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ORIGINAL ARTICLES / ORIGINALNI RADOVI

The laureates of the 2018 Nobel Prize in Physiology and Medicine are: left – Tasuku Honjo (born in Jaunary 27, 1942; Univeristy of Kyoto, Kyoto, Japan), and right – James P. Allison (born in August 7, 1948; Univeristy of Texas MD Anderson Cancer Center, Houston, USA).
They are awarded for their discovery of cancer therapy by inhibition of negative immune regulation.

Dobitnici ovogodišnje Nobelove nagrade za medicinu: levo- Tasuku Honjo (rođen 27. januara 1942; Kjoto Univerzitet, Kjoto, Japan) i desno – Đejms P. Alison (rođen 7. avgusta 1948; MD Anderson Centar za rak Univerziteta u Teksasu, Hjuston, SAD).
Oni su nagrađeni za otkriće terapije kancera pomoću inhibicije negativne imunske regulacije.

ORIGINAL ARTICLE

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Secondary lymphedema of the arm, the perception of the disease, self-efficacy and depression as determinants of quality of life in patients with breast cancer

Sekundarni limfedem ruke, percepcija bolesti, samoefikasnost i depresija kao determinante kvaliteta života obolelih od karcinoma dojke

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Abstract

Background/Aim. Secondary lymphedema of the arm is one of possible side-effects and complications of breast cancer and its treatment which can contribute and precipitate to a number of new psychosocial problems. The aim of this study was to examine the differences in quality of life of patients suffering from breast cancer, with arm lymphedema and those without lymphedema, and to determine the significance of the perception of the disease, depressive symptoms and self-efficacy contribution to overall quality of life. **Methods.** The research was designed as a cross-sectional study, which included 64 patients – 34 with arm lymphedema and 30 without lymphedema. Questionnaire FACT-B + 4 was applied to assess the quality of life, BIPQ for the perception of the disease, depression was measured by DASS-21 scale, while self-efficacy was tested by SGSE scale. *T*-test, Mann Whitney U Test, χ^2 test and hierarchical regression analysis were applied to data processing. **Results.** There was not any significant difference between the groups in the total score of quality of life ($t = 0.469$, $p > 0.05$), or in the individual subscales: physical well-being ($t = 0.535$, $p > 0.05$), social/family well-being ($t = 1.43$, $p > 0.05$), emotional well-being ($t = 1.35$, $p > 0.05$), functional well-being ($z = -0.243$, $p > 0.05$), breast cancer scale ($t = -0.839$, $p > 0.05$) and arm scale ($t = -0.514$, $p > 0.05$), while the perception of the disease ($\beta = -0.603$, $t = -5.958$, $p < 0.001$) and depression ($\beta = -0.411$, $t = -4.101$, $p < 0.001$) proved to be significant predictors of quality of life and explain 50.2% variance of overall quality of life. **Conclusion.** The results of our study indicate the importance of a comprehensive rehabilitation program, directed both at functional and psychosocial aspects.

Key words:
breast neoplasms; women; upper extremity; lymphedema; quality of life; perception; depression.

Apstrakt

Uvod/Cilj. Sekundarni limfedem ruke je jedan od mogućih neželjenih efekata i komplikacija karcinoma dojke kao i lečenja karcinoma dojke, koji može doprineti i dovesti do većeg broja novih psiholoških problema. Cilj ove studije bio je da se ispita postojanje razlika u kvalitetu života bolesnica sa karcinomom dojke koje imaju sekundarni limfedem ruke u odnosu na one koje ga nemaju i da se utvrdi značaj percepcije bolesti, depresivnih simptoma i samoefikasnosti za ukupni kvalitet života. **Metode.** Sprovedeno istraživanje je dizajnirano kao studija preseka, sa odgovarajućim kliničkim uzorkom kojeg su činile 64 bolesnice, od kojih 34 sa limfedemom ruke i 30 bez limfedema. Za procenu kvaliteta života primenjen je upitnik FACT-B+4, za percepciju bolesti BIPQ; depresivnost je merena skalom DASS-21, dok je samoefikasnost ispitana skalom SGSE. Za obradu podataka korišćeni su *t*-test, Mann Whitney *U* Test, χ^2 test i hijerarhijska regresiona analiza. **Rezultati.** Nije utvrđena značajna razlika između grupa kako u ukupnom skoru kvaliteta života ($t = 0.469$, $p > 0.05$), tako ni u pojedinačnim domenima: fizičko blagostanje ($t = 0{,}535$, $p > 0{,}05$), socijalno/porodično blagostanje ($t = 1.43$, $p > 0{,}05$), emocionalno blagostanje ($t = 1.35$, $p > 0{,}05$), funkcionalno blagostanje ($z = -0.243$, $p > 0.05$), simptomi izazvani karcinomom dojke ($t = -0.839$, $p > 0.05$), te tegobama ruke ($t = -0.514$, $p > 0.05$), dok su se percepcija bolesti ($\beta = -0.603$, $t = -5.958$, $p < 0.001$) i depresivnost ($\beta = -0.411$, $t = -4.101$, $p < 0.001$) pokazali kao značajni prediktori kvaliteta života i objašnjavaju 50,2% varijanse ukupnog kvaliteta života. **Zaključak.** Rezultati naše

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studije ukazuju na značaj sveobuhvatnog programa rehabilitacije, usmerenog kako na funkcionalne tako i na psihosocijalne aspekte.

Ključne reči:
dojka, neoplazme; žene; ruka; limfedem; kvalitet života; percepcija; depresija.

Introduction

Breast cancer, although one of the leading causes of morbidity and mortality in the world and in our country, due to the modern oncological treatment which in recent decades has given more positive outcomes, provides us with new approaches to dealing with patients, and primarily with focus on rehabilitation and promotion of quality of life of patients.

Health-related quality of life (HRQOL) refers to an individual's perception on impact which the disease and treatment may have on his/her functioning; considering the fact that it is a multidimensional construct, quality of life includes physical, functional, psychological and social aspects of life [1].

One of the possible side-effects and complications of breast cancer and its treatment, which can contribute and precipitate to a number of new psychosocial problems, is the occurrence of secondary lymphedema of the arm (SLEA).

SLEA is the result of a functional overload of the lymphatic system when the volume of lymph exceeds the existing transport capacity of the lymphatic system of the arm, caused by a mechanical insufficiency of the lymphatic system, usually as a result of surgery, radiation therapy, infection or trauma [2]. It occurs in 10%–30% of patients undergoing therapy for the treatment of breast cancer [3, 4]. Given the high incidence of this type of complication, increasingly longer life expectancy of women with breast cancer, and in connection with it, a tendency to improve quality of their life, there are more and more rehabilitation programs aimed at preventing and minimizing risk factors for the occurrence of secondary lymphedema of the arm, or treatment when already occurred [5–9].

SLEA is accompanied by subjective symptoms, increased risk of infection and damage of the brachial plexus. Swelling of the arm impedes the performance of activities of daily living, followed by changes in physical appearance, which can precipitate dissatisfaction with personal appearance, decline in self-esteem and self-confidence, increase of sexual problems. In fact, many patients associate swelling of the arm with malignant disease and previous or current experience [10]. This implies the reason why in dealing with these patients more attention is paid to their assessment of their quality of life and the psychological aspects of the disease (adaptation, distress, body image, etc.) [11].

It has been pointed out that the quality of life in the patients with lymphedema is significantly lower when compared to the patients who did not develop lymphedema [12–14], especially in the functional and physical domains. However,there are also studies that have indicated [15] that quality of life of women with lymphedema depended more on certain psychological factors such as coping with and experiencing lymphedema, social support and pain, than on the volume of swelling.

For successful adaptation to the disease, and thus the quality of life, the perception of the illness, or a way in which a patient sees the cause of the disease, its duration, symptoms and consequences of the disease influencing the emotional state, etc. , is of a great importance. People with the same disease often have different perception of their disease which largely depends on the personality traits, age, cultural context, the importance of social support, marital status; the experience of illness is significantly influenced by comorbidity [16]. According to the cognitive model of health, the common-sense model (CSM) of self-regulation [17], depending on the way the disease is represented in the mind of the patient, the person will use different strategies to cope with the disease and these strategies determine the outcome of adaptation. For successful adaptation to the chronic illness, it is of a great importance a positive perception of their personal capacities, that is, it is necessary for an individual to realize his/her personal capabilities to cope with various life situations [18]. The current findings suggest a positive relationship between quality of life and self-efficacy, and a negative relationship between self-efficacy and depression [19, 20] as well as longer life expectancy for those breast cancer patients who perceived their own efficiency higher [21, 22]. Further, maladaptive psychological conditions, such as depression, show undeniable influence on the quality of a person's life. Research conducted with women suffering from breast cancer showed a correlation between mild and moderate depression characteristics and a lower quality of life in all spheres, including sexual functioning [23, 24].

The aim of this study was to determine differences in quality of life in women with breast cancer-related lymphedema currently undergoing a rehabilitation program and those women with breast cancer without lymphedema. The aim was also to determine the contribution of psychological factors like perception of the disease, depression, and self-efficacy, in order to adapt a rehabilitation program to include interventions focused on the improvement of psychological status of such patients.

Methods

The research was designed as a cross-sectional study, with appropriate clinical sample consisting of 64 women suffering from breast cancer who underwent breast cancer surgery and who were at the time of the study (from December 2015 to May 2016) at rehabilitation treatment for SLEA (34 patients), or at one of the regularly scheduled controls of specialist physiatrist (30 patients) at the Oncology Institute of Vojvodina.

Study did not include patients treated with chemotherapy or radiotherapy at the moment of assessment, and patients with progressive disease (metastases) in order to eliminate the possibility of the impact of the underlying disease or side effects of therapy on the quality of life assessment.

Breast cancer-related lymphedema was quantified as mild, moderate, or severe (a difference of size in at least one measured level of arm was up to 3 cm, from 3.1 cm to 5 cm, at least 5.1 cm or more, respectively).

Functional Assessment of Cancer Therapy – Breast (FACT – B+4) was applied for assessment of quality of life of our patients. The questionnaire measured four domains of quality of life (physical well-being – PWB, social/family well-being – SWB, emotional well-being – EWB and functional well-being – FWB), a domain that includes symptoms and concerns related to breast cancer – BCS, as well as an additional domain which included difficulties caused by lymphedema – ARM. The respondents gave answers to a five-point Likert scale, where 0 meant – not at all, while 4 meant– very much. Internal consistency of this scale proved to be excellent both for the whole scale and for its subscales [25].

Perception of the illness was assessed using the Brief Illness Perception Questionnaire (BIPQ) [26]. The questionnaire measured 9 different domains of perception of disease, which respondent assessed by answering to a 0-to-10 scale (in the case of the first eight domains related to the impact of the disease on life, duration, control, treatment, symptoms, concern, understanding and impact of the disease on the emotional state), or by writing answers in case of the last domain that was qualitative and related to the perception of the causes of the disease. The perception of the disease can be measured individually by domain, and can be seen as a total score, or, as a measure that indicated perception of the disease as threatening [26]. In our research, we observed the total score of perception of the disease where a higher score indicated more negative perception of the disease. Metric characteristics of the scale in previous studies proved to be satisfactory [27].

Depression was assessed by using a questionnaire Depression Anxiety Stress Scale (DASS-21) [28]which measured the symptoms of depression, anxiety and stress, and can be used as a measure of distress if it is regarded as a total score, or as an individual measure of depression, anxiety, stress, if referring to subscales. The task of respondent was to assess the level of these affective states in the previous week on a four-point Likert type scale where 0 indicates no, and 3 mainly or almost always. In our research related to the assessment of depression we used scores of a subscale of depression. Internal consistency of the total scale and subscales proved to be acceptable, both in foreign and domestic research [29].

Self-efficacy was assessed by the General Self-Efficacy Scale (SGSE) [30]. This one-dimensional instrument consisted of 10 questions on a four-point Likert type scale from 0 (completely false) to 3 (completely true- This scale also showed good metric properties in previous studies [31].

Data was analyzed by using a statistical software package SPSS 21.0. The Student *t*-test, Mann Whitney *U* test, and χ^2 test were used to test differences between groups while Pearson product-moment correlation was used to test the correlation between the examined variables. To test the individual and the total contribution of psychological variables in prediction of quality of life, hierarchical regression analysis was conducted. In all analyzes, differences were interpreted as statistically significant if $p < 0.05$ and $p < 0.01$.

Results

Table 1 shows the sociodemographic data of our sample. As we can see, the sample of the patients with lymphedema differed due to the severity of lymphedema. There was no statistically significant difference between the groups when the age of patients was taken into consideration (*t*-test, $p > 0.05$). We examined the possible existence of the intergroup differences related to the level of education and the presence of other chronic diseases, and there was also no statistically significant difference (χ^2 test, $p > 0.05$). The differences between the two groups in all aspects of quality of life, the perception of the disease, depression and self-efficacy are shown in Table 2. As we can see, there was no statistically significant difference not only in the assessment of the total quality of life of women with lymphedema and those without it but also on individual subscales (*t*-test, Mann Whitney *U* test, $p > 0.05$), with having been noted that the scores of the patients in the control group were slightly higher on the scale referring to symptomatology related to breast cancer and problems with the arm. Table 3 shows simple correlations between the scales relating to the quality of life and the scales that measured the psychological status of the examined patients. Since all three psychological variables showed significant correlation with the total quality of life, we examined their role in prediction of quality of life. It is noticeable that there are three blocks of predictors (Table 4). In the first one, we examined the contribution of the perception of the disease in predicting quality of life. In the second block of predictors, we also added the condition of depression to the perception of disease while in the third one we added self-efficacy to the existing predictor variables. The first model was significant (F = (1.62) = 35.50, $p < 0.001$), and explained 36.4% of variance, and the perception of the disease proved to be an important predictor variable (β = -0.603, t = -5.958, $p < 0.001$). The second model was significant (F = (1.61) = 30.68, $p < 0.001$), and explained 50.2% of variance and the depression proved to be a significant predictor of quality of life (β = -0.411, t = -4.101, $p < 0.001$). The third model was also significant (F = (1.60) = 20.20, $p < 0.001$), and explained 50.3% of variance; however, self-efficacy did not prove to be a significant predictor variable (β = 0.035, t = 0.346, $p > 0.05$). We noted that the percentage of explained variance changed through the steps, that is, the first model explained only 36.4% (Fchange = 35.50. $p = 0.001$ $p < 0.001$), while after introducing depression, the percentage of the explained variance increased to 50.2% (Fchange = 16.81. $p = 0.001$, $p < 0.001$), and after introducing self-efficacy, the percentage of variance remained almost unchanged (Fchange = 0.120, $p = 0.730$, $p < .0001$).

Table 1

Baseline characteristics of the study sample

Characteristics	Breast cancer patients		*p*
	Lymphedema group (n = 34)	Control group (n = 30)	
Degree of SLEA, n (%)	mild 24 (73) moderate 4 (12) severe 5 (15)		
Age (years), mean ± SD (min-max)	60.20 ± 8.82 (39–75)	56.16 ± 10.18 (29–74)	0.316
Education (%)			
primary school	9	17	
secondary school	53	47	0.624
higher school	9	3	
college	29	33	
Other chronic disease (%)	56	47	0.462

***t*-test; χ^2 test; SD – standard deviation.**
SLEA – secondary lymphedema of the arm.

Table 2

Differences in Quality of Life between two groups

Quality of life scales	Breast cancer patients		*t* (Z*)	*p*
	Lymphedema group (n = 34)	Control group (n = 30)		
Total, mean ± SD	117.90 ± 19.85	115.48 ± 21.38	0.469	0.641
Physical well-being, mean ± SD	21.47 ± 5.04	20.80 ± 4.97	0.535	0.595
Social/family well-being, mean ± SD	22.60 ± 4.29	21.04 ± 4.42	1.428	0.158
Emotional well-being, mean ± SD	18.47 ± 4.69	16.83 ± 5.03	1.346	0.183
Functional well-being, mean ± SD; Med (min-max)	20.08 ± 3.68 20 (13–27)*	19.80 ± 4.85 20 (10–28)*	- 0.243*	 0.808
Breast cancer scale, mean ± SD	22.79 ± 6.48	24.10 ± 5.89	-0.839	0.405
Arm scale, mean ± SD	12.47 ± 3.48	12.90 ± 3.15	-0.514	0.609

***t*-test; *Mann Whitney *U* Test; Med (min-max) – median (minimum-maximum); SD – standard deviation.**

Table 3

Correlations between quality of life (QOL) subscales and possible QOL predictors

QOL Scales	Potential predictors scales	r	*p*
QOL (total)	illness perception	-0.603**	0.000
	depression	-0.594**	0.000
	self-efficacy	0.338*	0.006
Physical well-being	illness perception	-0.609**	0.000
	depression	-0.496**	0.000
	self-efficacy	0.205	0.103
Social/family well-being	illness perception	-0.219	0.082
	depression	-0.293*	0.019
	self-efficacy	0.240	0.056
Emotional well-being	illness perception	-0.608**	0.000
	depression	-0.573**	0.000
	self-efficacy	0.418**	0.001
Functional well-being	illness perception	-0.346*	0.005
	depression	-0.495**	0.000
	self-efficacy	0.270*	0.031
Breast cancer scale	illness perception	-0.433**	0.000
	depression	-0.443**	0.000
	self-efficacy	0.229	0.069
Arm scale	illness perception	-0.369*	0.003
	depression	-0.225	0.074
	self-efficacy	0.069	0.585

r –Pearson product-moment correlation coefficient; *$p < 0.05$; **$p < 0.001$.

Table 4

Hierarchical regression analysis: Illness Perception, Depression, and Self-efficacy as potential predictors of Quality Of life in breast cancer patients

Model	Predictors	B	*SE B*	*β*	R	R^2	F	*p*
1	Illness perception	-0.854	0.143	-0.603**	0.603	0.364	350.50	0.000
2	Illness perception	-0.604	0.142	-0.427**	0.708	0.502	160.82	0.000
	Depression	-10.984	0.484	-0.411**				0.000
3	Illness perception	-0.597	0.144	-0.422**	0.709	0.503	0.12	0.000
	Depression	-10.920	0.521	-0.397**				0.000
	Self-efficacy	0.088	0.253	0.035				0.730

Dependent variable: Quality of life ($p < 0.001$).**

Discussion

The first aim of this study was to examine whether there was a difference in the quality of life in the patients who developed lymphedema compared with those who also suffered from breast cancer, but with no swelling of the arm. According to the findings of the previous studies, the patients with lymphedema show significantly lower quality of life compared with those patients who did not develop it, both at the global level as well as in individual domains, particularly in the domain related to physical condition [12–14]. However, due to the fact that sometimes obtained findings were contradictory, several studies underlined the importance of the way in which the influence of lymphedema was measured and suggested to take into account during research both objective measures of lymphedema (the diagnosis of lymphedema and the size of the swelling) and subjective measures (symptoms that patients manifest as pain, mobility difficulties, bodily sensations, perceptions of lymphedema) [32, 33]. Our research did not show a significant difference in the quality of life of the patients diagnosed with lymphedema. There are a number of possible explanations for such a finding. First of all, for years, we were the only one in the country who implemented a program of early rehabilitation [9], through which, among other things, the patients were trained to prevent and recognize the symptoms, and, as quickly as possible, began treatment of secondary lymphedema if noticed, which probably led to early detection and prevention of severe forms of swelling of the arm. Therefore, our sample consisted mostly of the patients with mild lymphedema. Secondly, considering the fact that patients had been repeatedly on physical treatments of Complex Decongestive Physical Therapy (CDPT), we assume that they were automatically exposed to a smaller number of problems, due to the size of a reduction of the swelling and also because they were educated and encouraged how and in what way to use the swollen arm in their daily activities, and therefore, they adapted to their condition and successfully cope with it. Previous studies showed the benefits of participation in lymphedema treatment, in terms that physical treatment and size reduction of the swelling had an impact on physical condition, but a more positive assessment of the quality of life and the other aspects were probably influenced by the education of the patients [34–37]. Finally, in order to gain a clearer insight into the impact of lymphedema on quality of life and the effects of the treatment, future research should take into account the subjective symptoms and ailments caused by lymphedema and the perception of lymphedema, especially in the patients who have just faced the diagnosis of lymphedema. It would enable us to make more credible comparison and explanation of the reason why our patients do not experience any significant difference in quality of their life.

When the psychological aspects are taken into account, we obtained more than expected findings, which is that the perception of the disease and depression contribute greatly to the quality of life while the self-efficacy, which although did show significant correlation with the functional and emotional state, did not prove to be a significant predictor of quality of life. We already know that the perception of the disease, that is, developed beliefs that include knowledge about the disease and the symptoms manifested by the disease, cause, duration, consequences and controllability and emotions associated with them, contribute to the physical and psychosocial response to the disease, by encouraging the use of certain coping strategies [17]. The perception of the disease proved to be important for the the patient to cooperate during drug treatment, for any psychopathological manifestations as a response, and in population of patients suffering from breast cancer it proved to be an important predictor of quality of life up to 15 months after diagnosis of malignancy [38]. This indicates the importance of recognizing the way in which the patient perceive the disease in order to implement the earliest possible interventions aimed at a refutation of irrational or maladaptive beliefs and cognitive representations which patients may have regarding malignant diseases. When we talk about depression and depressive symptoms such as rumination, fatigue, problems with concentration, insomnia and general dysphoric mood, there are countless studies that indicate the impact of this emotional states on the decline in the quality of life in cancer patients [39]. Therefore, a successful treatment and promotion of quality of life in our patients critically depend on early recognition of symptoms and timely response. Within a program of early rehabilitation of patients with breast cancer, for few years now, our institute has been providing postoperative psychooncological support, which among others includes the distress screening and education of the patients about the early recognition of depression and anxiety symptoms. In this way, our patients get the possibility of treatment of possible maladaptive responses to the disease. Self-efficacy, or confidence of a person in his/her own capacity to overcome difficulties and to adequately manage the situation, proved to

be an important factor in adaptation to cancer and quality of life [40] and is associated with a lower intensity of negative emotions in threatening situations [41]. In our study, the predictive power of self-efficacy in improving quality of life was minor. Considering the fact that its importance for the quality of life is indisputable, it is assumed that it probably has a greater impact on the emotional and functional status than on the total quality of life, and produces more significant effect through the coping strategies and affect, which should be investigated by a mediation analysis in future research. These findings suggest that it is very important to take into account the psychological status of the patients (especially the way in which the cancer itself is experienced) when making a plan of rehabilitation treatment in order to promote quality of life of our patients. This is utterly important, because it is then, when their future quality of life is determined: a manner in which they would cope with the new stressful situation, the way he/she would feel, how they would evaluate their self-efficacy in further struggle with malignant disease and how much they would cooperate during the treatment. Given the fact that all of these psychological factors can be influenced in educational and supportive way as well as through cognitive-behavioral therapy, within rehabilitation treatment that focuses on the physical and functional status of our patients, from the very moment when faced the diagnosis of malignancy and the first responses to the disease are formed, it is indicative to conduct psychological assessment and support, and psychological support and continuous monitoring of those who are more vulnerable, thus improving quality of life of our patients in all aspects and at all stages of the disease and treatment.

Recommendations that may be suggested for the future research refer to providing a larger sample and a prospective monitoring of patients in the context of adaptation to the disease, depression and quality of life, assessing not only vulnerability factors but also factors of resilience and multidimensional observation of lymphedema, including both objective and subjective measures of lymphedema, with the ultimate goal to set up the most efficient program of oncological rehabilitation and providing preconditions for quality of life.

Conclusion

The perception of the disease and depression determine to a great extent quality of life of the cancer patients. Quality of life in patients with diagnosed lymphedema did not differ from patients without lymphedema. Our findings indicate the importance of a comprehensive rehabilitation program, primarily preventive rehabilitation program, both aimed at the functional and psychosocial aspects.

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Prognostic value of presepsin (soluble CD14-subtype) in diagnosis of ventilator-associated pneumonia and sepsis in trauma patients

Prognostička vrednost presepsina (solubilnog CD 14-podtipa) u dijagnozi pneumonija povezanih sa mehaničkom ventilacijom i sepse kod traumatizovanih bolesnika

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Abstract

Background/Aim. Presepsin (soluble CD14-subtype) is a fragment of CD14 produced in response to bacterial infections and a novel biomarker of pneumonia, sepsis and septic shock. The aim of this study was to compare sensitivity and specificity of persepsin, soluble CD14-subtype (sCD14-ST) with other biomarkers: procalcitonine (PCT), C-reactive protein (CRP) and leukocyte count (Le) in mechanically ventilated injured patients, as a marker of pneumonia, sepsis and septic shock. **Methods.** The prospective study was undertaken in trauma and surgery intensive care unit of the Emergency Center, the Clinical Center of Serbia from January to April 2013. The study included 39 trauma patients requiring mechanical ventilation, and who developed one of the following inclusion criteria: Systemic Inflammatory Response Syndrome (SIRS), ventilator associated pneumonia (VAP), sepsis and/or septic shock. On admission Acute Physiology and Chronic Health Evaluation II (APACHE II) Score and Injury Severity Score (ISS) were calculated. Seventy-two measurements of four biomarkers (presepsin, PCT, CRP and Le) were performed in 39 patients at the moments of diagnosis of SIRS, VAP, sepsis and/or septic shock (21 when SIRS diagnosis was established, 21 after the diagnosis of VAP, 18 at the moment of diagnosis of sepsis and the remaining 12 measurements were conducted while diagnosing the septic shock). The Sequential Organ Failure Assessment (SOFA) score was calculated at these points as well. **Results**. Patients were mainly severely injured (mean ISS = 24.2) and had moderately severe medical condition at admission (mean Apache II score, 14.5). Presepsin concentration significantly differed among all the four groups, except between sepsis and septic shock. The strongest positive correlation of presepsin evinced with PCT ($r = 0.741$, $p < 0.001$). The sCD14-ST indicated better performance in diagnosis of both VAP (AUC = 0.909) and sepsis (AUC = 0.899), compared to PCT (AUCs: 0.863, 0.885, respectively), CRP (AUCs: 0.703, 0.677, respectively) and Le (AUCs: 0.668, 0.700, respectively). **Conclusion.** This study revealed that sCD14-ST is a reliable biomarker for distinguishing sepsis severity. It also showed a good correlation with the infection development as well as worsening in injured patients.

Key words:
presepsin protein, human; pneumonia, ventilator associated; sepsis; shock septic; biomarkers; sensitivity and specificity; diagnosis.

Apstrakt

Uvod/Cilj. Persepsin (solubilni CD14-podtip) je fragment CD14 koji se produkuje kao odgovor na prisustvo bakterijske infekcije i predstavlja novi biomarker u dijagnostici pneumonije, sepse i spetičkog šoka. Cilj ove studije bio je da se uporedi senzitivnost i specifičnost presepsina (solubilnog CD14-podtipa) sa ostalim biomarkerima infekcije: prokalcitoninom (PCT), C-reaktivnim proteinom (CRP) i brojem leukocita (Le) kod povređenih bolesnika na mehaničkoj ventilaciji, kao markera pneumonije, sepse i septičkog šoka. **Metode.** Prospektivna studija sprovedena je u dve jedinice intenzivnog lečenja (JIL) (traumatološka i hirurška) Kliničkog centra Srbije u periodu od januara do aprila 2013. godine. U studiju je bilo uključeno 39 traumatizovanih bolesnika na mehaničkoj ventilaciji kod kojih se razvio neki od sledećih ishoda koji su bili i kriterijumi za

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uključivanje: sisemski inflamatorni odgovor (SIRS), pneumonija povezana sa mehaničkom ventilacijom (VAP), sepsa i/ili septički šok. Na prijemu u JIL *Acute Physiology and Chronic Health Evaluation II* (APACHE II) skor i *Injury Severity Score* (ISS) su računati za svakog bolesnika. Sedamdeset i dva merenja koncentracije četiri biomarkera (presepsin, PCT, CRP i Le) urađena su kod 39 bolesnika u trenutku postavljanja dijagnoze SIRS, VAP, sepse i/ili septičkog šoka. *Sequential Organ Failure Assessment* (SOFA) skor takođe je meren istovremeno. **Rezultati.** Većina bolesnika na prijemu bila je teško povređena (srednja vrednost ISS skora = 24.2) i bila je u srednje teškom stanju (srednja vrednost APACHE II skora = 14.5). Koncentracije presepsina značajno su se razlikovale između sve četiri grupe bolesnika, osim između grupa sa sepsom i septičkim šokom. Najsnažniju pozitivnu korelaciju presepsin je imao sa PCT ($r = 0.741$, $p < 0.001$). sCD14-ST pokazao je bolju prediktivnu vrednost u dijagnozi pneumonije (AUC = 0.909) i sepse (AUC = 0.899) u odnosu na PCT (AUCs: 0.863, 0.855), CRP (AUCs: 0.703, 0.677) i Le (AUCs: 0.688, 0.700). **Zaključak.** Ova studija je pokazala da je sCD14-ST pouzdan biomarker u određivanju težine sepse kao i da je u dobroj korelaciji sa nastankom infekcije i pogoršanjem stanja kod povređenih bolesnika.

Ključne reči:
presepsin protein, humani; pneumonija, respiratorom uzrokovana; sepsa; šok, spetički; biološki pokazatelji; senzitivnost i specifičnost; dijagnoza.

Introduction

Ventilator associated pneumonia (VAP) is one of the most common nosocomial infections appearing in the intensive care unit [1]. Up to 10% of patients receiving mechanical ventilation will eventually develop VAP, with an attributable mortality rate estimated between 1% to 1.5% [2]. In critically injured patients, incidence, morbidity and mortality of VAP are even higher [3].

Sepsis is infrequent but significant cause of death in patients with blunt injury. In modern era of intensive care medicine, delays in the initiation of antimicrobial treatment is not rare and while the mortality of patients with sepsis is gradually decreasing, it is still quite high [4]. A cause of this could be found in non-specific symptoms of the infection leading to an inappropriate empirical treatment and increased resistance profile of pathogens. In septic complication, however, it is important to bear in mind not only the fact that the sensitivity of blood cultures for the diagnosis of pneumonia sepsis is less than 25% but also that, when present, the organisms may originate from an extrapulmonary site of infection, in as many as 64% of cases, even if VAP is present [5].

Since clinical criteria have low sensitivity and specificity, there is no "gold standard" for diagnosing either VAP or sepsis [6]. Currently, procalcitonin (PCT) together with C-reactive protein (CRP) and different haematological parameters [white blood cell (WBC) count] are used as markers to diagnose sepsis or severe sepsis [7]. On the other hand, several invasive or semi-invasive methods can be used to diagnose VAP, with certain disadvantages in each of them [8]. Various scores and markers have already been proposed as the promising candidates for evaluating the response of VAP to antibiotic treatment [9]. A number of biomarkers, including soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), interleukin-1-beta, granulocyte colony-stimulating factor, macrophage inflammatory protein-1-alpha and others have been tested recently for patients with suspected or confirmed VAP. However, the obtained results were not promising, but they were even contradictory [10]. Therefore, defining a reliable marker that can enable fast diagnosis of VAP would be of great benefit.

The great efforts have been invested into the assessment of presepsin for sepsis diagnosis [11, 12]. Presepsin or soluble CD14 is a fragment of CD14, the lipopolysaccharide-binding protein complex receptor, produced in a response to bacterial infections. Presepsin is a novel biomarker [soluble amino-terminal fragment of the cluster of differentiation (CD) marker protein CD14] in sepsis that is released into the circulation during monocyte activation [13]. In recent meta-analysis results have demonstrated good overall diagnostic performance of presepsin in sepsis [14].

As far as we know, there are no clinical studies investigating the potential role of presepsin in VAP. Ideal biomarker for VAP will be accurately elevated in serum or broncho-alveolar lavage (BAL) fluid, will respond to adequate therapy and will not be altered in patient without infection complication initially. Also, ideally, biomarker results for VAP diagnosis should be available sooner than obtaining the quantitative culture of BAL fluid, which can take up to 48 hours [15]. Taking into account favorable kinetics of presepsin release as well as its half-life of approximately 4–5 h, this biomarker could be exactly the one we have been searching for.

The aim of this study was to estimate and compare the prognostic value of soluble CD14-subtype (sCD14-ST) with other laboratory (and CRP and Le) and standard biomarkers (PCT) in mechanically-ventilated injured patients as a marker of pneumonia and the severity of infection complications such as sepsis and septic shock.

Methods

Study design

The prospective study was undertaken in trauma and surgery intensive care unit of the Emergency Center, the Clinical Center of Serbia from January to April 2013. Trauma patients requiring mechanical ventilation and who developed one of the following inclusion criteria were included: systemic inflammatory response syndrome (SIRS), VAP, sepsis or septic shock. Exclusion criteria were as follows: the patients younger than 18 years of age, confirmed gastric aspiration, patient receiving antibiotic therapy in the previous 90 days, recent

hospitalization, residence in a nursing home or extended care facility, home therapy and immunodeficiency.

This study was approved by the Ethics Committee of the Faculty of Medicine at the University of Belgrade, Serbia (No. 29/IV-14).

Clinical assessment

Baseline demographic data included age and gender are given in Table 1. On admission, the Acute Physiology and Chronic Health Evaluation II (APACHE II) Score and the Injury Severity Score (ISS) were calculated. The clinical, laboratory and radiographic data were monitored on a daily basis for the development of pneumonia and/or sepsis. The diagnosis of pneumonia and sepsis was made by an intensive care physician. Chest X-rays were interpreted by a radiologist.

Levels of presepsin, PCT, CRP and Le were measured at the moment the clinical diagnosis of VAP was defined, when patients developed septic reaction or septic shock. The Sequential Organ Failure Assessment (SOFA) score was calculated at these points as well.

The biomarkers measurements

Blood was collected with/without heparin as an anticoagulant, using a conventional blood collection tube (Vacutainer, BD, Franklin Lakes, NJ, USA). Collected blood samples were centrifuged (15 min, 3000 rpmi) and plasma/serum aliquots were stored at -80°C, until the measurement was performed. All biomarkers of interest (PCT, CRP and Le) as well as other markers of sepsis were determined at the central laboratory of the Center of Medical Biochemistry, using commercially available methods following the instructions of the manufacturers. The study protocol was approved by the Ethics Committee of the Clinical Center of Serbia.

The presepsin concentration was performed with the Presepsin test (PATHFAST ®, Mitsubishi Chemical Medience Corp., Tokyo, Japan). The test principle is based on non-competitive chemiluminescent enzyme immunoassay (CLEIA). During incubation of the sample with alkaline phosphatase labeled anti-presepsin polyclonal antibody and anti-presepsin monoclonal antibody coated magnetic particles, the presepsin of the sample binds to the anti-presepsin antibodies forming an immunocomplex with enzyme labeled antibody and antibody coated magnetic particles. After removing the unbound substances, a chemiluminescent substrate is added. Following a short incubation, the luminescence intensity generated by the enzyme reaction is measured. The luminescence intensity is related to the presepsin concentration of the sample, calculated by the means of a standard curve [16].

Quantitative analysis of PCT was performed by using an automated electrochemiluminescence immune analyzer (Elecsys BRAHMS PCT, Roche Diagnostics, Mannheim, Germany). CRP was determined by nephelometry (BN II, Siemens, Erlangen, Germany). Both parameters, PCT and CRP, are routinely available from the central laboratory of our hospital. For their determination, serum samples were used. The leukocyte counts were established by automatic laser mediated counting.

Table 1

Patients characteristics

Characteristics of patients	Values
Age (year), mean ± SD	47.9 ± 15.2
Gender, n (%)	
males	33 (84.6)
females	6 (15.4)
APACHE II categories, n (%)	
1–10	12 (30.8)
11–20	18 (46.2)
21–30	9 (23.1)
31–40	-
> 40	-
APACHE II, mean ± SD	14.4 ± 6.0
Severity of injuries, n (%)	
mild (ISS < 9)	7 (17.9)
moderate (ISS: 9–15)	11 (28.2)
severe (ISS: 16–24)	14 (35.9)
profound (ISS: 25–75)	7 (17.9)
ISS, mean ± SD	24.2 ± 12.8
Infection status, n (%)	
SIRS	21 (29.2)
VAP	21 (29.2)
Sepsis	18 (25.0)
Septic shock	12 (16.6)

SD – standard deviation; Apache II – Acute Physiology and Chronic Health Evaluation; ISS – Injury Severity Score; SIRS – systemic inflammatory response syndrome; VAP – Ventilation Associated Pneumonia.

Definitions

Trauma was defined as presence of injury to more than one body area or system or the presence of major traumatic brain injury alone. The APACHE II is a severity-of-disease classification system applied within 24 h of admission of a patient to an intensive care unit (ICU).

SIRS is defined by the presence of at least two of the following signs: body temperature lower than 36 °C or higher than 38 °C, heart rate faster than 90 beats per minute, tachypnea (high respiratory rate) with more than 20 breaths per minute, leukocyte count lower than 4000 cells/mm³ or higher than 12,000 cells/mm³ or the presence of more than 10% immature neutrophils.

VAP is diagnosed when new persistent pulmonary infiltrates, not otherwise explainable, appear on chest X-rays > 48 h after admission to the ICU, with the presence of one systemic and two pulmonary criteria. Patients with pulmonary contusion show worsening of X-ray findings as diagnosed by a radiologist. The systemic criteria are as follows: fever >38 °C, leukopoenia (count lower than 4,000 WBC/mm^3) or leukocytosis (count higher than 12,000 WBC/mm^3); for adults older than 70 years of age, the systemic criteria also include altered mental status, with no other recognized cause. The pulmonary criteria are as follows: new onset of purulent sputum (or a change in the character of the sputum, increased respiratory secretions or increased suctioning requirements), worsening gas exchange (desaturations, increased oxygen requirements or increased ventilator demand), new onset or worsening cough, and dyspnoea, tachypnoea, rales or bronchial breath sounds. Positive chest X-ray is defined as a progressive or new infiltrate, consolidation, pleural effusion or cavitation. Sputum change is defined as the new onset of purulent sputum or a change in the character of the sputum [17]. Respiratory samples for a bacteriological examination were collected from BAL.

Sepsis was defined as the worsening of clinical condition with at least one acute sepsis-related organ dysfunction, with one of the clinical variables: significant edema or positive fluid balance, hyperglycemia (plasma glucose > 7.7 mmol/L) in the absence of diabetes, organ dysfunction variables: arterial hypoxemia (Pao2/Fio2 < 300), acute oliguria (urine output < 0.5 mL/kg/h for at least 2 h despite adequate fluid resuscitation), increase of the creatinine level > 44.2 μmol/L, coagulation abnormalities, ileus, thrombocytopenia, hyperbilirubinemia (plasma total bilirubin level > 70 μmol/L) and tissue perfusion variables: hyperlactatemia (> 1 mmol/L) or decreased capillary refill or mottling. In order to exclude the possibility of extra-pulmonary infection source, only the patients with bacteremia having the same pathogens isolated from the respiratory sample were included.

Septic shock was defined as sepsis accompanied by arterial hypotension (systolic blood pressure – SBP < 90 mm Hg, mean arterial pressure – MAP < 70 mm Hg, or a SBP decrease > 40 mm Hg in adults or less than two sd below normal for the age) [18].

Statistical analysis

Statistical analysis was done using STATA 12.0 software (Stata Corporation, College Station, Texas, USA). For continuous variables the Kolmogorov-Smirnov test was performed to assess the assumption of normality. Thus, normally distributed continuous data were presented as mean ± standard deviation (SD) and were compared between the groups by using the Student's *t*-test, and skewed data were presented as median [inter-quartile range (IQR)] and were compared using the Mann-Whitney *U* test. Bonferroni correction was applied for all the multiple comparisons. Categorical data were presented as frequency (percentage) and compared using χ^2 test or Fisher exact-test, where appropriate. Spearman's correlation test was used to assess the correlation between presepsin and other biomarkers and variables describing medical condition severity (ISS, SOFA and Apache II). Receiver operation characteristic (ROC) analysis was done and c-statistic was calculated for each inflammation marker, in order to examine their diagnostic accuracy. The comparison of four different area under curve (AUC) was done by using χ^2 test within ROCCOMP command (Delong, Delong and Clarke-Pearson method), with post hoc Bonferroni correction [19]. For all the comparisons *p*-values ≤ 0.05 were considered as statistically significant difference with 95% confidence interval (CI).

Results

Patients' and injury characteristics

In 39 patients (33 males and 6 females) who sustained trauma, spending more than 48 hours on mechanical ventilation, 72 measurements of four biomarkers were performed. Blood was drawn at the moment of SIRS, VAP, sepsis or septic shock diagnosis. Twenty one of all those measurements were performed when SIRS was diagnosed, other 21 measurements were conducted at the moment of VAP diagnosis, further 18 measurements at the moment of sepsis diagnosis and 12 measurements coincided with septic shock diagnosis (Table 1).

Study participants were predominantly middle-aged (mean age 47.9 ± 15.2 years) and males [n = 33 (84.6 %)]. They were mainly severely injured according to the mean ISS of 24.2. The majority of the patients had moderately severe medical condition on admission, as indicated by mean APACHE II score 14.5 (Table 1).

Biomarkers concentrations

Distribution of serum presepsin, PCT, CRP and Le in patients with SIRS, VAP, sepsis and/or septic shock are shown in Figure 1. Median, minimum and maximum concentrations of biomarkers is summarized in Table 2.

Comparisons of biomarkers between four groups are shown in Table 3. Presepsin concentration significantly differed among all four groups, except between sepsis and septic shock. These differences were highly significant ($p < 0.001$) with the exception of the difference between the

patients with VAP and sepsis, which was less prominent [median (IQR) 663.0 (271.5) vs. 988.0 (746.8); $p = 0.012$]. PCT was significantly higher in every consequent group, except for the septic shock group compared to sepsis [12.5 (4.4) vs. 11.5 (7.0); $p = 0.215$)]. CRP and Le significantly differed only between the SIRS and septic shock [124.0 (112.0) vs. 220.3 (143.4); $p = 0.012$] and [11.0 (8.3) vs.17.5 (7.0); $p = 0.024$, respectively].

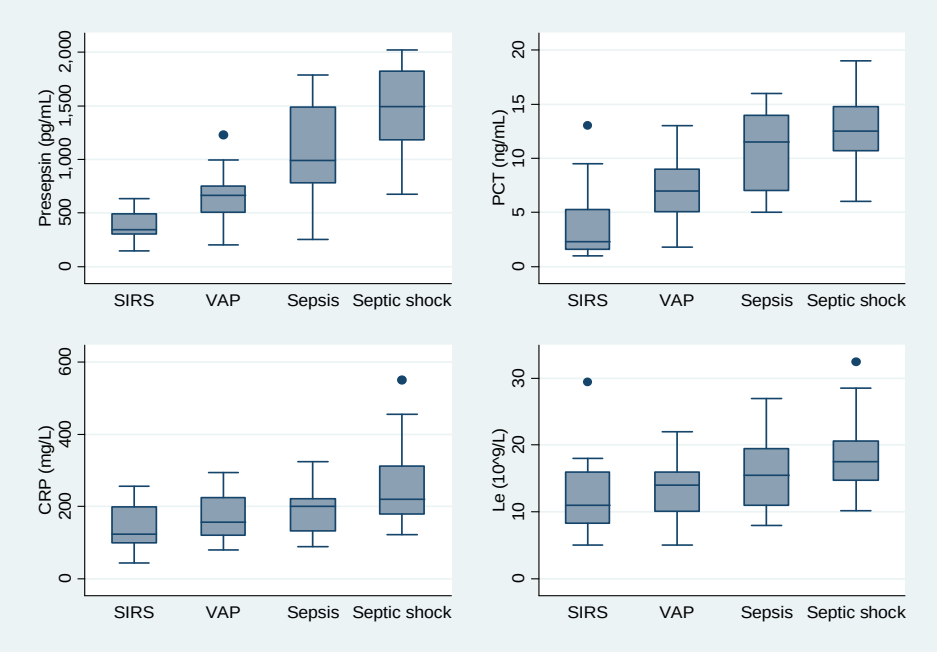

Fig. 1 – Distribution of presepsin, procalcitonin (PCT), C-rective protein (CRP) and leukocytes (Le) in 39 trauma patients at the moment of diagnosis of systemic inflammatory response syndrome (SIRS) (n = 21), ventilator associated pneumonia (VAP) (n = 21), sepsis (n = 18) or septic shock (n = 12).

Table 2

Concentration of presepsin, procalcitonin (PCT), C-reactive protein (CRP) and leucocytes (Le) in the trauma patients

Biomarker	SIRS	VAP	Sepsis	Septic shock
	Median (IQR) Min–Max	Median (IQR) Min–Max	Median (IQR) Min–Max	Median (IQR) Min–Max
Presepsin (pg/mL)	343.0 (202.0) 144–633	663.0 (271.5) 199–1230	988.0 (746.8) 252–1789	1494.0 (674.3) 674–2020
PCT (ng/mL)	2.3 (3.8) 1–13	7.0 (4.5) 1.8–13	11.5 (7.0) 5–16	12.5 (4.4) 6–19
CRP (mg/L)	124.0 (112.0) 43–256	157.0 (119.5) 79–294	200.0 (101.3) 88–324	220.3 (143.4) 122–550.4
Le (10^9/L)	11.0 (8.3) 5–29.4	14.0 (6.5) 5–22	15.5 (8.9) 8–27	17.5 (7.0) 10.2–32.4

SIRS – systemic inflammatory response syndrome; VAP – ventilator-associated pneumonia; IQR – interquartile range.

Table 3

Comparisons of presepsin, procalcitonin (PCT), C-reactive protein (CRP) and leucocytes (Le) between groups*

Biomarkers	Presepsin	PCT	CRP	Le
SIRS vs. VAP	< 0.001	0.024	0.630	0.406
SIRS vs. Sepsis	< 0.001	< 0.001	0.222	0.258
SIRS vs. Septic shock	< 0.001	< 0.001	0.012	0.024
VAP vs. Sepsis	0.012	0.012	0.587	0.202
VAP vs. Septic shock	< 0.001	< 0.001	0.378	0.144
Sepsis vs. Septic shock	0.132	0.215	0.185	0.285

***Mann-Whiney *U* test with Bonferroni correction.**
SIRS – systemic inflammatory response syndrome; VAP – ventilator-associated pneumonia.

Table 4

C-statistic of presepsin, procalcitonin (PCT), C-reactive protein (CRP) and leukocytes (Le) in prediction of infection or sepsis

Biomarker	Non-VAP vs. VAP			Non-sepsis vs. sepsis		
	AUC	95% CI	*p*	AUC	95% CI	*p*
Presepsin	0.908	0.842–0.975	0.000	0.899	0.818–0.980	0.000
PCT	0.863	0.765–0.961	0.000	0.885	0.810–0.960	0.000
CRP	0.703	0.57–0.835	0.007	0.677	0.553–0.800	0.011
Le	0.668	0.527–0.809	0.026	0.700	0.579–0.820	0.004

VAP – ventilator-associated pneumonia; AUC – area under the curve; CI – confidence interval.

Correlation between presepsin and other markers

The strongest positive correlation presepsin evinced with PCT ($r = 0.741$, $p < 0.001$) (Figure 2). Presepsin was in significant positive correlation with other inflammation markers and with SOFA score ($r = 0.575$; $p < 0.001$) which represents the extent of the organ function/failure and was measured at the moment of each infectious condition. The similar correlations with abovementioned variables were observed for PCT, while CRP correlated with APACHE II and ISS as well.

ROC analysis

Diagnostic accuracy of the four sepsis biomarkers (presented as the ROC curves) in discrimination of non-VAP (SIRS) and VAP conditions (VAP + sepsis + septic shock) are shown in Figure 3 while for non-sepsis (SIRS + VAP) and sepsis conditions (sepsis + septic shock), they are shown in Figure 4. The summary of the c-statistic or the AUC for those four markers is shown in Table 5. According to the AUCs, presepsin evinced better performance in diagnosis of both VAP (AUC = 0.908) and sepsis (AUC = 0.899), than PCT (AUC = 0.863; 0.885, respectively), CRP (AUC = 0.703; 0.677, respectively) and Le (AUC = 0.668; 0.700, respectively). However, the AUCs comparison revealed that AUC for presepsine was significantly greater only when compared to AUC for Le ($\chi^2 = 10.92$, $p = 0.006$) in discriminating between non-VAP and VAP conditions. Considering non-sepsis and sepsis conditions, AUC for presepsin was significantly higher than AUC for CRP ($\chi^2 = 11.40$, $p = 0.004$) and Le ($\chi^2 = 11.67$, $p = 0.003$). However, presepsin showed as good diagnostic accuracy as PCT.

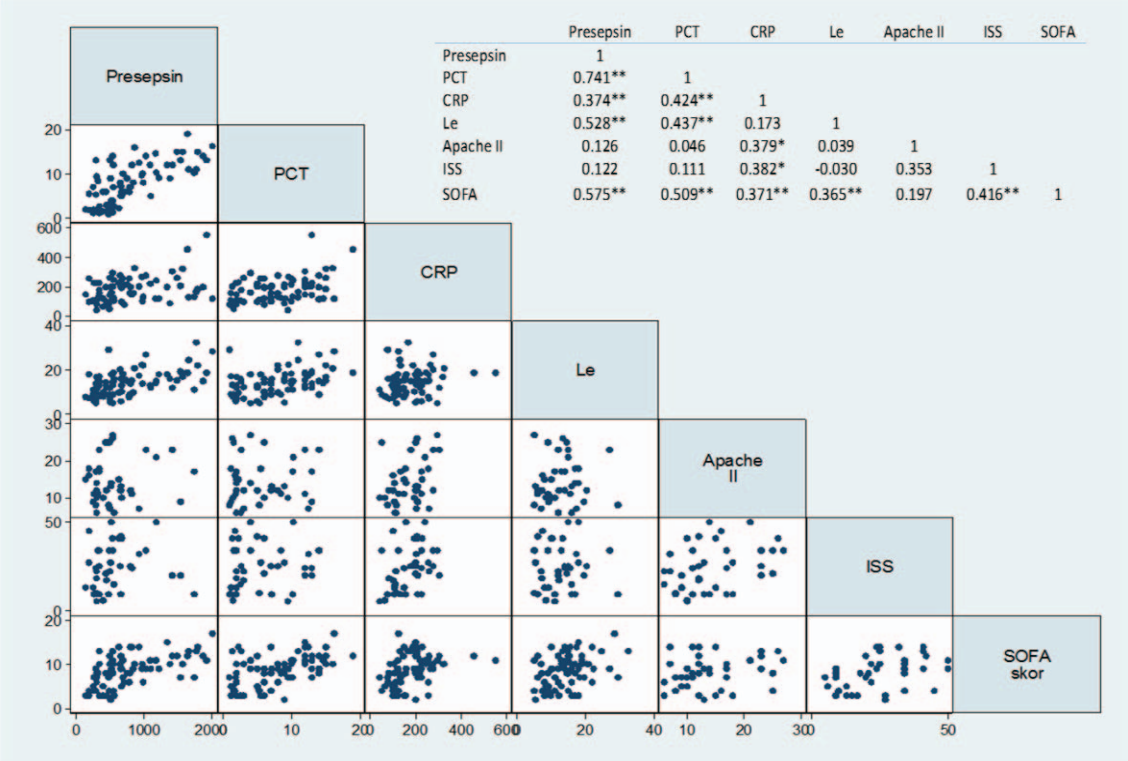

Fig. 2 – Correlation of presepsin with other inflammation markers, Acute Physiology and Chronic Health Evaluation (APACHE II) score, Injury Seventy Score (ISS) and Sequential Organ Failure Assessment (SOFA) score. (*$p < 0.05$; **$p < 0.001$).

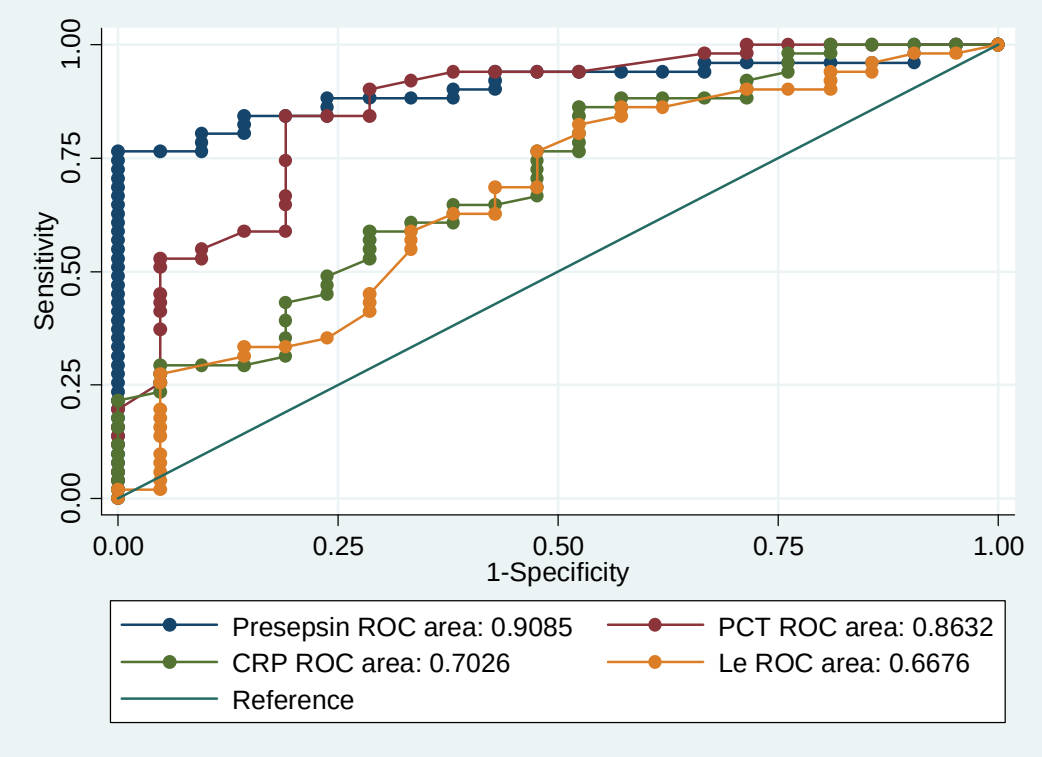

Fig. 3 – Receiver operation characteristic (ROC) curve for presepsin, procalcitonin (PCT), C-reactive protein (CRP) and leukocytes (Le) in the non-infection group [systemic inflammatory response syndrome (SIRS)] and the infection group [sepsis, ventilator-associated pneumonia (VAP) and septic shock].

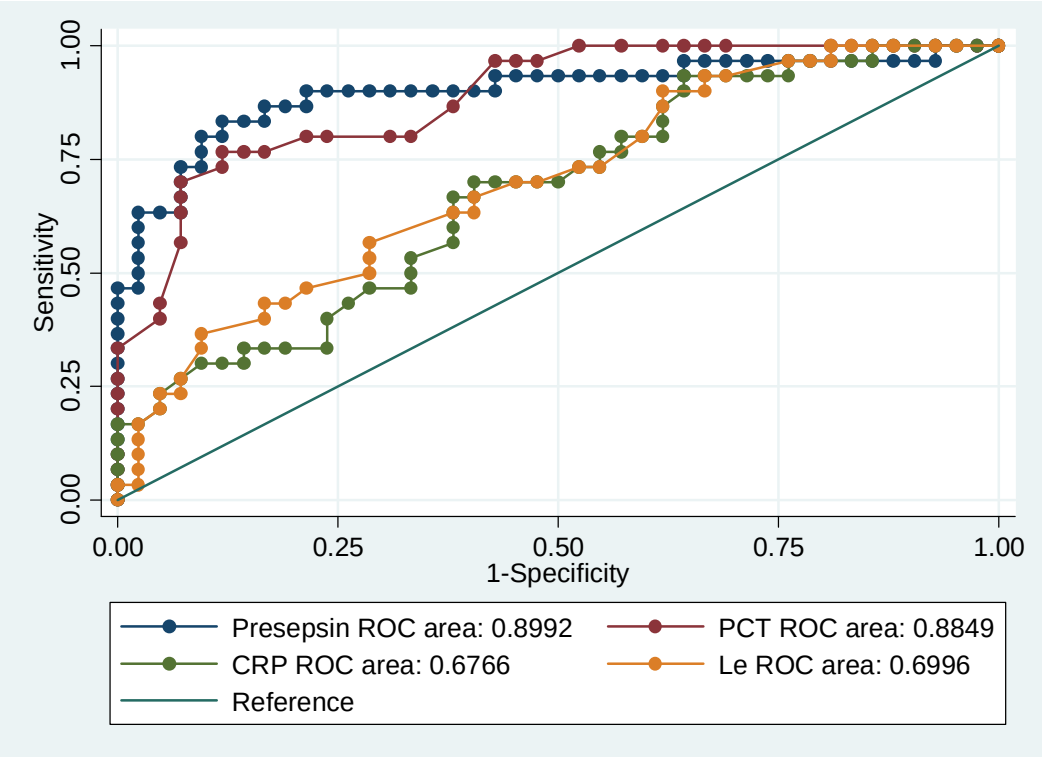

Fig. 4 – Receiver operation characteristic (ROC) curve for presepsin, procalcitonin (PCT), C-reactive protein (CRP) and leukocytes (Le) in the non-septic group [Systemic Inflammatory Response Syndrome (SIRS) and ventilator-associated pneumonia (VAP)] and the septic group (sepsis and septic shock).

Table 5
Comparisons of the area under curves (AUCs) with Bonferroni correction

Biomarker	Non-VAP vs. VAP	Non-sepsis vs. sepsis
Presepsin vs. PCT	0.414	0.744
Presepsin vs. CRP	0.228	0.004
Presepsin vs. Le	0.006	0.003
PCT vs. CRP	0.186	0.012
PCT vs. Le	0.162	0.001
CRP vs. Le	0.717	0.777

VAP – ventilator-associated pneumonia; PCT – procalcitonin; CRP – C-reactive protein; Le – leukocytes.

Discussion

The number of severely injured patients treated in the ICU and requiring prolonged mechanical ventilation is significant, leading to high incidence of VAP, as shown earlier [20]. The accurate diagnosis of infection, especially pneumonia, in the ICU settings is challenging and is linked with decrease in morbidity and mortality.

During VAP development the beginning of treatment cannot be accurately suggested. It is usually associated with the ICU rounds and changes in the chest X-ray findings, which can lead to delayed clinical decision-making. Moreover, using clinical decision, as suggested by many guidelines, can postpone appropriate antibiotic regimens. Lower or upper respiratory sputum sample techniques are still the most reliable diagnostic method for ventilator-associated pneumonia. However, it takes at least two days to obtain the results. The case is the same with blood cultures, which are frequently used for sepsis confirmation. Therefore, defining a reliable marker that can enable fast diagnosis of VAP would be of great benefit.

In the present study we evaluated prognostic value of presepsin in diagnosis of VAP and sepsis in critically injured patients requiring mechanical ventilation, i.e., its potential to differentiate the local infection (VAP) from SIRS, as well as to differentiate VAP from sepsis and septic shock. In our study levels of presepsin were significantly higher in patients who developed VAP compared to those with SIRS and significantly higher in patients with sepsis compared to those with VAP or SIRS. These results are in line with few studies that measured levels of sCD14-ST in blood samples for local infection diagnosis [21–23]. In study of Shozushima et al. [22] presepsin levels were significantly higher in patients with local infection, sepsis, and severe sepsis than in patients without infection. Similarly, Popov et al. [23] showed significantly higher values of presepsin in the group of patient with infectious complications than in those without infections following cardiac surgery. In our study, however, we measured biomarkers levels only after the clinical diagnosis of infection was made. Regularly measuring sCD14-ST levels can possibly hasten diagnosis, even before significant clinical changes are present (e.g., sputum, fever, chest X-ray change) [7].

Presepsin in our study evinced an excellent ability to differentiate both, non-VAP vs. VAP diagnoses and non-septic vs. septic diagnoses, as shown by the high area under the ROC curves (0.908 and 0.899, respectively). Considering pneumonia, presepsin was only investigated in severe community acquired pneumonia (sCAP) treated in the ICU. Similarly to our findings, in a study by Klouche et al. [24] presepsin evinced a great capacity to differentiate sCAP from severe sepsis and septic shock. They also observed significantly higher concentrations of presepsin in patients with pneumonia compared to those with the non-infectious respiratory failure. The similar was observed in a study by Liu et al. [25] where presepsin levels corresponded with level of infection, i.e., presepsin was higher in patients with sCAP than in those with CAP.

Presepsin was studied more profoundly in patients with sepsis [14, 26–28]. Its elevation represents a dose–response mechanism of the host reaction to pathogen. In our study presepsin level was significant for differentiating the severity of sepsis, as shown in the mentioned studies. In addition to this, we observed significant positive correlation of presepsin with SOFA score, a clinical score which depicts the degree of patients' organ failure. This finding suggests that presepsin is a marker which closely corresponds to sepsis pathogenesis. The association of presepsin level with severity of disease scores were also observed Shozushima et. al. [22] (CAP severity score) and Liu et al. [25] (APACHE II score).

The most meaningful result of our study was that presepsin indicated a better performance in the diagnosis of VAP and sepsis than commonly used laboratory markers (PCT and WBC). PCT is one of the most frequently used markers for diagnosis of local infection and sepsis complications [29, 30]. Although procalcitonin-guided strategy used to treat suspected bacterial infections could reduce antibiotic exposure, PCT tends to rise transiently in non-septic conditions and SIRS, like invasive trauma, surgery and physical exercise, as shown in the previous studies and which is not the case with presepsin [26, 31, 32]. In our study, in the presence of pneumonia and sepsis, the levels of PCT raised significantly, however diagnosis can be delayed due to the fact that PCT value increased 4 hours after infection and peaked one day after infection while presepsin concentration increased in 2 hours after infection, reaching the peak value after 3 hours [13]. In spite of the fact that presepsin in our study showed significant positive correlation with other laboratory markers of sepsis (PCT, CRP, WBC), presepsin expressed the strongest positive correlation with PCT. This finding is in accordance with other clinical studies that have also pointed out the im-

portance of PCT and the presepsin measurements in the patients with sepsis, as diagnostic and prognostic markers [33].

Conclusion

In the era of terrifying increase of pathogens resistance, great efforts have been made to define the new tools for excellence in early diagnosis of infections. Some of them are novel biomarkers which can hasten the process. Keeping in mind that the best preventive techniques for VAP are not specifically defined or are not available, real time control of infection relies on its diagnosis. Our study suggests that presepsin (sCD14-ST) is reliable biomarker for diagnosis of VAP in severely injured patients requiring mechanical ventilation, as well as for distinguishing sepsis severity in these patients.

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ORIGINAL ARTICLE

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Correlation of precancerous lesion incidence with duodenogastric reflux and N-nitroso compound duration at reflux and antireflux stomach surgery – An experimental study

Korelacija incidencije nastanka prekanceroznih lezija sa vremenom delovanja duodenogastričnog refluksa i N-nitroznih jedinjenja kod refluksnih i antirefluksnih operacija na želucu – eksperimentalna studija

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Abstract

Background/Aim. Duodenogastric reflux occurs after gastroenteroanastomosis, with or without resection of the stomach (Billroth I, Billroth II), after vagotomy and pyloroplasty, gastroduodenostomy as well as in conservatively treated patients. The aim of this study was to analyse the effect of carcinogenic N-nitroso compounds (MNNG) influence in conjunction with duodenogastric reflux, in the function of time for precancerous lesions development in gastric mucosa. A particular purpose of the study has been to suggest some effective surgical procedures, which could prevent the harmful effects of duodenogastric reflux on gastric mucosa, as a potential activator of carcinogenesis. **Methods.** The research included 90 experimental, male Wistar rats, divided into 3 groups. The two experimental groups were subjected to two surgical procedures: Billroth II gastric resection (group B2), and the Roux-en-Y reconstruction (group RY), respectively. The control group (group C) animals did not receive any surgical treatment. All groups were administered *per os* chemical cancerogen MNNG. All anastomoses were performed by extra mucosal suture with monofilament polypropilene 7–0, and 8–0. The animals were sacrificed consecutively, and subjected to total gastrectomy after 8, 16 and 24 weeks. **Results.** Pathohistological analysis was performed on defined regions of the rat stomach in three time period. The B2 group, at the end of our experiment, showed predominant incidence of severe lesions: hyperplasia (0%), gastritis (0%), metaplasia (6.7%), dysplasia (46.7%), early carcinoma (20%) and carcinoma (26.7%). At the end of our experiment, the RY and C groups showed the similarities of the obtained results related to time with predominant incidence of mild lesions: hyperplasia (13.3% *vs* 0%, respectively), gastritis (13.3% *vs* 13.3%, respectively), metaplasia (6.7% *vs* 13.3%, respectively), dysplasia (66.7% *vs* 66.7%, respectively), with an extremely low incidence of early carcinoma (0% *vs* 6.7%, respectively) and no incidence of carcinoma (0% *vs* 0%, respectively). **Conclusion.** Without the presence of duodenogastric reflux, MNNG causes a low degree of precancerous gastric lesions. However, direct contacts of MNNG with gastric mucosa, including the presence of duodenogastric reflux, induce precancerous lesions and carcinoma. The percentage of reversible changes decreases with time, while the percentage of irreversible lesions and carcinoma increases. A lack of distinction in the findings between the RY and C groups confirms a gastroprotective role of the Roux-en-Y procedure.

Key words:
duodenogastric reflux; gastric mucosa; precancerous conditions; stomach neoplasms; nitroso compounds; digestive system surgical procedures; disease model, animal; rats.

Apstrakt

Uvod/Cilj. Duodenogastrični refluks (DGR) se sreće posle gastroenteroanastomoze, sa ili bez resekcije želuca (Billroth I, Billroth II), posle vagotomije i piloroplastike, gastroduodenostomije, kao i kod neoperisanih bolesnika. Cilj rada bio je da se ispitaju kancerogena svojstva N-nitrozo jedinjenja (MNNG) u sadejstvu sa duodenogastričkim refluksom, u

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funkciji vremena, na razvoj prekanceroznih lezija u sluzokoži želuca. Poseban cilj bio je da se preporuči metoda hirurške prevencije štetnog delovanja DGR-a na sluznicu želuca, kao mogućeg aktivatora kancerogeneze. **Metode.** Istraživanjem je obuhvaćeno 90 eksperimentalnih pacova, muškog pola, Wistar soja, podvrgnutih dvema operativnim procedurama: Billroth II (grupa B2) i resekciji želuca sa Roux Y rekonstrukcijom (grupa RY). U kontrolnoj grupi (grupa C) životinje nisu bile operisane. Svim grupama je *per os* dat hemijski kancerogen MNNG. Sve anastomoze su bile urađene ekstramukoznim šavom, a kao šavni materijal korišćen je monofilamentni polipropilen 7–0, i 8–0). Nakon 8, 16 i 24 nedelje, životinje su žrtvovane i urađena je totalna gastrektomija. **Rezultati**. Patohistološki su analizirani definisani regioni želuca pacova u tri vremenska perioda. Na kraju eksperimenta grupa B2 je pokazala dominantnu incidencu ozbiljnih promena: hiperplazija (0%), gastritis (0%), metaplazija (6.7%), displazija (6.7%), rani rak (20%) i ispoljeni rak (kod 26.7% životinja). Na kraju eksperimenta utvrđena je sličnost dobijenih rezultata u funkciji vremena između RY i C grupe, sa preovladavajućom pojavom blažih promena: hiperplazije (13.3% i 0%, redom), gastritisa (13.3% i 13.3%, redom), metaplazije (6.7% i 13.3%, redom), displazije (66.7% i 66.7%, redom), upadljivo malom incidencijom ranog karcinoma (0% i 6.7%, redom) i odsustvom karcinoma (0% i 0%, redom). **Zaključak.** Dejstvo samo MNNG na sluzokožu želuca bez prisustva duodenogastričkog refluksa izaziva lakši stepen prekancerogenih lezija. Direktni kontakt MNNG sa gastričkom sluznicom, uz duodenogastrički refluks, indukuje nastanak prekanceroznih lezija i karcinoma. Protokom vremena procenat reverzibilnih promena se smanjuje, a procenat ireverzibilnih lezija i karcinoma povećava. Nedostatak razlike između RY i C grupe potvrđuje gastroprotektivnu ulogu Roux-Y procedure.

Ključne reči:
duodenogastrički refluks; želudac, sluzokoža; prekancerska stanja; želudac, neoplazme; nitrozo jedinjenja; hirurgija digestivnog sistema; procedure; bolest, modeli na životinjama; pacovi.

Introduction

Duodenogastric reflux (DGR), enterogastric reflux, bile reflux, alcaline reflux gastritis, or postresection gastritis, are all synonyms for the same phenomenon, which can be defined as the "return of duodenal content to the stomach through an incompetent pyloric valvula, i.e., from duodenum and intestines, through anastomosis, into the stomach" [1].

Bile presence in the stomach is not a normal finding, since pylorus prevents any significant DGR. Despite previous evidence of bile presence in some patients, these findings given sufficient significance [1]. Surgical interventions, which cause pylorus destruction, induce DGR in approximately 5% to 35% surgically treated patients. It is also possible that idiopathic DGR precedes surgical interventions; however, the percentage of such cases is very low [2].

DGR occurs after gastroenteroanastomosis (GEA), with or without resection of the stomach (Billroth I – B1, Billroth II – B2), after vagotomy and pyloroplasty, gastroduodenostomy as well as in conservatively treated patients. GEA by B 2 type resection and pylorus removal in B 1 type resection create conditions for the occurrence of continuous duodeno-biliary-pancreatic juice reflux into stomach, which triggers inflammatory-dystrophic-metaplastic gastric mucosal lesions and the consecutive damage to its physiological functions, thus creating conditions for the occurrence of other diseases [3]. In a previous paper, we published the findings of the effects of DGR on the occurrence of precancerous gastric lesions [4].

Apart from biliary salts and biliary acids, there are other significant microenvironmental factors suspected to contribute to precancerous lesion occurrence: decreased gastric acidity (hypochlorhydria and achlorhydria), presence of bacteria in gastric lumen as well as presence of nitrates and nitrites and, particularly, N-nitroso compounds [5]. The possibility of formation of N-nitroso compounds has been proven both *in vitro* and *in vivo* in the stomach of experimental animals, in noncarcinogenic secondary and tertiary amines and amides present in food as well as nitrogenic acid, i.e., nitrites and nitrites found in potable water. N-nitroso compounds can be formed from nitrites and some pharmaceuticals, such as antihistaminic drugs, tranquilisers, disulphiram, antibiotics, etc.. Some authors emphasize the possibility of formation of N-nitroso compounds in the patients subjected to long-term treatment with certain antagonists of H2 receptors (cimetidine, ranitidine), since cimetidine is a derivate of guanine which, in the presence of nitrite, can easily be transformed into mononitro-cimetidine, which is an analogue of MNNG [6, 7].

The aim of this experimental study was to determine the role of DGR, in conjuction with the duration of N-nitroso compound presence, in the development of gastric precancerous lesions, In particular, the purpose of the study was to suggest an effective surgical procedure for the prevention of harmful effects of DGR on gastric mucosa as a potential activator of carcinogenesis.

The purpose of the experiment was also to confirm that DGR, along with N-nitroso compound in correlation with time, is responsible for the incidence of precancerous gastric lesions after GEA.

Methods

This study was conducted at the Centre for Biomedical Research of the University of Niš, Serbia from April 2012 to February 2013. The experiment was done on 90 male Wistar rats, with average weight of 225 grams, obtained from the Vivarium of the Faculty of Medicine in Niš. All legal and regulatory principle referring to animal treatment were respected. The two experimental groups were subjected to two surgical procedures (GEA) – stomach resection Billroth II and by Roux-en-Y (RY) reconstruction. The control group was not subjected to any surgical intervention. Allocation to the experimental groups were random. Diagram of the study show flow of experimental animals through of the study (Figure 1).

Chemical carcinogen N-methyl-N-nitro-N-nitrosoguanidine (MNNG®), with the molecular formula $C_2H_5N_5O_3$, CAS Number 70–25–7, obtained from ABCR GmbH & Company, Karlshruhe, Germany, was administered to all experimental groups. The animals received MNNG in the concentration on 100 mg per liter of drinking water. MNNG was administered starting with the fifth postoperative day in the animals subjected to the surgeries. Non-operated animals received it since the first experimental day [8, 9].

Construction of the tested groups

Similar to the previous experiment [4], this research included 2 experimental and one control group, with 30 animals each. The first experimental group included 30 animals and was marked as B2 group, since gastric resection was done by Billroth II reconstruction, by omega winding (Figure 2a – painted, Figure 2b – in nature). In the B2 experimental group, the effects of surgically induced DGR and N-nitroso compounds (MNNG) on gastric mucosa were examined in correlation with time. The second experimental group, comprised of 30 animals, was marked as RY group, due to the performed resection of pyloric and antral gastric part by means of Roux-en-Y reconstruction (Figure 3a – painted, Figure 3b – in nature). As expected, the gastric mucosa in this group was examined for the influence of N-nitroso compounds (MNNG) without DGR. The control group included 30 animals which were not operated (marked as group C) with administered MNNG (Figure 4a – painted). In addition, each group included 3 sub-groups. 5, 10 and 15 animals were sacrificed in each group, respectively, in the week 8, 16 and 24 of experimental observation (Figure 1).

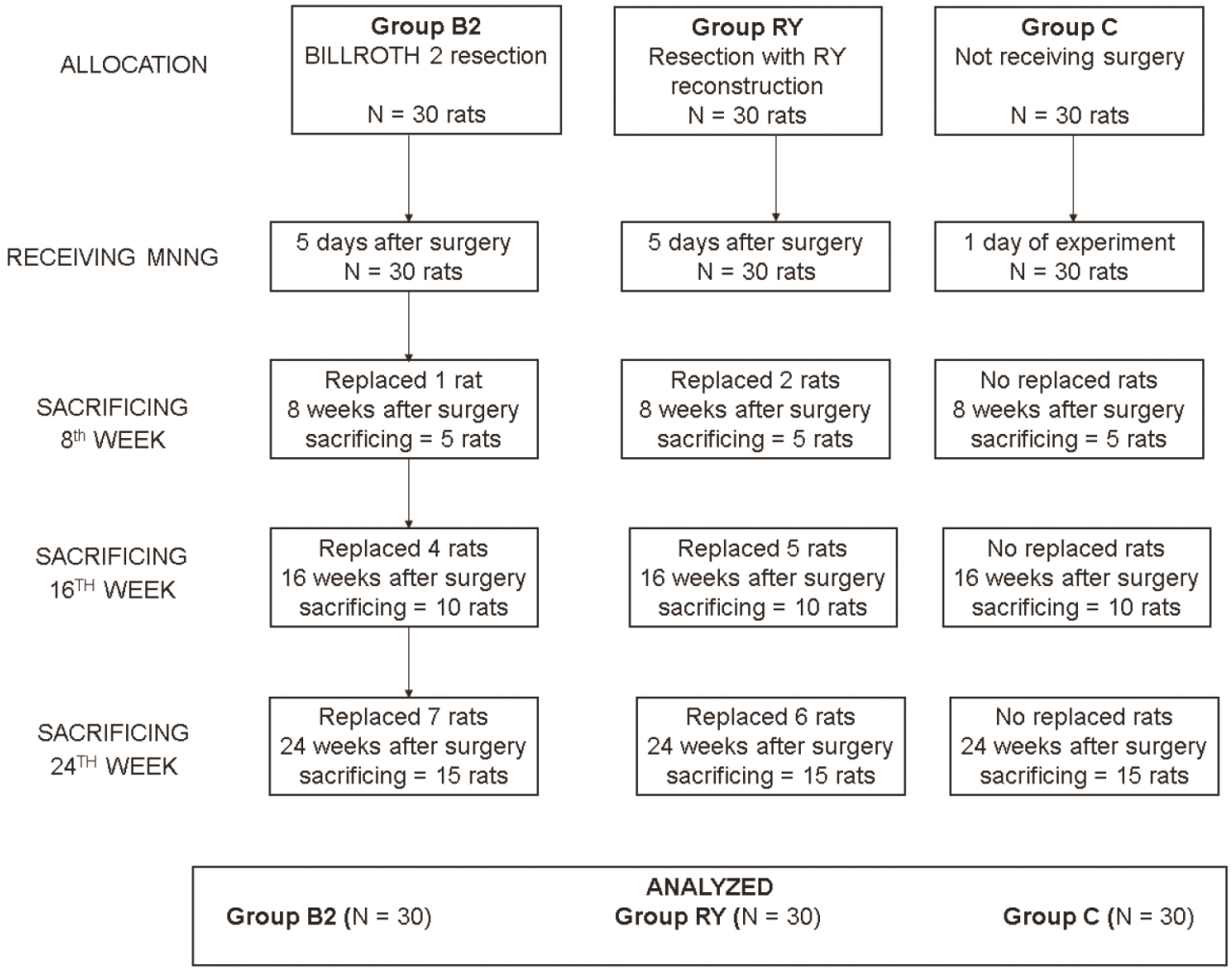
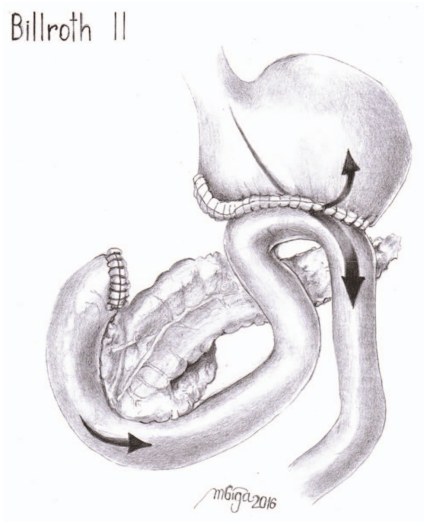


Fig. 1 – Flow of experimental animals through the study.



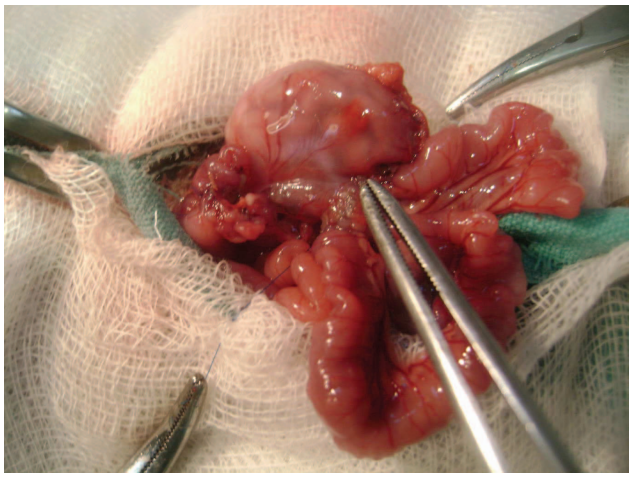
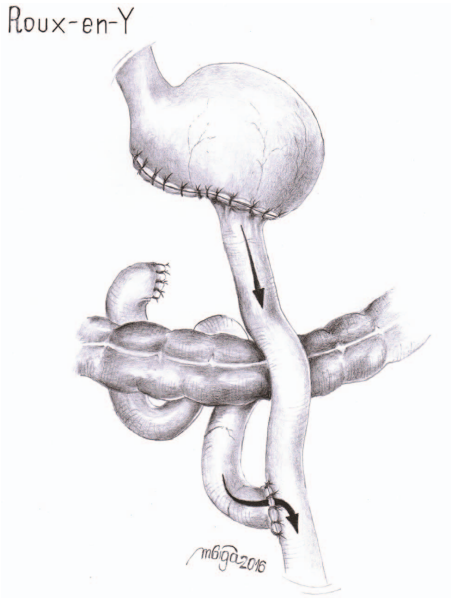
a)  b)

Fig. 2 – Gastro-jejuno anastomosis Billroth II: a) painted; b) in nature.



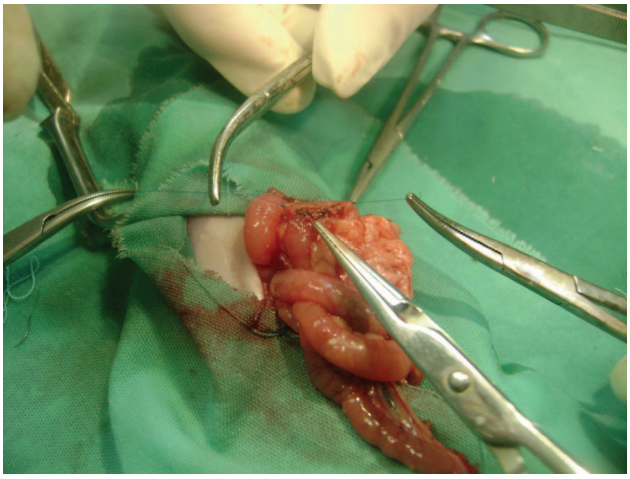
a)  b)

Fig. 3 – Gastro-jejuno anastomosis Roux-en-Y: a) painted; b) in nature.

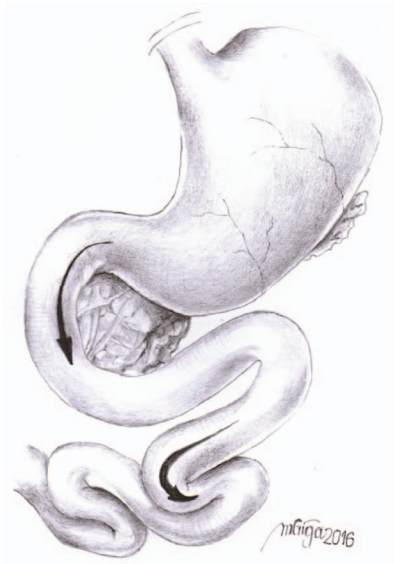


Fig. 4 – Unoperated animal (painted).

Suregery

The animals were anesthetized by ketamine hydrochloride (Ketamidor® 10%, Richter pharma AG, Wels, Austria), with the operatively administered dose of 0.1 mL per 100 g of body weight, intraperitoneally. All anastomoses were performed by extra mucous monofilament suture polypropylene 7–0 and 8–0.

Follow-up after the surgical procedures

The animals were placed individually in the prepared cages and were observed until they have recovered consciousness and ability to move. Ketorolac tromethamine was used as an analgesic in the dose of 1 mg *per* kg of body weight, intramuscularly, upon indication of pain. During the first 12 hours, the animals received solution of 5% glucosis *per os*, tap water and physiological solution in the equal ra-

tio. Food for experimental animals, mixed with physiological solution was introduced on the day 3 after the surgery. After 5 days, the surviving animals were moved to group cages, for the rest of the experimental period. The animals that showed signs of discomfort, poor nutrition, or disease were sacrificed and were replaced. The animals which died after the half time period of the study were not replaced. The final selection of experimental animals in groups was based on the common minimum number (30) of survived animals in each group.

Receiving of the carcinogens

The experimental animals received the solution of tap water and MNNG *per os*, in graded bottles of 250 mL. All bottles with H_2O and MNNG solution were lined with aluminium foil, in order to prevent the carcinogen decomposition from the influence of sunlight. All measurements of the substances used in the experiment were performed at the Biochemical Institute of the Faculty of Medicine, the University of Niš, Serbia, by making use of the analytical scale Denver Instrument Company, Type AA-200DS.

Measurement of gastric pH

In order to confirm DGR presence in the stomach of both, surgically treated and non-resected animals, gastric pH of the sacrificed animals was measured in two stomach regions (pericardial and para-anastomotic one in the resected animals and anthropyloric one in the non-resected animals). Increased pH values suggested bile presence (alkaline effect) in the stomach, indicating the presence of DGR. By means of gastrostomy of appropriate length, (Figure 5), universal pH indicator stripes with 0–14 measurement range (Merck®) were inserted. The stripes were kept in the stomach for approximately 6 minutes (as instructed by the producer), in order to determine the pH values by the etalon comparison method.

Fig. 5 – Stomach Ph measurement by universal indicator.

Sacrificing and histological processing

The animals were sacrificed by diethyl-ether overdose, in the designated intervals, followed by total gastrectomy. The stomach was, then, opened along the pylorus minor curve to the cardia and washed it with physiological solution. The stomach was fixed by 10% formaline for 48 h. The fixed stomach was cut into approximately 2 mm slices, from the previously defined regions (parastomal region and a part of the secretory fundus) as well as from the macroscopically visible pathological change (Figure 6). The histological processing of the fixed slice tissues was performed in the autotechnicon at the Institute for Experimental Medicine of the Faculty of Medicine, the University of Niš, Serbia. Paraffin, cross-sections 4.5 μm thick, were stained by the following methods: 1) classic hematoxylin and eosin (HE) method and 2) hystochemical methods: [a) alcian blue periodic acid schiff (AB-PAS), Ph 2, 5 for mucin, i.e., intestinal and pyloric metaplasia, dysplasia and carcinoma verification, b) Van Gieson – for collagen fiber, i.e., atrophic gastritis and scirrhous variant carcinoma (desmoplastic reaction) verification].

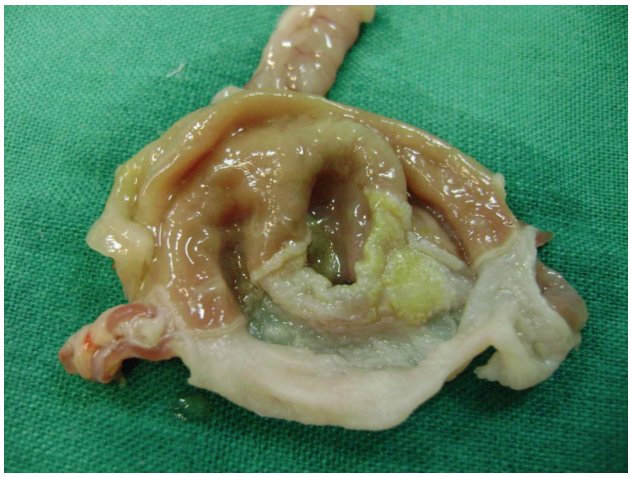


Fig. 6 – Visible changes in Bilroth II gastric resection (B2) group after sacrificing (16th week).

Histopathological classification

Due to a large number of precancerous lesions, histopathological types and sub-types [10], and with considerations of the evident discrepancies between the classification of Japanese pathologists on one side and the European and American pathologists on the other side [11], we opted as our protocol for the classification of identified alterations (lesions) systematization by Katić [12]. Our classification protocol were as follow: 1. normal gastric mucosa – includes a normal pathohistological finding of gastric mucosa, including anastomositis and inflammatory infiltrates, which are considered to be normal responses of the body to the surgical trauma (Figure 7); 2. hyperplasia (hyperplastic gastropathies) a key feature of hyperplastic gastropathy, commonly known as "hypertrophic gastritis" is the hyperplasia of foveolar and/or glandular epithelium, with an absence of inflammatory cells in the mucosa [it includes three types of gastropathy: glandular, foveolar and mixed (Figure 8)]; 3. gastritis – includes acute, chronic, specific, non-specific and cystic gastritis (Figure 9); 4. metaplasia – including intestinal, pyloric, enterocolonic, ciliated and combined metaplasia (Figure 10); 5. dysplasia – mild, medium and severe dysplasia in non-resected stomach [World Health Organization (WHO) classification] and adenomatous, microglandular, cuboid and cystic

dysplasia, characteristic for resected stomach (according to Borchard) (Figure 11); 6. early gastric carcinoma – defined as the carcinoma which is limited to mucosa or sub-mucosa, regardless of the presence of metastases in lymph nodes (Figure 12); 7. carcinoma – including adenocarcinoma, "signet ring cell" carcinoma and anaplastic carcinoma (Figure 13).

This study was conducted on animals. Ethical Committee of the Medical Faculty University of Niš approve this work on meeting in June 2010.

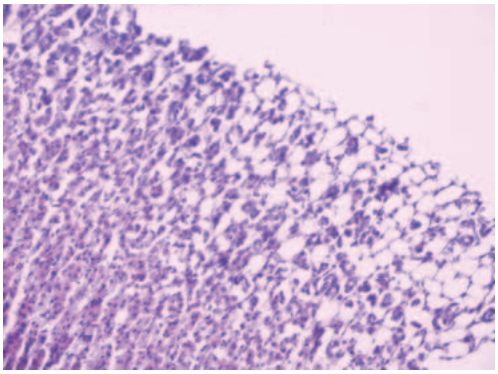


Fig. 7 – Normal gastric mucosa: corpus glands are covered by pariethal and main cells [hematoxylin and eosin (HE) ×400].

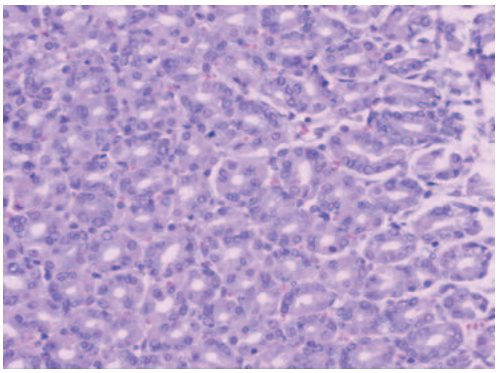


Fig. 8 – Foveolar hyperplasia: transversal section corpus [hematoxylin and eosin (HE) ×200].

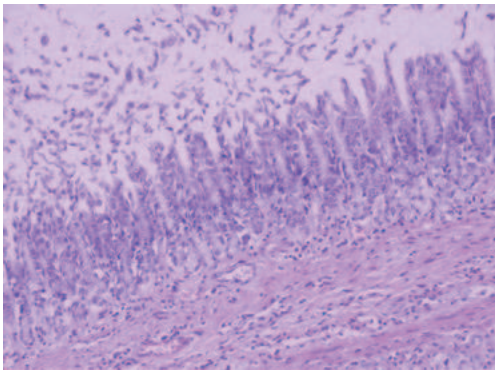


Fig. 9 – Cystic atrophic gastritis with a focal erosion [hematoxylin and eosin (HE) ×100].

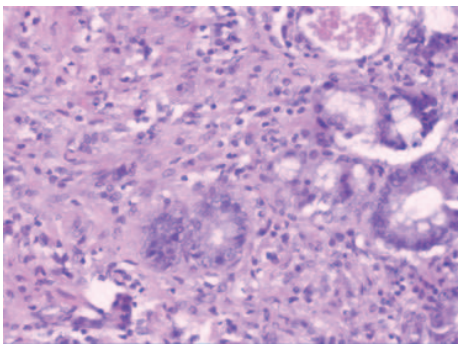


Fig. 10 – Mature intestinal metaplasia (precancerogenous lesion) [hematoxylin and eosin (HE) ×400].

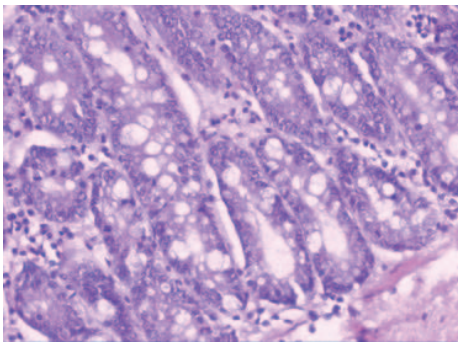


Fig. 11 – Multifocal displasia with intestinal metaplasia [hematoxylin and eosin (HE) ×400].

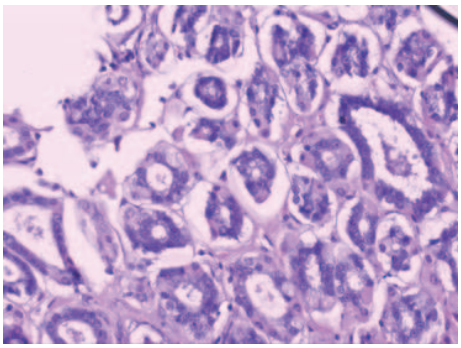


Fig. 12 – Early gastric carcinoma located intramucously, without propria invasion [hematoxylin and eosin (HE) ×400].

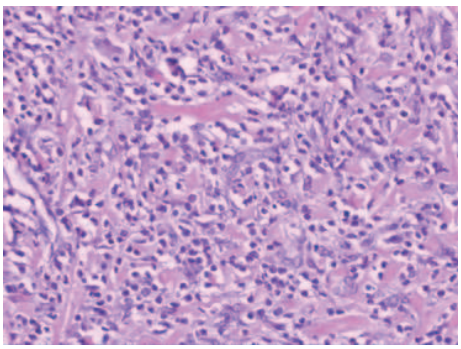


Fig. 13 – Anaplastic carcinoma with a pronounced stroma reaction [hematoxylin and eosin (HE) ×200].

Statistical analysis

The obtained research data were statistically processed by the descriptive method and by making use of appropriate statistical tests. The data base was created in the statistical program Microsoft Office Excel 2007, while the data processing was done by the SPSS program, version 12.0 (Statistical Package for Social Sciences). All statistical tests were considered acceptable if the probability of zero hypothesis was equal or lower than 5%. The following statistical test were used for data processing: χ^2-test, Fisher-Freeman-Halton test (Fisher's exact test), monofactorial analysis of variance (ANOVA) with correction by Brown-Forsythe and Tukey HSD test.

Results

The study was completed with 3 groups of 30 experimental animals each (90 rats), as plan in the design of the study. A total of 12 rats were replaced in the group B2 while 13 animals were replaced in the RY group. No replacement was required in the group C (Figure 1). The replaced animals died or had to be sacrificed prematurely, in the cases of presumed bad outcome. The replacement or lethal outcome occurred due to dehiscence of anastomosis, surgical wound infection, autophagy (laparotomic sutures eaten by the rats themselves), heterophagy (*viscera* consumed by other rats), or for some other unknown reason.

The determined gastric acid levels provided indirect information on the degree of DGR. The pH values were measured after the weeks 8, 16 and 24, and included the following values: the group B2: 4.8 ± 0.5, 5.4 ± 0.7 i 5.4 ± 0.7, the group RY: 3.9 ± 0.2, 4.2 ± 0.6 i 4.1 ± 0.5 and the group C: 3.8 ± 0.5, 4 ± 0.4 i 4.1 ± 0.5, respectively. The mean pH value in the B2 was found to be statistically significantly different from the same finding in the groups RY and C, while no significant difference was found between the RY and C groups, at any measurement point. Unifactor variance analysis confirmed a statistically significant difference among the experimental groups in the period after 8 weeks: $p < 0.01$ (F 2.12 = 10.11, $p = 0.003$), in the period after 16 weeks: $p < 0.001$ (F 2.27 = 17.2, $p < 0.001$) and in the period after 24 weeks: $p < 0.001$ (F 2.42 = 30.04, $p < 0.001$).

The statistical analysis of histopathological specimens taken after 8 weeks, in correlation with MNNG influence duration in the B2 group, showed a hyperplasia of gastric mucosa in 40%, while gastritis was found in 20% and metaplastic changes in 40% of the samples. At the second timeline, 16 weeks after the experiment, the histopathological findings indicated dysplastic changes of all three degrees (mild, moderate, severe) in 50%, while carcinoma was found in 10% of the samples. Metaplasia were noted in 30% of the samples whereas gastritis was identified in only 10% of the samples. In the animals sacrificed in week 24, the percentage of dysplasia was similar to the findings after 16 weeks (46.67%). The percentage of metaplastic changes was significantly decreased (6.67%), but the finding that was not expected included a twice more frequent incidence of early gastric carcinoma (20%) and even more frequently in the broader anastomosis area and its surroundings (adenocarcinoma, diffuse type signet ring cell carcinoma), or on the anastomosis itself (26.67%) (Table 1). Independence χ^2-test showed a statistically significant correlation between the histopathological findings within the B2 subgroup with administered MNNG during all three observed periods, $\chi^2_{(BM)}$ (10, n = 30) = 17.51, $p = 0.009$. Additional analysis showed a statistically significant correlation among groups observed for 8 and 24 weeks, $\chi^2_{(BM8\text{-}24)}$ (5, n = 20) = 13.05, $p = 0.002$. The findings in the group B2 in observed for 16 weeks, showed no statistical difference, in comparison with those other two groups, $\chi^2_{(BM8\text{-}16)}$ (4, n = 15) = 6.79, $p = 0.114$, $\chi^2_{(BM16\text{-}24)}$ (4, n = 25) = 6.04, $p = 0.16$.

In the RY group, the effects of nitrous compounds on gastric mucosa were examined in the absence of DGR (antireflux surgery), in correlation with time. After 8 weeks**,** hyperplastic changes were predominant in this subgroup (60%), whereas gastritis and metaplasia changes were found equally in 20% each.

Table 1

Comparative analysis of histopathological findings by time

Type of surgery (carcinogen)	Time (weeks)	Hystopathological findings, n (%)							Total
		0	1	2	3	4	5	6	
B2 (MNNG)		8	2 (40.0)	1 (20.0)	2 (40.0)				5 (100.0)
		16		1 (10.0)	3 (30.0)	5 (50.0)	1 (10.0)		10 (100.0)
		24			1 (6.7)	7 (46.7)	3 (20.0)	4 (26.7)	15 (100.0)
RY (MNNG)		8	3 (60.0)	1 (20.0)	1 (20.0)				15 (100.0)
		16	5 (50.0)		1 (10.0)	4 (40.0)			10 (100.0)
		24	2 (13.3)	2 (13.3)	1 (6.7)	10 (66.7)			15 (100.0)
Control (MNNG)		8	3 (60.0)	2 (40.0)					5 (100.0)
		16	3 (30.0)	3 (30.0)		4 (40.0)			10 (100.0)
		24		2 (13.3)	2 (13.3)	10 (66.7)	1 (6.7)		15 (100.0)

0 – normal mucosa, 1 – hyperplasia, 2 – gastritis, 3 – metaplasia, 4 – dysplasia, 5 – early carcinoma, 6 – carcinoma. B2 – Billroth II; RY – Roux-en-Y; MNNG – N-methyl-N-nitrosoguanidine.

After 16 weeks, MNNG, in absence of DGR, already led to the incidence of hyperplasia in 50%, mild dysplasia u 40% and metaplasia in 10%. In the week 24 of the experiments, the percentage of dysplasia, and, consequently, the degree of changes (moderate and severe) was at 66.67%. Thirteen percent showed hyperplasia and gastritis. Metaplasia was prevalent in 6.67%. The absence of early carcinoma and carcinoma was evident (Table 1). χ^2-test indicated a statistically significant correlation between the histopathological findings between RY subgroups with MNNG in all three periods, $\chi^2_{(RM)}$ (6, n = 30) = 10.72, p = 0.044. Additional analysis showed a statistically significant difference among the subgroups observed for 8 and 24 weeks, $\chi^2_{(RM8\text{-}24)}$ (3, n = 20) = 7.9, p = 0.029, while the 16-week subgroup did not show statistically signficantly difference, compared to other two subgroups, $\chi^2_{(RM8\text{-}16)}$ (3, n = 15) = 4.13, p = 0. 282, $\chi^2_{(RM16\text{-}24)}$ (3, n = 25) = 4.72, p = 0. 198.

In the group C, MNNG effects on the normal gastric mucosa were observed and correlated with the timeline of its application. After week 8, the findings included hyperplasia in 60% and gastritis in 40% of the samples. After week 16, mild or moderate dysplasia was found in 40%, while gastritis and hyperplasia were present in 30% of the samples. After 24 weeks, early carcinoma was found in 6.67%, dysplasia in 66.67% and the same percentage of gastritis and metaplasia (13.33%) (Table 1). χ^2-test showed statistically significant correlation between the histopathological finding between the non-operated animal subgroups with administered MNNG, in all three timeline periods, χ^2 (M) (8, n = 30) = 15.53, p = 0.009. Additional analysis showed statistically significant correlation between the histopathological findings between the subgroups observed for 8 and 24 weeks, χ^2 (M8–24) (4, n = 20) = 12.57, p = 0.002, while the 16-week subgroup did not show statistically signficantly difference, compared to other two subgroups, χ^2 (M8-16) (2, n = 15) = 2.67, p = 0.317, χ^2 (M16–24) (4, n = 25) = 7.11, p = 0.075.

Discussion

DGR is defined as the return of duodenal content into the stomach, through incompletent pylorus, i.e., from duodenum and intestines, through anastomosis into the stomach. It is probable that the excessive DGR is related to the carcinogenesis of the upper intestinal tract. The use of proton pump blocker decreased the incidence of gastric and duodenal ulcer and the need for its surgical treatment; however, if the medication treatment yields no results, surgical treatment is recommended [1, 13].

Partial gastrectomy causes an increase of intragastric pH, which, in turn, results in the excessive growth of nitrate-reducing bacteria, as well as atrophy of gastric mucosa, due to reduced gastrin levels and DGR. It is possible that mutagens have already been present, and/or have been formed from enterogastric content. In such a case, intragastric biliary acids would be a good source of amids. N-nitroso-taurocholic and N-nitroso-glycocholic acids are both mutagenic and carcinogenic. However, to date, little is known about the carcinogenic nature of N-nitroso biliary acids [14, 15].

The measurement of gastric juice pH values, in the course of the experiment, showed statistically significant differences in all three observed periods (8, 16 and 24 weeks), while the additional analyses showed that the mean pH values in the group B2 were statistically significantly different from the RY and C groups, in all three measurement periods, while no statistically significant differences were observed between the RY and C groups, at any measured period. The results of this study are conformant with the findings of Gronnier et all [16]. They confirmed that increased pH, due to DGR, was synergic and has a neutralizing effect on gastric acidity levels.

Increased cellular proliferation represents a high risk of carcinogensis. A possible mechanism of carcinogensis in DGR is, therefore, an increased number of cells at risk of repeated influence of carcinogen MNNG after a longer period (multi-shock process), due to chronic stimulation of cell proliferation. Moreover, increased cell proliferation can create clones of the previously affected cells and, thus, increase the possibility for another shock, or several new shocks of the same cell [17].

Gunassekaran et al. [18] suggested that the sum of 4 to 6 genetic events can be sufficient for tumour development and carcinogensis. Wogan et al. [19] added that the continuously increased cell proliferation, caused by DGR, can also accelerate further cell proliferation from the initiated cells, in order to lead to an incidence of carcinoma.

Decreased incidence of dysplastic changes in the B2 group (46.7%), compared to the RY group (66.7%) in our experiment was explained by the finding in the B2 group after 24 weeks, in which the total incidence of early carcinoma and carcinoma amounted to 46.7%, indicating that dysplasia occured more rarely than carcinogensis. The same percentage of dysplasia found in the RY and C group suggests that the absence of DGR has a key role on the occurence and growth of precancerous lesions and carcinoma, regardless of the carcinogen.

Øvrebø et al. [20], concluded that the superficial part of the mucosa, triggered by DGR, i.e., by billiary salts, further supports the penetration of MNNG in the proliferative gastric mucosa part.

Also, other authors argue that increased DGR caused mucosal changes that led to increased exposure to proliferating cell carcinogenesis. This is, largely, the reason for the increased cell proliferation and expansion of the proliferating mucosa part. The superficial part between the epithelial border and the proliferating work creates certain protection against the carcinogens entering from the gastric lumen into the mucosa. Mucous erosion in the antrum, probably represent an increased risk of carcinogenesis, by increasing the probability of cell proliferation and by reducing the density of the mucosa above the proliferative portion [21].

The specific finding in our study is the incidence of such a large percentage of cancer and dysplastic changes in the B2 group (conditionally refluxed) after 24 weeks of the experiment, since the time of DGR and MNNG action is relatively short, compared to other researchers, where the timelines of the experiments were 40, 90 and even 120 weeks [22–26]. These results

suggest that the time of influence of DGR in the presence of MNNG is in direct correlation with the severity of precancerous lesions and that the minimum time of the occurrence of cancer is 20 weeks after the start of the experiment. Our results are not in agreement with most of the authors who argue that the most distinctive changes in the stomach of experimental animals, under the effect of MNNG, occur in the 30th week after the initiation of the experiment [27, 28].

According to many authors, the period up to the 20th week of the experiment brings only the first stadium of the pathological changes. The second stadium, according to these authors, includes the period between experimental week 20 and 30 which is characterised by the occurrence of adenomatous hyperplasia with excessive glandular proliferation and mild atypia. Hyperplasia can be directed to the upper or lower part, including the penetration into the mucosa. The third stadium of the experiment is the period after week 30 of MNNG application, which is followed by the occurrence of adenocarcinoma [28]. It is important to emphasise that this group of authors applied carcinogen to the healthy animals, without surgically-induced DGR [29].

Characteristic for our experiment is, also, the occurence of adenomatous hyperplasia (40%), already after week 8 of MNNG application in the group B2 while 50% of dysplasia was already identified after week 16. It is assumed that the presence of the experimentally induced DGR and the preparation of gastric mucosa for its effects has influenced the quicker incidence of more severe precancerous lesions and, even, the occurrence of carcinoma.

Similar to our results, Mihailović [30] claims that as early as in the week 5 of the experiment, erosion with regenerative hyperplasia was observed, with an extension of the generative zone, cystic glands alterations and reduction of neutral zone and mucine. After week 24 of our experiment, there was a considerable incidence not only of adenocarcinoma, which is a characteristic histopathological finding in MNNG application, but also the appearance of diffuse form of signet ring cell carcinoma type as well as anaplastic forms. This finding is in accordance with Pritchard and Przemeck [28], who concluded that all types of cancer that exist in people can also occur in rats, except the mucoid type.

A considerable number of authors also reported the occurrence of metastases in the liver, lymph nodes and lungs of experimentally-induced carcinomas, after 30 weeks of the experiment [27, 30, 31]. The findings in our experiments can be, perhaps, explained by the fact that, in this experiment, MNNG added at a dose of 100 mg per liter of water [32, 33]. While some authors used the dose of 83 mg/L of drinking water in their studies. On the other hand, there are authors who state that with the increasing concentration of MNNG in drinking water of experimental animals, the incidence of gastric cancer remains unchanged, but the incidence of the small intestine carcinoma is increased [33].

Kobayasi et al. [34] examined the impact of DGR onto 4 groups of Wistar rats undergoing gastrectomy B2, B2 gastrectomy with conversion to Roux-en-Y after 24 weeks, B2 gastrectomy with conversion to Roux-en-Y after 36 weeks and the basic gastrectomy, Roux-en-Y reconstruction. The histological criteria selected in this experiment were similar to ours, with regard to the degree of cellular atypia for carcinoma diagnosis, lesions classified as hyperplasia or adenomatous hyperplasia in the application of carcinogens. These authors concluded, on the basis of histochemical assays, that the proliferative lesions were actually the phenotypical gastric cells, while the malignant lesions were actually the phenotypic intestinal cells. What is more important for our research, they concluded that proliferative lesions did not advance into carcinoma and that their incidence decreases if DGR was interrupted by conversion to Roux-en-Y. Rodrigues et al. [35] investigated the effect of DGR in 3 experimental groups of Wistar rats: G1 (control), G2 (subjected to GEA and, two weeks later, to ligature afferent loop), and G3 (GEA). After 36 weeks, the transit was restored by latero-lateral anastomosis between the afferent and efferent loops. The authors concluded that after 54 weeks the reflux through the pylorus favors the development of predominantly benign (precancerous) proliferative lesions in the gastric mucosa. The termination of reflux caused an inhibitory effect on the growth of these lesions, confirming the benign-reversibility of these lesions. Neoplastic lesions were rare in this experimental model.

Our findings lead to the conclusion that the presence of experimentally induced DGR in the group B2 led to the emergence of more serious (irreversible) histopathological changes, in terms of precancerous lesions and even the appearance of early gastric cancer and the rest of the stomach [36].

Pyloric sphincter destruction and, consequently, the loss of pyloric function, is related to the regurgitation of duodenal content. DGR contributes to the development of malignant processes. A high level of DGR directly related to the incidence of esophageal carcinoma and gastric stump carcinoma. Therefore, surgical procedures which prevent or decrease this reflux are highly important for ulcer prevention and treatment in human medicine [37]. In the light of these findings, the recommended surgical procedures are those that induce lower reflux, such as jejunal interposition, or Roux-en-Y anastomosis.

Conclusion

The sole effects of MNNG on gastric mucosa without DGR presence cause a mild degree of precancerogenous lesions. However, direct contacts of gastric mucosa with MNNG, in the presence of DGR induce the formation of precancerogenous lesions, by decreasing the percentage of reversible changes and increasing the percentage of irreversible lesions and carcinoma, over time. The duration of exposure to DGR in the presence MNNG is in direct correlation with the severity of precancerous lesions. A lack of statistically significant correlation between the RY and C group, strongly confirms the presented conclusions and endorses the protective role of Roux-en-Y procedure.

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Disclosures

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Notice

This experiment represents a different aspect from our previous research conducted at the Center for Biomedical Research, Faculty of Medicine, the University of Niš.

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Analysis of inpatient costs in patients with knee osteoarthritis treated by implantation of total condylar knee endoprosthesis

Analiza troškova bolničkog lečenja obolelih od gonartroze implantacijom totalkondilarne endoproteze kolena

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Abstract

Background/Aim. Total knee replacement is an elective and high cost surgical procedure which is performed more frequently as a result of increasing prevalence of knee osteoarthritis. The aim of this study was to provide insight into the value and structure of inpatient costs associated with total knee replacement in Serbia. **Methods.** This study was conducted as an in-depth, bottom-up, retrospective, case series analysis of services consumption patterns and costs associated with inpatient treatment of patients with knee osteoarthritis by implantation of primary total condylar knee endoprosthesis from perspective of the national Republic Health Insurance Fund. We obtained data on 97 patients treated with primary unilateral or bilateral total knee replacement in 2014 at the Clinic for Orthopaedic Surgery and Traumatology of the Military Medical Academy in Belgrade, a tertiary health care university hospital. **Results.** Mean age of entire study sample was 67.89 years. Majority of patients (60 patients; 61.9%) had unilateral implantation of total condylar knee endoprosthesis. Bilateral implantation was performed in 37 (38.1%) patients. Mean total inpatient cost per patient for both unilateral and bilateral implantation of total condylar knee endoprosthesis was EUR 2,709.1, ranging from EUR 1,685.2 to EUR 5,356.6. Mean total inpatient cost per patient was EUR 2,093.8 for unilateral implantation and EUR 3,706.8 for bilateral implantation. Two major cost drivers were surgery specific material and surgery. Cost of implants was the highest single cost driver in all observed groups of patients. **Conclusion.** Our findings imply that inpatient costs associated with implantation of primary total condylar knee endoprosthesis are substantial. It seems that the most important cost drivers are surgery and surgery specific material, with implants being the highest single cost driver. Further research should be focused on analyzing factors that influence these costs in order to develop effective strategies which could contribute to substantial savings in the future.

Key words:
arthroplasty, replacement, knee; inpatients; costs and cost analyses; cost control; knee prosthesis; serbia.

Apstrakt

Uvod/Cilj. Totalna artroplastika kolena je elektivna i skupa procedura koja se u današnje vreme sprovodi sve češće kao posledica rastuće prevalencije gonartroze. Cilj ovog rada bio je analiza vrednosti i strukture bolničkih troškova lečenja obolelih od gonartroze implantacijom totalkondilarne endoproteze kolena. **Metode.** Ova studija je sprovedena kao opservaciona retrospektivna studija troškova zasnovana na pristupu „od dna prema vrhu“. Istraživanu populaciju činilo je 97 bolesnika sa gonartrozom kojima je tokom 2014. godine urađena primarna unilateralna ili bilateralna implantacija totalkondilarne endoproteze kolena na Klinici za ortopedsku hirurgiju i traumatologiju Vojnomedicinske akademije u Beogradu. Bolnički troškovi su izračunati iz perspektive Republičkog fonda za zdravstveno osiguranje, na osnovu analize baze podataka nastale na osnovu anonimizirane fakturisane medicinske dokumentacije na otpustu. **Rezultati.** Prosečna starost ispitanika bila je 67,89 godina. Većini bolesnika (njih 60, tj. 61,9%) je izvršena unilateralna implantacija totalkondilarne endoproteze kolena. Bilateralna implantacija je izvedena kod 37 bolesnika (38,1%). Prosečan ukupni bolnički trošak po bolesniku za zajedno posmatrane unilateralnu i bilateralnu implantaciju iznosio je 2.709,1 € (opseg: 1.685,2–5.356,6 €). Prosečan ukupni bolnički trošak po bolesniku iznosio je 2.093,8 € za unilateralnu implantaciju, dok je za bilateralnu implantaciju iznosio 3.706,8 €. Dve kategorije sa najvećim troškovima bili su troškovi materijala korišćenog tokom operacije i troškovi same operacije.

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Troškovi endoproteze bili su pojedinačno najveći u odnosu na ostale podkategorije troškova. **Zaključak.** Rezultati ove studije ukazuju na to da su bolnički troškovi implantacije primarne totalkondilarne endoproteze kolena značajni i da najveće kategorije troškova čine upravo troškovi same operacije i materijala korišćenog tokom operacije, pri čemu su pojedinačno najveći troškovi endoproteze. Buduća istraživanja bi mogla biti usmerena na analizu faktora koji utiču na ove troškove, kako bi se razvile efikasne strategije za uštedu u budućnosti.

Ključne reči:
artroplastika kolena; hospitalizacija; cene i analize cena; cene, kontrola; koleno, proteza; srbija.

Introduction

Assessment of economic implications of orthopaedic surgeries is gaining more attention as musculoskeletal disorders demanding such procedures are on the rise [1]. Total knee replacement (also referred to as total knee arthroplasty) is an elective, expensive, but cost-effective surgical procedure which is being performed more frequently as a result of increasing prevalence of knee osteoarthritis (gonarthrosis) [2–7]. Osteoarthritis is a degenerative disease of cartilage and surrounding tissue associated with joint pain, stiffness and limitation of movement which affects about 10% of persons over the age of 60 years [8–11]. Knee replacement involving implantation of total condylar endoprosthesis is indicated when conservative treatment can not reduce knee joint pain and dysfunction and is associated with a substantial improvement in quality of life and pain relief in patients with knee osteoarthritis [5, 6, 12]. This procedure involves altering the articular surfaces in a way that enables replacement of total damaged knee joint with a prosthetic implant [4, 5]. Total condylar knee endoprosthesis resurfaces all three compartments of the knee (lateral, medial, and patellofemoral) [13].

The mean utilization rate of knee replacement in Organisation for Economic Co-operation and Development (OECD) countries was 150 procedures per 100,000 people in 2011 [14]. The number of performed total knee replacements has been increasing worldwide as rise in the prevalence of osteoarthritis follows the pace of acceleration of global population ageing and rising trends of obesity [12, 15–24]. The recently published Global Burden of Disease, Injuries and Risk Factors Study noted that disability-adjusted-life-years (DALYs) attributable to high body mass index (BMI) increased the most among the top five risk factors from 1990 to 2015 [22]. The demand for primary total knee replacements is projected to grow to 3.48 million procedures annually in the United States by 2030 [20]. However, accessibility to this procedure is inconsistent across the world and is affected by high cost and limited number of skilled personnel who can perform it [25]. Patients seeking care in publicly-funded institutions frequently spend weeks or months on the waiting list for provision of this surgery unless they are able to pay for private care [26]. For example, the mean length of time spent on waiting for this procedure within the Veterans Affairs Connecticut Healthcare System in the United States can be as long as two years [27]. These all have negative impact on patients as they experience great pain and suffer functional limitations while they await surgery [4].

The costs associated with total knee replacement are becoming increasing concern worldwide because they put a significant financial burden on most healthcare systems [12, 28]. This procedure was associated with one of the most noticeable increase for inpatient costs among all payer types in the United States [29]. Aggregate inpatient costs of total knee replacement reached United States dolar (USD) 9.2 billion in 2007, and they grew by 27.5% between 2004 and 2007 [30]. Previous studies reported that cost of implants, hospital room and operating room segment of care may account over 75% of inpatient costs [31–34]. However, the cost of implants is usually the highest and it can even reach up to 87% of overall inpatient cost [6, 29, 35]. The inpatient costs associated with total knee replacement varies across different regions. Costs reported in European studies vary from EUR 4,103 in public hospitals in Portugal [36] to EUR 15,358 in Italy [37]. A report published in the United States in 2015 noted substantial variation of hospital charges for knee replacement procedures ranging from USD 11,317 in Alabama to USD 69,654 in New York [38]. Such variations in cost of care are one of the main reasons for increasing number of patients from developed countries who travel to hospitals in emerging market countries like Taiwan, Thailand, India and Singapore where cost of this procedure can be 8 to 10 times less expensive than in the United States due to low labor and maintenance cost [39–42].

Nearly 20,000 patients were on the waiting lists for knee and hip replacement in Serbia in 2014 with reported increase of 20% compared to previous year [43]. Average length of time that patients spent waiting for these procedure was 311 days in 2014, which was 25 days less than was reported in 2013 [43]. It is likely to expect that demand for these procedures will continue to rise in Serbia with continuing population ageing [44]. The core fund in Serbia in charge for most inpatient care expenditures is Republic Health Insurance Fund which is a non-profit state owned institution subject to budget shortages [44–46]. Consequently, there is a need to assess major inpatient cost drivers in order to make more efficient health policy programs. Since health expenditures related to total knee replacement are substantial, it is crucial to understand cost of care provided across various settings in order to provide baseline data for pharmacoeconomic analyses in the future.

So far, there is a substantial knowledge gap on actual inpatient cost of total knee replacement in Serbia. Therefore, the aim of this study was to provide insight into the value and structure of inpatient costs associated with total knee replacement by implantation of total condylar knee endoprosthesis in Serbia.

Methods

Study design and patient selection

This study was conducted as an in-depth, bottom-up, retrospective, case series analysis of services consumption patterns and costs associated with inpatient treatment of patients with knee osteoarthritis by implantation of primary total condylar knee endoprosthesis from the perspective of the third party payer, i.e., from the national Republic Health Insurance Fund. Indirect cost and out-of-pocket patient's expenditure, as well as costs in settings other than inpatient, remained out of scope of this study. We obtained data on patients who were treated with primary unilateral or bilateral total knee replacement in 2014 at the Clinic for Orthopaedic Surgery and Traumatology of the Military Medical Academy in Belgrade, a tertiary health care university hospital. The source of data was an anonymised database consisting of electronic hospital discharge invoices. In total, 97 complete patient files were analysed. Data on age, gender and length of hospitalisation were also collected.

Structure and pricing of the used recourses

The official Republic Health Insurance Fund pricelist was applied at the time of the service provision. Average middle exchange rate for Euro (EUR) given by the National Bank of Serbia for 2014 was used to convert costs originally reported in the national currency Serbian Dinar (RSD): EUR 1 = RSD 117.2478 [47].

For the present study, total inpatient costs associated with the implantation of primary total condylar knee endoprosthesis were collected. Costs were separated into the following categories: general surgery related medical care (hospital admission day and consumables, rehabilitation services, and all other services such as social care, transport, counseling, epidemiological measures), surgery (surgical intervention and anesthesia), imaging diagnostics [classical imaging diagnostics – Roentgen, contrasts, films and consumables intended for imaging diagnostics services provision, computed tomography (CT) and ultrasound imaging diagnostics], surgery specific materials (implants, dressing material, consumables for surgical intervention and other consumables such as gloves, braunilas, tubes), laboratory analysis (general biochemistry and hematology, coagulation status analysis, microbiology related lab), medicines (parenteral and enteral nutritive solutions and systems, blood and its derivatives – transfusions, antibiotics, antimicotics, antiviral and antiprotozoal drugs, analgesics, thromboprophylactic medicines and all other drugs).

Statistical analysis

Categorical variables were presented as frequencies of certain categories, while continuous variables were summarized as mean and standard deviation, as well as median and minimum and maximum values. Patients were divided into two groups based on the type of implantation: unilateral (implantation performed on only one knee) and bilateral (implantation performed on both knees). The differences in continuous variables were assessed by Mann Whitney U test because data were not normally distributed. The χ^2 test was used to assess differences in categorical variables. The differences were considered significant if probability of null hypothesis was less than 0.05. Costs are presented as mean and median cost per patient including standard deviation, minimum and maximum values. All mean and median cost values refer only to those patients that have actually used a particular service, as some services were used by few patients. Share of cost of certain category in total inpatient cost was calculated and presented graphically. Statistical analyses were performed using Microsoft Office Excel 2007® and IBM SPSS® Statistics for Windows, Version 20.0 (IBM Corp, Armonk, NY, USA).

Results

Study sample consisted of 97 patients. Baseline characteristics of study sample are shown in Table 1. Mean age of entire study sample was 67.89 years, ranging from 41 to 83 years. There were 40 (41.2%) female patients and 57 (58.8%) male patients. Majority of patients (60, 61.9% patients) had unilateral implantation of total condylar knee endoprosthesis. Bilateral implantation was performed in 37 (38.1%) patients. There was no statistical difference in the mean age and mean duration of hospitalisation of patients who had unilateral implantation compared to patients who had bilateral implantation. However, fewer women had bilateral implantation compared to men.

Results of descriptive statistical analysis of cost domains are presented in Table 2.

Table 1

Baseline characteristics of study sample

Variable	Unilateral implantation (n = 60)	Bilateral implantation (n = 37)	Test value and significance of null hypothesis (*p*)	All patients (n = 97)
Age (years)	67.90 ± 8.77 [70 (41–83)]	67.86 ± 6.92 [67 (50–82)]	U = 1049.0; *p* = 0.650	67.89 ± 8.08 [69 (41–83)]
Gender				
female	30 (50.0)	10 (27.0)	χ^2 = 4.082; *p* = 0.043*	40 (41.2)
male	30 (50.0)	27 (73.0)		57 (58.8)
Mean duration of hospitalization (days)	8.05 ± 3.14 [7.5 (2–21)]	9.05 ± 4.99 [7 (2–26)]	U = 1051.0; *p* = 0.658	8.43 ± 3.95 [7 (2–26)]

Results are presented as mean ± SD [median (minimum-maximum)], or n (%); *significant difference.

Table 2

Cost domains of inpatient treatment of patients with knee osteoarthritis by implantation of total condylar knee endoprosthesis

Domain	Mean cost per patient ± standard deviation [Median cost per patient (minimum–maximum)][#] (EUR)		
	Unilateral implantation (n = 60)	Bilateral implantation (n = 37)	All patients (n = 97)
General Surgery Related Care	137.6 ± 65.2 [119.3 (39.5–350.6)]	157.8 ± 87.0 [145.0 (36.8–418.1)]	145.3 ± 74,5 [131.3 (36.8–418.1)]
hospital admission day and consumables	120.4 ± 56.3 [105.4 (26.4–329.0)]	135.4 ± 87.2 [118.6 (26.4–418.1)]	126.2 ± 69.7 [105.4 (26.4–418.1)]
rehabilitation services	27.3 ± 20.9 [21.1 (6.0–95.7)]	35.7 ± 19.7 [32.9 (5.8–83.7)]	30.5 ± 20.7 [25.7 (5.8–95.7)]
all other services (social care, transport, counseling, epidemiological measures...)	21.8 ± 10.3 [21.8 (14.5–29.1)]	N/A ± N/A [39.6 (39.6–39.6)]	27.7 ± 12.6 [29.1 (14.5–39.6)]
Surgery	356.5 ± 39.2 [378.5 (270.3–431.3)]	525.3 ± 44.6 [544.7 (419.2–634.9)]	420.9 ± 92.1 [391.0 (270.3–634.9)]
surgical interventions	216.1 ± 0.0 [216.1 (216.1–216.1)]	379.9 ± 0.0 [379.9 (379.9–379.9)]	278.6 ± 80.0 [216.1 (216.1–379.8)]
anesthesia	140.4 ± 39.2 [162.4 (54.1–215.2)]	145.4 ± 44.6 [164.8 (39.3–255.1)]	142.3 ± 41.2 [162.9 (39.3–255.1)]
Imaging Diagnostics	11.0 ± 9.6 [8.3 (5.5–68.7)]	16.9 ± 7.7 [16.5 (10.9–58.0)]	13.3 ± 9.3 [10.3 (5.5–68.7)]
classical imaging diagnostics – röntgen	9.4 ± 3.1 [8.3 (5.5–29.4)]	15.1 ± 2.1 [15.7 (10.9–18.6)]	11.6 ± 4.0 [10.3 (5.5–29.4)]
contrasts, films and consumables intended for imaging diagnostics services provision	N/A ± N/A [2.8 (2.8–2.8)]	N/A ± N/A [2.8 (2.8–2.8)]	2.8 ± N/A [2.8 (2.8–2.8)]
ct imaging diagnostics	N/A	N/A ± N/A [44.3 (44.3–44.3)]	N/A ± N/A [44.3 (44.3–44.3)]
ultrasound imaging diagnostics	48.5 ± 12.9 [48.5 (39.4–57.7)]	N/A ± N/A [18.6 (18.6–18.6)]	38.5 ± 19.5 [39.4 (18.6–57.7)]
Surgery specific materials	1,461.7 ± 232.0 [1,409.9 (1,084.0–2,683.9)]	2,808.1 ± 397.8 [2,795.1 (1,444.6–4,406.7)]	1,975.2 ± 724.3 [1,465.8 (1,084.0–4,406.7)]
implants	1,273.7 ± 223.8 [1.223.8 (917.0–2,473.4)]	2,525.7 ± 383.3 [2,468.9 (1,224.7–4,049.3)]	1,751.2 ± 677.9 [1,270.5 (917.0–4,049.3)]
dressing material	75.7 ± 31.7 [73.2 (37.0–293.9)]	119.6 ± 22.5 [120.6 (62.2–153.6)]	92.5 ± 35.6 [80.3 (37.0–293.9)]
consumables for surgical intervention (sutures, staplers, drains, antiseptics, etc.)	74.1 ± 50.7 [64.3 (53.4–451.4)]	121.6 ± 30.1 [128.6 (38.3–145.8)]	92.2 ± 49.6 [73.1 (38.3–451.4)]
other consumables (gloves, braunilas, tubes, etc.)	38.2 ± 17.1 [30.3 (5.8–73.3)]	41.1 ± 15.6 [37.5 (7.4–67.4)]	39.3 ± 16.5 [34.1 (5.8–73.3)]
Laboratory Analysis	20.5 ± 11.3 [17.8 (3.9–48.7)]	26.6 ± 19.9 [23.7 (2.6–94.4)]	22.8 ± 15.3 [19.8 (2.6–94.4)]
general biochemistry and hemathology	19.7 ± 11.0 [17.4 (3.9–48.7)]	25.7 ± 17.5 [23.7 (2.6–80.5)]	21.9 ± 14.0 [19.3 (2.6–80.5)]
coagulation status analysis	6.1 ± 5.9 [2.8 (1.8–14.0)]	N/A ± N/A [29.3 (29.3–29.3)]	9.4 ± 10.3 [3.7 (1.8–29.3)]
microbiology related lab	N/A ± N/A [7.0 (7.0–.7.0)]	N/A ± N/A [1.3 (1.3–1.3)]	4.2 ± 4.0 [4.2 (1.3–7.0)]
Medicines	108.4 ± 45.1 [97.2 (41.1–223.7)]	172.9 ± 64.0 [162.6 (63.1–351.0)]	133.2 ± 61.6 [120.3 (41.1–351.0)]
parenteral and enteral nutritive solutions and systems	16.3 ± 10.2 [14.7 (4.6–76.1)]	20.9 ± 18.1 [17.3 (2.7–119.8)]	18.1 ± 13.9 [15.6 (2.7–119.8)]
blood and its derivatives – transfusions	40.4 ± 25.6 [33.1(15.8–124.7)]	66.9 ± 31.8 [57.2 (19.5–160.2)]	54.5 ± 31.7 [52.3 (15.8–160.2)]
antibiotics, antimicotics, antiviral and antiprotozoal drugs	20.5 ± 9.9 [21.0 (4.3–47.8)]	24.3 ± 15.5 [21.7 (4.5–71.5)]	22.0 ± 12.4 [21.5 (4.3–71.5)]
analgesics	7.1 ± 11.1 [3.0 (0.6–59.7)]	9.1 ± 12.6 [3.5 (0.6–50.2)]	7.9 ± 11.7 [3.33 (0.6–59.7)]
thromboprophilactic medicines	42.7 ± 21.9 [36.0 (9.5–113.7)]	57.4 ± 27.2 [52.2 (1.7–124.2)]	48.3 ± 25.0 [42.9 (1.7–124.2)]
all other drugs	3.2 ± 2.9 [2.6 (0.3–13.2)]	4.1 ± 4.2 [3.2 (0.3–19.0)]	3.6 ± 3.4 [3.0 (0.3–19.0)]
Total inpatient cost	2,093.8 ± 253.0 [2,029.6 (1,685.2–3,358.2)]	3,706.8 ± 439.9 [3,669.2 (2,195.3–5,356.6)]	2,709.1 ± 855.7 [2,195,3 (1,685.2–5,356.6)]

#All values refer only to those patients that have actually used a particular service; N/A = not available; n = number; EUR = Euro.

Mean total inpatient cost per patient for both unilateral and bilateral implantation of total condylar knee endoprosthesis was EUR 2,709.1, ranging from EUR 1,685.2 to EUR 5,356.6. Mean total inpatient cost per patient was EUR 2,093.8 (range: 1,685.2–3,358.2) for unilateral implantation and EUR 3,706.8 (range: 2,195.3–5,356.6) for bilateral implantation.

Structure and percentage ratio of mean costs per patient are shown in Figure 1. Two major cost drivers were surgery specific material and surgery. Cost of implants was the highest single cost driver in all observed groups of patients (Table 2, Figure 2). The cost associated with imaging diagnostic services was the lowest (Table 2, Figure 1).

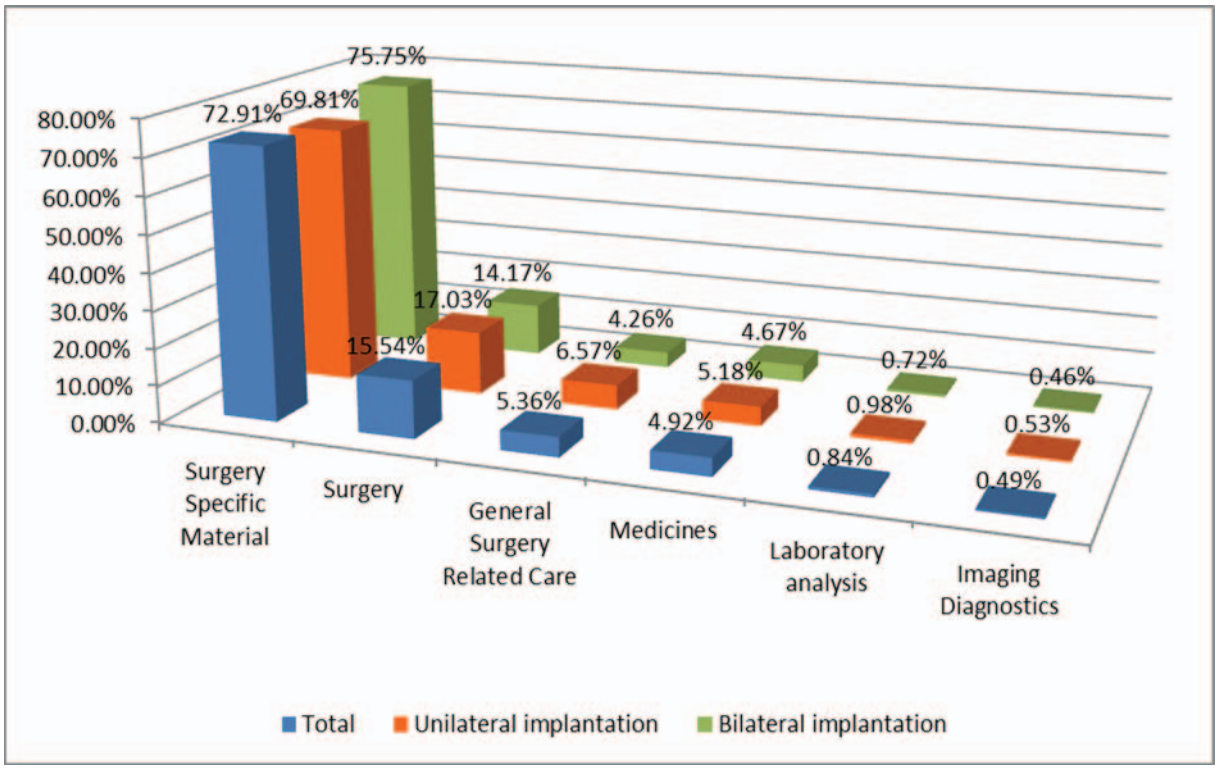

Fig. 1 – Structure and percentage ratio of mean costs per patient.

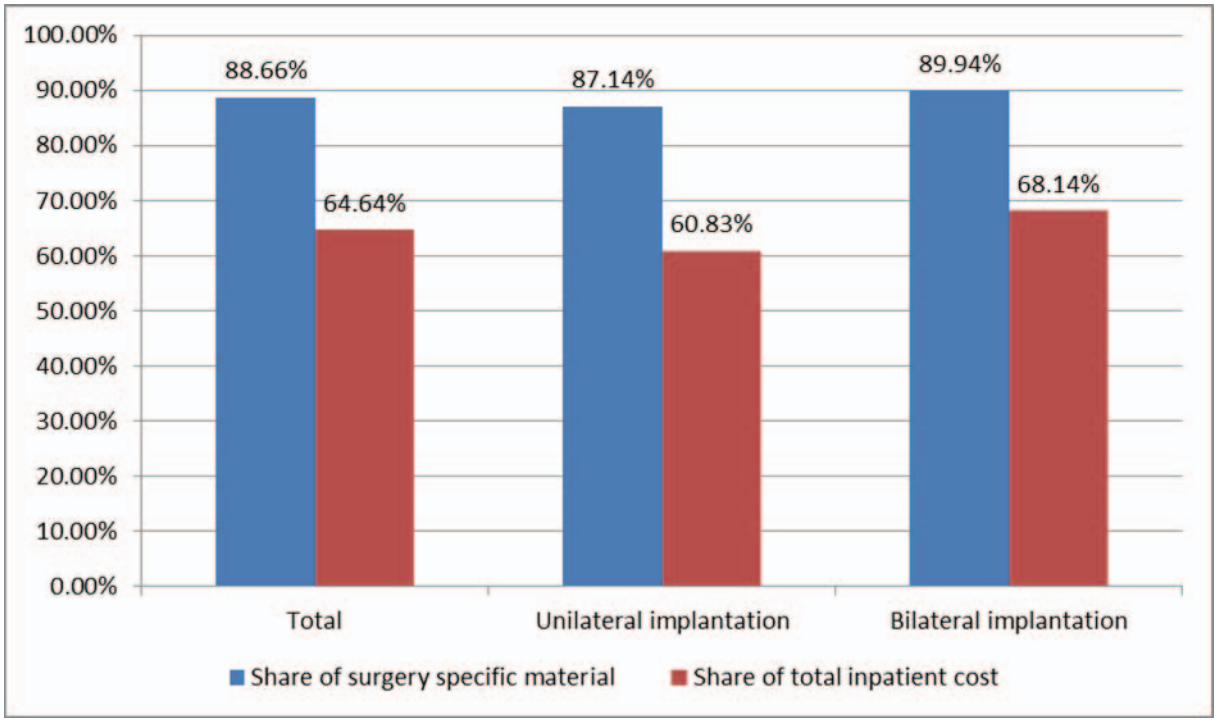

Fig. 2 – Share of implant cost in cost of surgery specific material and total inpatient cost.

Discussion

The value of previously reported mean inpatient cost of primary total knee replacement differs widely from country to country. When comparing results of different studies, it should be kept in mind that methods, data sources (e.g., hospital accounting system, reimbursement rates and charges, etc.) and categorization of costs may vary significantly. Mean total inpatient cost of total knee replacement estimated in our study is comparable with the mean inpatient cost in public (EUR 4,103) and private (EUR 5,226) hospitals in Portugal [36]. Studies conducted in Spain [48], France [49], Italy [37, 50] and United Kingdom [7] reported somewhat higher values. In France, mean hospital cost was EUR 7,404 [49]. In Spain, mean total inpatient cost was EUR 7,645 [48]. In Italy, mean hospital cost per knee and hip replacement procedure was EUR 6,952, whereas the mean cost of the surgical procedure was EUR 3,798, while that of the inpatient care was EUR 2,924 [50]. Another Italian study reported that average cost per patient (including hospital, rehabilitation and complication cost) was EUR 15,358 [37]. In the United Kingdom, on average each admission costs British Pound (GBP) 6,363 according to the analysis of patient level data and services valued at 2007–2008 prices from the Knee Arthroplasty Trial [7]. In China, the total cost for unilateral procedure was USD 8,173.25, whereas for bilateral procedure it was USD 14,257.64 in 2010 [51]. In Taiwan, mean total medical cost for unilateral procedure was USD 3,919 [52], whereas median cost of simultaneous bilateral procedure was USD 6,994.4 [53]. Early studies conducted in the United States reported that mean total inpatient cost for unilateral total knee replacement was USD 10,081 [54] during 1991–1994, USD 12,561 during 1991–1992 [55] and USD 15,673 during 2000–2008 [56]. These costs can be even higher if patients have concomitant diagnosis of depression and anxiety [57] or venous thromboembolism and bleeding [58]. In a cohort of Australian patients, mean inpatient cost of knee replacement in the first 30 days postoperatively was Australian dolar (AUD) 21,006 for a period 2011–2012 [19]. Estimated mean inpatient cost can range from Canadian dolar (CAD) 12,500 [59] to CAD 14,758 [60] in Canada. Higher reported costs in other countries may be attributed to the differences in the local healthcare systems, clinical, coding, administrative and costing practices in individual countries as well as patient demographics and surgeon practices.

As costs related to total knee replacement are substantial, it is important to develop strategies for their control and reduction. Orthopaedic surgeons should be the first and the most important patient advocates who will carefully evaluate hospital cost saving programs and ensure their enactment do not compromise treatment outcomes [6]. Several reports have highlighted that safe cost reduction can be achieved through the knee implant standardization process to reduce variation in implant selection and implementation of the clinical pathway programs which coordinate and standardize the activities of the physicians, nurses and other staff involved in providing care to the patients [6, 32, 61–64]. One study reported that substantial cost savings can be achieved when one versus two packets of bone cement is used in combination with a hand mixing technique with no difference in clinical outcomes [28]. There are also proposals to increase number of total knee replacements in an outpatient setting [65]. However, it is more complicated to monitor recovery process as patients do not stay overnight under supervision and there is a lack of high quality evidence that directly compares outcomes of outpatient and inpatient orthopaedic procedures [65]. It has also been shown that shortening of waiting times for surgery is cost-effective and may also be cost saving [66].

Growing body of literature has shown that the cost of implants contribute significantly to total cost of joint replacement procedures [6, 29, 51, 67]. Large share of implant costs (64.64%) in total inpatient cost is an important finding of our study that confirms previous observations. Robinson et al. [35] reported that share of implant cost can vary from 13% to as high as 87% of total inpatient cost. Some other studies reported lower share. For example Portuguese study reported 28%–33% [36] and the US study 29%–40% [54]. Mean implant cost in our study was EUR 1,751.2, which again is similar to the cost reported in Portuguese study (EUR 1,259 – EUR 1,447) [36] and Italian study (EUR 1,850) [50]. Fixed implant cost associated with total knee replacement was CAD 3,060 in Canada [59]. An early US study reported that the average implant cost was USD 3,963 for unilateral procedure and USD 7,428 for bilateral procedure [54]. Variation in share and value of implant cost may be attributable to the patient characteristics and hospital characteristics as well as different categorization of other costs [35]. In addition, factors that may have influence on final cost of the implants are costs of design, research, development and manufacture as well as the cost of support staff such as industry representatives [68].

The average prices of hip and knee implants have increased more than 100% over the past decade, although it would be expected to decrease with the increasing number of procedures if orthopaedic implant device companies followed conventional economies-of-scale principles [35, 67]. Proposed strategies for restraining implant cost are volume-discounted vendor contracts, single-price contracts, unilateral price caps, implant standardization programs as well as surgeons cost awareness discussions [6, 31, 32, 64, 69]. Access to information on the prices of devices should be available to orthopaedic surgeons, and there should also be incentives for their participation in cost reductions programs [70]. It has also been shown that innovative implants used for total knee replacement should decrease failure of this procedure by 50%–55%, or more, compared to standard implants to be broadly cost-effective [71]. In addition, the patents on many widely used implants have recently expired and introduction of generic implants has the potential for major cost savings [68]. Companies that put generic implant replicas on the market have been established, but formal independent systems which should evaluate their absolute equivalence are yet to be founded [68]. The new generic implants are similar to the originals by a process of reverse engineering and their equivalence has been assessed in terms of geometry, but the monitoring and their independent evaluation to verify biomechanical compatibility is essential in order to prove that they are as good and safe as the originals [68].

Our findings should be interpreted in light of some potential limitations. Our analysis was restricted to the direct medical costs of procedure in inpatient setting. We did not take into account out-of-pocket patient's expenditure, indirect costs and costs associated with post-discharge period in outpatient setting when rehabilitation and complication costs may be considerable. In addition, hospital discharge invoices to Republic Health Insurance Fund may be partly unreliable in some cases as data entering is usually left to nurses or clerks who may not have sufficient comprehension of this process which can lead to incorrect data entry. Certain fraction of invoices is even disputed by the Republic Health Insurance Fund. For example, in the first half of 2016, the Republic Health Insurance Fund through the control of regularity of invoicing and demands for drug reimbursement noted that the amount of incorrect claims had a value of RSD 8,256,642.95 (about EUR 67,072) [72]. In 2016, estimated expenditure of the Republic Health Insurance Fund on health care was RSD 204.3 billion (about EUR 1.66 billion) [73]. The pattern of services and materials acknowledged by the Republic Health Insurance Fund in some cases may also lead to the differences between what was invoiced and what patients really consumed, so our findings might have underestimated the true cost of some consumed services and materials. Another limitation is rather modest sample size. However as included patients represent entire population of knee osteoarthritis patients, who were treated over a period of entire year in one of the largest university hospitals in Serbia, they certainly provide valuable insight into the value and structure of inpatient cost of total knee replacement in this region.

Conclusion

Our findings imply that inpatient costs associated with implantation of total condylar knee endoprosthesis are substantial. It seems that the most important cost drivers are surgery and surgery specific material, with implants being the highest single cost driver. Further research should be focused on analyzing factors that influence these costs in order to develop effective strategies which could contribute to substantial savings in the future.

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Factors associated with maintenance of human Q fever in Vojvodina, Serbia

Faktori koji doprinose održavanju humane Q groznice u Vojvodini, Srbija

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Abstract

Background/Aim. Q fever occurs worldwide and can be found in humans as well as in domestic mammals. The aim of this study was to describe the epidemiological characteristics of Q fever and evaluate correlation between the Q fever occurrence and weather conditions. **Methods.** We conducted a descriptive epidemiological study using data of the Institute of Public Health of Vojvodina, Scientific Veterinary Institute, Novi Sad, and the Republic Hydrometeorological Service of Serbia, from 2006 to 2015. **Results.** Out of 272 human Q fever cases, 114 (41.9%) patients were aged between 20 and 39 years. Between January and May, a total of 193 (71.0%) cases of disease were registered. In the Central Banat and South Banat, a strong positive correlation between an increase in Q fever detection and an increase of average wind speed (ρ= 0.69719 and ρ=0.62303, respectively) was observed, while a strong negative correlation was determined between the average rainfall with the number of Q fever cases in the Central Banat, the South Banat and Srem district (ρ = -0.78033, ρ = -0.70675 and ρ = -0.70431, respectively). During a 10-year period, a strong positive correlation was found between the human Q fever cases compared to the number of cattle and sheep Q cases in the Srem district (r = 0.7989 and r = 0.7966, respectively). Direct contact with domestic animals was the most frequent route of Q fever transmission in Vojvodina. **Conclusion.** The timely sharing of information between the animal and human health sectors as well as between neighbouring countries is crucial for an appropriate and an early outbreak response, especially during windy and dry months of the year. Additionally, it is essential that people who are exposed to a risk of infection must be permanently educated about reducing the risk of transmission of infection.

Key words:
q fever; zoonoses; disease outbreaks; risk factors; serbia.

Apstrakt

Uvod/Cilj. Kju groznica je rasprostranjena širom sveta, a otkrivena je kako među ljudima, tako i među domaćim sisarima. Cilj ovog istraživanja je bio da se opišu epidemiološke karakteristike Kju groznice i da se proceni povezanost između pojave Kju groznice i klimatskih uslova. **Metode**. Sprovedena je deskriptivna epidemiološka studija upotrebom podataka Instituta za javno zdravlje Vojvodine, Veterinarskog instituta iz Novog Sada i Republičkog hidrometeorološkog zavoda Srbije, u periodu od 2006. do 2015. godine. **Rezultati**. Od ukupno 272 slučaja humane Kju groznice, 114 (41,9%) pacijenata su bili uzrasta od 20 do 39 godina. Od januara do maja registrovano je ukupno 193 (71,0%) slučaja oboljenja. U Srednjem i Južnom Banatu uočena je jaka korelacija pozitivnog smera između porasta broja obolelih od Kju goznice i porasta prosečne jačine vetra (ρ= 0,69719 i ρ=0,62303), dok je jaka korelacija negativnog smera, između prosečnih vrednosti padavina i broja registrovanih slučajeva Kju groznice, registrovana u Srednjem Banatu, Južnom Banatu i Sremu (ρ = -0,78033, ρ = -0,70675 i ρ = -0,70431). Tokom desetogodišnjeg perioda, utvrđena je i jaka korelacija pozitivnog smera između broja obolelih od Kju groznice i broja obolele stoke, odnosno obolelih ovaca u Sremskom okrugu (r = 0,7989 i r = 0,7966). Direktan kontakt sa domaćim životinjama bio je najčešći put prenosa Kju groznice u Vojvodini. **Zaključak**. Pravovremena razmena informacija između sektora za zdravstvenu zaštitu životinja i sektora za zaštitu zdravlja ljudi, kao i između susednih zemalja, od ključnog je značaja za odgovarajući i rani odgovor na epidemijsko javljanje oboljenja, posebno tokom vetrovitih i suvih meseci u godini. Osim toga, za osobe koje su izložene riziku od obolevanja, neophodno je sprovođenje stalne edukacije o smanjenju rizika od prenošenja infekcije.

Ključne reči:
kju groznica; zoonoze; epidemije; faktori rizika; srbija.

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Introduction

Q fever is a zoonosis caused by the gram-negative bacterium *Coxiella burnetii* (*C. burnetii*). Domestic animals are the main source of the infection. Humans are infected mainly through inhalation of aerosolized particles contaminated with *C. burnetii* excreted by an infected animal [1]. The disease is considered as a re-emerging or endemic zoonosis in many countries [2–4], including the Autonomous Province of Vojvodina (Vojvodina), the northern region of Serbia [5].

The disease was registered in many animal species, including mammals, birds and arthropods. In animals, *C. burnetii* infections are generally asymptomatic. However, *C. burnetii* infections in mammals can cause pneumonia, abortions, stillbirths as well as delivery of weak lambs, calves or kids, which are the most frequent clinical signs of the disease [6]. Furthermore, in the majority of cases, abortion occurs at the end of gestation without specific clinical signs. Just before abortion, in the last weeks of pregnancy, massive *C. burnetii* multiplication was detected in placenta, which is the most common source of the infection [7]. Additionally, infected animals shed *C. burnetii* in their feces, urine, milk and birth products. Milk shedding is more frequent and lasts longer in cows and goats than in sheep [6], but sheep shed more and longer in vaginal discharges than goats and can shed bacteria at subsequent pregnancies [8].

The contaminated products form aerosols that can be spread several kilometers by the wind. In contrast with urban areas, where Q fever occurs mainly as sporadic cases, in rural areas diseases usually occur as outbreaks [9].

Q fever is essentially an airborne disease. The predominant mode of spreading the infection occurs after inhalation of aerosols generated from infected placentas, body fluids or contaminated dust resulting from contaminated manure and desiccation of infected placenta and body fluids [10]. Ingestion of raw dairy products is a minor route of transmission. Transmission by direct contact with the skin or mucosal contact with contaminated products and vertical transmission of Q fever are very rare [9, 11, 12]. In humans, about 60% of the Q fever infections are asymptomatic [12]. In accordance with this, and because of the common nonspecific or atypical course of the disease [9], many of human Q fever cases are unrecognized. In patients who develop acute clinical features, Q fever usually presents with fever, chills, and headache (similar to a flu-like disease), or as atypical pneumonia or hepatitis [9, 12]. In about 5% of the cases, disease may become chronic leading to an often fatal endocarditis, chronic fatigue syndrome and repeated abortions [12, 13].

Several studies suggest the role of wind and rainfall in the transmission of the *C. burnetii* between ruminants and from ruminants to humans [14–18].

The aim of this study was to improve our understanding about the epidemiological characteristics of human Q fever in Vojvodina and particularly to determine possible relationships with occurrence of disease and weather conditions as well as to examine the relation between the number of animal and human Q fever cases.

Methods

Study area and population

The Autonomous Province of Vojvodina, with a population of almost 2 million, is located in the northern part of the Republic of Serbia (situated at the crossroads between Central and Southeast Europe). Vojvodina is divided into seven districts and 44 municipalities, and it is bordered by Croatia to the west, the Romania to the east, the Hungary to the north and the Bosnia and Herzegovina to the southwest. It has a multi-ethnic and multi-cultural identity with some 26 ethnic groups and six official languages. Overall, the climate is a moderate continental with a mean maximum temperature in July (the average monthly temperature is 21.4°C) and mean minimum temperature during January (the average monthly temperature is -1.3°C). During the colder part of the year east and southeast wind, koshava, dominates [19].

Collection of data of human Q fever cases

A retrospective, observational study was conducted. Data for this study were obtained from the communicable disease registration of the Centre for Disease Control and Prevention of the Institute for Public Health of Vojvodina (IPH) in the 10-year period (from 2006 to 2015) [20].

Data on human cases were collected as part of the routine system of infectious disease surveillance in Vojvodina. The questionnaire consists of questions that are related to the basic information on socio-demographic characteristics of patients, the date of onset and questions about all clinical signs/symptoms of the disease, hospitalization, and included the data about the potential sources and modes of Q fever transmission. Questionnaires of all registered human Q fever cases were filled by district epidemiologists in six local departments of the Public Health of Vojvodina and by epidemiologists in the Centre for Disease Control and Prevention of IPH.

Laboratory confirmation of human Q fever

All sera samples from the patients with suspicion of Q fever diseases were analysed at the Serbian Reference Laboratory for Q fever, the Center for Microbiology of the Department of Public Health of Zrenjanin. Human Q fever cases were confirmed by enzyme-linked immunosorbent assay (ELISA). Serologic evidence of a positive IgM and/or IgG antibody result to phase II antigen *C. burnetii* was used. Among all of the patients, those results of the first serology tests were equivocal or negative; to obtain a definitive laboratory confirmation of Q fever, paired serum samples at least two weeks apart were performed.

Veterinary data

Veterinary data were obtained from the Scientific Veterinary Institute, Novi Sad. Samples from cattle and sheep were examined during the regular annual reports and monitoring of animal health protection, ordered by the program of measures for each year on the territory of the Republic of Serbia. We provided only veterinary data from the Srem district of Vojvodina.

Laboratory confirmation of animal Q fever

According to the recommendation proposed by the World Organisation for Animal Health (OIE) and Manual of Diagnostic Test and Vaccines for Terrestrial animals (mammals, birds and bees) [21], ELISA method was used for detection of antibodies against *C. burnetii* in blood samples of cattle and sheep.

In addition, for the following purposes such as: identification of populations free from infection, contribution to eradication policies, identification of the prevalence of infection, surveillance and determination of immune status in individual animals or populations post-vaccination, ELISA method was considered as the diagnostic method of choice.

All animal samples were tested in the Scientific Veterinary Institute, Novi Sad.

Meteorological Data

Data on wind speed and rainfall were obtained from the Republic Hydrometeorological Service of Serbia [19].

Monthly average of wind speed and rainfall for the Central Banat, the South Banat and the Srem district were provided. We considered only the strong breeze (≥ 6 Beaufort wind force scale) which is the most common and strongest wind and blows for several consecutive days in Vojvodina.

Data analysis

Through descriptive epidemiological study, data were analysed chronologically, demographically and topographically for the observed period (from 2006 to 2015).

We used the basic statistical indicators, general and specific incidence rates. Incidence rates were calculated using the annual number of registered human cases as a numerator and the number of inhabitants in Vojvodina according to the two Censuses for the Republic of Serbia (2002 and 2011 year) as a denominator and multiplied by 100,000 persons per year.

Spearman's and Pearson's correlation coefficient were used to compare frequencies of qualitative data and determining the direction of association between two continuous variables, respectively. Average values of wind were shown as a monthly value of wind speeds above 6 of Beaufort scale and measured in meters per second (m/s). Average monthly rainfall for the observed 10-year period was measured in millimeters (mm).

Comparisons of categorical data between groups were made by Fisher's exact test (two-tailed). Differences were considered statistically significant if they were $p < 0.05$. Data analysis was performed using the SPSS version 22 software.

Results

Figure 1 summarises the general trend of Q fever incidence in Vojvodina during 2006–2015. A total of 262 serologically confirmed and 10 clinically classified cases of Q fever were reported with the highest incidence rates in 2006 (2.3/100,000) and in 2012 (3.7/100,000).

Most patients in the territory of Vojvodina 258/272 (94.9%,) were registered in three districts of Vojvodina: the Central Banat, the South Banat and Srem. Observing these mentioned districts, with the exception of 2011 and 2012, when cases of Q fever were more frequently registered in the Srem district, the disease was mainly detected in the Central Banat or South Banat districts. Although the outbreak occurrence of human Q fever in three districts registered during four years (2006, 2010, 2012 and 2013), a total of 152/258 (58.9%) patients were classified as sporadic Q fever cases (Figure 2).

Figure 3 presents a number of the registered human Q fever cases in three districts of Vojvodina. Outbreak cases in four outbreaks were predominantly opposite to sporadic human Q fever cases.

Figure 4 shows the age and sex distribution of the patients in Vojvodina. Q fever cases were reported in patients of all age categories older than 10 years. The youngest patient was 13 years old while the oldest one was 89 years old.

During 2006–2015, a majority of confirmed cases were registered among patients in the age groups from 20 to 59 years old, 229/272 (84.2%). A total of 197/272 (72.4%) cases were males. Further, among patients in each of the age group (10–19, 20–29 and 30–39, years), participation of male patients amounted above 80% of all reported cases.

When observing each year individually, over four years (2006, 2011, 2013 and 2015), most cases were registered in April, and in 2007 and 2009, the highest percentage of cases were detected in May. During 2008, 2010, 2012 and 2014 the highest percentage of the reported cases was detected in March, November, January and February, respectively (Figure 5).

Average monthly wind speed and rainfall data over the 10-year period were compared to cumulative total monthly number of human Q fever cases in three districts of Vojvodina (Figure 6). In these three districts, as the average monthly wind speed increased and monthly rainfall declined from February to April, cases of acute Q fever appeared to increase, and then dropped during June to November, when wind speed declined and rainfall increased.

There was a strong positive correlation detected between the increase in Q fever cases and the increase in wind speed in the Central Banat and South Banat (Spearman's $\rho = 0.69719$, $p = 0.01173$ and $\rho = 0.62303$, $p = 0.03045$, respectively), while no significant correlation between the number of Q fever cases compared with the average of the wind speed in the Srem district ($\rho = 0.5498$, $p = 0.06404$) was detected. There a statistically significant negative correlation between the average rainfall and the number of Q fever cases in the Central Banat, the South Banat and Srem ($\rho = -0.78033$, $p = 0.00275$; $\rho = -0.70675$, $p = 0.01017$, and $\rho = -0.70431$, $p = 0.01056$, respectively) was detected.

When the number of sick cattle and sheep was increased after 2011, we noticed the epidemic of Q human fever in 2012 in the Srem district of Vojvodina. Comparison between human Q fever cases with the number of cattle and sheep Q fever cases confirmed cases showing a strong positive correlation (Pearson's $r = 0.7989$ and $r = 0.7966$, respectively) (Figure 7).

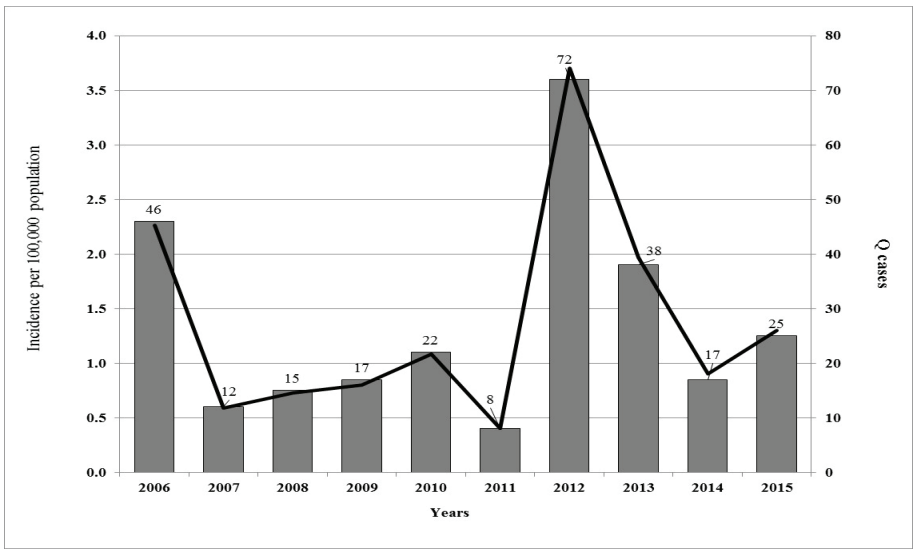

Fig. 1 – Human Q fever incidence in Vojvodina, Serbia, 2006–2015.

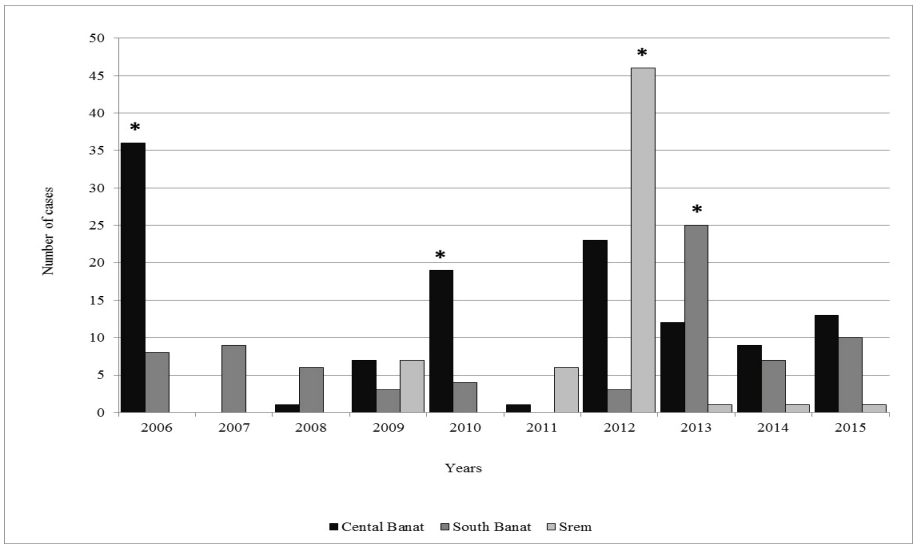

Fig. 2 – Reported human Q fever cases in three districts of Vojvodina, Serbia, 2006–2015. * – indicates the outbreak occurrence by districts and by years.

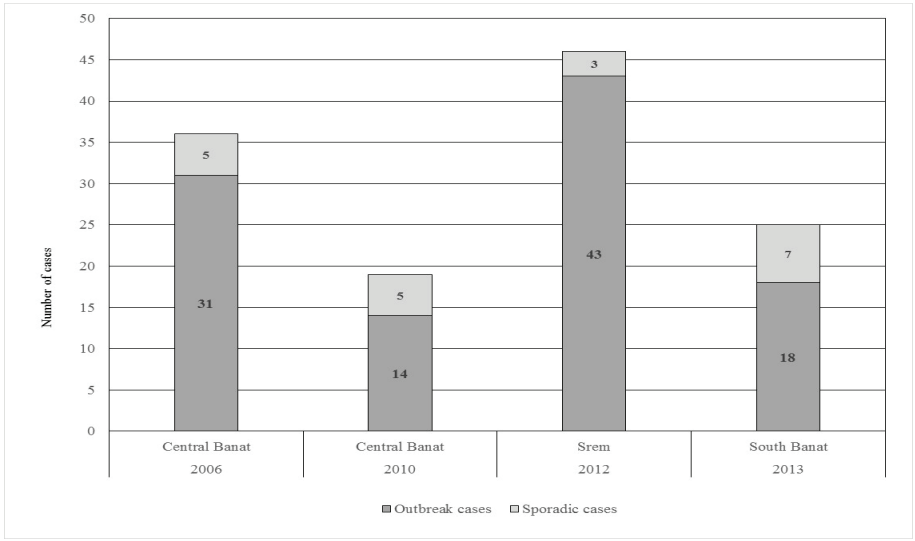

Fig. 3 – Reported human Q fever cases during four outbreak in three districts of Vojvodina, Serbia, 2006–2013.

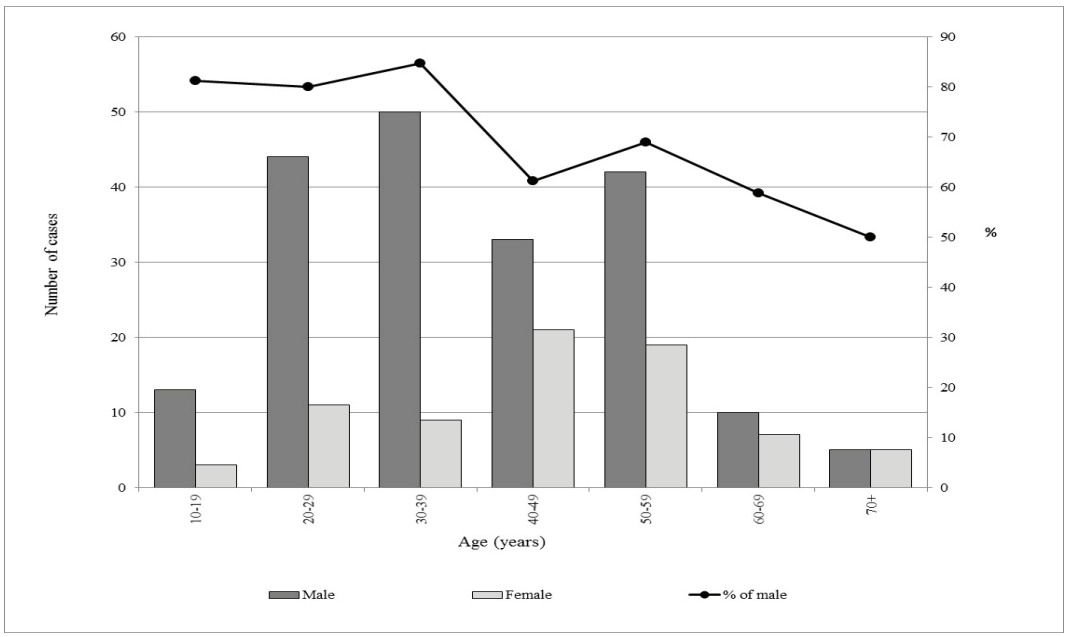

Fig. 4 – Age and sex distributions of Q fever cases in Vojvodina, Serbia, 2006–2015.

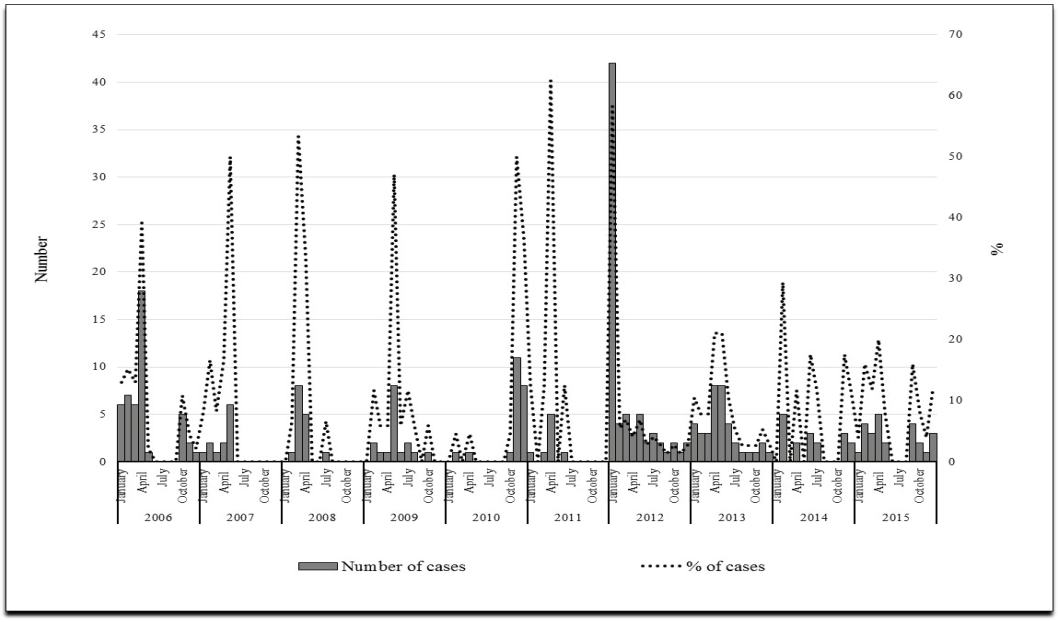

Fig. 5 – Seasonal distribution of human Q fever in Vojvodina, Serbia, 2006–2015.

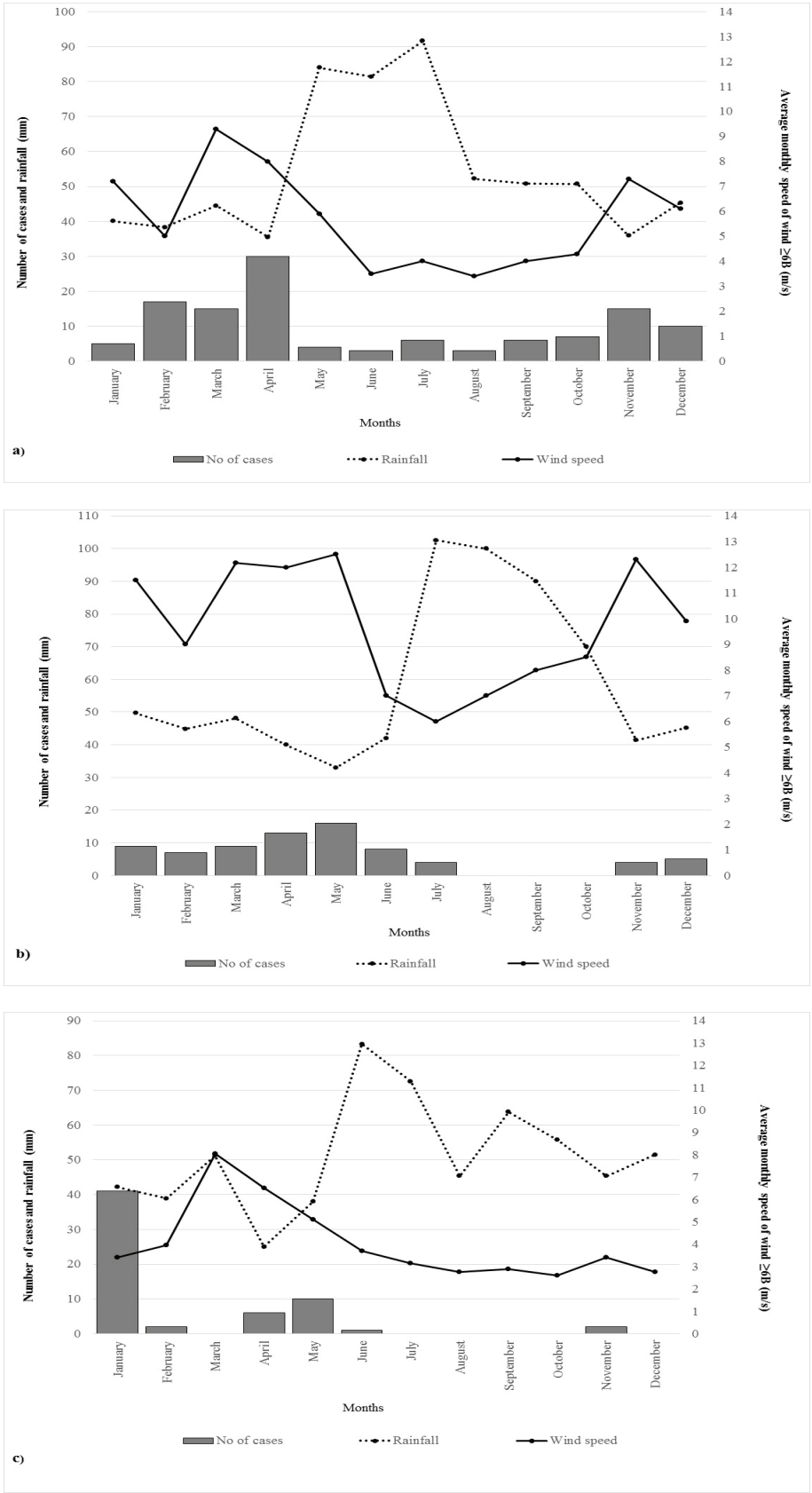

Fig. 6 – Correlation between average monthly wind speed (≥ 6 Beaufort wind force scale) and rainfall with human Q fever cases in three districts of Vojvodina, Serbia, 2006–2015: a) Central Banat, b) South Banat, c) Srem.

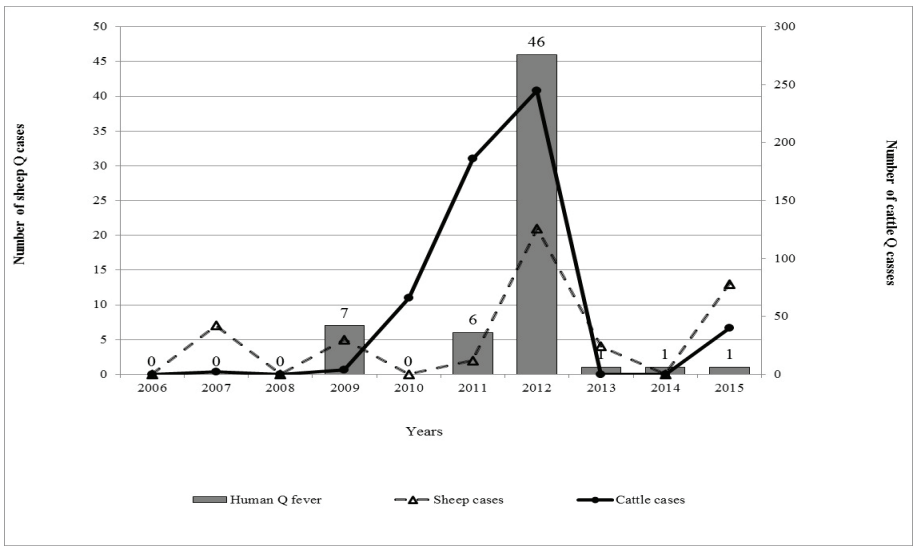

Fig. 7 – Reported human, cattle and sheep Q fever cases in the Srem district of Vojvodina, Serbia, 2006–2015.

Observed hospitalized and outpatients of Q fever cases, revealed no difference between sex and age groups. We found that hospitalized patients were more frequently registered in the South Banat and in urban areas of Vojvodina, while the patients who were more frequently registered at primary health care level (outpatients) were from the Srem district and from the rural areas of the Province. Hospitalized cases with Q fever were more likely to be registered during May-July and outpatients were more frequently detected during November-January (Table 1).

We analysed sources and modes of Q fever transmission from 2006 to 2015 among 236 patients in Vojvodina (Table 2). One half of the total number of cases (120) with available data had direct daily contact with their domestic animals during the maximum incubation period, which was the only possible way of spreading the infection, while every third patient had not been in any contact with animals. Out of the total number of patients, 14.8% cases had a contact with domestic animals in the neighbourhood, but only 2.6% of patients previously consumed cattle milk or sheep cheese. Further, we determined that contacts with domestic animals from the patients' neighbourhood were associated with hospitalization ($p < 0.0001$) (Table 2).

Table 1

Differences among hospitalized and outpatients with Q fever infection in Vojvodina, Serbia, 2006–2015

Characteristics	Total (n = 272)	Inpatients (n = 187)	Outpatients (n = 85)	*p* value*
	n (%)	n (%)	n (%)	
Gender				
male	197 (72.4)	137 (72.3)	60 (70.6)	0.6625
Age group (years)				
≤ 30	74 (27.2)	45 (24.1)	29 (34.1)	0.1056
30–59	173 (63.6)	121 (64.7)	52 (61.2)	0.5888
≥ 60	25 (9.2)	21 (11.2)	4 (4.7)	0.1123
Disctrict				
Srem	62 (22.8)	27 (14.4)	35 (41.2)	< 0.0001
Central Banat	121 (44.5)	79 (42.2)	42 (49.4)	0.2938
South Banat	75 (27.6)	73 (39.1)	2 (2.4)	< 0.0001
South Bačka	1 (0.4)	1 (0.5)	0 (-)	ND
West Bačka	5 (1.8)	5 (2.7)	0 (-)	ND
North Banat	5 (1.8)	2 (1.1)	3 (3.5)	0.1781
North Bačka	3 (1.1)	0 (-)	3 (3.5)	ND
Area				
urban area	98 (36.0)	83 (44.4)	15 (17.6)	< 0.0001
rural area	174 (64.0)	104 (55.6)	70 (82.4)	< 0.0001
Months				
November–January	91 (33.5)	51 (27.3)	40 (47.1)	0.0022
February–April	108 (39.7)	77 (41.2)	31 (36.5)	0.5052
May–July	52 (19.1)	44 (23.5)	8 (9.4)	0.0073
August–October	21 (7.7)	15 (8.0)	6 (7.0)	1.0000

***Fisher's exact test; ND: Not determined.**

Table 2

Sources and modes of Q fever transmission among inpatients and outpatients in Vojvodina, 2006–2015

Sources and modes of transmission	Total (n = 236)	Inpatients (n = 168)	Outpatients (n = 68)	*p* value*
	n (%)	n (%)	n (%)	
Contact with animals	120 (50.8)	71 (42.3)	49 (72.1)	< 0.0001
No contacts with animals	75 (31.8)	57 (33.9)	18 (26.4)	0.2842
Livestock in the neighbourhood	35 (14.8)	34 (20.2)	1 (1.5)	< 0.0001
Consuming cattle milk or sheep cheese produced from uncooked milk	6 (2.6)	6 (3.6)	0 (-)	ND

***Fisher's exact test; ND: Not determined**

Discussion

Epidemiological surveillance of human Q fever in Vojvodina was implemented from 1964. The highest incidence rate of Q fever in Vojvodina was registered in 1976, when 900 human Q fever cases were reported. This was the largest outbreak of Q fever in Europe. After this, Q fever has remained to have an endemo-epidemic character in Vojvodina and until 1990 it was presented as the leading zoonosis in Vojvodina [20]. The main reason for endemo-epidemic maintenance of Q fever was in the fact that the nomadic herds of sheep came from the western regions of former Yugoslavia to Vojvodina, particularly in winter months. This practice was present until 1990, before the Wars in Yugoslavia. Consequently, after the War (1991–1995), a serious decrease of livestock was detected, leading to the significant reduction of the number of human Q fever in Vojvodina (under 5 per 100,000 inhabitants) [22], but interestingly, it increased in the neighbouring countries (Croatia and the Bosnia and Herzegovina) [23, 24].

When observing the data from the European countries (28 Member States and four non-Member States), there was a decreasing trend of confirmed Q fever cases between 2009 and 2013. In 2013, a total of 648 confirmed cases of Q fever in humans were reported in the European countries with a notification rate of 0.17 per 100,000 population, and two deaths due to Q fever were reported by Germany and Latvia [25].

Our results showed that the incidence rate of human Q fever in Vojvodina during the last ten years ranged between 0.4 and 3.7 per 100,000 inhabitants with the evident cyclic incidence peaks which correspond to years in which outbreaks were recognized. Although there were four outbreaks registered, most of the patients in Vojvodina were classified as sporadic human Q fever cases.

Three districts of Vojvodina (the Central Banat, the South Banat and Srem) were identified as the most important endemic Q fever territories, and covered 95% of all registered cases in Vojvodina. Possible explanation for this may be the fact that all three districts bordered with certain neighbouring countries where the endemic Q fever zones were also registered [23, 24, 26]. Thus, the Srem district is bordered by Croatia and Bosnia and Herzegovina, while the Central Banat and the South Banat are bordered by Romania. Using the available data from the World Health Organization report, between 2006 and 2010, the incidence rate of Q fever in Croatia, Hungary and Romania during each year was under 1 per 100,000 inhabitants, while in Bosnia and Herzegovina, it varied from 0.5 to 1.9 per 100,000 population [26]. Further, the explanation for noticeable differences in the number of registered cases across certain districts of Vojvodina is probably because many physicians did not recognize the Q fever in a number of cases, and because of an exchange of livestock fund between these regions. Additional assumptions could be made regarding that the Serbian reference laboratory for confirmation of human Q fever disease is situated in the Central Banat (Department of Public Health of Zrenjanin, Vojvodina), where the largest number of Q fever cases in Vojvodina 121/272 (44%) was identified. Nonetheless, insufficient collaboration between different professions and disciplines, including epidemiologists, microbiologists and veterinarians, in different parts of Vojvodina, can be considered as the reason for the evidence variation of registered human Q fever cases.

It is common knowledge that Q fever among young population has been rarely reported due to the fact that it is manifested as a milder disease or as an asymptomatic infection [27]. In accordance with the above mentioned, we recognized the human Q fever cases only in the patients who were 10 years old and older and the largest number of cases was registered in the economically active citizens in Vojvodina.

Similar to the results of several published studies [1, 28, 29], we found that Q fever in Vojvodina occured more frequently among males than females. We have determined that nearly three quarters of our patients were males. Thus, our findings are in good agreement with the fact that males attributed to risk of occupational exposure, and that females may develop clinical illness less frequently than males because of the protective role of estradiol [30]. However, we did not find a significant difference in gender, between hospitalized and outpatients in Vojvodina.

Q fever infections in humans in Vojvodina were seasonal, with 193 (71%) reported cases in January through May, when lambing, sheep shearing and other activities in which humans are more frequently in contact with potentially infected animals, contributed to the transmission of infection. However, similarly to the findings of other authors [28], we also found that the human Q fever cases were notified throughout the year, suggesting that human Q fever infections may also have non-seasonal influences. Thus, cases in Australia did not show a distinct seasonal trend [31].

So far as it is known, the significance of each domestic animal in the epidemiology of Q fever is different in various regions and it depends on the number of animals, and the level of their infection [1, 10]. Because infection in animals is

usually asymptomatic or limited to abortion and production losses, identifying the source of human infection is sometimes difficult. If the infectious materials contaminate the environment, *C. burnetii* can survive for months, and is resistant to desiccation and temperature extremes [28].

In our territory, cattle and sheep were considered as the main animal reservoirs of *C. burnetii* [20]. Although we covered only the district of Srem in Vojvodina, we first precisely illustrated that the increase and decrease in the human Q fever cases were strongly correlated with a variation in the number of sick cattle or sheep. Similar results were reported by other authors [32, 33]. Besides, some authors suggested that the number of goats was in the positive correlation with the human Q fever cases [1].

As far as we know, this is the first study that demonstrated relationships between values of wind velocity and rainfall with the number of human Q fever cases in our country. Similar to the results of other several studies [15, 17, 18, 33]; our results showed that the spread of *C. burnetii*-infected aerosols was accelerated by low rainfall and high wind velocity. From the observed three districts of Vojvodina (the Central Banat, the South Banat and Srem), we found that the largest number of human Q fever cases were registered during the first four months of the year, when the highest values of wind speed were also registered. Also, during the 2010, when the outbreak of Q fever in the South Banat was registered [20], the largest number of cases was registered in November and December. Although it is known that the South Banat has the highest values of wind speeds in Vojvodina, the average wind velocity in December 2010 was higher than of all other wind speed values which were noticed in the same month through observed 10 years.

In addition, the smallest number of human Q fever cases was noticed between June and October, when the rainfall ranged 42 millimeters to 102 millimeters per month. This period is designated as the rainy part of the year in mentioned districts.

When observing the health care level where the patients were registered, we provided evidence that outpatients were more frequently registered in the Srem district than in the other districts of Vojvodina. We think that the reason for noticed differences could be due to enhanced awareness and better clinical recognition of the disease in the Srem district during outbreak of human Q fever in 2012. Furthermore, in accordance with the fact that the access to the secondary and tertiary health care settings is not equal for all citizens of Vojvodina, the patients in urban area were more likely to be hospitalized than outpatients, while the patients in rural areas were more frequently registered at the primary health care levels.

Although the reason for this is unclear, we have found that the inpatients were more frequently registered during May-July, while the outpatients were more frequently detected during November-January. We think that the reason for determining seasonal predictors for hospitalization perhaps is the fact that during spring and summer months, with the absence of many other acute respiratory infections, physicians become more aware of human Q fever. In accordance with this, they more often refer the patients to secondary or tertiary health care levels for definitive diagnostic confirmation.

The most common route of transmission from infected animals to humans is inhalation of aerosols or dust containing *C. burnetii*. However, we noticed that every third patient in Vojvodina had no contacts with animals and 36% of all human Q fever cases were in urban areas of Vojvodina. Our findings are in good agreement with the previously published evidence that the *C. burnetii* was highly infectious. *C. burnetii* can cause disease with a small inoculum (between one to five microorganisms) [3, 4], and may be transported by the wind far away from its original source [12]. The risk of *C. burnetii* infection in humans is highest within a 5 kilometers radius from the anticipated source [16].

Consumption of unpasteurized dairy products may also result in human infection [28]. In our study, only 2.6% patients previously consumed uncooked animal products.

Human Q fever infection in Vojvodina was previously described as the professional disease, affecting those in contact with livestock such as farmers and veterinarians [20]. However, due to the decline of economic activity, increasing trend of losing work among people who worked with animals, and consequently a reduction of professional exposure to animal potentially infected with Q fever [34], the human Q fever in the last ten years more frequently was registered among adult nonprofessional population in Vojvodina. Nevertheless, our results showed that a large number of human Q fever cases were registered among people in contact with domestic animals, and they presented one half of all other sources of human Q fever infection. Further, our data showed that patients in contact with animals were more frequently registered at the primary health care level (outpatients), while the patients who had contacts with domestic animals in their neighbourhood were associated with hospitalization. A possible explanation for this could be related to the fact that during outbreak years in Vojvodina, as a result of implementation of active surveillance of Q fever, mainly among people in the epidemic areas who had direct contact with domestic animals, a large number of outpatients were registered. Also, the largest number of patients in contact with domestic animals in their neighbourhood was registered during the interepidemic periods, and because they did not visit a physicians in time, they experienced complications as a consequence and they were more often hospitalized than others.

We recognize certain limitations that should be addressed in the future work. First, because most of Q fever infections remain asymptomatic or give nonspecific signs and symptoms, the true incidence rate of human Q fever in Vojvodina is probably higher. Although three districts of Vojvodina were recognized as endemic area in Vojvodina, we believe that many of cases across other four districts were underestimated. Second, despite the fact that we found a positive correlation between available data on the number of human and animal Q fever cases only in the Srem district of Vojvodina, more extensive studies in Vojvodina are required to elucidate this issue. Finally, in our questionnaire we did not predict taking data about the clinical presentation of the disease, and acute or chronic course of infection. Due to the mentioned limitations, we could not determine the final patient outcomes.

Conclusion

A better recognition of human Q fever cases, especially in rural area, can be improved through education of physicians on all health care levels. In addition, the timely sharing of information between the animal and human health sectors as well as among neighbouring countries is crucial for an appropriate and early outbreak response. People who are professionally exposed to a risk of infection must be educated about the disease.

To provide a more precise estimation of incidence rate among the population of Vojvodina, all patients with symptoms compatible with Q fever infection need to be tested or a seroepidemiological study would have to be done to include other asymptomatic cases.

A vaccination strategy for humans with a high risk occupation, and improving the vaccination coverage for animals, especially after the lambing season, can directly contribute to reduction of the number of Q fever cases, hospitalization, complications as well as to prevention of outbreaks in humans in Vojvodina.

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Conflict of interest

This investigation was carried out as part of routine activities without additional funding sources.

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Factors influencing no-reflow phenomenon in patients with ST-segment myocardial infarction treated with primary percutaneous coronary intervention

Faktori koji utiču na „no reflow“ fenomen kod bolesnika sa infarktom miokarda sa elevacijom ST-segmenta lečenih primarnom perkutanom koronarnom intervencijom

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Abstract

Background/Aim. It is not know which factors influence no-reflow phenomenon after successful primary percutaneous intervention (pPCI) in patients with myocardial infarction with ST elevation (STEMI). The aim of this study was to estimate predictive value of some admission characteristics of patients with STEMI, who underwent pPCI, for the development of no-reflow phenomenon. Worse clinical outcome in patients with no-reflow points to importance of selection and aggressive treatment in a group at high risk. **Methods.** This was retrospective and partly prospective study which included 491 consecutive patients with STEMI, admitted to a single centre, during the period from 2000 to September 2015, who underwent pPCI. Descriptive characteristics of the patients, presence of classical risk factors for cardiovascular disease, total ischemic time and clinical features at admission were all estimated as predictors for the development of no-reflow phenomenon. No-reflow phenomenon is defined as the presence of thrombolysis in myocardial infarction (TIMI) < 3 coronary flow at the end of the pPCI procedure, or ST-segment resolution by less than 50% in the first hours after the procedure. The significance of the predictive value of some parameters was evaluated by univariate and multivariate regression analysis. In univariate analysis, we used the χ^2 test and Mann Whitney and Student's *t*-tests. **Results.** No-reflow phenomenon was detected in 84 (17.1%) patients (criteria used: TIMI < 3 coronary flow) and in 144 (29.3%) patients (criteria used: ST-sement resolution < 50%). Patients older than 75 years [odds ratio (OR) = 2.53; 95% confidence interval (CI) 1.48–4.33; $p = 0.001$] and those who had Killip class at admission higher than 1 had increased risk to achieve TIMI-3 flow after pPCI. Killip class higher than 1 (OR 1.59; 95% CI 1.23–2.04; $p < 0.001$), left anterior descendent artery (LAD) as infarct related artery (IRA) and total ischemic time higher than 4 hour were associated with increased risk to failure of rapid ST segment resolution after pPCI. **Conclusion.** Older age and Killip class were main predictors of TIMI < 3 flow, and Killip class, LAD as IRA and longer total ischemic time were predictors for the failure of rapid ST segment resolution after pPCI.

Key words:
myocardial infarction; percutaneous coronary intervention; no-reflow phenomenon; risk factors.

Apstrakt

Uvod/Cilj. Nedovoljno je poznato koji faktori utiču na nastanak “no-reflow” fenomena nakon uspešne primarne perkutane intervencije (pPCI) kod bolesnika sa infarktom miokarda sa ST elevacijom (STEMI). Lošiji klinički tok i ishod lečenja kod bolesnika sa “no-reflow” ukazuje na značaj dobre selekcije i agresivnijeg lečenja u grupi sa visokim rizikom. Cilj studije bio je da se proceni prediktivna vrednost određenih karakteristika na prijemu kod bolesnika sa STEMI koji su lečeni PPCI za razvoj “no-reflow” fenomena. **Metode.** Radi se o retrospektivnoj i delom prospektivnoj studiji koja je obuhvatila 491 bolesnika sa STEMI, lečenih na Klinici za uregentnu internu medicinu Vojnomedicinske akademije u Beogradu, u periodu od 2000. godine do septembra 2015. godine pomoću pPCI. Deskriptivne karakteri-

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stike bolesnika, postojanje klasičnih faktora rizika za kardiovaskularne bolesti, vreme od početka bola do pPCI, kao i klinički status na prijemu procenjivani su kao mogući prediktori za razvoj fenomena "no-reflow". "No-reflow" fenomen definisan je kao tromboliza kod infarkta miokarda (TIMI) < 3 na kraju pPCI procedure ili rezolucija ST-segmenta za manje od 50% u prvih nekoliko sati nakon procedure. Značaj prisustva i prediktivne vrednosti ovih parametara, procenjivan je univarijantnom i multivarijantnom regresionom analizom. U univarijantnoj analizi, korišćen je χ^2, Mann Whitney i Studentov *t*-test. **Rezultati.** "No-reflow" fenomen nađen je kod 84 (17,1%) bolesnika (prema kriterijumu TIMI < 3 koronarnog protoka) i kod 144 (29,3%) bolesnika (prema kriteriumu ST – sement rezolucija < 50%). U grupi bolesnika starijih od 75 godina (*odds ratio* – OR = 2.53; 95% CI 1.48–4.33; $p = 0.001$), kao i kod onih koji su imali srčanu slabost definisanu kao Killip > 1 [(OR), 1.59; 95% *confidence interval* (CI) 1.23–2.04; $p < 0.001$], postojao je statitistički značajno veći rizik za razvoj "no-reflow" fenomena. Takođe, leva prednja descendentna arterija – *left anterior descending* (LAD), kao infarktna arterija (IRA) i ukupno ishemijsko vreme duže od četiri sata bili su povezani sa povećanim rizikom za razvoj "no-reflow" fenomena, praćeno preko parametra izostanak rezolucije ST segmenta za > 50% nakon PPCI. **Zaključak.** Starije osobe i srčana slabost i Killip > 1 bili su glavni prediktori TIMI < 3 protoka, a Killlip, LAD kao infarktna arterija i duže trajanje ishemije bili su prediktori za sporiju rezoluciju ST-segmenta nakon pPCI.

Ključne reči:
infarkt miokarda; perkutana koronarna intervencija; no-reflow fenomen; faktori rizika.

Introduction

There are several definitions of no-reflow phenomenon. Some authors defined it as an inadequate flow through the infarct related artery (IRA) after successful primary percutaneous intervention (pPCI), without mechanical obstruction [1].

Patients who develop this phenomenon have significantly worse short-term but also long-term prognosis. Therefore, identifying predictors of no-reflow phenomenon has a great clinical and prognostic significance.

It is known that many factors influence the development of no-reflow, such as demographic data, ischemic time, IRA, distal embolisation, microvascular obstruction or reperfusion injury [2].

Parameters that indicate existence of no-reflow phenomenon are flow through the infarct artery thrombolysis in myocardial infarction (TIMI) < 3 after successful pPCI, or resolution of ST-segment in electrocardiogram (ECG) that is less than 50% in the first hours after pPCI.

The main objective in this study was to estimate predictive value of several admission characteristics in patients with ST-segment elevation myocardial infarction (STEMI), who underwent pPCI, for development of no-reflow phenomenon.

Methods

This is a retrospective and partly prospective study which included 491 consecutive patients with STEMI admitted to the Clinic for Emergency Internal Medicine at the Military Medical Academy in Belgrade, Serbia, during the period from 2000 to September 2015. All patients included in the study were treated with pPCI with subsequent treatment in the cardiac intensive care unit (CICU), following current recommendations for the treatment of patients with STEMI in CICU [3]. Descriptive characteristics of the patients, presence of classical risk factors for cardiovascular disease, total ischemic time and clinical features at admission were all estimated as predictors for the development of no-reflow phenomenon. No -reflow phenomenon is defined as the presence of TIMI < 3 coronary flow at the end of adequate implantation of stent(s) after pPCI (no significant residual stenosis in the infarction related artery), or ST-segment resolution by less than 50% in ECG that was done one hour after the procedure. These two criteria were estimated independently on the same cohort of consecutive STEMI patients.

Numeric variables were described by the mean and standard deviation as measures of descriptive statistics for variables with normal distribution. For variables with non-normal distribution median and interquartile range were performed as a descriptive parameters. Categorical variables were described by number of cases and percentages. The Student's *t*-test was used for comparison of two groups for numerical variables with normal distribution. Mann Whitney U test was used for comparison of two groups for variables with non-normal distribution. The Pearson's χ^2 test was used for testing differences in categorical variables among groups.

To identify predictors, univariate and multivariate binary logistic regression was used. The variables that showed a statistically significant effect on the dependent variable in univariate logistic regression entered the model of multivariate binary logistic regression. The results are presented as the odds ratio (OR) with 95% confidence interval (CI) and *p* value.

Normality distribution was tested by Kolmogorov-Smirnov test.

The differences were considered significant if probability of null hypothesis was less or equal than 0.05 ($p \leq 0.05$). All calculations were performed by the SPSS, Version 20 (Statistical Package for the Social Sciences).

Results

No-reflow phenomenon was detected in 84 (17.1%) patients (criteria used: TIMI < 3 coronary flow) and in 144 (29.3%) patients (criteria used: ST-segment resolution < 50%).

Table 1

Basic demographic characteristics at admission of patients with STEMI treated with primary PCI in relation to TIMI flow at the end of the procedure and resolution of ST-segment in ECG in the first hour after the procedure

Demographic characteristics	TIMI 3 407 (82.9 %)	TIMI < 3 84 (17.1 %)	*p*	ST-segment resolution ≥ 50% 347 (70.7 %)	ST-segment resolution < 50% 144 (29.3 %)	*p*
Age (years), mean ± SD	61 ± 12	66 ± 13	0.002[a]	62 ± 12	64 ± 12	0.148[a]
Age intervals (years), n (%)						
< 59	188 (46.2)	26 (31.0)	0.011[b]	150 (43.4)	58 (40.4)	0.606[b]
59–74	149 (36.5)	30 (35.7)	1.000[b]	130 (37.5)	51 (35.3)	0.673[b]
> 74	70 (17.3)	28 (33.3)	0.002[b]	66 (19.1)	35 (24.3)	0.210[b]
Women, n (%)	107 (26.4)	21 (25.0)	0.892[b]	93 (26.8)	38 (26.5)	1.000[b]

TIMI – Thrombolysis In Myocardial Infarction; n – number in group; SD – standard deviation; *p* – statistical significance; [a]Student's *t*-test; [b]χ^2 – chi-square test; STEMI – ST elevation myocardial infaraction; PCI – percutaneous intervention; ECG – electrocardiogram.

Table 2

Basic characteristics at admission of patients with STEMI treated with primary PCI in relation to TIMI flow at the end of the procedure and resolution of ST-segment in ECG in the first hour after the procedure

Clinical characteristics	TIMI 3 407 (82.9 %)	TIMI < 3 84 (17.1 %)	*p*	ST-segment resolution ≥ 50% 347 (70.7 %)	ST-segment resolution < 50% 144 (29.3 %)	*p*
Risk factors, n (%)						
smoking	216 (53.0)	35 (42.5)	0.111[b]	189 (54.4)	65 (45.5)	0.099[b]
hypertension	277 (68.1)	63 (75.3)	0.235[b]	242 (69.8)	103 (71.6)	0.737[b]
hypercholesterolemia	256 (63.0)	52 (61.8)	0.892[b]	210 (60.4)	96 (66.7)	0.270[b]
diabetes	109 (26.7)	24 (29.3)	0.683[b]	87 (25.0)	45 (31.3)	0.166[b]
BMI (kg/m^2), mean± SD	26.4 ± 3.5	26.7 ± 3.8	0.685[a]	26.5 ± 3.5	26.2 ± 3.6	0.611[a]
Infarct related artery, n (%)						
LAD (left anterior descending)	165 (40.5)	36 (42.9)	0.717[b]	124 (35.7)	65 (45.1)	0.038[b]
ACX (circumflex artery)	74 (18.2)	13 (15.5)	0.639[b]	60 (17.3)	18 (12.5)	0.220[b]
RCA (right coronary artery)	157 (38.6)	34 (40.5)	0.806[b]	134 (38.6)	50 (34.7)	0.405[b]
Multivessel disease, n (%)	272 (66.9)	66 (79.0)	0.035[b]	240 (69.3)	99 (68.9)	1.000[b]
Kilip class > 1, n (%)	57 (14.1)	34 (40.5)	< 0.001[b]	45 (13.0)	40 (27.9)	< 0.001[b]
Ischemic time (hrs), median (IQR)	3.5 (2.5–6.0)	4.0 (3.0–6.5)	0.508[c]	3.0 (2.0–5.0)	5.0 (3.0–8.8)	< 0.001[c]
Overall stent length in IA, median (IQR)	23.0 (18.0–33.0)	24.5 (18.0–46.5)	0.114[c]	23.0 (18.0–32.0)	25.0 (19.0–36.5)	0.055[c]

TIMI – Thrombolysis In Myocardial Infarction; n – number in group; mean ± SD – mean ± standard deviation; IQR- Interquartile range; *p* – statistical significance; IA – infarct artery; [a]Student's *t*-test; [b]χ^2 – chi-square test; [c]Mann Whitney U test.
For abbreviations see under Table 1.

Patients with TIMI < 3 flow at the end of the procedure were significantly older than patients with TIMI 3 flow, in particular older than 74 years ($p = 0.002$). They have also had more often multivessel coronary disease ($p = 0.035$) and were more often admitted with signs of acute heart failure ($p < 0.001$). On the other side, patients with resolution of ST-segment less than 50% had more often LAD as IRA compared to patients with significant resolution of ST-segment in the ECG ($p = 0.035$). They have also had more often signs of acute heart failure at admission ($p < 0.001$), and borderline significance was achieved in comparison of the median overall stent lengths in infarction artery with longer overall stent length in this patients with absence of ST-segment resolution ($p = 0.055$). Other demographic and basic characteristics at admission are presented in Tables 1 and 2.

According to univariate binary regression analysis for TIMI flow criteria used, the best predictors of occurrence of flow TIMI < 3 at the end of the procedure were Killip class > 1 at admission (unadjusted OR = 4.14; 95% CI 2.74–6.95; $p < 0.001$), age over 74 years (unadjusted OR = 2.39; 95% CI 1.42–4.03; $p = 0.002$) and presence of multivessel disease (unadjusted OR = 1.86; 95% CI 1.05–3.30; $p = 0.035$). In multivariate binary regression ("stepwise" method) analysis, as the best independent predictor of flow TIMI < 3 was Killip class >1 (OR = 1.93; 95% CI 1.49–2.52; $p < 0.001$). Age > 74 was detected as the second most important predictor of TIMI < 3 (OR = 2.12; 95% CI 1.22–3.68; $p = 0.007$) (Table 3).

According to univariate binary regression analysis for ST-segment resolution criteria used, the best predictors of ST-segment resolution by less than 50% were: Killip class > 1 (unadjusted OR = 2.60; 95% CI 1.59–4.27; $p < 0.001$), ischemic time (unadjusted OR = 1.11; 95% CI 1.06–1.16; $p < 0.001$), overall stent length in infarction artery (unadjusted OR = 1.01; 95% CI 0.99–1.03; $p = 0.055$) and LAD as IRA (unadjusted OR = 1.55; 95% CI 1.03–2.32; $p = 0.038$) (Table 4). However, the best independent predictors of no-reflow according to this criteria, using the stepwise multivariate binary regression analysis, were ischemic time (OR = 1.15; 95% CI 1.08–1.22; $p < 0.001$) and Killip class > 1 (OR = 1.59; 95% CI 1.17–2.77; $p = 0.003$).

Discussion

The rate of no-reflow in our study is in the range of available literature data. Independent predictors of no-reflow in our group of consecutive STEMI patients were presence of acute heart failure at admission and total ischemic time. Older age and multivessel disease showed to be good predictors, as well.

These data are consistent with literature where percentage of no-reflow is between 11–40% [4].

Univariate and multivariate binary regression analysis for the best predictors of no-reflow (resolution of ST-segment less than 50% and TIMI < 3 flow) have shown that the Killip > 1 on admission is the best predictor of no reflow-phenomenon. This data correlates with the fact that the most difficult patients develop no-reflow [5].

Table 3

Unadjusted odds ratio (OR) and adjusted in univariate and multivariate analysis for the best predictors of TIMI flow less than 3

Parameters	Univariate binary regression analysis		Multivariate binary regression analysis		Multivariate binary regression analysis („stepwise" method)	
	unadjusted OR (95% CI)	*p*	adjusted OR (95% CI)	*p*	OR (95% CI)	*p*
Killip > 1	4.14 (2.47–6.95)	< 0.001	1.95 (1.49–2.54)*	< 0.001	1.93 (1.49–2.52)	< 0.001
Age 74 years	2.39 (1.42–4.03)	0.002	2.53 (1.48–4.33)**	0.001	2.12 (1.22–3.68)	0.007
Multivessel disease	1.86 (1.05–3.30)	0.035	1.67 (0.93–3.00)*	0.085	/	/

TIMI – Thrombolysis in Myocardial Infarction; OR – odds ratio; CI – confidence interval;*gender, age; ** gender; *p* – statistical significance.

Table 4

Unadjusted odds ratio (OR) and adjusted in univariate and multivariate analysis for the best predictors of resolution of ST-segment less than 50%

Parameters	Univariate binary regression analysis		Multivariate binary regression analysis		Multivariate binary regression analysis („stepwise" method)	
	unadjusted OR (95% CI)	*p*	adjusted OR (95% CI)*	*p*	OR (95% CI)	*p*
Killip > 1	2.60 (1.59–4.27)	< 0.001	1.59 (1.23–2.04)	< 0.001	1.59 (1.17–2.77)	0.003
Ischemic time	1.11 (1.06–1.16)	< 0.001	1.11 (1.06–1.17)	< 0.001	1.15 (1.08–1.22)	< 0.001
Overall stent length in IA	1.01 (0.99–1.03)	0.055	1.01 (0.99–1.03)	0.126	/	/
LAD as infarct artery	1.55 (1.03–2.32)	0.038	1.55 (1.03–2.33)	0.034	/	/

IA – infarct artery; LAD – left anterior descending; OR – odds ratio; CI – confidence interval;*gender, age; ** gender; *p* – statistical significance.*gender, age; *p* – statistical significance.

These data are particularly important in the case of elderly patients. According to the TIMI < 3 flow, elderly patients have a high risk to develop no-reflow.

Ischemic time greater than 4 hours, also represents an independent predictor of no-reflow phenomenon. This data is correlated with the data available in literature [6]. Additionally, data from literature, where ischemia-reperfusion period is listed as an essential parameter, visualization of thrombus mass in the IRA and target lesion length over 13.5 cm can also be considered as independent predictors of no-reflow phenomenon.

In the largest data reported (CathPCI registry), most obtained data coincided with our testing. Cardiogenic shock and time to PCI were also the most important parameters of no- reflow ($p < 0.001$). Angiographic criteria such as total length of the lesion, complexity of the lesions – type C and bifurcation lesions are also independent predictive factors. Adequately performed procedures are another important factors in the prevention of no-reflow phenomenon [7].

Consequently, we can conclude that elderly patients with anterior wall infarction, especially those with prolonged time of ischemia, are at the highest risk of no-reflow development. Patients with TIMI flow 0 or 1 before the pPCI intervention represent the high risk group. These factors are targeted for maximum therapeutic approach and special attention, due to a risk of no-reflow phenomenon. This is especially important because of the fact that this group of patients may have significantly worse prognosis.

Study limitation

This study was done in one sigle center, and we accept the limitations of the therapy which we use during the study. There is not fully systematized use of pharmacological therapy, and the effects are still unclear. Thus, the treatment strategy is not definied totally; we used standard therapy for all patients with no-reflow (glycoprotein platelet inhibitors, nitroglycerin, adenosine, verapamil, heparin), but further investigation is necessary.

Conclusion

Elderly patients with anterior wall infarction, especially those with prolonged time of ischemia, are at the highest risk of no-reflow development. Patients with TIMI flow 0 or 1 before the pPCI intervention represent the high risk group. These factors are targeted for maximum therapeutic approach and special attention because of the risk of no-reflow phenomenon. This is especially important due to fact that this group of patients may have significantly worse prognosis.

Signs of acute heart failure at admission and total ischemic time as well as older age above 74 years, and multivessel disease were significant predictors of the occurrence of no reflow phenomenon in patients with first STEMI who underwent primary PCI.

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Stress assessment in patients with clinically diagnosed sleep bruxism

Procena stresa kod bolesnika sa klinički dijagnostikovanim bruksizmom

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Abstract

Background/Aim. Many studies investigated association between stress, anxiety or personality traits and sleep bruxism (SB), but results are still contradictory. We aimed to investigate whether there is a relation between clinically diagnosed sleep bruxism and salivary cortisol levels as one of the major stress biomarkers and to examine psychological factors and personality traits specific to sleep bruxism. **Methods.** A total of 23 sleep bruxism patients and 42 healthy non-sleep bruxism adults participated in this study. Diagnose of sleep-bruxism was assessed by self-report and clinical examination and also confirmed by bed-partner. Morning saliva was collected from all participants for analyses of the cortisol level. Sleep bruxism patients underwent a psychodiagnostic personality interpretation using the Minnesota Multiphasic Personality Inventory – MMPI-202 test. **Results.** Statistically significant difference between levels of morning salivary cortisol in the group of SB patients and the control group was recorded ($t = 2.943$, $p < 0.01$). Analysis of the personality profiles indicated that the sleep bruxism patients avoid contact with unpleasant feelings, especially depression, suppress the aggression and censor the expression of anger and rage. **Conclusion.** This study showed that patients with sleep bruxism have higher levels of salivary cortisol. Personality traits such as depression, hypomania and suppressed aggression were found to be common characteristics in patients with sleep bruxism. Present findings might support the hypothesis that sleep bruxism and psychological states such as stress may be related, but the cross-sectional nature of this study does not allow us to draw conclusions about the causal relationship between stress, personality traits and sleep bruxism.

Key words:
sleep bruxism; stress, psychological; anxiety; personality; saliva; hydrocortisone.

Apstrakt

Uvod/Cilj. Mnoge studije ispitivale su povezanost između stresa, anksioznosti, crta ličnosti i bruksizma, ali su rezultati i dalje kontradiktorni. Cilj ove studije bio je da se ispita da li postoji povezanost između klinički dijagnostikovanog bruksizma koji se javlja tokom spavanja i koncentracije kortizola u salivi koja se smatra jednim od glavnih biomarkera stresa, kao i da se ispitaju psihološki faktori i crte ličnosti osoba sa bruksizmom. **Metode.** Studija je obuhvatila 23 bolesnika sa bruksizmom i 42 zdrava ispitanika bez znakova bruksizma. Dijagnoza bruksizma uspostavljena je na osnovu spostvene izjave i kliničkog pregleda kao i potvrde partnera. Uzorci jutarnje pljuvačke prikupljani su radi analize kortizola. Bolesnici sa bruksizmom podvrgnuti su psihodijagnostičkom ispitivanju pomoću Minesota multifaznog testa ličnosti – MMPI-202. **Rezultati.** U grupi bolesnika sa bruksizmom registrovana je značajno veća koncentracija kortizola u pljuvačci u poređenju sa pacijentima bez znakova bruksizma ($t = 2.943$, $p < 0.01$). Psihodijagnostička interpretacija profila ličnosti pokazala je da ispitanike sa bruksizmom karakteriše izbegavanje kontakta sa neprijatnim osećanjima, posebno depresijom i potiskivanje agresivnosti, odnosno izražena autocenzura na otvoreno ispoljavanje ljutnje i besa. **Zaključak.** Kod bolesnika sa bruksizmom registrovane su povećane koncentracije kortizola u salivi. Psihodijagnostička analiza ukazuje da su depresija, hipomanija i supresija agresije izražene kod bolesnika sa bruksizmom. Nalazi bi mogli da podrže hipotezu povezanosti bruksizma u toku spavanja i stresa, ali priroda ove studije ne dozvoljava izvođenje zaključaka o uzročnoj povezanosti stresa, crta ličnosti i bruksizma u toku spavanja.

Ključne reči:
bruksizam; stres, psihički; anksioznost; ličnost; pljuvačka; hidrokortizon.

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Introduction

Sleep bruxism (SB) is defined as an oral activity characterised by grinding or clenching of the teeth during sleep, usually associated with sleep arousal. According to the International Classification of Sleep Disorders SB is listed as the sleep-related movement disorder [1]. Similar oral activity can occur during wakefulness, but it is a different phenomenon and it is important to differ those two entities, both in clinical practice and in research. In order to avoid the possibility of misunderstanding concerning the diagnosis of bruxism, an international group of bruxism experts proposed that bruxism had two distinct circadian manifestations: it can occur during sleep (SB) or during wakefulness (awake bruxism). According to diagnosis, bruxism was graded as possible – based on self-report, probable – clinically diagnosed and definite – confirmed by polysomnographic recording [2]. The prevalence of the SB in general population is about 12.8 % [3].

Psychological factors such as stress, anxiety and personality traits were often discussed in the genesis of bruxism, both sleep and awake, and results are still contradictory. Many studies showed that patients with SB have a higher stress sensibility – they are mainly people who are easily provoked, unhappy, anxious and often ill-disposed towards the surroundings [4–7]. On the other hand, recent studies failed to confirm association between stress and the SB [8, 9].

The aim of this study was to investigate whether there is a relation between the SB and salivary cortisol (hydrocortisone) levels as one of the major stress biomarkers and to evaluate the influence of psychological factors specific to the SB.

Methods

Subjects

Among 200 patients examined at the Clinic of Prosthodontics, Faculty of Dental Medicine, University of Belgrade, Serbia, a total of 23 SB patients with occlusal sings of bruxism were selected to participate in the study. The group included 4 males and 19 females, aged between 20 and 34 (mean age 26.56) years. Control group consisted of 42 healthy adults, 13 males and 29 females, aged between 20 and 35 (mean age 26.3) years, with no signs of SB. Inclusion criteria were the presence of all permanent teeth, except third molars, and at the most two fillings in dental arch. Exclusion criteria were: pregnancy, neurological disorders, use of medications to suppress anxiety, and use of medications that could affect salivary cortisol level, sleep or motor activity. All subjects gave a written informed consent to participate in the study. The present study was approved by The Ethics Committee, Faculty of Dental Medicine, University of Belgrade.

Diagnostic criteria for sleep bruxism

All the selected patients were diagnosed with 'probable' SB[2], assessed by self-report and clinical examination, and also confirmed by a bed-partner with the aim to avoid former SB. Clinical examination included observation of toothwear – second, third or fourth degree evaluated by the abrasive scale by Hansson et al. [10]. In addition, Krough-Poulsen provocation test was performed [11]. Patients were instructed to move the mandible till the present bruxofacets were matched (interlocked) in position as "the key in the lock" and then to clench or grind their teeth in that position. If the pain in the masticatory muscles was provoked, the test was positive.

Psycho-diagnostics

The subjects of the SB group underwent a psychodiagnostic personality interpretation using the Minnesota Multiphasic Personality Inventory – MMPI-202 test. The MMPI is a standardized questionnaire developed by Hathaway and McKinley [12] in 1940 at the University of Minnesota. It was published in 1943 and since then it underwent a long path of development, verification, transformation and expansion.

We used the MMPI-202 test which is abbreviated version, modified and standardized for Serbian population [13]. Psycho-diagnostics was carried out at the Institute of Psychiatry, Clinical Center of Serbia, Faculty of Medicine, University of Belgrade, Serbia.

The MMPI-202 test contains 210 items and 15 scales. There are separate test forms and scoring for males and females. Three validity scales, **L**, **F**, and **K** (L – lie, F – infrequency, and K-correction) scales are used to check eligibility and willingness of subjects for this type of testing. Eight "pathological" scales of the MMPI-202 inventory reflects following psychopathological classes: **Hs –** hypochondriasis, **D** – depression, **Hy** – hysteria, **Pd** – psychopathic deviate, **Pa** – paranoia, **Pt** – psychasthenia, **Sc** – schizophrenia, and **Ma** – hypomania. Four additional scales are **Si** – scale of social introversion-extraversion, **An** – anxiety scale, **Ag** – aggression scale, **C** – list of critical statements. **An** – anxiety scale refers to the manifest anxiety while Pt – psychasthenia scale represents a latent anxiety. **Ag** – scale of aggression in the MMPI-202 is a latent aggression, and **Pd** is the manifested aggression. Scale **C** contains bizarre and clearly psychotic items, so is aimed to assess psychotic behaviour. It was shown in practice that a "typical" psychotic sections (**Pa-Sc**) is not necessarily a consequence of psychotic disorders, but also can be a consequence of excessive emphasized personality traits. The MMPI test was administered and evaluated by a clinical psychologist. All SB patients were tested and individual scores were assumed and showed in graphics for all control and clinical scales as a profiles for both gender, followed by comments.

Saliva sampling

Samples of the whole unstimulated morning saliva were collected in a sterile glass tubes with a lid using a passive drool technique. Samples were collected before breakfast or 60 minutes after the breakfast, latest at 9 am. Since acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth, patients were instructed to rinse their mouth with water 10 minutes before sample collection. Also, patients were in-

structed not to brush their teeth, smoke or drink coffee or alcohol 12 hours before sample collection, in order to avoid the influence of these factors on the cortisol levels. Specimens were centrifuged and frozen until the analysis.

Salivary cortisol analysis

The level of salivary cortisol was measured by chemiluminescence, using device "IMMULITE DPC", Los Angeles, US, third-generation. For *in vitro* test, barcode-labelled the Cortisol test units (LCO1) that contain polystyrene beads impregnated with anti-cortisol antibodies (polyclonal antibodies obtained from rabbits) were used. The Cortisol reagent wedge (LCO2) contained alkaline phosphatase conjugated to cortisol in buffer.

Statistical analysis

Statistical analysis was performed using the SPSS®21 software. The Leven's test was used to assess the equality of variances of subpopulations. Modified *t*-test was used to compare means between salivary cortisol levels both in the SB and control group.

Results

Mean salivary cortisol levels of the SB group and the control group are shown in Table 1. Since the equal variances were not assumed ($F = 23.601$; $p < 0.001$), we used modified *t*-test to compare the mean values of salivary cortisol levels between the groups. Results show that there was a statistically significant difference between salivary cortisol levels in the SB and the control group ($t = 2.943$; $p < 0.01$).

Table 1

Salivary cortisol levels in sleep bruxism (SB) patients and non-SB subjects (control group)

Group	Salivary cortisol levels (nmol/L)				
	n	mean ± SD	Leven's F test and probability (*p*)	*t*-test and probability (*p*)	95% confidence interval
SB group	23	45.75 ± 17.54	F = 23.601	***t* = 2.943**	38.4–53.1
non-SB group	42	34.42 ± 7.80	$p < 0.001$*	***p* < 0.01**	32.0–36.8

***t* - test, α < 0.05; n – number of patients; SD – standard deviation; SE – standard error.**

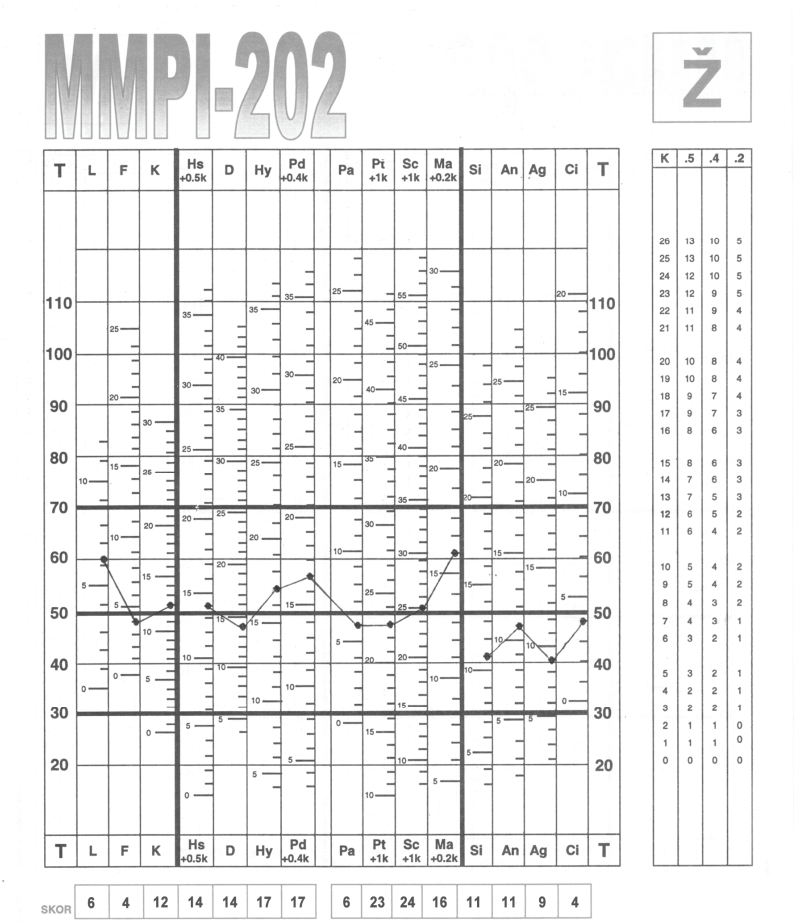

Fig. 1 – Female (Ž) respondents and personality psychological profiles. MMPI – the Minnesota Multiphasic Personality Inventory.

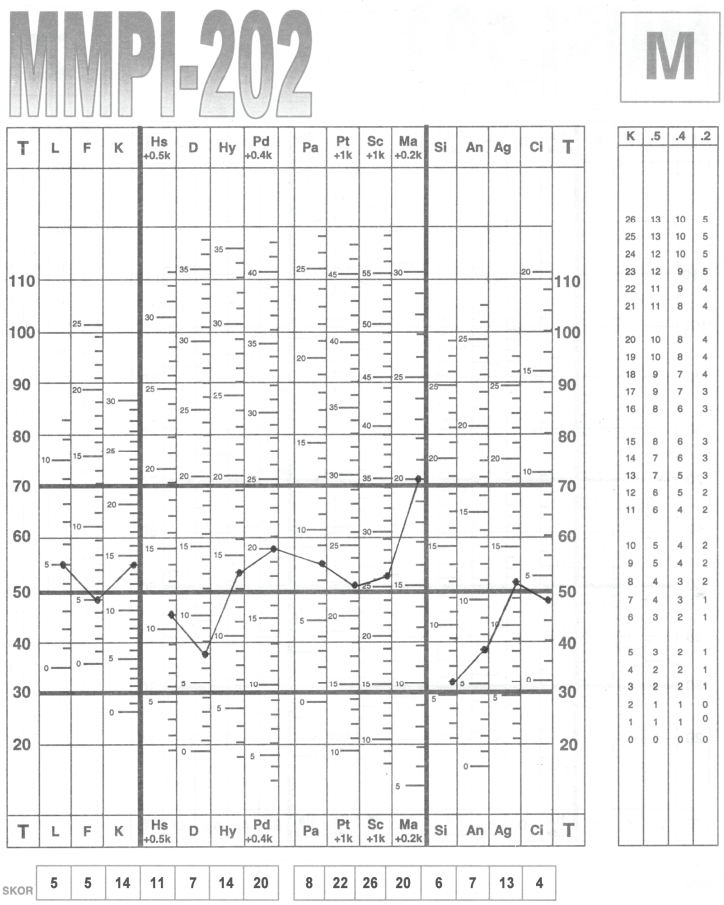

Fig. 2 – Male (M) psychodiagnostic profile according to MMPI-202.
MMPI – the Minesota Multiphasic Personality Inventory.

To examine the possibility of influence of psychological factors in the genesis of the SB, we evaluated psychological personality profiles of patients in the study group. The Psycho-diagnostic MMPI – 202 profile for female patients showed dissimulative trend. The overall profile scores were uniform but much decreased, reflecting a tendency of the patients to conceal a problem, presenting themselves in a different manner. The female respondents in our study were hiper-scrupulous, highly socialized and conventional. Characteristics of this type are pedantry, perfectionism and diligence. They perform every task very meticulously and with great energy and perseverance, but they are often dissatisfied with the outcome and prone to self-criticism, so the slightest failure may produce a depressive reaction. Their dissatisfaction and a constant tension in the race for success led them to a prolonged state of stress, which probably resulted in chronic discontent. Nevertheless, they avoided contact with depressive feelings (low D-14, T < 50) and suppress aggression (low Ag-9; T score 40), (Figure1).

The psycho-diagnostic MMPI – 202 profile for males was in favour of hyperactive, sociable personalities, dominate in the society. They evoke sympathy in the environment with their cheerfulness and penchant for humour. They tend to overrate their abilities and undertake numerous projects and simultaneous activities. Hyper-vigilance attention and highlighted inner restlessness (high Ma-20, T score above 70) was stressed within the male respondents (Figure 2). They tended to present themselves as healthy, energetic, confident and enterprising people. In fact, this type of reaction was the defense of the depressive feelings that might occur in stressful situations, i.e. hypomania as a defence against depression. This mechanism is called reaction formation.

Discussion

The aethiology of SB still remains unclear. Theories about the influence of peripheral factors such as occlusal interferences are mainly abandoned today [4, 14]. It is widely accepted that central factors could play an important role in the genesis of the SB. Many authors suggested that stress, physical and especially mental, was a generator of SB, while others point out that a certain personality characteristics (extreme responsibility, pedantry, perfectionism) were crucial factors for the occurrence of the SB [4–7]. However, association between psychological stress and the SB in literature is contradictory.

This study combined psychological assessment and quantitative stress assessment, using a biochemical stress parameter. Emotional stress is very difficult to objectify. Therefore, in psychiatry, emotional status is measured by issues of fear, anxiety, affect, arousal, behaviour influenced by emotions, jealousy, depression, ability to experience pleasure etc. In addition to psycho-diagnostic parameters, there are objec-

tive biochemical parameters indicating the higher levels of physical and psychological stress. It is well known that high levels of psychological stress cause increased secretion of "stress" hormones in the blood, saliva or urine [15]. Psychological stress initiates activation of the hypothalamus-pituitary-adrenal axis (HPAA), one of the systems involved in stress response. This results in the increased secretion of cortisol, epinephrine and norepinephrine in blood, urine and saliva. Therefore, in some studies on the SB objective parameters were used to asses stress [16–19]. In this study, levels of salivary cortisol were used to asses HPAA. The synthesis of cortisol has a very distinctive circadian rhythm. In fact, almost all of the daily amount of cortisol is synthesized in the early morning hours, just before waking. It has been shown that perceived chronic stress was related to an elevation of salivary cortisol after awakening [20, 21]. Therefore, we collected the morning saliva samples for analysis of salivary cortisol. Ninety percent of blood cortisol is bind to cortisol bind globulin (CBG) and only 10% is free cortisol which represents biologically active fraction of this hormone. There is a high correlation between free blood cortisol and salivary cortisol levels [15]. Owning to salivary cortisol stability, saliva samples can be stored at 5°C for up to 3 months and at -20°C to -80°C up to 12 months. Saliva sampling is non-invasive, stress-free and relatively easy procedure. All this advantages contribute to extensive use of salivary cortisol as a stress biomarker in researches [15]. We found statistically significant difference between levels of morning salivary cortisol in the SB group (45.75 n ± 17.5 nmol/L) compared to the control group (34.42 nmol/l ± 7.8 nmol/L) which indicates higher level of stress in individuals with the SB. In accordance to our results, several studies found higher levels of stress biomarkers in the SB patients. An electromyographic (EMG) study of Clark et al. [16] found a correlation between the increased levels of urinary epinephrine as stress biomarker and masseter muscle activity during sleep. Vanderas et al. [17] analysed urinary catecholamine levels in 314 children with clinically diagnosed bruxism. Results indicated a high association between urinary epinephrine and dopamine levels and bruxism, but it was not specified whether it was sleep or awake bruxism. Seraidarian et al. [18] also found the higher levels of urinary epinephrine, norepinephrine and dopamine in individuals with the SB. On the contrary, recent study on children with the SB failed to find a positive correlation between salivary cortisol and the SB [19]. These discrepancies in salivary cortisol levels, may be due to different stress response and reactivity of HPAA in children and adults [22].

Some authors suggested that the SB was a stress coping strategy [23]. The few recent studies investigated influence of chewing and clenching on salivary stress biomarkers. Tahara et al. [24] found a decrease of salivary cortisol levels during chewing and clenching, indicating that those actions promote relaxation in subjects under stress. Soeda et al. [25] found that the strong chewing force induced a greater reduction of salivary cortisol levels and released mental stress more than a weak one. Study by Takahashi et al. [26] indicates that stabilisation splint therapy reduces number of the SB episodes, but increases levels of salivary stress biomarker chromogranin A. This study is cross-sectional and does not evaluate changes in the salivary cortisol levels in the SB patients, but in further studies it would be interesting to examine whether reduction of repetitive jaw-muscle activity achieved through the long-term SB therapy influences salivary cortisol levels.

It should be also mentioned that different diagnostic criteria used in studies makes it difficult to compare results. In this study, sleep bruxism was graded as 'probable', since it was clinically diagnosed. This should be considered as a limitation of the study. Polysomnographic recording is a golden standard in the SB diagnostics [2]. However, it is often unaffordable and, therefore, not so often used in studies. With the aim to minimise diagnostic errors in this study, the presence of the SB episodes during sleep was confirmed by a bed-partner.

Early EMG studies reported association between stress and SB. The results of Rugh and Solberg [27] study showed that the intensity of the SB varied and these variations depended on the intensity of behavioural cognitive factors such as emotional daily stress and the intensity of stressful life events (illness, employment, problems at work, loss of job, family arguments, exam, physical exhaustion, etc.). On the contrary, an EMG study on 100 SB patients done by Pierce et al. [8] showed no correlation between self-reported stress and the SB. Similarly, another study showed that the SB was not strongly related to a daily stress [9]. However, a few recent studies on self-reported bruxism found that anxiety and stress sensitivity may be related with the SB [6–7, 28]. Results of the EMG study of Manfredini et al. [7] indicate that anxiety trait but not anxiety state, is actually important factor in the pathogenesis of the SB.

Regarding the results of the above mentioned studies, it could be suggested that personality characteristics and stress-coping strategies are more important factors in the genesis of the SB than the stress itself. Hence, psycho-diagnostic analysis of personality profiles of the SB patients in this study was performed to reveal the possible role of specific personality traits that might have significant impact on the maintenance of symptomatology of the SB. A common characteristic of personality profile of both males and females with the SB was the avoidance of contact with unpleasant feelings, especially depression, as well as suppression of aggression and censorship of the expression of anger and rage. Inability to discharge aggressive impulses in a socially acceptable manner and the inability of sublimation of aggression probably lead to long-term feelings of discomfort and therefore might influence the development of the SB. The results of this study are also consistent with the results of the study of Molina and dos Santos [29] suggesting that anger and hostility are related to the severity of bruxism. One of the possible explanations is the thesis that "A person is the sum total of his/her life experiences, each of which is registered in his/her personality and structured in his body" [30]. Life of each person consenquently affects its physical body. Self-image determines which feelings and impulses will be expressed. The estimated censorship does not allow the expression of depressive mood, even on a verbal level. Due to these difficulties to express unpleasant emotions, psychic energy accumulates in the oral region on the muscular level [31]. This could lead to discharge through the auto-aggressive actions such as bruxism. In

further studies, it would be interesting to compare personality profiles of the SB patients and non-SB subjects.

Conclusion

The results of this study indicate the higher level of salivary cortisol in patients with sleep bruxism. Personality traits distinguished in the psychological assessment, such as depression, hypomania and suppression of aggression might be the important factors that influence the developing of prolonged state of stress which might lead to generating a symptoms of sleep bruxism. However, the cross-sectional nature of this study does not allow us to draw conclusions about the causal relationship between stress, personality traits and sleep bruxism.

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Conceptual model of collaborative pharmaceutical practice in healthcare and social care for the elderly

Konceptualni model kolaborativne farmaceutske prakse u zdravstvenoj i socijalnoj zaštiti starijih osoba

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Abstract

Background/Aim. In the new millennium, the focus has been increasingly shifting to optimisation by enhancing the collaborative (common, joint) practice of healthcare professionals, for the purpose of achieving effectiveness and efficiency. Pharmacists are the last link in the healthcare services providing chain. The aim of this study was to present a critical analysis of the published models of the collaborative pharmacy practice along with development of a conceptual model of collaborative pharmacy practice in the healthcare and social care for the elderly population. **Methods.** Using two search algorithms that were created to search articles published in English, a comprehensive search of the bibliographic databases Web of Science and PubMed was undertaken (up to June 2015). Afterwards, articles were independently assessed by two authors, against predetermined inclusion and exclusion criteria. **Results.** Regulations on pharmacy collaboration are present in many developed countries. However, the implementation of the collaborative practice is still not widespread. Therefore, a conceptual model of the collaborative healthcare and social care of the elderly provides an insight into a multi-layer structure that has to be established in order to achieve a functioning system of the collaborative healthcare practice. The model concluded that aspirations towards teamwork, communication and above all – the system of regulators and payers, who acknowledge a healthcare collaboration, are crucial for establishment of a collaborative healthcare practice. **Conclusion.** This research provides a tool in the form of a guide and check-list for decision-makers and policy-makers in order to achieve the preferred effects generated from the collaborative practice by selecting the models and activities that need to be undertaken for implementation of the collaborative healthcare and social care of the elderly that is best suited for their country.

Key words:
delivery of health care; pharmacists; interdisciplinary communication; aged.

Apstrakt

Uvod/Cilj. U cilju postizanja efektivnosti i efikasnosti, u novom milenijumu se sve više govori o optimizaciji zdravstvene zaštite, kroz jačanje udružene prakse zdravstvenih profesionalaca. Farmaceuti predstavljaju poslednju kariku u lancu pružanja zdravstvene zaštite. Cilj ove studije bio je da prikaže kritičku analizu publikovanih modela kolaborativne farmaceutske prakse, kao i razvoj konceptualnog modela farmaceutske kolaborativne prakse u zdravstvenoj i socijalnoj zaštiti starije populacije. **Metode.** Koristeći dva algoritma koja su kreirana za pretragu, pretraženi su radovi (do juna 2015. godine) iz bibliografija elektronskih baza podataka Web of Science i PubMed. Nakon pretrage, radovi su nezavisno procenjeni u odnosu na predefinisane kriterijume za uključenje i isključenje, od strane dva autora. **Rezultati.** Regulativa o farmaceutskoj kolaboraciji je zastupljena u mnogim razvijenim zemljama. Međutim, implementacija kolaborativne prakse još uvek nije široko rasprostranjena. Stoga, konceptualni model kolaborativne farmaceutske prakse u zdravstvenoj i socijalnoj zaštiti starijih osoba obezbeđuje uvid u višeslojnu strukturu koja treba biti uspostavljena kako bi se osigurao funkcionalni sistem kolaborativne zdravstvene prakse. Model zaključuje da su aspiracije prema timskom radu, komunikacija i nadasve sistem regulatora i platioca koji prepoznaju zdravstvenu kolaboraciju, ključni za uspostavljanje kolaborativne zdravstvene prakse. **Zaključak.** Ovo istraživanje donosi alat u obliku vodiča i čekliste za donosioce odluka i regulativa, kako bi se istim omogućilo da postignu preferirane efekte prikupljenih kolaborativnih praksi, kroz označavanje modela i aktivnosti koje treba preduzeti za implementaciju one kolaborativne zdravstvene i socijalne zaštite starijih osoba koji su najprimenjiviji u njihovoj zemlji.

Ključne reči:
zdravstvena zaštita; farmaceuti; komunikacija, interdisciplinarna; stare osobe.

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Introduction

Despite the common interest in optimising the work and minimising the potential risks to patients, general practitioners and pharmacists generally strived to work independently with a minimal inter-professional contact in the past [1]. Furthermore, the relationship between the general practitioners and pharmacists was often described as being historically conflicting and rivalling, with major tensions associated with the commercial aspect of the open-type pharmacies [2].

Some papers [3–9] emphasise the need for communication and collaboration among healthcare professionals, for the purpose of ensuring much needed continuity and coordination in healthcare, all towards securing the most favourable outcome for patients. Taking into account the presence of a growing ageing population on the global level as well as the increased health and social problems the elderly are facing, it is suggested that future efforts of the healthcare and social care systems should be focused on ensuring the most adequate meeting of growing demand heading their way [10]. There is a consensus among the authors dealing with social pharmacy of the idea that healthcare and social care systems should be approached holistically. Similarly to such a paradigm, there is indeed an increase in the importance of promotion and enhancement of the models of collaborative healthcare and social practice, with particular focus on the elderly, as a particularly vulnerable group. In this respect, in the past ten years and simultaneously with improvement of the collaborative practice, there was a number of studies published [5–9], which have attempted to explain healthcare and social dimensions of the collaborative practice in an insufficiently empirical manner, according to some authors.

Alongside with increased number of publications which have their focus on the healthcare collaborative practices, there was an increased number of attempts to apply the ideas given in aforementioned publications. In Serbia, for example, some pharmaceutical services described in the Regulation of healthcare services nomenclature at primary healthcare, should be provided in collaboration with physicians [11]. Some of those services are: informing healthcare professionals by pharmacist about rational prescribing and use of medications and medical devices according to approved indications, current therapy guidelines, new knowledge about side effects, or market withdrawals of medications and medical devices, pharmaceutical waste collection and classification, etc. However, a payment model for appropriate reimbursement of pharmaceutical services still does not exist in Serbia.

When considering the collaboration of healthcare and social care systems intended for the elderly, it is impossible not to focus, first of all, on the inter-professional collaboration within the system and then between the two systems specified. Due to the fact that collaboration between healthcare and social care systems is covered by the literature (from the healthcare towards the social care system), in this paper, the emphasis will be placed on the healthcare practice first, to be followed by the healthcare and social care collaboration practice.

In 2003, the Ministry of Health of England initiated that issue and started devising the collaborative practice, with a vision that an open-type pharmacy should be recognised as an integral part of the National Health Service (NHS), and that pharmaceutical services should be better integrated in business operations of other providers of services belonging to the primary level of healthcare – the physicians in particular [12]. In 2005, that was followed by an introduction of conceptual changes for pharmacists, which were aimed at extending the role of pharmacists by ensuring their greater involvement in consultation services, which included the cooperation with physicians [13]. Then in 2008, the Department of Health in England stated that collaboration between pharmacists and physicians had not been developing at the expected pace and investment of additional efforts would be required in the future in order to ensure the proper course of the collaborative concept [14].

The collaborative system of healthcare is focused on a team approach to providing healthcare services to individuals and their families, which would eventually result in a higher level of continuous healthcare [15]. Its roots and development go back to the period of World War II, when healthcare professionals cooperated with each other and joined efforts to ensure proper treatments and cure for wounded soldiers [3]. Nevertheless, the adoption of this approach was delayed, due to an absence of laws and regulations, resistance of healthcare providers who felt threatened as well as a lack of any sort of compensation/reimbursement to third parties in such a healthcare system.

In Denmark, decision-makers and representatives of the country`s authorities have assumed a much needed political initiative to integrate all stakeholders within the system of social care and healthcare for the elderly in the last ten years. The political initiatives resulted in concrete administrative regulations. Such policy aims (emphasised by focusing the overall systemic efforts on the care for the elderly) represents the integral structure of reforms in the local community (municipalities). Described reforms included evaluation of the health and social status of elderly people in their homes, which was carried out by interdisciplinary teams, which would then initiate some improvements to the existing situation after the evaluation [Patient Centred Medical Home (PCMH) a collaborative practice model]. The efficiency of the activities specified above confirmed the necessity of a joint, collaborative action of both healthcare and social sectors, for the purpose of enhancing an adequate healthcare and social care [5]. As soon as the evidence started suggesting the interrelation between the absence of a continuous, coordinated and collaborated healthcare and negative outcomes, the collaborative practice among the healthcare workers of different professions has become a national target. A new, team-based approach connects healthcare professionals such as physicians, pharmacists and nurses. This approach of interdisciplinary collaborative teams provides the availability of patient information to all healthcare workers [16]. That way, they become aware of the overall expectations of patients in terms of the most positive outcome as well as of the expec-

tations of other healthcare employees, to provide, altogether, the full healthcare services and monitor them within the system.

The previous examples and theoretical considerations of issues of collaborative pharmacy practice in a modern society are very challenging to researchers, requiring a critical approach in the analysis of the published models of collaborative pharmacy practice. The purpose of this paper is to give a critical analysis of the proposed models of collaborative practice in the healthcare of the elderly population. Additionally, the paper contains a proposed conceptual model of the collaborative pharmacy practice in healthcare and social care of elderly population.

Methods

A comprehensive search of the bibliographic databases Web of Science and PubMed was undertaken (up to June 2015). Two search algorithms have been created by using combination of Medical Subject Headings (MeSH) and free-text with following Boolean operators: i) [collaborative *near/5 model* AND healthcare (MeSH)] OR [physician (MeSH) AND pharmacist (MeSH)] AND [social care AND healthcare (MeSH)] AND [elderly (MeSH) OR older people] OR [collaboration and *geriatrics* (MeSH)]; ii) [collaboration AND pharmacist (MeSH)] AND [physician (MeSH) OR general practitioner] AND (model OR relationship).

The desk analysis was used to search for all English language articles using the aforementioned databases. In order to select all potentially eligible publications, two reviewers (VOI, VM), assessed independently their title, abstracts and full text against following predetermined inclusion and exclusion criteria (Table 1).

After independent reading of two authors, records that did not fit inclusion and exclusion criteria were discarded. Any disagreement between reviewers was resolved by discussion and consensus.

The literature search (Figure 1) initially yielded 676 articles (Web of Science, n = 201; PubMed, n = 475). After removing 108 identified duplicates, 568 potentially relevant studies were remained for further screening. After screening, 535 publications were excluded based on their title and abstract. There were 7 publications that had unobtainable full copies. After 26 full copies assessed for eligibility and 7 publications additionally added through manual search of reference list, 23 publications were excluded after full copy screening. Once being assessed against inclusion and exclusion criteria, 10 articles remained.

Exploration and consideration was done in accordance to its contribution to development of the collaborative healthcare models: Disease Management (DM), Medication Therapy Management (MTM), PCMH and Accountable Care Organisations (ACO). A dynamic conceptual model of collaborative health care and collaborative pharmacy practice in healthcare and social care of the elderly was generated for better understanding of positive and negative effects on collaborative healthcare models, and for creating a guidebook for implementation of collaborative models into a healthcare system.

Results and discussion

Development of a conceptual model of collaborative healthcare and collaborative pharmacy practice in healthcare and social care for the elderly

The term of collaborative pharmacy practice is defined in different ways. As pharmacists are focused mainly on administering medications prescribed by the physicians, it can be said that the collaborative practice between pharmacists and physicians (and vice versa) is only sporadic, until the point when the patient`s security and positive outcomes became a dominant focus of the entire healthcare system. Being focused on medications is in the nature of the pharmacy profession, and that very focus has led to defining and creating examples of the collaborative pharmacy practice oriented towards the collaborative practice between physicians and pharmacists.

The collaborative practice that also involves pharmacists gradually gained impetus with support of regulatory bodies and positive evidence. The Collaborative Practice Act (CPA), extending to 46 USA states, allows pharmacists to start a voluntary collaboration with physicians and other healthcare service providers, in order to be able to provide a full set of healthcare services to patient [17]. In most US states, there are no special or additional requirements for joining that sort of agreements, besides owning a licence. Still, in several states of the USA, the American Society of Health-System Pharmacists (ASHP) requires a certain number of years (years of service) spent working at a clinic or a similar health facility upon finalising the licencing procedure.

Table 1

Inclusion and exclusion criteria

Inclusion criteria:		
i.	Language:	English.
ii.	Type of study:	Qualitative and quantitative study.
iii.	Processes analysed:	Inter-professional work, collaboration, attitudes towards team work, collaborative models, payment models and systems, system and legislative barriers, healthcare and social care services for the elderly.
iv.	Team structure:	At least one pharmwacist involved in a multidisciplinary team or collaborative process.
Exclusion criteria: Studies focused solely on the quantification of the inappropriate prescribing of drugs and incidence of dispensing errors.		

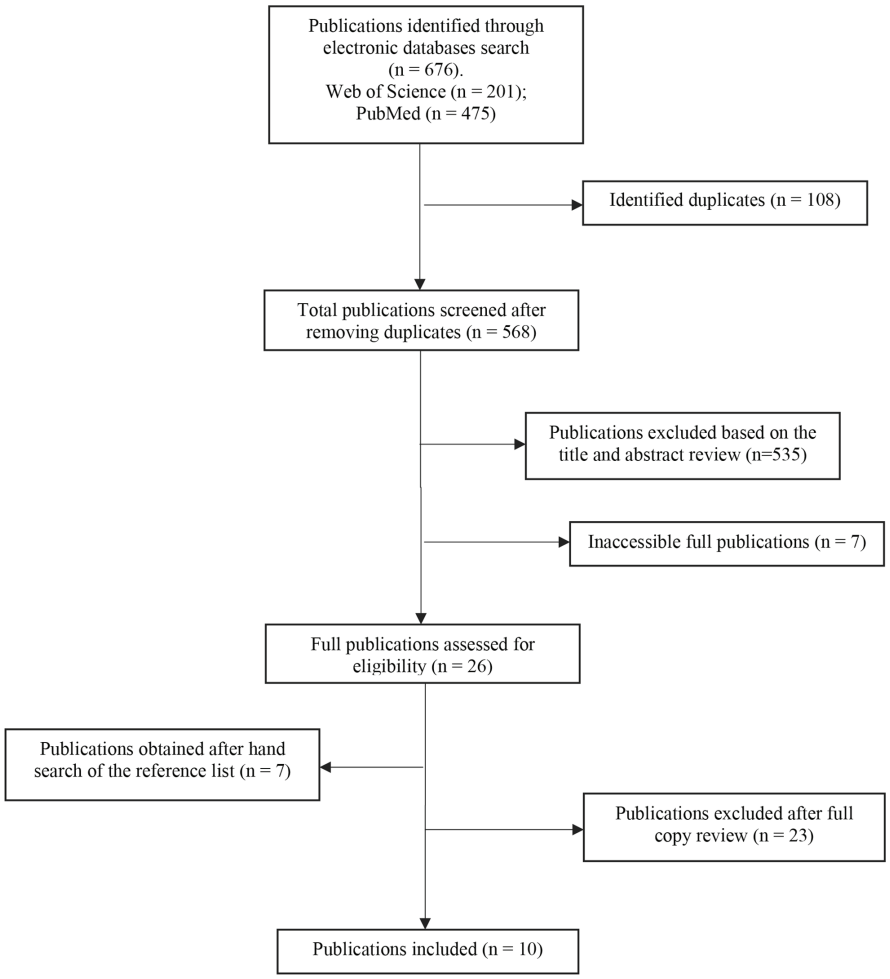

Fig. 1 – Literature search pathway.

The collaborative practice of pharmacists and physicians is focused on a medication therapy management, therapy adjustment, patient consultation, and eventually, identification, elimination and prevention of any interactions between medications or a drug-food interaction. Most forms/types of the collaborative practice involve pharmacists cooperating with one or several physicians, in the clinic/dispensary or hospital practice, i.e., some form of clinical healthcare or similar activity. Pharmacists in other countries have arrangements (agreements or contracts) with physicians similar to the CPA; in Quebec, Canada, for example, pharmacists are allowed to initiate and change the therapy prescribed by a physician as well as to demand laboratory analyses, if considered necessary [6]. Hence, the collaborative pharmacy practice may be understood as a physicians and pharmacists team gathering primarily (whether collocated or not) around the same goal, i.e., the best possible outcome for the patient. Before establishing any collaborative practice, healthcare professionals certainly need to show their willingness to overcome the traditional communication and inter-professional barriers (by changing their attitude about the exclusive professional work independence). It is also necessary to have a legislative, political and economic will to support such inter-professional association (by developing a legislative and economic infrastructure through implementation of regulations and payment modalities).

Due to the fact that the literature [7–8] present the four most represented models of healthcare and social collaborative practice, it is relevant to start analysing them in order to see the advantages and limitations of the described models. The static nature of these models is evident from the very beginning. Although Bradley et al. [7] explained in detail all the phases and activities that precede collaboration, very little attention was paid to the dynamics of collaborative relations through particular collaborative models that were already operational in the collaborative practice of several developed countries. In addition, visibility of individuals (patient and healthcare professional`s point of view) in collaborative models is quite infrequent, despite a growing number of authors who have recently described four most represented models of the collaborative healthcare practice (the MTM, primarily). In this regard, our team of authors agrees that it is necessary to conduct a further analysis of the four most operative models of healthcare and social practice from the perspective of the service provider, as well as the perspective of patients/elderly people and the system/payers.

It may be noted in Tables 2a and 2b that there are positive and negative aspects in each model of the collaborative practice as well as different aspirations of the involved participants towards team pooling. Harmonisation of diversity of aspirations among different participants in a collaborative practice as well as development of solutions based on compromise are the greatest challenges in organising the collaborative models in the practice of one country. Accordingly, the significance of understanding of each model by different providers of health and social care services is equally invaluable as by patients/elderly people and system/payers. In addition, the aforementioned models of collaborative practice in the healthcare and social care of elderly people are organised at different locations. Therefore, it would be beneficial to designate the places, where particular models of collaborative practice are organised, or locations and health facilities a patient needs to visit in order to receive the healthcare (or healthcare and social care services) within a collaborative practice model. Thus, new questions have emerged as well as the willingness to figure out the adequate responses after conducting a detailed analysis of the existing models. This ambition resulted in creating an idea and a need for conceptualising a new model, which would enable providing clear guidelines for the planned, gradual introduction of collaborative models into a practice of any country, by ensuring a profession-based connection of collaborative models and creating a dynamic model.

Table 2a

Analysis of the most represented healthcare and social collaborative practice models – perspective of service providers

Collaborative practice models	Scope of the model	Physician	Pharmacist	Social worker
ACO (organisations of healthcare providers)		Organisations of healthcare providers that agree on the payment based on the performance of services provided. Such organisations focus on the most positive outcomes for their patients and function within the Medicare programme.		
	+	Most optimal outcomes for the patient. Shared responsibility.	Acknowledgment of all additional services provided to the patient.	Involvement of a third party for payment. Private funds.
	-	More time consuming. If the level of performance is not met, payment is disputed.	Due to shared responsibility, the setting is team-oriented, but more effort invested in own services. Team autonomy in part.	Model not available to all patients. Lesser involvement of social workers.
PCMH (healthcare services provided at patient's home)		Focused on a team, collocated therapy for chronic diseases. Developed as a result of a lack of physicians. Includes teams composed of physicians, pharmacists, social workers, nurses, carers, nutritionists, etc. Provides outpatient services mainly.		
	+	Increased trust in colleagues owing to the team spirit. More optimal outcomes.	Increased quality of the provided service through a holistic approach to the patient's condition.	High and direct engagement of social workers. Assessment of the social status.
	-	Necessity of having a clear plan for service provision (according to items on the list).	Visiting patients on site. Loss of time. Necessity for recognising the model by the state programme.	Higher patient selectivity compared to the social status.
MTM		Medication Therapy Management including greater involvement of pharmacists in patient's therapy, both in pharmacies and on site. It includes: analysis of medication therapy, pharmacotherapeutic consultation, anticoagulation therapy management, immunisation, health and wellness programmes.		
	+	Delegating responsibility and trust to the pharmacist.	Therapy management for several chronic diseases. Person in charge of therapy prescription. Respect and trust.	Higher savings for the social care system.
	-	Taking away a part of autonomy. Frequent collocation.	Payment models and economic acknowledgement of additional services.	Non-inclusion of social workers.
DM		Education of patients about medications, continuous monitoring (by the physician and pharmacist) of patients with highly prevalent chronic conditions; in case of several treatment modalities; a possibility of self-care; carrying a significant economic burden. Multiway communication.		
	+	Transfer of patients' education to pharmacists.	One chronic disease. Patient education. Enhancement of the outcome.	Periodical use of the model.
	-	Complexified communication.	No possibility to request further analyses and prescriptions.	Non-inclusion of social workers.

ACO – Accountable Care Organisations; PCMH – Patient Centred Medical Home; MTM – Medication Therapy Management; DM – Disease Management.

Table 2b

Analysis of the most represented healthcare and social collaborative practice models – elderly and system perspective

Collaborative practice model – elderly	Scope of the model	Patient/elderly people	System/payers of services
ACO (organisations of healthcare providers)		Organisations of healthcare providers, who agreed on the payment based on the performance of services provided. Such organisations focus on the most positive outcomes for their patients and function within the Medicare programme.	
	+	Full healthcare service in one place. Plenty of time is dedicated to patients.	Directing the costs of system to a third party.
	-	A small number of patients are able to afford this type of healthcare.	It is necessary to include the third party as the payer, i.e., private funds.
PCMH (healthcare services provided at patient's home)		Focused on a team, collocated therapy for chronic diseases. Developed as a result of a lack of physicians. Includes teams composed of physicians, pharmacists, social workers, nurses, carers, nutritionists, etc. Provides outpatient services mainly.	
	+	Patients is able to receive the full healthcare and social service at their home.	A holistic approach to the condition of the patient reduces the costs by decreasing the likelihood of administering the wrong therapy.
	-	May disturb the peace and privacy of other tenants.	Increased costs for the system. Work of the organisation gets complicated and travel costs are higher.
MTM		Medication Therapy Management including greater involvement of pharmacists in patient's therapy, both in pharmacies and on site. It includes: analysis of medication therapy, pharmacotherapeutic consultation, anticoagulation therapy management, immunisation, health and wellness programmes.	
	+	A patient does not have to visit a physician to get the prescription for a chronic disease therapy.	Reducing the workload of physicians who are often unavailable. Reducing the time and procedure for receiving the appropriate therapy.
	-	Possible non-determination of the designated pharmacist who would monitor the patient's condition for a longer period of time.	More complicated payment and valorisation of the additional work.
DM		Education of patients about medications, continuous monitoring (by the physician and pharmacist) of patients with highly prevalent chronic conditions; in case of several treatment modalities; a possibility of self-care; carrying a significant economic burden. Multiway communication.	
	+	Patients are provided with the necessary education on diseases at the pharmacy.	Reducing costs for an organisation and making appointments. Increase in potential loss of earnings for employees due to long waiting periods at the physician's.
	-	Traditionally, patients have more confidence in physicians. Ensuring one's privacy.	The system is generally not familiar with provision of these additional services by pharmacists.

ACO – Accountable Care Organisations; PCMH – Patient-Centred Medical Home; MTM – Medication Therapy Management; DM – Disease Management.

Consideration of the proposed conceptual model of collaborative healthcare and pharmacy practice in elderly healthcare and social care

The conceptual model presented in Figure 2 suggests a holistic approach to the implementation of collaborative models that must be considered in a multi-way manner where each phase represents the activity already completed in previous phases. Therefore, the very analysis of the proposed conceptual model should be approached from all directions and in a multi-way manner, representing the natural dynamics of the system. Certainly, it should be emphasised at the very beginning of the analysis that the system of healthcare and social care could still function traditionally independently. In such case, any form of collaboration, in terms of its continuity and planned organisation, is actually made impossible. However, it might still be possible for a physician to call a pharmacist by the phone for an eventual

consultation regarding a particular medication, respecting his/her expertise in pharmacology. Nevertheless, that case does not constitute a form of collaborative practice, but actually a traditional isolated form of the healthcare practice. This case is presented in the bottom left corner of the pyramid in Figure 2. It represents a negative value (absence of a collaborative practice), if the pyramid is considered a coordinate system. Anything occurring prior to the origin is a number of preliminary activities that need to be undertaken, with a clear intention of having a collaborative association for enabling a collaborative practice to begin with. More details on the preliminary activities of collaboration were discussed by Bradley et al. [7], so the proposed conceptual model shown in Figure 2 does not describe them. Traditional isolation at the ground zero is followed by an initiation phase (provided there is a team cooperation aspiration). The initiation phase continues further and coincides with the phase of communication and collaboration. The highest level comprises the phase of integration of all providers of the healthcare and social care services and the system/payers.

Disease Management (DM) provides education to patients regarding medications and continuous monitoring of chronic diseases. It may be organised within a pharmacy, and represent a healthcare collaboration only. Although regular activities of pharmacists include consultation services about medications, DM comprises certain monitoring activities and supervision of chronic conditions of a particular patient. Educational seminars could also be organised in a planned manner, for a particular type of chronic disease on pharmacy premises, in a form of informative and confidential workshop. The same form of organisation may be used within a health centre, i.e., at the primary level of healthcare. Collaboration in disease management often includes delegating traditional activities of physicians to pharmacists (monitoring of chronic diseases), or, on the other hand, a joint association for organised consultations.

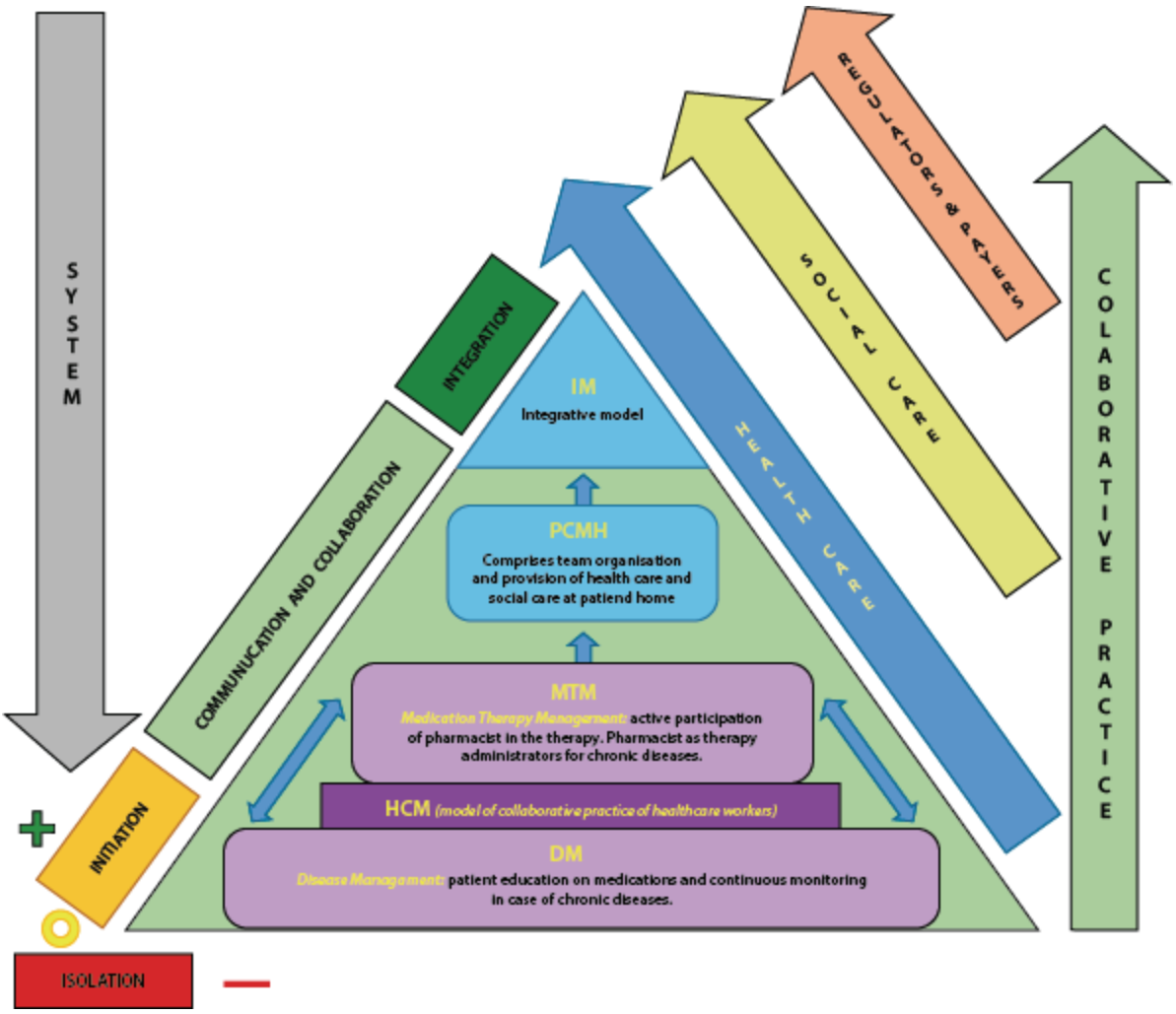

Fig. 2 – Conceptual model of collaborative healthcare and pharmacy practice in healthcare and social care for the elderly.
IM – Intergrative model; PEMH – Patient-Centred Medical Home; MTM – Medication Therapy Management; DM – Disease Management; HCM – Hospital Case Management.

Medication Therapy Management (MTM) is certainly the most thoroughly described model of the collaborative healthcare practice in relevant literature. MTM ensures an active participation of pharmacists in a therapy. Implementation of this model includes establishment of a very high level of inter-professional communication. It often happens that a pharmacist becomes an administrator of medications for a chronic disease (when the complete therapy management may be performed by a pharmacist, and following the diagnosis established by a physician) after taking an additional year of education at a faculty of medicine. In this model of the collaborative healthcare practice, a significant responsibility is given to the system/payers, which is/are required to establish a functional payment system for additional services provided by the pharmacists. If the very MTM is considered, patients could encounter this model at the pharmacy. In this phase of explanation of the collaborative model, it should be noted that MTM may be an integral part of Patient Centred Medical Home (PCMH) and a part of an Integrated Model, while the MTM would always include DM as well. Due to such a connection between a disease management and medication therapy models, these two constitute the hospital Case Menagment (HCM) model of collaborative practice of healthcare workers, because they are allowed to organise themselves independently from the social workers.

PCMH is a model of team organisation and provision of healthcare and social care services at patient`s homes. This form of practice is mainly focused on the elderly, those with difficulty walking and/or persons whose homes are located far away from the healthcare facilities (primary or secondary level). Patients/elderly people may use this model in their own homes, when a collaborative team visits them upon confirming the visit. It is obvious at this level that this model includes social care for the elderly in addition to the healthcare (as shown in a pyramid in Figure 2). Besides the healthcare workers (e.g.: physicians, pharmacists, nurses, therapists, etc.), the PCMH model teams also involve the social workers, who visit the homes of elderly persons and provide them with a range of necessary healthcare and social services as well. This model includes both MTM and DM.

The Integrative Model (IM) is a model with the highest level of collaboration. Actually, the IM is generally used as the Accountable Organisations (ACO) model, functioning within the Medicare. In addition to the health and social care, this level of collaborative practice includes a payer, i.e., the third party (mainly private funds) and naming it an integrative model was quite logical. The patient goes to an organisation – a health facility where he/she is provided with a full collaborative service. The model relies on a high level of communication, often supported by information systems, which connects all members of the team, who keep a joint record on the patient/beneficiary.

The conceptual model comprises both: pull and push strategies, or learning about the system needs for collaborative models and their support as well as a promotion of collaborative models and establishing positive regulatory provisions, etc. In this respect, it is obvious that the system functions in a multi-way manner, but primarily from the top of the pyramid (regulations and regulators) towards the bottom of the pyramid as shown in Figure 2.

Stimulations and obstacles to collaborative practice in geriatric care

Individual ageing is a natural and inevitable process that we all face constantly. Population ageing, unlike the previous one, is a unique phenomenon in a demographic history of mankind caused primarily by industrial, sexual revolution and absorption of women into the labour markets. Its ultimate outcomes, increased early childhood survival combined with the extended longevity in most nations jointly contribute to the growing share of elderly citizens in most contemporary societies [18]. Those demographic trends of further population ageing are present in European countries, and represent one of the greatest challenges encountered in the healthcare, social and economic systems of those countries. According to the Eurostat [19] information from 2014, the population above the age of 65 would increase approximately 50%, from 18.2% at the time (data from 2013) to 28.1% by 2050. In this respect, the share of the working-age population would also change, and thus, according to the current trends, by 2050, the ratio between the working-age population and population above the age of 65 would decrease from the current rate of 4 : 1 to 2 : 1 [19]. Therefore, in addition to a higher life-expectancy for the population [20], the age limit for working-age population would also change, and so the healthcare system for the elderly would have to operate at a higher system performance level [21]. This situation puts a positive pressure on the healthcare and social care systems regarding developing new and innovative models to be able to adequately respond to growing demands set before the healthcare and social care systems for the elderly. Taking into account that visibility of older people in rural area is even lower than the visibility of older people in urban areas, which is correlated with higher depression of older people in rural than in urban areas [22], it is highly important to achieve functional MTM collaborative practice, especially in rural areas. Due to that, a holistic approach to perceiving the problems shared by the aforementioned systems produced several collaborative and inter-professional models of pharmacy practice within the healthcare and social care for the elderly.

Collaborative practice in the field of geriatric care takes place in most cases only upon establishing some of the healthcare collaborative practice models as described above. Therefore, it should not come as a surprise that only few papers discuss this topic, although the importance of collaboration in geriatric care is unquestionable. The paper by Young et al. [23] is listed in the literature as a reference paper for establishing the foundations and guidelines for a further research into the problem of collaboration in the field of geriatric care. Young et al. [23] provided their contribution to the given sensitive issue by summing up all the stimulating and restricting factors encountered by the collaborative practice in the field of geriatric care.

Conclusion

The proposed collaborative practice model integrates dynamically the most represented models of the collaborative practice in the healthcare and social care for the elderly, providing a new insight into the described models from the perspective of service providers, patients/elderly people and systems/payers. Additionally, it is necessary to ensure understanding of multi-way relations within collaborative practice models (with healthcare and social care systems both of the payer and the regulator, as active participants in the background). Thus, a conceptual model should be considered prior to the actual implementation of the model of the collaborative practice in the healthcare and social care for the elderly, within the system of a country, in order to be more certain about selecting the models and activities that need to be undertaken in order to achieve the preferred effects generated from the collaborative practice.

If one considers the implementation of a collaborative model of the healthcare and social care practice by focusing on the elderly, the course of action should be based on an efficient overcoming of challenges as well as the establishment of a primary model of Medication Therapy Management in phases. Subsequently, the collaborative practice should be extended to include the implementation of the PCMH model which is the most significant model for the elderly population. Certainly, the basis for each model of collaborative practice focusing on the elderly is the pharmacy collaborative practice. That is why the pharmacy collaborative practice is the unavoidable, initial and conditional (*conditio sine qua non*) basis for the collaborative practice model focusing on the elderly.

Finally, it may be useful to conclude once again, that by inclusion of a positive regulatory pressure, together with an efficient implementation of the payment model for the collaborative pharmacy practice, all other obstacles on the path of implementing a collaborative pharmacy model should be overcome, taking into account the positive attitude towards the collaborative practice shared by a society, healthcare and social care professionals.

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S H O R T
C O M M U N I C A T I O N S

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Possibilities of retrograde intrarenal surgery in the treatment of renal lower pole stones in children

Mogućnosti retrogradne intrarenalne hirurgije u lečenju kalkulusa u donjem polu bubrega kod dece

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Abstract

Background/Aim. Renal stones located in the lower pole of kidney represent a serious challenge for surgical treatment in children. The options are: open surgery, extracorporeal shock-wave lithotripsy, percutaneous nephrolithotomy and retrograde intrarenal surgery. Reports about the endoscopic treatment in children are limited. The aim of the study was to evaluate the effectiveness of retrograde intrarenal surgery in pediatric patients with renal stones in lower pole of the kidney. **Methods.** We retrospectively analyzed the results of the retrograde intrarenal surgery in 24 patients with renal stones in lower pole, between April 2012 and April 2016. Flexible ureterorenoscopy in combination with holmium laser lithotripsy were performed. We considered stone fragment size 3 mm or less as a measure of sufficient fragmentation of the stone. **Results.** Mean duration of general anesthesia was 68 (range, 40–90) minutes. Duration of hospitalization was 1–3 (mean, 1.6) days. Complications were found after two (8.4%) surgical procedures: perirenal haematoma in one (4.2%) and urinoma in one (4.2%) patient. The stone was completely fragmented in 18 (75%) patients. In 3 (12.5%) patients the stone was incompletely fragmented and in 3 (12.5%) patients the stone was not fragmented. Double J stent was placed in 5 (21%) patients. Mean follow-up was 9 (range, 6–18) months. **Conclusion.** Retrograde intrarenal surgery in children is the least invasive, effective and safe surgical procedure for stones in lower pole of the kidney, with minimal complication rate. Unsuccessful treatment in some patients was due to loss of ureterorenoscope deflection with laser probe in working channel.

Key words:
child; kidney calculi; lithotripsy, laser; postoperative complications; treatment outcome.

Apstrakt

Uvod/Cilj. Kalkulusi lokalizovani u donjem polu bubrega predstavljaju veliki izazov u hirurškom lečenju kod dece. Mogućnosti su: otvorena hirurgija, ekstrakorporalna litotripsija, perkutana nefrolitotripsija i retrogradna intrarenalna hirurgija. Broj publikacija o endoskopskom lečenju urolitijaze kod dece je ograničen. Cilj istraživanja bio je da se utvrdi efikasnost retrogradne intrarenalne hirurgije kod pedijatrijskih bolesnika sa kalkulusima lokalizovanim u donjem polu bubrega. **Metode.** Retrospektivno su analizirani rezultati retrogradne intrarenalne hirurgije kod 24 bolesnika sa kalkulusima u donjem polu, u periodu od aprila 2012. do aprila 2016. godine. Primenjena je fleksibilna ureterorenoskopija u kombinaciji sa laserskom litotripsijom. Kao mera uspešne dezintegracije kalkulusa smatrana je veličina partikule od 3 mm ili manja. **Rezultati.** Dužina opšte anestezije prosečno je iznosila 68 (opseg 40–90) minuta. Dužina hospitalizacije iznosila je 1–3 (prosečno 1,6) dana. Komplikacije su zabeležene posle dve (8,4%) hirurške intervencije: perirenalni hematom kod jednog (3,1%) i urinom kod jednog (3,1%) bolesnika. Kalkulus je bio u potpunosti dezintegrisan kod 18 (75%) bolesnika. Kod 3 (12,5%) bolesnika kalkulus je bio delimično dezintegrisan i kod 3 (12,5%) bolesnika kalkulus nije bio dezintegrisan. "Double J" stent plasiran je kod 5 (21%) bolesnika. Prosečni period praćenja iznosio je 9 (opseg 6–18) meseci. **Zaključak.** Retrogradna intrarenalna hirurgija kod dece je najmanje invazivan, efikasan i bezbedan metod hirurškog lečenja kalkulusa lokalizovanih u donjem polu bubrega, sa

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niskom stopom pojave komplikacija. Razlog neuspešnog ishoda kod pojedinih bolesnika jeste gubitak savitljivosti ureterorenoskopa sa laserskom sondom u radnom kanalu.

Ključne reči:
deca; nefrolitijaza; litotripsija, laser; postoperativne komplikacije; lečenje, ishod.

Introduction

The surgical treatment of urolithiasis in children is basically similar to treatment in adult patients, but anatomic and physiologic specificities makes it more difficult in pediatric patients [1, 2]. It is very clear that the narrow urinary tract in children is one of the biggest problems [3]. Renal stones located in the lower pole of the kidney represent the biggest challenge for surgical treatment in all patients, especially in children [4]. The options are: open surgery, extracorporeal shock-wave lithotripsy, percutaneous nephrolithotomy and retrograde intrarenal surgery. Open surgery is, in general, an out-of-date technique. Shock-wave lithotripsy is very limited for lower pole stones. Percutaneous nephrolithotomy is effective, but more invasive technique than the endoscopic treatment. Retrograde intrarenal surgery is the least invasive technique, but reports on the treatments in children are limited [5–7].

The aim of the study was to evaluate the effectiveness of a retrograde intrarenal surgery in pediatric patients with renal stones, located in lower pole calices of the kidney. We also evaluated the limitations of the endoscopic treatment in lower pole of the kidney.

Methods

We retrospectively analyzed the results of the retrograde intrarenal surgery in 24 patients with renal stones located in the lower pole calices of the kidney (Figures 1 and 2). The patients were treated between April 2012 and April 2016 (10 girls and 14 boys, mean age 9.2 years (range 4–18 years).

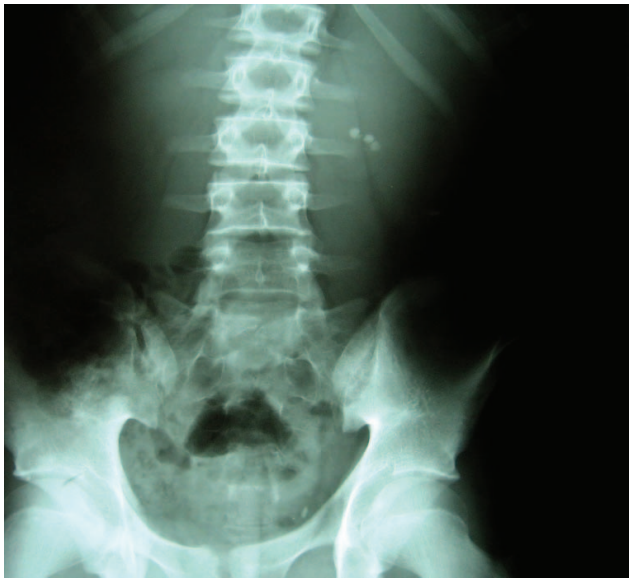

Fig. 1 – Stones in the lower pole of the left kidney, ureter and bladder (KUB) radiography.

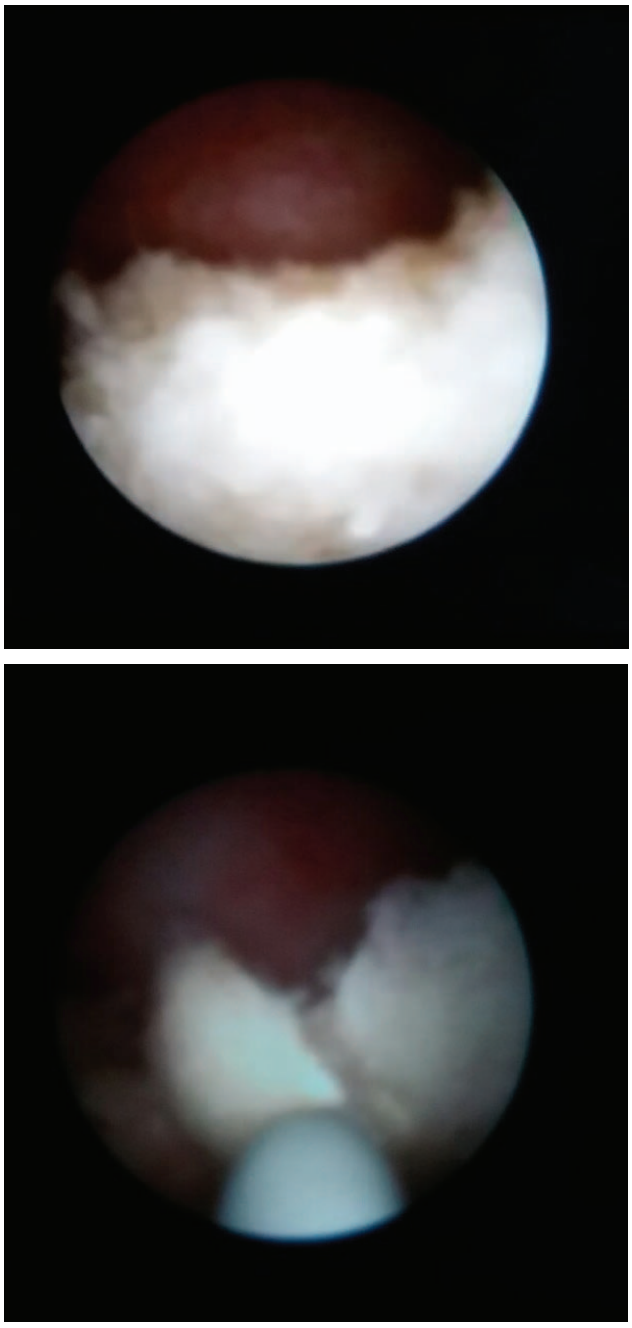

Fig. 2 – Endoscopic view of the retrograde intrarenal surgery in the lower pole of the kidney.

Patients with renal stones in other segments of the kidney (upper pole calices, renal pelvis) were excluded from the study. Flexible 7.5-F ureterorenoscopy with possibility of deflection of 270, in combination with holmium laser lithotripsy were performed in all patients, under the general anesthesia. Routine and bacteriological analysis of urine and kidney function tests were also evaluated. In all patients metabolic screening of urine was performed to find the cause

of stone formation. Ultrasound and kidney, ureter and bladder (KUB) radiography were performed to identify the location of the stone. Stones were measured by ultrasound. Patients received preoperative antimicrobial prophylaxis. After introduction through the ureteral orifice, flexible ureterorenoscope was placed through ureter to the renal pelvis. Then, the deflection of the flexible ureterorenoscope was performed in order to achieve an adequate stone visualization. We used a 365 μm and 230 μm probes with a 3 mV green helium light guide for transferring energy from the lithotripter to the stone. Micro laser fibers were used, generating 0.2 to 2 J at a frequency of 5 to 10 Hz. We considered stone size 3 mm or less as a measure for sufficient fragmentation of the stone. Some bigger particles were removed from the urinary tract by a stone-basket and smaller ones were left for spontaneous ejection. If ureteral wall damage was present, 4-F or 4.7-F double J stent was placed depending of the constitution and the age of the patient. We used to remove it after two to five days after the surgery. Ultrasound examination was performed in all patients during the first postoperative day. Depending on the severity of surgery, the patients were discharged between the first and the third postoperative day. After one, three and six months, patients were evaluated by urinalyses, kidney function tests, ultrasound and, in some cases, by KUB radiography. The size of the residual stone bigger than 3 mm was the indication for retreatment.

Results

Flexible ureterorenoscopy and holmium-laser nephrolithotripsy were performed in the total number of 35 procedures in 24 patients. There were 10 (41.7%) girls and 14 (58.3%) boys. Mean age was 9.2 years (range 3–18). In 20 (83.3%) patients a single stone was found and in 4 (16.7%) patients the multiple ones. Stones were located in left kidney in 9 (37.5%), in right kidney in 11 (45.8%) and in both kidneys in 4 (16.7%) patients. Mean stone size was 13 mm (range, 8–26 mm). Bacteriological findings of urine were normal in all patients (sterile urine culture) and also, kidney function tests (urea, creatinine) were within reference values (Table 1).

Table 1

Clinical profile of patients

Patients' characteristics	Values
Number of patients	24
female, n (%)	10 (41.7)
male, n (%)	14 (58.3)
Mean age (years), mean (range)	9.2 (3–18)
Single stone, n (%)	20 (83.3)
Multiple stones, n (%)	4 (16.7)
Stone side, n (%)	
left	9 (37.5)
right	11 (45.8)
bilateral	4 (16.7)
Mean stone size, mm (range)	13 (8–26)
Urine culture, n (%)	
sterile	24 (100)
UTI	/

n (%) – number (percentage) of patients; UTI – urinary tract infection.

Mean duration of general anesthesia was 68 minutes (range, 40–90 minutes). Duration of hospitalization was 1–3 days (mean, 1.6 days). Complications were found after two (8.4%) surgical procedures: perirenal haematoma in one (4.2%) and urinoma in one (4.2%) patient. The stone was completely fragmented in 18 (75%) patients. In 3 (12.5%) patients the stone was incompletely fragmented and in 3 (12.5%) patients the stone was not fragmented. Double J stent was placed in 5 (21%) patients. It was removed 2–5 days (mean 3.5 days) after the surgery. Mean follow-up was 9 months (range, 6–18 months) (Table 2).

Table 2

Results of surgical treatment

Parameters	Values
Number of procedures	35
Anesthesia duration (min), mean (range)	68 (40–90)
Retreatment, n (%)	11/24 (45.8)
Mean hospitalization (days), mean (range)	1.6 (1–3)
Complications, n (%)	2/24 (8.4)
perirenal haematoma	1/24 (4.2)
urinoma	1/24 (4.2)
Complete fragmentation, n (%)	18/24 (75)
incomplete fragmentation	3/24 (12.5)
no fragmentation	3/24 (12.5)
double J stent	5/24 (21)
Mean follow-up, (months), mean (range)	9 (range 6–18)

n (%) – number (percentage) of patients.

Discussion

Stones located in the lower pole calices of the kidney always represent one of the biggest problem in the surgical treatment of urolithiasis, especially in children [7, 8]. It is difficult to decide what kind of treatment is optimal in every particular case. Extracorporeal shock-wave lithotripsy (ESWL) is not sufficiently effective for stones located in that part of the kidney. On the other hand, there are many reports about very serious side effects of that kind of treatment in children after long term follow-up such as diabetes and hypertension. That is why many authors nowadays do not suggest ESWL as good choice of the treatment of renal stones in children [9, 10]. Also, some authors suggest combination of ESWL and retrograde intrarenal surgery for the most complicated cases [11].

When we talk about lower pole stones in children, the data in current literature are very limited. It is difficult to find guidelines or relevant suggestions on how to treat those patients [12]. Some authors suggest only observation/medical treatment option for asymptomatic patients [4]. Retrograde intrarenal surgery is mentioned like the best and less invasive surgical approach. However, that kind of treatment is associated with serious technical problems during flexible ureterorenoscopy in narrow urinary tract in children. When the stone is visualized and available for laser probe, the treatment is highly effective. In some patients, even if we visualize the stone, when the laser probe is inside the working channel, sufficient deflection of the flexible ureterorenoscope is lost. In that case the stone is not available for laser beam and the lithotripsy is impossible [12, 13].

Thus, the last observation represents limited success rate in the treatment of lower pole renal stones in children in our study. All other problems during flexible ureterorenoscopy were overcome, but problem of loss of ureterorenoscope deflection when the laser probe was inside the working channel could not be solved. We can state that was the only reason for unsuccessful treatment in one quater of our patients. Those patients were selected for percutaneous nephrolithotomy. The other option is open surgery, but that kind of treatment is no longer in the protocol for surgical treatment of nephrolithiasis in our institution [13–15].

The results of retrograde intrarenal surgery for the treatment of lower pole stones are different in various publications. Bozkurt et al. [4] report stone-free rate of 94%, while Kim et al. [8] report stone-free rate of 47%. In our series stone-free rate was 75%. Considering small invasiveness of that procedure in comparison with alternative surgical techniques, it represents a good result.

The occurrence of complications in retrograde intrarenal surgery is associated with stone composition, morphological and physiological conditions, constitutional characteristics of the patient, use of adequate equipment and surgeons' experience in endoscopic surgery. Ureteral perforation, urinoma, bleeding, renal puncture with instruments or accessories, postoperative hydronephrosis, urinary tract infection, urosepsis, etc. are possible complications [16–18]. None of them was found in our series. There were only two complications: perirenal haematoma in one patient and urinoma, also, in one patient. Those are, the so-called, "minor" complications, which do not affect the final outcome of the treatment (Grade II, Clavien-Dindo classification of surgical complications) [19]. Two days of prolonged hospitalization with bed rest and antibiotic intravenous therapy, were measures for the treatment of those patients. After three days ultrasound findings were correct.

Ureteral stenting after endoscopic lithotripsy was always controversial. In the past, that was a mandatory procedure, but recently it has been applied in fewer cases, required only in case of mucosal damage and in case there was a risk of ureteral stone particles obstruction [20]. In our series, five patients required double J stenting when the surgeon estimated that the degree of mucosal damage was significant. Double J stent was removed 2–5 days after the surgery and did not affect the final outcome of the treatment.

Conclusion

Retrograde intrarenal surgery in children is the least invasive surgical procedure for the treatment of stones, located in lower pole calices of the kidney. It is effective and safe kind of treatment, with minimal complication rate. In some patients retrograde intrarenal surgery is not effective because of specific anatomic conditions in lower pole, when the stone is not available for laser beam, even the deflection of flexible ureterorenoscope is maximal. In these patients the use of alternative surgical procedures should be considered, primarily percutaneous nephrolithotomy.

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The effect of physical therapy in the treatment of patients with cervical dystonia with or without concomitant use of botulinum toxin

Efekat fizikalne terapije u lečenju obolelih od cervikalne distonije sa ili bez istovremene primene botulinskog toksina

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Abstract

Background/Aim. Botulinum toxin is a basic, recommended method of treatment in controlling cervical dystonia (CD). Physical therapy has limited effect due to the nature of the disease that is a result of a disorder in structures and relationships of the basal ganglia. The aim of this study was to analyze the effect of physical therapy applied as monotherapy, or with parallel application of botulinum toxin in patients with CD. **Methods.** Randomized controlled clinical pilot study included 14 patients diagnosed with idiopathic CD. All patients were initially assessed by using the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) and Torticollis Rating Scale (Tsui scale). In the control group, composed of 5 patients, the treatment included only physical therapy. The experimental group (9 patients) was first given botulinum toxin, and physical therapy was applied after five days. Physical therapy was conducted five times a week in the period of two weeks at the Clinic. Patients of both groups were instructed to continue with the physical therapy at home. The effects of treatment were analyzed after 1, 3 and 6 months using TWSTRS and Tsui scale. **Results.** At the beginning of the investigation, the differences in TWSTRS and Tsui scale between the groups were not significant. In the control group, after 1 month, significant improvement was achieved in all three parts of the TWSTRS. After 3 and 6 months, the effects of physical therapy were reduced to control levels. In the experimental group, highly significant increase of all parameters of TWSTRS was noted after 1, 3 and 6 months. In the control group, highly significant decrease of changes in Tsui scale was noticed only after one month while in the experimental group, it was maintained after 3 and 6 months. **Conclusion.** Application of physical therapy provides a significant improvement in disease severity, but the effect is better and of longer duration when combined with the botulinum toxin.

Key words:
cervical dystonia, primary; physical therapy modalities; botulinum toxins; classification.

Apstrakt

Uvod/Cilj. Primena botulinskog toksina predstavlja osnovnu, preporučenu metodu lečenja u kontroli cervikalne distonije (CD). Fizikalna terapija ima ograničene domete s obzirom na prirodu bolesti koja je posledica poremećaja u strukturama i vezama bazalnih ganglija. Cilj ovog rada bio je analiza efekta fizikalne terapije primenjene u smislu jedinog terapijskog modaliteta ili uz paralelnu primenu botulinskog toksina kod bolesnika sa CD. **Metode.** Randomno, kontrolisano kliničko istraživanje po tipu pilot studije obuhvatilo je 14 bolesnika kod kojih je postavljena dijagnoza idiopatske CD. Kod svih bolesnika inicijalno je procenjena težina bolesti korišćenjem *Toronto Western Spasmodic Torticollis Rating Scale* (TWSTRS) i *Torticollis Rating Scale* (Tsui skala). U kontrolnoj grupi, sastavljenoj od 5 ispitanika, lečenje je podrazumevalo fizikalnu terapiju, a u eksperimentalnoj, sastavljenoj od 9 bolesnika, prvo je dat botulinski toksin, a posle pet dana je primenjena fizikalna terapija. Fizikalna terapija je primenjivana 5 dana nedeljno tokom 10 dana na Klinici. Bolesnicima obe grupe je receno da nastave sa fizikalnom terapijom u kućnim uslovima. Efekti terapije analizirani su nakon 1, 3 i 6 meseci korišćenjem TWSTRS i Tsui skale. **Rezultati.** Na početku istraživanja, razlike u TWSTRS i Tsui skali između grupa nisu bile značajne. U kontrolnoj grupi, nakon 1 meseca, postignuto je značajno poboljšanje u sva 3 dela TWSTRS. Nakon 3 i 6 meseci, efekat fizikalne terapije smanjen je do početnog nivoa. U eksperimentalnog grupi, postignuto je značajno poboljšanje svih praćenih parametara TWSTRS

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nakon 1, 3 i 6 meseci. U kontrolnoj grupi, značajno smanjenje parametara u Tsui skali primećeno je samo nakon 1 meseca, dok se u eksperimentalnoj grupi održavalo i nakon 3 i 6 meseci. **Zaključak.** Primena fizikalne terapije daje značajno poboljšanje težine bolesti, ali je efekat bolji i dugotrajniji ako se primenjuje uz botulinski toksin.

Ključne reči:
cervikalna distonija, primarna; fizikalna terapija, metodi; botulin, toksini; klasifikacioni indeksi.

Introduction

Cervical dystonia (CD) is the most frequent form of focal dystonia in adults, with typical repeated spastic (clonic) or prolonged (tonic) muscle contractions which cause non-physiologic movements, or positions of the head, neck and shoulder. Torticollis, lateral shift, and oscillatory movements can follow as a consequence of CD. This disease is more frequent in females, with the incidence of 1: 10, 000 [1].

Certain activities and stress can increase positional changes in CD. Temporary amelioration of the disease can be seen in the morning after rest, after antagonistic movements, or particular positions. CD typically worsens during the first five years of illness, with stabilization of the changes. Pain accompanies CD in 75% of patients, and its intensity sometimes correlates with the stage of CD. The disease can have spontaneous recovery, especially in younger patients, but it is usually short-term [1–4].

Treatment of CD includes physical procedures such as stretching, range of motion exercises, muscle relaxation, and cervical orthoses. Different medicaments are used for therapy, mainly anticholinergics, dopamine antagonists, benzodiazepines, and gamma-aminobutyric acid (GABA) agonists. The effect of medication is various, with average 40% of success [5]. Surgical therapy of CD includes dorsal ramisectomy, bilateral pallidotomy, or stimulation of globus pallidus, and it is advised in advanced forms of disease [6].

Botulinum toxin (BT) was successfully applied in the therapy of CD. Its high efficiency was proven, with few side effects. Both forms of BT are used, type A (Botox®, Dysport®), and B (Myobloc®). The mechanism of action is based not only on denervational relaxation, but also on the influence on muscle fibers, basal ganglia, thalamus, and cortex [7–10].

However, the duration of the effect of BTA in CD has not been sufficiently studied.

The aim of this paper was to analyze the effect of physical therapy applied as monotherapy or in combination with botulinum toxin type A in patients with CD.

Methods

This randomized controlled clinical pilot study included 14 patients with CD. The diagnosis of CD was based on criteria proposed by Albanese et al. [11] in 2013. The study was approved by the Institutional Ethic Committee. All the patients gave written informed consent.

The patients were randomly divided into two groups. The first, control one, enrolled five patients who underwent physical therapy treatment without BT application. Physical therapy included exercises to increase range of motion, muscle stretching, occupational and functional therapy. It was planned individually (according to the patient's clinical findings), and conducted five times a week in the period of two weeks.

The second, experimental group, included 9 patients treated with BT A (Dysport®, Ipsen Biopharm Ltd, UK). One ampule of BT, 500 IU, diluted with 2.5 mL sterile 0.9% NaCl just before the application, was used for application in few places of the neck muscles according to the level of spasticity. Ipsilateral *m. sternocleidomastoideus* was the predominant site. *M. splenius*, *m. scalenus*, *m. semispinalis*, and *m. levator scapulae* were addionally injected. Five days after the BT application, the patients in this group received the same physical therapy, as in the control group, five times a week in the period of two weeks.

One, three and six months after the therapy, the effects were analyzed using the Toronto Western Spasmodic Torticollis Rating Scale (TWSTRS) and the Torticollis Rating Scale (Tsui scale) [12–14].

TWSTRS consists of three parts. The first part is the torticollis scale (maximum score amounts 35 and represents the biggest changes) and includes rotation (range 0–4), *laterocollis* (range 0–3), *antero* and *retrocollis* (range 0–3), lateral shift (range 0–1), sagittal shift (range 0–1), duration (range 0–5 multiplied by 2), sensory ticks (range 0–29), elevation/shift in shoulder (range 0–3), range of motion (range 0–4), and time (0–4). The second part is the disability scale (maximum 30) which includes: work, activities of daily life, driving, reading, watching television and activities outside home (range for each of them 0–5). The third part is the pain scale (maximum 20) which consists of severity of pain (range 0–10), duration of pain (0–5), and disability caused by pain (range 0–5).

The Torticollis Rating Scale (Tsui scale) has four parts. The first one is amplitude of sustained movements (range 0–9) with rotation, shift, and antero or retrocollis (for each range 0–3). The next part is duration of sustained movements (range 0–2), as well as elevation of shoulder (range 0–2). Finally, there is head tremor (range 1–4, intensity x duration). Total torticollis is obtained by multiplying the first and the second part, and adding the third and fourth part of the scale.

The tests were filled in by professionals who were unaware of the treatment groups. Obtained data were processed using standard statistical methods (Students' *t*-test, Mann-Whitney test, and Kruskal–Wallis one-way analysis of variance).

Results

Fourteen patients aged 32–52 years, average age 42.3 ± 5.6 years, were included in the study. Females predominated

(females : males = 11 : 3), and CD lasted for 13.5 ± 6.4 months (range 9–27).

The values of TWSTRS in the control and the experimental group were without significant differences at the beginning of the study. In the control group, applied physical therapy produced significant improvement in all three parts, especially in pain subscale ($p < 0.01$), but also in torticollis and disability subscale ($p < 0.05$) after one month from the start of the treatment. Three and six months after the beginning of the treatment, the effect of physical therapy was reduced. Application of BTA and physical therapy resulted in highly significant increase of all the parameters of TWSTRS ($p < 0.01$) after one month. This effect was maintained later on. After three months, it was also highly significant, and after six months, most of the parameters were still improved ($p < 0.05$), except for anterocollis, lateral and sagittal shift, sensory ticks, and range of motion. Longer effect was noticed for disability and pain subscales (Tables 1 and 2).

At the beginning of the investigation, the differences in Tsui scale between the control and the experimental group were not significant. In the control group, highly significant decrease of changes was noticed after one month. Further follow-up demonstrated the loss of the positive effects. Contrary to this, BTA and physical therapy produced much better and longer improvement. Most of the improvements was initial ($p < 0.01$), with retaining slightly lower results after three and six months ($p < 0.05$) (Tables 3 and 4).

No side-effects of BTA treatment were noticed.

Discussion

Our study showed highly significant improvement of all the parameters of CD in the patients treated with BTA and physical therapy after one and three months, and significant improvement after six months. The results were better when compared with the patients who were treated with physical therapy only.

Our study also documented that physical therapy produced good, but short-term improvement in patients with CD. Contrary to this, BTA and physical therapy produced highly significant positive effects especially in the first three months. After six months, the effect was reduced, but still present.

Physical therapy of CD was insufficiently studied, without any randomized controlled study. However, physical treatment produced only insignificant improvement of movements in CD. Also, the impact of iontophoresis on CD was minimal [14].

Botulinum toxin type A is the most efficient medicamentous treatment of CD producing reversible denervation of neuromuscular junction by inhibition of acetylcholine release in presynaptic axons of motor plates. It is used to reduce pain, dystonic position, reduced range of motion, and tremor. Kinematic studies have confirmed that BT normalizes the speed and amplitude of neck movements in patients with CD [15].

Table 1

The values of TWSTRS scale in cervical dystonia after the treatment with physical therapy

Parameter	Before therapy	After 1 month	After 3 month	After 6 month
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Torticolis scale				
rotation	3.0 ± 0.2	2.6 ± 0.4†	2.8 ± 0.4	2.9 ± 0.2
laterocollis	2.4 ± 0.2	2.0 ± 0.2†	2.2 ± 0.3	2.3 ± 0.2
antero/retrocollis	2.3 ± 0.3	1.9 ± 0.2†	2.0 ± 0.2	2.1 ± 0.3
lateral shift	0.8 ± 0.1	0.5 ± 0.1†	0.6 ± 0.1	0.7 ± 0.2
sagittal shift	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2
duration factor	7.9 ± 1.0	6.3 ± 0.5†	6.0 ± 0.5†	6.3 ± 0.7†
sensory tricks	1.4 ± 0.2	0.9 ± 0.2†	1.3 ± 0.2	1.3 ± 0.2
shoulder elevation/shift	2.3 ± 0.3	2.1 ± 0.3†	2.1 ± 0.3†	2.1 ± 0.4†
range of movement	3.0 ± 0.6	2.7 ± 0.5†	2.7 ± 0.7	2.9 ± 0.5
time	3.0 ± 0.6	2.6 ± 0.4†	2.8 ± 0.5	2.9 ± 0.5
Disability scale				
work	3.7 ± 0.5	3.2 ± 0.5†	3.7 ± 0.4	3.6 ± 0.4
daily life activities	3.8 ± 0.5	3.2 ± 0.5†	3.5 ± 0.4	3.6 ± 0.5
driving	4.0 ± 0.7	3.4 ± 0.3†	3.5 ± 0.4	4.2 ± 0.5
reading	3.8 ± 0.4	3.0 ± 0.4†	3.7 ± 0.2	3.8 ± 0.4
watching television	3.8 ± 0.4	3.0 ± 0.4†	3.7 ± 0.2	3.5 ± 0.2
activities outside home	3.8 ± 0.6	3.2 ± 0.5†	3.5 ± 0.4	3.5 ± 0.2
Pain scale				
intensity	7.5 ± 1.0	5.6 ± 0.9*	6.4 ± 1.0†	6.5 ± 0.8†
duration	4.0 ± 0.5	3.1 ± 0.5*	3.2 ± 0.6†	3.7 ± 0.3
disability by pain	4.1 ± 0.5	3.1 ± 0.3*	3.0 ± 0.8†	3.7 ± 0.3

TWSTRS – Toronto Western Spasmodic Torticollis Rating Scale; SD – standard deviation;*$p < 0.01$; †$p < 0.05$.

Table 2

The values of TWSTRS scale in cervical dystonia after the treatment with physical therapy and botulinum toxin type A

Parameter	Before therapy	After 1 month	After 3 months	After 6 months
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Torticolis scale				
rotation	3.1 ± 0.4	1.9 ± 0.5*	2.1 ± 0.6*	2.6 ± 0.7†
laterocollis	2.5 ± 0.3	1.6 ± 0.4*	1.7 ± 0.4*	2.0 ± 0.6†
antero/retrocollis	2.2 ± 0.5	1.3 ± 0.5*	1.2 ± 0.4*	2.0 ± 0.7
lateral shift	0.7 ± 0.2	0.2 ± 0.1*	0.3 ± 0.1*	0.6 ± 0.2
sagittal shift	0.6 ± 0.3	0.3 ± 0.1*	0.3 ± 0.2*	0.4 ± 0.3
duration factor	8.0 ± 1.4	5.2 ± 1.6*	5.0 ± 1.8*	5.8 ± 2.2†
sensory tricks	1.3 ± 0.4	0.8 ± 0.6*	1.1 ± 0.3	1.0 ± 0.5
shoulder elevation/shift	2.4 ± 0.3	1.9 ± 0.6*	1.9 ± 0.8	2.1 ± 0.7†
range of movement	3.2 ± 0.9	2.1 ± 1.1*	2.3 ± 0.7*	2.7 ± 1.2
time	3.1 ± 0.6	2.0 ± 0.8*	2.1 ± 0.7*	2.4 ± 1.5†
Disability scale				
work	3.8 ± 0.9	2.3 ± 0.7*	2.4 ± 0.8*	3.1 ± 0.9†
daily life activities	3.9 ± 0.7	2.1 ± 0.5*	2.3 ± 1.2*	2.9 ± 1.1†
driving	4.2 ± 0.8	2.2 ± 0.8*	2.2 ± 1.0*	2.7 ± 1.1*
reading	3.6 ± 0.8	2.3 ± 0.6*	2.7 ± 1.1*	2.8 ± 0.9
watching television	3.7 ± 0.7	2.6 ± 1.0*	2.7 ± 1.0*	2.6 ± 0.9*
activities outside home	3.8 ± 0.6	2.2 ± 1.2*	2.4 ± 1.3*	2.8 ± 1.2†
Pain scale				
intensity	7.9 ± 1.5	4.7 ± 1.9*	4.6 ± 2.0*	5.8 ± 1.8†
duration	4.1 ± 0.9	2.0 ± 0.8*	2.2 ± 1.3*	2.8 ± 1.4*
disability by pain	4.2 ± 0.6	1.9 ± 0.7*	2.0 ± 1.8*	3.1 ± 0.9†

TWSTRS – Toronto Western Spasmodic Torticollis Rating Scale; SD – standard deviation; *$p < 0.01$; †$p < 0.05$.

Table 3

The values of Tsui scale in cervical dystonia after the treatment with physical therapy

Parameter	Before therapy	After 1 month	After 3 months	After 6 months
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Amplitude of movement				
rotation	2.5 ± 0.4	2.0 ± 0.3*	2.2 ± 0.4†	2.4 ± 0.3
shift	2.5 ± 0.4	2.0 ± 0.3*	2.4 ± 0.4	2.4 ± 0.3
antero/retrocollis	2.7 ± 0.3	2.2 ± 0.4*	2.2 ± 0.4†	2.4 ± 0.3
Duration of movement				
duration	2.0 ± 0.2	1.7 ± 0.2*	1.8 ± 0.3†	2.0 ± 0.2
Shoulder elevation				
elevation	2.0 ± 0.2	1.7 ± 0.2*	1.9 ± 0.3	1.9 ± 0.9
Head tremor				
intensity	1.8 ± 0.2	1.5 ± 0.2*	1.6 ± 0.3	1.7 ± 0.2
duration	1.8 ± 0.2	1.5 ± 0.2*	1.6 ± 0.3	1.7 ± 0.2

SD – standard deviation; *$p < 0.01$; †$p < 0.05$.

Table 4

The values of Tsui scale in cervical dystonia after the treatment with physical therapy and botulinum toxin type A

Parameter	Before therapy	After 1 month	Afetr 3 months	After 6 months
	mean ± SD	mean ± SD	mean ± SD	mean ± SD
Amplitude of movement				
rotation	2.4 ± 0.8	1.6 ± 0.7*	1.7 ± 0.9†	1.9 ± 1.2†
shift	2.6 ± 0.7	1.4 ± 0.9*	1.6 ± 1.0*	2.0 ± 1.0†
antero/retrocollis	2.7 ± 0.6	1.5 ± 1.0*	1.4 ± 0.9*	2.1 ± 0.9†
Duration of movement				
duration	1.9 ± 0.3	1.1 ± 0.2*	1.2 ± 0.3†	1.7 ± 0.2
Shoulder elevation				
elevation	2.2 ± 0.6	1.1 ± 0.6*	1.5 ± 0.4†	1.3 ± 0.9†
Head tremor				
intensity	1.7 ± 0.2	1.2 ± 0.2*	1.3 ± 0.3†	1.7 ± 0.2
duration	1.6 ± 0.3	1.2 ± 0.3*	1.4 ± 0.2†	1.5 ± 0.3

SD – standard deviation; *$p < 0.01$; †$p < 0.05$.

The treatment of CD using BT must be individual, according to pharmacokinetics and anatomy of the region. In some cases, electromyography (EMG) can be used to deliver BT precisely. The effect of BT in CD depends on the presence of contractures, and on the medical skills. There are no universal opinions concerning application site, doses, and efficiency of certain BT serotypes. On the other side, the symptoms and status of CD can be different [7–10]. Higher doses of BT and more local injections are needed for complex forms of CD. Initial dose of 500 IU of Dysport® can be increased if needed [16]. In our patients initial dose of 500 IU was sufficient to provide good therapeutic response.

The duration of effect is from twelve to sixteen weeks. In our study, the effect was maintained six months after the treatment which can be attributed to the exercises that patients practiced after the BTA application. The aim of the exercises was to achieve postural reducation and voluntary control of the head and neck position as well as passive muscular elongation for prevention of contracture.

Reported side effects after BTA include local pain, dryness of mouth, muscle weakness, and general weakness. In this study important side effects were not noticed.

Long-term application of BT indicated continuous improvement in 63% in a five-year period. The primary resistance was verified in 10%, while secondary one amounted to 8% [17, 18]. Reduced long-term efficiency of BT is explained by neutralizing antibodies [19–21]. They were found in 32% of children with cerebral pulsy usually after eight weeks of treatment [9].

The efficiency of BT in CD was verified in few double blind studies. They used clinical and video surveillance of symptoms, EMG, Tsui scale, and other scales [22, 23].

In this study, we used TWSTRS and Tsui scale that showed comparable values for follow-up in CD. Since TWSTRS includes disability and pain subscale, it gives important indicators of daily activities in CD and is therefore more valuable in clinical investigations.

TWSTRS showed good reliability in all the subscales, with bigger variability depending on the gravity of disease. Disability subscale is more sensitive than the subscales for pain and for severity of the disease as well [4, 23].

Tsui scale is used to measure the position of the head and shoulders, duration of movement, and head tremor in CD. Inter-test reliability was good, but the validity of this scale was not studied. Cervical Dystonia Impact Profile (CDIP-58) measures the 58 health parameters in CD, but it has not been widely used in investigations [4, 23].

Conclusion

TWSTRS and Tsui scale have confirmed highly significant improvement of all the parameters of cervical dystonia treated with botulinum toxin A and physical therapy after one and three months and significant improvement after six months.

The results were better and of longer duration compared to patients who were treated with physical therapy only. Future studies with larger number of patients and longer follow-up are necessary to confirm these initial positive effects.

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CASE REPORTS

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Central retinal artery occlusion following embolization in juvenile nasopharyngeal angiofibroma – A case report

Okluzija centralne arterije retine posle embolizacije juvenilnog nazofaringealnog angiofibroma

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Abstract

Introduction. Juvenile nasopharyngeal angiofibromas are highly vascular, locally aggressive lesions, that affect male adolescents. The surgery is the treatment of choice, although it shows a strong propensity to bleed during surgical removal. Preoperative embolization enables the surgical approach in a less bloody way and also a complete resection of the tumor. However, this procedure is not without complications. The most severe complication of this technique is a migration of an embolus into the intracranial circulation. **Case report.** We present a 9-year-old boy who lost vison on his left eye following preoperative embolisation of juvenile nasopharyngeal angiofibromas as a result of central retinal artery occlusion. A recent review of the literature reported only three previously documented cases of central retinal artery occlusion occurring after embolization for a nasopharyngeal angiofibroma. We want to point out the possibility of this rare but devastating complication and the importance of rapid and accurate diagnosis and treatment so that a visual outcome could be better when applying an early medical treatment. **Conclusion.** Described case of central retinal artery occlusion is a rare and unusual, iatrogenic vascular event, that could arise as a complication from embolisation of nasopharingeal tumors. However, physicians (ophthalmologists and ear-nose-throat surgeons) should be aware od this devastating complication, and the close evaluation of angiograms for detection of any vascular abnormality before and during the embolization is crucial.

Key words:
nasopharyngeal neoplasms; angiofibroma; child; diagnosis; embolization, therapeutic; otorhinolaryngologic surgical procedures; neoplasm recurrence, local; retinal artery occlusion; treatment outcome.

Apstrakt

Uvod. Juvenilni nazofaringealni angiofibrom je benigna, vaskularizovana, lokalno agresivna lezija, koja se obično javlja kod adolescentnih momaka. Terapija izbora je hirurško uklanjanje. Kako ovaj tumor pokazuje sklonost ka krvarenju tokom hirurškog uklanjanja, uvođenjem preoperativne embolizacije omogućuje se operacija sa manje krvi, ali i kompletna resekcija tumora. Međutim, ova procedura nije bez komplikacija. Najozbiljnija komplikacija embolizacije jeste migracija embolusa u intrakranijalnu cirkulaciju. **Prikaz bolesnika.** Prikazujemo slučaj devetogodišnjeg dečaka koji je izgubio vid na levom oku posle preoperativne embolizacije juvenilnog nazofaringealnog angiofibroma zbog okluzije centralne arterije retine. Pregledom literature našli smo svega tri objavljena slučaja okluzije centralne arterije retine nakon embolizacije nazofaringealnog angiofibroma. Ovim radom želimo da ukažemo na mogućnost ove retke i teške komplikacije, kao i na značaj brze i tačne dijagnoze, jer se bolji vidni ishod dobija kod ranog započinjanja lečenja. **Zaključak.** Opisani slučaj okluzije centralne arterije retine je redak, jatrogeni vaskularni akcident koji može da se javi kao komplikacija embolizacije nazofaringealnih tumora. Lekari (oftalmolozi i otorinolaringolozi) bi trebalo da budu svesni ove teške komplikacije, kao i toga da je ključno detaljno praćenje angiograma radi detekcije bilo kakve vaskularne anomalije, pre i tokom embolizacije.

Ključne reči:
nazofarinks, neoplazme; angiofibrom; deca; dijagnoza; embolizacija, terapijska; hirurgija, otorinolaringološka, procedure; neoplazme, lokalni recidiv; okluzija retinalne arterije; lečenje, ishod.

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Introduction

Juvenile nasopharyngeal angiofibromas (JNA) are highly vascular, benign but locally aggressive tumor-like lesions, that commonly affect adolescents [1]. The origin of JNA are still uncertain concerning its fibrous or vascular derivation. While some authors considered JNA as vasoproliferative malformation because of its extensive vascularisation, the others proposed JNA as a specific type of hemangioma [2]. Recent evidence based on immunohistochemical and electron microscopic examinations indicates that JNA represent vascular malformations derived from incomplete regression of the artery of the first branchial arch, rather than a true neoplasm. Nevertheless, the exact etiology of these lesions remains a matter of debate [3].

Traditionaly, the surgery is considered as a treatment of choice. However, because of its rich vascularisation, JNA shows a strong propensity to bleed during surgical removal [4]. The introduction of endoscopes and preoperative embolization of the feeding vessels, changed the surgical approach of these tumors by providing an operation in a less bloody way and complete resection of the tumor [5]. However, embolisation is not a procedure without complications. The most fearing complication would be migration of an embolus into the intracranial circulation, while the other complications include systemic reaction to the contrast, infection at the site of puncture, femoral hematoma and thrombosis, facial paralysis, skin necrosis, oronasal fistula [6].

We present a 9-year-old boy who lost his vison on his left eye following preoperative embolisation of JNA, as a result of central retinal artery occlusion (CRAO). Recent review of the literature reported only three previously documented cases of CRAO occurring after embolization for a nasopharyngeal angiofibroma [7–9]. We want to point out the possibility of this rare but devastating complication and the importance of rapid and accurate diagnosis and treatment so that a visual outcome could be better when applying an early medical treatment.

Case report

A 9-year-old boy was presented to the Ear-Nose-Throat (ENT) Clinic with a complaint of left nasal obstruction, repeated left-sided nose bleeds and snoring of few months duration. On the clinical examination, purplish mass filling the left nasal cavity was found. The triad of epistaxis, one-sided nasal obstruction and a mass in a nasopharinx are indicative for JNA so the diagnosis of this tumor was proposed. Computed tomography (CT) showed a heterodense, soft tissue lesion in the nasal cavity, with measurement 35 × 40 × 58 mm, displacing the nasal septum and extending partially to the sphenoid sinus. It was recommended to embolize the feeding vessels of the tumor using polyvinyl alcohol (PVA) particles as a part of preoperative preparation in order to reduce the size of the tumor and to reduce the possibility of bleeding during the surgery. Five days after the embolization process, the patient underwent an endoscopic angiofibroma excision. The histopathologic finding confirmed the JNA. Eight months after the operation, patient is presented with reccurence of the symptomatology. On control CT scan, the hyperdense mass filling the nasal cavity, measuring 17 × 27 × 16 mm was found. Comparing the clinical and imaging findings, a diagnosis of residual JNA was made. Endovascular angiography of the both carotid and vertebrobasillar system was carried out. At angiography, it was noticed that most of the blood supply to the lesion arises from the maxillar artery, so this artery was then embolised (Figure 1).

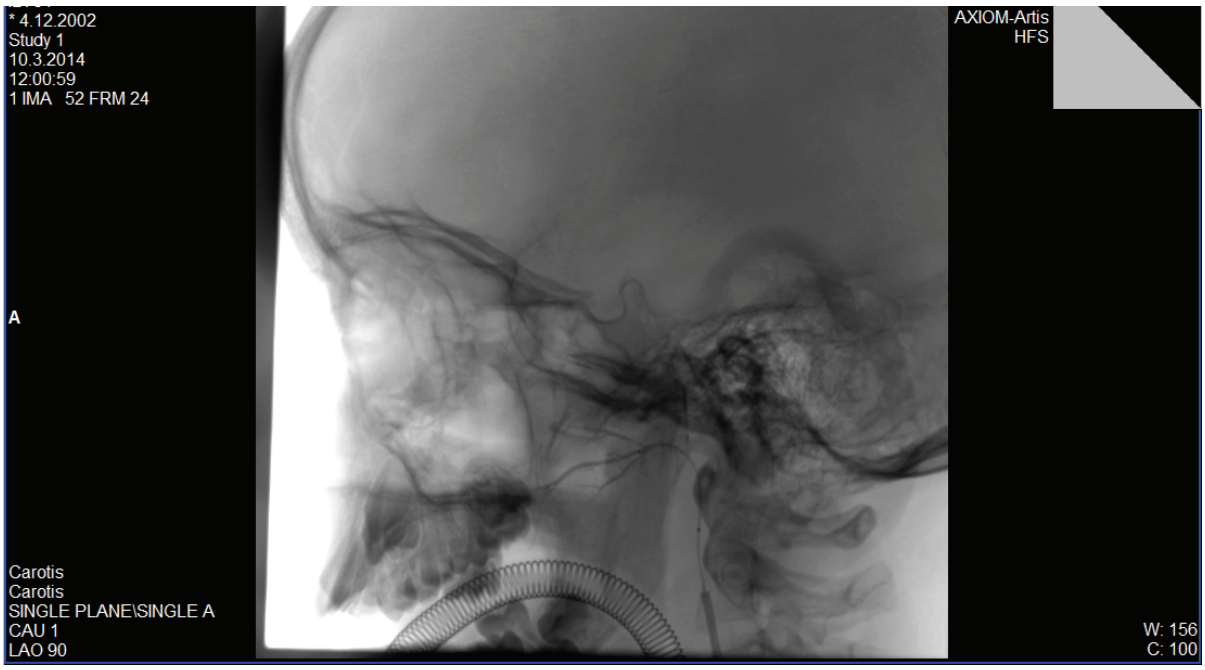

Fig. 1 – Angiogram of the external carotid vascular system showing the vascular angiofibroma. Note the appearance of a tumor blush.

Following the procedure, the patient awoke but with impaired conscious, somnolent, with highly positive meningeal signs such as stiff neck, positive Brudzinski and Kernig sign. His left pupil was dilated and nonresponsive to light. As the patient was somnolent and desoriented, he was examined by a neurologist, and it was performed an urgent CT scan and brain magnetic resonance imaging (MRI) and angiography (MRA). The CT scan showed the left hemisphere edema, while MRI showed microischemic lession and left subarachnoid front-parietal hemorrhage. Prompt treatment with antibiotics and antiedematous therapy with mannitol and dexamethasone was started. He was also examined by an ophthalmologist the following day after the vascular event. Left eye visual acuity (VA) was not perceptive of light and the relative afferent pupillary defect was positive in this eye. The patient's fundus examination showed whitish retinal edema and a cherry-red spot appearance of the macula with narrowed vessels (Figure 2).

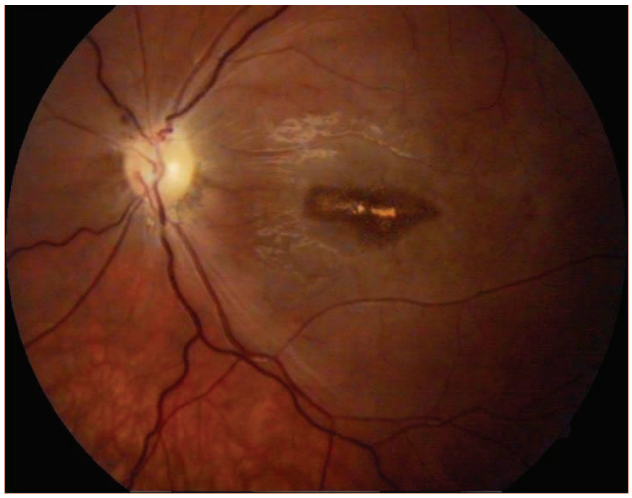

Fig. 2 – Fundus changes on the left eye one months after the vascular event. Note maintained whitish appearance of the macula with cherry-red spot and narrowed vessels.

A diagnosis of central retinal artery occlusion was made. Ocular massage was initiated and proceeded for 15 minutes. Topical timolol was given twice a day. The patient's general condition improved slowly with progressive resolution of neurological signs and partial resorption of cerebral edema on the brain CT scan. On the other hand, the visual loss on the left eye was still persistant. Two month after the vascular incident, the patient was conscious with no neurological deficit. The only problem he had was low vision on the left eye. After detailed discussion with the parents, it was decided to perform an operation again. Five days after the repeated embolization process, the patient underwent endoscopic angiofibroma excision and the tumor was removed completely. Ten months after the embolization, VA was still no light perception. Fundus changes on the left eye such as cherry-red spot disappeared while it was noted attenuated retinal arterioles and optic disc atrophy (Figure 3).

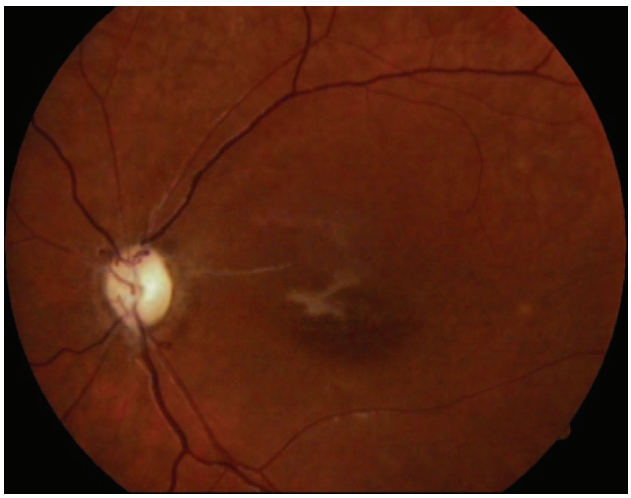

Fig. 3 – Fundus changes on the left eye three months after the vascular event. Note attenuated retinal arterioles, optic disc atrophy and dissapearance of cherry red spot.

Discussion

JNA represents the most often head and neck vascular malformation in males in pre-puberty period [2, 9]. Symptoms that occur are typical for JNA: progressive unilateral nasal obstruction accompanied with rhinorrhea and recurrent epistaxis. Depending of tumor extension to surrounding structures, rhinosinusitis, alteration in olfaction, proptosis, vison alteration, headache and neurologic deficit are also possible manifestation [10].

Although different treatment modalities are used for angiofibromas such as surgery, hormonal therapy, radiation and systemic chemotherapy, a surgical excision of the mass remains a treatment of choice [11]. As the JNA is highly vascularised tumor, blood loss during surgical resection is one of the major preoccupations during the operation. The appropriate surgical approach should be determined by performing the preoperative transcatheter embolisation of the tumor [2] in an attempt to decrease intraoperative bleeding and to make tumor resection more easy [12]. Because of the often bilateral vascular supply, both carotid systems should be angiographically evaluated [2]. However, preoperative embolisation is not without complications. The most severe complications, like cerebral infarcts and vision loss, have an incidence of less than 2% [5]. Central retinal artery occlusion and subsequent vision loss, as seen in our case, were reported only few times in literature after JNA embolization [7–9].

There are three mechanisms describing in which way PVA embolisation cause CRAO: the congenital variation of vasculature, over-forced injection which cause a reflux into the internal carotid system and presence of collateral vessels which arise from tumor [9]. The presence of collateral vessels could be masked by a tumor itself, but if it is recognized, the microcatheter should be advanced beyond the second portion of ophthalmic artery to prevent the embolic event [1].

Ramezani et al. [7] reported a case of a child with right sided JNA who developed CRAO following preoperative embolisation, probably due to the presence of suspicious collateral artery between the external carotid artery and oph-

thalmic vessels on the left side which had not been noticed before the embolisation. On the other hand, Casasco et al. [4] assumed that, in their case, a small amount of permanent liquid polymerizing agent entered the ophthalmic artery, which resulted in an acute loss of vision due to CRAO.

In our case, we did not find any vascular abnormal communication nor collateral which could explain the route of the embolus which affected the ocular circulation. We could assumed that the changes were caused by the PVA material pass through the cerebral circulation and internal carotid artery and its branches supplying the retina.

There are some authors who found the preoperative embolisation as a risk factor, with a higher rate of JNA recurrence [13, 14]. Petruson et al. [13] found that the recurrence rate in non-embolized patients was 8% and 41% among embolized patients. In their opinion, the only factor affecting recurrence was the age at the moment of making a diagnosis, i.e., the younger the patient was, the greater the risk for recurrence. Yet, they hope that the development of imaging and embolization techniques will contribute to reducing the recurrence rate. On the other hand, Ogawa et al. [6] found the embolisation as an effective technique for decreasing the tumor size and easier way for resecting it, thus lowering the recurrence rate of the tumor. They concluded it after reviewing the medical records of 170 patients who underwent preoperative embolisation for resection of JNA, confirming that recent development of embolization techniques, made embolisation even safer and more effective.

Nevertheless, this emergency vascular accident continues to be an undesirable and tragic event for anyone affected by it, especially because of the difficulties in preventing and managing CRAO [11].

Conclusion

Described case of CRAO is a rare and unusual, iatrogenic vascular event, reported only few times in literature (according to PubMed search), that could arise as a complication from PVA embolisation of nasopharingeal tumors. However, physicians (ophthalmologists and ENT surgeons) should be aware od this devastating complication and the close evaluation of angiograms for detection of any abnormality before and during the embolization is crucial. Since, visual prognosis would be much better with applying an early treatment, it is extremely important to set rapid and accurate diagnosis of CRAO and to treat all such patients within a few hours after the occlusion. On the other hand, all patients undergoing these procedures (or their parents) should be fully explained and informed about the risk of visual loss as it could strongly influence their future quality of life.

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CASE REPORT

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Urachal adenocarcinoma – case report and literature review

Adenokarcinom urahusa – prikaz bolesnika i pregled literature

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Abstract

Introduction. Urachal adenocarcinoma is extremely rare and comprises from 0.35% to 0.7% of all bladder tumors. The most common histologic subtype of urachal tumors is adenocarcinoma which can be associated with intestinal metaplasia and mucin production. **Case report.** We report a case of a 53-year-old patient who attented a urologist because of an intermittent haematuria lasting for three months. The ultrasound examination detected infiltration of the bladder at the fundus, 24 × 29 mm in diameter. By the same wall, next to the tumor, there was an oval hypoechoic lesion about 40 mm in diameter. Computed tomography scan showed a solid, echogenic, strictly limited tumor at the fundus of the bladder, anteriorly, 32 × 35 × 22 mm in diameter which was positive after contrast application. The patient underwent partial cystectomy with complete excision of the tumor lesion 1.5 cm in healthy tissue. Histopathological analysis showed diagnosis of *Adenocarcinoma mucinosum vesicae urinariae infiltrans*. Patohistological findings detected a part of the urachal wall with a thin layer of fibromuscular tissue, chronic inflammation, microcalcifications in the lumen, flattened and desquamated epithelium. One year after the surgery, there were no signs of primary disease or metastases in other organs. **Conclusion.** Urachal adenocarcinoma is extremely rare. Long term survival could be achieved by surgical treatment in the early stage of the disease which consists of complete resection of urachal carcinoma and partial or total cystectomy.

Key words:
urachus; adenocarcinoma; urinary bladder; diagnostic techniques and procedures; urologic surgical procedures.

Apstrakt

Uvod. Adenokarcinom urahusa je izuzetno redak i čini od 0.35% do 0.7% svih tumora mokraćne bešike. Najčešći histološki tip tumora urahusa je adenokarcinom, koji može biti udružen sa intestinalnom metaplazijom i produkcijom mucina. **Prikaz bolesnika.** Prikazujemo slučaj bolesnika starog 53 godine, koji je došao kod urologa zbog intermitentne hematurije koja se javljala u periodu od tri meseca. Na ultrazvučnom pregledu uočena je infiltracija mokraćne bešike uz fundus, dijametra oko 24 × 29 mm. Uz isti zid neposredno uz tumorsku promenu, bila je prisutna ovalna hipoehogena promena dijametra oko 40 mm. Nalaz kompjuterizovane tomografije pokazao je solidnu, ehogenu, jasno ograničenu tumefakciju na krovu mokraćne bešike, anteriorno, dijametra 32 × 35 × 22 mm koja se dobro prebojavala nakon aplikacije kontrastnog sredstva. Bolesniku je učinjena parcijalna cistektomija sa potpunom ekscizijom tumorske promene na 1,5 cm do u zdravo tkivo. Histopatološkom analizom postavljena je dijagnoza *Adenocarcinoma mucinosum vesicae urinariae infiltrans*. Na patohistološkom nalazu uočen je deo zida urahusa sa tankim slojem fibromuskularnog tkiva, hroničnim zapaljenjem u zidu, mikrokalcifikatima u lumenu, aplatiranim i najvećim delom deskvamovanim epitelom. Godinu dana nakon operacije nije bilo znakova recidiva tumora niti pojave metastaza u drugim organima. **Zaključak.** Adenokarcinom urahusa je izuzetno redak. Dugoročno preživljavanje se može postići hirurškim tretmanom u ranoj fazi bolesti, koji obuhvata kompletnu resekciju karcinoma urahusa i parcijalnu ili totalnu cistektomiju.

Ključne reči:
urahus; adenokarcinom; mokraćna bešika; dijagnostičke tehnike i procedure; hirurgija, urološka, procedure.

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Introduction

Urachal adenocarcinoma is extremely rare and comprises from 0.35% to 0.7% of all bladder tumors. It represents 22%–35% of all bladder adenocarcinomas [1]. The urachus is composed of three layers: outer muscular, middle layer consisting of a connective tissue and internal canal which is lined with transitional cell epithelium. It is located between transverse fascia anteriorly, parietal peritoneum posteriorly, cranial umbilicus and bladder caudal. Urachal neoplasms can arise in any of these layers, and can be epithelial or mesenchymal [2]. The criteria for the diagnosis of urachal cancer are not strictly defined. Most investigators agree with following: tumor in the dome of the bladder; absence of cystitis cystica and cystitis glandularis; predominant invasion of the muscularis or deeper tissues with a sharp demarcation between the tumor and surface of bladder urothelium. The presence of urachal remnants within the tumor and extension of tumor into the bladder wall, with involvement of the space of Retzius and anterior abdominal wall or umbilicus are also criteria for diagnosis. There is a lack of evidence for a primary neoplasm of another localization [1, 3]. In most cases, the adenocarcinoma of urachus presents with a higher stage of disease at the time of diagnosis because it develops on the outside of the bladder where it does not cause any symptoms. After the disease has progressed and grown into the bladder, dysuria, haematuria, abdominal and umbilical pain can occur secondarily [3]. Surgical treatment consists of partial or total cystectomy with *en bloc* resection of the median umbilical ligament and umbilicus [4]. Currently, there is no standard radiotherapy or chemotherapy regimen for the treatment of urachal cancers [5].

Case report

We report a case of a 53-year-old patient who attented urologist because of intermittent haematuria lasting for three months. The patient denied dysuric symptoms, fever, abdominal or umbilical pain. Case history did not mention a loss of body weight. Standard laboratory blood tests were normal. Physical examination showed regular findings. Abdomen was below the chest and there was no pain at superficial or deep palpation.

The ultrasound examination detected infiltration of the bladder at the fundus, 24 × 29 mm in diameter. By the same wall, next to the tumor, there was an oval hypoechoic lesion about 40 mm in diameter. Computed tomography (CT) scan of abdomen and pelvis (Figure 1) showed a solid, echogenic, strictly limited tumor at the fundus of the bladder, anteriorly, 32 × 35 × 22 mm in diameter, which was positive after contrast application. Superior to the tumor, intraabdominaly, outside the bladder, continued encapsulated hypodesic, strictly limited lesion about 66 mm in craniocaudal diameter. Superior lesion was in close relation with the intestinal wall, but there was no evidence of infiltration.

The patient underwent partial cystectomy with complete excision of the tumor lesion 1.5 cm in healthy tissue (Figure 2). Intraoperative and postoperative course was uneventful without complications. One year after the surgery there were no signs of primary disease or metastases in other organs.

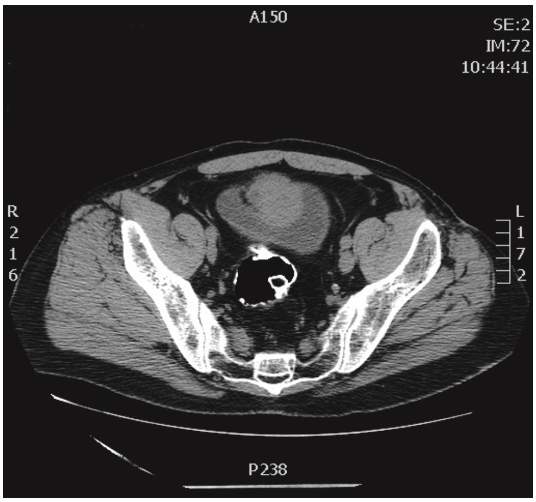

Fig. 1 – Computed tomography scan: a solid, echogenic, strictly limited tumor at the fundus of the bladder, anteriorly, 32 × 35 × 22 mm in diameter.

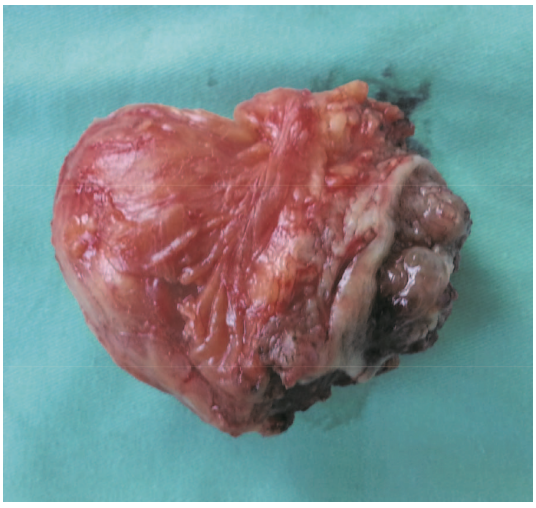

Fig. 2 – Surgical findings after partial cystectomy and complete excision of the tumor.

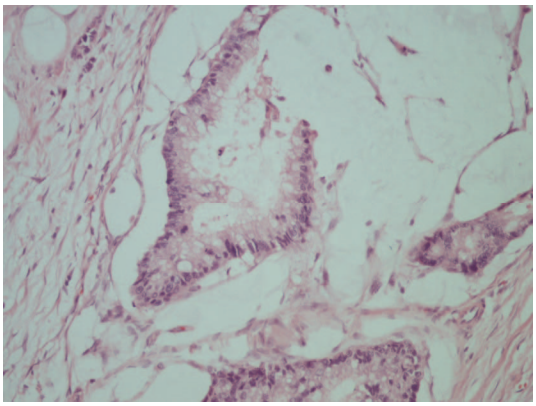

Fig. 3 – Adenocarcinoma mucinosum focus with extracellular mucin (hematoxylin eosin – HE × 200).

Histopathological (HP) analysis showed diagnosis of *Adenocarcinoma mucinosum vesicae urinariae infiltrans* (Figure 3). HP findings detected a part of the urachal wall with thin layer of fibromuscular tissue, chronic inflammation, microcalcifications in the lumen, flattened and desquamated epithelium (Figure 4). The level of histological malignancy showed grade 2. Tumor had infiltrated the connective and adipose tissue around the bladder, but had not penetrated the serous membrane. Invasion of the veins was registered at several places.

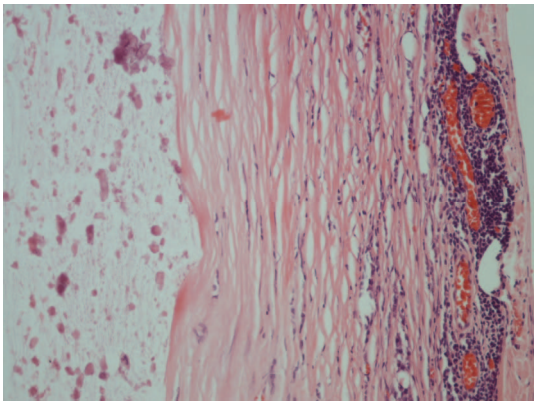

Fig. 4 – Part of wall of urachus with a thin layer of fibromuscular tissue (hematoxylin eosin – HE × 200).

Discussion

Urachal adenocarcinoma is extremely rare and in most cases it occurs in patients over 30 years of age, in 1 per 5 million people [6]. Gupta et al. [7] noticed a small number of patients younger than 30 years in whom the urachal adenocarcinoma was diagnosed. Based on a study by Ashley et al. [8], it was observed that the urachal carcinoma occured more frequently in older male than in female population. The most common symptoms are hematuria, dysuria, abdominal and umbilical pain. Ultrasound examination allows localization of the tumor and detection of highly echogenic calcifications as well as of the solid components of the tumor and lesions in the anterior abdominal wall. Williams and Chavda [9] pointed out the importance of CT or magnetic resonance imaging scans of the abdomen and pelvis, which may also provide information on local extent, lymph node involvement and metastases. Further metastatic evaluation could be obtained by chest radiography or bone scanning.

Urachal tumors can be of mesenchymal or epithelial origin. Gopalan et al. [1] noticed that the most common histologic subtype of urachal tumors was adenocarcinoma, which can be associated with intestinal metaplasia and mucin production. A very few number of studies [10] showed that squamous cell carcinoma, transitional cell carcinoma and anaplastic carcinoma can arise from the urachus. Prakash et al. [2] reported the case of a complex mucinous cystadenoma of urachus. Histopathologically, the tumor was characterized by the absence of cellular atypia as seen in adenocarcinomas. Urachal cystadenoma had a low malignant potential because it can result in *pseudomyxoma peritonei* if ruptured. Yu at al. [11] emphasized that the benign urachal neoplasms, such as adenomas, fibromas, fibroadenomas, hamartomas and fibromyomas, are extremly rare.

Criteria for the diagnosis of urachal carcinoma were established by Wheeler and Hill [12] and modified by Mostofi et al. [13]: tumor in the dome of the bladder; absence of cystitis cystica and cystitis glandularis; predominant invasion of the muscularis or deeper tissues with a sharp demarcation between the tumor and surface bladder urothelium; presence of urachal remnants within the tumor; extension of tumor into the bladder wall with involvement of the space of Retzius, anterior abdominal wall or umbilicus and no evidence of a primary neoplasm elsewhere.

Most of the patients at the time of diagnosis are in advanced stages of the disease because symptoms occur after tumor infiltration of bladder and/or other organs. Siefker-Radtke et al. [14] showed early peritoneal dissemination of urachal adenocarcinoma and metastases predilective in the bones, lungs and liver. Lee [15] reported the case of urachal adenocarcinoma that metastasized in both ovaries. If there is a bilateral mucinous adenocarcinoma of the ovaries, the presence of urachal adenocarcinoma as primary tumor must be ruled out.

The stage of the disease is determined by a system set up by Sheldon et al. [16]. The early stage of the disease implies the presence of a tumor in the urachal mucosa, while the advanced stage considers the presence of tumor in the bladder, abdominal wall, peritoneum and the presence of metastases in regional lymph nodes and distant sites. Mayo Clinic proposed a modification of this system [5].

Due to the low incidence of urachal adenocarcinoma, setting of standard treatment protocols is somewhat difficult. If the diagnosis is established in early stage of the disease, long term survival could be achieved by surgical treatment. Williams and Chavda [9] showed high survival rate of patients who underwent complete resection of urachal carcinoma and partial or total cystectomy. Asano et al. [17] pointed out the importance of intrapelvic and iliac lymphadenectomy, but consensus on this issue has not been reached, and lymphadenectomy is not routinely recommended. Elser et al. [5] reported the case of urachal adenocarcinoma which was resistant to multiple chemotherapy protocols. This is in concordance with literature data which indicate modest response of the tumor to chemotherapy and radiotherapy.

Conclusion

Urachal adenocarcinoma is extremely rare. The most common symptoms are hematuria, dysuria, abdominal and umbilical pain. Most of the patients at the time of diagnosis are in advanced stages of the disease because symptoms occur after the tumor infiltrated bladder and/or other organs. Long term survival could be achieved by surgical treatment in the early stage of the disease, which consists of complete resection of urachal carcinoma and partial or total cystectomy.

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CASE REPORT

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Treatment of subacute osteoporotic vertebral compression fractures with percutaneous vertebroplasty – A case report

Lečenje subakutnih kompresivnih fraktura osteoporotičnih pršljenova primenom perkutane vertebroplastike

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Abstract

Introduction. Percutaneous vertebroplasty (PVP), as a mini-invasive approach in the treatment of patients with osteoporotic vertebral compression fractures (OVCFs), provides stabilization of the spine and relives pain. The most commonly it is applied in the 3–6 weeks before bending of the spine. Complete cessation of pain is easier to achieve if you treat "less mature" fractures. The aim of the report is to show that PVP is effective and safe for old fractures too. **Case report.** A 77-old patient suffered from a stable compression fracture of 3th lumbar (L3) vertebral body after minor trauma. This fracture was clinically and radiologically diagnosed. The conservative treatment that included lumbo-sacral orthosis (LSO), analgesic drugs and physical therapy, was primarily applied due to permanent pain and type of fracture. After a period of two months, pain persisted, but it was localized in a thoracic spinal segment with radiologically diagnosed fractured bodies of 8th (Th8) and 10th (Th10), thoracic vertebra without neurological deficit. Thoraco-lumbo-sacral orthosis (TLSO) was prescribed and after six months the indication for vertebroplasty of the Th8 and Th10 vertebral body was given. The pain relief had been achieved and the patient was discharged from the Clinic for Orthopedics on the postoperative day 2, and was symptom free during the follow-up period. **Conclusion.** In patients with stable OVCFs, PVP is an effective therapy for reducing pain and improving mobility of 6 months old fractures.

Key words:
spinal fractures; fractures, compression; osteoporosis; pain; vertebroplasty; treatment outcome.

Apstrakt

Uvod. Perkutana vertebroplastika (PVP), kao mini invazivni pristup u lečenju bolesnika sa kompresivnom frakturom osteoporotičnog pršljena (KFOP), obezbeđuje stabilizaciju kičmenog stuba i otklanja bol. Najčešće se primenjuje u periodu od tri do sest nedelja, pre pojave krivljenja/pogrbljenja kičme. Potpuni prestanak bola je lakše postići ako se leče "manje zreli" prelomi. Cilj prikaza je da se pokaže da je PVP efikasna i bezbedna i kod starih fraktura. **Prikaz bolesnika.** Bolesnik starosti 77 godina, posle minimalne traume, zadobio je stabilnu kompresivnu frakturu tela trećeg lumbalnog (L3) pršljena koja je klinički i radiološki verifikovana. Zbog permanentnog bola i tipa frakture primarno je uključeno konzervativno lečenje (lumbosakralna ortoza-mider), analgetici i fizikalna terapija. Posle dva meseca bol je i dalje perzistirao, ali sada u torakalnom segmentu kičmenog stuba gde je dao radiološki konstatovane frakture tela osmog torakalnog (Th8) i desetog torakalnog (Th10) pršljena, bez neurološkog deficita. Ordinirana je konzervativna terapija, ali zbog perzistentnog bola 6 meseci kasnije postavljena je indikacija za operativno lečenje u smislu PVP tela Th 8 i Th 10. Bol je kupiran i

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bolesnik je otpušten iz klinike drugog postoperativnog dana. Kontrolni pregledi bili su uredni. **Zaključak.** Kod bolesnika sa stabilnom KFOP, PVP je efikasna terapija za redukciju bola i poboljšanje mobilnosti i kod preloma starih šest meseci.

Ključne reči:
kičmeni pršljenovi, prelomi; prelomi, kompresivni; osteoporoza; bol; vertebroplastika; lečenje, ishod.

Introduction

Painful vertebral compression fractures (VCFs) may be the consequences of different pathological factors such as osteoporosis, myeloma or vertebral metastases. The very common cause of these fractures is trauma, even the minor one, especially when associated with osteoporosis [1]. The most of these fractures are asymptomatic, but even in this cases and especially in symptomatic ones, quality of life may be notably changed due to height loss, kyphosis, back pain, and lost self-confidence regarding physical activities [2]. The conservative treatment including analgesic medications, rest and physical therapy is often ineffective on long-term basis, because of the persistent pain, decreased mobility and neurological complications [3, 4]. Operative management of VCFs has gained popularity as it produces rapid, significant and sustained improvements in back pain, function and quality of life. Surgical intervention is indicated for those patients with intractable back pain in whom conservative therapy failed, or where there is evidence of impending or existing neurologic deficit, or where the spinal deformity is extremely severe [5].

Percutaneous vertebroplasty (PVP) is one of the favored methods of treating painful VCFs. It encompasses augmentation of the vertebral body by injection of polymethylmethacrylate. Short-term results indicated that 75% to 100% of patients can have good to moderate pain relief after vertebroplasty. PVP is most effective in compression fractures less than 6 months old [5].

The pain relief is the primary goal of this treatment, beside the vertebral stabilization, better mobility and functional improvement [4]. Indications for the PVP are persistent and intensive back pain at the level of osteoporotic fractured vertebra when the Visual Analogue Scale (VAS) is 5 or higher [4, 6]; fracture not older than a year, with the best analgesic results with lesions not older than six months, increased risk for kiphosis [2, 7], vertebral fracture with less than 30% height loss [8]. Contraindications include coagulation disorders, allergies to bone cement or contrast, systemic or local infection [7], osteomyelitis and spondylodiscitis, tumor extension into epidural space [2], unstable or older fractures [7], asymptomatic fractures and fracture with spinal cord compression and resulting neurological signs [4, 6].

The aim of this paper was to show that PVP can successfully be used for six months old osteoporotic VCFs.

Case report

A 77-old patient was admitted with a chief complaint of the back pain, primarily localized in lumbosacral (LS) region after minor injury. The fracture of the body of third lumbar (L3) vertebra was clinically and radiologically diagnosed and estimated as stable, with no indication for surgical treatment. The lumbo-sacral orthosis (LSO), analgesic drugs and physical therapy were prescribed as conservative treatment for spine stability and pain reduction.

After a period of 2 months, the patient, denying any kind of trauma, was examined again due to back pain in the thoracic region. Pain in the LS spinal segment persisted, in spite of prescribed therapy and wearing of LSO. Vertebral injuries of thoracic vertebra 8 (Th8) and thoracic vertebra 10 (Th10) were radiologically diagnosed, with no neurological deficit on physical examination (Figure 1).

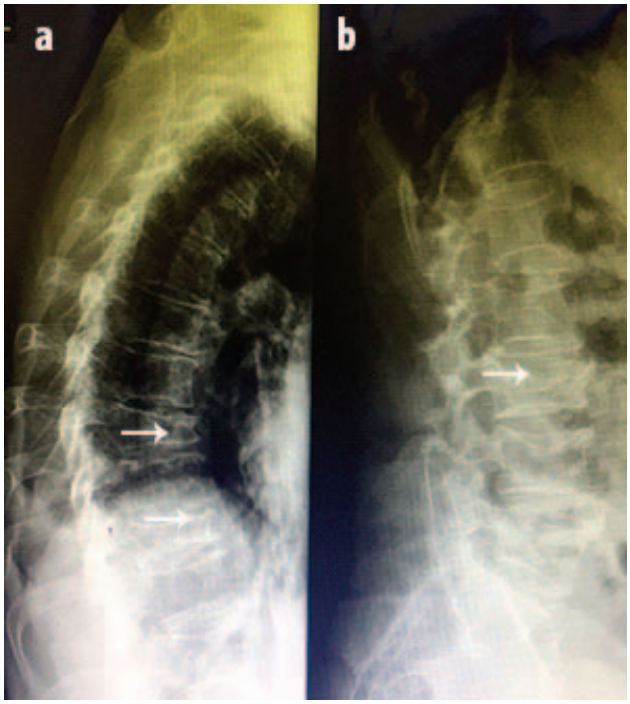

Fig. 1 – a) Radiographic left-lateral projection: thoracic (Th) vertebrae – de novo fractures of Th8 and Th10 vertebral body before vertebroplasty; b) Radiographic right-lateral projection: primarily fracture of lumbar third (L3) vertebral body.

Thoraco-lumbo-sacral orthosis (TLSO) was prescribed. After 6 months, the patient still felt pain in the injured region, predominantly in thoracic region, with the Visual Analogue Scale (VAS) score of 8. Nervous structures were intact and the strength of lower extremity muscle was preserved – score was 5. Earlier fracture of L3 was healed, but because of persisted pain, the indication for PVP of the Th8 and Th10 vertebral body was given. The patient was admitted to the Clinic for Orthopedics at the Clinical Center Kragujevac. After the usual and appropriate preoperative preparation and administration of 2% lidocaine (10 mL) as local anesthetic, standard PVP [2] of injured vertebral bodies of Th8 and Th10 was performed (Figures 2 and 3).

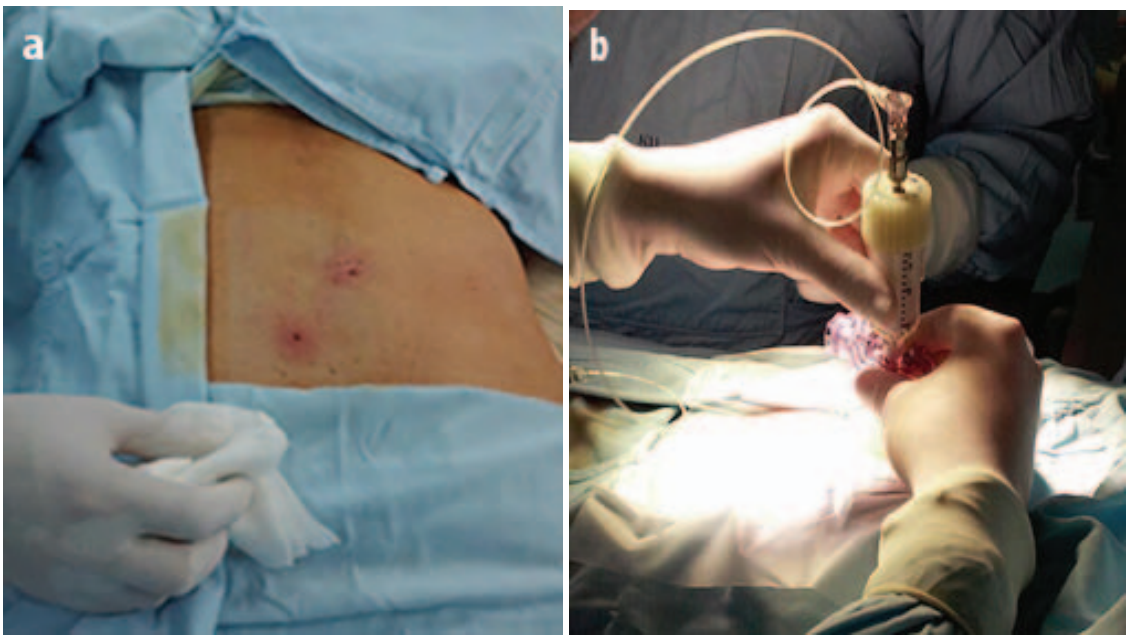

Fig. 2 – a) Operative field in the level of thoracic 8 (Th8) and thoracic 9 (Th9) vertebra; b) The cement application during the vertebroplasty.

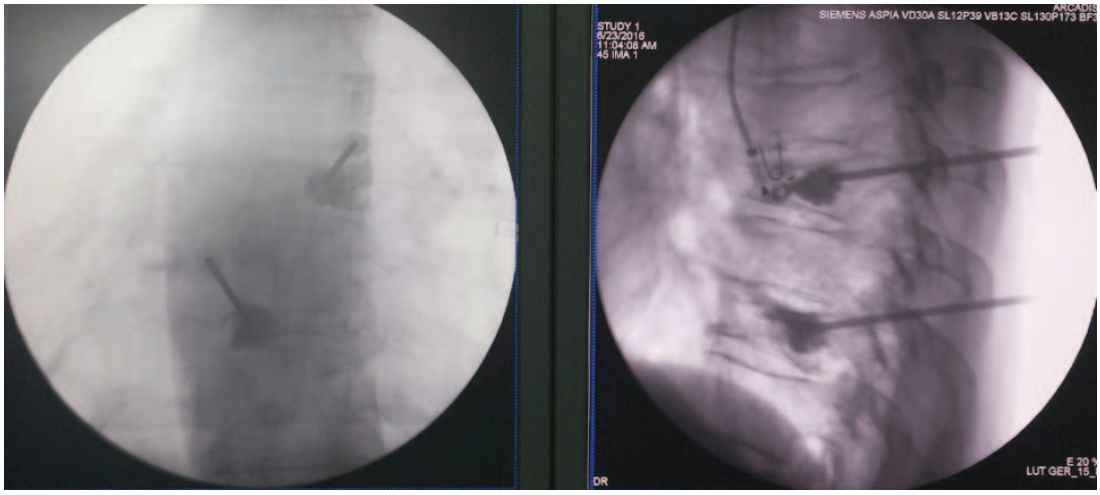

Fig. 3 – Radiographic imaging: Needle position in anteroposterior (AP) and lateral projection; Unipedicular approach to vertebral body.

Pathohistological findings showed fragments of cortical, partly grossly calcified bone, with elements of active bone marrow, with slight domination of granulocytes (eosinophils), with no elements of myeloproliferative or metastatic disease (Figure 4).

The patient's early postoperative course was uneventful; he achieved full vertical posture a day after the surgical treatment and was able to walk without assistance. He was discharged from the Clinic for Orthopedics on the second postoperative day without clinical symptoms, with recommendation to use TLSO brace. The patient was reviewed in clinic 5 days after the procedure, when he came without help, with no complaints and without prescribed TLSO brace. He was followed-up in two weeks intervals and was symptom free. Complete physical rehabilitation program was conducted and patient returned to his daily life activities.

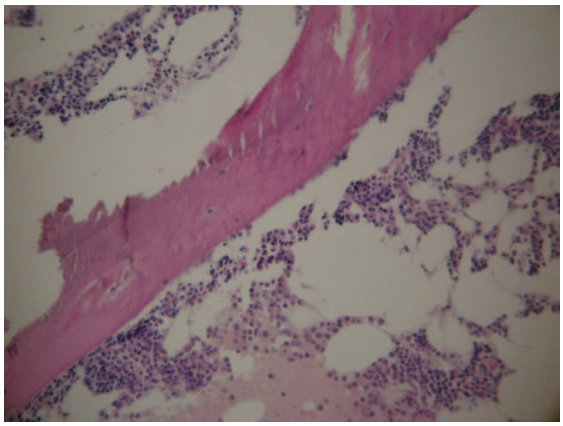

Fig. 4 – Fragments of cortical bone with elements of active bone marrow, with domination of granulocytes hematoxylin-eosin (HE x 400).

Discussion

The first PVP was performed in 1984 by French radiologists Galibert and Deramond for treatment of a painful hemangioma in the cervical spine of a young female patient. PVP is a technique in which a medical grade cement is injected through a needle into a painful vertebral body. This stabilizes the fracture, allowing most patients to discontinue or significantly decrease analgesics and resume normal activities. The success rate for this procedure in treating osteoporotic VCFs is 73%–90%. Significant complications of the procedure are less than 1% [5].

The presented patient underwent PVP in order to reduce the back pain caused by the vertebral fractures occurred after the minor trauma.

The symptoms associated with vertebral osteoporotic vertebral compression fractures (OVCFs) changes in quality of life. In the group of patients with clinical symptoms due to an OVCFs (one-third of all patients with a OVCFs), pain is the most striking feature of the fracture. In 80%–85% of the acute symptomatic patients, pain will disappear with conservative treatment within 6–8 weeks after initiation of the treatment [9].

In managing the presented patient, the surgeons opted for PVP, considering that conservative therapy was not effective in the pain relief in thoracic and lumbar spine. The pain reduction in our patient was complete after the conducted PVP. The results of most of the studies showed much better pain relief after PVP than after conservative treatment [4, 10]. The significant reduction of pain score from the first postoperative day is also the great advantage of PVP [11]. The adverse effects of analgesic drugs and long period in bed during the conservative medical management may contribute to worsening of the symptoms and the further demineralization of the bones [12, 13]. Several studies also reported improving kyphosis to a certain degree after PVP [11, 14]. It is also shown that minimally invasive procedures, such as this one, are cost-effective in comparison to nonsurgical treatment for osteoporotic and tumor related vertebral fractures [15].

Also, obtaining bone biopsies during PVP does not lead to increased morbidity and can verify the pathologic process underlying the VCFs [16].

According to Röllinghoff et al. [6] PVP should not be conducted in patients younger than 48 years. Also, patients over the 85 are not candidates for PVP, considering the low bone mineral density [16]. The presented patient in our case was 77 years old, so the age was not the contraindication for PVP. The fractured vertebrae of our patient were not with posterior dislocation, which would also be the contraindication for this surgical procedure [6, 12]. The presented PVP was conducted with satisfactory results six months after the reported trauma. In several conducted studies it was shown that better results were obtained when the procedure was done in the first months after the trauma [4], but the pain relief and normal life quality after PVP were also described in patients with one year old fracture of the spine [14]. In managing this patient, surgeons opted for unilateral transpedicular approach, which advantages over the bilateral approach are in the reducing the time required to perform the treatment, radiation exposure, risks of the side effects and the costs [15]. The one of the described common complications of PVP is the fracture of the non-treated vertebrae next to the treated one [16, 17]. This may be the consequence of the greater stiffness of the vertebra filled with bone cement and altered biomechanics and the load transfer of the spine [11]. According to the others, these new fractures are not the side effect of the PVP, but the result of the further deterioration of the osteoporotic spine and reduction of bone mineral density [3, 10, 16]. In presented case, no complications were encountered.

Several clinical studies and meta-analysis concluded that the PVP is very successful surgical method in reducing the pain in OVCFs, the complications of this technique are rare and that is more successful in pain relief and functional recovery than non-surgical therapy [12, 17]. PVP is effective in patients with chronic painful osteoporotic VCFs. Pain relief after PVP was immediate, sustained for one year and may be an important factor for reducing persistent pain. PVP for patients with chronic painful osteoporotic VCFs has not been extensively studied [18]. The majority of papers describe populations that are a case mix of "acute" (fracture age < 8 weeks) and "long-standing" (fracture > 8 weeks) OVCs. Subacute (> 2 month old) and chronic (> 6 month old) OVCFs are fractures which do not respond to at least 8 weeks of conservative treatment using analgesics, a short period of bed rest and a corset. Treatment of long-standing fractures remains controversial. Despite these preliminary reports, outcomes in patients with older fractures treated by PVP remain undefined. The most of the older fractures respond to PVP, although there may be fewer complete responses. However, treating patients earlier is still preferable because they are more likely to have complete eradication of pain and may retain more mobility [19].

Conclusion

In patient with compression fracture of vertebral body, when the pain relief cannot be achieved by application the conservative therapy, PVP, performed under local anesthesia, is the treatment of choice for spine stabilization and fast and lasting pain reduction even for OVCF six months old.

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Bilateral ovarian metastases of gallbladder carcinoma – A case report

Obostrane ovarijalne metastaze karcinoma žučne kese

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Abstract

Introduction. Gallbladder carcinoma is a rare malignancy with a poor prognosis because it is diagnosed late. There are only a few cases of ovarian metastasis from gallbladder carcinoma described in the literature. We presented a rare case of ovarian metastasis of gallbladder carcinoma and highlight the importance of differentiation between primary and metastatic ovarian tumors. **Case report.** A 55-year old women had cholecystectomy for suspected cholecystitis. However, histological findings showed invasive adenocarcinoma of her gallbladder. The patient refused further proposed treatment. Three months later, the same patient presented with abdominal pain and discomfort. Imaging diagnostic methods (magnetic resonance scan) showed no local tumour mass at the site of cholecystectomy, but large, bilateral, multilocular ovarian tumor of mixed consistency. During surgery, ovarian tumours and infiltration of omentum was found. A total abdominal hysterectomy with bilateral salpingo-oophorectomy and omentectomy was performed. Histological findings indicated adenocarcinoma but could not distinguish between a primary ovarian carcinoma and gallbladder metastatic tumor. An immunohistochemical examination clarified that the findings corresponded to metastatic ovarian adenocarcinoma from the gastrointestinal tract, i.e., adenocarcinoma originating from the gallbladder. Unfortunately, the patient did not successfully recover and died three months later. **Conclusion.** The presence of ovarian masses of unknown origin and a diagnostic dilemma between primary and metastatic tumor require careful clinical, radiological, intraoperative, and histological examination for the purpose of establishing a definitive diagnosis and providing optimal treatment.

Key words:
gallbladder neoplasms; neoplasm metastasis; ovary; diagnosis, differential; immunohistochemistry; treatment outcome.

Apstrakt

Uvod. Karcinom žučne kese je redak malignitet koji zbog kasnog dijagnostikovanja ima lošu prognozu. U literaturi je opisano samo nekoliko slučajeva ovarijalnih metastaza karcinoma žučne kese. Prikazan je redak slučaj bolesnice sa ovarijalnim metastazama karcinoma žučne kese sa akcentom na značaju diferencijalne dijagnoze između primarnih i metastatskih tumora jajnika. **Prikaz bolesnika.** Bolesnici starosti 55 godina zbog sumnje na zapaljenje žučne kese urađena je holecistektomija. Međutim, histološkom analizom utvrđeno je da se radilo o karcinomu žučne kese. Bolesnica je odbila predloženo dalje lečenje. Posle tri meseca javila se u ambulantu zbog bolova u trbuhu. Imidžing dijagnostikom (magnetna rezonanca) isključeno je prisustvo lokalnih tumorskih masa na mestu holecistektomije, ali su vizualizovani veliki obostrani tumori jajnika mešovite konzistencije. Intraoperativno, nađeni su tumori jajnika i infiltracija omentuma. Urađena je totalna histerektomija sa obostranom adneksektomijom i totalna suprakolična omentektomija. Histologijom je utvrđeno da se radilo o adenokarcinomu, ali je ostalo nejasno da li se radilo o primarnom adenokarcinomu jajnika ili metastazama karcinoma žučne kese. Tek imunohistohemijskom analizom utvrđeno je da se radilo o metastatskom karcinomu jajnika porekla gastrointestinalnog trakta, najverovatnije žučne kese. Nažalost, bolesnica nije uspela da se oporavi i preminula je nakon tri meseca. **Zaključak.** Prisustvo ovarijalnih tumora nepoznatog porekla i dijagnostička dilema između primarnog i metastatskih tumora zahtevaju pažljivo kliničko, radiološko, intraoperatvno i histološko ispitivanje, sa ciljem postavljanja definitivne dijagnoze i pružanja optimalnog lečenja bolesnika.

Ključne reči:
žučna kesa, neoplazme; neoplazme, metastaze; jajnik; dijagnoza, diferencijalna; imunohistohemija; lečenje, ishod.

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Introduction

Gallbladder carcinoma is a rare malignancy with a poor prognosis due to the fact that it is almost always diagnosed at a late stage of the disease [1, 2]. There are no differences in the clinical presentations of benign biliary disease and gallbladder carcinoma, which is why gallbladder carcinoma must always be kept in mind [2]. The tumour spreads most commonly by direct extension and may involve adjacent organs (liver, bile duct, duodenum, colon), while vascular invasion can cause distant metastasis into the liver, lungs, and other organs.

Approximately 5% to 15% of malignant ovarian tumors are metastases from other sites (genital or non-genital tract tumours) [3]. Making an accurate distinction between primary and metastatic ovarian tumors is of a great importance since mistakes in interpretation can lead to inadequate treatment and suboptimal outcomes. The origin of ovarian metastasis is usually the gastrointestinal tract with the best known metastasis being the Krukenberg tumor arising in 73% from gastric cancer [4]. Moreover, some researchers showed that the most common non-genital malignant tumors presenting with ovarian metastasis are colon cancer, followed by appendix and breast [5]. On the other hand, there are only a few cases of ovarian metastasis from gallbladder carcinoma described in literature [6–9].

Case report

A 55-year-old woman underwent a classic cholecystectomy for suspected cholecystitis. As the histologic examination of the gallbladder showed invasive adenocarcinoma, the Clinic Cancer Board indicated an exploratory laparotomy for radical resection, but the patient refused the proposed reoperation and any further treatment. Three months later, the same patient presented to a gynaecological outpatient clinic with lower abdominal pain and discomfort. A mass arising from her pelvis and extending to the umbilical level of the abdomen was palpated on a bimanual examination and presented as solid. The patient's blood count and liver function tests were within normal limits. A serum level of Ca-125 was 80.6 U/mL (reference range 0–21 U/mL). Pelvic ultrasonography showed large, bilateral, multiseptated ovarian tumors with solid components and the presence of a large amount of ascites in the abdomen. A magnetic resonance (MR) scan of her pelvis and abdomen was performed (Figure 1).

A chest radiograph revealed left-sided pleural effusion. After a preoperative evaluation, an exploratory laparotomy was performed. Intraoperative findings revealed about five litres of ascites and bilateral adnexal tumors. The left adnexal tumor was 20 × 15 cm in diameter, and the right one was 15 × 10 cm. Both tumors were of semisolid consistency and smooth greyish surface, and identification of the ovarian or tubal tissues was impossible. Tumor infiltration of the bladder wall and omentum was identified as well as massive adhesions. A total abdominal hysterectomy with bilateral salpingo-oophorectomy and resection of the visualized tumor masses and omentectomy were performed.

The postoperative course was uneventful, and the patient was discharged from hospital on the eighth postoperative day.

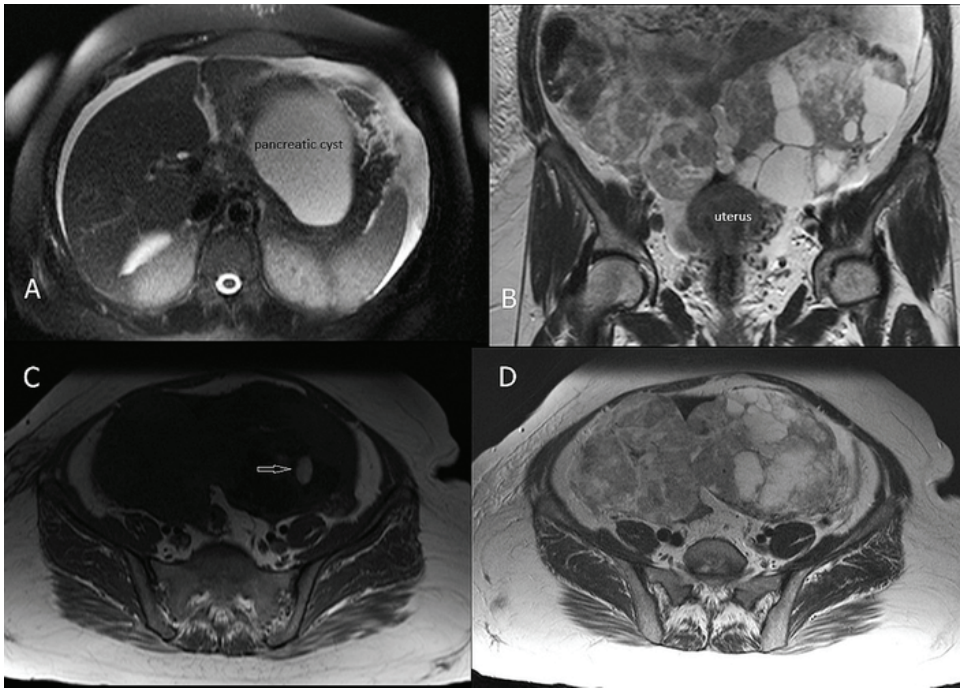

Fig. 1 – Magnetic resonance imaging findings: A) The local tumor mass was not seen at the site of the cholecystectomy. The T2w FS image reveals ascites as well as the presence of a big, encapsulated, well-defined, cystic mass in the pancreatic tail; B) Bilateral ovarian tumor masses, multilocular and with thick septa, of inhomogeneous signal intensity are seen in a coronal T2w image. Both tumor masses are well delineated without infiltration of adjacent structures; C) An axial T1w FS image showed the presence of small areas of haemorrhage in the tumor mass (indicated by an arrow); D) No enlarged parailiac lymph nodes are noted on the axial T2w image.

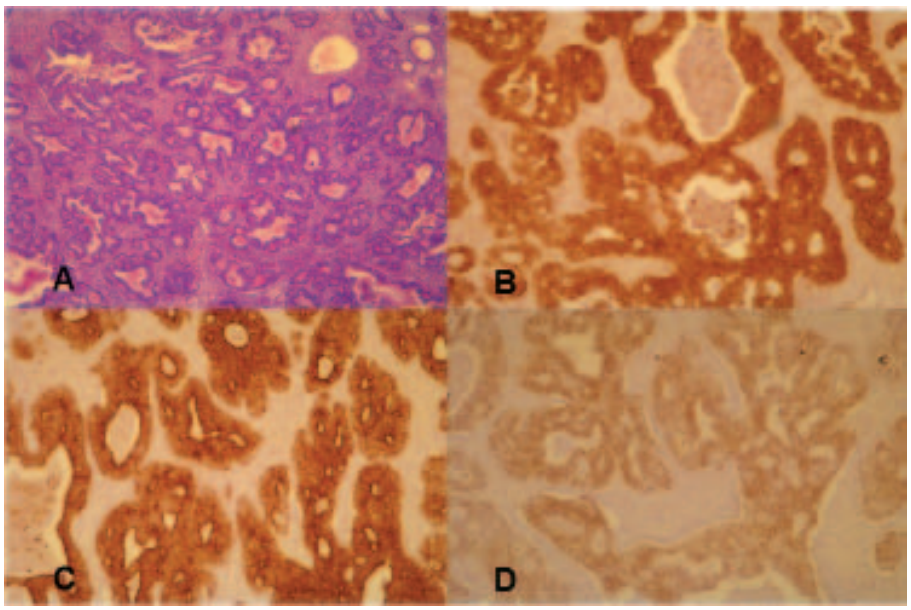

Fig. 2 –A) Small gland adenocarcinoma in a prominent desmoplastic stroma (hematoxylin and eosin, ×5); B) The tumor cells are diffusely and strongly positive for ß-catenin (×10); C) CK7 (×10), and D) CDX-2 (×10).

Histological findings were, on the basis of morphology, unable to determine whether the patient was a case of primary ovarian carcinoma or gallbladder metastatic tumor. The histologic specimen was routinely processed and embedded in paraffin, and sections were stained with hematoxylin-eosin for light microscopic examination (Figure 2A). The well-differentiated adenocarcinoma was formed of small tubular and rare microcystic glands surrounded by desmoplastic, or more often, oedematous stroma. The glands and microcysts had attenuated lining cells. Haemorrhage and necrosis were spread in the tumor, and residual ovarian structures were not presented. A review of the histological findings indicated adenocarcinoma, which had the same morphological characteristics as the gallbladder adenocarcinoma of the patient, and concluded that a definitive diagnosis required immunohistochemical examinations of specimens of both the gallbladder and the ovaries.

Immunohistochemistry was performed on paraffin sections with a broad spectrum of immunohistochemical stains (Figures 2B, 2C, 2D). The tumor cells showed diffuse strong labelling for CK7, CDX-2, and β-catenin, but only focal CK-20. Expression of ER, PR, WT-1, and Ca-125 was absent. Based on light microscopy appearance along with the immunohistochemistry staining, the final diagnosis of ovarian metastasis of primary gallbladder adenocarcinoma was made.

The definitive decision made by the Clinic Cancer Board after the surgical treatment was that specific oncological treatment was not indicated considering the disease severity and the overall condition of the patient, but that symptomatic and supportive therapy was indicated.

Unfortunately, the patient died three months after the gynaecological surgery due to an advanced stage of malignancy. The patient's family refused to give consent for the autopsy.

Discussion

The clinical presentation of gallbladder carcinoma mimics benign conditions, which is why gallbladder carcinoma is often diagnosed at an advanced stage and therefore has a poor prognosis. The patient we are reporting on was also misdiagnosed due to confusing symptomatology. Overall mean survival is a mere six months, while the five-year survival rate is only 5% [10]. The most common metastatic lesions are in the liver, pancreas, and common biliary duct [11].

Most metastatic ovarian tumors originate from the gastrointestinal tract, breast, and gynaecologic organs. Unlike colon or appendix carcinoma, or primary tumors of the upper gastrointestinal tract, which usually produce metastatic transformations of the ovary, ovarian biliary metastases are rare [1]. In general, patients with metastatic ovarian cancer are younger than patients with primary epithelial ovarian tumors, which proved to be true for our patient as well. The reported median age is between 35.7 and 55 years [4].

It is very important to distinguish metastatic ovarian tumours from primary ovarian cancer; otherwise, the patient may get insufficient treatment since imaging findings cannot make accurate distinctions. Available literature data suggest that bilaterality and semisolid, semicystic consistency point to metastatic ovarian tumors [3]. In 66% of cases, metastatic ovarian cancers are bilateral tumours that symmetrically enlarge the ovaries, retaining the overall ovarian outline [4], features which were present in our case as well.

Misinterpretation of metastatic ovarian tumors as primary ovarian neoplasms occurs more often when ovarian lesions are the first manifestation of the disease. In the presented case, the data on the previous surgery, histological diagnosis of the gallbladder adenocarcinoma, and

current local findings of the bilateral adnexal masses and their macroscopic appearance could have led to a reasonable conclusion of metastatic ovarian tumor. Nonetheless, the fact that there were neither local metastases nor recidivism of the primary carcinoma was misleading. Moreover, the presentation of unusually advanced ovarian vascular deposits, rather than local metastases of the gallbladder carcinoma, makes this case distinctive. Finally, only an immunohistochemical analysis was able to clarify any doubts and disregard the possibility of primary ovarian adenocarcinoma.

Conclusion

The presence of ovarian masses of unknown origin and the diagnostic dilemma between primary and metastatic tumors require careful clinical, radiological, intraoperative, and histological examination for the purpose of establishing a definitive diagnosis. Whenever adnexal masses are detected, it is necessary to conduct an evaluation of the patient's gastrointestinal tract.

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Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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