Методе Студија је обухватала 60 испитаника подељених у две групе стратификоване према полу и старости. Примењен је дијагностички протокол *RDC/TMD*, а коришћени инструменти за мерење интензитета бола били су: нумеричка скала бола, дигитална палпација, градуирана скала хроничног бола, визуелна аналогна скала (ВАС) и алгометрија.

Резултати Вредности индекса срчане снаге су статистички значајне, у негативној су корелацији са алгометријским мерењима (од -0,48 до -0,59) и позитивној, статистички значајној корелацији са вредностима ВАС (0,71).

Закључак Резултати овог истраживања наводе нас на закључак да између примењених инструмената за мерење интензитета бола код особа са мастикаторним МФБ постоји међузависност и да су ВАС и алгометрија објективније и прецизније методе мерења интензитета бола него мануелна палпација.

Кључне речи: миофасцијални бол; дијагностички инструменти; ВАС; алгометар; мануелна палпација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of injectable platelet-rich fibrin use in the initial treatment of chronic periodontitis

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SUMMARY

Introduction/Objective The objective of the study was to investigate whether there are differences in therapeutic effect between initial treatments of chronic periodontitis [scaling and root planning (SRP)] alone and SRP in conjunction with injectable platelet-rich fibrin (I-PRF) application, comparing clinical parameters after three months.

Methods Twenty-four patients with chronic periodontitis who had at least two sites with probing pocket depth (PPD) ≥ 5 mm on contralateral side participated in the study. Using a split-mouth design, the patients were treated with SRP + I-PRF (study group) or SRP only (control group). The clinical parameters, clinical attachment level (CAL), gingival margin level (GML), PPD, bleeding on probing, and plaque index, were recorded on both sides.

Results Compared to baseline, both treatment modalities demonstrated an improvement in investigated clinical parameters. The mean value of CAL was reduced from 1.97 ± 0.75 (0.25–3.31) to 1.07 ± 0.44 (0.12–1.78) in the study group, whereas it decreased from 1.81 ± 0.66 (0.42–2.96) to 1.48 ± 0.55 (0.22–2.30) in the control group. Similarly, the corresponding values for GML and PPD showed statistically significant difference between the groups ($p = 0.040$ and $p = 0.006$, respectively).

Conclusion Regardless the limited number of patients in the study, initial periodontal therapy in conjunction with injectable platelet-rich fibrin proved to display significant improvement in all clinical parameters compared to initial periodontal therapy alone.

Keywords: chronic periodontitis; injectable platelet-rich fibrin; initial treatment

INTRODUCTION

Periodontitis is a chronic multifactorial disease, characterized by the progressive destruction of periodontal supporting tissues. Periodontitis presents an inflammation developed by disorders of the host immune response to the infections caused by periodontopathogens [1]. Chronic periodontitis (CP) represents a form of destructive periodontal disease that is generally characterized by slow progression [2]. The World Workshop on the Classification of Periodontal and Peri-implant Disease and Condition in 2017 agreed that the disease previously described as "chronic" or "aggressive" would be grouped under a category "periodontitis" [3]. Periodontitis was regarded as the sixth most prevalent disease globally in 2010 and it affected approximately 50% of the adult population worldwide in 2014 [4]. Due to its high prevalence it is essential to constantly upgrade periodontal therapy.

The principal goal of the periodontal therapy is to restrain active inflammation during the disease and possibly provide support for the reconstruction of periodontal tissue defects [5]. Initial periodontal therapy, scaling and root planning (SRP) is not frequently resolute at repairing disease-related defects [6, 7]. The periodontal wound healing after SRP usually induces the development of a long junctional epithelium, which is responsible for frequent recurrence of a periodontal pocket [8]. To enhance the process of regeneration, the adjunctive therapeutic procedures have been added to the conventional therapy since the end of the last century.

Platelets have been applied in dentistry over the past three decades. These autologous regenerative tools are concentrated suspensions of supra-physiological amount of growth factors (GFs) and, when applied locally, can induce soft and hard tissue regeneration [8]. Platelets are important reservoirs of various GFs and cytokines, which are vital in wound repair and homeostasis [8]. The periodontal wound healing process implies a series of cell-to-cell interactions and molecular signals that are primarily mediated by cytokines and GFs. GFs control enhancing collagen production, cell proliferation and differentiation, as well as blood vessel formation [9].

Platelet concentrates have advanced from the first generation, platelet-rich plasma (PRP) to the second generation, platelet-rich fibrin (PRF). PRF, developed by Choukroun et al. [10], enables a scaffold enriched with platelets

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and GFs, as well as leukocytes. The concentrate is generated from a blood harvest without any artificial biochemical modifications and anticoagulants [10]. Previous research has demonstrated that PRF contains a greater amount of GFs than PRP. It induces higher fibroblast migration and expression of the transforming growth factor-β1, the platelet-derived growth factor, and the vascular endothelial growth factor [11]. Along with these factors, there is a higher concentration of the fibroblast growth factor, the insulin-like growth factor-1, the epidermal growth factor, and the platelet-derived epidermal growth factor. Thus, they ensure a better environment for regeneration and repair of the defects. Currently, PRF is widely utilized in the surgical treatment of periodontal intrabony defects, treatment of furcation defects, sinus lift procedures, and tissue engineering [12].

Since the standard PRF is not entirely appropriate for injection, a new injectable formulation of PRF (termed I-PRF) enables easier use of the platelet concentrate in a liquid state. After being generated during centrifugation, it maintains its liquid viscosity for about 15 minutes. [13, 14]. Initially, the PRF has been developed at high centrifugation speeds, enabling a formation of a fibrin clot, which could be utilized as a three-dimensional scaffold for the promotion of periodontal regeneration [15].

Generally, the assessment of periodontal therapy consists of a full-mouth periodontal examination, which enables estimation of the degree of tissue inflammation and destruction. This is conducted by objective measuring of clinical attachment level (CAL), gingival margin level (GML), probing pocket depth (PPD), bleeding on probing (BOP), plaque index (PI), and radiographs assessing the alveolar bone level [16].

So far, patients with CP have not been treated with I-PRF during SRP treatment. Therefore, the aim of this study was to determine the effects of local I-PRF application in conjunction with SRP, compared to application of SRP alone, on periodontal clinical parameters of CP.

METHODS

The randomized, split-mouth, controlled clinical trial recruited patients with CP from the Department of Periodontology, School of Dental Medicine, University of Belgrade. The trial evaluated clinical periodontal outcomes after the initial treatment with or without conjunction of I-PRF. This trial had been approved by the Ethics Committee of the Department of Periodontology, School of Dental Medicine, University of Belgrade. After being informed of the research methods, all the patients submitted their written consent for sharing their personal data and their participation in the study. The study was registered at ClinicalTrials.gov as NCT02898675 on September 12, 2016.

For three months, 30 adult patients were included in the study. The preconditions for participating in the study were a presence of minimum 3 mm CAL and horizontal bone loss of both quadrants of the mandible or maxilla, which were confirmed by full-mouth radiograph images.

The following criteria were used in the patient selection:

- Inclusion criteria: age of 20–75 years; a minimum of six teeth per quadrant; a minimum of two teeth in each quadrant with a probing depth ≥5 mm; BOP had to be at ≥ 40% tooth sites; no involvement of furcation; good general health;
- Exclusion criteria: periodontal therapy within the last 12 months; having surgical therapy; use of antibiotics over the last six months; ongoing drug therapy that might have an impact on the clinical signs and symptoms of periodontitis; pregnancy or nursing; current and former smokers.

Clinical charting

Clinical charting was performed immediately before the first treatment. The following examinations were carried out after three months. The research included the examination of all teeth and tooth sites, except the third molars and the tooth sites associated with furcation involvements of degree II and III [17]. The following variables were recorded from the mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual, and mesio-lingual surfaces of each tooth: CAL, GML, PPD, BOP, and PI.

The examiner, a specialist of periodontology, performed and noted all the examinations. Prior to the start of the study, the examiner gained the adequate level of competence and reproducibility skills in accordance with the various clinical parameters and indices that were going to be utilized [18].

Treatment procedures

In regard to screening examination, the patients were thoroughly informed on self-performed plaque control activities consisting of using the modified Bass brushing technique, a soft toothbrush, regular toothpaste twice a day, and inter-dental cleaning with inter-dental brushes once a day. A full-mouth SRP was conducted in all diseased sites by using local anesthesia, in one or two sessions, during the period of 24 hours. The standard of oral hygiene was checked at the baseline examination and during the recall visit after three months following the baseline treatment, and further instructions were provided when it was necessary. Three months following the completion of the baseline treatment, all the patients were recalled for professional supragingival plaque control and reinforcement of oral hygiene. Additionally, re-instrumentation was conducted by using the ultrasonic device in all the sites with a remaining PPD of ≥ 5 mm.

Preparation of I-PRF

Blood samples were taken into two 10 ml tubes and prepared for I-PRF preparation. The blood without anticoagulant was then centrifuged at 700 rpm for three minutes (60 g) at room temperature by a Duo Centrifuge (Process for PRF, Nice, France). The upper liquid layer was taken as I-PRF by using a syringe. Afterwards, by applying I-PRF into

periodontal pockets through perforations at the point of interdental space on individually formed occlusal splints, it was enabled to hold it there for a longer time. The I-PRF was applied in one quadrant (study group) of the chosen jaw (mandible or maxilla), whereas the physiological saline was inserted in the opposite side (control group). The splint was removed after 15 minutes. Treatment allocation was decided by a toss of a coin.

Statistical analysis

Mean values and standard deviation were calculated. The Mann–Whitney U-test was performed to determine whether the two groups had similar clinical measurements at baseline and whether one treatment produced better clinical results after a three-month follow-up. The Wilcoxon signed-rank test was used to analyze whether clinical measurements differed before and after treatment. For the whole statistical analysis, a significance level of 5% was used. Software package PASW Statistics Version 18.0 (SPSS Inc., Chicago, IL, USA) was used for all calculations.

RESULTS

All the patients' tooth sites did not display any clinical signs of deterioration after a three-month period. It proved to be uneventful healing, without any pain or any other discomfort in either of the treatment modalities. The only discomfort was experienced by three patients, due to repeated blood collection after failing to find an appropriate blood vessel. During the therapy, one patient no longer participated in the study since she got pregnant, and another one left the country. The remaining 24 subjects, i.e. 10 men and 14 women, finished the treatment protocol. The mean age was 37.29 ± 10.23 years, ranging 22–64 years.

At baseline, none of the assessed clinical parameters showed a statistically significant difference between the study and control groups (Table 1).

Throughout the study, a significant gain in CAL, GML, BOP, PI, and a significant reduction in PPD took place in the study group (Table 2), as well as in the control group (Table 3).

Three months after the therapy (Table 4), the mean value of CAL decreased from 1.97 ± 0.75 (0.25–3.31) to 1.07 ± 0.44 (0.12–1.78) in the study group, whereas it decreased from 1.81 ± 0.66 (0.42–2.96) to 1.48 ± 0.55 (0.22–2.3) in the control group. Similarly, the corresponding values for GML and PPD showed statistically significant difference between the groups (p = 0.040 and p = 0.006, respectively). The major difference was recorded with BOP – at the baseline examination, 57% of the surfaces in the study group and 61% of the surfaces in the control group showed BOP. After a three-month period, a marked improvement in the bleeding scores took place in both groups, so that 15% of the PDT group and 33% of the SRP group had positive scores (p = 0.00). Initially, PI was 0.61 ± 0.517 and 0.64 ± 0.19, respectively. After three months, plaque values were markedly reduced, and no statistically significant differences were recorded between plaque scores of surfaces treated by both therapy modalities (p = 0.012).

Table 1. The mean values of clinical parameters of both groups at baseline

Index	Study group X ± SD (min–max)	Control group X ± SD (min–max)	[a]p
CAL	1.97 ± 0.75 (0.25–3.31)	1.81 ± 0.66 (0.42–2.96)	0.404
GML	1.72 ± 0.6 (0.02–2.5)	1.86 ± 0.56 (0.75–2.54)	0.457
PPD	3.68 ± 0.72 (1.63–4.53)	3.68 ± 0.89 (1.67–4.96)	0.975
BOP	0.57 ± 0.21 (0.19–0.96)	0.61 ± 0.17 (0.31–0.94)	0.433
PI	0.61 ± 0.517 (0.29–0.92)	0.64 ± 0.19 (0.31–0.91)	0.413

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;
[a]Mann–Whitney test

Table 2. The mean values of clinical parameters of study group at baseline and after three months

Index	Baseline X ± SD (min-max)	After 3 months X ± SD (min-max)	[a]p
CAL	1.97 ± 0.75 (0.25–3.31)	1.07 ± 0.44 (0.12–1.78)	0.000*
GML	1.72 ± 0.6 (0.02–2.5)	0.62 ± 0.49 (-0.72–1.3)	0.000*
PPD	3.68 ± 0.72 (1.63–4.53)	1.73 ± 0.64 (1.03–2.98)	0.000*
BOP	0.57 ± 0.21 (0.19–0.96)	0.15 ± 0.18 (0–0.9)	0.000*
PI	0.61 ± 0.517 (0.29–0.92)	0.19 ± 0.23 (0–1.15)	0.000*

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;
[a]ANOVA;
*statistically significant

Table 3. The mean values of clinical parameters of both groups after three months

Index	Study group X ± SD (min–max)	Control group X ± SD (min–max)	[a]p
CAL	1.07 ± 0.44 (0.12–1.78)	1.48 ± 0.55 (0.22–2.3)	0.003*
GML	0.62 ± 0.49 (-0.72–1.3)	0.99 ± 0.57 (0.12–2.1)	0.040*
PPD	1.73 ± 0.64 (1.03–2.98)	2.31 ± 0.73 (1.22–3.58)	0.006*
BOP	0.15 ± 0.18 (0–0.9)	0.33 ± 0.12 (0–0.58)	0.000*
PI	0.19 ± 0.23 (0–1.15)	0.2 ± 0.89 (0.12–0.5)	0.112

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;
[a]Mann–Whitney test

Table 4. The mean values of clinical parameters of control group at baseline and after three months

Index	Baseline X ± SD (min–max)	After 3 months X ± SD (min–max)	[a]p
CAL	1.81 ± 0.66 (0.42–2.96)	1.48 ± 0.55 (0.22–2.3)	0.000*
GML	1.86 ± 0.56 (0.75–2.54)	0.99 ± 0.57 (0.12–2.1)	0.000*
PPD	3.68 ± 0.89 (1.67–4.96)	2.31 ± 0.73 (1.22–3.58)	0.000*
BOP	0.61 ± 0.17 (0.31–0.94)	0.33 ± 0.12 (0–0.58)	0.000*
PI	0.64 ± 0.19 (0.31–0.91)	0.20 ± 0.89 (0.12–0.5)	0.000*

CAL – clinical attachment level; GML – gingival margin level; PPD – probing pocket depth; BOP – bleeding on probing; PI – plaque index;
[a]ANOVA;
*statistically significant

DISCUSSION

Obviously, the initial treatment of CP aims to achieve the results that can ensure a long-term improvement in clinically measured parameters. This randomized clinical trial with a split-mouth design displayed the difference between the effects of SRP in conjunction with I-PRF *vs*. SRP alone

in terms of changing clinical periodontal outcomes during the initial treatment of CP.

The obtained results demonstrated that both therapeutic modalities could result in statistically significant improvement of all explored clinical parameters three months after initiating the therapy. At baseline, no significant differences in terms of PPD and CAL were recorded between the two groups. The positive clinical outcomes of the control group after three months correspond with the previous findings concerning clinical efficacy of SRP in treatment of CP. This indicates that in subjects with CP, SRP was successful in reducing PPD and improving CAL [19]. All the patients were trained to maintain oral hygiene regularly. This might have improved the clinical parameters in both groups throughout the study period.

Over the years, the conventional therapy of periodontitis (SRP) has been enhanced by using various adjunctive therapies, mostly by systemically or locally administered antibiotics and antiseptics [20]. Since their use involves some risk, they should be prescribed only for specific situations under optimal conditions. Although the influence of nonsurgical use of lasers on the initial treatment of CP has been considered recently, some studies have shown that its impact on PPD and CAL reduction is less effective than that of antibiotics [20].

Our research is currently focused on novel adjunctive regenerative methods of CP treatment. Although a liquid, injectable form of this platelet concentrate was discovered in 2006 by Choukroun, only the PRF in the form of fibrin membrane was applied during the surgical therapy of CP. For the first time, in this study we tried to adequately use the injectable form of PRF (I-PRF) for a non-surgical treatment of CP. I-PRF is suitable for periodontal pocket application due to its advantage of being in a liquid form.

The injectable form of PRF preparation is based on a slower and shorter centrifugation spin. Moreover, this protocol of centrifugation leads to a higher presence of regenerative cells with higher concentration of GFs, and cytokines, which together may enhance the healing potential of both bone and soft tissues [21].

Clinical trials use CAL to examine various therapeutic modalities that could either reduce the progression of periodontal disease or enable the regeneration of supporting structures. In our study, the progress was made in reducing CAL in the test group more than in the control group three months after the initial treatment ($p < 0.05$). The reduction matches the previous systematic reviews on SRP with different adjuncts, showing that a three-month therapy leads to the CAL value ranging 0.08–1 mm [22, 23]. Our results demonstrated CAL gain by es much as 0.9 mm, representing better outcome compared to the control group with only 0.33 mm reduction. CAL gain during SRP with I-PRF was far higher when compared to SRP alone.

The greater clinical value of CAL gain may be due to more rapid wound healing, less short-term gingival inflammation, and sustained reduction of periopathogenic bacteria [24]. A study by Dohan et al. [25] shows that I-PRF contains more GFs than PRF, which is six to seven times more loaded with GFs than PRP. In addition, those GFs are released steadily within 21 days [11]. The process is enabled due to the fact that after a short period of time, approximately 15 minutes, I-PRF is formed into a matrix scaffold [11]. The scaffold was proved to have a direct impact on the ability of human gingival fibroblasts to migrate, proliferate, release additional GFs and periodontal ligament cell growth, as well as to increase the differentiation of osteoblasts [26]. By preventing the down-growth of junctional epithelium to the root surfaces and suppressing its interference between the root and soft tissue, a new attachment on root surfaces can be formed.

Furthermore, antimicrobial and anti-inflammatory effects of PRF have also been described [27]. Dohan et al. [28] stated that PRF has immunological and antibacterial properties due to its leukocyte degranulation, and possess some cytokines that may induce angiogenesis and pro/anti-inflammatory reactions. The decrease of microorganism concentration in this area results in reducing inflammation. Reducing the inflammation level brings about the decrease of PPD, GML, and BOP values. The study of Van der Weijden and Timmerman [19] reported the mean PPD reductions ranging 1.29–2.16 mm during CP therapy with SRP alone. In our research, PPD in the study group was reduced by 1.95 mm after a three-month period, while the control group showed a significantly lower decrease ($p < 0.05$). At the same time, GML values in the both groups were reduced by 1.1 mm and 0.87 mm, respectively.

BOP was also reduced in both groups after three months. A successful treatment of CP implies a minimal number of sites with BOP (< 10%), with no probing depths ≤ 3 mm [29]. Our results displayed that BOP after SRP alone dropped to 33%, while it decreased to 15% after SRP + I-PRF. It is probably due to the presence of residual pockets deeper than 4 mm. BOP is proved to be a useful prognostic indicator in estimating periodontal tissue after a non-surgical therapy according to sensitivity and predictability calculations. This is further documented by the fact that presence of residual PPD ≥ 6mm in combination with BOP ≥ 30% was significantly associated with tooth loss [30].

Both groups in our study demonstrated reduced PI after three months and the improvement in oral hygiene.

CONCLUSION

Regardless of the limited number of patients, the results of the present study indicated that local application of I-PRF in conjunction with SRP, compared to SRP alone, had significant effect on periodontal clinical parameters in the treatment of CP.

Conflict of interest: None declared.

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Ефекти примене инјектабилног фибрина богатог тромбоцитима у иницијалној терапији хроничног пародонтитиса

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САЖЕТАК

Увод/Циљ Циљ овог истраживања био је да се утврди да ли постоји разлика у исходу иницијалне терапије хроничног пародонтитиса, на основу клиничких пародонталних параметара после три месеца, применом додатне апликације инјектабилног фибрина богатог тромбоцитима.

Методе У студију су укључена двадесет четири болесника са хроничним пародонтитисом који имају бар у две регије на контралатералним странама вилице дубину сондирања већу од 5 *mm*.

Употребом методе „подељених уста", болесници су третирани иницијалном терапијом хроничног пародонтитиса у комбинацији са инјектабилним фибрином богатим тромбоцитима (студијска група) или само иницијалном терапијом хроничног пародонтитиса (контролна група). Клинички параметри – ниво припојног епитела, ниво ивице гингиве, дубина сондирања, крварење на провокацију и индекс плака бележени су са обе стране.

Резултати У поређењу са почетним мерењима, оба терапијска облика су показала напредак у резултатима. Средња вредност нивоа припојног епитела се смањила са 1,97 ± 0,75 (0,25–3,31) на 1,07 ± 0,44 (0,12–1,78) у студијској групи, док је у контролној групи опала са 1,81 ± 0,66 (0,42–2,96) на 1,48 ± 0,55 (0,22–2,30). Слично томе, одговарајуће вредности нивоа ивице гингиве и дубине сондирања показале су статистички значајну разлику између група ($p = 0,040$ и $p = 0,0069$).

Закључак Без обзира на ограничени број болесника, иницијална терапија пародонтитиса у комбинацији са инјектабилним фибрином богатим тромбоцитима показује значајно побољшање клиничких параметара у односу на изоловану иницијалну терапију.

Кључне речи: хронични пародонтитис; инјектабилни фибрин богат тромбоцитима; иницијална терапија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Ruptures of trachea and bronchi diagnosed by virtual bronchoscopy with multidetector computed tomography and fiberoptic bronchoscopy – advantages and shortcomings of methods

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SUMMARY

Introduction/Objective Fiberoptic bronchoscopy often is too aggressive, which requires the use of other noninvasive diagnostic methods. The study presents research results on the diagnostic capabilities of virtual bronchoscopy with multidetector computed tomography and fiberoptic bronchoscopy in traumatic abnormalities of trachea and main bronchi.

Methods A total of 21 patients (six males and 15 females) at the ages of 11–82 years (50.65 ± 19.8) were studied by the methods of virtual bronchoscopy with multidetector computed tomography and fiberoptic bronchoscopy. The diagnostic capabilities of virtual bronchoscopy as compared to fiberoptic bronchoscopy were assessed by established criteria.

Results Ruptures of the trachea and/or bronchi were proven by fiberoptic bronchoscopy in 21 patients and by virtual bronchoscopy in 19 patients. The greatest frequency was reported for the post-intubation ruptures (15 patients, 71.42% with virtual bronchoscopy; 16 patients, 76.19% with fiberoptic bronchoscopy), followed by post-traumatic ruptures (three patients, 14.29%); ruptures of trachea and the left lower lobar bronchus as a result of an advanced neoplasm of the esophagus (one patient, 4.76%), diagnosed by both methods; mucosal erosion after instrumental manipulations (4.76%, after fiberoptic bronchoscopy).

Conclusion Achieved diagnostic accuracy in ruptures of trachea and bronchi by virtual bronchoscopy is 90.47% and by fiberoptic bronchoscopy it is 100%. In terms of localization, shape and size, almost complete correspondence of changes with those of fiberoptic bronchoscopy was found. The presence of abundant secretion in virtual bronchoscopy may be interpreted incorrectly and efficiency of virtual bronchoscopy decreases.

Keywords: diagnostic capabilities; traumatic abnormalities; trachea; bronchi

INTRODUCTION

Ruptures of trachea and bronchi are rare, difficult to diagnose, lack well-known clinical signs but are potentially life-threatening [1–10]. They affect more often female patients and patients aged 50 years or more [3, 4]. The outcome of the trauma is favorable if the diagnosis is established at an early stage and accompanied by rapid primary treatment because tracheal and bronchial ruptures are potentially rapidly lethal [9, 11]. At a later stage, the risk of tracheal stenosis, which is often insurmountable, increases [6]. This requires the use of new advanced diagnostic methods and adequately precise equipment. Some of the authors consider computed tomography (CT) to be an adequate means for assessing most of the abnormalities of the respiratory tract; however, multidetector computed tomography (MDCT) allows for multiplanar reformation, CT bronchoscopy, and virtual bronchoscopy (VB). VB as a noninvasive method allows for three-dimensional evaluation of the tracheobronchial tree. It is determined as a finer, more short-term method than fiberoptic bronchoscopy (FB) [12–15]. The diagnosis of ruptures of the trachea is often delayed or omitted, but it is still proven that the success rate associated with the improvement of care for patients has increased. The main causes pointed out for their appearance include blunt traumas, severe cough, vomiting or secondary iatrogenic injuries, post-tracheal intubations, etc. [4]. Research of diagnostic capabilities of VB with MDCT in tracheal ruptures is very scanty [6, 7, 9, 14, 15, 16].

The present study is aimed at assessing the advantages and shortcomings of VB with MDCT in diagnosing patients with ruptures of the trachea and bronchi.

METHODS

Clinical material and equipment

A total of 21 patients (six males and 15 females aged 11–82 years; 50.65 ± 19.80) were studied

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for ruptures of trachea and bronchi by the methods of VB with MDCT and FB for more than five years (2013–2020). The methods were carried out on 64 MDCT Siemens Definition AS (Siemens Healthineers, Erlangen, Germany) and on Olympus BF PE2 bronchoscope (Olympus Corporation, Tokyo, Japan), respectively. Syngo.via VB20 (Siemens Healthineers) workstation, with the capacity to track and match the images in the axial, coronary, sagittal planes, was used. Multiplanar reconstructions were performed by applying maximum intensity projection techniques and capabilities to archive and export images and video.

Optimized standard protocols were used in patients with various abnormalities of trachea and bronchi, and different ages. The best results were achieved with current strength 80–100 mAs, voltage 120 kV, 3 mm beam collimation, and reconstruction of 3 mm, rotation speed at 0.5 sec., pitch D-FOV large.

Protocols

Criteria for pathological changes in ruptures of trachea and bronchi used to compare the results of the diagnosis by FB and VB with MDCT include the following: localization of the rupture; disposition; shape of the rupture; length of the rupture (cm); distance of the rupture from the carina (cm); distance of the rupture from vocal ligaments (cm); length of changes resulting from the healing of the ruptures after medical treatment (cm); the number of affected tracheal rings.

Statistical analysis

The variation analysis of quantitative variables includes the following factors: for the medium-level – arithmetic mean; for dispersal – rank (value range from–to), standard deviation (SD). Non-parametric methods of analysis were applied by the Mann–Whitney test (U-test) for the comparison of two samples in unknown distributions (e.g. age, sex, various abnormalities of trachea and bronchi, etc.); Kruskal–Wallis test (H-test) for the comparison of more than two samples of different sizes. Two-way analysis of variance (Friedman's test, F-test) was used for determining the factor for variation of compared criteria. The Pearson (r) correlation coefficient was used to verify the association between the variables. The software products MS Excel (MS Office 2010; Microsoft Corporation, Redmond, WA, USA), BioDiversity Pro and Statistica 10 were used for the statistical processing of data [17, 18].

The study was done in accord with standards of the institutional committee on ethics.

RESULTS

Of 21 studied patients, 21 had proven ruptures of trachea and/or bronchi after FB, and 19 after VB. The greatest frequency was reported for the post-intubation ruptures (71.42% with VB – 15 patients, two males and 13 females; 76.19% with FB – 16 patients, two males and 14 females), followed by post-traumatic ruptures (14.29%) – three patients (two males and one female) and ruptures of trachea and the left lower lobar bronchus resulting from an advanced neoplasm of the esophagus (4.76% – one male), diagnosed by both methods; mucosal erosion after instrumental manipulations (4.76% – one male, after FB). No significant differences were found between the two methods in respect of frequency of occurrence of the separate categories of abnormalities ($p > 0.05$).

The results of VB reported 21 ruptures in 19 patients, and those of FB 23 ruptures in 21 patients. Ruptures with localization in the upper part of the trachea (16 patients with FB and 14 with VB) prevail, followed by those with localization in the middle part of the trachea (four patients each), and with ruptures localized in the upper and middle part of the trachea (one patient each). Two ruptures were recorded in one patient, one in the upper part and one in the lower part of the trachea, affecting the left lower lobar bronchus, reported by both methods. Nineteen patients diagnosed by FB and 17 patients diagnosed by VB had longitudinally located ruptures in the membranous part of the trachea. The shape of ruptures established by both methods was predominantly linear (17 patients diagnosed by FB and 16 patients diagnosed by VB), and in a small number of cases it was irregular (six and five patients diagnosed by FB and VB, respectively). Circular interruption of the trachea at the border between the cervical and mediastinal parts was found in one patient. Differences in the length of ruptures (in centimeters), established by both methods, were not significant (Table 1; $p > 0.05$). Similar results were obtained for the distances measured by both methods to *rima vocalis*, for the distances from the carina, for the size of changes after healing of ruptures following the medical treatment, and for the number of affected tracheal rings. Low-grade stenoses (stenoses of grade I) were found in 19% of the studied patients (one male – 5%, and three females – 14%). Negligible differences in the number of ruptures and the number of patients with true-positive results for ruptures are due to the total of two patients with false-negative results reported by VB against those reported by FB, decreasing the number of patients with ruptures and the reported ruptures by two each. In two patients (one male and one female), two ruptures were found by VB and FB each (Table 1).

Both methods reported a prolapse of mediastinal tissues in nine patients, as a result of changes occurred in them, reported during the study. In three of the patients we found a higher disposition of the distal linear surfaces corresponding to the granulations formed.

A total of four of the surveyed patients were operated on [three patients with closed thoracic trauma (posttraumatic) and one patient with iatrogenic post-intubation trauma of more than 3.5 cm, located on the membranous part of the trachea]. After the diagnosis confirmation with CT, FB, and VB, three of these patients (post-traumatic) were found to have large, extensive lesions and the presence of an intense pneumothorax, pneumomediastinum, leading to tracheal stenoses and the main bronchi, proven by CT, FB, and VB. One of these patients has established atelectasis on the right side. In three of the patients who were operated on,

Table 1. Criteria and factors for assessment of abnormalities in ruptures of the trachea and the bronchi

Criteria	Factors	Ruptures	
		Fiberoptic bronchoscopy	Virtual bronchoscopy
Localization (number of ruptures – 21/23, respectively)	the upper part of the trachea	14	16
	the middle part of the trachea	4	4
	the upper and middle part of the trachea	1	1
	the upper and lower part	2	2
Disposition (number of ruptures – 21/23)	along the length of the trachea in its membranous part	17	19
	in the area of cartilage rings in the transverse direction	3	3
	total circular interruption cervical/mediastinal part	1	1
Shape (number of ruptures – 21/23)	linear with smooth edges	16	17
	irregular with uneven edges	5	6
Length of rupture (number of ruptures – 21/23)	0.5–3 cm	14	16
	3–5 cm	5	5
	above 5 cm	2	2
	rank (average ± SD)	0.5–7.1 cm (2.82 ± 1.73)	0.5–7.1 cm (2.42 ± 1.68)
Distance *rima vocalis* – upper edge of rupture (cm) (number of ruptures – 21/23)	up to 3 cm	7	8
	3–5 cm	11	12
	above 5 cm	3	3
	rank (average ± SD)	0.1–13.7 (3.90 ± 2.81)	0.1–13.7 (3.71 ± 2.78)
Distance from carina (number of ruptures – 21/23)	up to 3 cm	2	2
	3–5 cm	7	7
	above 5 cm	12	14
	rank (average ± SD)	2.0–13 (6.38 ± 2.87)	2.0–13 (6.64 ± 2.94)
Length of changes after healing of ruptures (cm) (number of changes – 16/17)	up to 3 cm	12	13
	3–5 cm	3	3
	above 5 cm	1	1
	0 cm	6	7
	rank (average ± SD)	0.72–4 (1.45 ± 1.33)	0.72–4 (1.37 ± 1.31)
Number of affected tracheal rings (number of patients – 21/23)	1	11	13
	2	6	6
	3	3	3
	0	1	1
	rank (average ± SD)	1–3 (1.52 ± 0.81)	1–3 (1.47 ± 0.79)

intubation was performed under the lesion. This avoids the continuous movement of the ruptured part and achieves rapid recovery (healing of the lesions for four days). For the fourth patient, an operative suture was imposed. All other rupture patients were conservatively treated with dynamic clinical follow-up by a specialist. The investigated patients (including the patients who were operated on) have been followed-up clinically and radiographically (by CT, FB, and VB). All the patients with ruptures presented in the paper have been discharged from hospital clinically healthy.

DISCUSSION

The presented study evidence of ruptures of trachea and bronchi in patients diagnosed by VB and FB offers significantly higher results than those reported by other authors [19]. Traumatic abnormalities of the trachea and main bronchi are rarely met in medical practice (in about 0.8–2% of the cases), but they can still be serious life-threatening conditions. They can be treated successfully if diagnosed at the earliest possible stage. Tracheal and tracheobronchial ruptures represent a serious injury which is often neglected in the initial post-traumatic period. Ruptures create a risk of stenoses at a later stage, which is insurmountable [4, 6, 9, 10, 12, 16, 19–22].

FB has been established as a time-tested method ("golden standard") in the diagnostic practice, allowing for direct visualization in the lumen of the respiratory tract, detection, and diagnosis of pathological changes in the tracheobronchial tree [7, 23–26]. FB, however, as an invasive method, is inapplicable in patients in a serious condition, where it may lead to more serious complications and to aggravating the outcome of the treatment. In such cases, where FB is considered too aggressive (young children, elderly people with poor health status, etc.), it is obligatory to apply other safer and quick diagnostic methods, possessing the same or better efficiency [12, 14, 21, 22, 27].

VB as a relatively recent (the mid-1990s), non-invasive method based on the use of MDCT and the follow-up three-dimensional reconstruction of the respiratory tract allows for real visualization, high resolution of the tracheobronchial tree, assessment of the trachea and main bronchi wall integrity, as well as assessment of changes in their lumen, even in areas inaccessible to FB [1, 23, 27].

Studies on the diagnostic capabilities of VB in traumatic abnormalities of trachea and bronchi are scanty. There is almost no data on the advantages and shortcomings of VB and FB in ruptures of trachea and bronchi [12, 22, 23, 27].

VB is shown as a more practical, more short-term and more precise method than FB for the assessment of the trachea and the main bronchi, allowing for three-dimensional assessment of the tracheobronchial tree. VB is indicated as a better method than FB for diagnosing ruptures of the trachea in patients with pneumomediastinum [9].

As a result of the performed study, it is found that for all four groups of abnormalities, in 90.47% of ruptures of trachea and bronchi cases VB showed results utterly comparable to those of FB. Almost complete correspondence with FB was found in terms of localization, shape and size of the changes. The presence of abundant secretion may be interpreted incorrectly with VB. Age is not defining in

terms of the size of ruptures ($p > 0.05$). Significant differences between the length of the ruptures in both sexes (U-test, $p = 0.04$) were obtained. Significant differences were found between the length of the ruptures and the following: the distance to the carina (U-test, $p = 0.0$); the distance to the rima (U-test, $p = 0.04$); the length of changes after healing of the ruptures (U-test, $p = 0.01$); the number of affected tracheal rings (U-test, $p = 0.03$); the distances between the carina and rima (U-test, $p = 0.0004$). Significant differences were also found in terms of distances to the carina and the following: the length of the changes after healing of the ruptures (U-test, $p = 0.0$); the number of affected tracheal rings (U-test, $p = 0.0$). The differences were significant regarding the length of the rima and the length of the changes after healing of the ruptures (U-test, $p = 0.0$), as well as the number of affected tracheal rings (U-test, $p = 0.0003$). In general, significant differences were found between the length of ruptures and distances to the carina, the distances to the rima, the length of changes after healing, the number of affected tracheal rings (H-test, $p = 0.0$). The length of ruptures is a determining factor with impact on the values of distances to the carina, distances to the rima, the length of changes after healing of the ruptures and the number of affected tracheal rings (F-test, $p = 0.0$). Generally, a high negative correlation was reported between the length of the ruptures and the distances to the carina ($r = -0.74$), and high positive correlation was found between the length of changes after healing and the number of affected tracheal rings ($r = 0.84$). No significant differences were found between the results obtained by the application of either method (FB, VB; $p > 0.05$).

This was also reported by studies of other authors, according to which data obtained from FB and VB are comparable, but whereas the advantages of FB are the immediate symptoms of color, vascularity, and mobility, VB predominates in circumventing obstructions and in providing an excellent view, away from obstructive ruptures or stenotic segments, as well as in determining the optimal path for passing the instruments into ruptures outside the field of vision [1].

The results presented from the performed VB in ruptures of trachea and bronchi are of higher sensitivity and precision than those in studies of some of the other authors (68–89%) [23, 27]. This is most likely due to the optimization of standard operating protocols and the high-tech equipment used in recent years, with an individual approach to the technical specifications for each patient.

In recent years, a number of authors have applied the VB method to ruptures of the trachea and bronchi using optimized low-dose protocols that achieve good visualization of the bronchi of the sixth–seventh order [14, 22, 28, 29]. Thin-cut and ultra-high-definition studies allow lower and more final-order bronchi to be seen, with better resolution and low noise, but to perform the periodic follow-up of the patients with the possibility of attenuated treatment, the dose obtained is high and radiation-intensive [30].

The equipment and low-dose protocols used are in accordance with the goals and objectives of our study. With the applied optimized low-dose protocols, a good visualization of the bronchi of the sixth–seventh order is obtained. High comparability of the results with those of the performed FB is also achieved. This corresponds with the objectives of the study and is sufficient to determine the location of the ruptures, their shape, size, distance from the carina, and the treatment behavior – operative or non-operative attenuated treatment, with follow-up of patients.

According to data from the literature, confirmed also by the performed study, the diagnostic capabilities of FB and VB increase with the increase of the degree of obturation; however, VB has greater sensitivity than FB in more difficult cases of stenoses. VB is presented as a supplementary technique enhancing the capabilities for visualization with the improvement of the success rate of diagnosis and treatment in urgent conditions, especially in patients with life-threatening injuries.

This study gives reason to accept that VB is a successful method for diagnosing traumatic injuries of the trachea and the main bronchi. The success rate is closely related to the localization and size of ruptures and is higher in ruptures sized ≥ 0.5 cm (the least reported size in this study).

The results of performed studies give us the reason to summarize that VB will be more often relied on in establishing the therapeutic diagnostic algorithm in patients with abnormalities of the trachea and the bronchi. However, it should be remembered that the efficiency of the method decreases in the presence of abundant secretion in the lumen of airways. VB has also been applied as a non-invasive method for establishing the size of the changes in the course of the healing process. VB is essential for the screening of some chronic lung diseases, such as chronic obstructive pulmonary disease, pulmonary fibrosis, etc., found to accompany traumatic abnormalities of the trachea and the bronchi. Because of the accurate visualization of the tracheobronchial tree, the method can also be used for training. A limitation of the method is that it cannot determine changes in the mucosa in case of superficial injuries.

CONCLUSION

CT VB is a valuable method that complements the tracheal evaluation with axial cuts and multiplanar reconstructions. The non-invasive character of the method of VB allows for its application in life-threatening conditions and control of the healing process. VB provides similar visual information as FB but in a non-invasive way. Changes in bronchial mucosa may cause differences in the quantitative assessment of changes. The efficiency of VB decreases substantially in the presence of abundant secretion.

Conflict of interest: None declared.

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Руптуре трахеје и бронхија дијагностиковане виртуелном бронхоскопијом са мултидетекторском компјутеризованом томографијом и фиберoптичком бронхоскопијом – предности и недостаци метода

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САЖЕТАК

Увод/Циљ Фиберoптичка бронхоскопија често је превише агресивна, што захтева употребу других неинвазивних дијагностичких метода. У раду су приказани резултати истраживања о дијагностичким могућностима виртуелне бронхоскопије са мултидетекторском компјутеризованом томографијом и фиберoптичком бронхоскопијом у трауматским абнормалностима трахеје и главних бронхија.

Методе Укупно 21 болесник (шест мушкараца и 15 жена) у доби од 11 до 82 године (50,65 ± 19,8) проучаван је методама виртуелне бронхоскопије са мултидетекторском компјутеризованом томографијом и фиберoптичком бронхоскопијом. Дијагностичке способности виртуелне бронхоскопије у односу на фиберoптичку бронхоскопију процењене су утврђеним критеријумима.

Резултати Руптуре трахеје и/или бронхија доказане су фиберoптичком бронхоскопијом код 21 болесника и виртуелном бронхоскопијом код 19 болесника. Највећа учесталост забележена је након руптуре после интубације (15 болесника, 71,42% виртуелном бронхоскопијом; 16 болесника, 76,19% фиберoптичном бронхоскопијом), након чега следе посттрауматске руптуре (три болесника, 14,29%); руптуре трахеје и левог доњег лобарног бронха као последица узнапредовале неоплазме једњака (један болесник, 4,76%), дијагностификоване обема методама; ерозија слузокоже после инструменталних манипулација (један болесник, 4,76%, после фиберoптичке бронхоскопије).

Закључак Постигнута дијагностичка тачност код руптура трахеје и бронха виртуелном бронхоскопијом је 90,47%, а фиберoптичком бронхоскопијом је 100%. У смислу локализације, облика и величине, пронађена је готово потпуна подударност промена с променама фиберoптичке бронхоскопије. Присуство обилне количине секрета у виртуелној бронхоскопији може се погрешно интерпретирати и ефикасност виртуелне бронхоскопије се смањује.

Кључне речи: дијагностичке могућности; трауматске абнормалности; трахеје; бронхије

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

External validation of prostate health index-based nomogram for predicting prostate cancer at extended biopsy

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SUMMARY

Introduction/Objective Prostate Health Index (PHI)-based nomograms were created by Lughezzani et al. (2012) and Zhu et al. (2015) for predicting prostate cancer (PCa) at extended biopsy.
The aim of the study was to externally validate two nomograms in the Serbian population.

Methods This retrospective study comprised 71 patients irrespective of digital rectal examination (DRE) findings, with prostate-specific antigen level < 10 ng/ml, who had undergone prostate biopsies, and PHI testing. Data were collected in accordance with previous nomograms predictors. Independent predictors were identified by using logistic regression. The predictive accuracy was measured by the area under the receiver operating characteristic curve (AUC). The calibration belt was used to assess model calibration. The clinical utility was measured by using decision curve analysis (DCA).

Results There were numerous differences in underlying risk factors between validation dataset and previously available data. Analysis demonstrated that the DRE and PHI were independent predictors. AUCs for both nomograms, in patients with normal DRE had shown to have a good discriminatory ability (77.2–86.2%). In the entire population AUC of nomogram had exceptional discrimination (92.9%). Zhu et al. nomogram is associated with lower false positive predictions. The calibration belt for Zhu et al. nomogram was acceptable. Our DCA suggested that both nomograms are likely to be clinically useful.

Conclusion We performed external validation of two PHI-based nomograms predicting the presence of PCa in both the initial and the repeat biopsy setting. The PHI-based nomograms displayed adequate accuracy and justifies its use in Serbian patients.

Keywords: prostate cancer; prostate biopsy; external validation; nomogram; Prostate Health Index

INTRODUCTION

Prostate cancer (PCa) is the most prevalent cancer among male population in Europe and the sixth main cause of mortality due to cancer in men worldwide [1]. Contemporary guidelines recommend 10–12 core systematic transrectal-ultrasounds (TRUS)-guided prostate needle biopsy for early discovery of PCa [2]. Due to the lack of common risk factors specificity, and prostate biopsy treatment complications, several prediction tools were introduced to assist with the identification of those at highest risk of detecting PCa on prostate needle biopsy and avoid unnecessary biopsies.

Several nomograms have been developed to predict individual PCa outcomes that range from biopsy outcome prediction in men at risk of PCa, through prediction of increase in Gleason score grade between biopsy and radical prostatectomy pathology, to prediction of specific direction and location of extracapsular invasion at radical prostatectomy (RP) and mortality rate from hormone-refractory PCa [3]. The predictive accuracy (c-index) of the nomogram extended 73–76% in prediction of PCa detection. Furthermore, compared to extended biopsy schemes, earlier predictive nomograms (sextant biopsy) are less accurate in predicting the chance of PCa [4]. Discrepancies in disease risk factors may influence the performance of nomogram. Hence, they have to be approved before using in a specific geographic region and in contemporary patients. If a predictive tool is used for a population that differs from the one used for its development, it should be externally validated so that it can provide general and clinical appropriateness. In addition, nomograms should be reassessed regularly [5].

Recent studies have shown that Prostate Health Index (PHI), precursor prostate-specific antigen (PSA) isoform [−2]proPSA (p2PSA) derivative, may increase our capability to discriminate patients with and without PCa independently or in models [6–9]. Recently developed PHI-based nomograms incorporated several traditional PCa factors, along with PHI [10, 11].

Based on these considerations, the aim of the study was to externally validate two published PHI-based nomograms for predicting individual risk for PCa at extended biopsy within a Serbian population and compare their c-index.

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METHODS

Patient population

We validated two published PHI-based nomograms using patients who had undertaken TRUS-guided prostate biopsies and p2PSA testing, between May and December 2017 at the Clinical Centre of Kragujevac in accordance with the standards of the institutional committee on ethics. Inclusion criteria were PSA level < 10 ng/ml and at least 10 core biopsies undergone. This retrospective study comprised 71 patients irrespective of digital rectal examination (DRE) findings. The study was permitted by the institutional review boards (01/17/2608). Patients with incomplete data, acute bacterial prostatitis, and patients who had undergone previous endoscopic surgery of the prostate were excluded as well as those being treated with dutasteride or finasteride. Patients with chronic kidney disease, hemophilia, or previous polytransfusion were also excluded, as these conditions may change the concentration of p2PSA. Data were collected regarding the candidate predictors in accordance with previous nomograms. The Zhu et al. [11] nomogram is based on three criteria: age, prostate volume (PV) and PHI; the Lughezzani et al. [10] nomogram was constructed using the following predictors: age, DRE, PV, biopsy history, and PHI.

At presentation, blood samples were drawn prior to biopsy or any prostate manipulation using regular methods, and were processed and frozen at -70°C within eight hours for future analysis. Samples were defrosted and analyzed for tPSA and [–2]proPSA simultaneously using UniCelDxI 600 Access Immunoassay System (Beckman Coulter, Brea, CA, USA). The equation (p2PSA/fPSA) × √PSA was used to calculate PHI.

DRE were done by a urologist on all patients. DRE was assigned as normal, or suspicious/positive. In order to gain ultrasound data and prostate biopsy, Aplio 300 ultrasound device with 5–10-MHz probe (Canon Medical Systems Corporation, former Toshiba, Otawara, Japan) was used. PVs were calculated by measuring the gland in three dimensions, and using the following formula: 0.52 [length (cm) × width (cm) × height (cm)]. TRUS-guided prostate biopsies were performed according to a standardized extended scheme. After obtaining a median of 12 core biopsies (10–12 cores), it was assessed by local pathologists.

Statistical analyses

Descriptive statistics was used for predictor variables. Univariate and multivariate logistic regression analyses with Backward-Wald stepwise were used in order to identify and quantify the independent predictors of PCa. The results were expressed in odds ratios (ORs) with 95% confidential interval (CI).

For patients with a normal DRE the probability of PCa was calculated according to Lughezzani et al. [10] and Zhu et al. [11] nomogram PHI-based nomogram and compared with their outcome and for the entire population with a suspected and not suspected DRE, only the Lughezzani et al. [10] nomogram was applied. We assigned the points of each attribute of the patient by drawing a vertical line from that variable to the points' scale, then, sum all the points, and draw a vertical line from the total points scale to obtain the probability of PCa. The c-index was measured by the area under the receiver operating characteristic curve (AUC). We calculated AUC analysis and the Brier score for each nomogram, and compared AUCs by the DeLong test. The Hosmer–Lemeshow (HL) goodness-of-fit statistics was used to assess model calibration and we plotted a calibration belt [12]. The calibration belt is a fitted polynomial logistic function curve between the logit transformation of the predicted likelihood and result with surrounding 80% and 95% CI [13]. We also compared the specificities of PHI-based nomograms at 90% sensitivities using a bootstrap based method [14]. By using decision curve analyses (DCA), clinical usefulness was assessed [15]. All analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) or STATA version 13.0 (STATA Corp., College Station, TX, USA). Statistical significance was set at $p < 0.05$.

RESULTS

In Table 1 we presented the features of the patients used for each PHI-based nomogram and our validation cohort.

Table 1. Descriptive characteristics of the study population used for previous prostate health index-based nomograms and our external validation cohort

Characteristics	Lughezzani et al. [10] nomogram	Zhu et al. [11] nomogram	Validation cohort
Study period	July 2010 – July 2011	April 2012 – August 2014	May–December 2017
Patients, n	729	347	71
DRE, suspicious n (%)	129 (17.7)	0 (0)	20 (28.2)
PCa, n (%)	280 (38.4)	52 (15)	23 (32.4)
Age, mean ± SD/median (range)	64.3 ± 7.8	64 (21)	64.3 ± 5.4
Total PSA, ng/ml median (range)	6.39 (0.5–19.9)	6.89 (3.09)	5.06 (2.03–9.85)
Prostate volume, ml median (range/IQR)	58 (9–230)	40 (23.4)	50 (18–128)
p2PSA, pg/ml, median (range/IQR)	16.4 (0.1–137)	13 (10)	14.3 (3.2–34.2)
PHI, median (range/IQR)	41.2 (6.5–192.8)	32.7 (19.9)	33.3 (14.2–135.4)
Previous biopsy, n (%)	244 (33.5)	0 (0)	10 (14.1)
Number of biopsies, n	≥ 12	≥ 10	≥ 10

DRE – digital rectal examination; PCa – prostate cancer; PHI – prostate health index; PSA – prostate-specific antigen; p2PSA – precursor PSA isoform; SD – standard deviation

Table 2. Logistic regression analyses of previous nomogram predictors for prostate cancer detection in our validation cohort

Characteristics	Univariate analysis OR (95% CI)	p	Multivariable analysis OR (95% CI)	p
Age	1.105 (1.001–1.220)	0.048		
DRE	16.125 (4.562–56.990)	0.000	7.859 (1.193–51.786)	0.008
tPSA	1.409 (1.084–1.832)	0.010		
Prostate volume	0.963 (0.934–0.994)	0.018		
Biopsy history	0.258 (0.065–1.027)	0.055		
p2PSA	1.132 (1.052–1.218)	0.001		
PHI	1.130 (1.068–1.195)	0.000	1.126 (1.052–1.206)	0.001

DRE – digital rectal examination; CI – confidential interval; OR – odds ratio; PHI – prostate health index; p2PSA – precursor PSA isoform; tPSA – total prostate-specific antigen

Table 3. Predictive accuracy of different nomograms

Predictive accuracy	Lughezzani et al. [10] nomogram	Zhu et al. [11] nomogram
DRE Unsuspicious		
AUC (95% CI)	86.2 (73.6–94.2)	77.2 (63.3–87.8)
HL test χ^2, p-value	11.62, 0.169	1.29, 0.257
Calibration belt, test statistic, p-value	5.91, 0.015	1.10, 0.294
Brier score	0.111	0.094
DRE Unsuspicious/suspicious		
AUC (95% CI)	92.9 (86.9–98.8)	
HL test χ^2, p value	7.39, 0.495	
Calibration belt, test statistic, p-value	9.27, 0.002	
Brier score	0.116	

AUC – area under the receiver operating characteristic curve; CI – confidential interval; DRE – digital rectal examination; HL – Hosmer–Lemeshow test

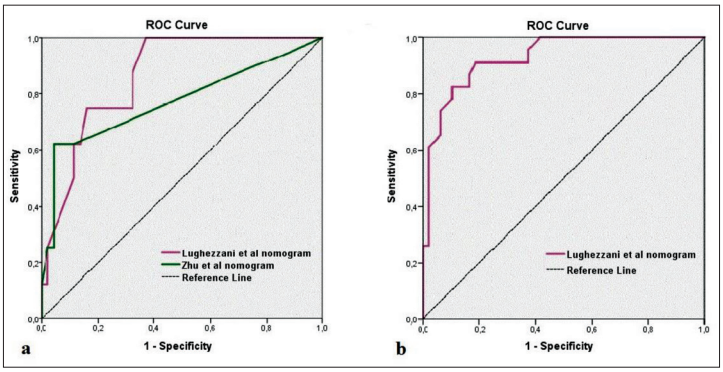

Figure 1. Receiver operating characteristic curve analyses of PHI-based nomograms in: a) patients with normal digital rectal examination; b) the entire validation cohort

Comparison between our validation dataset and the previously published data has shown numerous differences in underlying risk variables. The mean age was similar in all cohorts. Except disparity in study period, the proportion of men manifested with suspicious findings on DRE was also different (17.7% *vs.* 28.2%, p = 0.044), while Zhu et al. [11] included only patients with normal DRE. Chinese men had significantly smaller prostate glands (p < 0.001), the lowest p2PSA value, and the lowest detection rate. Similar to our validation cohort, Lughezzani et al. [10] included both initial and repeat biopsy, while Zhu et al. [11] nomogram was confined to initial biopsy. There was a notable difference between the original cohort and the validation cohort concerning repeated biopsies (p = 0.01). Our patients had significantly lower tPSA compared to Chinese men (p < 0.001). The highest median value of PHI was established in the European cohort.

The univariate logistic regression has shown that all of variables with the exception of biopsy history were significant predictors of PCa. However, only DRE and PHI sustained their prognostic significance during multivariable analyses (Table 2).

AUC for both nomograms, in patients with normal DRE showed to have a good discriminatory ability (77.2–86.2%) (Figure 1, Table 3), and in pairwise comparison of ROC curves the difference between areas of Zhu et al. [11] and Lughezzani et al. [10] nomogram (9%) was nonsignificant (p = 0.229). In the entire population, AUC of nomogram had exceptional discrimination (92.9%), and their c-index was not significantly lower (p = 0.312) comparing to patients with normal DRE. All HL tests had p-value higher than 0.05, indicating that there are no significant differences between the observed and expected outcomes and consequently all models suggest good overall calibration. The better (lower) value of Brier score was for nomogram by Zhu et al. [11].

We presented both nomograms calibration belt as related to the external validation dataset, in patients with normal DRE (Figures 2a and 2b), and in the entire population (Figure 2c). The predicted probability of the previously reported nomograms is represented on the x-axis, and the actual proportion of biopsy-proven PCa is represented on the y-axis. The calibration belt for Zhu et al. [11] nomogram was acceptable only, and showed deviations irrelevant from ideal calibration (Figure 2b). Conversely, for Lughezzani et al. [10] nomogram, the calibration curve calibrates poorly in all risk range, in the entire cohort (Figure 2c), and overestimated PCa in the first three risk deciles, in patients with normal DRE (Figure 2a).

In patients with normal DRE, at a 90% sensitivity, the specificity of the Zhu et al. [11] nomogram (88.4%) was significantly higher (p = 0.011) than the specificity of the Lughezzani et al. [10] nomogram (66.5%). This phenomenon indicates that Zhu et al. [11] nomogram is associated with lower false positive predictions.

In Figure 3, we presented the results of the DCA. All biopsy strategies suggest that if all patients are biopsied, all will avoid an unfavorable outcome. If the risk is higher than 8% and if patients agree to undergo further intervention, our DCA suggested that both nomograms have a chance to be suitable for that. However, Zhu et al. [11] nomogram (green line) lead to the higher net benefit compared with Lughezzani et al. [10] nomogram (purple line) in various threshold probabilities above approximately 18% (Figure 3a). However, their curves are partly overlapping. The reduction in the number of avoidable biopsies per 100

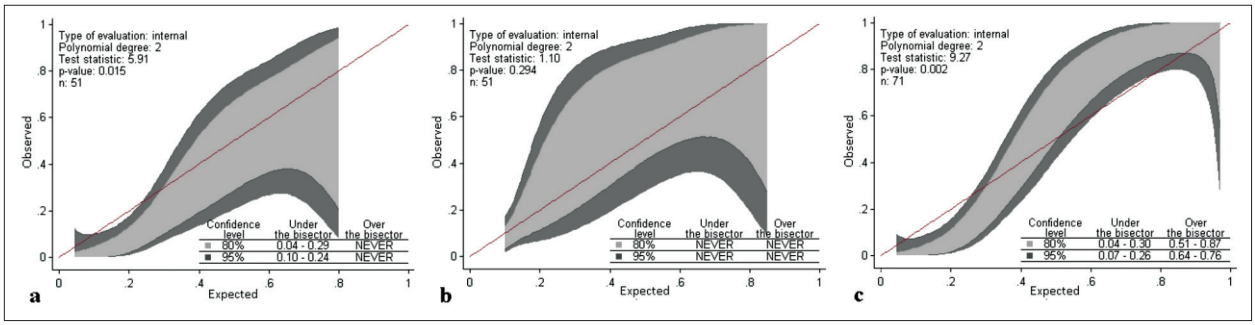

Figure 2. Calibration belt for the PHI-based nomogramsat two confidence level: a) Lughezzani et al. [10] nomogram in patients with normal digital rectal examination; b) Zhu et al. [11] nomogram in patients with normal digital rectal examination; c) Lughezzani et al. [10] nomogram in the entire validation cohort; the degree of the polynomial, the Wald statistics results and the number of patients are given in the upper-left quadrant; confidence intervals: 80% (light gray area) and 95% (dark gray boundaries)

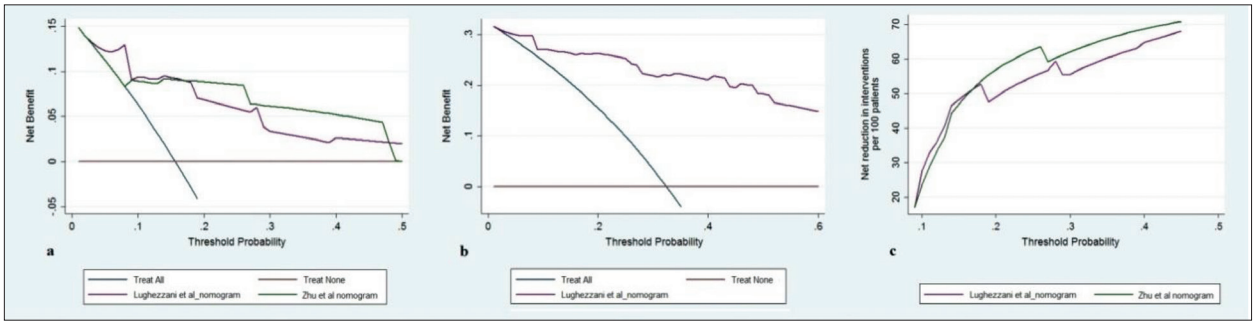

Figure 3. Decision curve analysis of the effect of PHI-based nomogram on the detection of prostate cancer: a) in patients with normal digital rectal examination; b) the entire population; c) net reduction in interventions per 100 patients is plotted against various threshold probabilities; net benefit is compared with 'Biopsied for all' strategy and 'Biopsied for none'

Table 4. Estimated specificity at fixed sensitivity of 90% for different nomograms and number of avoided biopsies versus the proportion of missed prostate cancer

Characteristics	Lughezzani et al. [10] nomogram			Zhu et al. [11] nomogram		
Sensitivity (90%)	Specificity (95% CI)[a]	Biopsy spread (%)	Missed (%)	Specificity (95% CI)[a]	Biopsy spread (%)	Missed (%)
DRE Unsuspicious	66.5 (49.3–85.8)	58	10	88.4 (76.7–95.4)	76	10
DRE Unsuspicious/suspicious	81.9 (54.2–97.9)	59	10			

DRE – digital rectal examination;
[a]BC_a bootstrap interval (1,000 iterations)

patients is net of false negatives, without a decrease in the number of patients with PCa who duly have PCa. In addition, in this case, Zhu et al. [11] nomogram (green line) outperformed Lughezzani et al. [10] nomogram (purple line) above approximately 18% (Figure 3c). For example, at a probability threshold of 20%, the use of the Lughezzoni et al. [10] and Zhu et al. [11] nomogram decreases the number of avoidable biopsies by about 45–55 per 100 patients, respectively, without missing any of PCa.

DISCUSSION

Various methods have been suggested to determine the likelihood of PCa, which may decrease the amount of avoidable prostate biopsies in the near future. We assessed the performance of an earlier developed PHI-based nomogram by studying three aspects of validity: discrimination, calibration, and clinical usefulness. In the present population, our external validation results validated a proper precision of the previously developed nomograms for predicting the likelihood of PCa in the initial and repeat biopsy setting. The superior diagnostic value of Zhu et al. [11] nomogram over Lughezzani et al. [10] nomogram was evidenced in patients with normal DRE. The clinical benefit of the PHI-based nomograms was additionally confirmed by DCA. These results suggest that previously developed nomograms may help clinicians and patients to make evidence-based choices for prostate biopsy based on patients' individual conditions.

Previous existing nomograms have established criteria associated with higher risk of PCa in the initial and repeat biopsy setting. They included age [4, 10, 11, 16–23], race [22], DRE [4, 10, 16–22], total PSA [4, 16–23], percent free PSA [4, 16, 18–21], PV [10, 11, 17, 20, 21, 22], PSAD [19, 23], hypoechoic lesions on ultrasound [19, 21], biopsy history [10, 23], family history [22], PHI [6, 7, 8, 10, 11], PHI density [9], PCa gene-3(PCA3) [22], and magnetic resonance imaging (MRI) [23]. Despite several variables having shown statistically significant prediction value in the

univariate analysis, only few sustained their independent value in the multivariate analysis. According to the analysis, encouraging prediction of PCa is possible based on DRE and PHI. Our findings were in accordance with earlier studies that PHI, as part of a multivariable approach, was the most accurate in predicting PCa at initial and repeat biopsy [6, 8].

Earlier developed predictive models or nomograms (sextant biopsy) are less precise in predicting the likelihood of PCa on initial biopsy [4]. Extended biopsy schemes changed the rate of PCa detection as well as the capability of typical risk factors, such as percent free PSA, to predict the likelihood of PCa on needle biopsy. Furthermore, concept of sampling density supported the idea to increase the number of core biopsies in order to improve the diagnostic yield [4].

The earlier developed PHI-based nomograms verified their capability to determine the presence of PCa at biopsy in their original cohort [10, 11]. Validation on diverse external data sets allows for assessment of the generalizability of the prediction tool to wider population than originally stated. Additionally, it is generally believed that external validation is more reliable than internal validation for prediction models, since it is insisted on transportability rather than reproducibility [24]. We are not the first researchers to carry out a validation between different PHI-based nomograms. When the nomogram applied to five external validation populations from European tertiary care centers, its yielded moderate predictive accuracies of 75.2% [5]. In our study, we found that the accuracy was better (77.2–92.9%) than the accuracy of many earlier ones (70–77%) which externally validated different nomograms [4, 16, 20, 22].

Calibration is one of the crucial features of every predictive model. Unfortunately, using the traditional approach of calibration (HL test, calibration plot), still shows several limitations. The traditional plot is not supplemented by any data on the statistical significance of deviations from the bisector [12]. On the other hand, the calibration belt is providing information on the direction, extent, and risk classes affected by divergences between the observed and predicted PCa [13]. In the analysis, only Zhu et al. [11] nomogram had acceptable calibration. This is probably due to varieties between populations. Except disparity in the study period, there were significant dissimilarities between the original and the validation cohort which include inclusion criteria (variety of PSA ranges, DRE findings), the incidence of PCa, proportion of men presenting with doubtful findings on DRE, PV, tPSA, p2PSA, PHI, and biopsy history. It indicates that certain patient characteristics are the difference in distribution between the validation sample and the development sample. It is questionable whether perfect calibration could be achieved in practice by any model. In addition, time variation may be a potential explanation why the previous models are not considered better than the recent ones. Although these differences most likely affect our calibration of PCa, they allow validity, and maybe generality, of a model to a more diverse and various populations. We also consider models originated from a specific country more convenient for local utilization [21].

In our DCA we confirmed clinical uselessness of these PHI-based nomograms. We also identified the range of threshold probabilities (< 10%) in which nomograms were of value. In patients with normal DRE, Zhu et al. [11] nomogram lead to the higher net benefit compared with Lughezzani et al. [10] nomogram in various threshold probabilities above approximately 18%. Furthermore, Zhu et al. [11] nomogram is associated with lower false positive predictions, when specificity is observed at fixed sensitivity. Superiority of Zhu et al. [11] nomogram could be partly explained by its derivation from men with normal DRE.

The most significant limitation of this study is small validation cohort from a single institution. The differences in population characteristics for both nomograms development and the validation cohort were the next difficulty. Furthermore, regardless of the use of a standardized comprehensive biopsy scheme, the PCa discovery rate may have been dissatisfactory in some of these patients. Lastly, diagnostic imaging is turning into an essential element of PCa diagnosis. Multiparametric MRI is helping clinicians with new information to better guide prostate biopsies [23]. However, we have shown that the nomogram remains highly predictive even in the different population and may be a significant tool to help clinicians in discriminating between patients with and without PCa. Nevertheless, when making decision about carrying out prostate biopsy we should consider multiple factors, including the patient's life expectancy, co-morbidity, and preference apart from risk of PCa. Secondary, it is also important to notice that clinicians could have lack of enthusiasm to use predictive tools. A United States survey has shown that only 35.5% of radiation oncologists and urologists currently use a decision aid in clinical practice [25]. We believe that a similar nomogram has not yet been developed or validated in the Serbian population.

CONCLUSION

In our study, we performed external validation of two PHI-based nomograms predicting the probability of PCa in both the initial and the repeat biopsy setting. The PHI-based nomogram displayed adequate accuracy and calibration properties. The satisfying performance of the nomograms in the validation cohort justifies its use in Serbian men.

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Екстерна валидација номограма заснованих на здравственом индексу простате у предвиђању карцинома простате при проширеној биопсији

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САЖЕТАК

Увод/Циљ Лугецани са сарадницима (2012) и Жу са сарадницима (2015) креирали су номограме засноване на здравственом индексу простате (*PHI*) у предвиђању карцинома простате при проширеној биопсији.

Циљ студије је да екстерно валидира ове номограме у српској популацији.

Методе Ова ретроспективна студија укључила је 71 болесника, независно од дигиторектalног налаза, са серумским нивоом антигена специфичног за простате мањим од 10 *ng/ml*, код којих је учињена биопсија простате и тестирање *PHI*. Прикупљани су подаци о претходно дефинисаним предикторима у номограмима. Коришћена је логистичка регресија за идентификацију независних предиктора. Предиктивна тачност процењена је пољем испод криве *ROC* (*AUC*). Калибрација номограма процењена је калибрационим појасом. Клиничка корисност је процењена анализом криве одлучивања.

Резултати Постојале су бројне разлике у предиспонирајућим факторима ризика наше валидационе базе података са претходно публикованим подацима из којих су изведени номограми. Анализа је показала да су дигиторектални налаз и *PHI* независни предиктори. Код болесника са нормалним дигиторектalним налазом *AUC* за оба номограма су показала добру дискриминациону способност (77,2–86,2%). У целој популацији *AUC* номограма је показао изузетну дискриминацију (92,9%). Номограм Жуа и сарадника је повезан са мање лажно позитивних предикција. Калибрациони појас за номограм Жуа и сарадника био је прихватив. Наша анализа криве одлучивања указује да оба номограма могу бити клинички корисна.

Закључак Спроведена је екстерна валидација два номограма заснована на *PHI* који предвиђају присуство карцинома простате при иницијалној или поновљеној биопсији. Номограми засновани на *PHI* показали су добру тачност и оправдавају употребу код болесника у Србији.

Кључне речи: карцином простате; биопсија простате; екстерна валидација; номограм; здравствени индекс простате

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Rational red blood cells administration – have we achieved a satisfactory level?

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SUMMARY

Introduction The important indicators of the quality of work in blood transfusion banks and health care facilities in general is the ratio of the cross-matched red blood cell (RBC) units, and the number of transfused RBC, known as cross-match to transfusion ratio (C:T).

The objective of this research was to provide an assessment of the quality of our work in a cross-sectional study, showing C:T ratios for certain areas of surgery or particular surgical indications.

Methods We analyzed the data related to the activities of the Department for Pre-Transfusion Testing and Blood Distribution at the Blood Transfusion Institute of Serbia during the September and November of 2017 period. In total, 341 patients were included in the study, for whom 1,067 RBC units were requested.

Results In pre-transfusion testing, 562 units were cross-matched and 249 units were transfused. The overall C:T ratio was 2.25. There are variations in C:T by departments. For the departments of abdominal surgery and reanimation, where uncrossmatched RBC units were requested, C:T was < 2. Other departments had C:T > 3 for almost all therapeutic areas.

Conclusion Our results show that the C:T ratio ranged 2.02–3.6, indicating the need to reevaluate the protocols based on which the blood is requested according to individual indications, to adequately prepare patients for surgery in order to reduce the risk of possible allogeneic transfusion, and to apply Patient Blood Management protocols, which include the use of alternatives to allogeneic blood transfusion.

Keywords: red blood cells administration; cross-match to transfusion ratio; Patient Blood Management

INTRODUCTION

Safe use of blood and blood components currently requires multidisciplinary collaboration among clinicians of different profiles such as surgeons, anesthesiologists, internists, and transfusion medicine specialist as the last instance that can affect the decision on administration of the particular blood component [1]. Although the use of transfusion remains an irreplaceable treatment modality for a large number of patients accompanied by a clear benefit through rapid correction of hemoglobin levels, and consequently of oxygenation, it is also associated with a range of risks of infectious and non-infectious nature [2]. Errors in transfusion medicine can be avoided in a large percentage and prevention is cost-effective, systematic, and applicable [3].

Hemoglobin binds 98% of oxygen; therefore, measurement of hemoglobin levels is to date the best and most commonly used test to estimate the necessity of RBC administration for the correction of anemia [4]. However, hemoglobin should not be the only parameter to be considered when deciding on potential RBC transfusion [5]. It should be noted that there are two approaches to the administration of RBC transfusion – a liberal one and a restrictive one. The liberal approach to transfusion is primarily based on hemoglobin levels, and it uses the hemoglobin level of 90 g/L as the threshold for RBC administration. In critically ill patients, as well as in bleeding patients, the restrictive approach uses hemoglobin threshold level below 70 g/L [6].

Managing the requirements for blood and blood components in relation to the needs of patients with Patient Blood Management (PBM) is an evidence-based multidisciplinary approach to treating patients with blood and blood components [7].

In order to establish a functional PBM system, close cooperation with doctors involved in the treatment of patients is particularly important, and the key moment is the training of health care staff. The aim of the above measures is to avoid any unnecessary transfusion [8]. Considering the experience of countries that have established the PBM system (Australia and New Zealand) and certain countries of the European Union, there is a clear benefit for both the patients and a country's health care system, which is reflected in the fact that PBM significantly affects the quality of the treatment of patients [9]. At this moment, there are no available data about PBM implementation in the surrounding states.

One of the important indicators of the quality of work of blood transfusion bank and health care facilities in general is the ratio of the number of requested RBC and the number of

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cross-matched RBC, known as C:T ratio (cross-match to transfusion ratio) [10]. This ratio should not exceed 2 [11]. Namely, the routine ordering of blood is usually carried out by the most junior clinical staff, who beside limited knowledge of the true nature or magnitude of the proposed surgery can cause at least three major problems. Firstly, in blood banks with a limited 'pool' of available blood, over-ordering actually leads to less blood being available for emergency transfusion. While in theory it is possible to recall blood that is out of circulation, this would inevitably lead to disruption of elective surgical lists. Secondly, if blood is cross-matched but not transfused, it is more likely to pass its expiry date and must be discarded. Third, cross-matching is costly. It would be easy to assume that the simple task of ordering the correct amount of blood for an elective surgical procedure is performed accurately in every hospital [12].

During 2012, according to the British Society of Hematology Guidelines, C:T ratio was listed as an important parameter for defining optimal administration of blood that implies the type of pre-transfusion analyses and the number of units for a particular type of surgical procedure [13].

The aim of our study was to provide an assessment of the quality of our work in a cross-section study by showing the C:T ratio for certain areas of surgery, i.e. particular surgical indications. Considering that PBM system has not been established in our country, this study represents one of the first of its kind in our country. However, we must note that the General Hospital in Pančevo has introduced its own protocols for the application of RBC, which has led to a significant reduction in blood consumption [14].

METHODS

In this retrospective study, the data related to the activities of the Department for Pre-Transfusion Testing and Distribution of Blood, Blood Components and Hemovigilance at the Blood Transfusion Institute of Serbia for the period of two months (September and November of 2017) were analyzed. During this period, 341 patients were monitored, for whom a total of 1,067 RBC units were requested.

The analyzed data refer to RBC administration at the Emergency Center of the Clinical Center of Serbia, including requests from the Department of General Surgery, Orthopedics, Neurosurgery and Reanimation. The data were collected based on requests for blood and blood components coming from those departments that were subsequently entered into the protocol, while one portion of the data was taken from the electronic database of the Department for pre-transfusion testing. The data collected included the departments where the patients were treated, the leading diagnosis at the time of blood request, requisition date, and the purpose of requisition (surgery or treatment). Patient data included first and last name, year of birth, hemoglobin level, blood type, number of requested RBC, number of cross-matched RBC units as well as the number of transfused RBC units. Since the data on hemoglobin levels were available in a small proportion of patients [only 29 (7.9%)], they were not taken into account during the statistical analysis performed to determine the C:T ratio.

The data collected were used to monitor the relationship between the number of requested and processed RBC units, depending on the therapeutic area, as well as to determine the C:T ratio. Institutional approval for the study was granted by the local research ethics committee in accordance with internationally accepted ethical standards.

Statistical analysis included methods of descriptive and analytical statistics using IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA). The significance of the difference for continuous variables with normal distribution was estimated using analysis of variance (ANOVA).

The value of $p < 0.001$ was considered to be statistically significant.

RESULTS

Table 1 shows an overview of the requested, cross-matched, and transfused RBC units per department. A total of 1,067 RBC units were requested for 341 patients. During pre-transfusion testing, 562 units were cross-matched and 249 were transfused. The overall C:T ratio was 2.25, which corresponds to the consumption of 44.36% of used RBC (Table 2).

Table 1. Number of issued *vs*. requested and processed red blood cells by departments

Emergency Center department	Number of patients	Requested	Cross-matched	Transfused
Surgery	223	654	255	126
Orthopedics	38	118	95	27
Neurosurgery	10	23	18	5
Reanimation	52	218	144	44
Reanimation without interaction	18	54	50	47
Total	341	1,067	562	249

Table 2. Cross-match to transfusion (C:T) ratio with regard to the departments

Emergency Center department	C:T
Surgery	2.02
Orthopedics	3.51
Neurosurgery	3.60
Reanimation	3.27
Reanimation without interaction	1.06
Total	2.25

ANOVA, $p < 0.001$

The largest number of RBC requests (654) were obtained from the Department of Surgery, where the highest number of patients (223) were treated. In this group, a corresponding C:T of 2.02 was obtained. The minimum number of RBC requests (23) was obtained from the

Table 3. Number of requisitions showing hemoglobin levels compared to the total number of patients and the purpose of blood requisition

Department	Number of requisitions with hemoglobin level n/N (%)	For treatment purposes	As part of surgical program	Hemoglobin level range
Surgery	23/223 (10.3)	19	4	53–91
Reanimation	0/52 (0)	0	0	0
Reanimation without interaction	0/18 (0)	0	0	0
Neurosurgery	0/10 (0)	0	0	0
Orthopedics	6/38 (15.79)	6	0	76–86
Total	29/341 (8.5)	25	4	53–91

Only two patients had hemoglobin level < 70

Table 4. Number of issued vs. requested and processed red blood cells with regard to the surgical procedure

Surgical procedure	Number of patients	Requested	Cross-matched	Transfused
Polytrauma	22	87	61	14
Subarachnoid hemorrhage, intracranial hemorrhage	7	25	10	2
Gastric ulcer, hernia, gallbladder and choledochal surgery, acute appendicitis, abdominal pain of unknown etiology, idiopathic jaundice	95	230	33	11
"Status post op"	34	103	33	8
Femoral fracture	18	63	53	17
Hip surgery	3	9	9	3
Total	179	517	199	55

Department of Neurosurgery, where the highest C:T was calculated to be 3.6 (Tables 1 and 2), $p < 0.001$; $p < 0.001$ is considered a statistically significant difference.

Table 3 shows the number of requisitions that listed the hemoglobin levels. The analysis showed that only 29/341 (8.5%) requisitions listed the hemoglobin level – 23/223 from the Surgery Department and 6/36 from the Orthopedics Department. For 25 patients, blood was requested to correct anemia, and for four patients as part of the surgical program (Table 3).

Tables 4 and 5 provide an overview of requested, cross-matched, and transfused RBC units by the most common surgical procedures. The highest number of RBC requests were obtained for abdominal surgery and for the treatment of hip surgery. In both types of surgery, C:T of 3.0 was recorded, while the highest C:T of 5.0 was obtained for cases of bleeding into the central nervous system that required surgical intervention – $p < 0.001$.

Table 5. Cross-match to transfusion (C:T) ratio calculated for departments with regard to the surgical procedure

Surgical procedure	C:T
Polytrauma	4.35
Subarachnoid hemorrhage, intracranial hemorrhage	50
Gastric ulcer, hernia, gallbladder and choledochal surgery, acute appendicitis, abdominal pain of unknown etiology, idiopathic jaundice	30
"Status post op"	4.12
Femoral fracture	3.11
Hip surgery	3.00
Total	3.61

ANOVA, $p < 0.001$

Although the International Classification of Diseases lists the diagnosis code "status post op," this term is quite broad, and it is used frequently at emergency center surgical departments as an indication for blood requisitions. During the examined period, there were 34 such requisitions. A total of 103 RBC units were requested, 33 were cross-matched, and eight were transfused; C:T ratio was 4.12.

DISCUSSION

This study has shown that there is a substantial variation in the estimated C:T values between the departments requesting the blood, and according to the type of surgical procedure.

The analysis of the pooled data for the studied time period related to the requisition and issuing of RBC blood components showed that the overall ratio of processed and transfused RBC resulted in C:T of 2.25 – very close to the recommended value of < 2. However, when analyzed structurally, there are differences in C:T between the departments. Thus, the department of abdominal surgery and reanimation had C:T < 2. The reanimation department also had C:T < 2 in cases where uncrossmatched RBC units were requested, but it should be noted that such circumstances mainly included massive transfusions accompanied by risk of a number of adverse reactions. By contrast, all other departments for almost all therapeutic areas had C:T > 3. This indicates a high degree of uneconomical blood administration and a subsequent risk of blood shortage for all patients in need due to irrational blood processing and consumption.

Among the first countries in the world that recognized the importance of PBM are Australia and New Zealand. By introducing PBM, these countries reduced the consumption of RBC units in patient treatment, subsequently reducing the cost of treatment and the transfusion risk, but also allowing more appropriate RBC distribution. In

accordance with their recommendations, the C:T ratio should not exceed 1.8. If it does, for patients for whom requisition was made, it is sufficient to determine the patient's blood type and antibody screening [7]. It should be noted that such a policy implies that a health care facility carrying out surgical procedures has its permanently available transfusion service.

In order to reduce the number of unnecessary cross-matched RBC units and to provide an adequate amount of RBC units, a recommendation was made in India to introduce the Maximum Surgical Blood Ordering Schedule (MSBOS) according to the C:T parameter. Research was conducted in a tertiary facility and it monitored patients planned for elective abdominal and neurosurgery. According to this recommendation, C:T should ideally be 1:1, but all values that are < 2.5 with the aim of lowering the index towards 2 are acceptable for the efficient RBC use [15]. In India, analyses were performed in orthopedic surgery, and C:T indexes were monitored, which indicated the benefits of lowering the C:T ratio to values < 2. Based on this, protocols were designed that suggest the optimal number of RBC components which should be prepared for various surgical procedures [16].

Given these circumstances, it is necessary to apply multidisciplinary approach to determine the criteria for blood administration according to therapeutic areas. Based on the data collected, therapeutic areas can be divided into two large groups: (1) those that need to be recorded and that require determination of blood type, and for which blood is almost never requested, and (2) those for which blood is requested and cross-matched, but is almost never used or is used very rarely, as well as those for which larger amount of blood loss is expected with certainty, which needs to be substituted by allogeneic transfusion. Currently, patients from the first group are tested for blood type according to the ABO and Rh system. For patients in the second group, blood type is determined without exception, as well as the indirect antiglobulin antibody screen [17].

Furthermore, it is necessary to clearly indicate on the requisition forms the diagnosis under which the blood is requested. The current principle which uses working diagnoses that are, although listed in the International Classification of Diseases, often unclear (e.g. "Status post op"), and very often without accompanying hemoglobin level, as shown by our research, does not provide enough data, seems confusing and often leads to wrong decisions on whether or not to prepare blood for such patients.

RBC transfusion in patients with anemia in whom compensatory mechanisms for adequate tissue oxygenation are reduced increases the capacity for oxygen transport [18]. A well-compensated anemia resulting from iron deficiency is the most common form of anemia and is not an indication for RBC administration itself, but as such requires the administration of iron via oral or intravenous route, with or without erythropoietin and with an assessment of the risk of adverse reactions [19]. Allogeneic transfusion has long been used to correct perioperative anemia. However, for the purpose of safety of blood transfusion itself, as well as due to limited resources and limited blood supply, modern transfusion tends to avoid this type of treatment for anemia [20]. It has been found that 30.4% (in some populations up to 75%) of patients had anemia of various grade in the preoperative period, that the risk of postoperative complications in these patients was 35% higher (most often infections), and that the 30-day risk for fatal outcome was increased by as much as 42% [21].

A restrictive transfusion strategy compared to a liberal strategy implies lower number of patients undergoing transfusion, as well as fewer RBC units used, while mortality, morbidity, and the number of myocardial infarction events remained unchanged. On the other hand, the liberal transfusion strategy did not show any benefit to patients [22].

Although our study has its limitations based on the facts that the study was retrospective and that the analysis covered a relatively short time interval, as well the obtained results were related to requested, cross-matched, and transfused RBC units but not using needed/not needed RBC, the estimated C:T ratios indicate the need to introduce and observe the procedures which would allow a more rational use of blood. Therefore, this study may be characterized as a pilot study, and results will be confirmed in a prospective study that will include an analysis of RBC administration over a longer period.

CONCLUSION

The results of our study examining the requested, cross-matched, and transfused RBC units indicate the need to introduce procedures that would allow rational use of blood. Considering the overall C:T ratio, it could be concluded that we performed close to the recommended value; however, data analysis by the department or by the type of surgical intervention shows that the C:T value varies 2.02–3.60, indicating that it is necessary to reevaluate protocols used for blood requisitions according to individual indications, to adequately prepare patients for surgery whenever possible to reduce the risk of possible use of allogeneic transfusion, and to establish PBM protocols that include the possibility of using alternatives to allogeneic blood transfusion.

Conflict of interest: None declared.

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Рационална примена еритроцита – да ли смо постигли задовољавајући ниво?

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САЖЕТАК

Увод Један од битних индикатора квалитета рада трансфузиолошке банке крви и здравствене установе у целини је однос броја обрађених јединица еритроцита којима је урађена интеракција и броја трансфундованих јединица еритроцита, однос *C : T*.

Циљ нашег истраживања је био да у студији пресека дамо процену квалитета нашег рада приказујући однос *C : T* за одређене хируршке гране, односно одређене индикације у хирургији.

Методе У ретроспективној студији извршена је анализа података која се односила на двомесечну активност Одељења за претрансфузиона испитивања и дистрибуцију крви, компонената крви и хемовигиланцу Института за трансфузију крви Србије (септембар и новембар) 2017. године. У наведеном периоду праћен је 341 болесник, за које је укупно требовано 1067 јединица еритроцита.

Резултати У претрансфузионом тестирању је обрађено 562, а издато 249 јединица еритроцита. Свеобухватни однос *C : T* је био 2,25, што одговара потрошњи од 44,36% искоришћених еритроцита. У односу на одељења постоје разлике у односу *C : T*. За одељења абдоминалне хирургије и реанимације, када је крв тражена без интеракције, утврђен је *C : T* < 2. Друга одељења су за готово све терапијске области имала *C : T* > 3.

Закључак Анализа података у односу на одељења или тип хируршке интервенције показује да вредност *C : T* варира од 2,02 до 3,6, што указује да је неопходно преиспитати протоколе по којима се крв требује према појединим индикацијама, адекватно припремити болеснике за операцију, како би се смањио ризик за евентуалну примену алогене трансфузије и применити протоколе управљања крвљу болесника, који подразумевају могућност примене алтернативних средстава алогеној трансфузији крви.

Кључне речи: примена еритроцита; однос броја еритроцита са урађеном интерреакцијом и броја трансфундованих еритроцита; управљање крвљу болесника

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Comparison between serum levels of interleukin-33 in children with allergic asthma before and after inhalatory corticosteroid treatment

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SUMMARY

Introduction/Objective Interleukin 33 (IL-33) has a very significant function in inflammatory and autoimmune mechanisms, but its significance in immunopathogenic mechanisms of different allergic diseases, including allergic asthma (AA), is becoming increasingly emphasized.
The objective of the study was to investigate serum levels of IL-33 in children with AA before applying inhalation corticosteroid therapy (ICS Th) and six months after it, correlating the gathered values of IL-33 with some clinical traits of the patient.

Methods The serum value of IL-33 has been determined in 61 children with AA before starting treatment and six months after treatment with ICS Th, and this was repeated in 30 healthy children.

Results Values of IL-33 in serum are significantly higher in children with AA that have not been treated with ICS Th during six months ($p = 0.00$; $p < 0.05$), which is also the case when comparing with healthy children ($p = 0.00$; $p < 0.05$). Serum values of IL-33 in children with AA after six months of ICS Th and in healthy children do not show significant difference ($p = 0.88$; $p > 0.05$). The correlation between serum values of IL-33 before applying ICS Th and the severity, degree of AA control, and the applied dose of ICS Th is statistically significant and positive.

Conclusion IL-33 values in the serum are significantly higher in children with untreated AA in those with poorly controlled AA. Six-month treatment with ICS Th leads to significant reduction of IL-33 serum levels, whose values are in positive correlation with the severity and control of AA.

Keywords: interleukin-33; asthma; anti-inflammatory medication

INTRODUCTION

Allergic asthma (AA) is a leading chronic disease in children, its incidence in the last decades is on the constant increase globally, as well as in Serbia. The newest prevalence estimates across the world indicate that 334 million people suffer from AA. It is estimated that the number of people with asthma will increase to over 400 million until 2025 [1].

Asthma is a chronic inflammatory disease of the airways that is characterized by episodes of reversible airway obstruction, bronchial hyperactivity, and chronic lung inflammation [2].

Interleukin-33 (IL-33) was initially identified in small veins with high endothelia when it was determined that it has similar molecular properties with certain members of IL-1 superfamily (IL-1α, IL-1β, IL-1Ra and IL-18) [3, 4, 5].

IL-33 can have pro- and anti-inflammatory and protective roles, so IL-33 represents a subject of numerous researches in order to clarify the precise role of this cytokine in inflammatory diseases [6–9].

Many studies had as its aim the investigation of the exact role of IL-33 and ST2 receptors in Th2 mediated disorders. In most cases results pointed out that IL-33/ST2 axes stimulates the Th2 inflammatory response [10, 11, 12].

In the AA etiopathogenesis, inflammatory cells and mediators that belong to the Th2 immune response, eosinophil and basophil granulocytes and mast cells have the key role. Environmental antigens like infections (virus, bacterial), allergens, and air pollution induce Th2 immune response that results with a release of appropriate cytokines by epithelial cells. The function and significance of individual cytokines, among them IL-33, in patients suffering from AA, especially in children, are not precisely known [13, 14].

Up until now, research emphasizes the importance of IL-33 in initiating the differentiation of naïve CD4+ T cells and their maturation into Th2 cells that through their own specific cytokine profile lead to the activation of eosinophil granulocytes that support allergic inflammation, or create predispositions in an individual for the development of asthma and its exacerbation [15]. The direct influence of mast cells leads to the releasing of TNF that specifically emphasizes antigen sensibilization. Indirectly through mast cells and IL-13, IL-33 induces eosinophilia and hyper-reactivity of airways [16, 17].

IL-33 is dominantly a product of tissue cells, although active leukocytes that are a classical source of other para-inflammatory cytokines present a significant source of this cytokine [5].

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Tissue damage that occurs as a consequence of infection of exposure to hypersensitive individuals to an allergen can lead to the release of IL-33 [15, 18].

Results of other studies conducted on those suffering from AA have shown an absence of correlation between serum IL-33 levels and other parameters of allergic inflammation (for instance eosinophils in blood, cumulative IgE in blood) in persons with low atopic status, while in those with high atopic status this correlation is present [19]. The explanation lies in the fact that IL-33 shows primarily the characteristics of a proinflammatory marker, while serum IgE and eosinophil granulocytes represent significant markers in the estimation of atopic status, but not the degree of inflammation [20, 21].

METHODS

We have performed a prospective study at the Institute for Child and Youth Health Care of Vojvodina in the period between September 2016 and March 2018. The study encompassed 61 children aged 6–18 years with the diagnosis of AA. The control group comprised 30 healthy children of the same sex and age as the children in the studied group.

Study protocol has been approved by the Ethical Commission (Faculty of Medicine in Novi Sad, Institute for Child and Youth Health Care of Vojvodina, Clinical Center of Vojvodina). Signed informed consent has been obtained from all the parents, and from children older than 10 years. The study has been performed adhering to the principles of the Helsinki Declaration.

Inclusion criteria in the study group were age of 6–18 years with a newly established diagnosis of light to mild AA, or subjects with light to mild AA diagnosed earlier but without ICS prophylaxis at least six months before inclusion into the study. Diagnosis and classification have been performed by Global Initiative for Asthma (GINA) guidelines [2].

Exclusion criteria were the following: existence of atopic dermatitis, urticaria, food allergies, chronic respiratory infections, uncontrolled gastro-esophageal reflux, eosinophilic esophagitis, parasite infection, any other chronic infection, allergen-specific immunotherapy (in the period prior to and during the study), acute infections and other acute illnesses, use of systemic corticosteroids immediately before and during the planed study.

Children that comprised the study group underwent two additional examinations after the initial one, three and six months after the treatment. Anamnestic and heteroanamnestic data for the first and the second control period were taken from all children in the study group. The type of difficulty, the need for using short-acting beta-2 agonist, and the frequency of its use were especially noted. We performed a clinical examination and specially noted the following: body mass and weight, nutrition levels (BMI, Z score, and percentiles), vital parameter values and transcutaneous oxygen saturation of hemoglobin as well as findings of a physical lung examination. All the examines underwent an investigation of lung functioning using the MasterScreenIOS spirometer (Jaeger, Germany) according to American Thoracic Society guidelines.

During the first examination and the follow-up six months later, all the participants underwent laboratory examinations that, amongst other things, encompassed determining levels of IL-33 in the serum. The measurement of IL-33 levels was performed at the laboratory of the Clinical Center of Vojvodina in Novi Sad. The levels were determined via direct sandwich enzyme-linked immunosorbent assay test that contains recombined human IL-33 and polyclonal antibodies specific for IL-33 (Human IL-33 Quantikine® ELISA, Research and Diagnostic Systems, Inc., Minneapolis, MN, USA). According to the specifications of the manufacturer, the minimal detectable level of IL-33 that can be determined by using this test is 0 pg/ml. All procedures have been performed by following the instructions of the manufacturer. The intensity of the colored reaction was determined by an automatized immunochemical analyzing device ChemWell (Awareness Technology, Inc., Palm City, FL, USA). Absorbance was measured at 450 nm filter via standard curve of serially diluted standards. By using the standard curve, we determined the levels of IL-33 in all 91 participants.

The therapeutic approach in asthma treatment to all the participants was in accordance to GINA recommendations for the treatment of mild and medium asthma, i.e. appropriately dosed ICS therapy was applied in all cases. During the study, on the follow-up examinations at three and six months from the beginning of ICS therapy, AA difficulty based on GINA recommendations was determined based on the level of control (controlled, partially controlled, uncontrolled) and treatment intensity (low, medium, high ICS dosages).

All the children in the control group underwent the following: anamnesis/hetero-anamnesis taken (except the information whether the child is being investigated for oversensitivity to any medication, the information pertaining to the exclusion from the study was especially noted, like the absence of difficulties and signs of acute infections two weeks before the examination, absence of chronic diseases). We performed a clinical examination and the following were especially noted: mass, height, nutrition level (BMI, Z score, and percentile), absence of clinical signs of infection. The level of IL-33 (pg/ml) in the serum was determined in the same way as in the investigated group.

Statistical analysis

Wilcoxon pair test, t-test for independent samples, Mann–Whitney U-test, median test, and χ^2 test were used for the determination of statistical significance. Spearman's rank correlation coefficient was used to determine correlation. The value of $p < 0.05$ was considered statistically significant. Data processing was performed by the statistical program package IBM SPSS Statistics, Version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Out of the total number of participants (n = 61) with an average age of nine years and six months, 32 (52.5%)

were boys and 29 (47.5%) were girls. The control group (n = 30) was made up of healthy children with an average age of nine years and eight months, 16 (53.3%) boys and 14 (46.6%) girls (Table 1).

The severity and degree of AA control that the participants experienced after three and six months of ICS Th as well as the ICS dose that the participants received during the first three months and between the third and sixth month of ICS Th are displayed in Table 1.

IL-33 level values (pg/ml) in the investigated group before the application of ICS Th and six months after ICS Th are presented in Table 2, in which values of serum IL-33 (pg/ml) in the control group are presented as well.

Participant grouping with regards to serum IL-33 levels that the participants had before and after ICS Th are presented in Table 3.

Children that suffered from AA before commencing ICS therapy had significantly higher values of IL-33 than healthy children of the same age ($U = 509$; $p = 0.00$; $p < 0.01$).

In children where AA treatment has not commenced, IL-33 serum values were significantly higher compared to those that underwent six months of ICS Th ($Z = -4.394$; $p = 0.00$; $p < 0.01$). It has been determined that children suffering from AA that have elevated values of IL-33 in the serum before ICS treatment also register higher IL-33 levels after six months of ICS therapy ($rs = 0.271$; $p = 0.04$; $p < 0.05$).

Results show that children with AA that have undergone six months of ICS Th do not show statistical differences in serum IL-33 levels of healthy children of the same age ($U = 897$; $p = 0.88$; $p > 0.05$) (Table 4).

In Table 4 we present serum values of IL-33 (before and after six months of ICS Th) referenced by AA severity and control level (the children had three and six months from the introduction of ICS therapy) and applied medication dosages (during the first and second trimester of ICS Th).

Table 1. Division of participants by asthma severity and control levels in the third and sixth month of inhalation corticosteroid therapy (ICS Th), as well as ICS dosages administered during the first three months and in the period from third to sixth month

Asthma severity and control levels	After 3 months of ICS Th		After 6 months of ICS Th	
	Number of participants	Percentage	Number of participants	Percentage
Asthma severity				
Light	46	75.4%	39	63.9%
Medium difficulties	15	24.6%	18	29.5%
Difficult	0	0%	4	6.6%
Asthma control				
Controlled	59	96.7%	45	73.8%
Partially controlled	2	3.3%	15	24.6%
Uncontrolled	0	0%	1	1.6%
ICS medication dose	During first 3 months		From third to sixth month	
Low	23	37.7%	47	77%
Medium	38	62.3%	14	23%

Table 2. Values of IL-33 serum levels (pg/ml) in the investigated group (before and six months after inhalation corticosteroid therapy (ICS Th) and in the control group

Investigated group	n	Min.	Max.	Mod	M	Mean	SD
Before ICS Th application	61	0.00	14.74	0.04	1.49	2.55	3.39
After 6 months of ICS Th application	61	0.00	7.8	0	0.26	0.83	1.39
Control group	30	0.00	2.68	0	0.26	0.57	0.63

Mod – most common value; M – medial value; SD – standard deviation; IL-33 – interleukin 33

Table 3. Participants grouped by interleukin 33 (IL-33) serum levels before and after six months of inhalation corticosteroid therapy (ICS-Th)

Participant groups based on IL-33 levels	n	%
Higher values before ICS Th	45	73.8
Higher values after ICS Th	13	21.3
Same values before and after ICS Th	3	4.9
Total	61	100

Table 4. Relationship between interleukin 33 (IL-33) values in the serum with allergic asthma (AA) severity and control level, and ICS dose

Clinical indicators of the inflammation level	Before ICS Th-IL-33 (pg/ml)		After six months of ICS Th-IL-33 (pg/ml)	
	rs	p	rs	p
AA severity – 3 months ICS Th	0.52**	0.00	0.23	0.08
AA severity – 6 months ICS Th	0.42**	0.00	0.45**	0.00
AA control – 3 months ICS Th	0.29*	0.02	0.14	0.28
AA control – 6 months ICS Th	0.39**	0.00	0.39**	0.00
Medication dose: first 3 months of ICS Th	0.08	0.56	-0.02	0.87
Medication dose: second 3 months of ICS Th	0.48**	0.00	0.16	0.20

ICS Th – inhalation corticosteroid therapy;
r_s – Spearman rank correlation coefficient;
*$p < 0.05$;
**$p < 0.01$

DISCUSSION

In our study, we analyzed the levels of IL-33 in the serum of children 6–18 years old with AA and in healthy children. The average levels of IL-33 were higher in children with AA before ICS therapy (2.550 ± 3.387 pg/ml). Lower values of IL-33 in the serum were detected in children after six months of ICS therapy, with an average value of 0.838 ± 1.394 pg/ml. The lowest level of serum IL-33 was measured in healthy children and it amounted to 0.573 ± 0.632 pg/ml.

It has been determined that IL-33 serum levels are significantly higher in children that suffer from AA before the beginning of treatment concerning healthy children. Similar findings have been found in the research by Bahrami et al. [22], according to which IL-33 levels in 61 children with asthma were compared with IL-33 levels of children in the control group, those without asthma, and the results were statistically significant. In the study, average values of IL-33 in the serum of children with asthma was 15.17 ± 32.3 pg/ml. Higher IL-33 serum levels in children that suffer from

asthma detected in the study by Bahrami et al. [22] than in those detected in our study can be explained by differences in inclusion criteria. In the research by Bahrami et al. [22], participants with AA were included regardless of the length (intermittent, persistent) and asthma severity (light, intermediary, severe) in contrast to our study, where participants with only the characteristics of persistent, light, and intermediary asthma were included. Other studies that compared IL-33 levels in children with AA with those in healthy children also produced similar results. A meta-analysis that encompassed eight previously conducted studies that cumulatively had 330 children with asthma and 248 healthy children shows that IL-33 serum levels were higher in children with asthma than in healthy children [23].

IL-33 serum values in healthy children that are in the control group of participants in our study are similar to those reported by other studies of pediatric populations. For instance, the average value of IL-33 in the serum of healthy children in the Iranian population was 0.61 ± 2.16 pg/ml [24]. But the results of research done on healthy adult population show higher IL-33 values that those detected in healthy children in our study. This fact suggests that patient age can be a significant factor in defining the normal span of IL-33 serum levels, and this is significant for interpreting laboratory findings in regular everyday practice.

Our study shows that after six months of ICS therapy in children that suffer from AA, there is a significant decrease in serum IL-33 levels. We did not find similar research in the literature available to us.

The influence of ICS on IL-33 in AA and their correlation is unclear. Studies performed on cell cultures have pointed out the significance of IL-33 for the creation of corticosteroid resistance. Namely, the research conducted by Kabata et al. [25] suggest that one of the potential mechanisms leading to corticosteroid resistance that can emerge in Th2-mediated inflammation of the air ways does so because of the influence of IL-33 on natural helper cells. These represent a sort of lymphoid cells of type 2 inborn immunity, i.e. resistance can emerge as a consequence of IL-33-mediated proliferation and production of type 2 cytokines from said cells. In vitro research on cell cultures shows that corticosteroids have a relatively efficient anti-inflammatory effect on IL-33-mediated inflammation [26].

In our study, children with AA that underwent six months of ICS treatment do not differ in IL-33 serum levels from healthy children of the same age. Studies that compared IL-33 serum levels before and after ICS therapy have not been found in the available literature. Similar values of IL-33 in the serum in children with AA after six months of ICS and in healthy children and the significant fall of IL-33 six months after ICS therapy can firstly be explained by the aforementioned anti-inflammatory effects of ICS. In addition, the results of our research can be explained by the characteristics of the participant group itself. Children included in our study had exclusively light and medium asthma severity, while patients with severe forms of asthma and patients that required additional treatment in order to control their illness (long-acting beta-agonist, combination of ICS and long-acting beta-agonist, systemic corticosteroids) were excluded from our study. Maintaining high values of IL-33 despite the application of ICS monotherapy can perhaps be expected in patients with the severe and/or steroid resistant form of AA, which is not the subject of our study.

The incidence of participants regarding the severity and the degree of AA control in our research is a direct consequence of inclusion and exclusion criteria of our study.

In our research the patients that had higher levels of IL-33 before ICS prophylaxis had a more severe form of AA during the follow up period, and worse control of asthma during the treatment period and they required higher dosages of ICS in the second trimester of treatment. Patients that after six months of ICS prophylaxis still had a severe form and worse control of AA had higher IL-33 levels in the serum. The connection of IL-33 and severity of AA is documented in other studies. Research performed by Bahrami et al. [22] on children with asthma has also shown a correlation between IL-33 in the serum and asthma severity. The lowest IL-33 serum levels were detected in children with mild asthma, somewhat higher values in children with medium severity asthma, and the highest ones in those with severe asthma [22]. In addition, studies conducted on adult populations have shown a significant difference in IL-33 values between patients with intermittent, light, medium severe, and severe persistent asthma [27]. Guo et al. [28] in a study conducted on 45 adult participants have shown a positive correlation between IL-33 levels in the serum and the thickening of the basal membrane in bronchial biopsy samples and asthma severity. Lower values of IL-33 after six months of ICS monotherapy application confirm the anti-inflammatory effect of ICS and its suppressive potential on pro-inflammatory cytokines. Lower degrees of control and severe form of AA in participants that had higher values of IL-33 before and after six months of ICS therapy show that IL-33 can be a useful marker when choosing the therapy type and dosage, i.e. contributing to the optimal treatment of AA.

CONCLUSION

Results of our research and the cited results of other studies suggest that serum IL-33 can represent a potent biomarker for the severity of AA. The great importance of determining IL-33 serum levels during diagnostic evaluation of AA before starting the treatment shows a potential for better defining the asthma phenotype and with it an earlier optimization of therapy.

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Поређење серумских нивоа интерлеукина-33 код деце са алергијском астмом пре и после шестомесечне инхалаторне кортикостероидне терапије

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САЖЕТАК

Увод/Циљ Интерлеукин-33 (ИЛ-33) има веома битну функцију у инфламаторним и аутоимунским механизмима, али се све више истиче и значај у имунопатогенетским механизмима различитих алергијских обољења, укључујући и алергијску астму (АА). Циљ овог рада је испитивање серумских вредности ИЛ-33 код деце са АА пре и после шест месеци примене инхалаторне кортикостероидне терапије (*ICS Th*) и корелације добијених вредности ИЛ-33 са појединим клиничким особинама ових болесника.

Методе Одређена је серумска вредност ИЛ-33 код 61 детета са АА пре започињања лечења и шест месеци после третмана са *ICS Th*, као и код 30 здраве деце.

Резултати Вредности ИЛ-33 у серуму су значајно веће код деце са АА која нису лечена у односу на децу са АА код којих је спровођена *ICS Th* током шест месеци ($p = 0,00$; $p < 0,05$), као и у односу на здраву децу ($p = 0,00$; $p < 0,05$). Серумске вредности ИЛ-33 код деце са АА после шест месеци *ICS Th* и код здраве деце не показују значајне разлике ($p = 0,88$; $p > 0,05$). Корелација између серумских вредности ИЛ-33 пре примене *ICS Th* и тежине, степена контроле АА као и примењене дозе *ICS Th* је статистички значајна и позитивна.

Закључак Вредности ИЛ-33 у серуму су значајно веће код деце са АА која нису лечена и код којих је АА лоше контролисана. Третман са *ICS Th* током шест месеци доводи до значајне редукције серумских нивоа ИЛ-33, чије вредности су у позитивној корелацији са тежином и контролом АА.

Кључне речи: интерлеукин-33; астма; дете; антиинфламаторни лекови

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Correlation of oxidative stress markers and semen parameters with the outcome of *in vitro* fertilization

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SUMMARY

Introduction/Objective The aim was to assess the effect of oxidative stress parameters on sperm count, motility, and morphology of spermatozoa, and the influence of different semen parameters on the outcome of *in vitro* fertilization (IVF) procedure – the number of fertilized oocytes, fertilization, and pregnancy rate of female partners.

Method In 52 male patients superoxide dismutase (SOD), malondialdehyde (MDA), and sulfhydryl (-SH) groups were determined in serum, before beginning the IVF cycle. Semen samples were collected after 2–3 days of sexual abstinence.

Results Patients were divided into two groups, the normozoospermic, and those with pathological sperm findings. The second group was divided into oligozoospermic, asthenozoospermic, and teratozoospermic groups. SOD activity was lower, while MDA and -SH groups, while not significantly, was higher in groups with sperm parameter disorders. Fertilization rate was significantly lower in the group of asthenozoospermia and teratozoospermia ($p = 0.034$), as well as delivery rate ($p = 0.020$). The group with oligozoospermia had significantly lower delivery rate ($p = 0.013$).

Conclusion Our study found higher fertilization and delivery rate in men with normozoospermia. However, no significant correlation between OS markers and semen parameters was found.

Keywords: *In vitro* fertilization; oxidative stress; sperm parameters

INTRODUCTION

Approximately 15% of couples of reproductive age have problems with infertility, and half of the cases are attributed to the male factor, while one of the mechanisms proposed for idiopathic male infertility is oxidative stress [1]. The diagnosis of male infertility is based on analysis of semen parameters: concentration, motility, and morphology of spermatozoa in the ejaculate.

Sperm cells are the first cells that are reported to be susceptible to oxidative damage. In the article printed in 1943, MacLeod [2] confirmed the rapid loss of sperm motility when incubated in medium with increased concentration of oxygen. Recent studies have also found an increase in reactive oxidative species (ROS) levels in 30–80% of infertile men [3, 4]. ROS are natural products of cellular metabolism, that at physiological concentrations are the essential requirements for the spermatozoa in the processes that lead to successful fertilization: ROS trigger sperm hyperactivation and capacitation [5]. Physiological oxidative conditions are necessary for the sperm maturation, binding to the zona pellucida, acrosomal reaction, and subsequent fusion of sperm and oocytes [6, 7]. That indicates that ROS itself has no adverse effect, except when the levels are elevated. Available data on the impact of oxidative stress on sperm are largely based on measuring the levels of malondialdehyde (MDA) in semen. Since ROS have both physiological and pathological functions, the human body has developed a defense system to maintain its concentration in a certain range. Due to the size and small volume of the cytoplasm, as well as the low concentrations of the enzyme cleaners, sperm has limited antioxidant defense properties [8]. Antioxidant profile in the blood in relation to the antioxidant profile and quality of spermatozoa is less investigated. Correlation between superoxide dismutase (SOD) levels in the blood and sperm number, as well as glutathione levels and sperm progressive motility, suggests that these parameters may be valuable biochemical markers in assessing reproductive and functional capacity of sperm [8].

Most of the studies examined ROS parameters in semen; there are only few studies that analyzed oxidative stress parameters in serum, or both in seminal plasma and serum. Serum oxidative stress markers as well as antioxidant profile showed correlation to sperm parameters, showing that serum can be a valuable tool in evaluation of oxidative stress in men [8–11].

The aim of this study was to examine the association between oxidative stress parameters, MDA, SOD, and -SH groups serum levels in males with semen parameters, as well as the influence of different sperm parameters and outcome of *in vitro* fertilization (IVF).

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METHODS

Study subjects and sample collection

The prospective clinical study was conducted at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia. We recruited 52 male patients, admitted for fertility treatment. All investigated patients agreed to participate in the study and signed an informed consent for all the undertaken procedures. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade. Inclusion criteria were age 24–45 years, body mass index 18–30 kg/m^2, no chronic illness, radiotherapy or chemotherapy. Patients with azoospermia were excluded from the study. Female partners were aged 18–40 years, body mass index 18–30 kg/m^2, with regular menstrual cycles from 25–32 days, without any medical disease or endometriosis stage III and IV. Infertility cause was categorized as male and unknown. The protocols of stimulation were determined individually. After ovarian stimulation and oocyte retrieval, methods of insemination were IVF, intracytoplasmic sperm injection, or a combined method. In assessing the quality of embryos, the Istanbul consensus clinical embryologists' criteria were used as the reference frame [12]. Embryo transfer was performed transcervically on day two or three after the oocyte retrieval. Pregnancy rate were calculated per embryo transfer. Pregnancy was diagnosed by positive serum β-hCG (> 50 IU/ml) 14 days after the embryo transfer.

Semen was obtained by masturbation technique after 48–72 hours of sexual abstinence. Samples were collected into sterile containers for immediate transportation to the laboratory. All semen samples were evaluated in the same laboratory. After liquefaction at 37°C for 30 min, routine semen analysis (liquefaction time, volume, pH, viscosity, sperm count, motility, and morphology) was carried out after liquefaction, according to the World Health Organization guidelines [13]. Samples were categorized based on the sperm count into normozoospermic and oligozoospermic, and based on sperm motility into normozoospermic and asthenozoospermic. Similarly, based on the sperm morphology, they were categorized as normal and teratozoospermic for analysis purposes. Based on semen analysis we divided patients into two groups: those with normal semen analysis (normozoospermia) and those with pathological sperm finding. The second group was divided into three groups: oligozoospermic, asthenozoospermic and teratozoospermic group.

Blood samples for oxidative stress parameters were obtained from each patient prior to stimulation commencement in the female partner. After separation, the serum/plasma was frozen and stored at a temperature of -70°C. After incomplete defrosting, preparation of the serum analysis was carried out by homogenization and centrifugation. The following parameters of oxidative stress groups were determined as described in detail in our previous study [14]: the concentrations of MDA, the activity of total SOD, and the concentration of total sulfhydryl (-SH).

Statistical analysis

For statistical analysis of the obtained data, the Statistical Package for the Social Sciences Version 22.0. (IBM Corp., Armonk, NY, USA) was used and differences were considered statistically significant at a probability level less than 0.05 for all tests. Results were presented as arithmetic mean and, ± standard deviation for variables with normal distribution and as median and interquartile range for other variables. Categorical variables are presented as relative or absolute frequency. Testing of distribution was carried out by Kolmogorov–Smirnov analysis. Comparison of the mean values of independent groups of data was performed by Student's t test and ANOVA analysis with Tukey's post hoc test for the differences between subgroups. For parameters without normal distribution, a test of significance between groups was performed using the Mann–Whitney test or Kruskal–Wallis test with post hoc Mann–Whitney test. Comparison of two dependent populations was performed by the Wilcoxon signed-rank test for data without normal distribution. Analysis of categorical values was performed using the χ^2 test.

RESULTS

Oxidative stress parameters: MDA, SOD, and -SH groups in males' serum and the outcome of IVF in female partner: number of fertilized oocytes, fertilization, and pregnancy rate are given in Table 1. In 52 men, we examined changes in the OS parameters and antioxidant protection depending on the disorder in sperm parameters and we compared them to normal semen analysis. No significant difference in the examined parameters between the groups was found, although the value of MDA were slightly lower in the group with normozoospermia compared to pathological findings (0.52 *vs.* 0.57, p = 0.254), while the value of SOD (26.18 *vs.* 24.12, p = 0.348) and -SH groups (0.46

Table 1. Oxidative stress parameters in serum of male partners and in vitro fertilization outcome in female partners

Parameters	Values
SOD (U/L)	25.17 (22.4–28.82)
MDA (μmol/L)	0.56 (0.48–0.65)
-SH groups (mmol/L)	0.44 (0.39–0.52)
Number of fertilized oocytes (n)	2.5 (1–6)
Fertilization (%)	54.5 (33.3–81.8)
Pregnancy rate (%)	41

SOD – superoxide dismutase; MDA – malondialdehyde; -SH – sulfhydryl groups; The median (25th and 75th percentile) are shown; the statistical analysis was made using the Mann–Whitney test

Table 2. Oxidative stress parameters in serum of men with normozoospermia and pathological sperm finding

Parameters	Normozoospermia n = 30	Pathological finding n = 20	p
SOD	26.18 (22.63–28.89)	24.12 (22.37–29.08)	0.348
MDA	0.52 (0.47–0.64)	0.57 (0.51–0.65)	0.254
-SH groups	0.46 (0.41–0.53)	0.43 (0.38–0.47)	0.138

SOD – superoxide dismutase; MDA – malondialdehyde; -SH – sulfhydryl groups; The median (25th and 75th percentile) are shown; the statistical analysis was made using the Mann–Whitney test

Table 3. Oxidative stress parameters in serum of male partners with normozoospermia and oligozoospermia. asthenozoospermia and teratozoospermia.

Parameters	Normozoospermic n = 30	Oligozoospermic n = 17	Asthenozoospermic n = 16	Teratozoospermic n = 12
SOD	26.18 (22.63–28.89)	24.12 (22.36–29.08)	23.9 (22.4–29.3)	26 (023.43–29.65)
MDA	0.52 (0.47–0.64)	0.57 (0.51–0.65)	0.58 (0.52–0.65)	0.58 (0.51–0.64)
-SH groups	0.46 (0.41–0.53)	0.43 (0.38–0.47)	0.41 (0.34–0.46)	1.38 (1.28–1.46)

SOD – superoxide dismutase; MDA – malondialdehyde; -SH – sulfhydryl groups;
the median (25th and 75th percentile) are shown; the statistical analysis was made using the Mann–Whitney test

Table 4. Number of fertilized oocytes and fertilization rate in female partners of men with normozoospermia and all others sperm parameters

Parameters	Normozoospermia n = 30	Pathological finding n = 20	p
Fertilized oocytes (n)	2.5 (1.8–5.6)	2.5 (1–6)	0.955
Fertilization (%)	68	50	0.102

vs. 12.43, p = 0.138) were slightly higher in the group with normozoospermia (Table 2). As shown in Table 3, when we compared the individual findings of normozoospermia with findings of oligozoospermia, teratozoospermia, and asthenozoospermia, there was no significant difference. We also compared the number of fertilized oocytes, fertilization rates, and outcome of pregnancies in female partners of examined male partners, depending on sperm parameters. A number of fertilized oocytes did not differ between groups. The fertilization rate was higher in male partners with normozoospermia, compared to abnormal semen analysis (68% *vs.* 50%, p = 0.102) (Table 4), as well as the pregnancy rate (44.1% *vs.* 40%, p = 0.756). In the group with normozoospermia, female partners who conceived had delivered a healthy child in 83%, comparing with 62.5% in female partners of men with all other sperm parameters, which was significantly lower (p = 0.034). When we separated group with disorder in sperm parameters, there was no significant difference concerning the number of fertilized oocyte (p = 0.864), as well as fertilization rate (p = 0.475) between normozoospermia and oligozoospermia. In female partners of men with asthenozoospermia and teratozoospermia there was a significantly lower fertilization rate comparing to normozoospermia group (p = 0.034) (Table 5). The group with oligozoospermia had a significantly lower delivery rate (p = 0.013).

DISCUSSION

In our study, we did not find significant difference in the examined changes in oxidative stress parameters and antioxidant protection between men with normal semen analysis and those with the disorder in sperm parameters. However, the value of MDA was higher in the group with pathological sperm finding, while the value of SOD and -SH groups were higher in the group with normozoospermia. When we compared oligozoospermia, teratozoospermia, and asthenozoospermia to normozoospermia group, results were similar. These trends in investigated parameters values are in accordance with some other studies [8, 9, 15]. Huang et al. [15] found higher concentrations of MDA in men with asthenozoospermia and oligoasthenozoospermia compared to normozoospermic men, showing similar finding compared to our study. Low levels of MDA in the seminal plasma were associated with an increased progressive motility of sperm and a positive correlation between elevated levels of MDA and abnormal morphology of sperm were found, which is consistent with the findings of other authors [16]. Different impairments of sperm cells as well as male infertility can be caused by increased lipid peroxidation. MDA is an indicator of the lipid peroxidation and may be a diagnostic tool in infertility, as well as a predictor of Assisted Reproductive Technology procedures success [17].

In results of studies that are comparable to our, correlation between the level of SOD in the serum and the sperm number was found in patients with pathological findings [8]. This group of researchers also found a significant correlation between the level of glutathione in the serum, and sperm progressive motility between patients with infertility and normal controls. In the study by Benedetti et al. [9] that estimated antioxidants profile in the plasma of fertile and infertile patients, lower antioxidants were found in infertile males, positively correlated with the concentration, motility, and morphology of spermatozoa. In the paper by Mahanta et al. [18] the level of lipid peroxides in the blood of the infertile group was significantly higher compared to the fertile one, while the activity of SOD and glutathione peroxidase in the blood was significantly lower compared to fertile men. Some studies have observed no difference in glutathione between fertile and infertile males [19], while others have observed significantly reduced glutathione

Table 5. Number of fertilized oocytes and fertilization rate in female partners of men with normozoospermia and oligozoospermia, asthenozoospermia and teratozoospermia

Parameters	Normozoospermic n = 30	Oligozoospermic n = 17	Asthenozoospermic n = 16	Teratozoospermic n = 12
Fertilized Oocytes	2.5 (1.8–5.6)	2.5 (1–6)	2.5 (1–5.8)	2 (1–3)
Fertilization %	68 (39.1–100)	50 (25.2–74.6)	35.7 (25–68.6)	50 (25–75)

The median (25th and 75th percentile) are shown; the comparison was made using the Mann–Whitney test

levels in the seminal plasma of the infertile men as compared to fertile ones [20, 21].

Although the analysis of sperm a routine procedure, we do not get information about the functional capacity of sperm, consequently, no semen parameter by itself can predict the success possibility of the Assisted Reproductive Technology procedures. However, the percentage of sperm with normal morphology is in positive correlation with fertilization and pregnancy rates in IVF [22, 23]. We observed significantly lower fertilization rate in patients with asthenozoospermia compared to a normal finding. Fertilization rates significantly vary in the findings of normospermia compared to pathological semen findings and some studies found no association [24]. In patients with severe teratospermia, oligo- or azoospermia, the DNA fragmentation and the degree of aneuploidies of sperm were significantly higher, as well as a higher percentage of aneuploidy in embryos was found [25, 26, 27]. Even in these findings, fertilization can occur, but the rate of miscarriages is significantly higher [28]. However, there is a wide variation among samples from one individual and therefore more sophisticated sperm function tests, or selection tests are needed for improving the outcome of IVF procedures [29].

The present study has some limitations; it had a small sample. In addition, in the future studies, we are planning to include information about lifestyle habits, such as eating habits, alcohol intake, smoking, use of supplements etc. Beside assessment of oxidative stress status from serum, sperm data would be beneficial too.

CONCLUSION

Our results suggest that abnormal semen parameters affect the outcome of IVF. Fertilization rate was lower in the group with asthenozoospermia, while the delivery rate is lower in oligozoospermia, asthenozoospermia, and teratozoospermia. However, no significant correlation between oxidative stress markers and semen parameters was found.

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Conflict of interest: None declared.

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Корелација маркера оксидативног стреса и параметара спермограма са исходом вантелесне оплодње

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САЖЕТАК

Увод/Циљ Циљ рада била је процена утицаја параметара оксидативног стреса на број, покретљивост и морфологију сперматозоида, као и утицаја различитих параметара спермограма на исход поступка *in vitro* фертилизације, број фертилисаних ооцита, стопе фертилизације и порођаја.

Методе Код 52 мушка партнера одређиване су вредности супероксид-дисмутазе, малондиалдехида и сулфидрила (*-SH* група) у серуму, пре започињања поступка *in vitro* фертилизације. Узорци семена сакупљани су после два-три дана апстиненције.

Резултати Болесници су били подељени у две групе: група са нормозооспермијом и група са патолошким налазом спермограма. Друга група је затим подељена на подгрупе: олигозооспермија, астенозооспермија и тератозооспермија. Активност супероксид-дисмутазе била је нижа, док су вредности малондиалдехида и *-SH* група биле више у групама са поремећеним параметрима спермограма, али не статистички значајно. Стопа фертилизације била је значајно нижа у групи са астенозооспермијом и тератозооспермијом ($p = 0,034$), а такође и стопа порођаја ($p = 0,020$). Група са олигозооспермијом имала је значајно нижу стопу порођаја ($p = 0,013$).

Закључак У овој студији пронађене су више стопе фертилизације и порођаја у групи са нормозооспермијом. Није било значајне корелације између параметара оксидативног стреса и спермограма.

Кључне речи: *in vitro* фертилизација; оксидативни стрес; параметри спермограма

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of hysteroscopic polypectomy on the concentrations of tumour necrosis factor-α in uterine flushings and serum in infertile women

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SUMMARY

Introduction/Objective The aim of this paper is to present changes of tumor necrosis factor-α (TNF-α) levels in uterine flushings and serum of infertile female patients before and after hysteroscopic polypectomy.

Methods A total of 82 infertile female patients were included in the study. The patients were divided into two groups. The first group was the experimental group and comprised 56 infertile women with endometrial polyps, whereas the second group was the control group of 26 infertile women who were not diagnosed with endometrial polyps.

Results The results of this research primarily suggest that TNF-α concentrations obtained from uterine flushings and serum of infertile patients diagnosed with endometrial polyps differed before and after a surgical procedure. In the control group of patients, there was no significant difference observed regarding TNF-α concentrations in serum and uterine flushings of women without endometrial polyps. A comparison between these two groups revealed differences in TNF-α concentrations in both venous blood and uterine flushings. These differences were considered statistically significant.

Conclusion Endometrial polyps are one of the causes of higher TNF-α levels in both uterine flushings and serum.

Keywords: cytokines; TNF-α; endometrial polyp; uterine flushings; serum

INTRODUCTION

Endometrial polyps are benign localized overgrowth of endometrial tissue, composed of glands, stroma, and blood vessels covered by epithelium. They develop once the endometrium becomes hypertrophic, which is a consequence of oestrogen stimulation [1, 2, 3]. They are diagnosed based on hysteroscopic, sonographic, or hysterosonographic findings, after a polyp, which can vary in size, has been detected in the uterine cavity [4]. There is a large group of protein molecules that mediate and regulate intercellular communication both in physiological and pathological conditions and these are called cytokines [5, 6, 7]. Cytokines are produced by different cells that induce chemotaxis, activation, proliferation, and differentiation of other cells. The endometrial tissue also produces cytokines. Cytokines have a significant role in the relationship between the decidua and the embryo during implantation [8, 9]. The most important physiological functions of cytokines include regulating cell growth and humoral immune response, regulation of hematopoiesis, controlling cell proliferation and differentiation, and wound healing [10–13]. Cytokines can act locally or systemically [14]. Cellular response to most cytokines is followed by gene expression, which results in the target cell gaining some new functions and can sometimes lead to cell proliferation [15]. Tumour necrosis factor-α (TNF-α) belongs to the family of cytokines that are considered the most versatile. Not only does TNF-α play a prominent role in the synthesis of DNA in the early stage of proliferation, but it also contributes to cell differentiation and tissue remodeling. This is essential in terms of embryonic attachment [16].

The objective of this paper is to present changes of TNF-α levels in serum and uterine flushings of infertile female patients before and after hysteroscopic polypectomy.

METHODS

This was an open cross-sectional study, which included 82 infertile female patients aged 22–42 years. The patients were divided into two groups. Firstly, the experimental group comprised 56 infertile women who were diagnosed with endometrial polyps, whereas the second group was the control group of 26 infertile women who were not diagnosed

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with endometrial polyps. The study was conducted at the Narodni front Obstetrics and Gynecology Clinic in Belgrade and the Centre for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, between May 2012 and November 2013. The inclusion criteria were as follows: a history of infertility (≥ 1 year), age (22–42 years), and regular menstrual cycles, not taking hormonal contraceptives or hormonal medications that could affect the endometrium for the last six months, an endometrial polyp detected by either two-dimensional (2D) ultrasound or saline infusion sonohysterography, no other endometrial pathology observed by a transvaginal ultrasound. The exclusion criteria for both groups of participants were the presence of submucous myomas, endometriosis, endometrial cancer, uterine anomalies, history of uterus and fallopian tubes surgery, history of ovulation induction failure, age > 42 years. Furthermore, the research was approved by the Ethics Board of the Narodni front Obstetrics and Gynecology Clinic on May 24, 2012; ref. No.: 04-24/3-1. The diagnosis of polyps was threefold. They were diagnosed by 2D transvaginal ultrasound examinations conducted in the first stage of the cycle, by hysterosonographic examinations, or during hysteroscopy itself. After the intervention, all the patients were followed up for one month by taking the samples of peripheral venous blood and determining the levels of the same cytokines [17]. An ultrasound check-up was also performed. Hysteroscopy was performed in a fully equipped operating room using completely adapted instruments. The patients were under general anesthesia during hysteroscopy. Preoperative preparation involved complete diagnostics and routine tests: cervical and vaginal swabs; swabs for chlamydia, ureaplasma, and mycoplasma; Pap smear; 2D ultrasound; blood typing; hematology and biochemical analyses; preoperative assessment by an internist and an anesthetist; giving an informed consent for surgical treatment. The patients underwent surgical procedures prior to the mid-proliferative stage of the cycle, soon after menstrual bleeding had ceased. Hysteroscopy can be performed regardless of the phase of the cycle, providing the patients have been prepared by taking oral hormonal contraceptives. Diagnostic hysteroscopy was performed in the control group of infertile women to detect any pathological changes in the uterine cavity that could cause infertility. Endometrial biopsy was performed in patients with normal hysteroscopic findings. The endometrial samples were histopathologically examined to detect any abnormalities of the endometrium. During diagnostic hysteroscopy, saline is used for uterine distension, which enables the uterine cavity to be visually examined with 30° angle lens. In this way, uterine horns, the fundus, anterior and posterior uterine walls, and the lateral sides of the uterus are visualized. At the level of the intrauterine horns, the hysteroscope allows a panoramic view of the uterine cavity, followed by the visualization of the cervical canal. Due to the muscular structure of uterine walls, the distension pressure of ≥ 40 mmHg is required. Operative hysteroscopy was performed in the experimental group by using an operative continuous flow hysteroscope with a resectoscope that uses bipolar electrical current. The resectoscope has a cutting loop electrode, which is used for removing endometrial polyps. The procedure for determining the concentrations of TNF-α in uterine flushings was as follows: a Cusco's speculum was inserted once the patient was placed in the lithotomy position, which enabled the visualization of the cervix. After the cervix had been flushed with sterile saline, a pediatric 8F Foley catheter was placed through the cervical canal and into the uterine cavity. Then, 10 ml of sterile saline was injected through the catheter and immediately aspirated without contamination. Uterine flushings were centrifuged at 2,500 × g for 10 minutes and the supernatant was aspirated and stored at -20°C. Afterwards, the ELISA method was used to detect TNF-α and determine its concentration. This was conducted at the Centre for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac. When it comes to measuring serum TNF-α concentrations in the experimental group of patients, ~5 ml of peripheral venous blood was sampled one month before and one month after hysteroscopic surgery. The blood samples were collected into heparinized vacuum tubes and centrifuged at 2,500 × g for 10 minutes. The supernatant was aspirated and stored at -20°C. Again, the ELISA method was used to detect TNF-α and determine its concentration. Sample size calculation was performed by using a formula for computing a large sample implemented in the PASS software 11.0 (NCSS, LLC, Kaysville, UT, USA). Descriptive and analytical statistics were used to process the obtained data. Descriptive methods included absolute and relative numbers, measures of central tendency (mean, median), and measures of dispersion (standard deviation, inverse variance). Analytical statistics methods involved comparison tests (parametric, non-parametric). All results were analyzed using the IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA) software package. The obtained results were compared to previously reported data. Finally, the conclusions were reached by analyzing and interpreting these results.

RESULTS

The study included 56 patients with polyposis (68.3%) and 26 patients without polyposis (31.7%). The age interval was 22–42 years. The mean age in the group of patients diagnosed with polyposis was 35.2 ± 5.1, while the mean age in the group of patients without polyposis was 33.2 ± 4.9 years. No significant difference was observed regarding the age ($p = 0.061$). In the group of patients diagnosed with polyposis, there were 32 patients with primary infertility (57.1%), while in the group of patients without polyposis, there were 17 patients with primary infertility (65.4%). The statistical analysis revealed no significant differences between the two groups observed regarding primary infertility ($p = 0.479$). The serum concentrations of TNF-α before hysteroscopic polypectomy were significantly lower in the experimental group of patients than the serum concentrations of TNF-α detected after hysteroscopic polypectomy

Table 1. Serum concentrations of tumor necrosis factor-α in the experimental (Exp) group of patients before and after hysteroscopic polypectomy

Exp group of patients	Mean value
Serum before hysteroscopy	15.6220 (10.48–28.06) [pg/ml]
Serum after hysteroscopy	22.7302 (13.57–40.79) [pg/ml]
t-test	Values
Hypothesis mean difference	0
Df	110
t stat	-6.946
P (T ≤ t)	0.0001
T critical two-tail	1.980

Table 2. Serum concentrations of tumor necrosis factor-α in the control group of infertile patients before and after hysteroscopy

Control group of patients	Mean value
Serum before hysteroscopy	13.5908 (6.70–29.26) [pg/ml]
Serum after hysteroscopy	14.5127 (4.12–42.56) [pg/ml]
t-test	Values
Hypothesis mean difference	0
Df	110
t stat	-0.917
P (T ≤ t)	0.361
T critical two-tail	2.871

Table 3. Concentrations of tumor necrosis factor-α in serum and uterine flushings in the experimental (Exp) group of infertile patients before hysteroscopic polypectomy

Exp group of patients	Mean value
Serum before hysteroscopy	15.6220 (10.48–28.06) [pg/ml]
Flushings	12.6046 (6.43.43–23.81) [pg/ml]
t-test	Values
Hypothesis mean difference	0
Df	110
t stat	4.450
P (T ≤ t)	0.0001
T critical two-tail	1.980

in the same patients. There was a statistically significant difference in the serum concentrations of TNF-α in infertile patients before and after hysteroscopic polypectomy ($p < 0.005$). This is shown in Table 1 and Figure 1.

The serum concentrations of TNF-α before hysteroscopic polypectomy were higher in the control group of infertile patients than the serum concentrations of TNF-α detected after hysteroscopic polypectomy in the same patients. However, this difference was not statistically significant ($p > 0.005$). This is shown in Table 2 and Figure 2.

The serum concentrations of TNF-α before hysteroscopic polypectomy were higher in the experimental group of infertile patients than the concentrations of TNF-α detected in uterine flushings of the same patients. The difference was statistically significant ($p < 0.005$). This is shown in Table 3 and Figure 3.

The serum concentrations of TNF-α before hysteroscopy were lower in the control group of infertile patients than the concentrations of TNF-α detected in uterine flushings of the same patients. The difference was not statistically significant ($p > 0.005$). This is shown in Table 4 and Figure 4.

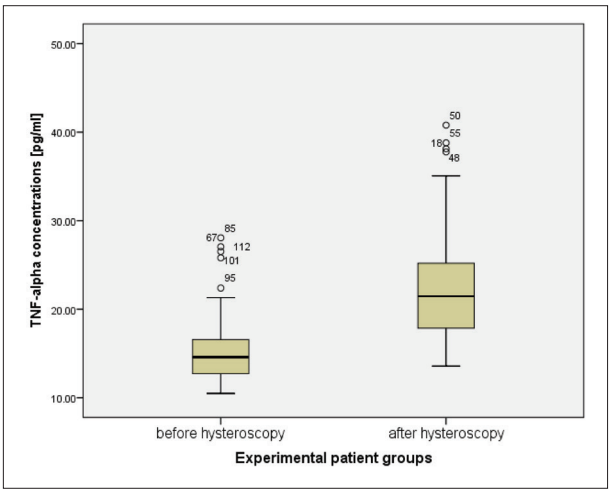

Figure 1. Serum concentrations of tumor necrosis factor-α (TNF-α) in the experimental group of patients before and after hysteroscopic polypectomy

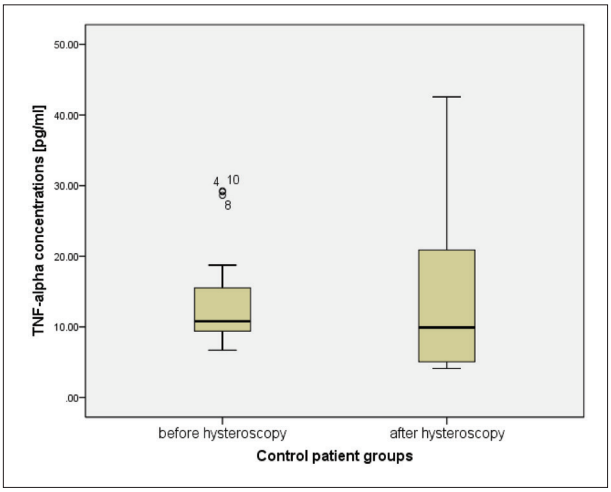

Figure 2. Serum concentrations of tumor necrosis factor-α (TNF-α) in the control group of infertile patients before and after hysteroscopy

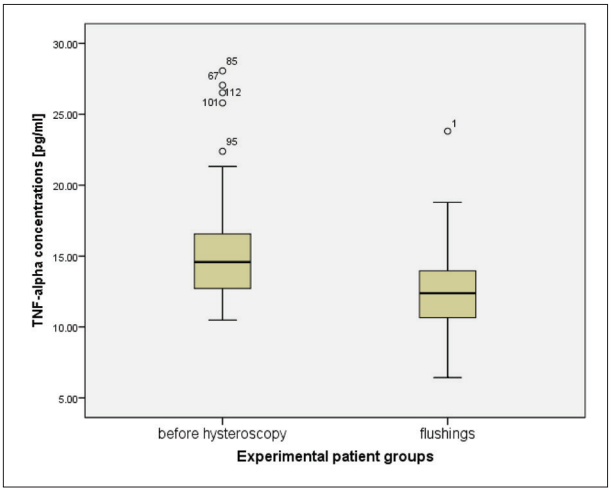

Figure 3. Concentrations of tumor necrosis factor-α (TNF-α) in serum and uterine flushings in the experimental group of infertile patients before hysteroscopic polypectomy

Table 4. Concentrations of tumor necrosis factor-α in serum and uterine flushings in the control group of infertile patients before hysteroscopy

Control group of patients	Mean value
Serum before hysteroscopy	13.5908 (6.70–29.26) [pg/ml]
Flushings	15.6673 (8.31–27.61) [pg/ml]
t-test	Values
Hypothesis mean difference	0
Df	47
t stat	-1.140
P (T ≤ t)	0.260
T critical two-tail	2.011

Table 5. Correlation of tumor necrosis factor-α concentrations in uterine flushings and serum in the experimental (Exp) group of infertile patients before and after hysteroscopic polypectomy

Pearson correlations	Flushings (Exp)	Serum before (Exp)	Serum after (Exp)
Flushings (Exp)	1	-0.18	0.108
Serum before (Exp)	-0.18	1	-0.132
Serum after (Exp)	0.108	-0.132	1

Table 6. Correlation of tumor necrosis factor-α concentrations in uterine flushings and serum in the control (Ctrl) group of infertile patients before and after hysteroscopy

Pearson correlations	Flushings (Ctrl)	Serum before (Ctrl)	Serum after (Ctrl)
Flushings (Ctrl)	1	0.482	0.696
Serum before (Ctrl)	0.482	1	0.398
Serum after (Ctrl)	0.696	0.398	1

The correlation between the TNF-α concentrations detected in all three samples retrieved from the experimental group of patients was examined. The peak values show the sample self-correlation. The correlation values between the samples indicate that there is not a correlation between the detected concentration values of TNF-α. In clinical terms, this means that polyps are associated with endometrial changes even after they have been removed. However, this does not indicate a correlation with serum either before or after polypectomy. This is shown in Table 5 and Figure 5.

The correlation between TNF-α concentrations in all three samples retrieved from the control group of patients was examined. The peak values show the sample self-correlation. The correlation values between the samples indicate that there is a correlation between the detected concentration values of TNF-α, which shows that the detected values are in accordance with similar statistical distributions. In clinical terms, this means that post-hysteroscopy concentration values of TNF-α in the endometrium and serum show a correlating distribution. The obtained results show a correlation between uterine flushings and serum prior to hysteroscopy. There is also a high correlation between uterine flushings and serum after hysteroscopy. This is shown in Table 6 and Figure 6.

DISCUSSION

Endometrial polyps are among the factors that are linked to certain conditions including infertility and pregnancy

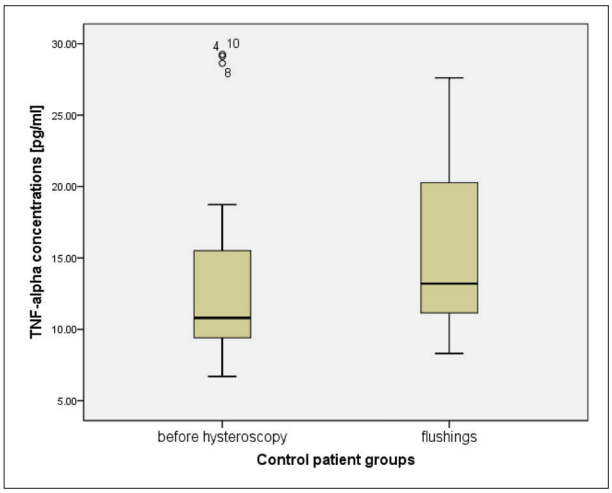

Figure 4. Concentrations of tumor necrosis factor-α (TNF-α) in serum and uterine flushings in the control group of infertile patients before hysteroscopy

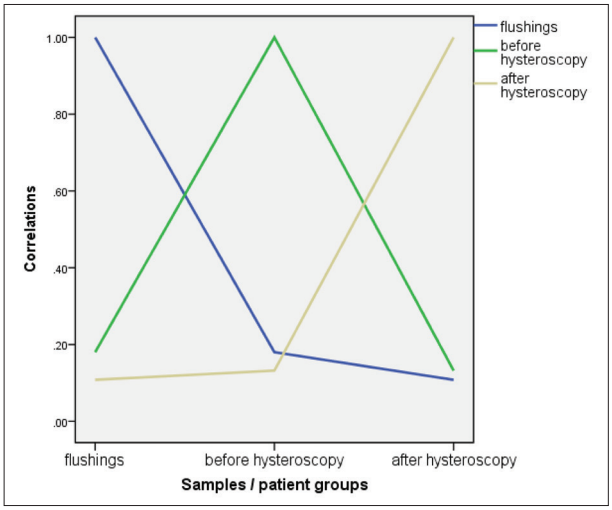

Figure 5. Correlation of tumor necrosis factor-α concentrations in uterine flushings and serum in the experimental group of infertile patients before and after hysteroscopic polypectomy

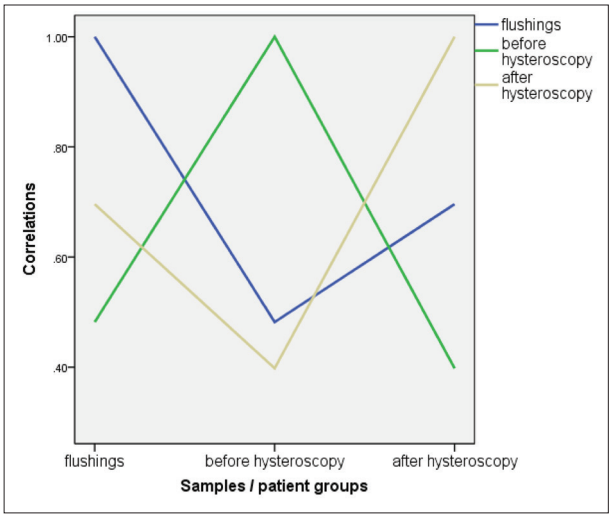

Figure 6. Correlation of tumor necrosis factor-α concentrations in uterine flushings and serum in the control group of infertile patients before and after hysteroscopy

loss in the early stages. The frequency of polyps in overall female population is 9–25% [18]. Even though polyps are asymptomatic in many cases, they can result in menstrual cycle disorders (e.g. intermenstrual bleeding). It has been proved that endometrial polyps can lead to infertility and early pregnancy loss. Nevertheless, the pathophysiological processes related to infertility caused by polyps have not been completely elucidated yet. It is believed that polyps may be the cause of abnormal bleeding, thus affecting the endometrial environment or they can have a negative impact on implantation conditions [19, 20]. It is assumed that infertility can be caused by a polyp of 1 cm in diameter. Uterine cavity assessment and endometrial polyp removal are routinely performed in infertile women. There has been evidence suggesting that hysteroscopic polypectomy resulted in increased pregnancy rates in infertile patients who had undergone the surgical procedure [21]. The human endometrium produces cytokines that act as important mediators between the embryo and decidua in the process of implantation. TNF-α belongs to the family of cytokines that are considered to be the most versatile. Not only does TNF-α play a prominent role in the synthesis of DNA in the early stage of proliferation, but it also contributes to cell differentiation and tissue remodeling [22]. Furthermore, TNF-α facilitates apoptotic processes, which initiates menstrual bleeding [23, 24]. In spite of infertility being perceived as a possible consequence of endometrial polyps, the fact is that the influence of endometrial polyps on endometrial implantation factors has not been sufficiently investigated yet. Based on the samples obtained before and after hysteroscopic polypectomy, the present study has shown that endometrial polyps found inside the uterine cavity affect TNF-α concentrations in both uterine flushings and serum of infertile women. The TNF-α concentrations obtained from the serum after hysteroscopic polypectomy were significantly higher in comparison to the serum concentrations of TNF-α before hysteroscopic polypectomy. These results are in line with previously reported findings of other authors [8]. The authors were able to prove that TNF-α secretion increased during the menstrual cycle after polypectomy had been performed and that it reached its peak during the mid-luteal menstrual cycle phase. TNF-α has multiple functions and its varying concentrations in different phases of the cycle suggest that it has an impact on the endometrium and has a complex role in the preimplantation of the embryo, which is a prerequisite for a successful implantation [22]. This is why it is important to follow up TNF-α concentrations after polypectomy. Apart from fostering DNA synthesis, TNF-α also improves cell differentiation and endometrial tissue remodeling. This is critical for a successful implantation. Abnormal TNF-α expression may further aggravate infertility linked to polyps and cause pregnancy loss in the early stages. Moreover, the mechanism behind a lower TNF-α synthesis caused by endometrial polyps is still not evident. What differentiates endometrial polyps from the surrounding endometrium is a massive fibrous stroma along with thick-walled dilated blood vessels. Such abnormal endometrial architecture can affect implantation regulators, i.e. it can result in their impaired secretion [25, 26]. It is important to point out that in the stromal component of endometrial polyps there is a lower number of hormone receptors. Therefore, the glands and stroma of polyps do not respond to progesterone stimulation, which may be the cause of abnormal secretion of progesterone in the endometrium [27, 28, 29]. A study that measured TNF-α concentration in uterine flushings of 12 patients before and after hysteroscopic polypectomy showed an increase in TNF-α concentrations after polyp removal with TNF-α values reaching the peak in the mid-luteal menstrual cycle phase [25]. These results are in line with previously reported findings by other authors [22]. There is a limited number of foreign publications dealing with this subject. No studies examining TNF-α concentration in uterine flushings and serum of infertile women with and without endometrial polyps have been published in Serbia to date. Similarly, no studies examining the effect of polypectomy in relation to TNF-α concentration in uterine flushings and serum have been published in Serbia thus far. The limitation of the previously reported studies including the present study is the small sample size.

CONCLUSION

TNF-α values in uterine flushings and serum before a surgical procedure were significantly higher in patients diagnosed with endometrial polyps in comparison to the TNF-α values obtained from women who were not diagnosed with endometrial polyps. TNF-α concentration in uterine flushings and serum of women diagnosed with endometrial polyps were significantly lower after surgery. TNF-α concentration in the group of women without endometrial polyps, i.e. the control group, were not significantly different before and after hysteroscopy. Furthermore, no significant difference was observed in the serum TNF-α concentrations in infertile patients who had undergone hysteroscopy in comparison to the infertile women who had no endometrial polyps.

Conflict of interest: None declared.

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Ефекат хистероскопске полипектомије на концентрацију фактора туморске некрозе алфа у испирку материце и серуму код инфертилних жена

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САЖЕТАК

Увод/Циљ Циљ овог рада је да прикаже промене нивоа фактора туморске некрозе алфа (*TNF-α*) у испирку утеруса и серуму инфертилних болесница пре и после хистероскопске полипектомије.

Методе Студија је обухватала 82 инфертилне болеснице. Болеснице су биле подељене у две групе. Прва група је била експериментална група коју је сачињавало 56 инфертилних болесница са ендометријалним полипом. Друга група је била контролна група коју је сачињавало 26 инфертилних жена без ендометријалног полипа.

Резултати Резултати овог истраживања примарно указују да се концентрације *TNF-α* у серуму и у испирку утеруса инфертилних жена са ендометријалним полипом разликују пре и после хистероскопске полипектомије. У контролној групи нису уочене значајне разлике у концентрацијама *TNF-α* у испирку утеруса и серуму болесница без ендометријалног полипа. Поређењем ове две групе уочена је статистички значајна разлика у нивоима *TNF-α* у испирку утеруса и серуму болесница.

Закључак Ендометријални полипи су један од узрока повећаних нивоа *TNF-α* у испирку утеруса и серуму болесница.

Кључне речи: цитокини; *TNF-α*; ендометријални полип; испирак утеруса; серум

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Efficiency and safety of intrathecal morphine for analgesia after hysterectomy

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SUMMARY

Introduction/Objective This prospective, randomized study was done to determine the efficiency and safety of the administration of intrathecal morphine chloride combined with the local anesthetic levobupivacaine given to female patients subjected to hysterectomy to ensure postoperative analgesia.

Methods The study sample consisted of 50 patients who were to undergo hysterectomy with adnexectomy and they were divided into two groups of 25 each. The patients in group A were given the combination of 0.3 mg of morphine chloride with 1.7 ml of 0.5% levobupivacaine immediately before the surgery, whereas the patients in group B were intravenously administered 5 mg of morphine chloride before the end of surgery, and after the surgery at certain time intervals. The postoperative pain was assessed at the first, sixth, 12th, and 24th hour by Numeric Rating Scale (NRS). Side effects, such as nausea, vomiting, itching and respiratory depressions were followed as well.

Results The postoperative pain was less expressed at any time interval both at rest and on exertion in group A ($p < 0.001$) and therefore the need for additional analgesia was less in group A ($p < 0.001$). The subjective feeling of satisfaction with postoperative analgesia was statistically significant in group A ($p < 0.001$).

Conclusion Intrathecal administration of morphine chloride combined with levobupivacaine ensures better postoperative analgesia after hysterectomy than intravenous morphine chloride, their side effects being equally frequent.

Keywords: hysterectomy; intrathecal morphine; postoperative analgesia

INTRODUCTION

Acute pain is the body's mechanism to signal tissue injury and danger [1]. The intensity of postoperative pain after hysterectomy ranges from moderate to severe. The efficient pain control leads to the earlier mobilization of patients and their faster recovery, thus leading to shorter hospitalization and lower treatment expenses [2–5]. Controlling acute pain after surgery is important not only in the immediate postoperative phase but also to prevent chronic postsurgical pain, which can develop in as many as 10% of patients [6].

The aim is to achieve good analgesia with minimum side effects. Currently, it cannot be said that there is an ideal mode of analgesia. Intravenous opioids and other analgesics are most frequently used drugs to alleviate the postoperative pain during the first 24 hours. The use of opioids for patients who have surgery presents a particularly challenging problem requiring clinicians to balance managing acute pain in the postoperative period and minimizing the risks of persistent opioid use after surgery [7].

Another good technique is epidural analgesia. A systemic review has shown that the continuous epidural analgesia is superior to the patient controlled analgesia (PCA) in the patients having had a major intra-abdominal surgery [8]. However, this procedure is a bit more invasive because a catheter must be inserted, which has to remain in the epidural space for at least 24 to 48 hours.

Intrathecal administration of local anesthetics with small doses of opioids is an attractive analgesic technique because the drugs are given directly into the liquor, close to the structures of the central nervous system through which these drugs act [9].This procedure is simple, easy to be performed, with a relatively low risk of failure. It is expected to decrease the postoperative pain as well as the necessity for additional analgesia. It has been shown to be effective in the alleviation of postoperative pain after prostatectomy, transurethral resection of the prostate and hepatectomy [10, 11, 12]. The most frequently used opioid is morphine. Analgesic effect of morphine administered intrathecally may persist for up to 48 hours [13]. A disadvantage of this technique is the occurrence of side effects such as nausea, vomiting, itching, urine retention, and respiratory depression, being the most severe complication [14–17].

This study was aimed at determining the efficiency and safety of administration of intrathecal

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morphine combined with a local anesthetic given to ensure analgesia in the patients to undergo hysterectomy.

METHODS

This prospective, randomized study included 50 patients from the elective surgical program performed at the Department of Surgical Oncology, Institute of Oncology of Vojvodina in Sremska Kamenica. The patients underwent hysterectomy with bilateral adnexectomy and lymphadenectomy. The study, which had been approved by the Ethics Committee of the Institute of Oncology of Vojvodina, was conducted from September 1, 2017 to February 15, 2018. The patients were divided into two groups according to the applied technique of postoperative analgesia:

Group A (25 subjects) – the patients having been given morphine chloride intrathecally in the combination with levobupivacaine before the surgery;

Group B (25 subjects) – the patients given morphine chloride intravenously before the end of surgery (when suturing began) and after the surgery.

The inclusion criteria were:

- Age ≥ 18 years
- American Society of Anesthesiologists (ASA) classification I or II
- Diagnosed neoplasm of the uterine body or cervix

The exclusion criteria were:

- Allergic reaction to morphine, local anesthetic, and metamizole
- Age < 18 years.

The patients meeting criteria were given both verbal and written details about the study method and aims as well as about the technique of premedication and anesthesia for the planned surgical intervention. The ones who had given their written consent to participate were included in the study sample.

The group A patients were given 0.3 mg of morphine chloride intrathecally with 1.7 ml of 0.5% levobupivacaine (L3–L4 space, 26G needle) 15 minutes before anesthesia, and the group B patients were intravenously given 5 mg of morphine chloride before the end of surgery when suturing began. Anesthesia in both groups of patients was general and balanced. All patients were premedicated with midazolam (0.05 mg/kg intravenously), and propofol (1.5 mg/kg) was used to introduce anesthesia along with opioid analgesic fentanyl (1.5 μg/kg). Rocuronium (1 mg/kg) was administered for muscle relaxation for intubation as well as during the surgery at a dose adequate to maintain the relaxation. Anesthesia was maintained with sevoflurane (1 minimum alveolar concentration). The lung ventilation was ensured with the gas mixture O_2:N_2O 40:60 so that $EtCO_2$ < 38 mmHg. Electrocardiogram, blood pressure, heart frequency, SpO_2, $EtCO_2$ and body temperature were monitored during the surgery. Boluses of fentanyl (50 μg) and of rocuronium (10 mg) were given when necessary. Anesthetics were administered in such a way so to provide satisfactory anesthesia, blood pressure values and heart frequency in the values ± 30% as compared with the values prior to anesthesia. Drugs for reversal of neuromuscular blockade (neostigmine 0.02 mg/kg and atropine 0.01 mg/kg) were given at the end of surgery. In addition, metoclopramide 10 mg intravenous was administered at the end of surgery, immediately before the patients woke up, to prevent nausea and vomiting. The group B patients were given metoclopramide at eight-hour intervals during the first 24 hours after the surgery.

The group A patients were given intravenous 2.5 g (maximum 5 g/24 h) of metamizole when necessary for postoperative analgesia in case of pain exceeding grade 4 according to the numeric rating scale (NRS), whereas morphine chloride (5 mg intravenously/6–8 hours) and metamizole 2.5 g (maximum 5 g/24 h) were administered alternatively after the surgery to the group B patients and 2–5 mg of intravenous morphine chloride was given when pain exceeded grade 4 according to NRS. The dose of additional morphine chloride in group B was dependent on the age and body weight of the patients, according to the protocol of our clinic.

Numeric values of postoperative pain intensity assessed according to NRS at rest, while coughing and active moving were recorded at intervals of one, six, 12, and 24 hours. These values ranged from 0 to 10 (0 – no pain, 10 – the most severe possible pain). In addition, the patients were asked to keep records of their subjective feeling of satisfaction (SFS) with analgesia, assessing it as: 1 – poor, 2 – medium, 3 – good, and 4 – excellent. Complications such as nausea, vomiting, itching, and respiratory depression were also recorded. All patients had urinary catheter so we could not follow urinary retention.

The data were analyzed and processed by IBM SPSS statistics 10.0 software (SPSS Inc., Chicago, IL, USA) and given in tables and figures created in Word and Excel Microsoft Office 2003 packs. The results were presented using standard statistical methods: frequency (f), arithmetic means (x), standard deviation (SD), value intervals (maximum and minimum), and percentages (%). The patients' characteristics were compared with Student's t-test and χ^2 test, $p < 0.05$ being the statistically significant value.

RESULTS

The study sample consisted of 50 female patients divided into two groups of 25 each. There were no statistically significant differences in age, body weight, ASA classification and the length of surgery between the group A and B (Table 1).

Table 1. Patient characteristics

Parameters	Group A	Group B	p
Age (years ± SD)	56.6 ± 11.1	57.7 ± 9.8	0.707
Weight (kg ± SD)	73.6 ± 13.2	75.5 ± 13.8	0.624
ASA I n (%)	2 (8)	3 (12)	1.000
ASA II n (%)	23 (92)	22 (88)	
Duration of the operation (min ± SD)	174.2 ± 38.8	150 ± 49.7	0.061

ASA – American Society of Anesthesiologists classification I or II

Table 2. Mean NRS (at rest) one, six, 12, and 24 hours after surgery

Parameters	Group A				Group B					
	Mean	Min	Max	Standard deviation	Mean	Min	Max	Standard deviation	t-test	p
NRS 1 h	1.04	0	5	1.485	4.32	0	9	1.97	-6.640	< 0.001
NRS 6 h	0.08	0	1	0.277	2.44	0	5	1.356	-8.523	< 0.001
NRS 12 h	0.04	0	1	0.2	1.44	0	3	1	-6.842	< 0.001
NRS 24 h	0	0	0	0	1.2	0	4	1	-6.000	< 0.001

NRS – Numeric Rating Scale

Table 3. Mean NRS on exertion, one, six, 12, and 24 hours after surgery

Parameters	Group A				Group B					
	Mean	Min	Max	Standard deviation	Mean	Min	Max	Standard deviation	t-test	p
NRS 1 h	1.48	0	6	2.044	6	2	10	1.848	-8.201	< 0.001
NRS 6 h	0.20	0	3	0.707	3.88	2	6	1.301	-12.424	< 0.001
NRS 12 h	0.08	0	2	0.400	2.76	1	5	0.879	-13.87	< 0.001
NRS 24 h	0.00	0	0	0.000	2.24	1	5	0.969	-11.552	< 0.001

NRS – Numeric Rating Scale

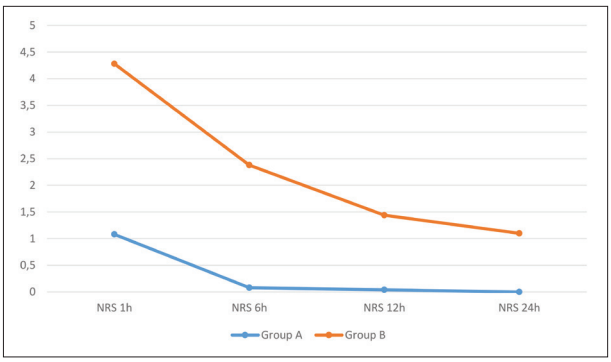

Figure 1. Mean NRS at rest, one, six, 12, and 24 hours after surgery

NRS – Numeric Rating Scale

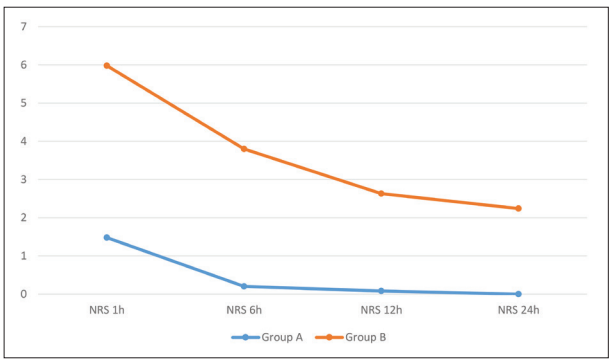

Figure 2. Mean NRS on exertion, one, six, 12, and 24 hours after surgery

NRS – Numeric Rating Scale

Table 2 and Figure 1 show the mean values of pain at rest assessed according to NRS during 24 hours after the surgery. A statistically significant difference was recorded. Patients in group A, who had received morphine chloride intrathecally, had the values lower than the numeric values found in the group B patients, and the difference was even more expressed after the first and sixth hour than after the 12th and 24th hour.

Table 3 and Figure 2 show the mean values of pain on exertion assessed according to NRS during the period of 24 hours after the surgery, with a statistically significant difference to the benefit of the group A patients.

The results of cross comparison of the need for additional analgesia an hour after the end of surgery are shown in Table 4. As it can be seen, only two patients, who had received morphine chloride intrathecally, needed additional analgesia, whereas the majority the group B patients needed additional analgesia. This difference was statistically highly significant ($\chi^2 = 12.834$; $p < 0.001$).

Tables 5, 6, 7 and 8 show the development of nausea as a side effect of analgesic. No statistically significant difference was found between the groups in various time intervals during the first 24 hours after the surgery.

There was no statistically significant difference in vomiting as a side effect of analgesic during the period of 12 hours after the surgery, as shown in Tables 9, 10, and 11. None of the patients had this problem 24 hours after surgery.

Table 4. Need for additional analgesia one hour after surgery

Additional analgesia after 1h	Total	Group A	Group B	χ^2	p
No n (%)	33 (66)	23 (92)	10 (40)	12.834	< 0.001
Yes n (%)	17 (34)	2 (8)	15 (60)		
Total n (%)	50 (100)	25 (100)	25 (100)		

Possible side effects of opioid analgesia are itching and respiratory depression. However, none of our patients developed any of them.

Table 12 shows the SFS with analgesia. Our patients assessed analgesia as: 1 – poor, 2 – medium, 3 – good, and 4 – excellent.

DISCUSSION

There were not statistically significant differences between the two groups regarding age, body weight, ASA classification and the length of surgery. We opted for the dose of 0.3 mg of morphine chloride based on a meta-analysis and a literature review [18, 19].

The results of pain assessment show that pain, both at rest and on exertion, was statistically significantly less expressed in patients having received morphine chloride intrathecally (Group A). The difference was even more

Table 5. Nausea one hour after surgery

Parameter			Group A	Group B	χ^2	p
Nausea 1h	No	n	23	20	1.495	0.417
		%	92	80		
	Yes	n	2	5		
		%	8	20		
Total		n	25	25		
		%	100	100		

Table 6. Nausea six hours after surgery

Parameters			Group A	Group B	χ^2	p
Nausea 6 h	No	n	23	21	0.758	0.667
		%	92	84		
	Yes	n	2	4		
		%	8	16		
Total		n	25	25		
		%	100	100		

Table 7. Nausea 12 hours after surgery

Parameters			Group A	Group B	χ^2	p
Nausea 12 h	No	n	23	20	1.495	0.417
		%	92	80		
	Yes	n	2	5		
		%	8	20		
Total		n	25	25		
		%	100	100		

Table 8. Nausea 24 hours after surgery

Parameters			Group A	Group B	χ^2	p
Nausea 24 h	No	n	25	22	3.191	0.235
		%	100	88		
	Yes	n	0	3		
		%	0	12		
Total		n	25	25		
		%	100	100		

Table 9. Vomiting one hour after surgery

Parameters			Group A	Group B	χ^2	p
Vomiting 1 h	No	n	24	24	0.000	1.000
		%	96	96		
	Yes	n	1	1		
		%	4	4		
Total		n	25	25		
		%	100	100		

Table 10. Vomiting six hours after surgery

Parameters			Group A	Group B	χ^2	p
Vomiting 6 h	No	n	24	24	0.000	1.000
		%	96	96		
	Yes	n	1	1		
		%	4	4		
Total		n	25	25		
		%	100	100		

Table 11. Vomiting 12 hours after surgery

Parameters			Group A	Group B	χ^2	p
Vomiting 12 h	No	n	23	23	0.000	1.000
		%	92	92		
	Yes	n	2	2		
		%	8	8		
Total		n	25	25		
		%	100	100		

Table 12. The subjective feeling of satisfaction (SFS) with analgesia

Parameters			Group A	Group B	χ^2	p
SFS	2 (medium)	n	0	4	29.684	0.001
		%	0	16		
	3 (good)	n	1	16		
		%	4	64		
	4 (excellent)	n	24	5		
		%	96	20		
Total		n	25	25		
		%	100	100		

prominent immediately after the surgery. Only two patients from group A asked for additional analgesia after the first postoperative hour and they received 2.5 gr of intravenous metamizole. The patients from this group required additional analgesia only 27 hours after the moment of intrathecal administration of morphine chloride. However, as many as 15 patients from group B asked for additional analgesia after the first hour and they received 2–5 mg of morphine chloride intravenously. The average consumption of morphine chloride after 24 hours was 21.7 mg in group B, which confirms the statement made by El Sherif et al. [20] who claimed that intrathecal morphine has about 100 times more potency than intravenous morphine. Jacobson et al. [21] believed that intrathecally administered morphine yields effective long-lasting analgesia (over 20 hours) and that the dose from 0.3 mg to 1 mg should ensure good analgesia without the major complication – respiratory depression. A team of Danish researchers has found in their study that it took 27 hours on average from the administration of 0.1 mg and 0.2 mg of intrathecal morphine given for Cesarean section until the first postoperative analgesic [22]. They recommend 0.1 mg as a dose of choice. Slappendel et al. [23] have compared various doses of intrathecal morphine in the patients having had hip replacement and their conclusion was that the dose of 0.1 mg ensures good analgesia with minimum side effects. An Egyptian team of researchers performed a study on safety and analgesic efficiency of intrathecal morphine (0.2, 0.5, and 1 mg) in patients undergoing a major abdominal surgery for cancer. They concluded that 1 mg of morphine resulted in superior analgesia 48 hours after surgery in comparison with 0.2 and 0.5 mg, without a significant difference in the frequency of side effects [24]. Urban et al. [25] have found that the dose of 20 μg/kg of intrathecal morphine considerably decreases the need for additional postoperative analgesia after lumbar fusion

surgery. It can be concluded that the dose of morphine, which is necessary for good postoperative analgesia, depends on the type of surgical procedure.

The following side effects were monitored: nausea, vomiting, itching, and respiratory depression. Since all patients had the urinary catheter, urinary retention, as a side effect, was not followed.

Nausea was less expressed in the patients who had received morphine chloride intrathecally; however, the difference was not statistically significant. Vomiting was equally frequent in both groups. It should be emphasized that the group A patients were given metoclopramide at the end of surgery as well as in the postoperative period if necessary because of nausea and vomiting, whereas the group B patients were given metoclopramide at the end of surgery and eight hours after the surgery. Similar results have been reported in other studies as well [26, 27].

None of our patients reported itching nor was respiratory depression observed as a side effect of morphine. Itching is often mentioned in literature as a frequent side effect of intrathecal morphine, particularly in obstetrics, where gestation hormones may lead to certain changes on opioid receptors [28]. Some researchers from the Netherlands have shown that intrathecal administration of morphine for laparoscopic colon resection is followed with itching more often [29].

Based on their study Jacobson et al. [21] have concluded that nausea, vomiting, and itching develop irrespectively of the dose given, whereas respiratory depression occurs after a dose exceeding 1 mg. In 2009, Gehling and Tryba [18] performed a meta-analysis of randomized controlled studies, which had dealt with the application of intrathecal morphine combined with spinal anesthesia. Their analysis included the following surgical procedures: Cesarean section, orthopedic surgery, gynecologic surgery, arthroscopy, transurethral prostatectomy, and hemorrhoidectomy. Morphine was administered in the doses ranging 0.025–2.5 mg. It has been found that the doses ≥ 0.3 mg increase the incidence of itching. The incidence of respiratory depression in the patients given a dose < 0.3 mg was 1%, whereas it was 9% in case of a dose ≥ 0.3 mg. The authors believe that intrathecal morphine requires prophylaxis and treatment of side effects. In addition, the respiratory function must be continuously followed for 24 hours. The same must be done in the patients who received systemic opioids. There is no evidence to corroborate the extended monitoring of patients receiving low doses of morphine.

The SFS with analgesia may be the most important factor when opting for postoperative analgesia. As many as 96% of the group A patients assessed analgesia as excellent, whereas only 20% of those from group B were of that opinion, which is in discrepancy with a similar study which showed almost equal satisfaction of patients with analgesia [29].

Finally, we should also mention a study performed by an American team of researchers on pregnant women who underwent Cesarean section delivery [30]. The study sample was divided according to the wish of the pregnant women when asked whether they wanted 0.1 mg or 0.2 mg of morphine after having been adequately informed about the advantages and shortcomings of such analgesia. The need for additional analgesia was found to be bigger in the patients who had received 0.2 mg of morphine. Therefore, it can be concluded that postoperative analgesia is a multifactorial problem, where the subjective feeling of the patients themselves and their fear of surgery, postoperative pain, and side effects are of uppermost importance.

Potential limitations to our study are a small sample and a subjective assessment of pain intensity according to NRS. It is difficult to observe a rare side effect in a small study sample. A similar study including a higher number of subjects would contribute to the strength of our conclusions. Urinary retention could not be followed as a side effect in this study because of routine placement of urinary catheter.

CONCLUSION

According to our study, it can be concluded that the combination of 0.3 mg of morphine chloride and 1.7 ml of 0.5% levobupivacaine provides very good analgesia after hysterectomy with adnexectomy and lymphadenectomy lasting for 24 hours after the surgery. Side effects do not differ from those associated with intravenous administration of opioids. Nausea and vomiting can be treated with antiemetics. Although respiratory depression was not observed in our study sample, intrathecal morphine must be administered with great caution and respiratory function must be monitored during the first 24 hours. Finally, our study has shown that the patients who received morphine chloride intrathecally were more satisfied than those who were given morphine intravenously.

Conflict of interest: None declared.

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Ефикасност и сигурност примене интратекалног морфина за аналгезију после хистеректомије

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САЖЕТАК

Увод/Циљ Циљ ове проспективне, рандомизоване студије је да одреди ефикасност и сигурност примене интратекалног морфин-хлорида у комбинацији са локалним анестетиком левобупивакаином за обезбеђивање постоперативне аналгезије код болесница које се подвргавају хистеректомији.

Методе Педесет болесница којима је индикована хистеректомија са аднексектомијом и лимфаденектомијом је подељено у две групе од по 25 испитаница. Испитаницама из групе А је непосредно пре операције интратекално апликована комбинација 0,3 *mg* морфин-хлорида и 1,7 *ml* 0,5% левобупивакаина, док су оне из групе Б интравенски добијале 5 *mg* морфин-хлорида пре завршетка операције и постоперативно у одређеним временским интервалима. Постоперативни бол је оцењен првог, шестог, 12. и 24. сата, уз помоћ нумеричке скале бола. Праћена су и нежељена дејства у виду мучнине, повраћања, свраба и респираторне депресије.

Резултати Постоперативни бол је у свим интервалима праћења, како у миру тако и приликом напора, био мање изражен у групи А ($p < 0,001$); због тога је и потреба за додатном аналгезијом била мања у групи А ($p < 0,001$). Субјективни осећај задовољства постоперативном аналгезијом је статистички значајан у групи А ($p = 0,001$).

Закључак Инратекално давање морфин-хлорида у комбинацији са левобупивакаином обезбеђује бољу постоперативну аналгезију после хистеректомије, у односу на интавенски морфин-хлорид, уз подједнаку учесталост нежељених дејстава.

Кључне речи: хистеректомија; интратекални морфин; постоперативна аналгезија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Clinical characteristics and surgical treatment of dacryocystitis – a ten-year retrospective study

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SUMMARY

Introduction/Objective Nasolacrimal duct obstruction with consequent epiphora and the development of dacryocystitis (DC) represents a common pathological entity in the clinical practice of ophthalmologists and maxillofacial surgeons. The etiology of DC is multifactorial and still has not been clarified in detail. It is considered that ascending infections from the nasal cavity and paranasal sinuses, injuries and surgical interventions in the middle third of the face, dacryoliths, tumors of the lacrimal sac and surrounding structures may be some of the etiological factors of nasolacrimal duct obstruction.
The aim of this study is to present clinical characteristics and surgical treatment of DC.

Methods A retrospective study was carried out. It covered a period of 10 years during which 49 patients with clinically verified DC were treated after surgical examination and complete diagnostics. Out of the total number, 37 patients underwent surgery.

Results The occurrence of predisposing factors was present in 80% of the patients – rhinitis and the inflammation of paranasal sinuses in 27 patients (72%), injuries and surgical interventions in the middle third of the face in nine patients (24%), whereas lacrimal sac and nasolacrimal duct tumors were noted in three patients (8%). Surgical failure, which was manifested in terms of recurrent DC and epiphora, was noted in six cases (16%).

Conclusion Regarding the possible complications of inadequately administered antibiotic therapy and a broad spectrum of pathological entities which comprise the differential diagnosis, dacryocystorhinostomy with an adequate histopathological analysis and appropriate antibiotic therapy in the acute stage represents a right way for the treatment of DC.

Keywords: dacryocystitis; predisposing factors; differential diagnosis; surgical treatment

INTRODUCTION

Nasolacrimal duct obstruction (NLDO) with consequent epiphora and the development of dacryocystitis (DC) represents a common pathological entity in the clinical practice of ophthalmologists and maxillofacial surgeons [1].

The etiology of DC is multifactorial and still has not been clarified in detail. It is considered that ascending infections from the nasal cavity and paranasal sinuses, injuries and surgical interventions in the middle third of the face, dacryoliths, tumors of the lacrimal sac and surrounding structures may be some of the etiological factors of NLDO [2].

The acute dacryocystitis (ADC) is characterized by the appearance of hyperemia and a painful swelling in the medial canthus region, as opposed to the chronic form (CDC) which is characterized by a persistent painless swelling in the mentioned region with signs of mucopurulent exudation from the lacrimal punctum, epiphora, chronic conjunctivitis, and episodes of exacerbation of the chronic process.

The congenital form of DC is statistically the rarest form found in 5% of infants [3]. It is a very serious disease characterized by a high mortality rate if not treated adequately.

The initial treatment of ADC implies a systemic and local administration of antibiotics, incision, and drainage of the lacrimal sac content, which leads to decompression, evacuation of content and possible microbiological analyses. The absence of treatment of the acute stage may lead to complications such as preseptal and orbital cellulitis, meningitis, and cavernous sinus thrombosis.

The final treatment involves dacryocystorhinostomy (DCR), which can be external or endonasal. Both procedures, external dacryocystorhinostomy (ext-DCR), described by Addeo Toti in 1904, and endonasal dacryocystorhinostomy (endo-DCR), described by Caldwell in 1983, have undergone numerous modifications over time [4].

The aim of this study is to present clinical characteristics and surgical treatment of DC.

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METHODS

A retrospective study was carried out. It covered a period from 2006 to 2015 during which 49 patients with clinically verified DC were treated after a surgical examination and complete diagnostics. Out of the total number, 37 patients underwent surgery.

All patients were surgically treated under general anesthesia at the Maxillofacial Surgery Clinic, Faculty of Medicine, University of Niš.

The analysis included the sex and age of patients, existence of chronic diseases, and occurrence of predisposing factors, i.e. existence of rhinitis, sinusitis, as well as injuries or surgical interventions in the middle third of the face. It also studied clinical characteristics of DC in terms of acute or chronic presentation of the process, localization, histopathology, microbiological analyses, recurrence, and postoperative complications of all patients, which involved epiphora, or recurrent DC. All patients underwent ext-DCR under general endotracheal anesthesia along with keeping a silicone tube for two months (Figures 1 and 2).

Classic ext-DCR with mono or bicanalicular silicone intubation, depending on the clinical manifestation of DC, was performed in all patients. The purpose of the above surgical procedures is based on the removal of the cystic sacs and the *de novo* formation of the nasolacrimal duct, which allows the normalization of the function of the lacrimal apparatus. The minimal period of postoperative monitoring was 18 months.

A multi slice computerized tomography was performed preoperatively in four patients with suspected lacrimal sac tumor in order to determine the extent of process and to plan further treatment.

This paper was approved by the institutional ethics committee, and written consent was obtained from the patients for the publication of this study and any accompanying images.

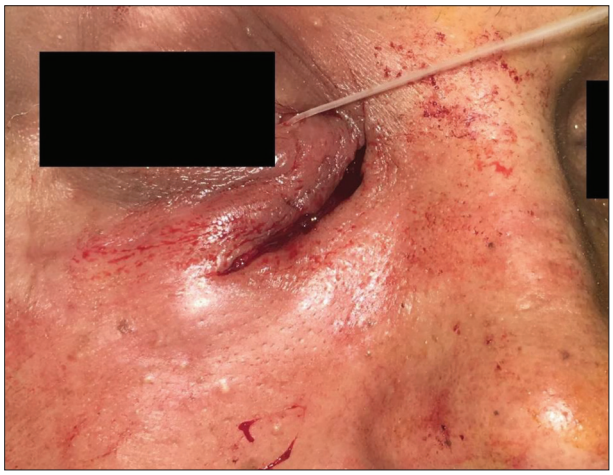

Figure 1. Condition after silicone single-channel tube placement; the photograph is used with the permission of the subject

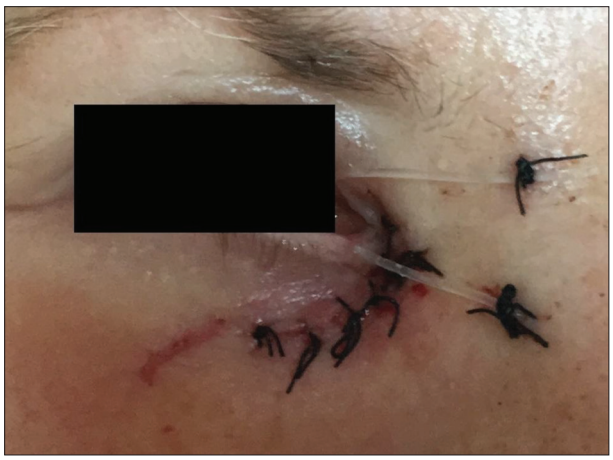

Figure 2. Condition after silicone double-channel tube placement; the photograph is used with the permission of the subject

RESULTS

The mean age of the mentioned group of patients was 56, with the age interval ranging from 27 to 72. Considering sex, 28 patients (75%) were female, whereas nine patients included in the study (25%) were male.

The presence of chronic systemic diseases was determined in 30 patients (81%). Chronic arterial hypertension was present in 20 patients, diabetes mellitus in 10, chronic obstructive pulmonary disease in eight, glaucoma in five, and hyperthyroidism and rheumatoid arthritis in four patients.

The occurrence of predisposing factors was present in 80% of the patients (Table 1); 18 patients consulted a doctor in the acute stage of the disease. They were treated with broad-spectrum antibiotics (2nd or 3nd generation cephalosporins and clindamycin) until clinical and laboratory results indicated the regression of the signs of infection. Incisions in the sac region were made in 10 cases. The ADC was more frequent in younger patients.

Table 1. The occurrence of predisposing factors

Predisposing factors	Patients (n, %)
Rhinitis and the inflammation of paranasal sinuses	27 (72%)
Injuries and surgical interventions in the middle third of the face	9 (24%)
Lacrimal sac and nasolacrimal duct tumors	3 (8%)

Initially, a chronic process was present in 31 patients. The congenital form of DC was not included in the study.

DC was more common on the left than on the right side (Figure 3).

Microbiological analyses indicated dominant presence of gram-positive flora. *Staphylococcus aureus*, staphylococcal pneumonia, and *Staphylococcus epidermidis* were isolated in 85% of cases; equally present both in the acute and chronic process. Methicillin-resistant *Staphylococcus aureus* (MRSA) was isolated in two cases. Gram-negative bacteria, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Neisseria* and *Klebsiella* were isolated in 10 patients, exclusively in ADC. Three microbiological findings of CDC were negative.

Surgical failure, which was manifested in terms of recurrent DC and epiphora, was noted in six cases (16%).

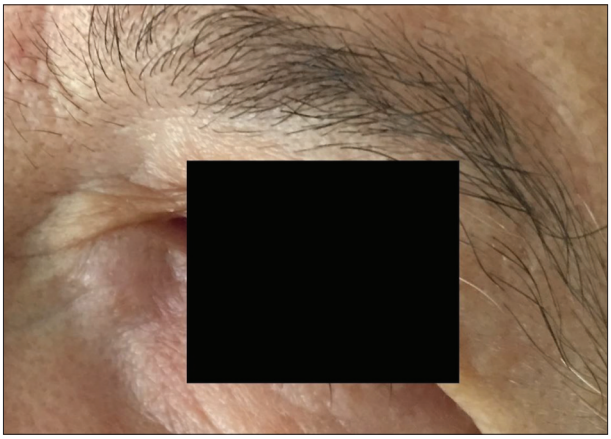

Figure 3. Chronic form of dacryocystitis; the photograph is used with the permission of the subject

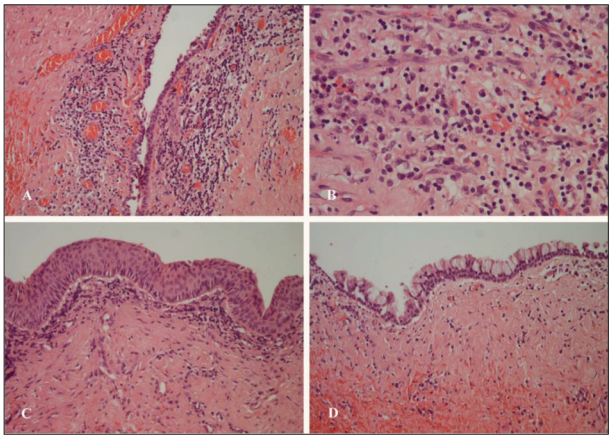

Figure 4. Pathohistological image of chronic dacryocystitis; the epithelium is usually cubical, with two layers (A) (H&E, ×10); the submucosa contains a large number of blood vessels (neovascularization), as well as a thick, chronic inflammation infiltrate (lymphocytes, plasmocytes, histocytes) (B) (H&E, ×40); the epithelium can show squamous metaplasia (C) and goblet cell hyperplasia foci (D) (H&E, ×20)

In three patients after DCR, a recurrence of the disease appeared on average four weeks after the surgery. Initially, the patients in question were diagnosed with ADC, which was treated with broad-spectrum antibiotics and incisions. Given that microbiological findings indicated the presence of gram-negative bacteria, no recurrences were noted after the administration of therapy based on the antibiogram.

In two cases, the anamnesis showed the existence of long-standing episodes of CDC treated out-patiently at another medical institution. After DCR had been performed, a recurrence of the underlying disease appeared two weeks after the stitches removal. Considering that microbiological analyses indicated the existence of MRSA, the patients were treated with intensive antibiotic therapy with vancomycin, after which the signs of infection diminished. Reinterventions were carried out after the regression of infection, after which no recurrences were noted.

In one case, recurrences appeared two weeks after the accidental loss of a silicone tube. No recurrences were noted after the reintervention and placing a new silicone tube.

Histopathological (HP) analyses after DCR indicated that chronic nongranulomatous inflammation was reported in 34 cases (91%) (Figure 4), the presence of papilloma in two cases, whereas lacrimal sac adenocarcinoma was reported in one case.

DISCUSSION

DC is the inflammation of the lacrimal sac clinically presented in the ADC and CDC. The process is more frequent in females above the age of 40, contrary to the congenital form, which is equally present in both sexes and represents 1% of the total number of all types of DC [5]. A more frequent occurrence of DC in females than in males is explained by a smaller diameter of the nasolacrimal duct and therefore bigger chances for the appearance of a pathway and consequent infection. The ADC is more common in the young. Similar results were presented in the study by Eshaghiet et al [6].

Greater incidence of DC on the left compared to the right side is a consequence of a sharper angle between the lacrimal sac and the nasolacrimal duct, therefore creating a greater possibility for the disruption of drainage, pathway, and a consequent infection, which is in correlation with the results of the study [7].

Ext-DCR, which uses transcutaneous access to enable exquisite visibility of the operative field, more control over intraoperative complications, and a shorter surgical course, is a surgical method of choice in treating DC. The success of the mentioned technique ranges from 80% to 96%, which is also in correlation with the results of our study [8].

In all cases, a silicone tube was placed despite the research conducted by Feng et al. [9] who concluded that success of the initial ext-DCR both with and without placing a silicone tube was identical.

The process of endo-DCR, which statistically shows identical success as the aforementioned procedure, has never been carried out in our institution due to the lack of technical possibilities [10].

Microbiological analyses indicated the presence of combined bacterial flora, i.e. the presence of both gram-positive and gram-negative bacteria.

A study, which included microbiological findings from 84 ADC and CDC, reported that *Staphylococcus aureus* was the most common gram-positive bacteria present in 28.8% of cases, equally present both in acute and chronic processes [11]. The existence of MRSA, which has been statistically increasing since 1998, is related to frequent episodes of exacerbation of the chronic form of the disease and the appearance of recurrences after DCR [12]. Gram-negative bacteria are in most cases associated with the ADC, foudroyant clinical course, and frequent recurrences, with *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Neisseria*, *Klebsiella*, and *Escherichia coli* being the most common ones [13].

In a study which retrospectively encompassed 377 HP findings after performed DCR, Anderson et al. [14] reported a dominant presence of chronic nongranulomatous

inflammation (321, 85.1%), granulomatous inflammation including sarcoidosis (eight, 2.1%), lymphoma (seven, 1.9%) and a total of five malignant tumors. The authors of the study stressed that the clinical course of the mentioned malignancies completely corresponded to the clinical image of CDC and suggested an obligatory HP analysis after each DCR. The dominant presence of chronic inflammation (85.1%) marked rhinitis and inflammation of paranasal cavities as possible etiological factors of DC. The results of the aforementioned study are in correlation with our results.

A study by Lefebvre et al. [15], which included 49 patients with performed DCR, reported surgical failure in seven cases (13%). Surgical failure occurred in patients with MRSA, gram-negative bacteria, rhinosinusitis, lymphoma, early loss of a silicone tube and Crohn's disease. In our study, the reasons of DCR failure were associated with MRSA infection in two cases, gram-negative bacteria infection in two cases, and an accidental loss of a silicone tube in one case. The occurrence of surgical failure associated with MRSA and gram-negative bacteria is also emphasized in studies by other authors [16].

The available literature suggests that the recommended silicone tube retaining time is at least two months after DCR [17]. Accidental loss or early removal is associated with the appearance of NLDO. In our study, all patients had the tube removed after two months, except for one patient, i.e. a case of accidental loss. In a study, which included 25 patients with evidently high risk of postoperative failure after DCR, Sodhi PK et al. [18] suggested the removal of a silicone tube to be six months after surgery.

CONCLUSION

DC is a common pathological entity in everyday clinical practice, more frequent in women above the age of 40.

Given the possible complications, inadequately administered antibiotic therapy and a broad spectrum of pathological entities, which comprise the differential diagnosis, DCR with an adequate HP analysis and appropriate antibiotic therapy in the acute stage represents a right way for the treatment of DC.

The success of the mentioned procedure, which statistically varies from 80% to 90%, confirms our choice of therapy in the treatment of DC.

Conflict of interest: None declared.

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Клиничке карактеристике и хируршки третман дакриоциститиса – десетогодишња ретроспективна студија

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САЖЕТАК

Увод/Циљ Опструкција дренаже назолакрималног канала са последичном епифором и развојем дакриоциститиса представља учестали патолошки ентитет у клиничкој пракси офталмолога и максилофацијалних хирурга. Етиологија дакриоциститиса је мултифакторијална и још увек није разјашњена до детаља. Сматра се да асцедентно ширење инфекције из носног кавитета и параназалних синуса, повреде и хируршке интервенције у пределу средње трећине лица, дакриолити, тумори лакрималног сакуса и околних структура могу бити неки од етиолошких фактора опструкције дренаже назолакрималног канала.

Циљ ове студије је приказивање клиничких карактеристика и хируршког лечења дакриоциститиса.

Методе Обављена студија је ретроспективна. Обухватила је период од 10 година, у којем је после хируршког прегледа и комплетне дијагностике лечено 49 болесника са клинички евидентним дакриоциститисом, од којих је оперисано њих 37.

Резултати Појава предиспонирајућих фактора се среће код 80% болесника – присуство ринитиса и запаљења параназалних синуса код 27 болесника (72%), повреде и хируршке интервенције у пределу средње трећине лица код девет болесника (24%), док је појава тумора лакрималног сакуса и назолакрималног дуктуса уочена код три болесника (8%). Оперативни неуспех који се манифестовао појавом рекурентног дакриоциститиса и епифоре уочен је код шест (16%) случајева.

Закључак С обзиром на могуће компликације неадекватно ординиране антибиотске терапије и широк спектар патолошких ентитета који чине диференцијалну дијагнозу, дакриоцисториностомија уз адекватну хистопатолошку анализу и одговарајућу антибиотску терапију у акутној фази представља сигуран начин за терапију дакриоциститиса.

Кључне речи: дакриоциститис; предиспонирајући фактори; диференцијална дијагноза; хируршко лечење

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effect of intraocular lens material and postoperative therapy on the posterior capsule opacification development after the senile cataract surgery

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SUMMARY

Introduction/Objective The most frequent postoperative complication of a successfully performed phacoemulsification cataract surgery is the development of posterior capsule opacification (PCO). It is caused by the proliferation and migration of the remaining residual epithelial cells.
The objective of this study was to investigate the influence of two different intraocular lenses and two different anti-inflammatory drugs on the development of PCO in one-year follow-up period.

Methods Investigation included 120 patients (120 eyes), equally divided into four groups. The first two groups included patients who used non-steroid anti-inflammatory drug (NSAID) postoperatively, while the other groups had corticosteroid therapy. The first and third group got hydrophilic intraocular lenses (IOL), the second and fourth group had hydrophobic IOL. Software program EPCO 2000 was used for the analysis of PCO. Student's t-test, Wilcoxon test, and ANOVA were used for data analysis and $p < 0.05$ value was accepted as statistically significant.

Results After the first three postoperative months, patients from NSAID groups had mean PCO score 0.25 ± 0.03, which was statistically significant higher ($p = 0.042$) comparing to corticosteroid groups. At the end of the investigation, the best result in PCO preventing was seen in the group of patients with hydrophobic IOL and corticosteroid therapy, with the mean PCO score of 0.47 ± 0.08.

Conclusion This study has revealed that IOL made of acrylic hydrophobic material seemed to be the right choice when choosing intraocular lens to prevent PCO development. On the other hand, NSAID and corticosteroid therapy have showed similar results in preventing postoperative intraocular inflammation. This fact can be very useful in situations when corticosteroids must be used with great caution.

Keywords: posterior capsule opacification; intraocular lens; nonsteroidal anti-inflammatory drugs; corticosteroids

INTRODUCTION

Cataract represents blur of the eye lens, which affects everyone over the age of 65. This process is physiological and occurs due to the morphological and biochemical processes of the eye lens that appear with aging. Cataract developed in this manner is known as a senile cataract [1]. The only possible cataract treatment is a surgical one, and that procedure is entitled phacoemulsification [1, 2]. Cataract surgery is one of the most commonly performed surgical procedures worldwide [3]. Although it represents a routine procedure, this surgery is neither without risk, nor without complications. Those complications could be temporary and mild, such as corneal edema, or temporary postoperative intraocular pressure increase, but also very serious like posterior capsule rupture, suprachoroidal hemorrhage, and postoperative endophthalmitis [3]. The most frequent postoperative complication of a successfully performed cataract surgery is the development of posterior capsule opacification (PCO), also known as the secondary cataract [4] (Figure1). It could provoke the decrease of the best-corrected visual acuity, reduction of contrast sensitivity, glare occurrence, or monocular diplopia [4].

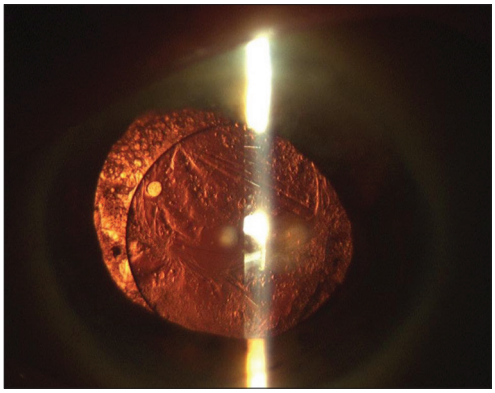

Figure 1. Posterior capsule opacification

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PCO is caused by proliferation and migration of the remaining residual epithelial cells. These cells are divided into "A" cells, which are situated under the anterior lens capsule, and "E" cells situated near the lens equator [1]. Phacoemulsification breaks down the blood-aqueous barrier and releases inflammatory cytokines. This local inflammatory reaction activates "E" cells that proliferate, migrate, and lead to PCO [1, 2]. Many methods are used to reduce inflammation and cells migration. They are performed during the phacoemulsification, such as emphasized hydrodissection, in-the-bag intraocular lenses (IOL), implantation, capsulorhexis size, or postoperative by picking adequate IOL and anti-inflammatory therapy [5–8].

The aim of this study was to investigate the influence of two different intraocular lenses and two different anti-inflammatory drugs on the development PCO in the one-year follow-up period.

METHODS

This study was a prospective, randomized study, conducted at the Clinic of Ophthalmology, Kragujevac Clinical Centre, Serbia, done from June 1, 2017 until June 1, 2018. It included 120 patients (120 eyes), who were selected for the cataract surgery. After the successfully performed phacoemulsification, patients were divided into four groups according to the implanted IOL and postoperative anti-inflammatory therapy.

The main inclusion criterion was the existence of the senile cataract. The patients with other types of cataracts, such as traumatic, iatrogenic, complicated, or presenile cataract were excluded from the study. The patients with previous history of intraocular surgery, trauma, inflammatory diseases of anterior eye segment, zonular weakness, glaucoma were also excluded. Those patients who were on a chronic topical, intraocular, or systemic anti-inflammatory therapy were excluded. The study involved only participants who underwent uncomplicated phacoemulsification.

With the approval of institutional Committee on Ethics and according to the tenets of the Declaration of Helsinki, all the patients gave their written consent at the beginning of the investigation.

A complete ocular examination was performed before the surgery as well as at every postoperative visit for every patient. That included visual acuity, intraocular pressure measurement, slit lamp evaluation, retinal examination, and ocular ultrasonography. Five days before the surgery, topically 0.3% solution of ofloxacin was administrated, five times per day.

The patients were randomized by picking two unmarked, opaque envelopes. The first envelope determined which IOL would be implanted. We used two acrylic, single-piece, square-edged IOL: hydrophilic – Eyecryl plus 600 (Biotech visioncare, Luzern, Switzerland) and hydrophobic – SA60AT (Alcon-Couvreur NV, Puurs, Belgium). The second envelope was about postoperative therapy: NSAID – nepafenac ophthalmic suspension 0.1% or dexamethasone phosphate 0.1%.

When all preoperative protocols were satisfied, the phacoemulsification was performed by two experienced surgeons. Phaco machine for all surgeries was Stellaris (Bausch & Lomb, Rochester, NY, USA). Adequate mydriasis was achieved using topical phenylephrine hydrochloride ophthalmic solution 2.5%. Tetracaine eye drops was the only anesthetic drug used during the surgery. Paracentesis and clear corneal incisions were made. Viscoelastic sodium hyaluronate ophthalmic solution 1.4% fulfilled the anterior chamber and continuous curvilinear capsulorhexis, hydrodisection and nucleus rotation followed. Then the nucleus was cracked and aspirated using the "stop and chop" technique. Irrigation and aspiration were performed to aspirate the remaining lens cortex. Capsular bag was fulfilled with viscoelastic and intraocular lens was implanted with adequate injector. When the viscoelastic was removed, intracameral solution of cefuroxime with 1 mg / 0.1 ml balanced salt solution was injected. Corneal incisions hydrated by balanced salt solution using a blunt injection needle. Postoperatively patients instilled topically 0.3% solution of ofloxacin five times daily, for one postoperative week, and one of two possible anti-inflammatory drugs, four times a day, during the first postoperative month.

After the randomization and phacoemulsification, 120 patients were equally divided into four groups (n = 30). The first two groups included patients who used postoperatively non-steroid anti-inflammatory drug with the difference that patients in the first group got hydrophilic intraocular lens, and the patients in the second group got hydrophobic intraocular lens. The other two groups were the corticosteroid groups. Hydrophilic IOL were implanted in group three, while the patients from the fourth group got hydrophobic IOL (Table 1).

Table 1. Distribution of the groups

Groups	Types of intraocular lenses	Medication used
Group I	Eyecryl plus 600	nepafenac ophthalmic suspension 0.1%
Group II	SA60AT	nepafenac ophthalmic suspension 0.1%
Group III	Eyecryl plus 600	dexamethasone phosphate 0.1%
Group IV	SA60AT	dexamethasone phosphate 0.1%

After the discharge from the Clinic, follow-up examinations were performed one, three, six and 12 months after the cataract surgery. During these visits, digital high-resolution images were taken for each patient during the slit lamp examination in a full mydriasis and retroillumination. All images were analyzed by using Evaluation of Posterior Capsule Opacification 2000, a standard software program for analysis of PCO [6]. The boundaries of each opaque area noticed at the posterior capsule were marked using a computer mouse. According to the density of these areas, opacification was scaled from grade 0 to 4. Posterior capsule without any opacification was considered as grade 0. Other grades included minimal (grade 1), mild (grade 2), moderate (grade 3), and severe (grade 4) PCO. The PCO score for each area was calculated by multiplying the

opacification density grade with the fraction of the capsule area. The sum of all these individual PCO scores defined the total PCO score for the analyzed image.

Statistical analysis was done by using SPSS Statistics for Windows software (IBM Corp., Armonk, NY, USA). The significance at different time intervals during the study was tested with the Student's t-test or by the Wilcoxon equivalence test in case where the distribution was not normal. Examination of the incidence of opacification in dependence on the type of intraocular lens was done by using the χ^2 test and ANOVA ($p < 0.05$ value was accepted as statistically significant).

RESULTS

The mean age of the examined patients was 76.4 ± 6.8 years (range 66–88 years) without statistical significance among the groups. A total of 64 females and 56 males were equally divided into four groups. During the study, four patients from corticosteroid groups had temporary intraocular pressure increase, which was efficiently treated with antiglaucomatous eye drops. Two patients developed postoperative macular edema (both from the corticosteroid groups) and one patient died, so they were excluded from the investigation.

At the first follow-up, one month after the phacoemulsification, the mean PCO score among the groups was I = 0.12 ± 0.03, II = 0.08 ± 0.02, III = 0.06 ± 0.01, IV = 0.05 ± 0.01 (Table 2). Groups II–IV had first grade opacification, while some patients from the first group developed second grade opacification. Statistically significant difference was noticed between the first and other groups, as well as between NSAID and corticosteroid groups ($p = 0.032$).

Table 2. The mean posterior capsule opacification score during one year of follow-up period

Group	1 month	3 months	6 months	12 months
I	0.16 ± 0.03	0.26 ± 0.04	0.48 ± 0.10	0.64 ± 0.12
II	0.08 ± 0.02	0.23 ± 0.03	0.37 ± 0.05	0.49 ± 0.06
III	0.06 ± 0.01	0.21 ± 0.03	0.42 ± 0.08	0.57 ± 0.09
IV	0.05 ± 0.01	0.18 ± 0.05	0.32 ± 0.04	0.47 ± 0.08

At the next visit, more participants from NSAID groups had worse mean PCO score (I = 0.26 ± 0.04; II = 0.23 ± 0.03) compared to those with topical corticosteroid (III = 0.21 ± 0.03; IV = 0.18 ± 0.05), with calculated statistical significance ($p = 0.042$). Comparing all four groups separately, statistically significant difference was detected only between groups I and IV ($p = 0.03$).

After six months postoperatively, the mean PCO score in the fourth group was statistically different from other groups (I = 0.44 ± 0.10; II = 0.37 ± 0.05; III = 0.42 ± 0.08; IV = 0.32 ± 0.04). The difference between hydrophilic IOL groups was not significant, $p = 0.069$.

Twelve months after the cataract surgery, the fourth group had the lowest mean PCO score, 0.47 ± 0.08. The mean PCO score in other groups was I = 0.64 ± 0.12, II = 0.49 ± 0.06 and III = 0.57 ± 0.09. No statistically significant difference was found between groups II and IV ($p = 0.061$). Statistical significance was noted between the first and other groups, as well as between hydrophobic *vs.* hydrophilic groups ($p < 0.001$).

DISCUSSION

According to many previous studies, PCO still remains the most common complication of successfully performed cataract surgery [6, 9, 10]. The only known treatment of formed PCO is Nd:YAG capsulotomy. This procedure is not without risk. Some of the possible complications are IOL damage, retinal detachment, macular edema, intraocular pressure increase [11]. Therefore, all researchers agree that the best treatment of PCO is prevention [10, 11].

Corticosteroids are well known to have anti-inflammatory effect, but they can cause severe ocular side effects: intraocular pressure increase, cataract development, disturbance of the corneal wound healing [12]. For this reason, not a small number of phaco surgeons are interested in some alternatives. NSAID for ocular use are mostly administrated in the management of ocular inflammation with non-infectious origin. During the postoperative period, they reduce anti-inflammatory reaction, and consequently the development of PCO [13]. Corticosteroids block the release of arachidonic acid by the suppression of the enzyme phospholipase A2. That action stops the production of inflammatory mediators, such as leukotrienes and prostaglandins [14]. NSAID act through the inhibition of the enzyme cyclooxygenase, which causes the suspension of prostaglandin production. Thereby, NSAID are mostly in use as antipyretic, anti-inflammatory, and analgesic drugs [15].

Intraocular lens material and design have an important impact on preventing PCO. Acrylic material is associated with reduced PCO rate by causing a lower postoperative inflammation than the previously used materials [9]. In addition, lenses with sharp edge design have better outcomes by the inhibition of lens epithelial cells' (LECs) migration [16].

After the appropriate surgical technique, our results indicated that the satisfactory PCO prophylaxis could be provided by implanting acrylic hydrophobic IOL. These results are in accordance with earlier studies [9, 10, 11, 17]. Intraocular lenses made of hydrophobic material can adhere to collagen membrane and fibronectin. That creates less space between IOL and posterior lens capsule, making it difficult for LECs to migrate and to develop PCO [18]. Some investigators advocate that the difference between these two materials is associated with less sharp edges of the hydrophilic lenses [9]. During the manufacture of hydrophilic IOL, they are primarily produced dehydrated, and then rehydrated which can lead to the loss of sharpness [19].

The results we collected highly indicated a strong anti-inflammatory potential of administrated corticosteroids in the first three postoperative months. This fact is similar to some earlier studies [14, 15]. During the final six months of the study, it seemed to be, that IOL material had the main influence on preventing the PCO development.

Anti-inflammatory drugs have a huge effect on controlling the inflammation in early postoperative period. LECs cannot be completely removed during phacoemulsification even using advanced surgical techniques. After a few months, because of the chronic inflammation, LECs start to proliferate and migrate towards the lens posterior capsule. During that period, IOL block the further migration of the LECs. Therefore, the finest results in preventing the PCO development can be reached by the synergistic act of anti-inflammatory therapy and aqueous intraocular lens implantation.

CONCLUSION

PCO still represents the most frequent postoperative complication of the uncomplicated cataract surgery. This condition causes decreased visual acuity and patients' dissatisfaction. In accordance with the results presented in this study, we believe that the adequate prevention of PCO forming is provided by the implantation of acrylic hydrophobic IOL in a capsular bag. Similar scores in PCO development one year after the phacoemulsification in hydrophobic IOL groups with NSAID or corticosteroid, provide the new possibilities in the prevention of postoperative inflammation. These results can be particularly useful in situations when corticosteroids must be used with great caution, such as glaucoma patients, the presence of active infection, or conditions with the delayed corneal healing.

Conflict of interest: None declared.

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Ефекат материјала интраокуларног сочива и постоперативне терапије на развој замућења задње капсуле сочива после операције сенилне катаракте

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САЖЕТАК

Увод/Циљ Најчешћа постоперативна компликација успешно изведене операције катаракте факоемулзификацијом је развој замућења задње капсуле сочива. То је проузроковано пролиферацијом и миграцијом преосталих резидуалних епителних ћелија.

Циљ ове студије је испитивање утицаја два различита интраокуларна сочива и два различита антиинфламаторна лека на развој замућења задње капсуле сочива током једногодишњег периода праћења.

Методе Истраживање је обухватило 120 болесника (120 очију), подједнако подељених у четири групе. Прве две групе укључивале су болеснике који су користили постоперативно нестероидни антиинфламаторни лек (*NSAIL*), док су остале групе добиле кортикостероидну терапију. Прва и трећа група добиле су хидрофилна интраокуларна сочива (ИОС), а друга и четврта хидрофобна ИОС. За анализу замућења задње капсуле сочива коришћен је софтверски програм *EPCO 2000*. Студентов т-тест, Вилкоксонов тест и *ANOVA* коришћени су за анализу података, а вредност $p < 0,05$ је прихваћена као статистички значајна.

Резултати После три постоперативна месеца болесници из група *NSAIL*-а имали су средњу вредност замућења задње капсуле сочива 0,25 ± 0,03, што је било статистички значајно више ($p = 0,042$) у поређењу са кортикостероидним групама. На крају студије, најбољи резултати у спречавању настанка замућења задње капсуле сочива забележени су у групи болесника са хидрофобним ИОС и кортикостероидном терапијом, са средњом вредношћу 0,47 ± 0,08.

Закључак Ова студија је показала да су интраокуларна сочива израђена од акрилатног хидрофобног материјала била прави избор приликом одабира интраокуларног сочива у спречавању развоја замућења задње капсуле сочива. С друге стране, *NSAIL* и терапија кортикостероидима су показале сличне резултате у спречавању постоперативне, интраокуларне инфламације. Ова чињеница може бити веома корисна у ситуацијама када се кортикостероиди морају употребљавати са великим опрезом.

Кључне речи: замућење задње капсуле сочива; интраокуларна сочива; нестероидни антиинфламаторни лекови; кортикостероиди

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Impact of illness acceptance on the quality of life in cancer patients after surgical treatment

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SUMMARY

Introduction/Objective In Poland, cancers are the second most common cause of death. One in four Poles will have cancer in their life, and one in five will die from it.
The purpose of the study was to assess the acceptance of illness and its impact on the quality of life in surgically treated cancer patients.

Methods The study included 123 cancer patients who had undergone surgical treatment between April and May of 2017. The most common were colon (33.3%) and breast cancer (31.7%). Sixty-five percent of the patients were only treated surgically. The Acceptance of Illness Scale, the WHOQOL-BREF quality of life questionnaire, and an original survey were used.

Results Sixty-two patients (50.4%) presented high illness acceptance levels. More than half of the patients rated their quality of life as good (41.5%) or very good (13%). A positive correlation was found between the acceptance of illness and the quality of life scores in the physical health ($R = 0.351$, $p < 0.001$), psychological ($R = 0.422$, $p < 0.001$), social relationships ($R = 0.525$, $p < 0.001$), and environment ($R = 0.533$, $p < 0.001$) domains. In the physical and psychological domains, the correlation had moderate strength, while correlations with the social relationships and environment domains were strong.

Conclusion Higher illness acceptance levels were associated with higher quality of life. Acceptance of illness was not associated with patient age, type of treatment, or repeated surgery. Patients who lived alone had significantly lower quality of life and significantly lower acceptance of illness. Patients who had undergone their first surgery perceived their quality of life in the environment domain significantly lower.
Keywords: acceptance of illness; quality of life; cancer; surgical treatment

INTRODUCTION

As lifespan extends, diseases associated with patients' age are an increasingly common medical problem. According to estimates, one in four Poles will have cancer in their life, and one in five will die from cancer. In 2017, malignant neoplasm caused 98,456 deaths in Poland [1]. In Poland, the morbidity rate of cancer is relatively low with 254 cases per 100,000 inhabitants, but the mortality rate of cancer is relatively high, with 237 cases per 100,000 inhabitants [1]. In 1972, Kubler-Ross et al. [2] reported that many patients reacted similarly to the diagnosis, and these reactions are a natural part of adaptation to this difficult life situation. Cancer and its treatment can influence a patient's life during the diagnosis and treatment but also years after completion of the treatment. Cancer makes one think of pain, suffering, disability, and often of the loss of one's job and the resulting deterioration of the financial stability. The diagnosis and oncological treatment can result in social isolation and fear of death, and patients often report the feeling of stigma. Literature reports highlight the importance of illness acceptance and its positive impact on the quality of life (QoL). Acceptance of illness consists in adopting a positive attitude towards a specific situation or belief. It supports the patient and prevents QoL deterioration in chronic illness [3, 4, 5]. The ability to accept illness is an important issue in the QoL of cancer patients. Patients must learn how to cope not only with the symptoms, but also with the changes in the QoL, constraints of independence, and the change of their roles in families and society [6, 7].

The purpose of the study was to assess the acceptance of illness among surgically treated cancer patients, as well as its impact on their QoL and health satisfaction.

METHODS

Inclusion criteria were the following: cancer diagnosis; surgical treatment; age > 18 years; good psychological condition; consent to participate in the study.

Exclusion criteria were as follows: lack of cancer or lack of surgical treatment; age < 18 years; lack of consent to participate; the presence of significant auditory or visual impairments; cognitive impairment precluding the completion of the questionnaire.

Out of 150 patients who had met the inclusion criteria, 27 respondents did not complete the questionnaires correctly, or they refused to

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participate in the study without giving any reason. The study was performed on 123 patients (73 women and 50 men) who underwent surgical treatment for cancer, at the Department of Surgical Oncology of the Wroclaw Regional Specialist Hospital between April and May of 2017. Our study was planned to be an observational and cross-sectional research.

Three questionnaires were used in the study: the Acceptance of Illness Scale (AIS), adapted by Juczyński for use with Polish patients, the WHOQOL-BREF QoL questionnaire, and a survey questionnaire developed by the authors, comprising five items concerning the patients' socio-demographic characteristics (i.e. sex, age, residence, professional activity, and family situation), and four items concerning their clinical status (i.e. type of cancer, treatment methods, number of surgeries, and comorbidities). All the surveys were anonymous.

The AIS is a measure of illness acceptance. The scale comprises eight statements evaluating the negative impact of health impairment. Each statement is scored using a five-item scale. The respondent selects a number: 1 – strongly agree, 2 – agree, 3 – undecided, 4 – disagree, 5 – strongly disagree; "1" corresponds to poor adaptation to the illness, while "5" to complete acceptance of it. The total score for one patient ranges 8–40 points. Three score groups were identified: group 1 – low acceptance of illness (8–19 points), group 2 – moderate acceptance of illness (19–29 points), group 3 – good acceptance of illness (30–40 points).

The WHOQOL-BREF questionnaire measures the QoL in four aspects or domains: physical/somatic (activities of daily living, ability to work, energy, mobility, dependence on medication, pain and discomfort, sleep and rest), psychological (body image, negative and positive feelings, religion, self-esteem, learning, memory, concentration), social relationships (personal relationships, social support, sexual activity), and environment (financial resources, physical and psychological safety and security, freedom, health and social care, opportunities for acquiring new information and skills, home environment, participation in and opportunities for recreation and leisure activities). The questionnaire comprised 26 items, rated using a five-point Likert scale. Scores in each domain may range 4–20 points. Higher scores correspond to better QoL. Additionally, the WHOQOL-BREF comprised two separate questions, concerning the respondents' overall perceived QoL (question 1) and overall perceived health (question 2) [8].

Bioethics section

The study was approved by the Local Bioethics Committee of the Wrocław Medical University (approval No. KB – 228/2017), and the written informed consent was obtained from all the study participants.

Statistical analysis section

The collected data were analyzed in three stages. First, the results of the authors' own survey were presented. Then the patients' AIS score and QoL (WHOQOL-BREF scores) were evaluated, and finally correlations between the AIS and QoL scores were analyzed. Differences between variables were verified using the non-parametric Mann–Whitney U-test and Kruskal–Wallis test. Variable distribution normality was verified using the Shapiro–Wilk test. Correlations were analyzed using Spearman's correlation coefficients. For all the tests, the significance threshold of p-value ≤ 0.05 was used. Calculations were performed using the Excel (Microsoft Office, Microsoft Corporation, Redmond, WA, USA) and Statistica (TIBCO Software Inc., Palo Alto, CA, USA) software.

RESULTS

The majority of the respondents were in the 45–64 years age group (47.1%), lived in urban areas (79.7%), with their families (76.5%), and were professionally active (52.9%). The most common diagnosis was colon cancer (33.3%), followed by breast cancer (31.7%), ovarian cancer (8.9%), and melanoma (5.7%). Most patients (65%) were treated only surgically. For 65.9% of the patients this had been the first surgery for the cancer, while 34.1% had undergone multiple surgeries. The most common co-morbidities were hypertension (65.4%), diabetes mellitus (30.8%), and osteoarticular disorders (25.6%) (Table 1).

Table 1. The demographic data of the study group

Variable	Study group (n = 123)
Sex	
men	50 (40.7%)
women	73 (59.3%)
Age	
21–44	29 (23.6%)
45–64	58 (47.1%)
> 65	36 (29.3%)
Place of residence	
city	98 (79.7%)
country	25 (20.3%)
Professional activity	
professionally active	65 (52.9%)
retired	33 (26.8%)
disability pensioners	16 (13%)
unemployed	9 (7.3%)
Family status	
live with families	94 (76.5%)
live alone	26 (21.1%)
live in residential care institution	3 (2.4%)
Type of cancer	
colon cancer	41 (33.3%)
breast cancer	39 (31.7%)
ovarian cancer	11 (8.9%)
melanoma	7 (5.7%)
thyroid cancer	5 (4.1%)
kidney cancer	5 (4.1%)
prostate cancer	3 (2.4%)
pancreatic cancer	3 (2.4%)
lung cancer	2 (1.6%)
other	7 (5.7%)
Treatment	
surgery only	80 (65%)
with chemotherapy	31 (25.2%)
with radiotherapy	15 (12.2%)
with hormone therapy	6 (4.9%)
Number of surgeries	
first	81 (65.9%)
multiple	42 (34.1%)
Co-morbidities	
hypertension	51 (65.4%)
diabetes mellitus	24 (30.8%)
osteoarticular disorders	20 (25.6%)
thyroid disorders	13 (16.7%)
heart disease	13 (16.7%)
psychological disorders	7 (9%)
kidney diseases	3 (3.8%)

Acceptance of illness was determined for the entire group. Sixty-two patients (50.4%) had high illness acceptance scores, 33.3% had moderate, and 16.3% had low scores. The mean AIS score for the entire group was 28.33, standard deviation was 8.02, and the median score was 30, which indicates on overall moderate acceptance of illness. The lowest score was 9, the highest was 40. In terms of pathology, the acceptance of the illness was better for patients with breast cancer (mean score of 31.06; median score of 32.5) and worse for patients with colon cancer (mean score of 18.21; median score of 20).

No statistically significant age-related differences were found in the acceptance of illness scores (Kruskal–Wallis test: χ^2 $c^2 = 1.554$; df = 2; p = 0.460). Acceptance of illness did not differ between patient groups distinguished by the type of treatment. In patients treated only surgically, the median score was 29.5, while in the combination treatment group it was 30. The difference was not statistically significant (Mann–Whitney test: U = 1677; p = 0.819). AIS scores were slightly higher in patients having undergone their first surgery for cancer than in those having undergone multiple surgeries (median scores were 30 and 26.5, respectively), but the difference was not statistically significant (Mann–Whitney test: U = 1376.5; p = 0.083).

Marked differences in AIS scores were found when considering the patients' family situation. Patients who lived alone had lower acceptance levels (Me = 19) than those who lived with others (Me = 32). The difference between these groups was statistically significant (Mann–Whitney test: U = 825; p = 0.011) (Figure 1).

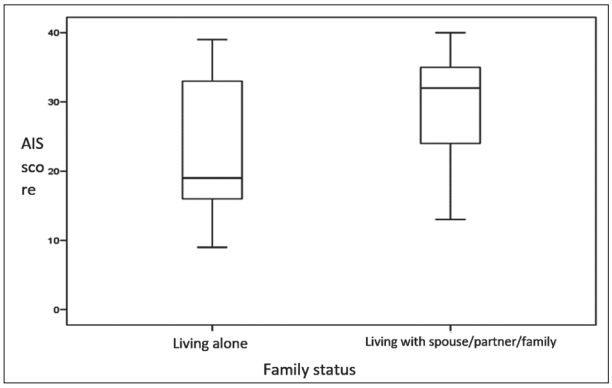

Figure 1. Acceptance of illness and family status

More than half of the patients rated their QoL as good (41.5%) or very good (13%). Poor or very poor QoL was reported by 9.7% and 4.1% of the patients, respectively. Many patients (31.7%) stated that their QoL was neither good nor poor. The highest QoL was reported in the social relationships domain (mean = 14.89; SD = 3.236; median = 16; max. score = 20; min. score = 4). This was followed by the environment domain (mean = 14.51; SD = 3.270; median = 15; max. = 20; min. = 7), in the psychological domain (mean = 13.59, SD = 2.541; median = 14.00; max. = 18; min. = 7), and the lowest QoL was found in the physical health domain (mean = 12.26; SD = 2.142; median = 13; max. = 18; min. = 7). In terms of pathology, the QoL was better in the somatic domain for patients with breast cancer (mean = 14.25; median = 15.5) than for patients with colon cancer (mean = 10.2; median = 11.25). In contrast, the QoL was worse in the social domain for patients with breast cancer (mean = 9.55; median = 10.5) than for patients with colon cancer (mean = 15.75; median = 16.5).

No statistically significant differences were found between age groups with regard to the QoL in the physical health (Kruskal–Wallis test: χ^2 $c^2 = 1.367$; df = 2; p = 0.505), psychological ($c^2 = 5.656$; df = 2; p = 0.059), or social relationships domain ($c^2 = 2.783$; df = 2; p = 0.249). There was, however, a statistically significant difference in the environment domain ($c^2 = 6.138$; df = 2; p = 0.0461) (Table 2).

An additional test demonstrated that patients aged 21–44 years rated their QoL in the environment domain lower than those aged 45–64 years (p = 0.046). In the former group, the median score was 14, while in the latter it was 16.

QoL in the social relationships domain differed significantly between patients living alone and those living with others (Mann–Whitney test: U = 897.5, p = 0.036). Those who lived alone rated their QoL in the domain lower (Me = 13) than those who lived with their families or partners (Me = 16).

The use of treatment other than surgical did not affect the respondents' QoL. No statistically significant differences were found with any of the combined treatment categories (p > 0.05).

With regard to the number of surgeries, a statistically significant difference was found concerning the environment domain score (Mann–Whitney test: U = 1,249, p = 0.015). The patients who had undergone their first surgery rated their QoL in the domain lower than those who had undergone multiple surgeries. In the former group, the median score was 13.5, while in the latter it was 16. For other domains, there were no statistically significant observations (p > 0.05).

To investigate whether acceptance of illness may affect the QoL of patients undergoing surgical cancer treatment,

Table 2. Correlations between respondents' age and quality of life (QoL)

QoL domain	21–44 y/o			45–64 y/o			more than 65 y/o			Test result
	Me	Min.	Max.	Me	Min.	Max.	Me	Min.	Max.	
Physical health (scale: 4–20)	13	7	15	13	8	15	12	7	18	$\chi^2 = 1.367$ df = 2 p = 0.505
Psychological (scale: 4–20)	14	7	16	15	8	18	13	9	18	$\chi^2 = 5.656$ df = 2 p = 0.059
Social relationships (scale: 4–20)	16	5	20	16	4	20	15	5	20	$\chi^2 = 2.783$ df = 2 p = 0.249
Environment (scale: 4–20)	14	7	18	16	8	20	14	8	20	$\chi^2 = 6.138$ df = 2 p = 0.046

df – degrees of freedom; Me – median

correlations were calculated for AIS and WHOQOL-BREF scores. Spearman's correlation coefficients were used (as the Shapiro–Wilk test demonstrated that the variables were not distributed normally).

Statistically significant results ($p < 0.001$) were obtained for all domains, which indicates that the acceptance of illness is correlated with the QoL in four domains: physical health (R = 0.351), psychological (R = 0.422), social relationships (R = 0.525), and environment (R = 0.533) (Table 3). In all the domains, the correlation was positive, indicating that higher levels of illness acceptance were associated with better QoL in the patients studied. The strongest correlation with AIS was found for the environment and social relationships domains, while the weakest one was found for the physical health domain. Correlations were also analyzed between the AIS and the QoL scores and comorbidities, but no statistically significant results were found.

Table 3. Correlation between the Acceptance of Illness Scale (AIS) and the Quality of Life (QoL) scale

Acceptance of Illness Scale (AIS)		
QoL domain	Spearman's correlation coefficient	
	R	p
Physical health (scale: 4–20) * AIS score	0.351	< 0.001
Psychological (scale: 4–20) * AIS score	0.422	< 0.001
Social relationships (scale: 4–20) * AIS score	0.525	< 0.001
Environment (scale: 4–20) * AIS score	0.533	< 0.001

DISCUSSION

A patient's attitude towards the diagnosis and illness determines his or her attitude and adherence to treatment [9]. Overall, the respondents presented "moderate" acceptance of illness levels, with a mean AIS score of 28.3. Similar findings were reported by Czerw et al. [6] (mean score of 27.56). Other findings from the cited study are also similar to the present results, i.e. the patients' age did not affect AIS scores in either study [6]. Most patients (56.16%) had a moderate acceptance level in a study by Karczmarek-Borowska et al. [10] (compared to 50.4% in the present study), though contrary to Czerw et al. [6], the study found that patients younger than 60 present higher acceptance scores than the older ones. Slightly lower scores were found among cancer patients in a study by Kołpa et al. [11] (25.35 points) and leukemia patients in a study by Wiraszka and Lelonek [12] (23.27). Despite the initial presumption that most cancer patients would have low illness acceptance scores, more than one half of the respondents were found to present high illness acceptance (50.4%), and low acceptance was only found for 16.3%. Similar results were obtained by Pawlik and Karczmarek-Borowska [13], who found 46.29% of breast cancer patients to accept their illness, and by Czerw et al. [14], who reported the mean AIS score of 28.45 among breast cancer patients. Higher acceptance levels were found in a study by Łuczyk et al. [15], where 39.43% of breast cancer patients obtained high scores. Religioni et al. [16] also studied prostate cancer patients, who obtained a mean score of 30.39, and therefore were also found to have a "high" level of illness acceptance (although 30 is a borderline score between moderate and high). In patients with colon cancer, Czerw et al. [17] found the mean AIS score of 27.74, which is also similar to the present findings. In our study, 50.4% of the respondents had high acceptance scores, while only 16.3% had low scores.

The standardized WHOQOL-BREF questionnaire allows for studying patients' QoL directly. Findings similar to ours were reported in a study on women by Lutgendorf et al. [18], according to which the patients also predominantly described their QoL as good. Slightly lower results were found in a study by Applewhite et al. [19], who compared thyroid cancer patients to patients with various other cancers (colon cancer, breast cancer, gliomas, and gynecologic tumors). In the entire group, the overall QoL was found to be moderate (a score of 5.56 on a scale of 0–10, with 10 denoting the highest QoL) [19]. An analysis of the available Polish literature on the subject shows that women with breast and gynecologic cancers perceive their QoL as good, with a score of 146.99 points in the LQ-C30 questionnaire before treatment, and 138.59 points after treatment. This indicates that the perception did not change over the entire period of treatment using various methods, as reported by Pietrzyk et al. [20]. A similar observation was made in our study, when comparing QoL between patients treated only surgically and those in whom the surgical treatment was combined with other methods. It is difficult to determine why, despite often very radical treatment, patients maintain relatively good QoL.

The second item of the WHOQOL-BREF concerns the patients' overall perceived health. Our finding may indicate that despite the burden of cancer, patients experience considerably less negative emotions than one could expect.

As described above, the WHOQOL-BREF questionnaire comprises four sections, reflecting the respondents' QoL in specific domains: physical or somatic health, psychological, social relationships, and environment. Our findings reveal small differences in the QoL scores for each domain of a patient's life. Notably, however, the lowest scores were found in the physical health domain, which may be due to the limitations associated with cancer, such as weakness or lifestyle changes recommended to patients after surgery. The highest scores were found in the social relationships domain, indicating that patients felt supported. The importance of social support in adaptation to illness among cancer patients was highlighted by Wyszomirska et al. [21], who also remarked that the availability of support in difficult situations, as perceived by the patient, may be even more important. Moreover, as stated by de Walden-Gałuszko [22], good psychological QoL in cancer patients depends on their internal development, which increases one's psychological capacity. She also states that development in these aspects not only enhances patients' QoL, but may even make their life fuller and richer than it had been before they fell ill.

One of the many aspects of the present study involved the impact of the respondents' age on their QoL. The obtained results demonstrated statistically significant differences only with regard to QoL in the environment domain. The additional test demonstrated that patients aged 21–44 rated their QoL in the environmental domain lower (Me = 14) than those aged 45–64 (Me = 16). Entirely different findings were reported by Viganò and Morais [23], describing patients unable to perform daily activities due to considerable weakness, which may directly affect QoL and tolerance of cancer treatment in elderly individuals. Yet another situation is reported in a study by Tobiasz-Adamczyk et al. [24], demonstrating significant differences between younger and older patients with regard to their perception of changes in their physical status, daily functioning, and performance of social roles. Younger patients experienced more restrictions due to disease symptoms. In older patients, limitations in daily living were found to be correlated to anxiety levels [24].

No statistically significant correlations between comorbidities and the AIS and QoL scores were found in our study. This may be related to the low prevalence of multimorbidity in the study group. Contrary results were obtained by Zielińska-Więczkowska and Żychlińska [25], though respondents in their study were aged above 60 years. These authors found patients with comorbidities to have higher cancer acceptance.

The relationship between acceptance of illness and the QoL was an important objective of our study. All the authors emphasize the impact of illness acceptance on patients' lives. In addition to interventions to improve their clinical condition, an improvement of patients' QoL requires psychological support, as the psychological domain was the one in which patients obtained lower scores (13.59) compared to the social relationships (14.89) and environment (14.51) domains. However, studies on the subject seldom include cancer patients, as these patients are a distinct group that is often difficult to work with. The present results demonstrate correlations between the acceptance of illness and the QoL in all the analyzed domains. Namely, the higher the patient's illness acceptance level, the higher their QoL, and vice versa – patients with better QoL have more acceptance for their illness. Zielińska-Więczkowska and Żychlińska [25] found a similar association for the psychological domain, whereas Ślusarska et al. [26], studying lymphoma patients, reported higher values for all the domains.

Our study has certain limitations. Due to the small number of different cancer types, our paper was not focused on one particular type of cancer, but on surgical patients with cancer in general.

CONCLUSION

1. Acceptance of illness was not associated with patient age, type of treatment, or repeated surgery. Higher illness acceptance levels were associated with higher QoL scores in all four domains in surgically treated cancer patients.

2. Patients who lived alone had significantly lower QoL scores in the social relationships domain and significantly lower acceptance of illness.

3. Patients who had undergone their first surgery perceived their QoL in the environment domain as significantly lower compared to those who had undergone multiple surgeries. Patients aged 21–44 years had significantly lower QoL scores in the environment domain.

Conflict of interest: None declared.

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Утицај прихватања болести на квалитет живота после хируршког лечења код болесника оболелих од рака

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САЖЕТАК

Увод/Циљ Рак је у Пољској други најчешћи узрок смрти. Један од четири Пољака током живота има рак, а један од пет умре од њега.

Сврха ове студије је да се процени прихватање болести и њен утицај на квалитет живота хируршки лечених болесника оболелих од рака.

Методе Студија је обухватила 123 болесника оболела од рака, хируршки лечена између априла и маја 2017. године. Најчешћи су били рак дебелог црева (33,3%) и рак дојке (31,7%). Шездесет пет посто болесника је лечено искључиво хируршки. Коришћени су Скала прихватања болести, упитник о квалитету живота *WHOQOL-BREF* и оригинална анкета.

Резултати Шездесет два болесника (50,4%) испољила су висок ниво прихватања болести. Више од половине болесника је свој квалитет живота оценило као добар (41,5%) или врло добар (13%). Пронађена је позитивна корелација између прихватања болести и резултата у бодовању квалитета живота у доменима физичког здравља ($R = 0,351$, $p < 0,001$), психолошког стања ($R = 0,422$, $p < 0,001$), друштвених односа ($R = 0,525$, $p < 0,001$) и окружења ($R = 0,533$, $p < 0,001$). Са физичким и психолошким доменима корелација је умерена, док је корелација са доменима друштвених односа и окружења јака.

Закључак Виши ниво прихватања болести повезан је са бољим квалитетом живота. Прихватање болести није у вези са старосном доби болесника, начином лечења или понављањем операције. Болесници који су живели сами имали су значајно нижи квалитет живота и значајно нижи степен прихватања болести. Болесници који су подвргнути првој операцији квалитет живота у домену окружења оценили су као значајно нижи.

Кључне речи: прихватање болести; квалитет живота; канцер; хируршко лечење

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The influence of pulmonary rehabilitation on the exacerbations of chronic obstructive pulmonary disease in Serbia

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SUMMARY

Introduction/Objective The chronic obstructive pulmonary disease (COPD) exacerbations have a major impact on outcomes of COPD patients. Pulmonary rehabilitation (PR) interrupts the vicious circle caused by exacerbations. It has not been widely implemented as standard of COPD treatment yet.
The aim of study was to examine the effectiveness of PR in prevention of exacerbations.

Method The prospective observation study included stable COPD patients between January 2015 and December 2018. The effects of PR on exacerbation rates were evaluated using univariate and multivariate logistic regression analysis, taking into account age, comorbidity, vaccination status (against seasonal flu), body mass index (BMI).

Results Study included 1,674 patients (956 males, age 65.93 ± 8.45, current or ex-smokers 94.9%; 21 ≥ BMI 1,406 patients, 84%, FEV_1 < 80% 1,448 patients, 86.5%). The PR rate was 48.1%. There was significant difference in PR status with respect to age (p = 0.020), comorbidities (p = 0.015), FEV_1 (p < 0.001), respiratory symptoms using COPD assessment test (CAT) score (p < 0.001), vaccination against seasonal flu (p < 0.001). Exacerbations occurred more frequently in non-PR patients (415 (51.6%) *vs*. 641 (73.7%), p < 0.001). In multivariate analysis, PR (RR 0.421; 95% CI (0.307–0.577); p < 0.001) and BMI ≥ 21kg/m^2 (RR 0.605; 95% CI (0.380–0.965); p = 0.035) were independent protective factors and CAT score >10 (RR 2.375; 95% CI (1.720–3.280); p < 0.001) and FEV_1 < 80% (RR 2.021; 95% CI (1.303–3.134); p = 0.002) were independent risk factors from exacerbations.

Conclusion Patients who successfully completed PR treatment had significantly less frequent exacerbations compared to patients that not pass through PR program.

Keywords: AECOPD; COPD; CAT score; pulmonary rehabilitation

INTRODUCTION

The acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are challenging for all physicians. After exacerbation, patient is at increased risk of re-exacerbation and hospitalization [1, 2]. Since there is no solid evidence that any intervention decreases chronic obstructive pulmonary disease (COPD) mortality, treatment of COPD has two goals. First is the control of symptoms, second is reduction and prevention of COPD exacerbations [3].

The main non-pharmacologic COPD therapy is the pulmonary rehabilitation (PR). PR reduces dyspnea and fatigue and improves psychological status of patients. It is evidence-based program that helps improve the well-being of patients. There are many national to worldwide guidelines [Global Initiative for Chronic Obstructive Lung Disease, American Thoracic society (ATS), and European respiratory society (ERS)], which recommend PR for COPD (Evidence Level A) [4, 5, 6].

The PR is one of the most cost-effective therapies for COPD. Despite this fact and the recommendations of the international and national guidelines, PR has not yet become well-recognized standard of care of COPD and also because a lack of medical staff specifically qualified in PR (physiotherapist, pulmonologist) in Europe [7, 8]. In addition, many patients had denied taking the PR programs.

The PR effects among COPD patients have been demonstrated in most of the studies coming from developed countries as opposed to developing or undeveloped countries where there has not been much research regarding this issue. Serbia is among these countries, where there has been no research on the effects of PR on COPD exacerbations, since 2007 [9]. This problem continues to be a great burden on the health care system budget because of other outlays. This study has risen from the need for continued education in COPD patients and the medical community regarding PR.

The aim of this study was to examine the frequency and effectiveness of the PR among COPD patients in Serbia. In addition, we examined the influence of patient related factors and PR on reducing COPD exacerbations.

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METHODS

Prospective cohort study was conducted over four years and it included consecutive ambulatory patients with COPD (January 2015 – December 2018), at the Polyclinic department of the Institute for Pulmonary Diseases of Vojvodina (IPDV) in Sremska Kamenica, Serbia. We collected basic demographics data and medical histories of the patients with an established COPD diagnosis. The criteria for being included in the study were patient aged over 40, COPD diagnosis (based on a post-bronchodilator FEV_1/FVC ratio of < 0.70) at least once a year.

The patients were divided into two groups according to PR status and followed during a one-year study. The demographic data included sex, age, smoking habits (packs per year), and body mass index (BMI). PR was conducted at the Polyclinic department of IPDV. Status of PR, COPD assessment test (CAT), spirometry test (forced expiratory volume in first second, FEV_1), six-minute walking distance (6MWD), comorbidity and vaccination against seasonal flu were obtained from the patient files and medical history at IPDV, but also as given by the patient. Exclusion criteria were active tuberculosis, cancer, unstable cardiovascular diseases, neurological and musculoskeletal disorders, patients who passed away or did not finish the PR course.

Every outpatient had the PR course according to the ATS-ERS statement and recommendations [5]. The course lasted three weeks, one to three times per year. The 60-minute exercise session was conducted every day, consisted of aerobic and muscle strength training for upper and lower extremities [10]. The patients were also advised to exercise at least twice a week on their own after finishing PR program. Physiotherapists were previously instructed to homogenize the type and duration of all activities.

The study encompassed a once-per-year monitoring of each patient. The major outcomes were moderate and/or severe exacerbations during the one-year follow up. Moderate exacerbation requires treatment with systemic corticosteroids or antibiotics; severe requires hospitalization or evaluation in the emergency department [11].

All research procedures and patients were in accordance with the standards of the Committee on ethics as well as in accordance with good clinical practices and declarations of the Helsinki committee and its later amendments or comparable ethical standards. The research was approved by the IPDV Ethics Committee.

Descriptive statistics were generated for all study variables, including mean and standard deviation for continuous variables and relative frequencies for categorical variables. The χ^2 test was used to determine whether there was a significant difference between the expected frequencies and the observed frequencies in one or more categories. The predictive values of evaluated variables for COPD exacerbations were evaluated with univariate and multivariate logistic regression analysis. All univariate statistically significant predictors were included in multivariate logistic regression analysis. All probability values were calculated by assuming a two-tailed α value of 0.05 with confidence intervals at the 95% level. All statistical analyses were performed with SPSS for Windows version 17 (SPSS Inc., Chicago, IL, USA).

RESULTS

The study included 1,674 patients (956 males, age 65.93 ± 8.45, current or ex-smokers 94.9%, packs-years 44.31 ± 25.09). The average duration of COPD was 7.54 ± 5.32 years (range 1–38 years). The average BMI was 27.24 ± 4.89 (range 16.8–41.3), 268 patients had BMI below 21 (16%). Most of the patients, according to FEV_1, were in stages 2–4 (1,448; 86.5%), every second was stage 2, every third in stage 3 (Table 1).

A total of 804 patients (48.1%) completed PR course, minimum once per year (Table 2). Exactly 33 (4.1%) patients dropped out the PR due to comorbidities (heart failure, locomotor disability). Unfortunately, 14 patients passed away in both group (seven in both groups, PR and non-PR); five due to severe exacerbation with respiratory failure, one due to pneumonia, four due to heart failure, five at home.

There were 1,473 patients with comorbidities; the most frequent were arterial hypertension (n = 1,241; 74.1%), ischemic heart disease (n = 432; 25.8%), diabetes mellitus (n = 357; 21.3%) and arrhythmia (n = 363; 19.2%). One comorbidity was present in 596 patients (35.6%), two in 474 (28.3%) and three or more in 366 (21.8%). There were 238 (14.3%) patients without comorbidities (Table 2).

Patients aged under 65 years [420 (52.2%) *vs*. 384 (47.8%); p = 0.020], those with comorbidities [721 (50.2%) *vs*. 715 (49.8%); p = 0.015], patients with FEV_1 > 80% [144 (63.7%) *vs*. 82 (36.3%); p < 0.001], patients with CAT < 10 [344 (51.3 *vs*. 332 (48.7%); p < 0.001], those vaccinated against seasonal flu [301 (57.6%) *vs*. 222 (42.4%); p < 0.001] and those walked less than 350 m on 6MWD [210 (66.2%) *vs*. 108 (33.8%); p = 0.035] were more often treated with PR (Table 2). There was no statistically significant difference in the frequency of PR implementation according to the sex, smoking status, BMI categories, number of previous exacerbation, and number of comorbidities.

During the previous year (prior to entering the study), 1,402 patients (83.7%) had COPD exacerbations. After one year of monitoring, 1,056 patients (63.08%) had exacerbations. Exacerbations more frequently occurred in patients who were not treated with PR compared to those who had undergone PR [641 (73.7%) *vs*. 415 (51.6%), p < 0.001]. Patients who passed the PR program had less frequent COPD exacerbations among all analyzed categories of age, presence of comorbidities, categories of BMI, immunization against seasonal flu, and results of 6MWD test (p < 0.01) (Table 2).

In a univariate analysis, significant protective factors against exacerbations were PR, BMI ≥ 21 kg/m^2, and vaccination, while significant risk factors were smoking, number of previous exacerbations > 2, CAT score > 10, and FEV_1 < 80%. In multivariate analysis, PR and BMI ≥ 21 kg/m^2 were independent protective factors and CAT score > 10, FEV_1 < 80%, and number of previous exacerbations > 2

Table 1. Patient characteristics, pulmonary rehabilitation, and acute exacerbation of chronic obstructive pulmonary disease

Characteristic	n (%)	Pulmonary rehabilitation		p
		Yes (804)	No (870)	
Male	956 (57.1)	443 (46.6)	513 (53.4)	0.268
Female	718 (42.9)	360 (50.2)	358 (49.8)	
Age < 65	804 (48.1)	420 (52.2)	384 (47.8)	0.020
Age ≥ 65	870 (51.9)	384 (44.1)	486 (55.9)	
Non-smoker	84 (5.1)	38 (45.2)	46 (54.8)	0.740
Smoker and ex smoker	1590 (94.9)	766 (48.2)	824 (51.8)	
BMI* ≥ 21	1406 (84)	685 (48.7)	721 (51.3)	0.363
BMI < 21	268 (16)	119 (44.4)	149 (55.6)	
Comorbidities	1436 (85.7)	721 (50.2)	715 (49.8)	0.015
Without comorbidity	238 (14.3)	83 (34.9)	155 (65.1)	
CMBD* – one	596 (35.6)	285 (47.9)	311 (52.1)	0.612
CMBD – two	474 (28.3)	233 (49.3)	241 (50.7)	
CMBD ≥ three	366 (21.8)	193 (52.3)	173 (47.7)	
FEV_1* ≥ 80%	226 (13.5)	144 (63.7)	82 (36.3)	< 0.001
FEV_1 < 80%	1448 (86.5)	660 (45.6)	788 (54.4)	
CAT* ≥ 10	998 (59.6)	460 (46.1)	538 (53.9)	< 0.001
CAT < 10	676 (40.4)	344 (51.3)	332 (48.7)	
Number of patients with previous exacerbations >2 (n = 1,402)	298 (17.8)	137 (45.9)	161 (54.1)	0.615
Number of patients with previous exacerbations ≤ 2	1104 (65.9)	520 (47.1)	584 (52.9)	
6MWD* ≥ 350 m	1356 (81.9)	594 (43.8)	762 (56.2)	0.035
6MWD < 350 m	318 (18.1)	210 (66.2)	108 (33.8)	
Vaccination	523 (31.2)	301 (57.6)	222 (42.4)	< 0.001
Vaccination – no	1151 (68.8)	493 (42.8)	658 (57.2)	

BMI – body mass index; CMBD – comorbidity; FEV1 – forced expiratory volume in the first second; CAT – chronic obstructive pulmonary disease assessment test; 6MWD – six-minute walking distance

Table 2. Frequency of chronic obstructive pulmonary disease* exacerbations in several patient groups according to pulmonary rehabilitation* status

Characteristic	n (%)	Pulmonary rehabilitation		p
		Yes (804)	No (870)	
AECOPD*	1056	415 (51.6)	641 (73.7)	< 0.001
Moderate	758 (71.8)	334 (44.8)	424 (55.2)	
Severe	51 (4.8)	28 (55.6)	23 (44.4)	
Both severe and moderate	247 (23.4)	53 (22.6)	194 (77.8)	
None	618 (100)	389 (48.4)	229 (26.3)	< 0.001
Age < 65	528 (50)	228 (54.5)	300 (77.7)	< 0.001
Age ≥ 65	528 (50)	187 (48.8)	341 (70)	< 0.001
Non-smoker	63 (5.9)	29 (73.3)	34 (73.9)	0.332
Smoker and ex smoker	993 (94.1)	386 (50.4)	607 (73.6)	< 0.001
BMI* ≥ 21	868 (82.2)	362 (52.8)	506 (70.2)	< 0.001
BMI < 21	188 (17.8)	53 (44.5)	135 (90.6)	< 0.001
Comorbidity	942 (89.2)	394 (55.8)	548 (75.2)	< 0.001
Comorbidity no	114 (10.8)	21 (24.1)	93 (61.2)	< 0.001
FEV1 ≥ 80%	139 (13.2)	60 (41.6)	79 (96.3)	< 0.001
FEV1 < 80%	917 (86.8)	355 (53.7)	602 (76.4)	< 0.001
CAT ≥ 10	595 (56.4)	199 (43.3)	396 (73.6)	< 0.001
CAT < 10	461 (43.6)	216 (62.8)	245 (73.8)	0.018
6MWD* ≥ 350 m	855 (80.9)	302 (42.5)	553 (85.6)	< 0.001
6MWD < 350 m	201 (18.1)	113 (53.8)	88 (81.5)	< 0.001
Vaccination yes	300 (28.4)	157 (52.3)	143 (64.7)	0.008
Vaccination no	756 (71.6)	258 (52.6)	498 (75.1)	< 0.001

AECOPD – acute exacerbation of chronic obstructive pulmonary disease; BMI – body mass index; FEV_1 – forced expiratory volume in the first second; CAT – chronic obstructive pulmonary disease assessment test; 6MWD – six-minute walking distance

Table 3. Predictors of chronic obstructive pulmonary disease* exacerbations according to logistic regression analysis

Univariate analysis	RR	95% CI	p
Pulmonary rehabilitation	0.409	0.305–0.547	< 0.001
Age ≥ 65	0.880	0.662–1.170	0.379
Smoking (previous and actual)	2.204	1.182–4.111	0.013
BMI* ≥ 21 kg/m^2	0.513	0.334–0.788	0.002
Comorbidities	1.340	0.872–2.058	0.182
FEV_1* < 80%	3.101	2.071–4.645	< 0.001
CAT*score ≥ 10	3.380	2.512–4.549	< 0.001
Number of previous exacerbations > 2	5.928	3.404–10.324	< 0.001
6MWD*	1.169	0.768–1.574	0.294
Vaccination	0.737	0.550–0.987	0.040
Multivariate analysis			
Pulmonary rehabilitation	0.421	0.307–0.577	< 0.001
BMI* ≥ 21 kg/m^2	0.605	0.380–0.965	0.035
FEV_1* < 80%	2.021	1.303–3.134	0.002
CAT* score ≥ 10	2.375	1.720–3.280	< 0.001
Number of previous exacerbations > 2	4.222	2.372–7.514	< 0.001

FEV_1 – forced expiratory volume in the first second; CAT – chronic obstructive pulmonary disease assessment test; BMI – body mass index; 6MWD – six-minute walking distance

were independent risk factors from exacerbations, while vaccination (p = 0.086) was not (Table 3).

DISCUSSION

The results of this study demonstrated that COPD patients receiving PR experienced significant reduction in COPD exacerbations compared to non-PR patients during one year follow up. The observed effects were more pronounced in patients with comorbidities, low BMI, CAT ≥ 10, and vaccination against seasonal flu.

A Cochrane meta-analysis by Puhan et al. [12] has shown the results of 20 studies regarding the efficacy of the PR in reducing the AECOPD. In our study, the effects on AECOPD were comparable to other studies. Schuler et al. [13] on 383 COPD patients noted a decreased number of exacerbations (moderate and severe) one year after PR. Katajisto and Laitinen [14] showed the decreasing of hospitalization due to exacerbation after PR, but the study was limited by small number of patients. Seymour et al. [15] analyzed 60 patients, the proportion of patients that experienced an exacerbation in previous period resulting in an unplanned hospital attendance was 57% in the non-PR group and 27% in those receiving PR. Meta-analysis from Moore et al. [16] showed that results from randomized controlled trials suggest PR reduces AECOPD rehospitalization but results from the cohort studies did not. This was probably caused by varying standard of PR programs and the heterogeneous groups of COPD patients.

Compared to our study, Hassan et al. [17] demonstrated similar results in number of comorbidities (85%). Crisafulli et al. [18] showed that every second patient, from 2,962 patients, had at least one comorbidity, while in our study, it was 35.6%. Two years later, 2010, Crisafulli et al. [19] demonstrated reducing AECOPD among moderate and severe COPD patients with comorbidities (316 patients) after having completed the outpatient exercise-training program, which we confirmed. Franssen and Rochester [20] had similar results in 2014. Carreiro et al. [21] showed there is no association between the number of comorbidities and PR outcomes, a finding that we also observed.

There is a great variety of duration in PR programs worldwide, 3–9 weeks [4, 5, 22], Crisafulli et al. [19] used three-week PR duration per course, just like we did in our study. Houchen-Wolloff et al. [23] had the similar number of patients (823; 54.3% out of 1,515) who had completed PR. In many developed countries (United Kingdom, Canada, Sweden) only 0.4–1.2% of all COPD patients have access to PR [24, 25, 26]. But also, many of the patients refuse to take the PR programs. IPDV started with outpatient PR courses in 2014. Our study showed that younger patients (< 65), patients without respiratory symptoms and better FEV_1 above 80%, who are active, are more likely to accept PR programs in order to improve their health status and avoid sick leave. Similarly, patients with comorbidities and those vaccinated against seasonal flu are more familiar with the problems that carry exacerbations and are more likely to accept interventions that reduce the risk, as Ilic et al. [27] showed. Mihaltan et al. [28] recently showed that physical activity levels were low in his study that comprised 2,190 patients (multinational COPD cohort, which also included Serbia). Our patients, who are less mobile (under 350 m of 6MWD), probably wanted to improve their strength and daily activities with PR that Garrod et al. [29] proved in their study. After PR program, there were significant improvements in reduction of AECOPD among patients both younger and older, BMI < 21 and ≥ 21, CAT < 10 and ≥ 10, patients who could walk < 350 m and ≥ 350 m of 6MWD.

This study has some limitations. First not all COPD patients were given the option of PR, as, unfortunately, some specialist did not explain the true value of PR or did not say anything to their patients. Also, many physicians, on the primary health care level, did not know about PR program for COPD. Second limitation is related to observational study design. As this was not a randomized controlled trial the baseline group were unbalanced. Nevertheless, the PR turned to be significant negative predictor of exacerbations when adjusted for confounding factors. Third, there were probably varying criteria for hospitalization or observation in the emergency room at health institutions. Despite these limitations, to our knowledge, this is a first longitudinal study investigating PR effects in exacerbations of COPD in this region (Southeastern Europe – Western Balkans). We believe our study is important as it underlines that in resource-limited settings there is a great area for improvement in COPD care using low-cost interventions such as PR.

CONCLUSION

Patients who successfully completed the PR treatment had significantly less frequent COPD exacerbations compared to patients that do not pass through PR program. Multivariable analyses confirmed that CAT score > 10, FEV_1 < 80% and number of previous exacerbations > 2 were independent risk factors, while PR program and BMI ≥ 21 were independent protective factors from COPD exacerbations. From the aforementioned, the study demonstrates that there is a great need for consistent information and education of all COPD patients and physicians with emphasis on prevention of exacerbation and progression of disease.

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Утицај респираторне рехабилитације на појаву егзацербација хроничне опструктивне болести плућа у Србији

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САЖЕТАК

Увод/Циљ Егзацербације хроничне опструктивне болести плућа (ХОБП) имају велики утицај на ток болести. Плућна рехабилитација (ПР) прекида зачарани круг услед понављаних егзацербација. Међутим, ПР још увек није широко заживела као стандардни део терапије.

Циљ рада је био да се утврди ефективност ПР у спречавању егзацербација.

Метод Проспективна опсервациона студија је укључила стабилне болеснике са ХОБП (јануар 2015 – децембар 2018) у Поликлиничкој служби Института за плућне болести Војводине, Сремска Каменица. Повезаност ПР и егзацербација ХОБП, као и старости, индекса телесне масе (*BMI*), коморбидитета, вакцинације против сезонског грипа, испитивана је у универијантној и мултиваријантној логистичкој регресионој анализи.

Резултати Студија је обухватила 1674 болесника (956 мушкараца, старости 65,93 ± 8,45, 94,9% пушача и бивших пушача; 21 ≥ *BMI* 1406 болесника, 84%; FEV_1 < 80% 1448 болесника, 86,5%). Утврђена је значајна разлика у ПР статусу у односу на старост ($p = 0,020$), коморбидитете ($p = 0,015$), FEV_1 ($p < 0,001$), респираторне симптоме коришћењем упитника ХОБП (*CAT)* ($p < 0,001$), вакцинацију ($p < 0,001$). Егзацербације су се чешће јављале код болесника који нису били на ПР [415 (51,6%) *vs*. 641 (73,7%), $p < 0,001$]. У мултиваријантној анализи, независни протективни предиктори појаве егзацербације били су плућна рехабилитација [*RR* 0,421; 95% *CI* (0,307–0,577); $p < 0,001$] и *BMI* ≥ 21 kg/m^2 (*RR* 0,605; 95% *CI* (0,380–0,965); $p = 0,035$). Независни фактори ризика за појаву егзацербација су били *CAT* > 10 [*RR* 2,375; 95% *CI* (1,720–3,280); $p < 0,001$] и FEV_1 < 80% [*RR* 2,021; 95% *CI* (1,303–3,134); $p = 0,002$].

Закључак Болесници који су успешно завршили ПР имали су значајно мање егзацербација у поређењу са болесницима који нису били на ПР.

Кључне речи: *AECOPD*; *COPD*; *CAT* скор; плућна рехабилитација

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Appearance and characteristics of the gunshot wounds caused by different fire weapons – animal model

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SUMMARY

Introduction/Objective Gunshot residue (GSR) on the skin of a victim are important evidence, with far better precision, for reconstructive questions in the forensic investigation of cases involving gunshot wounds.

The aim of this experimental study was to analyze if there was any significant difference in macroscopic characteristics of wounds that were caused with different types of weapons from three different distances.

Methods This study was conducted at the Department of Ballistic and Mechanoscopic Expertise, Federal Police Directorate. Experiments were done on pigskin and 55 samples were made. Shooting was conducted using a system for safe firing. Samples of the pigskin were shot by firing projectiles from four different weapons and from three different distances, (contact wound, five centimetres and 10 centimetres).

Results At the contact range, wounds caused by automatic rifle had horizontal, vertical diameters larger than those made by pistols. Diameters on the wounds that were caused with different pistols, were similar. At the range of five centimetres, the narrowest part of contusion ring significantly differs even through pistol wounds. Diameters at the range of 10 centimetres are in favor of these results. Gunpowder residue scattering area was statistically different depending of type of weapon ($p = 0.004$).

Conclusion Wound diameters and surface area are useful for differentiation between pistol and rifle caused wounds. It is unsecure method for determination of pistol caliber or fire range. GSR have much greater potential for future analyses, but even GSR cannot be used to determine pistol caliber.

Keywords: gunshot wounds; gunshot residue; macroscopic examination; caliber; fire range

INTRODUCTION

Throughout history, ballistics experts and forensic medicine experts have classified gunshot wounds with respect to range by a variety of methods. All of these methods include inspection and comparison with test firings or patterns of gunshot residue (GSR) at the wound site [1]. Firearm-related injuries are a leading cause of morbidity and mortality in the world. In many shooting cases, bullets hit surfaces of various parts of the human body (often the head) directly. For assessing the shooting distance, most of the forensic literature describes only visual/microscopic methods for examination of the wound appearance and discharge particle patterns around. Shooting distances from human body surfaces can be divided roughly into four ranges: contact, near contact range, intermediate range and distant range [2, 3]. In contact wounds, the muzzle of the weapon is held against the surface of the body at the time of shooting. The appearance of tearing, scorching, soot, or the imprint of the muzzle characterizes contact wounds. In near contact wounds, the muzzle of the weapon is not in contact with the skin, being held a short distance away (a few centimeters). A characteristic of this kind of gunshot is a wide zone of powder soot overlaying seared blackened skin around the entrance wound. Intermediate range gunshot wound is one in which the muzzle of the weapon is held away from the body at the time of discharge, but is still close enough, so that gunpowder expelled from the muzzle can produce "powder tattooing" on the skin [4].

An impact velocity of only 150 to 170 fps is required to penetrate the skin. Most entrance wounds, regardless of the range, are oval to circular with a punched-out clean appearance and are often surrounded by a zone of reddish damaged skin (the abrasion ring). While powder tattooing of the skin implies a close-range wound, the fact that there are different forms of propellant powder makes this unreliable finding. In addition, indicative of a close-range injury is a cherry hue appearance of underlying muscle due to carboxyhemoglobin, formed by carbon monoxide release during combustion [5].

Wound diameters and visual analysis of dispersion of GSR only are used in practice, like

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some kind of screening method, just to check if it fits to the known story from crime scene, fire range etc. Previous studies have distinguished that the caliber of the bullet that caused the entrance wound in the skin cannot be determined by the diameter of the entrance. A .38-caliber (9 mm) bullet can produce a hole having the diameter of a .32 caliber (7.65 mm) bullet and vice versa. The size of the hole is not only due to the diameter of the bullet, but also to the elasticity of the skin and the location of the wound. An entrance wound in an area where the skin is tightly stretched will have a diameter different from that of a wound in an area where the skin is lax. Bullet wounds may be slit-shaped in areas where the skin lies in folds or creases [2].

The size of the entrance hole in bone cannot be used to determine the specific caliber of the bullet that perforated the bone though it can be used to eliminate bullet calibers. Thus, a bullet hole of 7.65 mm in diameter would preclude it having been caused by a 9 mm (.38 caliber) weapon. Bone does have some elasticity, however, so that a 9 mm bullet may produce an 8.5 mm defect.

Previously, researchers tried to prove the potential usage of wound size, its surface area, but results were very inconclusive. GSR on the skin of a victim is important evidence, with far better precision, for reconstructive questions in the forensic investigation of cases involving gunshot wounds [3]. Powder soot may help to differentiate between entrance and exit wounds, draw conclusions on the muzzle-to-target distance and on the muzzle-target angle [5, 6]. GSR consists of particles composed of antimony, barium, and lead that arise from the condensation of primer vapors and soot debris consisting of carbon and metallic fragments [3, 6]. In recent times, there have been no studies that tried to determine, or exclude, the type of weapon or distance between body, and weapon with only wound characteristics.

The aim of this experimental study was to analyze if there was any significant difference in macroscopic characteristics of wounds that were caused by different types of weapons from three different distances.

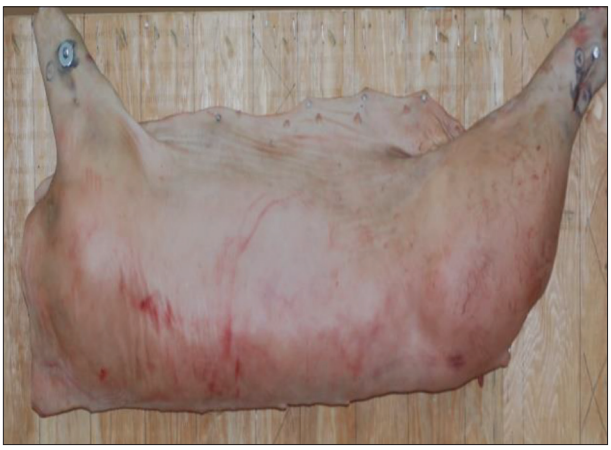

Figure 1. Pigskin is used as a subject in this study due to its similarity with human skin

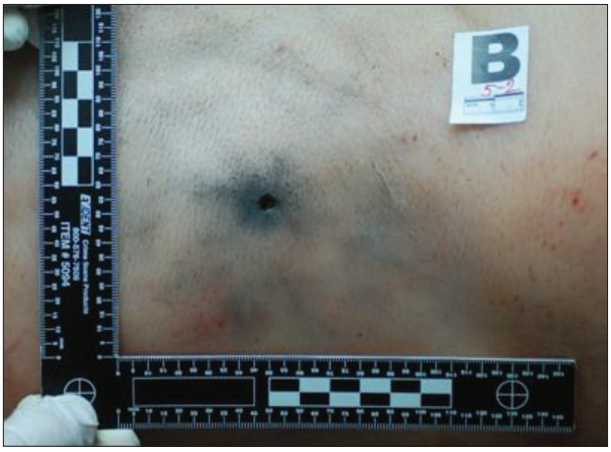

Figure 2. Sample of pigskin, shoot from CZ M70 pistol

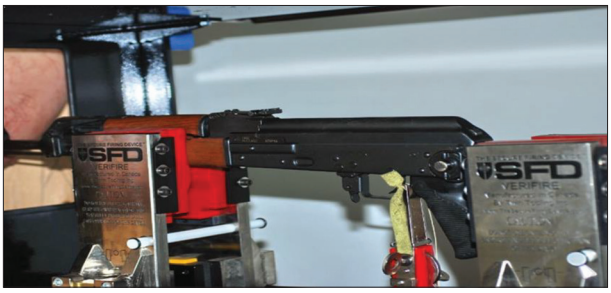

Figure 3. System Verifier – The Secure Firing Device, Twin Tooling, Canada

METHODS

This study was conducted at the Department of Ballistic and Mechanoscopic Expertise, Center for Forensic and Information Support, Federal Police Directorate. This study is performed in accordance with the ethical principles in compliance with the law on the protection of animals of Bosnia and Herzegovina. The study was approved by the Ethical committee of the Medical Faculty, at the University of Sarajevo, and used data is part of the author's PhD thesis (Figures 1 and 2).

The sample subject is pig (Figure 1). In total, 30 shooting pigskins were used, on which 60 shootings were made, but five of them were not included in the analyses due to technical error. Part of the pig's body size is approximately 120 × 45 × 20 m composed of skin, subcutaneous and muscle tissue, areas of the chest and abdomen, which is attached to a solid surface. Shooting was conducted using a system for safe firing from the firearm Verifire (The Secure Firing Device, Twin Tooling Inc., Gormley, Canada) (Figure 3). Samples of pig skins were shot by firing bullets from four different weapons and from three different distances (contact wound, and near contact wound, centimeters cm and 10 centimeters) (Figure 4). The weapons used in the experiment were most commonly used in the Balkan region in last 10 years according to the Federal and local police. Characteristics of weapons and projectiles are presented in (Table 1, Figure 3). Because it was conducted under experimental conditions, and used firearm devices, all samples were included in analyses (Figure 3, Table 1).

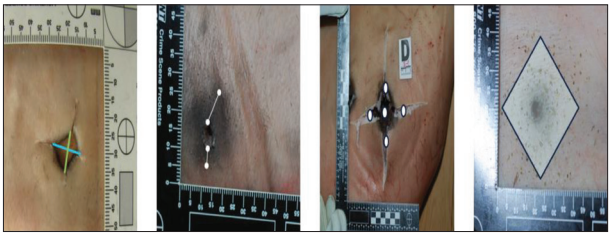

Figure 4. Examined characteristics of the wound; dimensions of the wound, contusion ring and the scattering area of gunshot powder particles were measured after shooting; based on these dimensions we have calculated the wound area; the size of the wound was determined using five points; one central point, was taken and around it the others; in one clockform, up to three, six, nine, and 12 hours; the values of surface area were calculated using the rhombus as a model

After shooting, the dimensions of the wound, contusion ring (CR), and the area of scattering of gunshot powder particles were measured. Based on these dimensions we have made calculation of the wound area. As a model of surface, rhombus was taken into account (Figure 4).

Statistical analysis

Results are presented as count (percent) or median (interquartile range) depending on data type. Fisher's exact test was used to assess significant differences between groups regarding nominal variables. Mann–Whitney U-test was used to test the differences between different weapons regarding interval data. No adjustment method for p values was used due to the small sample size and experimental nature of the study. All data were analyzed using SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA) and R Foundation for Statistical Computing version R 3.4.2. (R Core Team, Vienna, Austria).

RESULTS

In total, 55 wounds were analyzed, caused with four different weapons and from three different distances. Distribution based on the range was very similar, with no statistically significant difference in distribution, Fisher's Exact test p = 0.992 (Table 2).

First, we tested if there was any significant difference in any of examined characteristics of the wound in total, without considering range of firing. No significant difference was found in the horizontal or vertical diameter of the wound, nor the surface between four different calibers. CR in the narrowest and in the widest diameter had significantly different values; furthermore, the area of GSR was significantly different between tested calibers (Table 2.). We compared wound characteristics caused by pistols, and based on that, we have concluded that the widest and narrowest parts of CR significantly differs (widest p = 0.002, narrowest p = 0.005), as do GSR scattering area p = 0.036.

At the contact range, wounds caused with automatic rifle had horizontal, vertical diameters, significantly larger than wounds made by pistols ($p < 0.05$ *vs.* tested pistols). Diameters on gunshot wounds that were caused with different pistols, were very similar and none of them was statistically different ($p > 0.05$) (Table 3).

Table 1. Weapons of the experiment

Weapons	Caliber (mm)	Ammunition	Mark missiles	Manufacturer	Notation of sample
Pistol *Crvena zastava* M70	7.65	7.65 × 17 mm (0.32 AUTO)	PPU 0.32 AUTO	*Prvi partizan*, Užice, Serbia	A
Pistol *Crvena zastava* M57	7.62	7.62 × 25 mm	PPU 2001	*Prvi partizan*, Užice, Serbia	B
Pistol *Češka Zbrojovka* Model CZ 85 B	9 Luger	9 × 19 mm Luger	PPU 9 mm Luger	*Prvi partizan*, Užice, Serbia	C
Automatic rifle *Zavod Crvena zastava* M70AB2	7.62	7.62 × 39 mm	IK 91	*Igman*, Konjic, Bosnia and Hercegovina	D

Table 2. General characteristics of examined wounds

Parameters	Weapon			
	[a]Pistol CZ M70 (n = 14)	[b]Pistol CZ M57 (n = 13)	[c]Pistol CZ 85B (n = 13)	[d]Rifle CZ M70AB2 (n = 15)
Range				
Contact	4 (28.6%)	3 (21.4%)	3 (21.4%)	5 (33.3%)
5 cm	4 (28.6%)	5 (35.7%)	5 (35.7%)	5 (33.3%)
10 cm	6 (42.8%)	5 (35.7%)	5 (35.7%)	5 (33.3%)
Wound horizontal diameter (mm)	4.3 (2.7)	4 (1.65)	3.2 (2.5)	4.5 (15)
Wound vertical diameter (mm)	4.7 (1.3)	4.5 (1.5)	4 (2)	4.5 (18)
Surface area (mm^2)	21.2 (16.5)	20 (10.5)	12 (26.1)	20 (376.5)
Wideset part of CR (mm)	4.3 (3)[b,d]	9 (5.8)[a,d]	4 (7)[d]	20 (15)[a,b,c]
Narrowest part of CR (mm)	2.2 (2)[b,d]	4.5 (2)[a,c]	1.7 (1.5)[b,d]	4 (12)[a,c]
GSR scattering area (mm^2)	2034 (2037)[c,d]	1606 (1595)[c,d]	903 (724)[a,b,d]	4108 (2740)[a,b,c]

CR – contusion ring; GSR – gunshot residue;
[a] significant difference compared to Pistol CZ M70 at level $p < 0.05$;
[b] significant difference compared to Pistol CZ M57 at level $p < 0.05$;
[c] significant difference compared to Pistol CZ 85B at level $p < 0.05$;
[d] significant difference compared to Rifle CZ M70AB2 at level $p < 0.05$;
data are presented as median (interquartile range) or count

Table 3. Comparison of wound diameters based on the type of a gun and range

Parameters	Weapon			
	[a]Pistol CZ M70	[b]Pistol CZ M57	[c]Pistol CZ 85B	[d]Rifle CZ M70AB2
Contact (n)	4	3	3	5
Horizontal diameter (mm)	6.8 (3.1)[d]	6.5 (3)[d]	6 (1)[d]	19.5 (2)[a,b,c]
Vertical diameter (mm)	5 (3)[d]	5 (1.5)[d]	7 (3)[d]	26 (7.5)[a,b,c]
Wound surface area (mm^2)	30.7 (40.2)[d]	28 (15.7)[d]	42 (13)[d]	507 (193)[a,b]
Widest part of CR (mm)	4.5 (1.5)[d]	13 (12)	11 (4)	12 (2)[a]
Narrowest part of CR (mm)	2.5 (1)[b,c]	6 (7)[a]	8 (2)[a,d]	4 (1)[c]
GSR scattering area (mm^2)	567.5 (144.2)	1,000 (76.6)	627 (487)	1,575 (483)
5 cm (n)	4	5	5	5
Horizontal diameter (mm)	4.2 (1.8)	4 (1)	3 (0.7)	4 (1.8)
Vertical diameter (mm)	4.7 (2.3)	4 (2.5)	4 (1)	4.5 (0)
Wound surface area (mm^2)	21.2 (13.9)	20 (10)	12 (6)	18 (5.6)
Widest part of CR (mm)	6.5 (2.1)[d]	8.1 (1)[d]	6 (5)[d]	29 (6.5)[a,b,c]
Narrowest part of CR (mm)	3 (0.3)[b,d]	4.5 (1)[a,c,d]	2 (0.5)[b,d]	15 (0)[a,b,c]
GSR scattering area (mm^2)	2,144.7 (602)	1,710 (2,480.6)	558 (771)[d]	4,180 (1208)[c]
10 cm (n)	6	5	5	5
Horizontal diameter (mm)	4 (2)[c]	3.3 (1)	3 (1.8)[a]	3 (0.5)
Vertical diameter (mm)	3.8 (2.5)	4.5 (1.2)[c,d]	2.8 (1)[b]	2.5 (1)[b]
Wound surface area (mm^2)	17.6 (14)	18 (5.6)[c]	8 (4.6)[b]	8.7 (3)
Widest part of CR (mm)	2.7 (0.5)[b,d]	12 (5.8)[a,d]	3 (0)[d]	21 (8.5)[a,b,c]
Narrowest part of CR (mm)	1 (1)[b,d]	4 (2)[a]	1.5 (0.3)[d]	3.5 (1)[a,c]
GSR scattering area (mm^2)	2,534.5 (2,676.1)	2,012.5 (964)[d]	1,053 (350)[d]	4,444.0 (302.5)[b,c]

CR – contusion ring; GSR – gunshot residue;
[a] significant difference compared to Pistol CZ M70 at level $p < 0.05$;
[b] significant difference compared to Pistol CZ M57 at level $p < 0.05$;
[c] significant difference compared to Pistol CZ 85B at level $p < 0.05$;
[d] significant difference compared to Rifle CZ M70AB2 at level $p < 0.05$

Furthermore, wound surface area from automatic rifle was significantly larger than surface areas created with different pistol calibers (*vs.* CZ M70 p = 0.016, *vs.* M57 p = 0.036; *vs.* CZ 85 B 9 mm p = 0.036). At the contact, the values of widest and narrowest part of CR around the wound in total are significantly different (p = 0.003 and p = 0.004 retrospectively). We found that values of CR at close range (contact) had similar widest part diameter when firing from pistol with 7.62 mm, pistol with 9 mm or with automatic rifle with 7.62 mm caliber (p > 0.05). Gunpowder residue scattering area significantly differs between weapons when firing from close contact (p = 0.007). Pistol CZ M70 7.65 mm had smallest GSR scattering area, while wounds from automatic rifle had biggest GSR scattering area, but the size was very inconsistent.

At the range of 5 cm, there was not any significant difference in the diameters of the wound, or even in wound surface: horizontal diameter (p = 0.526); vertical diameter (p = 0.898), surface area (p = 0.903). The widest part of CR was significantly larger when wounds were caused with an automatic rifle (p = 0.001). Furthermore, there was not any difference between wounds caused by pistols. The narrowest part of CR was statistically different between wounds (p = 0.015). The narrowest part of CR was different on pistol wounds. Gunpowder residue scattering area was statistically different when firing with different weapons from 5 cm range (p = 0.007), with wounds from automatic rifles standing out.

In addition, diameters at the range of 10 cm are in favor of these results, with very similar results (p > 0.05). Horizontal diameters between pistol CZ M70 and pistol CZ85B were significantly different. Vertical diameter of wound caused with pistol CZ M57 (7.62 mm caliber) is significantly larger when it is caused with a 9 mm pistol or an automatic rifle. At the range of 10 cm, wounds had significantly different diameters of widest part of CR (F = 17.819, p = 0.001). Regarding the narrowest part of CR there was no statistically significant difference (F = 3.608, p = 0.063). Gunpowder residue scattering area was statistically different depending of type of weapon (F = 10.231, p = 0.004). What is interesting is that there was no statistically significant difference between GSR surface area around wounds that were caused by pistols.

Analyses of wounds caused by the same caliber but from different ranges were tested. Wounds caused by a 7.65 mm caliber pistol, had similar dimensional characteristics, and range of firing did not have any influence. Wounds caused by pistols CZ M57 with 7.62 mm caliber had significantly different horizontal diameter (p = 0.001). There was significant difference between horizontal diameters when firing with direct contact on skin and from 5 cm range (p = 0.04), also comparing wounds from direct contact between pistol and skin and those from 10 cm range, there was significant difference (p = 0.007). Horizontal diameters of wounds did not statistically differ when comparing those from 5 cm and 10 cm range.

A 9 mm caliber pistol caused much smaller wounds when firing from 5 or 10 cm than those that were caused from direct contact (*vs.* 5 cm p = 0.001; *vs.* 10 cm p = 0.001). In addition, vertical diameter was significantly smaller on wounds caused from 10 cm range than from direct contact (p = 0.004). Surface area of the wound is decreasing with the increase of the distance (p = 0.001).

The widest part and narrowest part of CR differed when using a 7.65 mm caliber pistol, measuring from three different fire ranges (p = 0.005). GSR surface area had significantly different values (p = 0.002), with trend of GSR area increasing with increase in distance. GSR surface area had significant change in value due to the change of fire range (p = 0.049). This was due to the smaller size of GSR scattering area when firing at the direct contact.

Statistically different values of widest (p = 0.007) and narrowest part of CR (p < 0.001) were measured on wounds caused with 9 mm pistol from different distances. GSR scattering area significantly differ based on distance (p = 0.002). An automatic rifle had statistically different values of widest and narrowest part of CR, based on distance (p = 0.002 and p = 0.057 respectively). There was no difference between wounds that were caused from 5

and 10 cm ($p > 0.05$). GSR surface area also significantly differ between different distances, as surface widens with the increase of distance ($p = 0.027$).

DISCUSSION

A small number of papers is done on this topic. In practice, we are searching for efficient, practical, and cheap methods that could be used for determination of firing distance and caliber.

Berryman et al. [7] compared wound diameters in head injuries, with diameters measured on skulls. They have concluded that there is no significant difference between .22 (5.6 mm) caliber and .25 (6.35 mm) caliber at close range wounds, while the .38 caliber (9 mm) wounds were significantly different ($p < 0.001$).

In our experiment, no matter which weapon we used, there was no significant difference between 5 and 10 cm range. Both of these values are categorized in near contact range, but diameters are decreasing with the increase of distance. There have been no papers testing close-range wounds so far.

Sahu et al. [8] had similar gunshot patterns in wound caused by a 9 mm pistol, in their study on a cotton cloth sheet. Horizontal diameter was wider for all the patterns at 5 cm range, but at 10 cm blackening was more dominant.

In our study, we used the geometrical shape of a rhombus. Matoso et al. [9] in their study have proven that different morphologies in the entrance holes are produces by three different calibers, using the same skull at the same shot distance of 10 cm. A 9 mm caliber wound was irregular and triangular, while a 10 mm caliber wound was round.

At the contact, the comparison of wounds caused by different weapons, the values of widest and narrowest part of CR around the wound in total, are significantly different ($p = 0.003$ and $p = 0.004$ retrospectively). Independently, we found that CR at close range had similar widest part diameter when firing from a 7.62 mm pistol, a 9 mm pistol or with a 7.62 mm caliber automatic rifle ($p > 0.05$).

Gunpowder residue scattering area differ significantly between weapons when firing from close contact ($p = 0.007$). Turillazzi et al. [10] showed that at 0.2 cm distance there was circumferential blackening with soot deposited in zone around entrance, while at 5 cm, a wide zone of powder soot overlying seared blackened skin was evident in the wound. Median area was not significantly different between 7.65 mm and 9 mm caliber. These results are in accordance with our results. The authors have proven that GSR deposits in the skin surrounding the entrance wounds strictly correlate with the shooting distance. In our study, GSR surface area had significantly different values ($p = 0.002$) when comparing calibers, with the trend of GSR area increasing with increased distance. This is explained by the fact that both ranges of 5 and 10 cm are categorized as near contact range. Intermediate range has a smaller GSR area, and in contact wounds with 0° angle GSR is in the wound channel [10].

The narrowest parts of CR could be used for determination between calibers, even between pistols. There is almost no difference between 7.65 mm caliber and 9 mm caliber.

Creating computer software for calculating wound area is one of the future goals. Petruk et al. [11] discussed multispectral method and means for determining the distance of the shot on the skin tissues. Using the computer model, they made an output of the expert system to generate diagnostic solution in the form of the distance to the target. They made a neural network. Multispectral improved method and means for determining the distance of shooting on the basis of the study gunshot injuries of the skin tissues, which allows to register the skin damage biological tissue forensic expert and use the findings as an evidence base.

The possibility to use unburned propellant powder for shooting-distance determination is analyzed in multiple articles. Hofer et al. [12] have concluded that infrared luminescence inspection of gunshot residue is an easy and reliable method for the detection of propellant particles in target tissue for about 80–90% of ammunition types. The quantification of unburned propellant particle densities can be used to draw shooting distance curves. The curve slope strongly depends on the morphology of the propellant particles. Muzzle-to-target distances could be determined up to 1.5 m for pistols and up to 3 m for revolvers.

Nowadays, GSR is the most used method for determining fire range. Even micro computed tomography analyses are based on GSR. Giraudo et al. [13] has described, "By increasing the firing distance, micro computed tomography analysis demonstrated a clear decreasing trend in the mean GSR percentage, particularly for shots fired from more than 15 cm. For distances under 23 cm, the powder particles were concentrated on the epidermis and dermis around the hole and inside the cavity, while at greater distances, they were deposited only on the skin surface. Statistical analysis showed a nonlinear relationship between the amount of GSR deposits and the firing range, well explained by a Gaussian-like function." In our study, GSR area is also in correlation with the firing range.

Hlavaty et al. [14] have analyzed histologic findings when estimating the fire range. They have proven that although variations existed, dark material of GSR was histologically identified in many skin and soft tissue, as well as bone sections at all ranges with tested calibers. These nonparallel results decrease the dependability of histology for range of fire estimation and reinforce using gross observation.

This study included a small number of samples and only three ranges. In future studies, intermediate range and long-range gunshot wounds should be taken into account and analyzed. In addition, we made this experimental study on pigskin, and more precise data would be collected from an experiment done on cadaver skin.

CONCLUSION

A new study should be conducted on a larger sample, which would include not only experimental conditions,

but also the real conditions. Computer software that automatically analyzes wound dimensions should facilitate the work. Based on this small sample, vertical and horizontal diameters, and wound surface area are useful for differentiation between pistol and rifle wounds from contact and near close range. It is an insecure method for determination of pistol caliber or fire range.

GSR has much greater potential for future analyses, but even GSR cannot be used to determine pistol caliber. It can be used to determine rifle inflected wounds, as it had significantly higher values then GSR scattering area around the pistol-inflicted wounds. In case there is a known weapon, GSR scattering area can be used to determine range. Since real-time shots were made at various angles, it is necessary to introduce a correction coefficient.

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Изглед и карактеристике рана нанесених пројектилима из различитог ватреног оружја – анимални модел

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САЖЕТАК

Увод/Циљ Расип барутних честица (РБЧ) на кожи жртве је важан доказ, са високом прецизношћу, коришћен да би се добили одговори о реконструкцији догађаја, те да би се објасниле ране изазване ватреним оружјем.

Циљ ове експерименталне студије је била анализа макроскопских карактеристика рана насталих пројектилима из ватреног оружја, а зависно од калибра и врсте оружја те удаљености.

Метод Студија је спроведена у Одељењу за балистичка и механоскопска вештачења Федералне управе полиције. Експеримент је спроведен на 55 узорака свињске коже. Експериментална пуцњава је вршена помоћу система за сигурну пуцњаву. Пуцање је вршено са три удаљености: контакт, 5 *cm* и 10 *cm*.

Резултати Приликом прислона оружја, ране настале пуцањем из аутоматске пушке имале су хоризонталне и вертикалне дијаметре знатно веће од оних нанесених пуцањем из пиштоља. Дијаметри рана узрокованих различитим пиштољима су имали сличне карактеристике, без значајне разлике. На удаљености од пет центиметара најужи део нагњечног прстена је имао различите вредности и међу ранама нанесеним испаљивањем пројектила из пиштоља. Дијаметри рана изазваних пројектилима са удаљености од 10 *cm* иду у корист претходно наведеним резултатима. РБЧ је статистички значајно различит и овисан о врсти оружја ($p = 0,004$).

Закључак Дијаметри и површина ране корисни су показатељи у разликовању између рана нанесених пројектилима из пиштоља односно аутоматске пушке. Метода је несигурна у утврђивању калибра и удаљености пуцања. РБЧ има много веће могућности за будуће анализе, али и оне не могу бити коришћене за утврђивање калибра пројектила испаљеног из пиштоља.

Кључне речи: ране нанесене ватреним оружјем; расип барутних честица; макроскопски преглед; калибар; удаљеност пуцања

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PRELIMINARY COMMUNICATION / ПРЕТХОДНО САОПШТЕЊЕ

"Jack of all trades" spotted in the Clinical Centre of Vojvodina – first detection of *Aeromonas hydrophila* from urinary tract infection samples

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SUMMARY
Aeromonas hydrophila is representative of group within the *Aeromonadaceae* family that mainly causes infections in humans. Aeromonads can induce meningitis, septicemia, respiratory and hemolytic uremic syndrome as well as gastroenteritis. Regarding diverse mechanisms involved in virulence and metabolic adaptation of *Aeromonas hydrophila* to various hosts and environments it is often introduced as "Jack of all trades." Here we report the first isolation of *Aeromonas hydrophila* from urine samples of three patients from the Urology Department within the Clinical Centre of Vojvodina. Epidemiological survey identified contact with surface water as the only mutual risk parameter. Following that, novel antibacterial agents against *Aeromonas hydrophila* are discussed.
Keywords: *A. hydrophila*; urinary infection; resistance; antimicrobials

Aeromonas hydrophila is detected in a broad variety of aquatic systems and it is regarded as an opportunistic pathogen. It is a Gram-negative bacterium and a representative of a group within *Aeromonadaceae* family that mainly cause infections in humans – motile aeromonads [1]. The presence of motile aeromonads was reported in a wide spectrum of food industry products (fish, meat, vegetables, milk, etc.), with predominance of *Aeromonas hydrophila* [2].

It is known that aeromonads have different virulence factors and can induce meningitis, septicemia, necrotizing fasciitis, respiratory and hemolytic uremic syndrome, and finally gastroenteritis in humans, where *Aeromonas hydrophila* was noted as the most common causative agent [1, 3–7]. Zhou et al. [8] reported that *Aeromonas hydrophila* induces extra-intestinal infections more often in patients with malignancy.

Food contamination is a mainly consequence of using water contaminated with *Aeromonas spp.*, as well as of overall poor hygiene in the working area during food processing or storage. It should be mentioned that milk is an exceptional medium for *Aeromonas hydrophila* growth, because of its nutrient composition, pH, and moisture content. Moreover, detection of *Aeromonas hydrophila* was reported in cheese, raw and pasteurized milk [2].

Since *Aeromonas hydrophila* has adopted diverse mechanisms involved in virulence and metabolic adaptation to various hosts and environments, it is often introduced as "Jack of all trades" [9]. Regarding the wide presence and pathogenicity of *Aeromonas spp.* strains, extensive and protracted use of antibiotics in treatment and prevention finally resulted in evolution of antimicrobial resistant strains, mainly in aquatic microbial pathogen group [2, 5, 10, 11]. Furthermore, Daood [12] reported a possible link between rising antibiotic resistance of aquatic strains and decreasing effect of antimicrobials in patients with *Aeromonas hydrophila* infections. Indeed, carbapenem-resistant *Aeromonas spp.* was reported recently in humans [13]. Besides that, *Aeromonas hydrophila* resistance to chlorine, low temperature, and the ability to form biofilm is described [14].

Here we report the first isolation of *Aeromonas hydrophila* from urine samples of three patients from the Urology Department within Clinical Centre of Vojvodina. Epidemiological survey was conducted in all three patients, where contact with surface water was identified as the only mutual risk parameter. All three samples were inoculated on blood agar, endo agar, and chromogenic agar, and were examined after an incubation period of 18–24 hours. Due to the atypical growth over the plates, Matrix Assisted Laser Desorption Ionization – Time of Flight mass spectrometry was used (MALDI Biotyper, Bruker, Billerica, MA, USA), where the presence of *Aeromonas hydrophila* was confirmed. Standard susceptibility to antimicrobials was determined via Kirby–Bauer test, known as the disc diffusion method; therefore, all three patients were treated with fluoroquinolones and reached remission.

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Considering the rising problem of multi-resistant *Aeromonas hydrophila*, several approaches have been recently suggested in order to outflank acquired bacterial resistance mechanisms.

Vijayakumar et al. [11] have demonstrated remarkable effect of fucoidan, marine brown seaweed extract, coated with gold nanoparticles. The tested substance showed larger inhibition zone compared to chloramphenicol (23.2 mm *vs.* 17.3 mm), as well as impressive biofilm inhibition activity against *Aeromonas hydrophila* [11]. Considering the treatment of aquatic organisms, as well as human patients, Rama Devi et al. [10] conducted an experiment with approach to target the quorum sensing system of *Aeromonas hydrophila* by introducing rosmarinic acid. They reported effect that includes significant inhibition of biofilm formation and production of virulence factors in three *Aeromonas hydrophila* isolates (AH 1, AH 12, and MTCC 1739) [10]. Stanković et al. [15] introduced millipede *Pachyiuli hungaricus* defensive secretion compounds as potential antimicrobial agents. Even lowest concentrations of isolated compounds (0.20–0.25 mg/ml) were reported as effective in *Aeromonas hydrophila* growth inhibition. They identified a total of 44 compounds, within which 2-methyl-1,4,-benzoqinone and 2-metoxy-3-methyl-1,4-benzoquinone were most dominant. Antimicrobial activity of Serbian Propolis against *Aeromonas hydrophila* was demonstrated by Ristivojević et al. [16] by a minimum inhibitory concentration assay, where zones of inhibition appeared at 0.05 mg/disc, while at a concentration of 0.20 mg/disc inhibition zone diameter was larger than 12 mm. Ramena et al. [17] tested antimicrobial activity of various plant extracts against *Aeromonas hydrophila*. In most cases, antimicrobial effect was insufficient compared to oxytetracycline, where only clove and cinnamon extracts showed inhibition zone diameter of 10.36 mm and 9.76 mm at a concentration of 50 mg.

In conclusion, considering effect of reported compounds against *Aeromonas hydrophila*, wide range of questions arise regarding compound isolation and stability, standardization and effectiveness in animal models respecting pharmacokinetics and pharmacodynamics parameters that will finally influence potential usefulness of novel agents. It should be noted that multi-resistant *Aeromonas hydrophila* strains are still not present in samples from Clinical Centre of Vojvodina, although the possibility for the emergence of resistant strains remains.

Ethical approval: All procedures described in this paper involving human participants were in accordance with the ethical standards and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest: None declared.

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„Мајстор свих заната" детектован у Клиничком центру Војводине – прва изолација бактерије *Aeromonas hydrophila* из узорака уринарних инфекција

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САЖЕТАК

Aeromonas hydrophila је представник групе унутар фамилије *Aeromonadaceae*, која најчешће доводи до обољења људи, укључујући менингитис, септикемију, респираторни и хемолитички уремијски синдром, као и гастроентеритис, где се *Aeromonas hydrophila* сматра најчешћим узроковачем. Захваљујући различитим механизмима развоја вируленције и метаболичке адаптације наспрам различитих домаћина и утицаја спољашње средине, често се назива „мајстором свих заната". У овом раду извештавамо о првој изолацији бактерије *Aeromonas hydrophila* из узорака урина три болесника Клинике за урологију Клиничког центра Војводине. Епидемиолошком анкетом је контакт са површинским водама идентификован као једини заједнички фактор ризика. Остатак текста је посвећен прегледу нових приступа антимикробне терапије против бактерије *Aeromonas hydrophila.*

Кључне речи: *Aeromonas hydrophila*; уринарне инфекције; резистенција; антибиотици

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

A case report of a hybrid procedure (visceral and partial aortic arch debranching) in the treatment of a challenging aneurysm of the thoracoabdominal aorta in the endovascular era

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SUMMARY
Introduction This paper aimed to present a hybrid approach as a less invasive and acceptable treatment.
Case outline Because of respiratory failure, the patient was deemed at high risk for open repair. Standard thoracic endovascular aortic repair (TEVAR) was unfeasible, so the patient underwent the hybrid procedure – partial aortic arch debranching at the first stage, followed by visceral debranching and endovascular exclusion of thoracic aortic aneurysm as a final procedure. The postoperative course was uneventful and the patient was discharged 10 days after TEVAR and visceral debranching.
Conclusion Staged hybrid procedure with combined debranching of the aortic arch and visceral arteries is feasible and should be considered as an alternative treatment option in patients with high-risk for open repair.
Keywords: endovascular procedure; aortic aneurysm; dissecting aneurysm

INTRODUCTION

Hybrid treatment with visceral and arch vessel debranching followed by thoracic endovascular aortic repair (TEVAR) has been used to treat high-risk patients and therefore allows treatment in this population group [1, 2, 3]. This paper aimed to present a hybrid approach as a less invasive and acceptable treatment.

CASE REPORT

A 56-year-old male was admitted to our hospital due to chronic dissecting aneurysm of the thoracic aorta with the maximum diameter of 8 cm (Figure 1). Visceral and infrarenal part of the abdominal aorta was free of dissection and aneurysmal disease. A few years ago, he suffered acute aortic dissection. Considering coexisting chronic obstructive pulmonary disease (forced expiratory volume in the first second was 50%), the patient's condition carried high risk for open repair. Multidetector computed tomography with angiography (MDCTA) imaging showed that standard TEVAR was unfeasible because of inappropriate characteristics of the proximal and distal sealing zone, so staged hybrid procedure was selected. Partial debranching of the aortic arch and visceral arteries provided an adequate length of both sealing zones of 25 mm. To achieve adequate proximal sealing zone for stent-graft deployment in the Ishimaru zone 1 of the aortic arch, we performed extrathoracic paratracheal carotid–carotid bypass in a right-to-left manner in conjunction with carotid–subclavian bypass using 8 mm tubular Dacron graft. After the transverse section of the left common carotid artery, the proximal part was ligated, while the distal part was anastomosed to the Dacron graft in an end-to-side fashion. The end-to-side anastomosis was also created to the left subclavian artery. We always endeavor to ligate the subclavian artery. However, in this case, the prevertebral part of the subclavian artery was difficult to approach. The left subclavian artery originated from the proximal landing zone, 12 mm away from the entry tear. Hence, it was expected that stent-graft would cover the artery origin preventing the endoleak from the left subclavian artery. Otherwise, endovascular occlusion of the artery would have been indicated. The procedure was performed under general anesthesia. The carotid shunt was not used because the retrograde flow from common carotid arteries was pulsatile. The postoperative course was uneventful, so two days later we performed visceral debranching via the midline laparotomy. The right common iliac artery was selected as an adequate inflow vessel for visceral debranching. Celiac trunk (CT) revascularization was accomplished via bypass to the common hepatic artery (CHA)

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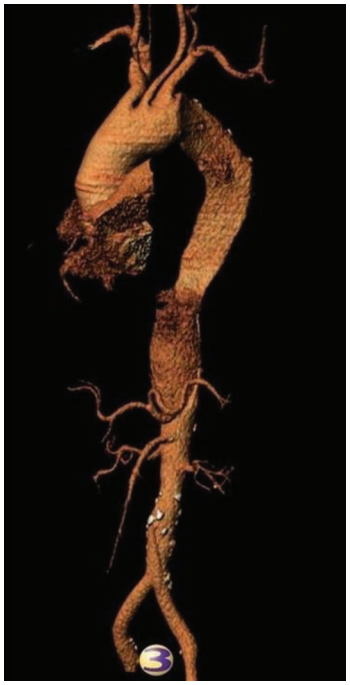

Figure 1. Multidetector computed tomography with angiography in volume rendering reconstruction; a chronic dissecting aneurysm of the thoracic aorta

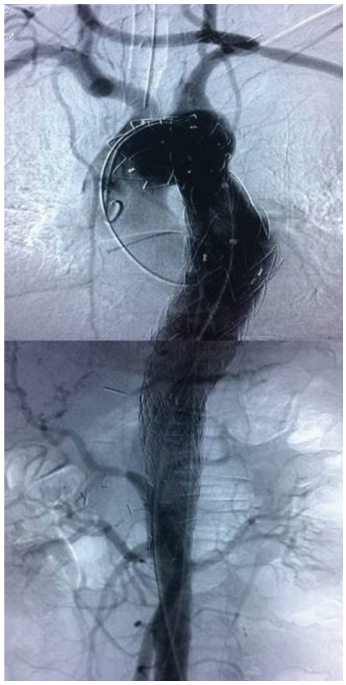

Figure 2. Completion angiography after thoracic endovascular aortic repair

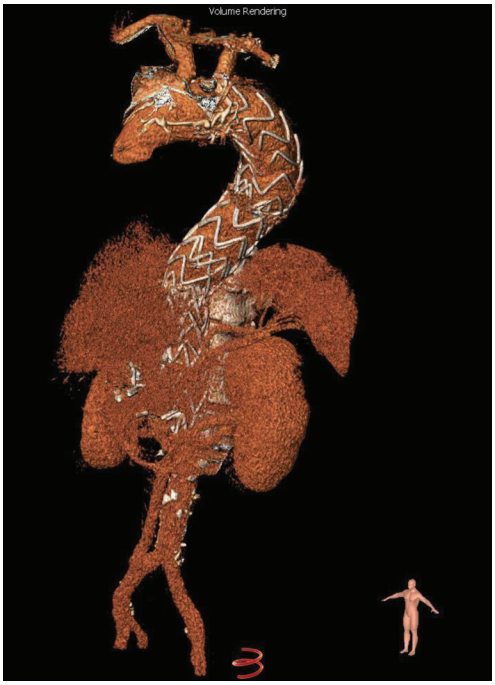

Figure 3. Multidetector computed tomography with angiography in volume rendering reconstruction, five years after thoracic endovascular aortic repair

in an end-to-side fashion. CT and CHA were approached through the gastrohepatic ligament in the lesser sac, while superior mesenteric artery (SMA) was identified at the base of the transverse mesocolon proximally to the origin of the middle colic artery. We used a jump graft (tubular Dacron graft of 8 mm) from the SMA to the CHA. The graft was passed retroperitoneally from the right common iliac artery to the SMA and then behind the pancreas to the CHA. The CT and SMA were ligated after completion of the bypass to avoid retrograde filling of the aneurysm sac. TEVAR was performed immediately after visceral debranching via the left transfemoral approach (Figure 2).

The thoracic aortic aneurysm was excluded using three Valiant Thoracic Stent Grafts (Medtronic Vascular, Santa Rosa, CA, USA) deployed above the renal arteries upwards to the innominate artery using the distal-to-proximal implantation technique which enables the deployment of a larger diameter stent-graft into a smaller diameter graft. Oversizing at the proximal and distal landing zone was 10–15%. To prevent paraplegia, cerebrospinal fluid drainage was used during the following 72 hours to maintain the intrathecal pressure below 10 mmHg and mean arterial pressure was targeted at ≥ 80 mmHg. The postoperative course was uneventful and the patient was discharged 10 days after TEVAR and visceral debranching. After five years of follow-up, MDCTA showed the regular position of the stent-graft and regular patency of the supra-aortic and visceral debranching (Figure 3).

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

DISCUSSION

In this paper, we report a case with a chronic dissecting thoracic aortic aneurysm who successfully underwent hybrid procedure. In terms of age and respiratory comorbidity, our patient was considered high risk for conventional open repair of a thoracic aortic aneurysm, which would require thoracophrenolumbotomy, single-lung ventilation, and aortic cross-clamping. A less invasive hybrid approach was indicated as an acceptable treatment. Its theoretical advantages implicate avoiding extensive two-cavity exposure, aortic cross-clamping, and extracorporeal circulation. Dissecting aortic aneurysm involved the thoracic part from the left subclavian artery to the CT. In order to achieve the adequate length of sealing zones and endograft apposition to the inner aortic curvature, partial arch debranching and retrograde bypass to the CT and the SMA were necessary. We opted for a two-stage hybrid treatment. Extra-thoracic partial debranching of the aortic arch was performed as a first procedure. We used the pretracheal route for crossover right-to-left carotid–carotid bypass, which we considered easier for tunneling and with more space for anastomosis creation. Other authors prefer the retropharyngeal route as a shorter and more direct path across the neck [4]. Also, in elective cases in which the left subclavian artery is going to be covered by endograft, we always perform a left carotid to subclavian artery bypass to prevent spinal cord ischemia, stroke, and arm claudication. Visceral debranching did not include renal arteries revascularization, so TEVAR was performed during the same procedure. In cases that require renal arteries clamping and revascularization, some authors recommend that TEVAR should be performed a

few days after visceral debranching to allow the kidneys to recover from the ischemia associated with the bypass before subjecting them to the nephrotoxic iodinated contrast during TEVAR [5]. Because of extensive coverage of the thoracic aorta, our patient was deemed as a high risk for spinal cord ischemia, hence prophylactic cerebrospinal fluid drainage was used as recommended by current guidelines of the European Society for Vascular Surgery [6].

Instead of hybrid procedures, in some institutions total endovascular repair is possible using custom-made and physician-modified fenestrated and branched devices, as well as parallel graft techniques (chimney/periscope).

Published mid-term results of endovascular renal and visceral artery revascularization are encouraging. Recently published studies showed a high technical success rate of 94–96% and primary five-year visceral arteries patency of 93% after fenestrated and branched endografts; however, in the same period, reinterventions were necessary in half of the cases [7, 8]. Also, Tsilimparis et al. [7] showed favorable outcomes of this technique, which is associated with lower mortality and morbidity (acute renal failure and mesenteric ischemia) in comparison to the hybrid procedure. High device costs and long manufacturing of custom-made fenestrated and branched devices limit this technique in cases with huge and symptomatic aneurysms. Also, these devices are not available in many centers in developing countries. Alternative "off the shelf" endovascular techniques, like parallel graft techniques and physician-modified fenestrated stent-grafts, involve the use of standard grafts with good short-term and mid-term results [9–15]. Considering the anatomy of our case, we deemed that the diameter and shape of the true lumen were inadequate to accommodate two chimney grafts for the CT and the SMA. Furthermore, we assessed that periscope graft in these arteries would be associated with acute angulation and risk for thrombosis.

Fenestrated stent-grafting in the aortic arch is technically challenging due to difficulties in stent-graft orientation and fenestration positioning. *In situ* fenestration techniques might avoid these problems, but because of possible issues with stent-grafts durability and ischemic complications during temporary carotid arteries coverage, this technique has not been widely adopted [16, 17, 18]. In recent years, arch branched endografts have been developed as a new technique for revascularization of supra-aortic branches. Despite encouraging initial results, further studies are required to standardize this technique [19, 20, 21]. Chimney technique to extend the proximal landing zone in TEVAR is feasible in the majority of vascular centers, with satisfied midterm outcomes, but not without a risk of major complications [22–25]. Zhao et al. [26] compared the outcomes of the chimney and hybrid technique for the reconstruction of a single arch branch and found that type Ia endoleak rate was significantly higher in the chimney group. Supra-aortic branch angulation and excessive oversizing were determined as a major risk factor for type Ia endoleak. Furthermore, compression and fracture of chimney graft, as well as retrograde type A aortic dissection, can lead to lethal consequences [27, 28]. Frequently performed excessive oversizing and ballooning of endografts, as well as more than one chimney graft, carry the greatest risk for retrograde dissection. In our institution, fenestrated and branched endograft techniques are not an option for aortic repair yet. Although the problem of the proximal landing zone in our case could be resolved by two chimney grafts for the left subclavian and the common carotid artery, we did not consider this technique because of the abovementioned reasons.

In selected cases of difficult aortic arch anatomy and risk for retrograde type A dissection after TEVAR, a frozen elephant trunk can be an alternative method for arch repair [29]. Sometimes, when the aortoiliac segment is not suitable for inflow, antegrade visceral debranching from the ascending aorta might be performed [30].

Staged hybrid procedure with combined debranching of the aortic arch and the visceral arteries is feasible and should be considered as a treatment option in patients with high-risk for open repair.

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Приказ хибридне процедуре (висцералног и парцијалног 'дебранчинга' лука аорте) у третману комплексне анеуризме торакоабдоминалне аорте у ендоваскуларној ери

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САЖЕТАК

Увод Циљ овог рада био је да прикаже хибридни третман као мање инвазивну и прихватљиву опцију лечења.

Приказ болесника Због значајног респираторног коморбидитета болесник је био неприхватљивог ризика за класичну отворену хирургију анеуризме торакалне аорте. Такође, стандардни ендоваскуларни третман није био могућ због неадекватне проксималне и дисталне зоне фиксације, те је болесник третиран хибридном процедуром. Процедура је рађена у два акта. У првом акту учињен је парцијални 'дебранчинг' (енгл. *debranching* – одвајање грана од главног стабла) лука аорте. У другом акту учињен је висцерални 'дебранчинг', после чега је анеуризма торакалне аорте искључена имплантацијом стент-графта. Постоперативни ток је протекао без компликација и болесник је отпуштен десетог постоперативног дана.

Закључак Комплексна хибридна процедура у два акта која укључује 'дебранчинг' аортног лука, а потом и висцералних артерија, представља могућу терапијску опцију код болесника са високим ризиком за отворено хируршко лечење.

Кључне речи: ендоваскуларна процедура; анеуризма аорте; аортна дисекција

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Larrey diaphragmatic hernia in an adult

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SUMMARY

Introduction Larrey hernia is a very rare type of the left sided parasternal congenital hernia with the incidence of 1–3% of all anterior diaphragmatic hernias.

Case report The paper describes Larrey congenital diaphragmatic hernia in an adult female patient, aged 70 years. Seventeen years earlier, the patient had had problems with intermittent left side chest pain, hypertension, dyspnea, shortness of breath, fatigue, and abdominal discomfort. She had no past surgical history, and no traumatic rupture of the diaphragm. An open surgical repair was conducted, reducing the herniated organs back into the abdominal cavity, and closing the diaphragmatic defect, repaired with a non-absorbable suture. During the immediate postoperative period, as well as six months later, the patient had a remarkable postoperative recovery.

Conclusion Larrey hernia represents an extremely rare kind of the anterior diaphragm hernias, which can be symptomatically manifested in older persons over 65 years of age. The treatment in the cases of asymptomatic and symptomatic Larrey hernias is a surgical intervention.

Keywords: Larrey hernia; Morgagni hernia; adult diaphragmatic hernia; open repair

INTRODUCTION

Larrey hernia represents an exceptionally rare type of left sided congenital diaphragmatic hernia, with an incidence of 2% of all anterior diaphragmatic hernias with intra-abdominal organ prolapse into the chest cavity, through the so-called "Hiatus Larrey" or the left sternocostal triangle. It usually symptomatically manifests later in life. In addition to the left-sided one, there is also a hernia called Morgagni right-sided anterior congenital hernia, which occurs more frequently, in 90% of the cases, and a hernia called Morgagni–Larrey hernia, which is bilateral, and the incidence of which is around 8% (Figure 1) [1, 2, 3].

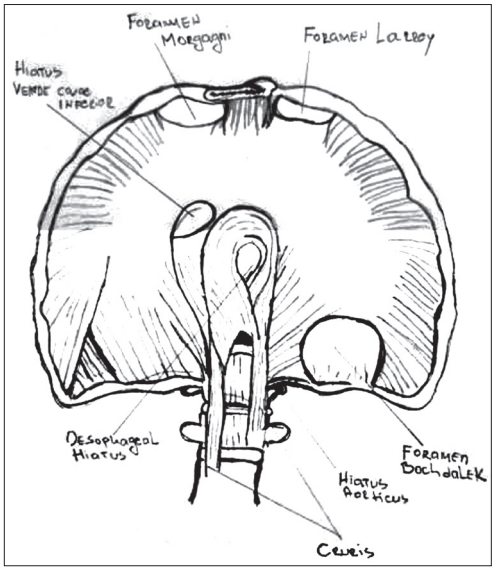

Figure 1. Anatomical locations of the common types of diaphragmatic hernia

CASE REPORT

We report a case of a 70-year-old woman, suffering from intermittent left-side chest pain, hypertension, dyspnea, shortness of breath, fatigue, and abdominal discomfort. She had had no past surgical history and no traumatic rupture of the diaphragm. The difficulties occurred 17 years earlier, in the form of intermittent heart palpitations and arrhythmia symptoms. During the previous six months, she had complained about vague thoracic and abdominal discomfort with mild dyspnea in exertion.

On admission, the woman was hemodynamically stable, and the laboratory analyses were within normal limits. She was examined at The Clinic for Digestive Surgery. Preoperative computed tomography (CT) revealed a large segment of transversal colon herniating into the left hemithorax, causing a partial compression of the lung (Figure 2). The patient was operated on under general anesthesia, upper and midline laparotomy was done with a herniated omentum, and the transverse colon was reduced into the abdomen (Figures 3 and 4).

A 6 × 3 cm defect was identified just behind the xiphisternum, through which a part of the omentum and transverse colon herniated into the left hemithorax.

After confirming viability by inspection and palpation, the contents were reduced back into

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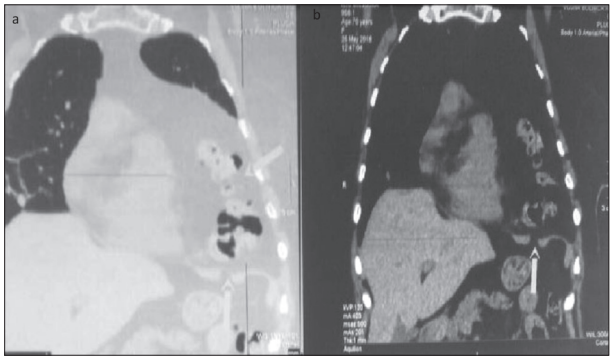

Figure 2. a) Preoperative sagittal computed tomography scan of the chest and abdomen with Larrey left side parasternal congenital diaphragmatic herniation of the abdominal contents into the thoracic cavity; b) preoperative sagittal computed tomography scan with the arrow showing anterior Larrey defect and anterior hernia

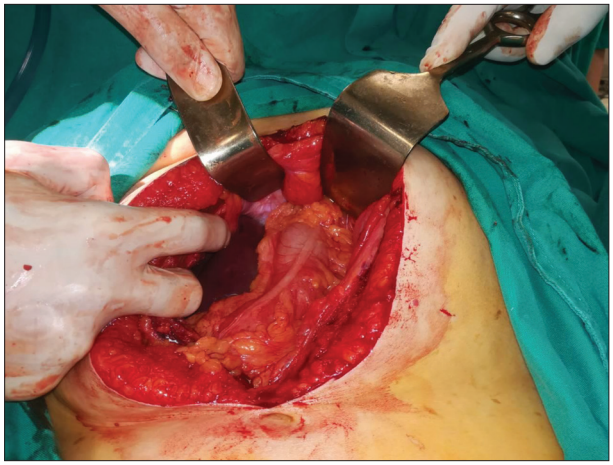

Figure 3. Intraoperative photograph showing diaphragmatic herniation of transverse colon and omentum trough the hernial defect into the thorax

the abdomen. The diaphragmatic defect was repaired and the plication of the diaphragm was performed with an interrupted suture (Figure 5).

The postoperative course was uneventful, and the patient was discharged from hospital on the eighth postoperative day. Six months after the operation, during a control examination, the patient was feeling well and a remarkable reduction of symptoms was noticed. The control CT scan was normal (Figure 6).

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

DISCUSSION

Diaphragmatic hernia is a weakness of the diaphragmatic wall that allows the passage of the abdominal organs into the chest cavity. According to the cause of occurrence, it is mainly congenital, but in 7% of the cases it can also be acquired as a consequence of trauma with a rupture of the diaphragm. According to the place of origin, congenital hernias can be classified as Bochdalek hernia, located posterolaterally, and Morgagni–Larrey or Morgagni–Larrey

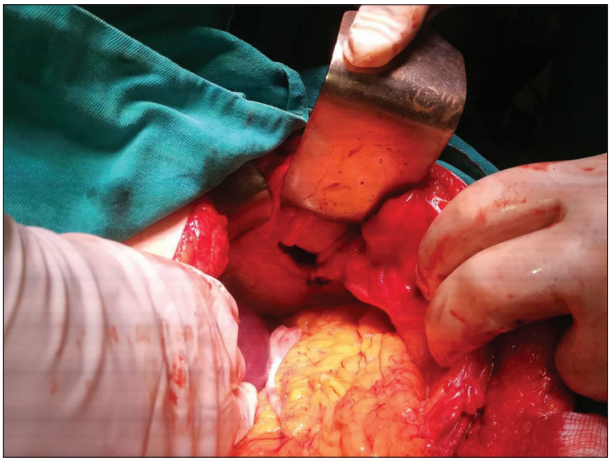

Figure 4. Intraoperative photograph showing the hernial defect on the left side of the retrosternal part of the diaphragm

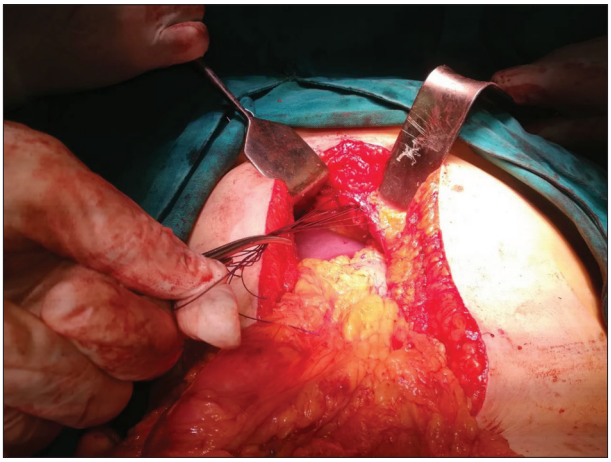

Figure 5. Primary closure of the retrosternal diaphragmatic defect using multiple interrupted sutures

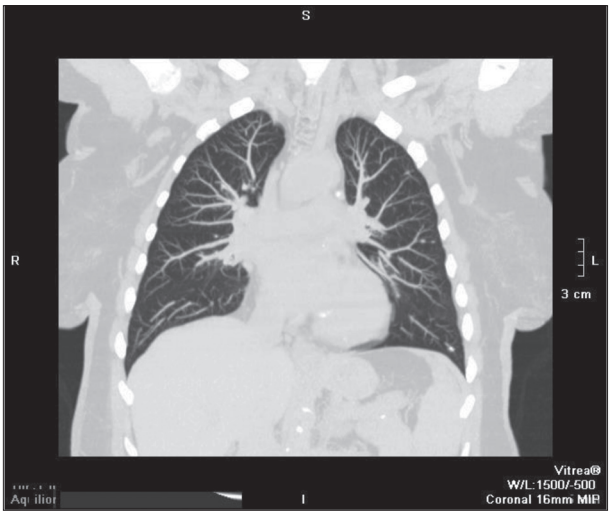

Figure 6. Six months after the operation control computed tomography scan was normal

anterolateral hernia, located parasternally, retro-chondrosternally, or in the retrocostoxyphoid region [4].

Larrey hernia represents an exceptionally rare type of congenital diaphragmatic hernia. It occurs as a consequence of a failure of fusion in the anterior portion of the

pleuroperitoneal membrane, resulting in the appearance of the left-sided defect in the retrosternal part of the diaphragm, called "Hiatus Larrey," or the left-sided sternocostal triangle [1]. It was named after the surgeon of Napoleon Bonaparte, Dominique Jean Larrey (1766–1842), who first described it in 1829, while analyzing the alternative ways in the treatment of pericardial tamponade [4]. Alongside Larrey's hernia, a more frequent congenital diaphragmatic hernia is the Morgagni's one, localized on the right side, and named after the famous Italian anatomist and pathologist from Bologna, Giovanni Battista Morgagni. He described it in his study *De sedibus et causis morborum per anatomen indagatis* (*On the seats and causes of disease investigated by anatomy*) in 1761 [5]. In the case where hernia is present bilaterally, it is called Morgagni–Larrey [6].

The incidence of congenital hernias is 0.3–0.5/1,000 in newborns, while Bochdalek hernia occurs in 1/2,200 childbirths. In cases of the front, or anterolateral ones, it is 1/1,000,000 childbirths [7]. Depending on the existence of the hernia sac, it is possible to classify them into so-called "true" and "false" hernias. The "true hernias," where hernia sacs are present, occur as a consequence of the disorder in the development of the diaphragm during the fetal period, when the closure of the pleuroperitoneal hiatus occurs, but the migration of the muscle is missing. In this case, the increased intra-abdominal pressure moves organs from the abdominal into the chest cavity, together with peritoneal evagination, which represents a hernia sac. The "false hernias" occur in the embryonic phase of development, when the closure of the pleuroperitoneal hiatus is missing, so the movement of intra-abdomen organs into the chest cavity is not followed by the peritoneum, with a subsequent lack of the hernia sac [4].

In general, the symptoms of anterolateral hernias occur during childhood, with respiratory symptomatology, while in only around 2–3% of the cases they manifest in adults, mainly around the age of 58 in the female population, and around the age of 50 in the male population [8].

In the review and analysis of 298 cases, Horton et al. [9] indicate that 28% of patients had no symptoms, while in 75% of the examinees there were some disturbances, such as pain and dyspnea. In the study by Abraham et al. [10], where the highest number of Larrey hernia cases were analyzed, 50% of the examinees were asymptomatic at presentation. The most common contents of hernia were stomach, transverse colon, omentum, and spleen. The conditions for the appearance of clinical disturbances are the increased intraabdominal pressure, pregnancy, obesity, chronic constipation, chronic obstructive lung disease, bronchial asthma, etc. [2, 10]. The clinical manifestation of Larrey hernia can also be developed as an acute condition in 25% of the cases. It occurs due to incarceration, i.e. volvulus of the abdominal cavity organs into the chest (stomach, transversal colon, omentum, small intestine, etc.), with gangrene formed and with perforation [6, 10]. The most common content of the anterolateral hernias is the omentum and the transversal colon, which was the case with our patient [11].

The diagnosis is set by a plain chest X-ray. However, the golden standard for diagnosing Larrey hernia is contrast CT. In cases where it is necessary to differentiate the unclear pictures of mediastinal or parasternal masses, it is also possible to apply the magnetic resonance imaging of the chest and abdomen [12]. The treatment of Larrey hernias, asymptomatic and symptomatic, is essentially surgical, performed in a timely fashion to prevent complications, such as incarceration, obstruction, strangulation, or volvulus with gangrene of the bowel. There are two possible approaches: transabdominal and transthoracic. In the series of 298 patients, from the aforementioned study by Horton et al. [9], 49% of the patients were treated trough thoracotomy, 30% through laparotomy, 17% laparoscopically, and 0.7% thoracoscopically. The transthoracic approach has an advantage in the expressed adhesions of the hernia and the hernia sac, with pericardial pleural and other mediastinal structures. It also refers to the patients who had a previous abdominal operation. The transabdominal approach presents an advantage in the cases where it is necessary to explore the opposite side of the diaphragm, due to the suspicion of the existence of Morgagni, or Morgagni–Larrey hernia, and to explore the remaining part of the abdominal cavity, owing to the suspicion of other associated diseases, particularly in cases of acute conditions with strangulation of the abdominal cavity organs [12, 13]. A combined approach should also be mentioned, which implies that the operation began with one approach (transabdominal or transthoracic), and then continued with another, in the conditions of non-reducible hernia, gangrene with perforation of the hollow viscus, etc. In addition to the so-called "open approach," it is possible to make a minimally invasive, i.e. laparoscopic or thoracoscopic approach. The well-known advantages of the minimally invasive treatment, including the faster postoperative recovery, as well as the shorter hospitalization period and minimal scarring, urged an increased number of surgeons to treat the patients in this manner in the last several years [1, 14]. The robotic surgery, whether transabdominal or transthoracic, represents another technological progress with well-recognized advantages: ergonomics, preciseness of instruments, as well as easy operation in narrow spaces [15, 16]. With regard to the treatment with a small-diameter hernia opening (less than 16 cm^2), it is possible to perform a primary suture, while in larger defects (larger than 20–30 cm^2), the plastics of the diaphragm opening is induced with the help of various kinds of meshes, to avoid tension [14]. The data from the literature indicate that polypropylene meshes are mainly applied, but the possibility of using other types of meshes has not been excluded [17].

Larrey hernia represents an extremely rare kind of the anterior diaphragm hernias, which can symptomatically manifest in persons over the age of 65 years. This kind of hernia should be taken into account in older patients suffering from long-term respiratory problems, palpitations, fatigue, difficulties in discharging, swollen abdomen, and occasional pain. The plain chest X-ray and CT represent choices of diagnostic procedures that could discover the existence of the anterior left diaphragm hernia with high precision. The treatment of asymptomatic and symptomatic Larrey hernias is a surgical intervention.

Conflict of interest: None declared.

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Лареова дијафрагмална кила код одрасле особе

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САЖЕТАК

Увод Лареова хернија је врло редак облик предње левостране парастерналне конгениталне киле са инциденцом 1–3% свих предњих дијафрагмалних кила.

Приказ болесника Приказан је случај појаве Лареове киле код женске особе старе 70 година. Седамнаест година имала је проблеме са повременим боловима у левој страни грудног коша, хипертензију, диспнеју, замор и нелагодност у трбуху. Болесница није пре тога оперисана нити је имала повреду дијафрагме. Оперисана је отвореним приступом, са враћањем органа из грудног коша у трбушну дупљу, са затварањем дијафрагмалног отвора нересорптивним концем. Током непосредног постоперативног периода и шест месеци после њега болесница је имала уредан клинички ток.

Закључак Лареова кила је изузетно ретка врста предње дијафрагмалне киле, која се симптоматски може манифестовати и код особа старијих од 65 година. Третман код асимптоматских и симптоматских Лареових кила је хируршка интервенција.

Кључне речи: Лареова кила; Моргањијева кила; дијафрагмална кила код одраслих; отворени приступ

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Upper limb robotic neurorehabilitation after pediatric stroke

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SUMMARY

Introduction Pediatric brain stroke is a rare condition, with the incidence of 1.2–13/100,000. The most common consequence is hemiparesis with unilateral hand impairment. There is level 4 evidence that robotics may improve the function of upper limbs. In this paper, we present the effect of combined robotic rehabilitation and kinesitherapy on the distal portion of the arm in the chronic phase of hemiparesis in childhood.

Case outline In a 7.5-year-old girl the treatment with robotic neurorehabilitation was administered in the chronic phase of post-stroke rehabilitation, 18 months after the stroke, involving individualized kinesitherapy for 30 minutes, and virtual reality-based rehabilitation using the robotic Smart Glove for 30 minutes. The rehabilitation protocol was administered for 12 weeks (five times a week). The results of therapeutic evaluation showed that the level 2 of Manual Ability Classification System remained unchanged until the end of treatment, while the grade assigned for the spasticity of flexors in the forearm and fingers was 2 at the treatment onset, 1+ after four weeks of therapy, and 1 after eight and 12 weeks of therapy. Qualitative improvement of arm function through the increase of the overall value of the Quality of Upper Extremity Skills Test was evidenced at each evaluation testing, being the greatest after the first four weeks of rehabilitation (4.83%).

Conclusion The result of our study suggests that combined robotic rehabilitation and kinesitherapy can improve the functional motor performance of the arm involved in the chronic recovery phase after a pediatric stroke.

Keywords: pediatric stroke; upper limb; robotics; rehabilitation

INTRODUCTION

A brain stroke is a devastating disease predominantly occurring in the elderly; however, it may occur in children as well. In general, pediatric stroke can be divided into arterial ischemic stroke (AIS) and hemorrhagic stroke. The division is the same as in the adult population, but the difference lies in its etiology [1].

The incidence of pediatric stroke ranges 1.2–13/100,000 inhabitants and it is considered a rare condition in the pediatric population [2]. The incidence rate of childhood AIS has been 1.6/100,000 per year [3]. However, it is a worrisome fact that the prevalence of pediatric stroke has risen by around 35% between 1990 and 2013 [4].

The risk factors for AIS in the pediatric population are arteriopathy, cardiac disease, cardiac surgery/interventions, sickle cell disease, infections, thrombophilia, etc. [5].

The signs and symptoms of acute stroke in children are similar to those in adults. The most common symptoms include hemiparesis and hemifacial weakness in 67–90%, and speech or language disturbances in 20–50% [6]. Clinical presentation of childhood stroke varies depending on the age of the child, with younger children usually having motor deficits, while older children commonly have a combination of language disorders and motor deficits [7]. It has been proposed in some studies that the recovery patterns and pathways differ between children and adults affected by stroke [8].

In spite of these differences in the aspects of etiology and recovery, therapeutic approaches for pediatric stroke are still largely based on the treatment of stroke in adults [2]. It should be stressed that stroke in childhood presents a serious rehabilitation challenge since in a high percentage of the affected it leads to physical, cognitive, and psychosocial disability. These deficits have a deep impact on independent functioning, everyday activities, and the quality of life of the affected children. Since there is a lack of randomized controlled studies that would address the issue, the optimal treatment is still debated upon, and most of the rehabilitation recommendations are based on expert consensus or weak evidence [5, 9, 10]. Recovery of the arm function is one of the main goals of rehabilitation attempts after childhood stroke; the upper limb function is essential in the performance of everyday activities and has a significant impact on independent functioning and the overall quality of life of the affected

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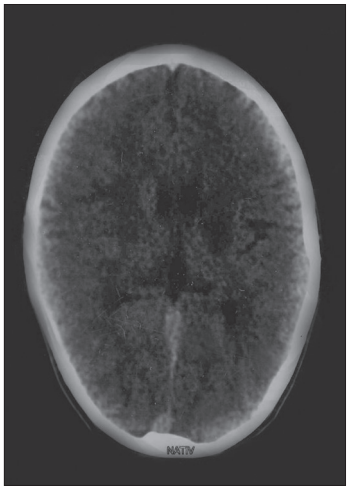

Figure 1. Multidetector Computed Tomography angiography in the axial plane showing a hypodense zone in the basal ganglia and internal capsule of the left side

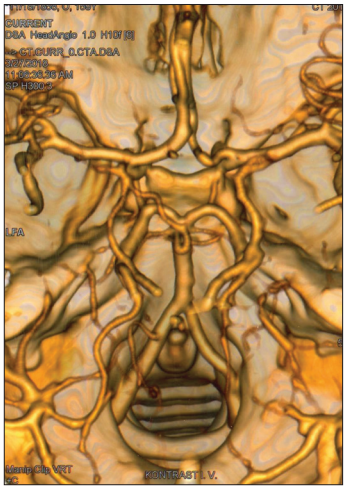

Figure 2. Multidetector computed tomography angiography, presented in 3D volume rendering technique, showing patency of intracranial arteries

Figure 3. RAPAEL Smart Glove™

children. The described abilities are largely determined by the distal function of the upper limb.

The first published systematic review of the papers dealing with the effectiveness of non-pharmacological rehabilitation interventions in motor and cognitive impairments after pediatric stroke has indicated that the available evidence supports the use of robotics in the rehabilitation of an upper limb [11].

CASE REPORT

In a 7.5-year-old girl, the treatment with robotic neurorehabilitation was administered in the chronic phase of post-stroke rehabilitation. At the age of five years and 10 months, after an hour-long strong headache, right-sided dissociated-type hemiparesis had developed (plegic arm and severely paretic leg). Multidetector Computed Tomography (MDCT) angiography in the axial plane showed a hypodense zone in the basal ganglia and internal capsule of the left side (Figure 1). MDCT angiography scanning, performed 2.5 months after the stroke, showed patency of intracranial arteries (Figure 2). An early rehabilitation treatment was introduced on the fourth post-stroke day, followed by an intensive rehabilitation treatment at the Physical Medicine and Rehabilitation Clinic, continuing rehabilitation with periodical out-patient treatment. Prior to robotic rehabilitation treatment, it was established that there were no cognitive impairments nor speech impairments.

The rehabilitation protocol was administered on the distal part of the paretic arm, consisting of individualized kinesitherapy (exercises to increase the motion range, to stretch shortened muscles, to strengthen agonist muscles) for 30 minutes, and virtual reality (VR)-based rehabilitation using the robotic Smart Glove (SG) for 30 minutes, under constant supervision of trained, licensed therapists. The rehabilitation protocol was administered for 12 weeks (five times a week).

The RAPAEL Smart Glove™ (Neofect, Yong-in, Korea) is a high technology device designed for rehabilitation of the distal portion of an upper limb after brain stroke (Figure 3). The glove represents a sensory device, supported by a computer system, able to follow/detect and measure the range of movements of the distal portion of the arm: forearm (pronation/supination), wrist (flexion/extension, radial/ulnar deviation), and fingers (flexion/extension of each of the fingers). The training games are divided in accordance with the aforementioned movements of all joint segments.

In each game, the patient is asked to perform a task associated with a particular movement. The games simulate the activities of daily living, and owing to the algorithm the SG adjusts individually the optimal game difficulty level (for games such as catching a butterfly, chopping food, playing drums, squeezing oranges, fishing, table sweeping), with visual feedback information.

The observed parameters of therapeutic evaluation are spasticity and functional motor status of the arm.

Spasticity was assessed according to the Modified Ashworth Scale [12].

The manual ability was classified according to the Manual Ability Classification System (MACS) [13]. Quality of Upper Extremity Skills Test (QUEST) was used for the assessment of the achieved functional motor level of the arm [14].

The measurements of the above parameters were performed before the treatment, and four, eight, and 12 weeks after the treatment started. There were no adverse events during the intervention and during the measurement of outcomes.

The results of the therapeutic evaluation showed that the level 2 of MACS remained unchanged until the end of treatment, while the grade assigned for the spasticity of

flexors in the forearm and fingers was 2 at the treatment onset, 1+ after four weeks of therapy, and 1 after eight and 12 weeks of therapy. The total value of QUEST at the first testing was 79.71%; 84.54% after four weeks; 88.73% after eight weeks; and 90.18% at the completion of therapy. The greatest increase of QUEST subscore was evident in the domain of grasping, for as high as 14.12% in relation to the initial value.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

DISCUSSION

There have not been many papers dealing with the issue of long-term arm recovery after pediatric stroke, in contrast to the adult populations, for which it has been established long ago that the distal portion of the upper limb is the last part of the body to recover [15]. In recent years, the interest in this problem has slightly increased. A study of the problem of pediatric AIS has been published, dealing with the motor functional outcomes and recognition of early poor outcome predictors aiming at adequate early interventions and long-term rehabilitation. The results suggest that fine motor functioning, adaptive behavior, the performance of the activities of daily living, and the overall quality of life are all lower compared to population norms. It has been found that pre-school children have poorer motor outcomes [16].

In the design of the rehabilitation protocol we abided by the recommendations of the Royal College of Paediatrics and Child Health (2017): Stroke in childhood and Royal College of Physicians Intercollegiate Stroke Working Party (2016), stating that the time from stroke should not prevent us from considering intensive training and that it is necessary to engage in training for at least 45 minutes every day for as long as patients are willing to participate, showing some measurable benefits from the treatment [5, 17]. The rehabilitation protocol in our study lasted 60 minutes, five times a week for 12 weeks. Due to common cold and family reasons, the patient did not attend treatment four times.

Considering the use of robotics, we should stress that it is a technologically innovative approach so that standard protocols and measurement indicants in the assessment of robotic neurorehabilitation have not yet been reported in the literature. It is, however, interesting for the children, it motivates them quickly to actively participate in the performance of movements that simulate everyday activities, with very important feedback incorporating vision, hearing, proprioception. Our opinion is that the lack of randomized studies is the reason for level 4 evidence that robotics may improve the function of upper limbs in children with hemiplegia and spasticity [11]. In particular, this level has been determined based on a paper in which a significant beneficial effect was achieved in hemiplegic children in terms of movement coordination and spasticity, which were maintained for as much as a month after robotic therapy [18]. The decision that our rehabilitation protocol should involve both kinesitherapy and robotic therapy was based on the fact that the therapy with SG system was possible with voluntary movements only and did not involve assisted movements, which were indicated and administered in the therapy even before the use of robotics in our patient. In fact, we decided to try the approach with robotics when there had not been any functional motor improvement of the arm during five months' monitoring period and with occasional kinesitherapy.

Our selection of measurement indices involved spasticity, the functional motor status of the arm, and participation in the activities of daily living. Spasticity reduction supported various functional outcomes so that after four weeks of treatment spasticity score was reduced by a half, and the QUEST score increased by as much as 4.83%. Each evaluation testing showed a qualitative improvement of the arm as a whole, with the greatest QUEST subscore increase in the domain of grasping, as we expected to a degree.

The termination of the therapy was based on the recommendations by the Royal College of Pediatrics and Child Health (2017): Stroke in Childhood, when the girl lost interest for games involving SG and when the functional improvement of the arm status in the last period was only 1.45% [5]. The level of manual ability of the arm in daily living activities improved, as her parents stated, but remained at level 2 by the MACS classification.

It can be interesting to consider the paper by Frascarelli et al. [19], who reported in 2009 on clinical improvement of control and coordination after the use of robotics in children with movement disorders, but were unable to establish which of the training variables had the greatest impact on recovery. Ten years have passed from the publication of this paper, but we still do not have an answer to the dilemmas reported in the paper. Many important questions remain open, among which the key problems are the treatment protocol definition, the optimal duration of intensive training, and whether the use of robotics can shorten this period and improve the outcome. It has been established so far that the use of robotics cannot replace the usual individual exercise techniques in children, but it has been proven that it could contribute to functional recovery [11]. The role of VR-based rehabilitation remains to be confirmed in the future in further studies, which would hopefully provide higher levels of evidence. The result of our study suggests that combined robotic rehabilitation and kinesitherapy are able to improve the functional motor performance of the arm involved in the chronic recovery phase after a pediatric stroke.

Conflict of interest: None declared.

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Неурорехабилитација руке роботиком после можданог удара код деце

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САЖЕТАК

Увод Педијатријски мождани удар спада у ретка стања са инциденцијом од 1,2–13/100.000. Најчешћа последица је хемипареза са оштећењем функције руке. Постоје докази нивоа 4 да роботика може побољшати функцију горњих екстремитета.

Циљ приказа је ефекат комбиноване примене роботичке рехабилитације и кинезитерапије на дистални део руке у хроничној фази хемипарезе у дечјем узрасту.

Приказ болесника Код девојчице старе 7,5 година, 18 месеци после педијатријског можданог удара примењен је рехабилитациони протокол дисталног дела руке: кинезитерапијски програм у трајању од 30 минута и роботска рехабилитација применом *Smart Glove* у трајању од 30 минута. Протокол је примењиван у трајању од 12 недеља, пет пута недељно. Резултати терапијске евалуације су показали да је функционални моторички ниво руке остао непромењен, да је спастицитет према модификованој Ашвортовој скали флексора подлактице и прстију од почетне вредности 2 после четири недеље терапије износио 1+, а после осам и 12 недеља 1. Квалитативно побољшање функције руке кроз пораст укупне вредности Теста за процену спретности горњих екстремитета евидентирано је на сваком евалуацијском тестирању; највеће је било после прве четири недеље рехабилитације (4,83%).

Закључак Резултати нашег истраживања показали су да комбинована примена роботске неурорехабилитације уз кинезитерапију побољшава функционално моторички опоравак руке у хроничној фази после педијатријског можданог удара.

Кључне речи: мождани удар; деца; горњи екстемитет; роботика; рехабилитација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

The breast necrosis caused by oral anticoagulant therapy

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SUMMARY

Introduction Described in 1943 for the first time, breast necrosis during anticoagulant therapy is only rarely encountered in clinical practice.

The objective of the article is to describe a patient who underwent anticoagulant therapy and developed breast necrosis during it.

Case outline A 57-year-old female patient was admitted to hospital with pain in her left breast, which upon examination showed to be erythematous, swelled, and hard. She had started experiencing the symptoms a few days earlier, and denied having had a fever. Over the previous four weeks she had received anticoagulant treatment (acenocoumarol) as popliteal embolectomy prophylaxis. The breast was firm, edematous, of limited mobility, and with no pectoral muscle infiltration. The breast ultrasound showed a homogeneous mass, with no signs of fluid retention or suspicious lesions. Upon admission, the patient began receiving intravenous antibiotic treatment and underwent blood tests. The second day upon admission, the patient's breast revealed a clearly demarcated area of necrotic skin. Surgical treatment was indicated. The surgery was performed in two stages, the first of which included a partial resection of the necrotic breast tissue, and the second simplex mastectomy. Histological analysis showed severe superficial necrosis, with underlying diffuse deep venous thrombosis and marked arteritis of medium and small vessels. Focal areas of extensive necrosis were found deep in the breast parenchyma.

Conclusion Considering that breast necrosis is extremely rare, it is usually not suspected initially. Learning about the patient's undergoing anticoagulant therapy is of crucial importance for reaching the right diagnosis. Breast abscesses should also be ruled out. Surgery is the treatment of choice, as changes to the breast tissue are usually irreversible.

Keywords: anticoagulants; necrosis; gangrene; breast; hemorrhage

INTRODUCTION

Breast necrosis and breast gangrene are synonyms of a condition which is rarely encountered in clinical practice. Breast necrosis was first described by Cutler in 1924 [1]. Flood et al. were the first to write about a case of breast necrosis caused by oral anticoagulant therapy [1]. Apart from the breast being affected, medical literature mentions the same condition manifesting as tissue necrosis affecting thighs, fingers and toes, the face, the back, and other locales. In 35% of all the cases described, necrosis was multifocal. According to the existing literature, 15% of all the cases of necrosis associated with the administration of anticoagulant therapy concerned breast tissue [2, 3]. In 1952, Verhagen wrote about soft tissue necrosis resulting from the administration of anticoagulant therapy, reporting on 13 cases, in one of which breast tissue was affected [4]. In 1971, Hagensen described a case of necrosis of breast fibroadenoma as a distinct clinical and pathological entity [1]. Oral anticoagulant therapy is commonly given as venous thromboembolism treatment and prophylaxis. Searching the available databases (PubMed/MEDLINE) yielded around 40 published articles reporting on cases of breast necrosis caused by oral anticoagulant therapy. The illness develops suddenly and is accompanied by acute pain. The patient's clinical condition indicates an emergency. The patient's confirmation that she had been receiving anticoagulant therapy is extremely important, as it suggests the possibility of breast necrosis. Breast necrosis is progressive and leads to dry breast gangrene. The etiology of breast necrosis remains unclear [1, 2, 5]. The sporadic nature of the condition cannot supply enough data on the this rare phenomenon [6].

CASE REPORT

A 57-year-old female patient was admitted to hospital with symptoms indicating a problem in the left breast. The symptoms had unexpectedly appeared several days earlier. The patient complained about her left breast being painful, red, and swollen. In providing medical history information, the patient reported being on anticoagulant therapy (acenocoumarol, 1 mg per day) for a month, during which she had not checked her international normalized ratio value (INR), as instructed by her vascular surgeon. The patient began receiving thromboembolism

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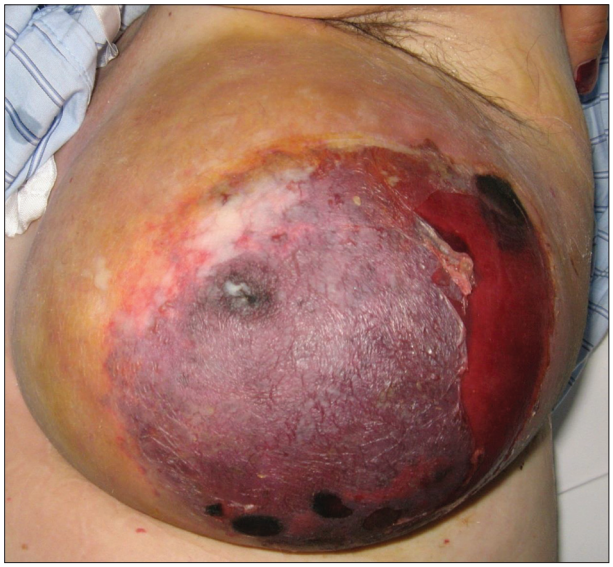

Figure 1. Breast necrosis upon admission

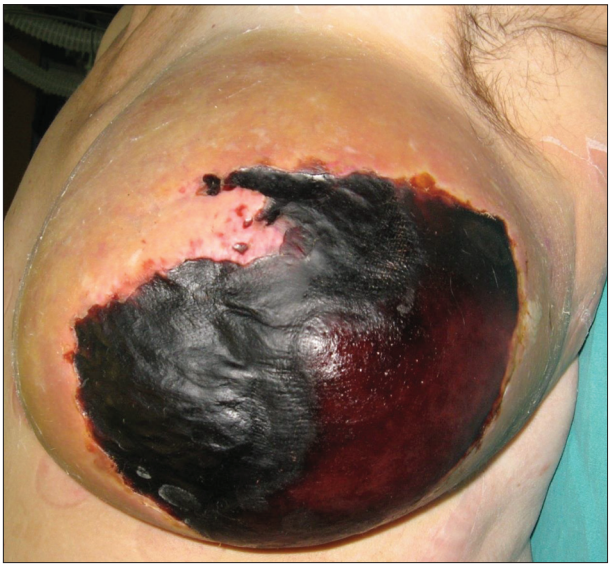

Figure 2. Breast necrosis with demarcation line, second day upon admission

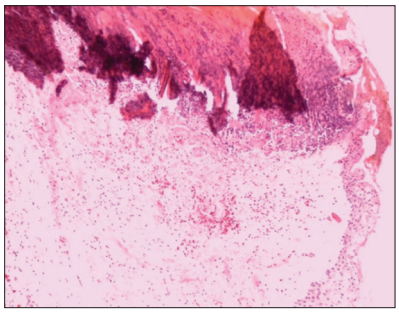

Figure 3. A loss of cellular details in breast tissue (H&E, 20×)

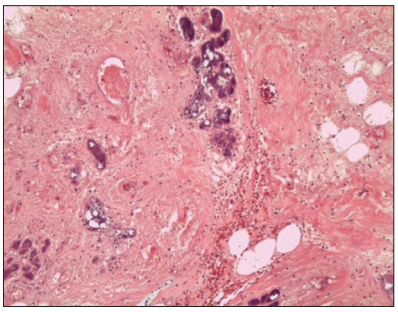

Figure 4. Necrosis areas of the breast parenchyma (H&E, 20×)

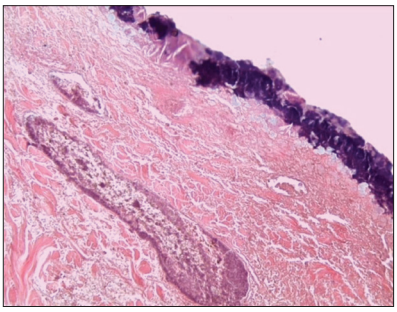

Figure 5. Inflammatory changes within and surrounding small arteries (H&E, 20×)

prophylaxis after a popliteal artery embolectomy. She also reported having developed a rectal cancer, which had been removed surgically – the patient had undergone rectum resection nine months earlier.

Upon admission, the patient's left breast was very swollen, erythematous, with the tissue feeling hard throughout and showing no signs of fluctuation (Figure 1).

Upon admission to hospital, the patient began receiving parenteral antibiotic and analgesic therapy. Her INR was tested, yielding 1.16. Following a consultation with a vascular surgeon, the patient was taken off the anticoagulant therapy, which was replaced with low-molecular-weight heparin (enoxaparin sodium, 20 mg). Basic blood chemistry tests were done. Blood count values were as follows: white blood cells 8.5×10^9L, red blood cells 3.8×10^{12}L, hemoglobin 108 g/L, hematocrit 0.341, Platelets 341; biochemistry tests were as follows: glucose 7.2 mmol/L, blood urea nitrogen 3.2 mmol/L, creatinine 59 mmol/L, total protein 61.7 g/L, total bilirubin 9.8 mmol/L, direct bilirubin 2.8 mmol/L, aspartate transaminase 17 U/L, alanine transaminase 5 U/L, lactate dehydrogenase 202 U/L, gamma-glutamyl transferase 58.8 U/L; electrolytes were as follows: Na 134.8 mmol/L, K 3.25 mmol/L, Cl 95 mmom/L, Ca 2.2 mmol/L, P 0.9 mmol/L. A radiologist was consulted, who performed a breast ultrasound, which confirmed the presence of edema and homogeneous breast mass, with no fluid accumulation or visible suspect solid or cystic lesions. The ultrasound examination revealed no pectoral muscle infiltration. On the second day after admission to the hospital, an area of necrotic tissue on her left breast became clearly demarcated (Figure 2). Surgery was indicated, and the operative procedure performed involved partial resection of the left breast and necrectomy. Partial resection of the breast was performed because mastectomy, which was surgically performable, would have left a skin defect and made closure by primary intent impossible. Following the surgery, the edema in the left breast decreased in size, in parallel with intravenous continuous analgesia and regular blood tests (electrolytes, INR). Post-operatively, the edema in the rest of the left breast reduced, making it possible to undertake a second surgical procedure and closure by primary intent. Next, the patient underwent mastectomy with closure by primary intent. The wound became infected post-operatively, but was successfully treated after an antibiotic sensitivity test was done and the best antibiotic regimen was chosen.

Based on histopathological analysis, the patient was diagnosed with hemorrhagic infarct of the breast skin, subcutaneous tissue, and parenchyma, as well as arterial thrombosis of the breast. Histological examination showed

a loss of cellular detail in the skin and subcutaneous tissue of the breast (Figure 3), as well as small areas of hemorrhage and necrosis that engulfed glands and stromal breast tissue. In addition to superficial necrosis, much wider areas of necrosis of the breast parenchyma was present (Figure 4). Diffuse subcutaneous venous thrombosis was present in the arteries and blood vessels of small and medium size, as well as distended veins in deeper tissues (Figure 5). Inflammatory changes were seen within, surrounding the wall of small arteries, some containing fibrin thrombi. Chronic edema and infiltrates were observed in the dermis and acute inflammation inside the walls of numerous small arteries. The patient made a recovery and was discharged from hospital after 26 days.

DISCUSSION

On average, the risk of breast necrosis among patients receiving anticoagulant therapy ranges 0.01–0.1% [2]. As for the cases of breast necrosis, i.e. breast gangrene caused by oral anticoagulant therapy described in medical literature, the condition generally appeared shortly after patients began receiving oral anticoagulant therapy [1, 4, 7]. The severe complication of anticoagulant therapy is hemorrhagic skin necrosis [8]. In the majority of cases, this period was three to six days, although in some of the cases breast necrosis developed 15 years after the patients received anticoagulant therapy [9]. In our case, the condition began four weeks after oral anticoagulant therapy administration. Some of the cases describing breast necrosis involved low-molecular-weight heparin therapy [7]. In our case, the patient did not receive low-molecular-weight heparin therapy at the time of the disease onset. The condition mostly affects middle-aged women between 55 and 65 years, which was also our case [9, 10]. One patient suffered breast necrosis after a rectal cancer surgery, which corresponds with our patient's medical history [1, 5]. Paraneoplastic syndrome occurs in approximately 10% of cancer patients. However, skin necrosis has not been described as a complication of paraneoplastic syndromes [11]. In most of the cases described, the patients were also obese, unlike our patient. Chan et al. [12] published new findings in their paper about the role of proteins C and S and the role of antithrombin III in the coagulation process, stating that these findings would enhance our understanding of the mechanisms leading to the condition. The authors also claimed hypercoagulable states following the introduction of anticoagulant therapy were a major factor contributing to the condition [12, 13]. A number of possible causes of breast necrosis have been proposed, such as trauma, previous treatment with anticoagulant therapy, specific vitamin deficiencies, etc. [9]. However, despite the efforts of medical researchers and workers to understand the etiopathogenesis of this condition, its causes remain unclear; it is indisputably known that a range of factors contribute to its development. The fact that the condition is very rare explains why relatively little attention is paid to identifying its causes [1, 5, 7]. Surgery was the preferred medical treatment in almost all of the cases described. In some of these cases, the operative procedure was done in two stages, as was the case with our patient [14, 15].

The incidence of breast necrosis caused by anticoagulant therapy is very low, and when such patients are admitted to hospital, breast inflammation is initially suspected. The information about the sudden development of the condition indicates that it might be acute, and the clinical description that it might be an emergency requiring immediate treatment. Timely diagnosis is essential for reducing morbidity. Another important piece of information concerns previous anticoagulant therapy administration, as it may help the physician suspect breast necrosis. In the majority of cases described in medical literature, the condition progressed very fast, leading to a clearly demarcated area of necrotic tissue, which made the physicians opt for necrectomy rather than needle biopsy of the breast.

NOTE

The case was presented in the form of a poster presentation at the 2nd Congress of Pathologists in Bosnia and Herzegovina with International Participation, which was held in Banja Luka, May 10–12, 2012. The abstract titled "Haemorrhagic infarction with skin necrosis of the breast complicating anticoagulant therapy" has been printed in the Abstract Book. The article was not published or sent to other medical journals for publishing.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

Conflict of interest: None declared.

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Некроза дојке узрокована оралном антикоагулантном терапијом

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САЖЕТАК

Увод Некроза дојке као последица примене антикоагулантне терапије је изузетно ретка појава у клиничкој пракси. Први случај у литератури описан је 1943. године.
Циљ рада је приказ болеснице која је под антикоагулантном терапијом развила клиничку слику некрозе дојке.

Приказ болесника Болесница старости 57 година примљена је на клинику са болном и клинички увећаном, еритематозном и напетом левом дојком. Тегобе су трајале неколико дана уназад и негирала је фебрилност. Анамнестички је наводила оралну антикоагулантну терапију (аценокумарол) четири седмице уназад, која је укључена после урађене транспоплитеалне емболектомије. Дојка је била тврда и едематозна, делимично покретна и без знакова инфилтрације пекторалног мишића. Ултрасонографија је показала хомогену масу без издвајања слободне течности и суспектних солидних промена. По пријему је укључена парентерална антибиотска терапија и урађена иницијална лабораторијска обрада. Други дан по пријему дошло је до демаркације зоне некрозе на кожи леве дојке. Индиковао се хируршки третман. Оперативни захват је урађен у два акта – први са парцијалном ресекцијом некротичног ткива дојке, а други са тоталном аблацијом остатка леве дојке. Хистолошки налаз је показао суперфицијалну некрозу са поткожном дифузном венском тромбозом на артеријама и крвним судовима мале и средње величине. У паренхиму дојке пронађене су фокалне области екстензивне некрозе.

Закључак С обзиром на то да је појава некрозе дојке изузетно ретка, најчешће се иницијално и не мисли на овај ентитет. Податак о антикоагулантној терапији је кључан у постављању сумње на ово обољење. Диференцијално дијагностички се мора мислити и на апсцес дојке. Начин лечења је примарно хируршки, а промене настале у дојци су најчешће иреверзибилне.

Кључне речи: антикоагулантна терапија; некроза; гангрена; дојка; хеморагија

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

A rare mechanism of subconjunctival dislocation of anterior chamber intraocular lens after blunt ocular trauma

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SUMMARY
Introduction Dislocation of intraocular lens (IOL) after ocular trauma is an emergency situation requiring prompt surgery. Dislocation of IOL into the subconjunctival space or pseudophacocoele is a rare event after blunt ocular trauma.
Case outline We report a case of a pseudophakic patient with a dislocation of the anterior chamber IOL (ACIOL) into subconjunctival space following blunt trauma to the right eye. A 76-year-old man presented with ACIOL dislocation into subconjunctival space, adapted old scleral wound and preserved corneal integrity. Fifteen years previously he suffered right eye globe rupture after blunt trauma, which was surgically closed and ACIOL was inserted.
Conclusion This case highlights a rare mechanism of ACIOL dislocation into subconjunctival space following blunt ocular trauma with preserved corneal integrity and adapted old scleral wound. In cases of post-traumatic aphakia, IOL dislocation into the subconjunctival space should be suspected.
Keywords: ocular trauma; intraocular lens; subconjunctival lens dislocation; eye globe rupture; epiretinal membrane

INTRODUCTION

Ocular trauma may cause significant ocular morbidity and is usually associated with the displacement of the natural lens in a phakic eye and of the intraocular lens (IOL) in a pseudophakic eye [1]. Biedner et al. [2] first defined the term "pseudophacocoele" for the subconjunctival extrusion of an IOL associated with eyeball rupture following blunt ocular trauma. Pseudophacocoele represents a rare clinical condition and therefore scarce data on patients' visual outcomes are reported. Blunt eye trauma may lead to IOL subluxation, dislocation into the vitreous cavity, anterior chamber or suprachoroidal space, or IOL may be extruded from the eye in the subconjunctival space, most commonly through the surgical wound [3].

We present a rare case of blunt ocular trauma in which an anterior chamber IOL was dislocated into the subconjunctival space with preserved integrity of bulbar wall despite the ocular trauma.

CASE REPORT

A 76-year-old man was admitted to our Clinic for Eye Diseases and presented with subconjunctival anterior chamber intraocular lens (ACIOL) dislocation following blunt trauma. He reported that he had suddenly lost conciseness, fallen down, and struck his right eye against a bedside table a month earlier. Medical history included transient ischemic attacks with sudden loss of conciseness. His ocular history included right eye globe rupture due to a similar blunt ocular trauma that he experienced 15 years previously. At that time, the globe rupture was surgically treated and the scleral wound was closed at another eye clinic. Later that year the ACIOL was inserted into his right eye at the same eye clinic. He had undergone a cataract operation with implantation of a posterior chamber intraocular lens (PCIOL) in his left eye several years prior to the latest blunt trauma.

The patient's best corrected visual acuity was 6/60 on the right eye and 6/6 on the left eye. Intraocular pressure of the right eye was 13 mmHg and 16 mmHg of the left eye. Slit lamp examination of the anterior segment in his right eye showed mild hyperemia of the bulbar conjunctiva and displaced ACIOL in the inferotemporal subconjunctival space (Figure 1). Examination of the sclera revealed an intact and adapted old scleral wound at 12 to 3 o'clock, with incarcerated uveal tissue at 2 o'clock (Figure 2). Corneal leucoma in the limbal zone from 10 to 2 o'clock was present. Observation of the anterior chamber demonstrated the presence of vitreous body and hyphema presented as a reddish tinge. Also, the presence of traumatic aniridia was revealed in his right eye (Figure 3). Eye fundus examination demonstrated mild partial hemophthalmos and epiretinal membrane in the macula. Fundus photography revealed the presence of an

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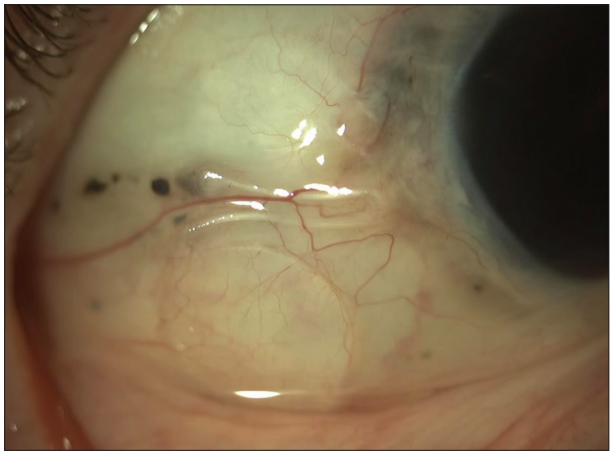

Figure 1. Dislocated anterior chamber intraocular lens in inferotemporal subconjunctival space

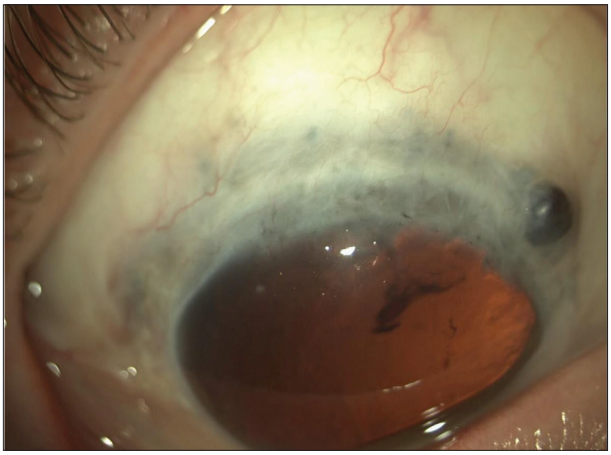

Figure 2. Intact and adapted scleral wound with the incarcerated uveal tissue at 2 o'clock

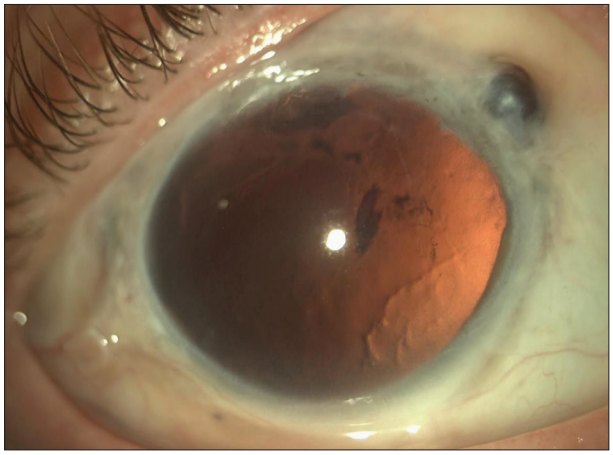

Figure 3. Corneal leucoma from 10 to 2 o'clock in the limbal zone, traumatic aniridia, the presence of vitreous body and hyphema in the anterior chamber

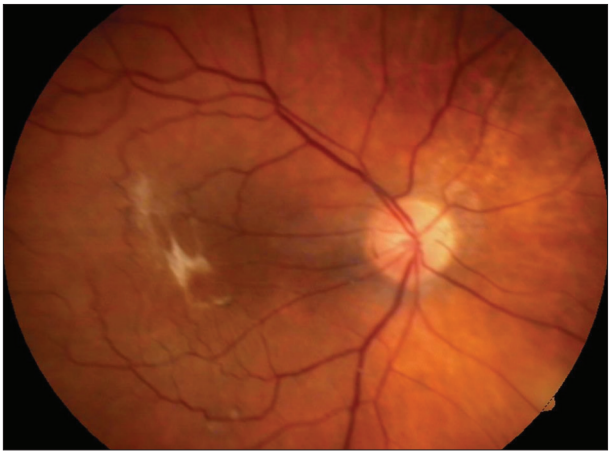

Figure 4. Fundus photography of an epiretinal membrane in the macula

epiretinal membrane in the macula in his right eye (Figure 4). Examination of the left eye showed the presence of PCIOL and hypertone fundus with no other abnormalities. Dislocated ACIOL in his right eye was operatively extracted from subconjunctival space under local anesthesia.

DISCUSSION

Pseudophacocoele or dislocation of IOL into the subconjunctival space after blunt ocular trauma is a rare entity and can lead to serious ocular complications [4]. In this case report we describe a rare mechanism of an ACIOL being dislocated into the subconjunctival space after blunt ocular trauma with no evidence of the dehiscence of an old scleral wound which occurred after the rupture of the eye globe following trauma 15 years previously. Scleral wound was adapted and intact, with incarcerated uveal tissue, and covered by conjunctiva. The integrity of the cornea was preserved.

Most commonly, IOL may be extruded from the eye into the subconjunctival space through the surgical wound. The presence of a large surgical wound increases the risk of IOL extrusion after blunt trauma, as presented by Aziz et al. [5], in which the authors hypothesized that IOL was dislocated into the subconjunctival space through the large corneal wound following the anterior–posterior compression and equatorial expansion of the globe. Kumawat et al. [6] reported a case of post-traumatic pseudophacocoele where no scleral wound dehiscence could be noted. In the reported case, previous scleral wound from cataract surgery may have opened up and led to the dislocation of IOL, but may have sealed spontaneously. We hypothesize that anterior–posterior compression and equatorial expansion of the globe may be a possible mechanism of ACIOL dislocation in our patient. Of note, our patient's visual outcome was surprisingly good considering the fact that he had experienced two serious blunt ocular traumas associated with complications including traumatic aniridia, aphakia, and epiretinal membrane.

We present a case of ACIOL dislocation in the inferotemporal part of the subconjunctival space following blunt ocular trauma with a rare mechanism of ACIOL dislocation since the bulbar wall integrity was preserved. We believe that in our patient previous corneal wound from cataract surgery may have opened up, caused the dislocation of the ACIOL, after which the wound sealed spontaneously as the patient was presented to our clinic a month after the

blunt eye trauma. The presence of epiretinal membrane in the macula, aphakia, and traumatic aniridia may be treated by vitrectomy with membrane peeling accompanied by transconjunctival intrascleral fixation of intraocular lens or by intrascleral insertion of a three-piece lens and silicon iris prosthesis [7, 8, 9].

ACIOL dislocation into the subconjunctival space after blunt ocular trauma should be suspected while examining cases of post-traumatic aphakia.

ACKNOWLEDGMENT

All procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendment. Written consent to publish all shown material was obtained from the patient.

Conflicts of interest: None declared.

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Редак механизам супконјунктивалне дислокације предњекоморног сочива после контузионе повреде ока

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САЖЕТАК

Увод Дислокација интраокуларног сочива као последица трауме ока је стање које захтева хитну хируршку интервенцију. Дислокација артифицијалног сочива у супконјунктивални простор или псеудофакоцела је ретка и неуобичајена последица после тупе повреде ока.

Приказ болесника Код болесника старог 76 година је утврђена дислокација артифицијалног предњекоморног сочива у доњетемпорални супконјунктивални простор после контузионе повреде десног ока настале после губитка свести и пада. Код болесника је утврђен очуван интегритет булбуса са добро адаптираном старом раном на склери у коју је инкарцериран део увеалног ткива. Болесник је 15 година раније имао трауматску руптуру булбуса на десном оку која је била хируршки збринута и тада му је уграђено артифицијално предњекоморно сочиво.

Закључак У раду је приказан редак механизам дислокације артифицијалног предњекоморног сочива у супконјунктивални простор после контузионе повреде ока са очуваним интегритетом рожњаче и добро адаптираном старом склералном раном. У случајевима посттрауматске афакије треба имати у виду и могућност дислокације интраокуларног сочива у супконјунктивални простор.

Кључне речи: траума ока; интраокуларно сочиво; супконјунктивална дислокација сочива; руптура булбуса; епиретинална мембрана

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Perspectives on mental health services during the COVID-19 epidemic in Serbia

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SUMMARY
World Health Organization (WHO) declared the COVID-19 outbreak a public health emergency of international concern. Mental health strategies implemented due to the coronavirus epidemic must include the assessment of mental health status of people in different subpopulations influenced by the COVID-19 outbreak, identifying persons who are at high risk of manifesting behavior disorders, suicide attempt, and aggression in aim of providing appropriate mental health care interventions for those in need. The target population of these mental health strategies is categorized in four different groups: the most vulnerable people with mental health problems, isolated people with symptoms of atypical acute respiratory infection, individuals who have been in close contact with the previous two categories, and people affected by the preventive and restrictive measures.
Keywords: COVID-19; coronavirus; mental health

THE COVID-19 EPIDEMIC IN SERBIA

In January 2020 the World Health Organization (WHO) declared the outbreak of the coronavirus disease 2019 (COVID-19 outbreak) to be a public health emergency of international concern. The WHO stated that there is a high risk of COVID-19 spreading to countries around the world. In March 2020, WHO made an assessment that COVID-19 can be characterized as a pandemic disease [1].

This infectious disease is caused by a new virus, severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) which can be transmitted between persons via close contacts, with developing acute respiratory distress syndrome and acute respiratory failure requiring treatment in intensive care units [2, 3]. Based on current epidemiological studies, the incubation period could be 1–14 days, mostly 3–7 days, with the greatest contagiousness during the latency period [4]. A recent clinical study has found that the most common clinical manifestations of the COVID-19 are fever, cough, fatigue, sputum production, shortness of breath, sore throat, and headache [5]. A minority of patients also manifested gastrointestinal symptoms, such as diarrheal syndrome and vomiting [5].

According to the officials of the Ministry of Health and the Dr. Milan Jovanović Batut Institute for Public Health of Serbia (official website: www.covid19.rs), the first case of COVID-19 in Serbia was reported on March 5, 2020, and confirmed on March 6, 2020. Until April 29, 2020, 78,942 persons had been tested, of which there were 8,724 confirmed cases, 1,292 recoveries, and 173 deaths (1.98%). On March 15, a state of emergency was declared in the country and numerous measures were introduced (self-isolation / home quarantine of persons older than 65 years, closure of educational institutions, lockdown for the entire population, etc.) with the aim to reduce social contacts between people. Numerous public health measures have been taken to prevent the spreading of the disease, among which significant actions and recommendations for protecting and improving mental health of the entire population.

MENTAL HEALTH PROBLEMS DURING THE COVID-19 EPIDEMIC

Mental health strategies must include three key points: 1) assessing the mental health status of people in different subpopulations influenced by the COVID-19 outbreak; 2) identifying persons who are at high risk of manifestations of behavior disorders, suicide attempt, and aggression, and 3) providing appropriate mental health care interventions for those in need. Another aspect will focus on the target population, which will be categorized in four different groups: 1) the most vulnerable people with mental health problems (e.g. hospitalized patients with severe physical conditions, front-line healthcare professionals and administrative workers); 2) isolated people with symptoms of

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atypical acute respiratory infection; 3) individuals who have been in close contact with the previous two categories; and 4) people affected by the preventive and control measures [6].

Recent studies show that confirmed and suspected cases of COVID-19 may experience loneliness, denial, anxiety, insomnia, despair, with developing symptoms of depression, obsessive-compulsive disorder [7, 8]. Also, strict quarantine and mandatory contact tracing by epidemiological professionals could cause stigmatization, social rejection, financial loss, and discrimination [9]. Some of them may also have increased risk of aggression and suicide attempt [7].

On the other hand, healthcare professionals, especially those working in a hospital that treats patients with confirmed or suspected COVID-19, are susceptible to mental health problems. They may experience fear of contagion and spreading the virus to their families, friends, and colleagues [10]. Recent studies show that health professionals who worked in COVID-19 units and hospitals during the epidemic reported depression, anxiety, fear, and frustration [11, 12].

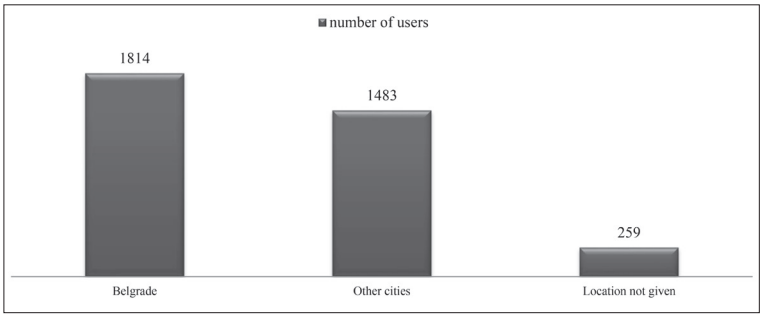

Figure 1. Number of users of the National Line for Psychosocial Support During COVID-19 Epidemic in Serbia according to residency

Table 1. Users' demographic characteristics and reasons for calling the National Line for Psychosocial Support During COVID-19 Epidemic in Serbia

Demographic characteristics and problems	n (%)
Sex	
male	1,205 (33.9)
female	2,262 (63.6)
no data	89 (2.5)
Age	
X ± SD; med (min–max)	55.4 ± 16.6; 60 (16–98)
Age group (years)	
≤ 30	295 (8.3)
31–40	398 (11.2)
41–50	409 (11.5)
51–60	494 (13.9)
61–70	441 (12.4)
71–80	897 (25.2)
≥ 81	121 (3.4)
no data	501 (14.1)
Reason for calling	
health advice	583 (16.4)
question about COVID-19	89 (2.5)
anxiety and tension	1,166 (32.8)
existing psychiatric disorder	651 (18.3)
information about control visit	121 (3.4)
information about entering the country	28 (0.8)
question about spending time in isolation	228 (6.4)
advice regarding work	53 (1.5)
other	637 (17.9)

NATIONAL LINE FOR PSYCHOSOCIAL SUPPORT DURING COVID-19 EPIDEMIC IN SERBIA

National Line for Psychosocial Support During COVID-19 Epidemic in Serbia was organized by the Ministry of Health, with the support of the Dr. Laza Lazarević Clinic for Mental Disorders, Belgrade, and has been operating since March 17, 2020. This service offers phone consulting by crisis response teams (psychiatrists, psychiatric nurses, and psychologists) assigned to provide mental health assistance, psychosocial support, and advice on mental health for both patients and healthcare professionals. Using the database from National Help Line Service for this study has been approved by the Ethics Committee of the Dr. Laza Lazarević Clinic for Mental Disorders Belgrade (No. 3732/20).

Since April 29, 2020, 3,556 persons from all parts of Serbia have used this national help line service. According to available data, over 50% of all users were from Belgrade, while only 7.3% of them did not say from which location they called (Figure 1). In 41.7%, calls were from other cities, such as Pančevo, Novi Sad, Niš, Kragujevac, Zaječar, Kikinda, Leskovac, Paraćin, Kraljevo, etc. (Figure 1). Among the users, 63.6% were female, while 2.4% did not state their gender (Table 1). The mean age of the callers was 55.4 ± 16.6 (the youngest user was 16 and the oldest was 98 years old), and most users were in the age group from 71 to 80 years old (25.2%) – Table 1. The majority of users called because of anxiety and a feeling of tension due to the COVID-19 epidemic (32.8%) – Table 1.

PLAN FOR MENTAL HEALTH PROTECTION DURING THE COVID-19 EPIDEMIC IN SERBIA

The National Health Service in Serbia plans psychosocial support directed to patients affected by COVID-19, especially for those in collective quarantines, such as the Belgrade Fair temporary hospital. Mobile teams of mental health professionals should be located at quarantine points, and provide the necessary psychosocial assistance, ensuring appropriate safety of patients through adequate equipment and counseling. The mobile team would consist of a psychiatrist, a psychiatric nurse, and mental healthcare associates (psychologist, social worker, specialist for the handicapped). This team would be formed among employees of mental health institutions. Mobile teams should be provided with medicines and necessary medical equipment to ensure first aid treatment. Target groups for this type of support would be persons with existing mental health disorders or complains of psychiatric problems (tension, insomnia, irritability, feeling low, fear, etc.) and persons

with mental health disorders under medicinal treatment or with psychological support. Also, these activities will have the role of preventing as well as suppressing symptoms of mental disorders and behavioral disorders by providing psychosocial support and pharmacotherapy.

Due to the specific nature of the current situation and epidemiological measures, psychological assistance to all persons infected with COVID-19 can also be provided through on-line psychological support (via applications such as Skype, Viber, or Whatsapp). In this way, direct contact between mental health professionals and persons infected with COVID-19 would be avoided, and the necessary psychological support would be provided. Psychological support online (via the same applications) is also planned for persons who have lost their loved ones (family members, friends) during the COVID-19 epidemic. The aim of this type of psychological support would be to assist and facilitate the process of grief.

In addition to these measures, psychosocial support should be provided for frontline workers in the COVID-19 centers – namely, healthcare workers who are expected to experience burnout syndrome. Mental health professionals would be organized in mobile teams and, if necessary, sent to where they are most needed. Team members should be the same for all future activities. Considering the overload of work the employees are faced with, it would be reasonable to regularly monitor and support them, providing a telephone line for their needs. Therefore, the target group for this undertaking in the domain of mental health would be doctors, nurses, and other workers in COVID-19 centers. The role of the mobile team would be support and counseling of the employees, recognition and prevention of burnout syndrome, as well as assistance in developing and maintaining successful stress management strategies.

Another aspect of the current situation is that it will certainly affect persons with chronic mental problems. They may experience a relapse due to external factors, but also due to self-initiated discontinuation of prescribed pharmacotherapy. The trend of decreasing the number of regular control visits of all patients has already been detected, as well as of those who are administered with long-acting antipsychotics. This can lead to exacerbation of mental disease and a concomitant loss of control over behavior and aggression directed toward oneself and others. For the purpose of prevention, it is suggested that teams should be formed within existing psychiatric facilities and medical centers, consisting of mental healthcare professionals and mental healthcare associates. They should contact patients on long-acting antipsychotics, or their families, by phone, in order to arrange home visits by the mobile team, where appropriate. The mobile team should consist of a psychiatrist and a psychiatric nurse, provided with medicines and necessary medical equipment to ensure first aid treatment. The task of the team would be to help in the prevention and suppression of mental and behavioral disorders by administering pharmacotherapy.

CONCLUSION

A mental health strategy during the COVID-19 pandemic is necessary in order to protect, preserve and improve public health of all categories of the population. The measures already taken and planned are expected to significantly reduce the possibility of deepening normal crisis response, the possibility of relapse in persons with mental disorders, to facilitate the grieving process for people who have lost loved ones during the pandemic, to enable the identification and timely rehabilitation of combustion syndromes in health and other workers at COVID centers, as well as general support for all those who have difficulties in psychological functioning in a crisis situation.

Conflict of interest: None declared.

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Унапређење служби за ментално здравље током епидемије *COVID*-19 у Србији

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САЖЕТАК

Светска здравствена организација је прогласила избијање вируса *COVID*-19 хитним случајем за јавно здравље од међународне важности. Стратегије менталног здравља због епидемије изазване вирусом корона морају укључити процену стања менталног здравља људи у различитим субпопулацијама под утицајем избијања *COVID*-19, идентификовање особа које су под високим ризиком од манифестација поремећаја у понашању, покушаја самоубиства и агресије, као и пружање одговарајућих интервенција заштите менталног здравља за оне којима је помоћ потребна. Циљна популација стратегије менталног здравља категорисана је у четири различите групе: најосетљивије особе са проблемима менталног здравља, изоловане особе са симптомима атипичне акутне респираторне инфекције, индивидуе које су биле у блиском контакту са претходне две категорије и особе изложене превентивним и контролним мерама.

Кључне речи: *COVID*-19; вирус корона; ментално здравље

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови**,** наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°*C*), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публи-

кације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

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