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Pierre-Auguste Renoir (February 25, 1841 – December 3, 1919): A women nursing baby (oil on canvas; 41.20 × 32.50 cm), Scotish National Gallery, Edinburgh, Scotland, United Kingdom.

The World Breastfeeding Week marks every year from 1 to 7 August in more than 170 countries to protect, promote and support breastfeeding and improve the health of babies around the world. This year's World Breastfeeding Week is aiming to support women to combine breastfeeding and work. Regardless of where they work, women should be allowed to exercise their right to breastfeeding. That is why a theme of the World Breastfeeding Week 2015 is: Breastfeeding and work – Let's make it work!

Pijer-Ogist Renoar (25. februar, 1841–3. decembar, 1919): Žena doji bebu (ulje na platnu; 41,20 × 32,50 cm), Škotska nacionalna galerija, Edinburg, Škotska, Ujedinjeno kraljevstvo.

Svetska nedelja dojenja obeležava se svake godine od 1. do 7. avgusta u više od 170 zemalja sa ciljem da zaštiti, promoviše i podupre dojenje, i da poboljša zdravlje beba širom sveta. Ovogodišnja Svetska nedelja dojenja ima za cilj da podrži žene u nastojanju da kombinuju dojenje i rad. Bez obzira gde rade, ženama bi trebalo omogućiti da ostvare svoje pravo na dojenje. Zbog toga je tema Svetske nedelje dojenja u 2015. godini: Dojenje i rad – Učinimo da to radi!

ORIGINAL ARTICLE

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Relationship between of short-course preoperative radiotherapy and serum albumin level and postoperative complications in rectal cancer surgery

Odnos između kratkotrajne preoperativne radioterapije i vrednosti albumina u serumu i postoperativnih komplikacija u hirurgiji karcinoma rektuma

Bratislav Trifunović*†, Jovan Kršić*, Mihailo Bezmarević*, Dragan Grbović*, Dejan Zeljković*, Branimir Nešković*, Ivan Soldatović‡, Rade Prelević§, Darko Mirković*†

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Abstract

Background/Aim. The identification of risk factors could play a role in improving early postoperative outcome for rectal cancer surgery patients. The aim of this study was to determine the relationship between short-course preoperative radiotherapy (RT), serum albumin level and the development of postoperative complications in patients after anterior rectal resection due to rectal cancer without creation of diverting stoma. **Methods.** This retrospective study included patients with histopathologically confirmed adenocarcinoma of the rectum with the clinical stage of T2-T4 operated on between 2007 and 2012. All the patients underwent open anterior rectal resection without diverting stoma creation. Preoperative serum albumin was measured in each patient. Tumor location was noted intraoperatively as the distance between the inferior tumor margin and anal verge. Tumor size was measured and noted by the pathologist who assessed specimens. Some of the patients received short-course preoperative RT, and some did not. The patients were divided into two groups (group 1 with short-course preoperative RT, group 2 without short-course preoperative RT). Postoperative complications included clinically apparent anastomotic leakage, wound infection, diffuse peritonitis and pneumonia. They were compared between the groups, in relation to preoperative serum albumin level, patient age, tumor size and location. **Results.** The study included 107 patients (51 in the group 1 and 56 in the group 2). There were no significant difference in age ($p = 0.95$), gender ($p = 0.12$) and tumor distance from anal verge ($p = 0.53$). The size of rectal carcinoma was significantly higher in the group 1 than in the group 2 (51.37 ± 12.04 mm *vs* 45.57 ± 9.81 mm, respectively; $p = 0.007$). The preoperative serum albumin level was significantly lower in the group 1 than in the group 2 (34.80 ± 2.85 g/L *vs* 37.55 ± 2.74 g/L, respectively; $p < 0.001$). A significant correlation between the tumor size and the serum albumin level was found ($p = 0.042$). Overall, postoperative complications were observed in 13 (25.5%) patients in the group 1 and in 10 (17.8%) patients in the group 2 without significant difference between the groups ($p = 0.18$). A significantly lower level of serum albumin was found in patients with postoperative complications and in those who died. A significant difference in anastomotic leakage occurrence between groups was found ($p = 0.039$). Male gender and the lower level of serum albumin were significant predictors for anastomotic leakage occurrence ($p = 0.05$ and $p = 0.002$, respectively), but preoperative RT had no significant impact on it. **Conclusions.** A lower serum albumin level, but not short-course of preoperative RT, was significantly associated with postoperative complications development after rectal resection without diverting stoma.

Key words:
rectal neoplasms; radiotherapy, adjuvant; surgical procedures, operative; treatment outcome; postoperative period; risk factors; serum albumin.

Apstrakt

Uvod/Cilj. Identifikacija faktora rizika mogla bi biti važna za poboljšanje ranog postoperativnog ishoda kod bolesnika operisanih zbog karcinoma rektuma. Cilj ove studije bio je da se odredi odnos između kratkotrajne preoperativne radioterapije (RT), vrednosti albumina u serumu i nastanka postoperativnih komplikacija kod bolesnika podvrgnutih prednjoj resekciji rektuma zbog rektalnog karcinoma bez kreiranja protektivne stome. **Metode.** Ova retrospektivna studija obuhvatila je bolesnike sa histološki potvrđenim adenokarcinomom rektuma i kliničkim stadijumom T2-T4, operisa-

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nih u periodu 2007–2012. godine. Kod svih bolesnika učinjena je prednja resekcija rektuma otvorenim hirurškim pristupom bez kreiranja protektivne stome. Kod svakog bolesnika određena je preoperativna vrednost albumina u serumu. Lokalizacija tumora zabeležena je intraoperativno kao udaljenost donje ivice tumora od analne ivice. Dimenziju tumora merio je i zabeležio patolog koji je pregledao resecirane uzorke. Jedan broj bolesnika primio je kratkotrajnu preoperativnu RT, dok drugi nisu. Bolesnici su bili podeljeni u dve grupe (grupa 1 sa kratkotrajnom preoperativnom RT i grupa 2 bez kratkotrajne preoperativne RT). Postoperativne komplikacije: klinički evidentna dehiscencija anastomoze, infekcija hirurške rane, difuzni peritonitis i pneumonija, upoređivane su između grupa u odnosu na vrednost serumskog albumina merenog preoperativno, godine starosti bolesnika, dimenzije i lokalizaciju tumora. **Rezultati.** Studija je obuhvatila 107 bolesnika (51 u grupi 1 i 56 u grupi 2). Nije bilo značajne razlike između grupa u godinama starosti (p = 0,95), polu (p = 0,12) bolesnika, i udaljenosti tumora od analne granice (p = 0,53). Dimenzija rektalnog karcinoma bila je značajno veća u grupi 1 nego u grupi 2 (51,37 ± 12,04 mm *vs* 45,57 ± 9,81 mm, p = 0.007). Vrednost preoperativno merenog albumina u serumu bila je značajno niža u grupi 1 nego u grupi 2 (34,80 ± 2,85 g/L *vs* 37,55 ± 2,74 g/L, p < 0,001). Nađena je statistički značajna korelacija između dimenzije tumora i vrednosti serumskog albumina (p = 0,042). Ukupno, postoperativne komplikacije zabeležene su kod 13 (25,5%) bolesnika u grupi 1 i kod 10 (17,8%) u grupi 2, bez statistički značajne razlike između grupa (p = 0,18). Statistički značajno niži nivo serumskog albumina nađen je kod bolesnika sa postoperativnim komplikacijama i kod onih koji su umrli. Uočena je statistički značajna razlika u pojavi dehiscencije anastomoze između grupa (p = 0,039). Muški pol i niža vrednost serumskog albumina bili su statistički značajni prediktori pojave dehiscencije anastomoze (p = 0,05 odnosno p = 0,002), dok kratkotrajna preoperativna RT nije imala statistički značajnog uticaja na njenu pojavu. **Zaključak.** Ustanovljena je povezanost nižih vrednosti serumskog albumina, ali ne i kratkotrajne preoperativne RT, sa nastankom ranih postoperativnih komplikacija nakon resekcije rektuma zbog rektalnog karcinoma bez kreiranja protektivne stome.

Ključne reči:
rektum, neoplazme; radioterapija, adjuvantna; hirurgija, operativne procedure; lečenje, ishod; postoperativni period; faktori rizika; albumin, serumski.

Introduction

Colorectal cancer is a significant cause of morbidity and mortality and one of the most common malignant disease worldover [1]. The actual treatment regimen for rectal cancer is en-bloc surgical excision of affected segment of the bowel with tumor-specific mesorectal excision. The importance of circumferential lateral spread in local disease recurrence led to the introduction of total mesorectal excision (TME) by Heald in 1982, which ensures resection of the complete mesorectum [2–4]. The adjuvant therapy, such as chemotherapy or irradiation may be used for more advanced rectal cancers [5]. Randomized studies have shown benefits of radiotherapy (RT) over surgery alone [6–8]. Preoperative RT has some advantages over postoperative adjuvant RT that include: better local disease control, reduced therapeutic toxicity, increasing the possibility of sphincter preservation surgery, and better overall outcome in recently published studies [9–13]. Short-course irradiation (25 Gy in 5 fractions) with immediate surgery is frequent regimen in the preoperative treatment of patients with resectable rectal cancer [8, 14–16]. However, there are concerns of neoadjuvant therapy effects on the early post-operative morbidity [17, 18]. Serum albumin has been considered as a marker of nutritional status [19, 20], but a number of studies consider it as an inflammatory marker rather than indicator of nutrition [21]. Studies have shown its predictive value in surgery for colon cancer [22, 23], but still, there is a lack of such evidence in surgery for rectal cancer and its complications [24].

Tumor size, in particular the maximal horizontal tumor diameter is an important prognostic parameter for patients with colorectal cancer. Whereas prognostic influence is strong within the colon, it appears to be of minor value within the rectum [25].

A number of possible complications and the risk of perioperative mortality burden patients undergoing resection for colorectal cancer [26]. Despite advances in surgical techniques, better understanding of the impact on preoperative bowel preparation, prophylactic antibiotics and better postoperative care, colorectal surgery is associated with the mortality rate of 3% to 6% and morbidity of 20% to 40% [27, 28]. The fact that colorectal cancer is a disease of the elderly, with only 5% of cases recorded among those below 40 years of age, even further complicates outcome [29, 30]. The results of systematic review of the outcome of surgery for colorectal cancer in elderly patients showed a progressive increase of postoperative morbidity and mortality with advancing age [31]. Wound infections, intra-abdominal abscesses, and/or anastomotic leakage are the most common complications. In literature data, there is a wide range of morbidity and mortality rates associated with anastomotic leakage [32]. In the past, an incidence of anastomotic leakage varied from 3.4% to 40% [33, 34]. A clinical leakage rate after anterior rectal resection varies from 3% to 11%, and its occurrence depends on various factors [35–38]. The advantages of diverting stoma after rectal resection are still under debate. Some authors propose creation of a diverting stoma only in low rectal resections, neoadjuvant chemoradiotherapy (CRT) and in patients with significant comorbidities, but others do not recommend a stoma at all [39–41]. Late postoperative complications are functional derangements and incontinence, especially in patients with neoadjuvant RT [42, 43]. Sexual and bladder functions may also be affected due to injury of autonomic nerves [44].

The identification of risk factors could play a role in improving early postoperative outcome. The literature (Medline, PubMed) has not conclusive data regarding postoperative complications between patients with and without short-course of preoperative RT without diverting stoma.

The aim of this study was to determine the relationship of preoperative serum albumin level and short-course preoperative RT with the development of early postoperative complications in patients with anterior rectal resection immediately after RT without creation of diverting stoma.

Methods

This retrospective study included patients with elective oncological resection for rectal adenocarcinoma between January 2007 and December 2012 at the Clinic for General Surgery, Military Medical Academy, Belgrade, Serbia. The study included all operated patients with histologically confirmed adenocarcinoma of the rectum with the clinical stage T2 to T4 (cT2-T4Nx disease) of the International American Joint Committee on Cancer (AJCC 6th edition) TNM classification [45] who underwent anterior resection of rectal cancer without creation of a diverting stoma, with or without short-course preoperative RT. Preoperative clinical staging was assessed by physical and *per* rectal examination, and by various imaging modalities (computed tomography scan, magnetic resonance imaging, endoscopic ultrasound). The inferior tumor margin was located no farther than 15 cm from the anal verge, measured by sigmoidoscopy. Preoperative serum albumin level was measured in each patient and a value noted one day before surgery was included in the study. Short-course preoperative RT included the total dose of 25 Gy administered in 5 fractions during 5 days according to the International Commission on Radiation Units and Measurements 50 guidelines [46]. In the patients who received short-course preoperative RT rectal resection was performed within 2 to 7 days after RT. Every patient received preoperative mechanical bowel preparation and antibiotic prophylaxis (metronidazol 500 mg and ceftriaxone 2 g). In all the patients standard oncological anterior resection of a rectum with TME (if tumor was located in the middle and low parts of the rectum), and partial mesorectal excision (if tumor was located in the upper part of the rectum) was performed by open approach with single or double stapling anastomosis technique, without the creation of diverting stoma. Pelvic contact drain was placed in each patient. Tumor location was noted intraoperatively as the distance from the inferior tumor margin to the anal verge. Tumor size was measured and noted by the pathologist who assessed specimens. The patients were divided into two groups (the group 1 with short-course preoperative RT followed by resection and anastomosis and the group 2 with resection and anastomosis without short-course preoperative RT). Postoperative complications included clinically apparent anastomotic leakage (the presence of stercoral contents to the pelvic contact drain), wound infection, diffuse peritonitis and pneumonia. If conservative treatment of the patients with anastomotic leakage failed, they were reoperated (Hartmann's procedure in all of them). Postoperative complications were assessed between the groups, as well, in relation to preoperative serum albumin level, age, tumor size and tumor location.

Statistical analysis was performed with SPSS software (Statistical package for the social sciences version 18.0, Chicago, IL, USA). Mann-Whitney *U*-test, Student's *t*-test and χ^2-test were used to test the significance of differences between the two groups. Correlations between parameters were tested with Pearson's correlation. Logistic regression was used to evaluate the influence of parameters on postoperative complications development. The results were expressed as median (range), mean ± standard deviation and number (%). *P*-values < 0.05 were considered statistically significant for all comparisons.

Results

Patient demographic data and clinical characteristic are listed in Table 1.

Table 1

Demographic and clinical data of the patients with rectal cancer

Variable	Group 1 (n = 51)	Group 2 (n = 56)	*p* value	Total (n = 107)
Age (years)	67 (27–89)	66 (28–83)	0.95	67 (27–89)
Sex, male	31 (60.8)	42 (75.0)	0.115	73/34
Clinical tumor stage				
T2	2 (3.9)	9 (16.1)		11
T3	45 (88.2)	47 (83.9)		92
T4	4 (7.8)	0	0.007**	4
Tumor distance from the anal verge(cm)	9 (4–15)	9 (5–15)	0.53	9 (4–15)
Tumor size (mm)	51.37 ± 12.043	45.57 ± 9.811	0.007*	51 (26–72)
Serum albumin (g/L)	34.80 ± 2.85	37.55 ± 2.74	< 0.001*	36 (27–43)

Data are presented as median (range), mean ± standard deviation and number (%); **t*-test for equality means, **χ^2-test – statistically significant difference.

The study included a total of 107 operated patients due to rectal cancer, 51 with short-course preoperative RT (group 1) and 56 without it (group 2). The majority of patients had the T3 stage of rectal cancer in both groups, however only 4 (3.7%) patients had the T4 stage (all in the group 1) with a significant difference between the groups (χ^2-test 7.777, p = 0.007). No significant difference in age (p = 0.95), gender (p = 0.115) and tumor distance from the anal verge (p = 0.53) between the groups was found.

The size of rectal carcinoma was significantly higher in the group 1 (51.37 ± 12.04 mm) than in the group 2 (45.57 ±

9.81 mm), $p = 0.007$. The preoperative serum albumin level was significantly lower in the group 1 than in the group 2 (*t*-test = -5.09, $p < 0.001$).

Overall postoperative complications including anastomotic leakage (conservatively or surgically treated), wound infection, diffuse peritonitis and pneumonia were observed in 23 (21.5%) patients; in 13 (25.5%) patients in the group 1, and in 10 (17.8%) patients in the group 2 without significant difference between the groups ($p = 0.18$). Postoperative complications are presented in Table 2.

vative treatment was successful in 5 of them. Seven patients with anastomotic leakage (group 1) required reoperation (Hartmann's procedure in all of them). Four of those patients died of diffuse peritonitis ($p = 0.101$). There was a significant difference in anastomotic leakage occurrence between the groups ($p = 0.039$). There were no patients with anastomotic leakage who required reoperation in the group 2 *vs* 7 patients in the group 1 with a significant difference (χ^2 8.224, $p = 0.004$), but without differences in conservatively treated anastomotic leakage between the groups ($\chi^2 = 0.024$, $p = 1.0$).

Table 2

Observed postoperative complications in both groups of patients

Variable	Group 1 (n = 51)	Group 2 (n = 56)	*p* value	Total (n = 107)
	n (%)	n (%)		n (%)
Anastomotic leakage – surgically treated	7 (13.7)	0	0.004	7 (6.5)
Anastomotic leakage – conservatively treated	5 (9.8)	5 (8.9)	0.98	10 (9.3)
Diffuse peritonitis	4 (7.8)	0	0.048	4 (3.7)
Wound infection	14 (27.5)	8 (14.3)	0.092	22 (20.5)
Pneumonia	5 (9.8)	2 (3.6)	0.254	7 (6.5)

***p* – values are calculated for χ^2-test.**

The overall postoperative mortality rate was 5.6% (6 patients). In the group 1, five (9.8%) patients died in the postoperative period during hospitalization. Four of them died as consequences of postoperative complications (diffuse peritonitis), and one as consequences of acute pulmonary thromboembolism and respiratory failure. In the group 2 one (1.8%) patient died as consequences of severe pneumonia complicated by sepsis in the postoperative period. In a patient with anastomotic leakage and diffuse peritonitis who died a handsewn suture on anastomosis was placed and diverting ileostomy was created as the second operation, with a subsequent Hartmann's procedure as the third operation. In the other 3 patients with anastomotic leakage and diffuse peritonitis who died the Hartmann's procedure was the second operation. No significant difference was found in the mortality rate between the groups (χ^2 3.242, $p = 0.101$). No relationship between age gender and overall occurrence of postoperative complications was found. However, tumor distance from the anal verge had the influence on the development of anastomotic leakage in all the patients ($p = 0.026$), mainly in those with anastomotic leakage conservatively treated with success ($p = 0.03$). In comparison between the tumor stage and the overall postoperative mortality rate a significant difference was found. Of 4 patients with cT4 tumor stage 3 died, of the patients with cT3 tumor stage 2 died, and one patient died of those with tumor cT2 stage. A significant difference between tumor stage and postoperative mortality rate was found ($p = 0{,}011$). Preoperative serum albumin levels were significantly lower in the patients with postoperative complications. A lower level of serum albumin was found in the patients with anastomotic leakage who were operatively treated ($p < 0.001$), in patients with anastomotic leakage treated conservatively ($p = 0.048$), in the patients with postoperative peritonitis ($p < 0.001$), in the patients with wound infection ($p < 0.001$), in the patients with postoperative pneumonia ($p < 0.001$), as well in the patients who died ($p < 0.001$). Anastomotic leakage was observed in 12 (23.5%) of the patients (group 1), while conser-

There were 22 (20.6%) patients with wound infection; 14 (27.5%) patients in the group 1 and 8 (14.3%) patients in the group 2 without a significant difference between the groups ($p = 0.92$). Although there were more patients with postoperative pneumonia in the group 1, no significant difference between the groups was found ($p = 0.254$). A significantly lower serum level of preoperatively measured albumin was found in the group 1 than in the group 2, $p < 0.001$.

No significant correlation between preoperative level of serum albumin, patients age, tumor cT stage and tumor distance from the anal verge was found, while a significant correlation between tumor size and the serum albumin level was found ($p = 0.042$). Male gender and a lower level of serum albumin were significant predictors of anastomotic leakage occurrence ($p = 0.05$ and $p = 0.002$, respectively), while short-course preoperative RT had no significant impact on it (Table 3).

Table 3

Statistics for the variables in the binary logistic regression equation (dependent variable – anastomotic leakage)

Predictors	Sig. (p)	Exp(B)*	95% C.I. for EXP(B)	
			Lower	Upper
Group	0.543	0.647	0.159	2.635
Gender	0.054	0.186	0.034	1.028
Tumor distance from the anal verge	0.170	0.809	0.598	1.095
Serum albumin level	0.002	0.691	0.549	0.870

***Exp(B) – estimated odds ratio in binary logistic regression models. The variability is 38% (Negelkerke R Square), calibrated (Hosmer and Lemeshow test $X^2 = 5.318$; $p = 0.723$) with classification power of 86%.**

Discussion

TME is the standard surgical treatment for rectal cancer in the distal two-thirds, and partial mesorectal excision for tumors in the proximal part of the rectum [3, 47]. After implementation of short-course preoperative RT or long-course

CRT, local recurrence rates were reduced to less than 10%. However, only one trial demonstrated the overall survival benefit in patients with short-course RT [8, 48]. In tumor stages T2-T4 and potentially curative resections (R0), short-course preoperative RT followed by immediate surgery could provide a lower local or systemic recurrence rate with acceptable risks of the occurrence of postoperative complications [49]. Also, the advantages of this regimen of neoadjuvant RT are lower costs and patient convenience, especially in older patients, such as in our study population. There were two reasons why some patients with advanced clinical T stage in our study population did not receive short-course preoperative RT: rectal cancer in the upper third of the rectum and the absence of tumor penetration throughout the mesorectal fascia confirmed by endoscopic ultrasound and/or magnetic resonance imaging. Although there were a lot of patients in our retrospective review who did not receive short-course preoperative RT, it was a relatively small number if compared to the number of all the operated patients for rectal cancer in our institution in a 3-year duration period.

There are no strong evidences that diverting stoma prevents anastomotic leakage, however, it is certain that it reduces septic complications resulting from anastomotic leakage and the overall postoperative mortality rate [50]. We included only patients with rectal resection and no diverting stoma in order to evaluate the impact of short-course preoperative RT and the serum albumin level on early postoperative complications.

In our study the overall postoperative mortality rate was high (5.6%, 6 patients), but 4 of them died because of anastomotic leakage and subsequent diffuse peritonitis. Comparing the results of the Stockholm I trial [51, 52] that showed mortality rate of 8% in the preoperative RT group *vs* 2% in the group without preoperative RT, we can agree that the TME for rectal cancer after short-course preoperative RT does not lead to an increase in the postoperative mortality rate. In the Stockholm III trial [53] a mortality rate in patients with short-course preoperative RT followed by immediate surgery was 0.8%, whereas in 75 patients with anterior rectal resection a stoma was created in 41% of them. In our study only patients without diverting stoma creation were included.

Malnutrition in patients with rectal cancer is caused by several factors. Cancer-induced higher metabolism, reduced dietary intake and body nitrogen loss due to increased whole protein turnover can lead to cancer cachexia [54–57]. Hypoalbuminemia is accepted to be a good malnutrition indicator in many studies involving the patients with cancer [58–60]. Also, low serum albumin level is associated with poor tissue healing, decreased collagen synthesis in surgical wounds including gastrointestinal anastomosis [61–63]. Explanation for why tumor size, but not the stage, is relevant to hypoalbuminemia still remains unknown. It is possible that large tumors cause more gastrointestinal symptoms, leading to poor nutritional intake and/or partial gut obstruction. Our findings showed a significant correlation between the tumor size and the serum albumin level, but the preoperative level of serum albumin did not correlate with age, tumor cT stage and tumor distance from the anal verge. Also, statistical analysis showed that lower level of serum albumin and tumor size were significant risk factors for postoperative complications following rectal cancer surgery. A preoperative serum albumin level was significantly lower in patients who developed postoperative complications. A significantly lower serum albumin level and larger size of rectal cancer in the group 1 may contributed to the occurrence of anastomotic leakage in addition to irradiation related toxicity in this group of patients.

It was reported that a significant difference was not found between patients with immediate surgery and patients with delayed surgery after short-course preoperative RT in terms of postoperative complications and reoperations. However, the patients with immediate surgery after short-course preoperative RT had a higher rate of postoperative complications [49, 53]. In our study, there were no patients in the group 2 who required reoperation. An increase in the anastomotic leakage rate after preoperative RT was observed by several authors, both after short-course and after long-course preoperative RT. A two to three fold increase in the incidence of anastomotic leakage is generally reported after RT[64–66] which is roughly the result of our study, also. The necessity for reoperation in our patients with anastomotic leakage in the group 1 may suggest that irradiation have more influence on anastomosis healing than serum preoperative albumin level. This could be supported by the fact that there were 5 patients in both groups with anastomotic leakage successfully conservatively treated and 7 patients in the group 1 who required reoperations with a significant difference ($p = 0.004$). However, logistic regression showed that independent factors for the development of postoperative complications including anastomotic leakage, were male gender and a lower level of serum albumin, but no irradiation. Anyway, it is certain that, low level of serum albumin, higher size of rectal cancer and short-course preoperative RT have influence on postoperative complications occurrence.

Our findings correlate with the two large population-based prospective studies [58, 59]. These studies reported that a decrease in serum albumin level from concentrations greater than 46 g/L to less than 21 g/L was associated with the exponential increase in mortality rates from less than 1% to 29%, and in morbidity rates from 10% to 65%. Moreover, a lower level of serum albumin was a better predictor of some types of morbidity, especially sepsis and major infections.

In gastrointestinal reconstructions after rectal resections, a significant difference in anastomotic leakage was not found in comparison between handsewn and stapled technique, but the level of anastomosis was important predictive factor for leakage [67], as confirmed in our study. We found that tumor distance from the anal verge influenced development of anastomotic leakage in all the patients ($p = 0.026$). Surgical site infection, including wound infection in open colorectal surgery varies from 2% to 25% with a higher incidence rate in rectal surgery [67, 68]. The preoperative RT, steroids and stoma creation are associated with a higher rate of surgical site infection in rectal resection [69]. Also, low serum albumin level may facilitate wound infection development [61, 63]. Although we had more patients with wound infection in the group 1 than group 2 (27.5% *vs*

14.3%, respectively), but there was no significant difference between groups. Comparing our results with the results of the Stocholm III we had same incidence rate of wound infection. However, a higher incidence rate of wound infection in the Stockholm III trial of 28% *vs* the overall incidence rate of wound infection of 20.6% in our patients could be contributed by both preoperative irradiation and stoma existence.

The results of this study indicate that there was a significantly increased rate of postoperative complications in the patients with lower serum level of albumin preoperatively.

The rate of postoperative complications was also increased in the patients with short-course preoperative RT, but without a statistical significance.

The main disadvantage of this study is a relatively small number of patients and the lack of data including operation duration, blood loss and blood transfusion which may affect the occurrence of early postoperative complications.

Conclusion

Short-course preoperative radiotherapy did not significantly increase the rate of postoperative complications, but a significantly higher rate of anastomotic leakage occured in the male patients and in the patients with a lower level of serum albumin. The patients with a lower serum albumin level had a significantly higher rate of postoperative complications.

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A novel microscopic method for analyzing Gram-stained vaginal smears in the diagnosis of disorders of vaginal microflora

Nov mikroskopski metod za analizu preparata bojenog po Gramu u dijagnozi poremećaja vaginalne mikroflore

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Abstract

Background/Aim. The Nugent's score is still the gold standard in the great majority of studies dealing with the assessment of vaginal flora and the diagnosis of bacterial vaginosis (BV). The aim of this study was to show that the analysis of Gram-stained vaginal samples under microscope at the magnification of ×200 (a novel microscopic method – NMM), as a fast and simple tool, easily applicable in everyday practice, better reflects complexity of vaginal microflora than the Nugent's methodology (×1000). **Methods.** Gram-stained vaginal smears from 394 asymptomatic pregnant women (24–28 week of pregnancy) were classified according to the Nugent's microscopic criteria (immersion, magnification ×1000). The smears were then reexamined under immersion but at magnification ×200. All samples were classified into 6 groups according to semiquanititative assessment of numbers (cellularity) and the ratio of rod (length < 1.5 μm) and small bacterial (< 1.5 μm) forms: hypercellular (normal full – NF), moderately cellular (normal mid – NM), hypocellular (normal empty – NE), bacterial vaginosis full (BVF), bacterial vaginosis mid (BVM), and bacterial vaginosis empty (BVE). Also yeasts, *coccae*, bifido and lepto bacterial forms as well polymorphonuclear (PMN) leukocytes were identified. **Results.** According to the Nugent's scoring, BV was found in 78, intermediate findings in 63, and yeasts in 48 patients. By our criteria BV was confirmed in 88 patients (37 BVF, 24 BVM, and 27 BVN). Generally, both tools proved to be highly concordant for the diagnosis of BV (Lin's concordance correlation coefficient = 0.9852). In 40% of the women mixed flora was found: yeasts in 126 (32%), *coccae* in 145 (37%), bifido forms in 32 (8%) and lepto forms in 20 (5%). Almost a half of BV patients had also yeasts (39/88). Elevated PMN numbers were found in 102 (33%) patients with normal and in 36 (41%) women with BV. **Conclusion.** The newly described methodology is simpler to apply and much better reflects diversity of vaginal microflora. In this way it may be more valuable to molecular biologists and their attempts based on quantitative polymerase chain reaction (PCR) to define formulas for molecular diagnosis of bacterial vaginosis.

Key words:
vaginal smears; microscopy; diagnosis; vaginosis, bacterial.

Apstrakt

Uvod/Cilj. Nugentov skor još uvek važi za zlatni standard u velikoj većini studija o proceni vaginalne flore i dijagnozi bakterijske vaginoze (BV). Cilj ovog rada bio je da se ustanovi da je mikroskopska analiza vaginalnog brisa preparata bojenog po Gramu na uvećanju ×200, brza i jednostavna tehnika, lako primenljiva u svakodnevnoj praksi i da bolje odražava kompleksnost vaginalne flore od metodologije po Nugentu. **Metode.** Preparati bojeni po Gramu kod 394 asimptomatske trudnice (24–28 nedelja trudnoće) klasifikovani su na osnovu Nugentovih mikroskopskih kriterijuma (imerzija, uvećanje ×1000). Slajdovi su ponovo analizirani pod imerzijom na uvećanju ×200. Na osnovu polukvantitativne procene broja (celularnost) i odnosa štapićastih (dužina > 1,5 μm) i malih bakterijskih formi (< 1.5 μm) sve ispitanice bile su podeljene u 6 grupa: hipercelularni (*normal full* – NF), srednje celularan (*normal mid* – NM), hipocelularan (*normal empty* – NE), „*bacterial vaginosis full*" (BVF), „*bacterial vaginosis mid*" (BVM), i „*bacterial vaginosis empty*" (BVE). Takođe, identifikovane su gljivice, koke, bifido i lepto bakterijske forme kao i polimorfonuklearni leukociti (PMN). **Rezultati.** Na osnovu Nugentovih kriterijuma, BV nađena je kod 78 ispitivanih trudnica, intermedijerni nalaz kod 63, i gljivice kod 48. Na osnovu naših kriterijuma BV je potvrđena kod 88 ispitanica (37 BVF, 24 BVM, i 27 BVN). Generalno, oba pristupa pokazala su visoku podudarnost u dijagnozi BV (Linov koeficijent podudarnosti korelacije =

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0,9852). Kod 40% žena pronađena je mešovita flora: gljivice kod 126 (32%), koke kod 145 (37%), bifido forme kod 32 (8%) i lepto forme kod 20 (5%). Skoro polovina žena sa BV imala je, takođe, gljivice (39/88). Povećan broj PMN je nađen kod 102 (33%) trudnica sa normalnim nalazom i kod 36 (41%) trudnica sa BV. **Zaključak.** Nova metodologija jednostavnija je za primenu i mnogo bolje odražava raznolikost vaginalne mikroflore. Ovakav pristup možda bi bio korisniji molekularnim biolozima i njihovim pokušajima da na osnovu kvantitativne lančane reakcije polimeraze (*polymerase chain reaction – PCR*) dođu do molekularne „formule" za dijagnostiku BV.

Ključne reči:
vaginalni brisevi; mikroskopija; dijagnoza; vaginoza, bakterijska.

Introduction

The Nugent's score is still the gold standard in the great majority of studies dealing with the assessment of vaginal flora and the diagnosis of bacterial vaginosis (BV) [1–8]. This being despite many, more or less obvious, shortcomings of the test: time consuming, a complicated numerical summing with narrow intervals, a need for experienced personnel, inutil in everyday practice, a need for standardizing surface of the microscopic field of view, and evaluation of only three bacterial morphotypes. In our opinion, the most important of the shortcomings is the inability to distinguish between *Lactobacillus iners* and *Gardnerella vaginalis* due to their great phenotypic resemblance (length, shape, Gram-staining properties) [9, 10]. Yet, the two bacteria make the mainstay of the Nugent's score. Not of less importance is the fact that observing 5–20 fields of view under the magnification ×1000, the actual scanned surface makes only a tiny fraction of the slide surface thus being a source of sampling error.

Over the past 2 years we have developed a novel method of viewing Gram-stained slides at magnification of ×200 in an attempt to eliminate most of the above cited drawbacks of the Nugent's score. It very simple, learning curve is steep and does not require any special microbiologic or gynecologic knowledge.

Introduction of new identification techniques (in particular, nucleic acid-based tests) independent from culture led to a true revolution in our understanding of the vaginal microflora. These studies discovered new species of bacteria, showed that the vaginal microbiome is more heterogenous and dynamic than thought previously [11–17]. Results of the Ravel's seminal study were surprising showing that lactobacilli are not prevailing bacteria in over 27% asymptomatic patients [18]. Moreover, in their cohort 97 had BV (according to Nugent's criteria) and 48% of examined women had vaginal pH > 4.5. Many questions need to be answered. First, are the women who do not have lactobacilli as a dominant flora, healthy or have asymptomatic BV? If they are healthy, then diagnosis of BV based on Nugent's criteria is frequently false, but if have asymptomatic BV, then the prevalence of this entity is much higher than we think. Second, is the acidic vaginal environment a prerequisite for a healthy vagina: what is then the value of Amsel's criteria in diagnosing BV if almost a half of asymptomatic women have pH > 4.5 [18, 19]? In this work we shall try to answer indirectly to the first question considering the value of Nugent's criteria in diagnosis of BV.

Methods

Gram-stained vaginal smears of 394 asymptomatic pregnant women (24–28 weeks of pregnancy) originally classified according to the Nugent's criteria (viewed under immersion, magnification ×1000) were reviewed and reclassified according to our new protocol (immersion, magnification ×200). The Nugent's scoring system was described previously [1]. The diameter of the image areas on our microscope (Leica DM 2000 LED, Ocular 10 × 22, Lens 100× 1, 25), was measured using a stage micrometer with a 0.01 mm interval scale (D = 0,21 mm) and the area was calculated using the formula $A = r^2 xphi = 0.35\ mm^2$. Calibrations of Nugent scoring system and counting of bacterial morphotypes were done as previously described Larson et al. [20]. In brief, score intervals 0–3 represented normal flora, 4–6 intermediate and 7–10 BV. The scoring system was based on counting three morphotypes: *Lactobacillus* spp., *Garduerella vaginalis* or *Bacteroides* (small Gram-variable rods or Gram-negative rods) and curved Gram-variable rods. As the slides were first viewed under immersion (magnification ×1000), in order to get a clear view at repeated viewing at magnification ×200, we had to put a drop of immersion oil – obviously not necessary if slides are viewed for the first time. The slides were viewed at two ends and in the middle along the shorter axis, e.g. perpendicularly to the direction of smear: 100 to 150 fields of view were viewed and it took at most 5–10 minutes. Apart from epithelial cells and above described bacterial morphotypes, polymorphonuclear leukocytes, yeasts, trichimonas, coccae, lepto forms, the degree of cytolysis, spermatozoids etc. were also identified. The shortest length still recognizable as a rod at the magnification ×200 is 1.5 µm. Based on this fact, under magnification ×200, it is easy to recognize the predominance of either rod forms (RFs, > 1.5 µm, laktobacilli) or non-rod forms (NRFs, < 1.5µm, Bactrerial Vaginosis Associated Bacteria – BVAB). The former is considered a normal finding. Numbers of RFs and NRFs were assessed semiquantitatively in this way: numerous bacteria covering most of the slide surface (in between, around and over epithelial cells – EC) were designated as "full"; bacterial forms rare or absent in between EC but found mostly around and at EC were designated as "mid"; and almost absent bacterial forms with only rare elements seen around and at EC were designated as "empty". Depending upon the ratio of RFs: NRFs, these three categories were further subdivided each into "normal" and "bacterial vaginosis" subgroups: the predominance of either RFs or NRFs, respectively. In this way all slides may be categorized into 6

groups: three normal (normal full – NF; normal mid – NM; and normal empty – NE) (Figure 1a, b, c) and three bacterial vaginosis varieties (BV – full, BVF – BV mid, BVM, and BV – empty – BVE) (Figure 1d, e, f) *Coccae* are generally Gram-positive, round, measuring 0.2 to 2 μm, but may be also Gram-negative, of irregular shape, larger but may always be distinguished from bacterial vaginosis/associated bateria (BVAB). We also had a group designated "*coccae*" into which were classified women with numerous, strongly Gram-positive, round and usually small bacteria, resembling dots, easily distinguished from BVAB (Figure 1g). Bifido forms (as in I-like group by Verhelst et al. [21]) were identified as Gram-positive forms, irregularly stained, shorter or longer, often irregular in shape with a tendency of branching with clubbed or curved endings (Figure 1h). All forms longer than 20 μm irrespective of Gram-staining were classified into the lepto forms (Figure 1i). PMN numbers were determined also semiquantitatively at ×200 during the same slide analysis and the women were divided into 4 categories: group 0 – PMN absent or much less numerous than EC; group 1 – PMN seen on more than 50% of field of view (FV) but their numbers still less than that of EC; group 2 – PMN seen on most FV and their numbers equal or higher than numbers of EC; group 3 – PMN seen on most FV and their numbers much higher than numbers of EC. The groups 0 and 1 were considered normal as to PMN number, and the other two groups were considered pathological.

We believe that our semiquantitative classification into 6 groups avoiding any intermediate group better reflects the complexity of vaginal microflora than the Nugent's criteria (Figure 1a-i).

Differences between the groups were calculated by paired *t*-test. The concordance between Nugent's and our classification systems was determined by the Lin's concordance test [22].

Results

According to the Nugent's criteria, BV was confirmed in 78 patients, 63 women had intermediate scores, and 253 patients had normal findings. At ×1000 magnification we detected yeasts in 48 women: 15 had BV, 7 were in the intermediate group and 26 had normal findings. When we used our own criteria, the diagnosis of BV was made in 88 patients, whereas normal findings were ascribed to 306 patients. Comparative results of microscopic analysis of Gram-stained specimens viewed under immersion at ×200 and ×1000 are given in Figure 2.

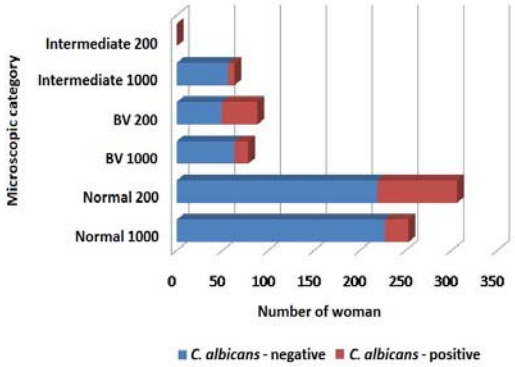

Fig. 2 – Microscopic categories of findings (BV – bacterial vaginosis)

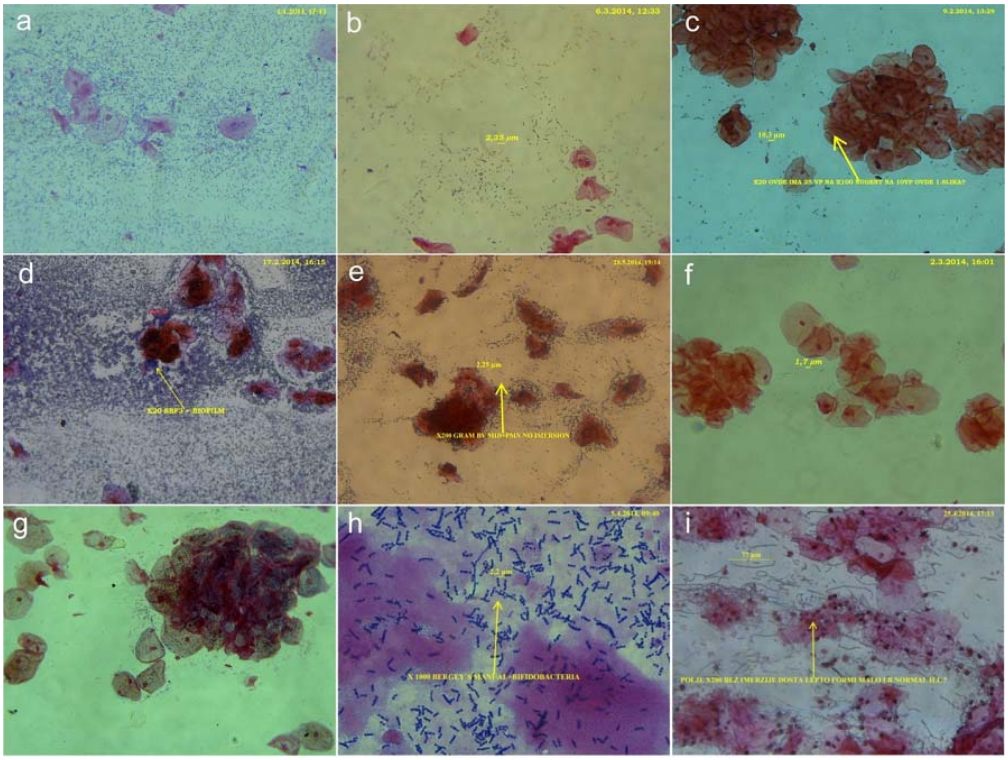

Fig. 1 – Characteristic microscopic classes of Gram-stained vaginal smears viewed under immersion with ×200 magnification:
a) normal full (NF); b) normal mid (NM); c) normal empty (NE); d) bacterial vaginosis full (BVF); e) bacterial vaginosis mid (BVM); f) bacterial vaginosis empty (BVE); g) *Coccae*; h) Bifido forms (×1000 magnification); i) Lepto forms.

When different classes of women, identified with our method of observation at ×200 magnification, were compared to diagnoses made by the Nugent's criteria, a substantial agreement was calculated by the Lin's method [concordance correlation coefficient (ρc) = 0.9852] (Figure 3).

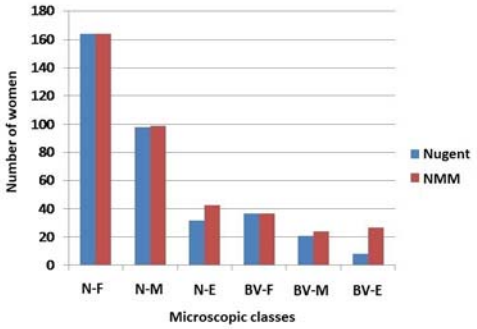

Fig. 3 – Comparison of microscopic classes.
N – normal; F – full; M – mid; E – empty; BV – bacterial vaginosis. NMM – our method.

As seen in Figure 3, the lowest concordance was found for cell-poor samples ("empty"), both normal and BV. Roughly, 40% of women (with both normal and BV findings) had mixed infections: yeasts (32%), *coccae* (37%), bifido (8%) and lepto forms (5%). The highest proportion of mixed infections was detected in cell-poor samples ("empty") (63%), and the lowest proportion in hypercellular ("full") (27%) samples with normal findings (p = 0.032) (Figure 4).

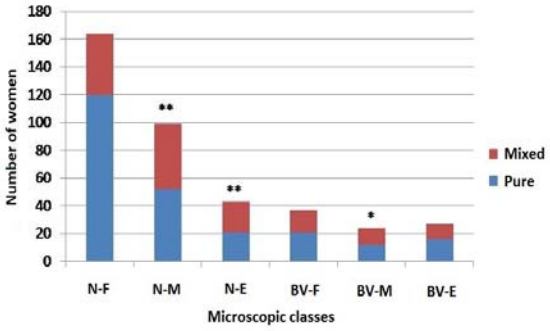

Fig. 4 – Microscopic classes of vaginal smears of women assessed by our method.
Cases of mixed infections or coinfections are presented within each group. With asterisks are denoted classes in which the ratio of mixed infections is significantly higher than in women with normal hypercellular smears (the first bar).
****p < 0.01, *p < 0.05. N – normal; BV – bacterial vaginosis; F – full; M – mid; E – empty.**

When the intermediate group identified by Nugent was analyzed across our criteria (Figure 5), it was clear that the great majority of the women belong to the hypocellular ("empty") samples but not a single to the group with a full-blown BV. As for elevated PMN numbers, there was no significant difference between the women with normal (33%) and BV samples (41%) (p = 0.205). However, significantly more PMN had women with both normal and BV moderately cellular samples ("mid") in comparison to those with normal hypercellular ("full") specimens (Figure 6). More than 50% of patients whose Gram-stained samples contained coccae, bifido and lepto forms had also elevated PMN numbers.

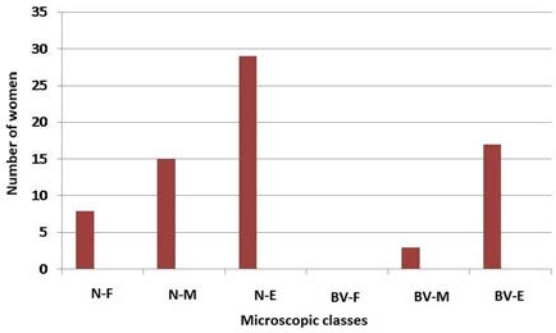

Fig. 5 – Distribution of women of the intermediate group by Nugent across microscopic classes determined by our method.
N – normal; F – full; M – mid; E – empty; BV – bacterial vaginosis.

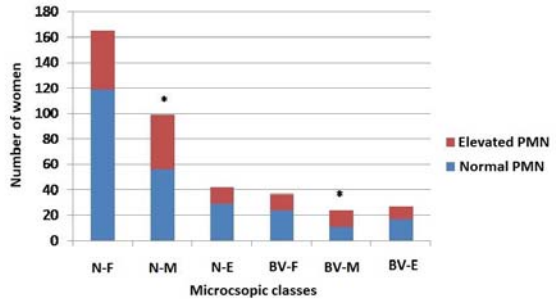

Fig. 6 – Distribution of women with normal and elevated polymorphonuclear leukocyte (PMN) numbers across microscopic classes determined by our method.
With asterisk are denoted microscopic classes with significantly higher proportion of elevated PMN numbers in comparison to normal (N) and bacterial vaginosis (BV) classes with hypercellular full (F) or paucicellular empty (E) samples.

Discussion

Our observations confirm both quantitative and qualitative variety of Gram-stained samples calling for more precise classifications based not solely on three bacterial morphotypes but extended to other important bacteria types seen under a microscope as well as PMN. Among our 88 women with BV, in 39 (44%) coinfection or mixed infection with *Candida albicans* was identified. We endorse the terms coinfection or mixed infection as proposed by Sobel et al. [23] as his definition of mixed vaginitis implies that at least two or more pathogenic processes, rather than two pathogens *per se*, coexist in the vagina, each contributing to symptoms and signs. This is very plausible as a means to distinguish between coinfections and mixed infections. But from a practi-

cal standpoint it is not much helpful as among these women we may encounter all possible combinations of clinical and laboratory signs: homogenous whitish vaginal discharge; granular, cheesy vaginal discharge; completely asymptomatic women; pruritus and burning; either positive or negative test with 10% potassium hydroxide (KOH); vaginal pH either lower or higher than 4.5; microscopy for yeasts negative but culture positive and *vice versa*. There are so many combinations making distinction between coinfection and mixed infection on these grounds a daunting task.

It is obvious that scanning Gram-stained samples at ×200 includes a much larger area than at x1000 so that things are seen differently, both quantitatively but also qualitatively. For example, more yeasts are detected if slides were viewed under ×200 according to our methodology. Further if women without BV, yeasts or *T. vaginalis* are considered "healthy", within this group we identified 66 patients with *coccae*, 30 with bifido forms and 18 with lepto forms. We feel these findings should not be ignored in future analyses. Besides much higher surface scanned, it can be done in 5–10 minutes as we do not need to include cumbersome counting of individual bacteria as in the Nugent's method. Hence, it is pretty much faster.

In hypercellular and moderately cellular samples (F and M groups) there is a rather high concordance in BV diagnosis according to the Nugent's and our criteria, 100% and 88%, respectively (Figure 2). However in cell-poor ("empty") samples for both BV and normal findings, concordance is much lower, 30% and 28%, respectively. A previous interobserver study draw attention to these patients: "however, some issues need to be looked at carefully. First, we found major discrepancies in scoring when lactobacilli morphotypes were few in number. This is of major importance in the scoring system since the score intervals are very narrow with a difference of only a few bacteria" [24]. In general, analyzing Gram-stained samples for BV with ×200 magnification is comparable to the Nugent's method with ×1000 magnification (Lin's concordance correlation coefficient 0.9852).

The most problematic are actually women with cell-poor samples where the concordance is lowest. Avoiding counting of bacterial morphotypes on a tiny fraction of a sample as in the Nugent's method may actually be helpful. It is known that two very important bacteria (*Lactobacillus iners* and *Garduerella vaginalis*) cannot be distinguished microscopically – it may be just one of the reasons to question the value of the Nugent's score as the gold standard [9–10]. Our poor-cell and, often, Nugent's intermediate group samples are characterized by conspicuous inhomogeneity of the smear on the slide. The area of our slide is 25.4 × 76.2 mm = 1935 mm^2, and if we assume that the cell smear takes up one third of the area slide (about 600 mm^2), when these samples are viewed under immersion at ×1000 we scanned 5–20 fields (Nugent) from a total of 17 143 fields (600/0.035). When viewed under ×200 according to our methodology, we scanned 100-150 fields from a total 686 fields (600/0.875), or ¼ of all stained slide surface [20]. When coupled with the smear inhomogeneity may explain a vast source of error [20]. Moreover, recent PCR assays report the heterogeneous character of intermediate flora, with some of them suggesting a molecular profile more similar to that of BV than to normal samples [25, 26]. That's why we should investigate other, more appropriate reference (gold) standards for vaginal infections.

One possible way of looking for the best microscopic criteria would be the application of Q-PCR which would in an objective way measure the presence and ration of lactobacilli and BVAB [8, 10, 25, 27–31]. Our method offers semi-quantitative assessment of bacterial forms which may be useful for comparative studies with Q-PCR. The Menard's et al. [8] study is very interesting in this regard. Q-PCR showed good agreement ($\kappa = 0.81$) and high sensitivity (100%) and specificity (93%) in relation to the Nugent's and Amsel's criteria. Yet, 10 (40%) women in this study had discordant results for the Amsel criteria and the Nugent score. The Nugent scoring system is excellent in diagnosing samples as either normal or BV, but the intermediate flora presents problems [20, 32, 33]. Vaginal smears with intermediate flora may be considered as heterogeneous flora that may include both normal and BV flora. The molecular criteria's lower positive predictive value of 73% suggests that may represent true-positives for the molecular condition of BV that were missed by traditional diagnostic tools (Nugent's and/or Amsel's criteria). The false-negatives of both standard methods reported above may support this explanation. The PCR quantification of *Gardnerella vaginalis* and *Atopobium vaginae* clearly defines a reproducible and standardized molecularly defined BV, irrespective of the clinical and microscopic characteristics of vaginal flora. As may be seen from our results (Figure 5) most women classified by Nugent into the intermediate group belong to our poor-cell ("empty") samples, both normal and BV.

It is the semiquantitive aspect of our classification of Gram-stained samples that may be more useful for better assignment of the results of Q-PCR. In particular as Q-PCR analysis has the goal to set cut-off values for densities of lactobacilli and/or BV-associated bacteria (*Garduerella vaginalis*, *Atopobium vaginae*, *Eggerthella, Prevotella*, BVAB2 and *Megasphaera* type 1) that would enable more objective and precise distinction between normal and pathological vaginal flora. It is logical that cut-off values of individual microorganisms differ in patients with different cellularity (our classes "full", "mid" and "empty"): thus prior microscopical classification may greatly assist in defining the cut-off values. If women with "hypercellular" and "hypocellular" BV are merged into one group it would be much difficult to define a reliable cut-off value distinguishing BV and non-BV flora.

Apart from bacterial morphotypes defining BV, other (*coccae*, bifido and lepto forms) probably deserve further attention. Although both groups of Ison and Hay [2] and Verhelst et al. [21] reserved a special class for *coccae*, there is not a single published study dealing specifically with these bacteria in vaginal fluid. Further, Verhelst et al. [21] emphasized the importance of atypical Gram-positive rods classified into the I-like group (bifido or corynebacterium form). Women in

this group ran a higher risk for preterm labor. Others have not studied these patients.

In our study the long (lepto) forms were defined as longer than 20 μm; they may correspond to genera *Leptotrichai amnionii, Sneathia sanguinegens, Leptothrix*, *Actinomyces* or even others? In the Bergey's manual the length of lactobacilli is cited as up to 10 μm. If we accept the fact than the percentage of lepto forms in our women would be even higher. Their potential importance is being realized only recently: Leptotrichia was found to be associated with BV and its presence correlate with clinical symptoms of BV [6]. Moreover, it produces lactic acid [6].

As we suppose that elevated PMN reflect a state of inflammation, it is of note that, although all of our patients were asymptomatic, more than 50% of women whose Gram-stained samples contained *coccae*, bifido and lepto forms had also elevated PMN numbers (Figure 6). This fact reinforces our hypothesis that studying these forms may also give us important data about vaginal microflora and its health status.

Despite considerable body of research and recent advances, BV remains an enigmatic condition. Molecular techniques have revealed the complex microbiology of BV confirming that it is most likely a syndrome caused by different communities of vaginal bacteria, i.e. a dysbiotic condition. Future studies on BV and its associated adverse outcomes should determine if specific combinations of microorganisms are associated with different adverse events [25, 27, 34, 35].

Conclusion

We would hypothesize and conclude that Gram-positive rods seen on the Gram-stained samples which we generally lump into lactobacilli, differ in length and thickness, and staining intensity; this probably reflects various rods of lactobacilli or even other bacterial species. There are many types of bacterial vaginosis depending upon the predominant bacterial vaginosis-associated bacteria: e.g. in women with prevailing *Garduerella vaginalis* there are many clue cells, whereas in patients with more abundant *Atopobium vaginae* or other bacterial vaginosis-associated bacteria clue cells are very rare or absent. There is an interesting constellation (at ×200) of numerous very short and thin rod-forms (length 1.5–2.5 μm), but at ×1000 it is clear that actually predominate non-rod forms. We suppose that this finding corresponds to a high number of *Lactobacillus iners*. If coupled with elevated PMN numbers, there is a high (70–90%) probability to encounter also *Candida albicans*. This is the commonest variety of mixed infection or coinfection (*bacterial vaginas* and *Candida albicans*); in all "borderline" cases (our poor-cell or "empty" classes) we opt for the application of probiotics and/or acidification of vagina rather than the application of antibiotics.

Future studies should check whether these groups do differ on clinical grounds, too. Moreover, it may be useful to molecular biologists to devise a "molecular formula" for the diagnosis of bacterial vaginosis based on quantitative polymerase chain reaction and the ratio of lactobacilli and bacteria associated with bacterial vaginosis.

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Changing body structure components and motor skills in Military High School students within one year

Promena komponenti strukture tela i motoričkih sposobnosti kod učenika Vojne gimnazije tokom jedne godine

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Abstract

Background/Aim. Proper growth and development of adolescents in the morphological, functional and psychosocial aspects is the imperative of the educational process. The aim of this study was to determine the status and changes in the indicators of morphological characteristics, motor skills and lifestyle habits among the students of the Military High School in Belgrade. **Methods.** The study included 217 students aged 15 to 18 years (from the first to the fourth grade). The two measurements performed at the intervals of one year were used to determine: the body structure by means of 10 variables and motor skills by 4 variables, while life habits were determined by 25 variables. **Results.** The differences in the indicators of morphological characteristics were recorded in all the groups, being the highest in the first year of schooling. During the period of growing up, a reduction of fatty component in percentage values was found, as well as an increase of muscle mass. The progressive growth of motor skills in the first, second and the third grade was recorded in the manifestation of power, and endurance improved only in the first year. In terms of dietary habits, there was no difference among the groups. **Conclusion.** The obtained results indicate proper morphological and motor development and the formation of lifestyle habits. The data obtained will serve as a basis for health and functional prevention and upgrading in terms of improvement of the process of military education.

Key words:
students; military personnel; adolescents; serbia; physical education and training; body cmposition; attitude to health; questionnaires.

Apstrakt

Uvod/Cilj. Pravilan rast i razvoj adolescenata u morfološkom, funkcionalnom i psihosocijalnom pogledu jeste imperativ vaspitno-obrazovnog procesa. Cilj ovog rada bio je da se ustanovi stanje i promene u pokazateljima morfoloških karakteristika, motoričkih sposobnosti i životnih navika kod učenika Vojne gimanazije u Beogradu. **Metode.** Istraživanjem je bilo obuhvaćeno 217 učenika, uzrasta od 15 do 18 godina (od prvog do četvrtog razreda). U dva merenja u razmaku od godinu dana određivani su telesna struktura pomoću 10 varijabli, motoričke sposobnosti pomoću četiri, a životne navike pomoću 25 varijabli. **Rezultati.** Razlike u pokazateljima morfoloških karakteristika evidentirane su kod svih grupa i bile su najveće u toku prve godine školovanja. Tokom odrastanja, ustanovljeno je sniženje procentualnog iznosa masnog tkiva, a povećanje mišićnog tkiva. Progresivan rast motoričkih sposobnosti do treće godine evidentiran je u ispoljavanju snage, dok je nivo izdržljivosti poboljšan samo u toku prve godine. U pogledu navika u ishrani nije bilo razlika između grupa. **Zaključak.** Rezultati istraživanja ukazuju na pravilan morfološki i motorički razvoj i formiranje životnih navika kod ispitivanih učenika. Dobijeni podaci će poslužiti kao polazna osnova za zdravstvenu i funkcionalnu prevenciju i dalje usavršavanje procesa obrazovanja u vojnom školstvu.

Ključne reči:
studenti; vojni kolektiv; adolescenti; srbija; fizičko vaspitanje i trening; telo, sastojci; stav prema zdravlju; upitnici.

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Introduction

Modern way of life in all generations has actualized particular lifestyle habits that contribute to increased risk of inappropriate adolescence development and potential health problems in adulthood.

Studies have shown that some of the causes of such a situation can be found in early youth and adolescence, in terms of the formation of inadequate lifestyle habits and the decrease in physical activity, and their transfer into adulthood [1, 2].

Nilsson et al. [3] stated that for proper growth and development, adolescents need at least 60 minutes of physical activity of moderate and high intensity every day. For example, in Hungary in 1987 schoolchildren watched television 35 to 45 minutes, and in 2005, 69 to 84 minutes, while their physical activity outside of school, which was 3 to 3.8 hours *per* week in 1987, reduced to 1 to 1.5 hours in 2005 [4]. Furthermore, Sabo [5] states that the body composition even in preschool children is violated and deviates from the standard of posture status.

Generally, it is necessary to follow the development of the entire body structure, as well as lifestyle habits, especially at younger ages, and according to the results determine the course of action. In his study Gajević [6] says that the process of programming and implementation of a system for monitoring morphological and motor skills of children and adolescents requires determination of the level, dynamics and structure of current phenomena, related to gender, age, social, ethnographic, geographic and other characteristics.

In military organization, the morphological and functional status is of great importance, serving, along with the educational work, as the basis for building a person with a high level of emotional stability and cognitive abilities [7], which are necessary for safe and successful doing tasks in specific jobs (pilots, sailors, special units, etc.).

In order to improve preventive healthcare and functional abilities, it is necessary to diagnose the existing condition and find ways to further educate adolescents, focusing on the problem, in order to reduce the consequences and avoid risks of transferring bad habits in adulthood.

It is indicative that studies on adolescents mostly deal with topics of excessive body weight (obesity) and related conditions as the risk for occurring and developing certain diseases. There are few studies, dealing with adolescent population showing the results of the morphological status and motor skills consistent however, with health standards, except in the case of the study with athletes.

The examinees in this study were the students of the Military High School, which prepares them for further education at the Military Academy, and they should, therefore, be properly guided in terms of nutrition, physical activity and lifestyle habits.

Thanks to specific boarding conditions of life and work, including a strict daily schedule that determines the time for eating, classes, rest, learning, sports activities and sleep, it might be expected that all those positive impacts in the support of proper growth of adolescents in terms of morphological and functional parameters and dietary habits will be shown with the students of the Military High School. The aim of this study was to perform measurements of morphological characteristics in four generations of students of the Military High School for a period of one year, to test their motor skills and to determine their dietary habits, as initial research with a view of taking the necessary preventive measures that would be incorporated into the military educational process.

Methods

This longitudinal study included 217 male students of the Military High School in Belgrade, divided into four groups: students of the first year (I, n = 68); the second year (II, n = 58); the third year (III, n = 54) and the fourth year (IV, n = 37).

The first measurement of the indicators of morphological status and testing of motor skills was carried out at the beginning of the school year, and the next one at the beginning of the next school year. The survey on the lifestyle habits was conducted in the period of the second measurement.

Measurement of the indicators of morphological characteristics was conducted in the laboratory of the Military Academy, using a BIA "InBody 720" and altimeter "SECA bodymeter 206" with a precision of 1 mm, while the abdominal circumference (AC) was measured with a flexible tape at the level of the navel with a precision of 1 mm.

Indicators of morphological characteristics were determined on the basis of directly measured variables: AC, expressed in cm; body height (BH) expressed in cm; body mass (BM) expressed in kg; body fat mass (BFM) expressed in kg; skeletal muscle mass (SMM) expressed in kg; visceral fat area (VFA) expressed in cm^2; total water (TW) expressed in L.

To assess the morphological characteristics, derived variables were also used: percent of body mass (PBF), calculated as BFM/BM, expressed in %; body mass index (BMI), calculated as BM/BH^2, expressed in kg/m^2; percent of sceletal muscle mass index (PSMM), calculated as SMM/BM, expressed in %.

A survey on dietary and lifestyle habits was conducted through a questionnaire, which consists of two parts [8]. The first part includes 14 questions about eating habits (type of food consumed, the frequency of daily meals and preferences related to food and beverages), where the answers are validated from 0 to 3. The second part of the survey is related to lifestyle habits (alcohol and cigarettes, physical activity and leisure time), and responses are validated from 0 to 4.

To assess motor skills, the tests that are regularly conducted every year to check the physical ability [9] were used: pull-ups (number of repetitions *per* minute); sit-ups (number of repetitions *per* minute); standing long jump (expressed in cm); 1,600 metar run (expressed in sec).

Statistical analysis was performed in Statistical Package for the Social Sciences (SPSS) version 19.0. The significance of differences of numerical variables within the groups was determined using general linear model – repeated measures and between the groups using *t*-test. To test the categorical variables, χ^2-test was used. Differentiation of students based

on numerical and categorical variables was determined by canonical discriminant analysis.

Results

Statistically significant differences between the first and second measurement of the variables that define the morphological characteristics were registered in all the variables, but not in all the groups (Table 1).

meter run, a significant increase was registered only in the group I (Table 2).

Canonical discriminant analysis included all 39 variables (Table 2). There are three functions: morphological area (0.738, $p < 0.0001$), motor area (0.559, $p = 0.001$) and the area of lifestyle habits (0.507, $p < 0.05$). Under "original", the first group of 45 (66.2%) students have the results that confirm they really belong to their group, while 42 students (72.4%) have the results that show they belong to the

Table 1

The results of morphological variables for the students of the Military High School measured in 2012 and 2013

Variables	Study grades											
	I			II			III			IV		
	2012 x̄ ± SD	2013 x̄ ± SD	Δ	2012 x̄ ± SD	2013 x̄ ± SD	Δ	2012 x̄ ± SD	2013 x̄ ± SD	Δ	2012 x̄ ± SD	2013 x̄ ± SD	Δ
Morphological												
AC (cm),	76.66 ± 6.17	81.46 ± 7.12**	4.80	79.23 ± 6.49	82.71 ± 6.56**	3.48	79.94 ± 5.39	83.53 ± 6.08**	3.58	81.80 ± 5.71	81.46 ± 5.26	-0.34
BH (cm)	176.91 ± 6.48	179.10 ± 6.48**	2.18	178.14 ± 6.09	179.25 ± 6.34**	1.11	179.47 ± 6.36	180.07 ± 6.34	0.59	179.31 ± 6.45	180.03 ± 6.49	0.73
BM (kg)	65.24 ± 8.79	69.64 ± 9.07**	4.40	68.47 ± 9.10	70.09 ± 8.36**	2.45	71.53 ± 8.70	74.33 ± 9.05**	2.79	74.04 ± 9.94	74.15 ± 9.12	0.11
BFM (kg)	7.77 ± 4.00	8.19 ± 3.85	0.42	7.87 ± 4.44	7.27 ± 3.98	-060	8.75 ± 4.47	8.74 ± 4.22	-0.01	9.73 ± 5.28	7.79 ± 3.87**	-1.94
SMM (kg)	32.26 ± 4.07	34.76 ± 4.30**	2.50	34.22 ± 3.76	36.22 ± 3.76**	2.00	35.63 ± 3.72	37.4 ± 4.11**	1.61	36.70 ± 4.37	37.94 ± 4.37**	1.24
PBF (%)	11.64 ± 4.43	11.52 ± 4.37	-0.12	11.15 ± 4.91	9.94 ± 4.68**	-1.21	11.93 ± 4.59	11.55 ± 4.25	-0.38	12.80 ± 5.10	10.31 ± 4.21**	-2.49
BMI (kg/m^2)	20.83 ± 2.53	21.69 ± 2.40**	0.86	21.57 ± 2.57	22.08 ± 2.38**	0.50	22.21 ± 2.46	22.89 ± 2.40**	0.69	23.01 ± 2.68	22.86 ± 2.37**	-0.15
PSMM (%)	49.55 ± 2.57	50.01 ± 2.58*	0.46	50.17 ± 2.92	51.22 ± 2.75**	1.04	49.96 ± 2.56	50.22 ± 2.50	0.27	49.74 ± 3.02	51.27 ± 2.54**	1.53
VFA (cm^2)	29,54 ± 17.81	32.02 ± 19.31	2.48	30.16 ± 22.67	26.42 ± 20.00*	-3.74	33.61 ± 20.67	34.15 ± 20.39	0.54	37.74 ± 25.18	28.51 ± 19.99**	-9.23
TW (L)	42.18± 4.95	45.06 ± 5.20**	2.88	44.36 ± 4.55	46.68 ± 4.53**	2.32	45.74 ± 4.86	47.95 ± 5.05**	2.21	47.13 ± 5.37	48.70 ± 5.45**	1.57
Motor												
PU (n), x̄ ± SD	5.40 ± 4.42	6.53 ± 4.16**	1.13	8.55 ± 5.17	10.05 ± 4.87**	1.50	9.98 ± 4.33	11.65 ± 4.03**	1.67	12.24 ± 4.28	12.27 ± 4.07	0.03
SU (n), x̄ ± SD	43.09 ± 7.17	50.29 ± 5.25**	7.21	46.69 ± 4.77	48.38 ± 5.01*	1.69	51.93 ± 6.01	51.65 ± 6.61	-0.28	50.89 ± 6.02	50.51 ± 4.21	-0.38
SLJ (cm), x̄ ± SD	205.96 ± 21.35	219.57 ± 17.36**	13.62	222.41 ± 15.77	231.41 ± 17.39**	9.00	230.04 ± 16.28	239.17 ± 17.18**	9.13	240.65 ± 15.90	243.22 ± 16.83	2.57
1,600 m run (sec)	427.88 ± 43.88	389.54 ± 27.68**	-38.34	374.88 ± 59.81	376.58 ± 31.22**	1.70	367.61 ± 25.21	364.04 ± 26.58	-3.57	372.03 ± 31.38	363.35 ± 26.50	-8.68

AC – abdomen circumference; BH – body height; BM – body mass; BMF – body fat mass; SMM – skeletal muscle mass; VFA – visceral fat area; TW – total water; PBF – percent of body mass; BMI – body mass index; PSMM – percent of smooth muscle mass index; PU – pull-ups; SU – sit-ups; SLJ – standing long jump; n – number; Δ – mean difference; x̄ ± SD – mean ± standard deviation; *$p < 0.05$; **$p < 0.01$.

BH was significantly higher in the second measurement in all the groups, and the values of BM were significantly different between the first and second measurement in the first three groups.

Significantly lower BFM values in the second measurement were registered only in the group IV, PBF was significantly lower in the groups II and IV, as well as VFA values.

The SMM values in the second measurement were significantly higher in all the groups, while the PSMM values were higher in the groups I, II and IV. The differences in the results of motor skills were significantly higher in the groups I, II and III (pull-ups), in the groups I and II (sit-ups) and in the groups I, II and III (standing long jump), while for the 1600

students of the other group, and so on. Under "cross validated", 34 (50%) first-year students, based on functions, really belong to the first group, 29 (50%) students to the second, 22 (40.7%) students to the third, 72.2% of the students belong to the group III and 78.4% to the group IV.

In terms of categorical variables from the survey on dietary and lifestyle habits, the following was shown: significantly fewer number of the group IV students than the students of other groups consumed sweets [χ^2-test 20178, degrees of freedom (df) 9, $p < 0.017$]; significantly fewer consumers of cigarettes (smoker, n = 27) than other students (n = 190) consumed fruit (χ^2-test 15,717, df 3, $p < 0.001$), and more of them consumed alcohol (χ^2-test 5,362; df 1, $p < 0.021$); consumers of tobacco and alcohol (the "smoking and alcohol group n =

Table 2

Canonical prediction for the students of Military High School

Classification results		Predicted group membership					Total
		Group	I	II	III	IV	
Original	Count (%)	I	45 (66.2)	13 (19.1)	7 (10.3)	3 (4.4)	68 (100)
		II	7 (12.1)	42 (72.4)	5 (8.6)	4 (6.9)	58 (100)
		III	5 (9.3)	4 (7.4)	39 (72.2)	6 (11.1)	54 (100)
		IV	2 (5.4)	2 (10.8)	4 (10.8)	29 (78.4)	37 (100)
Cross-validated	Count (%)	I	34 (50.0)	18 (26.5)	12 (17.6)	4 (5.9)	68 (100)
		II	16 (27.6)	29 (50.0)	6 (10.3)	7 (12.1)	58 (100)
		III	8 (14.8)	9 (16.7)	22 (40.7)	15 (27.8)	54 (100)
		IV	3 8.1)	6 (16.2)	9 (24.3)	19 (51.4)	37 (100)

51) practise training on an annual basis significantly less (χ^2-test 11,935, df 4, $p < 0.018$) than other students (n = 166).

It was found that the students of all the four examined group belong to the "smoking and alcohol" group – 18 from the group I, 10 from the group II, 14 from the group III and 9 from the group IV.

In addition, it was found that the group of students the consumers of tobacco and alcohol (alcsmoker, n = 51), compared to other students (n = 166), had significantly higher values of BH, SMM and TW (Figure 1).

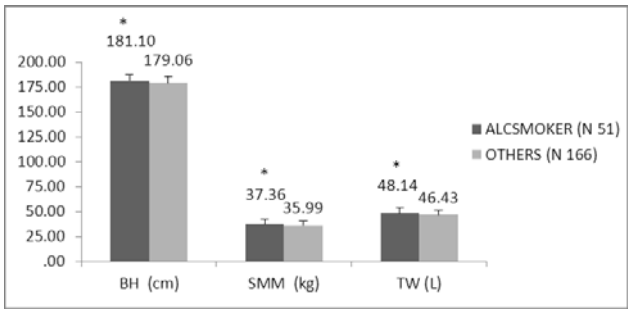

Fig. 1 – Mean differences in morphological variables: body height (BH), skeletal muscle mass (SMM) and total water (TW) between the alcsmokers (consumers of alcohol and cigarettes) and other students measured.

Discussion

When enrolling in the Military High School, students are selected according to the physical status and they are in excellent health, which is regularly monitored during training. During training they live under boarding conditions, have four regular meals and their daily activities and meals are carefully planned. They have two classes of physical education a week and are encouraged to get involved in sports activities in their leisure time, according to personal preferences. Their psychophysical development takes place within institutional educational system and the impact of newly acquired friends in the microenvironment. Maturation during this period involves constant change, both physical and psychosocial, so it is a big responsibility of teachers and professors to enable that the process of four years of schooling sets the right path.

Based on the results of one-year follow-up, there are significant differences in most indicators of morphological and motor characteristics. These results were expected, since this is the age group of 15 to 18 years, a period when adolescents grow up both mentally and physically. The greatest differences in body height were registered during the first year of schooling, or in the period between 15 and 16 years of age (average increase 2.18 cm). Studying the dynamics of the increase in body height, Rogol et al. [10] found that, after a relatively steady increase of 5 to 6 cm/year during childhood, there is a rapid growth (peak height velocity) in boys around the age of 14, then a slowdown due to connecting epiphysis of long bones, followed by the stabilization of height at about 17 years of age. Our study also confirmed a slowdown, expressed in absolute values of the differences between the mean values in the first and second measurement in the first three groups (group I – 2.18 cm; group II – 1.11 cm, group III – 0.59 cm), while in the group IV a larger value of this indicator (0.73 cm) was registered compared to the group III. Similar results regarding the body height in schoolchildren were also obtained by Gharib and Shah [11].

In addition to body height, in the first year, the biggest differences were recorded in BM, SMM and AC values. As an accompanying indicator of the general body growth, an increase in TW of 2.88 L was also recorded. These data show a greater increase in body size at the age of 15 to 16 compared to older age groups, when there is also an increase in body volume. Korovljev et al. [12] point out that in the phase of puberty it is the body volume that increases, influenced by an increase in transversal sizes of the skeleton, which at the age of 15 correlates with diameters of bones, and in later adolescence the correlation decreases. Sinobad [13] gives the amount of muscle mass in male high school students of 1st, 2nd, 3rd and 4th year, of 33.8 kg and 34.5 kg, but also the percentage reduction in SMM from 50% to 48%. In contrast, in the Military High School students, significant differences in the fat component of body mass was not observed in either of the groups. Moreover, in the groups II and III, lower values were registered in the second, as compared to the first measurement. In the group IV, BFM values were significantly reduced (1.94 kg), PSMM values were significantly increased in the groups I, II and IV, while PBF values were significantly decreased in the groups II and IV. The reduction in the fat component in absolute and percentage values and the increase in absolute and percentage values of muscle mass in the period of adolescence, from 15 to 18 years of age, were rare, unless in the athletes. In Portugal, for example, overweight and obesity are present in boys from 13 to 17 years of age with 23.6% [14]. In this regard, Zanovec et al. [15] found out that the respondents with a high level of physical activity had a significantly lower percentage of fat component and more lean-tissue mass compared with a group of low-level activities.

A significant improvement in motor skills of the Military High School students was mostly registered in the groups I, II and III, with a plateau in the group IV, except in the case of 1,600 m run, where the only significant difference was noted in the group I, where at the end of the school year, the students, under the influence of a systematic work improved the result by 38 seconds. In contrast, pull-ups and sit-ups values were significantly higher in the groups I, II and III, but not in the group IV. These results can be explained by the fact that the Military High School students have compulsory physical education twice a week. Besides that, 22.6% of the students practice sports once or twice a week, 71.9% three times or more, while only 5.5% of the students are not systematically, but occasionally involved in sports activities in their free time. About 80% of the students practice sports seven or more months during the year. Data on the incidence of sports show no significant differences between the groups, indicating a consistent educational, organizational and motivational impact on the students' affinity towards sports activities. Even 47.1% of the students of the "smoking and alcohol" group (n = 51) were registered to be involved in sports activities for 9 months a year or more.

The survey on dietary habits in most of the items does not show significant differences among the groups, which can be explained by the authority of the system and organized concern about the students' diet. In their study on the Belgrade adolescents of both sexes, Đorđević-Nikić et al. [8] show that about 36% of them skip breakfast and that 27.4% are classified in the sedentary group. Our data show that the Military High School students do not skip breakfast. According to Mota et al. [14], skipping breakfast is associated with the development of overweight/obesity, and the incidence of meals is in inverse proportion to overweight/obesity. Talking about the educational work with adolescents, Pearson et al. [16] emphasize that adolescents, who describe their parents as authoritative, consume more fruit and have better and healthier diets than those who describe their parents as indulgent and negligent. These data are important in connection with the claims by Sweeting et al. [17] that the dietary habits are formed by the age of 15 and are very little changed until 18.

According to Pastor et al. [18], the national education program on the diet quality gave a positive result in reducing incidence of obesity, the component of metabolic syndrome in adolescents of both sexes from the age of 12 to 16. As the proposal at a strategic level in order to improve the health of population, Enes and Slater. [19] suggest reducing diet that contains fat, such as pizza, chips and popcorn, and encourage the use of sugar free natural juices.

In a certain way, the Military High School as an institution replaces adolescents their family, assuming the role of an authoritative parent and organizing meals without differences in relation to the age of the students. This approach provides a balanced attitude towards food and gives results, which are confirmed in the morphological characteristics, especially in progressive SMM increase and PBF reduction.

The only item in the survey on dietary habits that provides a significant difference among the groups is eating dessert. Much more, the group IV students "occasionally" eat dessert compared to students of other groups, where most of them consume dessert "frequently". Students assigned to smoker group (n = 27) eat significantly less fruit. These data can be taken as indicative. According to Pearson et al. [20], children and adolescents eat significantly less fruit than recommended, and eating fruit in childhood can have the function of protection against cancer in adulthood. The parent – adolescent relationship has a major influence in the development of healthy lifestyle habits, [20] which is of special significance for the Military High School students, as in the course of their education, the system assumes the role of parents.

The students who belong to alcsmoker group (23.5%), compared to the other students, showed significantly better morphological characteristics, evaluated through BH, SMM and TW values. Interestingly, the students of all four groups belong to alcsmoker group. Those are students who are taller and have more muscle mass, and are therefore likely to have felt the need to take a symbolic step into the adult world, accepting a distinctive social model. In addition, they are very young and are not chronic smokers or alcoholics. Cigarette smoking and alcohol drinking (mostly beer) is still at the level of tasting and pleasure testing. In terms of motor skills, evaluated through tests of strength, explosiveness and endurance, they are not significantly different from those of the other Military High School students. Wider community shapes the environment in a way that adolescents recognize a symbol of adulthood in alcohol and cigarettes – "being a man means to light a cigarette and drink beer". A number of authors who have studied this phenomenon highlight that the habits acquired in adolescence are transferred in later periods of life [2, 8, 14, 16, 20], and the educational imperative of the whole society is in understanding and addressing issues of social perception.

In the boarding lifestyle, besides the impact of the institution, the formation of lifestyle habits is also influenced by peers. In their study, Simpinks et al. [21] state two theories that explain the reasons for finding friends. The social theory supports the view of becoming close and making friends based on common activities in a given period, whereas the theory of selection is based on recognizing the similarities. The selection based on similarity can have positive and negative consequences, as mutual induction, i.e. interaction, enhances the impact of either healthy or unhealthy lifestyle habits, depending on the shared content of friendship. In this sense, it is necessary that teachers in boarding conditions are skilled people who know how to recognize the formation of negative tendencies and groups, in order to act accordingly following pedagogical principles.

Including indicators of morphological characteristics, motor abilities and responses to the survey, through canonical discriminant analysis, we estimated belonging to the current class, or find an answer to the question on whether students in different groups according to their characteristics belong to the age group they are in. The results show that 66% of the group I students belong to the right group, and for the other groups, the percentage is as follows: 72.4% (group II), 72.2% (group III) and 78.4% (group IV). Based on the results correlated, 50% of the students from the groups I and II, 40.7% of the students from the group III and 51.4% of the students from the group IV belong to the same group. These data indicate that there is a fine differentiation of morphological and motor skills in relation to age, and that those negative effects, which in the formation of the final image are integrated under the influence of media, peers and the general state of society, have a minimal impact. This is primarily attributable to a uniform educational and organizational influence in teaching and extracurricular activities, which is a system solution used in the Military High School, with the aim of forming a physically and mentally healthy person.

Conclusion

The study observed a decrease in fat component in body structure and increase in muscle mass, along with an increase in motor skills in the Military High School students for a period of one year. Lifestyle habits related to diet do not differ among the students of different ages. The observed variables show that the institutional educational system applied in the Military High School gives good results aimed at forming a mentally and physically healthy person. The results of this study provide a basis for further research, which will be directed towards improving the process of military education.

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Factors contributing to communication skills development in cochlear implanted children

Faktori koji doprinose razvoju komunikacijskih veština kod dece sa kohlearnim implantatima

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Abstract

Background/Aim. Over the last 10 years more than 300 persons received cochlear implant in Serbia and more than 90% of the recipients were children under 10 years of age. The program of cochlear implantation includes postoperative rehabilitation in which cognitive, integrative and developmental methods are used. The study was conducted to reveal factors affecting communication performance (CP) of cochlear implanted (CI) children. Special attention was focused on the influence of the duration and intensity of rehabilitation and hearing age on further development of communication skills**. Methods.** A group of 30 CI children (13 boys and 17 girls) aged 2 to 5 years was enrolled in the study. All of the children had average intelligence and no other developmental disorder. They lived in families and attended rehabilitative seances 3 to 5 times a week. Their parents/caregivers answered structured questionnaire about functioning after pediatric cochlear implantation (FAPCI) and the results were the subject of detailed statistical analysis. **Results.** Analysis of variance did not show any difference between the boys and the girls regarding FAPCI achievements ($F_{(1, 28)} = 2.909$; $p = 0.099$) and age aberration in CP score ($F_{(1, 28)} = 0.114$, $p = 0.738$). Correlation analysis showed a statistically significant difference in FAPCI scores related to hearing age and duration of rehabilitation. Regression analysis (enter method) showed that model consisting of indipendent variables significantly contributed to prediction of overall FAPCI scores and Adjusted R^2 value could explain 32% difference in communication skills of participants in this study. **Conclusion.** Communication skills of CI children evaluated by FAPCI are falling behind normatives for normal hearing children 18.6 months on the average. Hearing age, duration and intensity of rehabilitation have positive predictive value for communication skills development. Later identification of hearing loss and later cochlear implantation lead to delayed development of communication skills.

Key words:
cochlear implants; child; communication; questionnaires.

Apstrakt

Uvod/cilj. U poslednjih 10 godina, kohlearna implantacija (KI) urađena je kod oko 300 osoba u Srbiji, od kojih 90% čine deca ispod 10 godina. Program KI praćen je odgovarajućom rehabilitacijom u kojoj se koristi saznajni, integrativni i razvojni metod. Ovo istraživanje ispitivalo je faktore koji doprinose razvoju komunikacijske veštine (KV) kod dece posle KI. Posebno smo ispitivali doprinos dužine i intenziteta procesa rehabilitacije i slušnog uzrasta razvoju ovih sposobnosti. **Metode.** Ispitali smo 30 KI dece (13 dečaka i 17 devojčica) uzrasta od 2 do 5 godina. Sva deca bila su prosečnih intelektualnih sposobnosti, bez udruženih smetnji u razvoju, živela su u porodičnom okruženju, a bila su uključena u program rehabilitacije od 2 do 5 puta nedeljno. Instrument u ovom istraživanju bio je *Functioning after Pediatric Cochlear Implantation* (FAPCI) upitnik za roditelje/staratelje. **Rezultati.** Poređenje rezultata KI ispitanika dobijenih FAPCI upitnikom sa normativima uspostavljenim za decu bez implantata pokazuju da razvoj njihovih komunikativnih veština (*communication performance* – CP) u proseku kasni 18,6 meseci. Među ispitanom decom nisu utvrđene statistički značajne polne razlike u CP, a one nisu zabeležene ni s obzirom na razliku u aberacijama u odnosu na uzrast u CP skoru ($F(1, 28) = 0.114$; $p = 0.738$). Rezultati korelacione analize pokazuju da je postignuće na FAPCI statistički značajno povezano sa slušnim uzrastom i dužinom trajanja rehabilitacije. Rezultati regresione analize *stepwise* izdvajaju slu-

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šni uzrast kao jedini značajan prediktor ukupnog skora na FAPCI upitniku, a vrednost prilagođenog R^2 pokazuje da se njime objašnjava oko 32% razlika u komunikacijskim veštinama ispitanika. **Zaključak.** Slušni uzrast, trajanje i intenzitet rehabilitacije pozitivno doprinose razvoju komunikativnih veština kod KI dece, dok kašnjenje u uspostavljanju dijagnoze i sprovođenju KI ometa ovaj aspekt razvoja.

Ključne reči:
kohlea, implantat; deca; komunikacija; upitnici.

Introduction

Before cochlear implantation (CI) was introduced speech and language achievement of severely and profoundly deaf children was far worse than in their hearing peers [1]. Speech and language of profoundly deaf children amplified by conventional hearing instruments was specific, with lot of restrictions and distortion. Their knolwedge was scarce and their language lacking grammar and syntax, their speech was concrete with poor articulation, they were reluctant to communicate verbally [2]. Introduction of cochlear implantation in the deaf chidren rehabilitation process had tremendous impact on educational choices and future perspectives. Before cochlear implants deaf children were mainly educated in special schools or in mainstream schools implementing special curriculum [3].

Since cochlear implantation in Serbia started 10 years ago more than 300 deaf persons received cochlear implant, mostly children under 10 years of age (90%) [4]. Rehabilitation treatment in the begining was based on auditory training principles and experience [5] and elements of program for improvement of auditory attention, such as NEAP (Nottingham Early Assessment Package) [6] etc. Nowadays we combine cognitive, integrative and developmental method. It is based on immitation of phases of motor, cognitive, sensitive and communication development in normal hearing and typically developing children [7]. Rehabilitation begins as soon as the hearing loss is detected. Depending on the age at implantation it lasts for several years, until the school begins. It could be continued after the child enters school if any further support is needed. The children in this study were enrolled in continuous rehabilitation and the outcomes were directly related to the hearing age, duration and intensity of rehabilitation. Our previous studies have shown that hearing age alone, without rehabilitation, does not lead to favorable results regarding speech and hearing [3]. Longer hearing age improves auditory perception, but not the overall communicative performance. Speech and hearing rehabilitation of CI children gives meaning to the sounds perceived through CI and than analyzed by the cortex [8].

Inclusive education has been introduced and became obligatory in Serbia in 2009. Children with developmental disorders are mainstreamed [9]. Since 2011 all of the children are included in mainstream schools regardless of the degree of the handicap [10]. In spite of the legislation, personal assistants for such children have not been provided yet, so that the deaf children from mainstream schools are depending on support of rehabilitation centers. Long before the new law on inclusive education was introduced, we used to send well rehabilitated deaf children to the mainstream schools years before the CI era in Serbia, so that we have more than three decades of experience on inclusive education for deaf.

There are numerous empiric studies which have proved the impact of CI on development of communication skills in children. Results of those studes have proven that CI improves perception of sound and speech as well as speech production, linguistic maturity and reading skills [9–11]. Some of the studies referred to speech understanding in deaf children without visual clues and they have shown that CI children were capable of understanding questions [12]. Negative impact of deafness affects mostly communication skills, but there is also considerable impact on personality of a deaf person [13] which is attributed to the lack of abstract categories. Investigators have shown a statistically significant difference between deaf children with cochlear implants and hearing aids regarding their ability to learn acquire and use abstract categories [14]. Rehabilitation results are evaluated through overall achievement of deaf and hard of hearing children in communication skills, education and fulfillment of individual desires and needs [15].

Objectives of clinical studies usually address some elements such as perception and certain segments of speech-language development. The need for more humanistic approach has induced studies on overall communication skills and quality of life of cochlear implant recipients. Medical publications define quality of life (QoL) as the capacity to have normal functional life and feel good about everyday activities [16, 17]. In people using aids or having permanent disability category of "health related QoL" (HRQoL) is used. Zaidman-Zait and Smith [10], examined improvements in children's HRQoL as a result of cochlear implantation. They assessed the HRQoL *via* condition-specific items concerning the relative benefits and problems associated with implant use, the child's behavior and social activities. Eleven parents and their children with cochlear implants (age range 6–20 years) reported both significant improvements in the child's HRQOL and minimal negative effects of the cochlear implant. When parents rated the items, the areas rated as having the greatest benefit were hearing environment sounds, speech perception, and speech production. Overall communication skills, child's sense of safety, self-esteem, vocabulary or language skills and relationship with family were rated as a benefit for the child.

The aim of this study was to investigate if hearing age, duration and intensity of rehabilitation are related to development of communication performance (CP) in CI children. Precisely, the study encompassed the factors affecting communication development in CI children, addressing particularly the impact of hearing age, duration and intensity of rehabilitation tretament.

Methods

The study encompassed 30 CI children (13 boys and 17 girls) of chronological age 2 to 5 years. All of the children in the study had average intelligence and no other developmental disorder. They lived in families. All of them were enrolled in speech and hearing rehabilitation 2 to 5 times a week. The instrument used in this study was the questionnaire Functioning after Pediatric Cochlear Implantation (FAPCI) for parents and caregivers [16].

FAPCI represents a psychometrically-validated unidimensional scale of communicative performance. Each of the 23 items contributes monotonically to the overall score on the scale. Scoring completed FAPCI surveys is best done using the protocol. The FAPCI instrument is a psychometrically validated survey that is used to evaluate the real-world verbal communicative performance of children aged 5 years or younger using cochlear implants. This instrument was designed to fill a gap in our current approach to the assessment of cochlear-implanted children, and FAPCI scores reflect a child's ability to communicate in real-world settings (e.g. at home or when interacting with family members). A special advantage of the FAPCI instrument is that detailed examples of communication situations are described thus helping the parents or caregivers to assess communicative behavior of their children.

The survey was conducted in 2013, Clinical Center of Serbia, Clinic for ENT&HNS, Audiology Rehabilitation Department, Belgrade, Serbia.

Descriptive statistics methods have been used for data analysis, Pearson's correlations for assessment of correlation between variables, unifactor analysis of variance (ANOVA) to assess differences between groups and multiple regression analysis to define predictive value of certain variables for final FAPCI scores.

Results

Patients characteristics are presented in Table 1.

The ANOVA results did not show a significant gender difference between the boys and the girls in FAPCI scores ($F_{(1, 28)} = 2.909$, $p = 0.099$) or age aberration in CP scores ($F_{(1, 28)} = 0.114$, $p = 0.738$), although the girls had slightly higher average FAPCI scores and slightly lower age aberration scores than the boys in this study (Table 2).

The results of correlation analysis (Table 3) showed that hearing age and rehabilitation duration affected FAPCI scores significantly. Correlation rang is moderate, but positive, suggestive of improvement of communicative skills with longer hearing age and rehabilitation.

It should be noted that some variables that were not among FAPCI achievement parameters showed significant connections (Table 3), such as high positive correlation between chronological age at the diagnosis and chronological age at implantation, as well as hearing age and duration of rehabilitation.

Table 1

Characteristics of patients (n = 30) included in the study

Patients characteristics	x̄	SD	Range
Chronological age (months)	41.83	10.71	25–58
Chronological age at onset (months)	16.93	9.21	1–33
Chronological age at CI (months)	27.27	10.23	11–44
Hearing age (months)	13.97	9.21	5–41
Rehabilitation duration (months)	22.70	9.61	9–50
CI rehabilitation intensity (frequency)	4.00	1.29	1–5

CI – cochlear implants.

Table 2

Descriptives for FAPCI total score and age aberration in communication performance (CP) score (by gender)

Data	FAPCI total score			Age aberration in CP score (months)		
	Gender		Total	Gender		Total
	male	female		male	female	
n	13	17	30	13	17	30
Mean	65.92	79.12	73.40	19.46	17.94	18.60
SD	19.788	21.857	21.676	14.89	9.69	12.01
Range	29–95	29–105	29–105	0–49	4–42	0–49

FAPCI – functioning after pediatric cocholear implantation.

Table 3

Correlations among study variables

Variables	2	3	4	5	6	7	8
1. FAPCI total score	-0.510**	0.170	-0.030	-0.232	0.478**	0.463**	-0.138
2. Age aberration in CP score	1	0.754**	0.411*	0.692**	0.083	0.202	0.089
3. Chronological age		1	0.425*	0.586**	0.492**	0.591**	-0.050
4. Chronological age at onset			1	0.663**	-0.289	-0.226	0.166
5. Chronological age at CI				1	-0.393*	-0.140	0.343
6. Hearing age					1	0.851**	-0.399*
7. Rehabilitation duration						1	-0.131
8. CI rehabilitation intensity							1

***p < 0.05; ** p < 0.01.**

FAPCI – functioning after pediatric cocholear implantation; CP – communication performance; CI – cochlear implants.

The results of regression analysis (enter method) showed that the model obtained by combination of those independent variables contributed to the prediction of the overall FAPCI score, and value of adjusted R^2 could explained 28% of difference in communication skills of subjects (Table 4). Although regression coefficient for none of the predictor variables reached statistical significance, the highest Beta value was observed for hearing age. It has positive value, suggesting that increase in hearing age leads to increase in communication skills.

Table 4

Summary of multiple regression analyses with the FAPCI total score and age aberration in CP score as dependents (enter method)

Multiple regression analysis	*β*	*t*	*p*		*β*	*t*	*p*
Regression 1				*Regression 2*			
Chronological age	-0.759	-0.987	0.334	Chronological age	1.346	2.737	0.012
Chronological age at CI	0.866	1.130	0.271	Chronological age at CI	-0.391	-0.797	0.434
Hearing age	1.291	1.757	0.093	Hearing age	-0.695	-1.478	0.154
CI rehabilitation intensity	-0.133	-0.683	0.502	CI rehabilitation intensity	0.114	0.916	0.370
F	3.540				16.587		
do	4.22				4.22		
p	0.022				0.000		
ΔR^2	0.281				0.706		

Regression 1: FAPCI total score as a criterion. Regression 2: Age aberration in CP score as a criterion.
FAPCI – functioning after pediatric cocholear implantation; CP – communication performance; CI – cochlear implants.

The results of regression analysis (stepwise method), choosing the set of most useful predictors, were somewhat different. Regarding the overall FAPCI score a statistically significant model is elicited using regression ($F_{(1, 25)} = 13.142$; $p = 0.000$) and that could explain 32% of variance with hearing age as a unique significant predictor ($\beta = 0.587$, $t = 3.625$, $p = 0.000$).

Discussion

The average age aberration in CP score in this group of children was 18.6 months (SD = 12.01) indicating that they fall behind their hearing peers from a normative sample. Normal hearing and typically developing children reach maximal FAPCI scores by the age of 3 years. Other authors did not specify the delay of CI children, although it has been proved in numerous test [17, 1]. The majority of the children in this sample were impanted between 1 and 3 years of age 24/30 (89%). FAPCI scores did not reflect significant differences regarding age at implantation in this study, although numerous studies have proven a considerable progress in communication skills in early implanted children [18–21]. A wide range of differences in this sample (from 0 to 49 months) reflects heterogenous structure regarding communicative skills of the children in this study, some of them being extremely delayed, while the others achieve age appropriate normatives for normal hearing children. The key issue is to establish the factors leading to such huge differences between CI children. Sometimes it could be due to preoperative rehabilitation and communicative achievements before the implantation or dynamics of auditory perception maturation in the first months after switch-on of CI [22]. The variability of data on communication skills obtained through this study confirms other authors experience [23] that there is still a great challenge in clinical evaluation of the outcome of cochlear implantation. Early implanted children enrolled in intensive postoperative rehabilitation have varible communication skills due to numerous individual (intelligence, motivation, personality type) or environmental (family, society) factors.

The correlation between varibales in this study shows a moderate negative correlation between FAPCI and age abberation in CP scores, higher age abberation in CP score corresponds to lower FAPCI score. High age abberation in CP scores could be a predictor of poor communicative performance. Unlike overall FAPCI score, there is high positive correlation between age abberation in CP score and chronological age and age at implantation. A higher age abberation in CP score corresponds to higher chronological age and age et implantation in this group of children. There is a moderate positive relation between age abberation in CP scores and age at diagnosis (higher abberation in later detected children). This finding supports the conclusion that late detection of hearing loss followed by late cochlear implantation is responsible for the major delay in communication skills development in CI children.

It should be emphasized that some variables apart from FAPCI achievement have shown considerable correlation, especially age at diagnosis and age at implantation, as well as hearing age and duration of rehabilitation. Comparative study of 22 children with cochlear implant and adequate sample of hearing impaired children with hearing aids [24] shows that hearing age and rehabilitation affect considerably better achievements in children with CI.

Multiple regression analysis was applied to evaluate relative predictive value of single variables for FAPCI score. Predictors included chronological age, chronological age at implantation, hearing age and frequency (intensity) of rehabilitation. It should be emphasized that some predictors were omitted from a final pedictor set, because of the high correlation with other indipendent variables, such as chronological age (high correlation with chronological age at implantation) and duration of rehabilitation (extremeley high correlation with hearing age). Apart from that, following preliminary results of casewise diagnostics exclusion of 3 participants from

final analysis due to extremely low FAPCI scores has been suggested and done.

Regression analysis (enter method) showed that the model consisting of those indipendent variables contributed considerably to prediction of overall FAPCI scores and adjusted R^2 could explain 28% of differences in communicative performance between the children in this study. Although regression coefficient of none of the predictor variables did not reach statistical significance, the highest Beta value was observed for hearing age variable. It has positive value suggestive of increase in communication skills through increase in hearing age. Review of the literature [3, 21, 22] has shown that among all the investigated variables hearing age had always extremely positive impact on communication development in CI children. Development dynamics of auditory skills following cochlear implantation is clearly defined and increase of hearing age is followed by certaininity in listening and development of communication skills in all users of cochlear implant enrolled in rehabilitation.

Regression using the same set of predictors provides a statistically significant model and explanation for 70% of differences between the subjects if age abberation in CP score is used as prediction criterion. In this model, the only statistically significant value of Beta coefficient was obtained for chronological age of a child, which means that increase in chronological age leads to a detected delay in comparison with the normative group. This result emphasizes the significance of the chronological age of CI children; higher chronological age induces bigger delay from normative values for normal hearing children [18].

The results of stepwise regression analysis, using the most versatile set of predictors a slightly different. If a FAPCI total score is used as criterion, regression provides a statistically significant model explaining 32% of variance, with hearing age being a single significant predictor, whereas regression using age abberation in CP score as dependent variable, in statistically significant model which explains 72% intersubject variability, depict both chronological and hearing age of a child as significant predictors. The sign of Beta coefficients corroborates previous conclusions: increase in hearing age improves FAPCI scores and decreases delay compared to normative group, whereas increase in chronological age increases delay from normatives for normal hearing children.

Apart from a small number of CI children in this study, cetain limitations could be attributed to the normative data we have used [18]. Normative data were not standardized and validated for Serbian population, although the authors find that the FAPCI is not language specific and could be successfully used to depict develomental characteristics of deaf implanted children.

Conclusion

Based on the data obtained in this study on the assessed sample it could be concluded that cochlear implantation has a significant, positive contribution to the development of communication skills of deaf children. Data evaluation shows that the early diagnosis and early intervention implemented in clinical practice, corroborate by a high correlation of chronological age at the diagnosis and chronological age at implantation. Communication skills of cohlear implanted children increase accordingly with increasing hearing age and the duration of rehabilitation.

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Addendum

FAPCI Instrument [17]

Item	Response format
How often does your child respond to phrases that s/he overhears from a nearby conversation?	F
Given an unlimited set of possible choices, how many age-appropriate items would your child be able to point to when they are presented in spoken language without visual cues?	Q
How many age-appropriate 2-step spoken commands presented without visual cues does your child understand?	Q
When riding in a car, my child is able to understand...	E
When listening from a different room of the house, my child is able to understand...	E
When in a noisy environment, my child is able to understand...	E
When using the telephone with a familiar caller, my child is able to understand...	E
How often does your child appropriately answer simple questions presented in spoken language without visual cues?	F
How many age-appropriate items would your child be able to identify with spoken language when they are pointed to?	Q
How much of your child's speech would an adult who is not familiar with your child understand?	Q
How does your child typically respond when greeted by a familiar person?	E
How many people's names does your child use in spoken language?	Q
Which statement best describes your child's singing?	E
What is the main way that your child communicates his/her wants when not coached by an adult?	E
How many of the following types of words/phrases does your child use in spoken language: what, where, why, inversion questions, which?	Q
How many of the following types of words/phrases does your child use in spoken language: words to describe size or color, numbers to describe how many, words to describe quantity, plural endings, possessive ending?	Q
How often does your child ask simple questions using spoken language?	F
How often does your child talk about his/her experiences during the day or about a past event using simple spoken sentences?	F
How often does your child use the past tense in spoken language?	F
How often does your child use the negative in a 2–3 word spoken phrase?	F
How often does your child correctly use pronouns in spoken language?	F
How often does your child correctly use prepositions in spoken language?	F
How often does your child initiate a spoken conversation with another child?	F

Detailed instructions, specific examples, and tips for responding to all items are provided with questions in the actual FAPCI instrument. Response format: F = Frequency-based questions (response levels of "never", "rarely", "sometimes", "frequently", and "always"); Q = Quantity-based questions (response levels with either specific quantities or "almost none (0-4%)", "few (5-24%)", "some (25-49%)", "most (50-95%)", or "almost all (96-100%)"); E = Example-based questions (response levels contain a description or an example of a behavior, and levels correspond to an ordinal scale of functioning adjudicated by the authors).

ORIGINAL ARTICLE

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Efficacy of nanocrystalline bone substitute biphasic calcium phosphate/poly-DL-lactide-co-glycolide for periodontal intrabony defects filling

Efikasnost nanokristalnog zamenika kosti bifaznog kalcijum-fosfata-poli-DL-laktid-ko-glikolida za popunjavanje infrakoštanih defekata parodoncijuma

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Abstract

Background/Aim. Different bone substitutes have been used for filling and reparation of intrabony defects. The aim of this study was to compare nanocrystalline material, biphasic calcium phosphate poly-DL-lactide-co-glycolide (BCP/PLGA) with deproteinised bovine bone (DPBB) and β-tricalcium phosphate (β-TCP) in the treatment of periodontal intrabony defects. **Methods.** The study included 24 patients with bilateral, intrabony defects in the region of the upper first and second premolar, and the upper first molar. On one side of the mouth, DPBB (BioOss®) was used to fill defects in upper premolars while β-TCP (RTR®) was used for the upper first molar. BCP/PLGA was applied into periodontal defects of the upper premolars and upper first molar of the opposite side. **Results.** The comparison of the BCP/PLGA and the β-TCP group, 6 months following filling of defects, showed a statistically significant reduction of periodontal pocket depth (PPD) and the position of the cement-enamel junction (CEJ) in the group with BCP/PLGA, when compared to the β-TCP group. The reduction of PPD and CEJ was similar in the groups treated with BCP/PLGA and DPBB. **Conclusion.** Significant reductions of PPD and CEJ were registered in the group with BCP/PLGA when compared to the β-TCP group.

Key words:
bone resorption; bone substitutes; oral surgical procedures; biocompatible materials; nanoparticles; treatment outcome.

Apstrakt

Uvod/Cilj. Različiti koštani zamenici koriste se za punjenje i reparaciju infrakoštanih defekata. Cilj rada bio je da se uporedi nanokristalni materijal – bifazni kalcijum-fosfat poli-DL-laktid-ko-glikolid (BCP/PLGA) sa deproteinizovanom govođom kosti (DPBB) i β-trikalcijum fosfatom (β-TCP) u terapiji humanih parodontalnih infrakoštanih defekata. **Metode.** U studiju su bila uključena 24 pacijenata sa bilateralnim, infrakoštanim defektima u regiji gornjeg prvog i drugog premolara i prvog gornjeg molara. Slučajnim izborom defekti su punjeni DPBB (BioOss®) – gornji premolar i β-TCP (RTR®) – prvi gornji molar. BCP/PLGA bio je postavljan u parodontalne defekte gornjih premolara i prvog gornjeg molara sa suprotne strane. Plak indeks (PI), indeks krvarenja gingive (BI), pozicija cementno-gleđnog pripoja (CEJ) i dubina parodontalnog džepa (PPD) mereni su preoperativno i šest meseci nakon operacije. **Rezultati.** Poređenjem BCP/PLGA i β-TCP grupe utvrđena je statistički značajna redukcija PPD i CEJ šest meseci nakon operacije. Redukcija PPD i CEJ bila je slična u grupama koje su tretirane sa BCP/PLGA i DPBB. **Zaključak.** Značajno smanjenje PPD i CEJ registrovano je u grupi sa BCP/PLGA u poređenju sa β-TCP grupom.

Ključne reči:
kost, resorpcija; kost, zamenici; hirurgija, oralna, procedure; biokompatibilni materijali; koloidi; lečenje, ishod.

Introduction

Modern periodontal therapy is based on the use of natural or synthetic biomaterials for the filling and reparation of intrabony defects. Different bone substitutes have been used for this purpose with various rates of success [1–3]. Deproteinised bovine bone (DPBB) is considered the most tested bone substitute in periodontal surgery. It has been suggested that

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DPBB possesses osteoconductive properties [4], allowing the fast attachment of osteoblasts and deposition of new bone [5]. A significant reduction in depth of intrabony defects has been observed after implantation of DPBB [6]. Scabbia and Trombelli [7] have indicated that implantation of DPBB produced significant improvement in terms of cement-enamel junction (CEJ) and the reduction of periodontal pocket depth (PPD) of human intrabony defects.

It has been shown that β-tricalcium phosphate (β-TCP) promotes osteogenesis and bone regeneration in intrabony defects in dogs with no signs of inflammatory reaction [8]. One of the major drawbacks of β-TCP is its rapid resorption. Jensen et al. [9] showed almost complete resorption of β-TCP, 8 weeks after filling osseous defects in minipigs.

A new biocomposite material, biphasic calcium phosphate (BCP) poly-DL-lactide-co-glycolide (PLGA) – (BCP/PLGA), in which BCP granules are coated with bioresorbable PLGA polymer, was introduced as a bone substitute by Ignjatovic et al. [10]. A high level of osseous regeneration was obtained when using BCP/PLGA to fill intrabony defects in 10 patients [10]. Rapid and strong osteoconductivity was observed after implantation of hydroxyapatite PLGA (HAp/PLGA) for reparing critical size radius defects in rabbits [11].

There is however no bone substitute material which provides complete regeneration of intrabony periodontal defects. Until now, there has been no study that compared BCP/PLGA with other bone substitutes for filling of intrabony defects. The aim of this study was to evaluate the clinical outcome of a biocomposite material use of BCP/PLGA and to compare it with DPBB and β-TCP in the treatment of human periodontal intrabony defects.

Methods

Materials

Aqueous calcium nitrate [$Ca(NO_3)_2$] solution was added to a solution of ammonium phosphate [$(NH_4)_3PO_4$] at 50°C over a period of 60 min, while stirring at the rate of 100 rpm. The solution was then subjected to heat treatment for 60 min at 100°C. The obtained gel was dried at room temperature and calcined at 1,100°C for 6 h. In our previous studies, we showed that the obtained calcium phosphate powder consisted of HAp and β-TCP. Based on the previously described methodology, the mass content was calculated at 80% HAp and 20% β-TCP[12]. This type of calcium phosphate is called BCP and is used for the production of BCP/PLGA composite biomaterial [12]. BCP powder, whose roentgenograms and microstructure were examined in our previous papers [13], was synthesized by precipitation.

PLGA (50:50) (Sigma Chemical Company, USA) was used as a polymer component. Granules of BCP were added into completely dissolved polymer, in amounts of 80 mass%. The solution was mixed at the speed of 30 rpm, and then methanol was added. After solvent evaporation, the particles were dried at room temperature for 24 h. The resulting granules of BCP/PLGA composite biomaterial were sterilized by γ rays (25 kGy) before use.

For crystallites sizes of Hap from BCP/DLPLG the Rietveld method was used [14].

Composite production via the solvent–nonsolvent procedure provides a covering of BCP with PLGA polymer [12–15].

Characterisations of materials

Wide angle X-ray structural (WAXS) analysis of the BCP/PLGA composite biomaterial was carried out using a Philips PW 1710 diffractometer. Microstructure characterization was carried out using a JSM 5300 scanning electron microscope. The particle size distribution of powders was measured using a Mastersizer 2000 (Malvern Instruments Ltd.) and Shirocco dispersion unit.

Patients selection and surgical procedure

The study protocol was approved by the Ethical Committee of the Faculty of Dentistry, University of Belgrade (document number 123/2). The study included 24 patients, 14 women and 10 men, suffering from severe periodontal disease. The patients included in the study signed informed consent forms. The inclusion criteria were: otherwise healthy patients (ASA I) between 18–45 years of age who were non-smokers, and had not undergone any dental treatment in the last 2 weeks. The study exclusion criteria included: nursing mothers, pregnant women, those using any contraceptive medication or methods and patients using any analgesic agents within 24 h of the treatment. In addition, every patient had bilateral, three-wall, intrabony defects in the region of the upper first and second premolar, and upper first molar, ≥ than 4 mm deep. This was a randomized, split mouth study, which included three groups: the group I – study group, (open flap debridement + BCP/PLGA), and two control groups: the group II (open flap debridement + deproteinised bovine bone, DPBB), and the group III – (open flap debridement + β-TCP). The intrabony defects were located on both the mesial and distal sides of the upper premolars and first molars.

The operative procedure started after local anaesthesia consisting of 2% lidocaine with adrenaline 1: 80 000 (Xylonor®, Septodont, France) was applied. The incision was intrasulcular and full thickness mucoperiosteal flaps at both vestibular and palatal sides were raised in order to expose entire intrabony defects. All granulation tissue was removed from intrabony defects and the roots were completely scaled and planed by hand and ultrasonic instruments.

After the open flap debridement was carried out, intrabony defects on the mesiopalatal or disto-palatal side of the upper premolars on one side of the mouth were filled with DPBB (BioOss®, Geistlich, Wolhausen, Switzerland), while β-TCP (RTR®, Septodont, France) was used on the mesiopalatal or disto-palatal side of the upper first molar. During the same appointment, BCP/PLGA was applied into periodontal defects of the upper premolars and upper first molar of the opposite side of the mouth. The implanted material was firmly packed into intrabony defects with a sterile amalgam rammer. Finally, the mucoperiosteal flaps were repositioned and sutured primarily with single interdental sutures (Ethicon®, Mersilk 4-0, USA).

The plaque index (PI), bleeding index (BI), position of the cement-enamel junction (CEJ) and PPD were recorded preoperatively as well as 6 months after the operation. All the measurements were made by the same investigator using the same type of manual periodontal probe (PCP 12, Hu-Friedy, Chicago, IL, USA) with a probe tip diameter of 0.4 mm. The PI was scored as: score 0 for no plaque; score 1 for a film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye, but only by using a disclosing solution or probe; score 2 for moderate accumulation of deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen with the naked eye; and score 3 for abundat soft matter within the gingival pocket and/or on the tooth and gingival margin. The BI was also registered before the treatment and 6 months following the operation and scored as: score 0 for no bleeding; score 1 for a single discreet bleeding point; score 2 for several isolated bleeding points or a single line of blood appears; score 3 where the interdental triangle fills with blood shortly after probing; and score 4 for profuse bleeding occurring after probing with blood flowing immediately into the marginal sulcus.

One month before the operative procedure, basic periodontal therapy, which included motivation and oral hygiene instructions as well as scaling and root planning with periodontal probes, was administered. In addition, where traumatic interdental contact was present, occlusal corrections were carried out. Furthermore, all inadequate fillings were replaced at least a month before.

Antibiotic therapy (Amoksicilin®, Galenika, Beograd, Serbia) was prescribed for one week postoperatively. Detailed hygiene instructions were given to every patient.

Statistics

Statistical analysis was carried out using IBM SPSS for Windows (version 19.0). The continuous variables, such as CEJ and PPD, were shown as a mean ± SD. On the other hand, variables such as PI and BI were presented as category variables. The significance of differences for PI and BI between baseline and 6-month data, and between the groups 6 months following the treatment, was evaluated with a $\chi 2$-test. The significance of differences for CEJ and PPD between baseline and the 6-month period was evaluated using the Wilcoxon test, while the Man Whitney *U*-test was used to determine differences for CEJ and PPD between groups 6 months after the treatment. The level of significance was set at $p < 0.05$.

Results

Figure 1a shows the X-ray diffraction (XRD) patterns of BCP/PLGA composite biomaterials. The most intense peaks at 2θ = 29°(2 1 0) and 31.8°(2 1 1) originate from HAp and that at 2θ = 31° (0 2 10) from (β-TCP). The crystallites sizes of HAp from BCP/DLPLG obtained from the XRD patterns were 98 nm.

Figure 1b shows a scanning electron microscope (SEM) image of the BCP/PLGA composite obtained according to experimental procedure. BCP granules are coated with the polymer and their average diameter is about 100 μm.

Dry powder of BCP/PLGA was analyzed with the aim of establishing particle size distribution. Figure 1c) shows the particle size distributions for BCP/PLGA powder: d10 = 1.519 μm; d50 = 100.973 μm and d90 = 464.050 μm. The highest and the most important present fraction (d50) has particles with the diameter of 100 μm. The results of particle size distribution are in accordance with SEM studies (Figure 1.b). Only 10% of all particles (d10) have a maximum diameter of 1.52 μm.

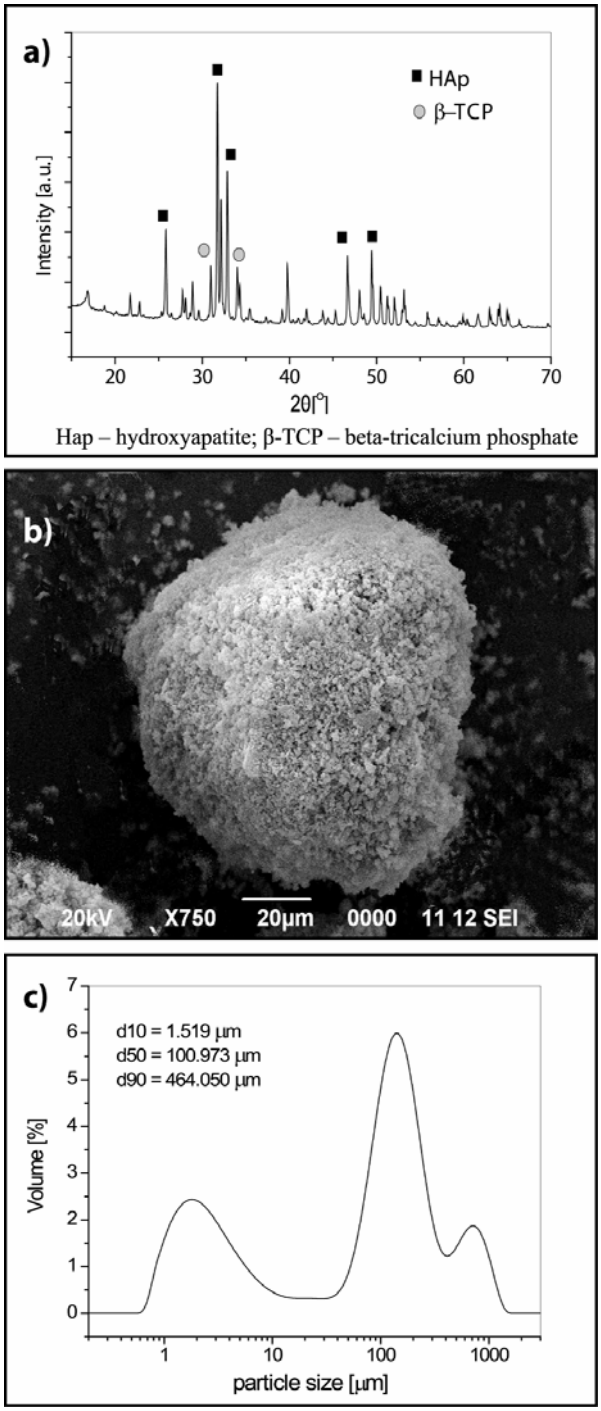

Fig. 1 – a) X-ray diffraction pattern of biphasic calcium phosphate/poly-DL-lactide-co-glycolide; b) Scanning electron microscope image of BCP/PLGA; c) Particle size distributions of BCP/PLGA.

The PI and BI were significantly lower in all the three tested groups 6 months following the operation when compared to preoperative values (Tables 1 and 2). There were no tested teeth with the BI score of 4 before the treatment or 6 months following the operation.

The results of this study also demonstrated statistically significant reduction in PPD and CEJ in all the three tested groups, 6 months following the treatment (Figure 2).

The comparison of BCP/PLGA and β-TCP, 6 months following filling of defects, showed a statistically significant re-

Table 1

Comparison of plaque index (PI) before the treatment and 6 months after the operation

Tooth/Time of record	Teeth, n (%)				Total	p (χ^2 test)
	Score 0	Score 1	Score 2	Score 3		
16 baseline	-	14 (58.3)	8 (33.3)	2 (8.3)	24 (100)	< 0.0001*
16 after BCP/ PLGA	17 (70.8)	7 (29.2)	-	-	24 (100)	
15 baseline	-	14 (58.3)	8 (33.3)	2 (8.3)	24 (100)	< 0.0001*
15 after BCP/ PLGA	17 (70.8)	7 (29.2)	-	-	24 (100)	
14 baseline	-	14 (58.3)	8 (33.3)	2 (8.3)	24 (100)	< 0.0001*
14 after BCP/ PLGA	17 (70.8)	7 (29.2)	-	-	24 (100)	
24 baseline	-	11 (45.8)	11 (45.8)	2 (8.3)	24 (100)	< 0.0001*
24 after DPBB	18 (75.0)	6 (25.0)	-	-	24 (100)	
25 baseline	-	11 (45.8)	11 (45.8)	2 (8.3)	24 (100)	< 0.0001*
25 after DPBB	18 (75.0)	6 (25.0)	-	-	24 (100)	
26 baseline	-	11 (45.8)	11 (45.8)	2 (8.3)	24 (100)	< 0.0001*
26 after β-TCP	18 (75.0)	6 (25.0)	-	-	24 (100)	

***Statistically significant difference; BCP/PLGA – biphasic calcium phosphate/poly-DL-lactide-co-glycolide. DPBB – deproteinised bovine bone; β-TCP – β-tricalcium phosphate.**

Score: 0 – no plaque; 1 – a film of plaque adhering to the free gingival margin and adjacent area of the tooth, seen only by using a disclosing solution or probe; 2 – moderate accumulation of deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface seen with the naked eye; 3 – abundat soft matter within gingival pocket and/or on the tooth and gingival margin.

Table 2

Comparison of bleeding index (BI) before the treatment and 6 months after the operation

Tooth/Time of record	Teeth, n (%)				Total	p (χ^2 test)
	Score 0	Score 1	Score 2	Score 3		
16 baseline	1 (4.2)	9 (37.5)	7 (29.2)	7 (29.2)	24 (100)	< 0.0001*
16 after BCP/ PLGA	10 (41.7)	14 (58.3)	-	-	24 (100)	
15 baseline	1 (4.2)	7 (29.2)	11 (45.8)	5 (20.8)	24 (100)	< 0.0001*
15 after BCP/ PLGA	10 (41.7)	13 (54.2)	1 (4.2)	-	24 (100)	
14 baseline	1 (4.2)	9 (37.5)	9 (37.5)	5 (20.8)	24 (100)	< 0.0001*
14 after BCP/ PLGA	12 (50.0)	12 (50.0)	-	-	24 (100)	
24 baseline	1 (4.2)	8 (33.3)	10 (41.7)	5 (20.8)	24 (100)	< 0.0001*
24 after DPBB	13 (54.2)	11 (45.8)	-	-	24 (100)	
25 baseline	1 (4.2)	6 (25.0)	10 (41.7)	7 (29.2)	24 (100)	< 0.0001*
25 after DPBB	12 (50.0)	12 (50.0)	-	-	24 (100)	
26 baseline	1 (4.2)	8 (33.3)	9 (37.5)	6 (25.0)	24 (100)	< 0.0001*
26 after β-TCP	11 (45.8)	13 (54.2)	-	-	24 (100)	

For abbreviations see under Table 1;

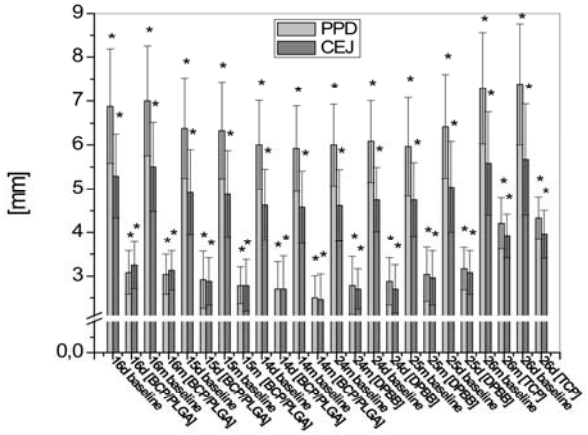

Fig. 2 – Reduction in periodontal pocket depth (PPD) and the cemento-enamel junction (CEJ) 6 months following the operation (d – distal side; m – mesial side; * – statistically significant; $p < 0.0001$). BCP/PLGA – biphasic calcium phosphate/poly-DL-lactide-co-glycolide.

duction of PPD and CEJ in the group with BCP/PLGA, when compared to the β-TCP group (Figure 3). The reduction in PPD and CEJ was similar in groups treated with BCP/PLGA and DPBB (Figure 3).

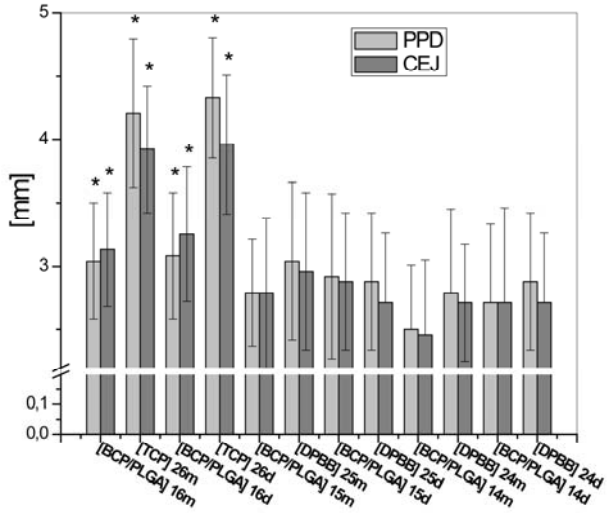

Fig. 3 – Comparison of periodontal pocket depth and level of cemento-enamel junction six months following the operation between tested groups (d – distal side; m – mesial side;* – statistically significant; $p < 0.0001$). BCP/PLGA – biphasic calcium phosphate/poly-DL-lactide-co-glycolide. TCP – beta-tricalcium phosphate; DPBB – deproteinised bovine bone.

Figure 4 shows one of 24 cases in this study. Figure 4a shows the intrabony defect on the mesial side of the upper first molar on X-ray film before the operation. Figure 4b shows the clinical appearance of the intrabony defect before the treatment and Figure 4c demonstrates the intraoperative view of the same defect. The defect was filled with BCP/PLGA powder (Figure 4d). After reconstruction, the mucoperiostal flap was sutured with silk (Figure 4e). In the retroalveolar X-ray image, made 6 months after intervention, the new-formed bone in the defect on the mesial side of upper first molar is obvious (Figure 4f).

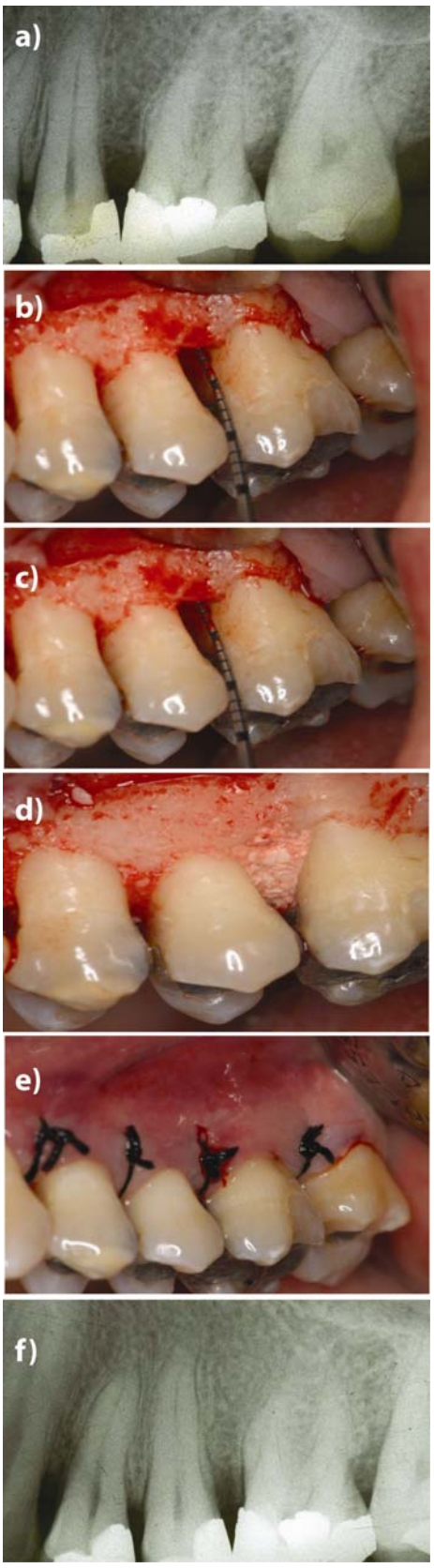

Fig. 4 – Filling the intrabony defect with biphasic calcium phosphate/poly-DL-lactide-co-glycolide (BCP/PLGA): a) X-ray image before the treatment; b) Clinical appearance of intrabony defect; c) Intraoperative view of intrabony defect; d) Applied BCP/PLGA; e) Sutured reconstruction; f) X-ray image after 6 months.

Discussion

The results of this study indicate that BCP/PLGA was more successful than β-TCP in periodontal intrabony defects filling when assessed six months following the treatment. In comparison to DPBB, the results obtained with BCP/PLGA were slightly better in terms of PPD and CEJ level reduction, but did not differ statistically. The improvement in PPD and CEJ obtained with the nanocrystalline bone substitute BCP/PLGA, could possibly be explained by its structure. XRD patterns show no peaks for PLGA polymer because this polymer is amorphous, which is in accordance with the XRD studies of PLGA of our previous research [13, 15]. BCP coated with polylactide polymer enables protein adhesion prior to cell adhesion to the proteins on biomaterial [16, 17]. It was shown that free surface energy decreased by adding BCP to the polymer component, and, consequently, allowed better interaction with surrounding cells [18]. Likewise, the bet-

ter results realised with BCP/PLGA in comparison to β-TCP could be explained by the preventive effect of the polymer component against removal by the immune system [19]. In addition, it was already shown that BCP/PLGA enhanced osteoblast differentiation and production of alkaline phosphatase as a sign of its activity [12].

Implantation of β-TCP into intrabony defects demonstrated a significant reduction in PPD and CEJ after 6 months when compared to baseline levels. The results of our study are consistent with those of Saini et al. [20] who have demonstrated significant clinical improvement in the treatment of human intrabony defects filled with β-TCP. Neamat et al. [8] in the study on dogs' periodontal intrabony defects showed that β-TCP promoted osteogenesis and bone regeneration. On the other hand, our results obtained with β-TCP were not as positive as those achieved with BCP/PLGA. A possible explanation could be β-TCP's rapid resorption. Due to the initial progressive degradation phase and the accumulation of phagocytising cells accompanying degradation of the material, the implant particles may not integrate with the bone [21].

Our results showed that PPD and CEJ were similarly reduced after filling intrabony defects with DPBB and BCP/PLGA. This is in accordance with the study of Kim and Kim [22] who presented similar bone formation after using DPBB and HAp/PLGA in critical size rat calvarial defects. The studies with Biooss® as a bone substitute showed intrabony defect filling and reduction of PPD [6], as well as osseous regeneration around dental implants [4]. Cordaro et al. [23] showed that bone-to-graft contact was greater with DPBB compared to β-TCP (48% *vs* 34% respectively), and, according to this finding, it can be concluded that DPBB has the higher osteoconductivity. Although we did not directly compare DPBB and β-TCP, good results obtained with DPBB as well as with BCP/PLGA may be explained by higher osteoconductivity than that achieved by β-TCP. Resorption of DPBB particles still remains a controversial issue. DPBB seems to be inert and stable over time and to remain sequestered in bone, marrow, and fibrovascular tissue (for up to 10 years) [24]. Schlegel and Donath [25] showed histological signs of DPBB particles 6 years following bone defects fill. On the other hand, Thaller et al. [26] demonstrated resorption of DPBB, which underwent normal physiologic bone remodelling in the rabbit calvarial model.

We found clinical and statistical improvement in terms of PPD and CEJ reduction in all the three groups 6 months following the operation. These results, obtained with each of the bone substitutes, are likely to be a consequence of the three-wall anatomy of intra-bony defects. The findings could be explained by the rich blood supply and growth factors deriving from each of three bony walls. This is in accordance with the study of Ellegaard and Löe [27], who reported that defect resolution was greater in a three-wall defect site than that in two-wall defects.

It is interesting to note that PI and BI showed statistically significant improvement 6 months following implantation of each bone substitute. The explanation for this may be better oral hygiene, closely connected with the reduction of PPD and CEJ.

Conclusion

The results of the present study indicate that both nanocrystalline biphasic calcium phosphate/poly-DL-lactide-co-glycolide and deproteinised bovine bone grafting biomaterials have clinically and statistically significant improvement in terms of cemento-enamel junction gain and periodontal pockel depth reduction when used for the treatment of intraosseous defects. In addition, it should be emphasised that (BCP/PLGA) demonstrated clinically and statistically significant reduction of periodontal pockel depth and cemento-enamel junction levels when compared to β-tricalcium phosphate 6 months following the treatment.

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Outdoor and indoor ozone level – A potential impact on human health

Nivo ozona u otvorenom i zatvorenom prostoru – mogući uticaj na zdravlje ljudi

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Abstract

Background/Aim. Air pollution outside and inside is still one of the most sensitive issues. The aim of this study was to assess the ozone level in ambient air and working premises in terms of its possible influence on human health. **Methods.** The study was based on the results obtained in Lithuanian conditions. Continuous ozone measurement data from the rural monitoring station in Preila over the period 1995–2011 were analyzed. More than 180,000 hourly values were examined according to the requirements in the Directive 2008/50/EC. The World Health Organization (WHO) and European Union indicators the Sum of Ozone Means Over 35 ppb (SOMO 35), the maximum daily 8-hour mean concentration of ozone higher than 100 and 120 μg/m^3 were estimated. Indoor ozone concentrations in copying and welding rooms were evaluated. The ozone concentration was measured with the ozone analyzer $O_3$41M. **Results.** The frequency distribution of ozone hourly concentrations at the Preila station showed that less than 1% of the data were higher than 120 μg/m^3 and 6% of them higher than 100 μg/m^3, that could have the adverse effect on human health, during 1995–2011. The investigations made in working premises showed that near a copying machine the ozone concentration can reach 330 μg/m^3, however in the room, i.e. 0.5 m from the machine, the average ozone concentration during automatic copying was 165 μg/m^3 and during manual copying it was 50 μg/m^3. Measurements in a welding room showed that the ozone concentration was in the range of 380–1,850 μg/m^3 at the distance of 25 cm from the electrode and at the distance of 1 m from the source the ozone concentration decreased 2.5 times. **Conclusion.** The danger of the ambient ozone level to human health practically was not observed in Lithuanian conditions. However, almost 6% of the data exceed the new WHO guideline of 100 μg/m3 during the measurement time. Indoor ozone during welding reached a higher level than during copying that can cause human health problems.

Key words:
ozone; air pollution; air pollution, indoor; welding; health; risk assessment.

Apstrakt

Uvod/Cilj. Zagađenje vazduha u otvorenom i zatvorenom prostoru i danas je jedno od najosetljivijih pitanja. Cilj ove studije bio je da se proceni nivo ozona u vazduhu i radnim prostorijama u pogledu moguceg uticaja na ljudsko zdravlje. **Metode.** Studija se zasnivala na rezultatima dobijenim u litvanskim uslovima. Analizirani su podaci kontinuiranog merenja ozona iz ruralnog monitoring centra u mestu Preila u periodu 1995–2011. Više od 180 000-časovnih vrednosti ispitane su u skladu sa zahtevima iz Direktive 2008/50/EC. Procenjivani su pokazatelji Svetske zdravstvene organizacije i Evropske unije *the Sum of Ozone Means Over* 35 ppb (SOMO 35), maksimalna dnevna 8-časovna srednja koncentracija ozona viša od 100 i 120 μg/m^3. Takođe, procenjivane su koncentracije ozona u zatvorenom prostoru gde su vršeni kopiranje i zavarivanje. Koncentracija ozona merena je analizatorom ozona $O_3$41M. **Rezultati.** Distribucija učestalosti koncentracije ozona po satima na Preila stanici pokazala je da je manje od 1% izmerenih vrednosti bilo više od 120 μg/m^3 i 6% izmerenih vrednosti 100 μg/m^3, što bi moglo imati negativan uticaj na ljudsko zdravlje tokom 1995–2011. Istraživanja rađena u radnim prostorijama pokazala su da pored aparata za kopiranje koncentracija ozona može da dostigne 330 μg/m^3. Međutim, u sobi, na 0,5 m od aparata, prosečna koncentracija ozona tokom automatskog kopiranja bila je 165 μg/m^3, a tokom ručnog kopiranja 50 μg/m^3. Merenja u sobi gde je vršeno zavarivanje pokazala su da je koncentracija ozona bila u opsegu 380–1 850 μg/m^3 na rastojanju od 25 cm od elektrode, a na rastojanju od 1 m od izvora koncentracija ozona bila je snižena 2,5 puta. **Zaključak.** Opasnost od ambijentalnog nivoa ozona za ljudsko zdravlje praktično nije uočena u litvanskim uslovima. Međutim, skoro 6% od izmerenih vrednosti prelazi novu smernica Svetske zdravstvene organizacije od 100 μg/m^3 tokom vremena merenja. Nivo ozona u zatvorenom prostoru za vreme zavarivanja dostizao je višu vrednost nego tokom kopiranja, što može biti štetno po zdravlje ljudi.

Ključne reči:
ozon; vazduh, zagađenje; vazduh, zagađenje u zatvorenom prostoru; zavarivanje; zdravlje; rizik, procena.

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Introduction

Air pollution is a major environmental health problem affecting many peoples in the world. The pollutants can be emitted from various sources into the boundary level of atmosphere. The large problem is also air pollution in the indoor environment where people spend the largest part of time.

Ozone is the one of the main pollutants the level of which is under regulations of many organizations. It is a secondary pollutant and mostly is formed through photochemical reactions appearing in areas with a high traffic density during warm periods of the year. The average ozone concentrations can be higher during spring and summer months, thus increasing the potential toxicity and harmful effect on human health [1].

The maximum daily 8-hour mean concentration, determined from 8-hour running averages over the day, is the parameter on which the European Union (EU) ozone long-term and target value for the protection of human health is based. EU in the Directive 2008/50/EC set the long-term objective for the protection of human health (daily maximum 8-hour average concentrations of 120 μg/m^3) that should not exceed 120 μg/m^3 on more than 25 days *per* calendar year averaged over 3 years. The target value was exceeded in approximately 22% of the area of 27 EU member states territory and affected approximately 16% of the total population in this territory in 2010 [2]. As it was pointed in the report the situation in 2010 was not fundamentally different but in contrast to the last three summers there were some exceedances in northern Europe and the average number of threshold exceedances increased slightly in northwestern, central and eastern Europe.

The World Health Organization (WHO) declares stricter requirements, reduced guideline for ozone from the existing level of 120 μg/m^3 to 100 μg/m^3, since the latest studies have shown health effects at ozone concentrations below 120 μg/m^3 but without clear evidence of a threshold [3]. This concentration should provide adequate protection of public health, though some health effects in some sensitive individuals may occur below this level.

The WHO recommended another indicator for human health impact – the Sum of Ozone Means Over 35 ppb (SOMO 35). SOMO 35 is an indicator of the accumulated ozone concentration in excess of 35 ppb (70 μg/m^3) during the whole year. The decision to select this value was based on some arguments and one of them told that a statistically significant increase in mortality risk estimates was observed at ozone concentrations above 50−70 μg/m^3. The largest contributions to SOMO 35 are made during the summer months, although some contributions can also be during colder months as March or October.

People spend about 90% time indoors where they receive most of their exposure to pollutants [4]. Investigation results relating high outdoor concentrations of pollutants with human health effects can be inaccurate due to the impact of indoor exposure. Typical indoor ozone concentrations vary between 2−20 μg/m^3. Exposure to air pollutants is strongly influenced by microenvironmental phenomena. For example, at an air exchange rate of 2/h, the indoor ozone concentration is about 1/3 of the outdoor ozone concentration due to indoor reactions [5]. These reactions increase indoor concentrations of products including formaldehyde, other carbonyl species, aerosols, and carboxylic acids. Therefore, personal exposure to ozone decreases while exposure to these byproducts increases [6].

Not only many materials but also some technological processes can be a source of pollutant emission as well. The old copying machines, which produce higher pollutant concentrations compared to the new design copying machines, are often used in the offices of developing or poor countries. However, the increase of indoor pollutant emissions due to the abundance of new sources (e.g., ozone generators, electrostatic air filters, laser printers and some technological processes) is also evident [7, 8]. The threshold value of pollutants in workplace air can vary depending on the regulations and guidelines in different countries; e.g., the ozone threshold value of 100 or 200 μg/m^3 is typically set. According to the Lithuanian hygiene standard HN 23 : 2007 the threshold limit value of ozone concentration in workplace air is 200 μg/m3, given the average of eight hours, and the maximum permissible ozone concentration, given the average of 5–10 minutes, is 600 μg/m^3. Considering the fact that exposure occurs both indoors and outdoors for all the pollutants it is reasonable to propose using the same air quality guidelines for both indoor and outdoor exposures [2].

Unceasing interest in atmospheric ozone is great mainly because of its effect on human health and the role in climatic change. Inhaled ozone can aggravate chronic diseases such as emphysema, bronchitis and asthma. Studies indicate that various pulmonary function parameters can be reduced by exposures to ozone concentrations in the range of 160−360 μg/m^3 for a period of 1−8 hours [2]. Ozone concentration level in Lithuania is lower in comparison to southern and western part of Europe [9–13]. The hourly mean of ozone concentration higher than 120 μg/m^3 was observed rarely. An increase in the 8-hour average ozone concentration by 100 μg/m^3 is expected to induce the 25% increase in symptom exacerbation among adults and asthmatics involved in normal activities as well as the 10% increase in hospital admissions for respiratory conditions [9].

Weschler [13] argues that mortality and morbidity associated with ambient ozone may be due, in part, to exposure to indoor ozone and its byproducts. Based on data from US urban communities during a 14-year period [2], it is estimated a 0.52% increase in daily mortality for a 20 μg/m^3 increase in the previous week‘s local ambient ozone level.

The aim of this study was to evaluate the level and variations of ozone concentration in Lithuanian ambient air and different workplaces from the viewpoint of the potential influence on human health.

Methods

Ozone concentrations were monitored by using a commercial UV ozone analyzer O341M (Environment s.a.). The

lower detection level of the instrument is 2 μg/m3 and the concentration measurement range is 0−2000 μg/m^3. Concentration of ozone was measured continuously and obtained data were saved according to experiment requirements as one minute or one hour averages. The air was sucked through the Teflon lines and the sample flow rate was 1.5 L/min.

The hourly ozone data from the Preila rural monitoring station (55°55' North and 21°00' East) over the period 1995−2011 were used in the analysis. More than 180,000 hourly values were examined. The criteria, according to the requirements in the Directive 2008/50/EC, were used for checking validity when aggregation of data and calculation of statistical parameters were performed. Continuous outdoor ozone measurements were hourly averaged when valid data were collected over less than 75% of the time (i.e. 45 minutes). The applied frequency distribution for hourly ozone data showed that the distribution has a Gaussian distribution shape during the analysed period. Trends in annual ozone concentration means were tested statistically using Mann-Kendall analysis. The Mann-Kendall test is a non-parametric test that has the advantage of robustness against outliers and can be applied to non-normally distributed data with missing values. Sen's non-parametric method was used to estimate the slope of an existing trend.

The indicator SOMO 35 was calculated according to recommendations by using the equation (1) [12].

$$SOMO\ 35_{measured} = \Sigma i\ \max(0, (Ci - 70)) \quad (1)$$

A correction to full time coverage has been applied:

$$SOMO\ 35_{estimate} = (SOMO\ 35_{measured} \bullet N_{period}) / N_{valid} \quad (2)$$

where N_{valid} is the number of valid daily values and N_{period} the number of days *per* year. The corrected values of SOMO 35 are calculated and used when sufficient valid daily measurements are available. For practical reasons, data coverage of at least 75% is required (i.e. N valid > 273) and the days with missing data should not concentrate in one season.

The indoor ozone investigation was carried out in the working room without sources of ozone as well as in the premises where ozone is emitted during the technological processes (i.e. copying, welding). The standard office room was selected for measurement of inflow of air with ozone from outdoors to indoors. Electric devices emitting ozone during their operation (i.e. laser printer, copying machine, etc.) were not in operation during the experiment. The area of a window was 1.44 m^2. Ozone concentration was measured at the points located at different distances from the window (0, 1, 2, 3, 4, 5 m) and at a different height (0, 0.9, 2.8 m) one minute after opening the window.

The ozone concentration was measured in a room of 16 m^2, where the source of emission was a copying machine. The maximum intensity of the copying machine was 120 copies per min. The indoor ozone concentration was investigated at a different height (0.1; 1.0; 2.5 m) and at a particular distance from the copying machine (0.1; 0.5; and 1.5 m).

Horizontal and vertical distribution of ozone concentration was investigated in a welding room of 95 m^2. Ozone emission from the welding machine was measured at a different distance from the electrode (0.25, 0.5 and 1 m) and at a different height of the room (0, 0.25, 1,5 m). Welding was carried out with the electrode of 4 mm diameter.

Results and Discussion

Ground-level ozone monitoring in Lithuania has been performed since 1982 [10] and the upward trend of annual mean concentration was established, however the ozone level danger for human health was practically not observed. The frequency distribution of the ozone hourly concentrations at the Preila station showed that less than 1% of the data were higher than 120 μg/m^3 and 6% of data higher than 100 μg/m^3 during 1995−2011 (Figure 1).

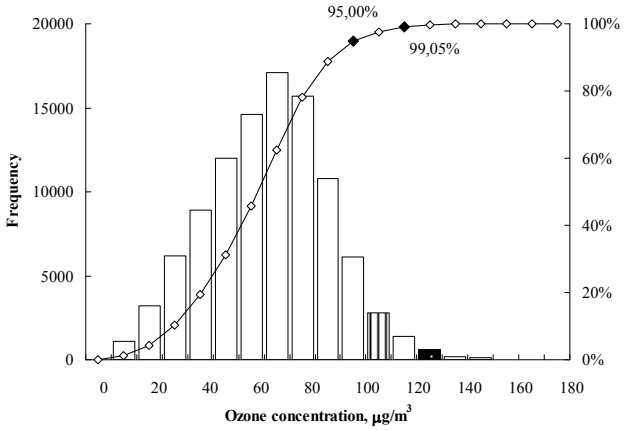

Fig. 1 – The frequency distribution of hourly ozone concentrations, Preila, 1995−2011.

The results of SOMO 35 calculated and corrected according to equation (2) and the number of days when 8-hour mean was more than 100 and 120 g/m^3 are presented in Figure 2. Data showed that values of these indicators can differ significantly from year to year.

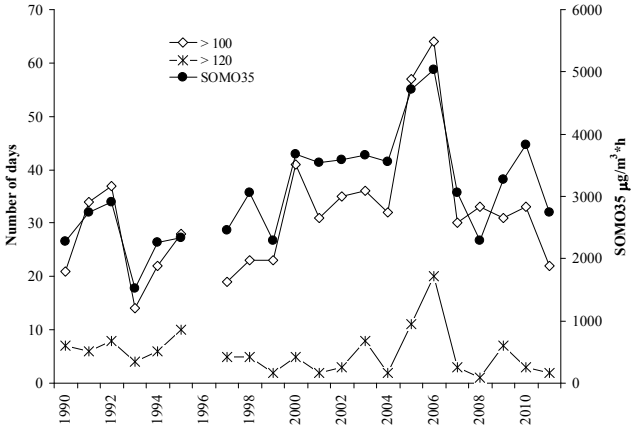

Fig. 2 – The variation of the sum of ozone means over 35 ppb (SOMO 35) and the number of days with ozone concentration > 120 μg/m^3 and > 100 μg/m^3.

The ozone inflow through the open window into the room was measured in summer when outside concentration reached 120 μg/m^3. Ozone concentration in the office room was equal to zero before opening the window. The ozone concentrations were measured at different distances from the window. The outdoor temperature was 25°C, and the indoor temperature was lower, i.e. 22°C.

The obtained results showed that the ozone concentration varied significantly at a distance up to 2 m from the window. Further distribution of ozone in the room was almost uniform, and equal to 54 ± 4 μg/m^3, i.e., ozone concentration was almost two times lower than the ozone concentration measured at the initial moment (i.e. outdoors).

Ozone that enters the office room from outdoors completely decays within 1.5 h. During the experiment, half of the total quantity of ozone in the office room decayed within 7 minutes. The obtained results coincide with the results of other authors [13]. It was established that the ratio of indoor ozone concentration (75 ± 5 μg/m^3) and outdoor ozone concentration (120 ± 11 μg/m^3) was equal to 0.63 ± 0.04.

The contribution of ozone emitted during technological processes (copying, welding) to the ozone concentration level in the working premises was estimated. The measurements of vertical dispersion of ozone emitted from the copying machine were performed near the floor, at the height of the copying machine (1 m) and near the ceiling (2.5 m). A variation of horizontal ozone concentration was established at the following distances from the source: 0, 0.5, 1 and 1.5 m (Figure 3).

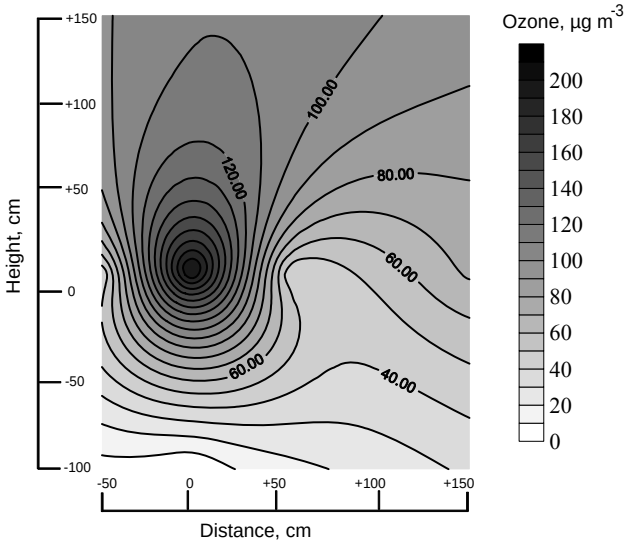

Fig. 3 – Spatial dispersion of ozone concentration in a copying room.

The ozone concentration in the copying room was about 4 μg/m³ at night, whereas during operation of copying machines it varied from 10 to 330 μg/m³. The copying process mostly was not intensive (from 1 to 20 copies/min) during the experiment, thus the measured ozone concentrations ranged from 30 to 50 μg/m³. With the increase in intensity of copying process, the indoor ozone concentration increased. The maximum ozone concentration was measured during automatic copying (the average concentration was 165 μg/m³) while during manual copying the measured ozone concentration was lower (the average concentration was 50 μg/m³). Therefore, it may be stated that the intensity of copying process has a major impact on the ozone concentration level in office premises.

The maximum ozone concentration (330 μg/m³) was measured at the height of the copying machine. The ozone emitted by the copying machine is rising towards the ceiling. The surface ozone concentration near the floor varied from 7 to 20 μg/m³, and near the ceiling it varied from 10 to 110 μg/m³. The results of the ozone concentration distribution at different distances from the copying machine are presented in Figure 3.

Welding is another technological process when high ozone concentration can occur. Ozone can be produced by ultraviolet light from the welding arc, and also in greater quantities by gas metal arc welding (GMAW) or short-arc, gas tungsten arc welding (GTAW) or heli-arc, and plasma arc cutting. The exposure to ozone generated in GMAW and plasma arc welding may produce excessive mucus secretion, headache, lethargy, eye irritation and irritation and inflammation of the respiratory tract. In extreme cases, excess fluid and even hemorrhage may occur in the lungs. The irritant effects of gas on the upper respiratory tract and the lungs can be detected with some delay.

The investigations of ozone distribution during welding showed a very high ozone level (Figure 4) in the room. Due

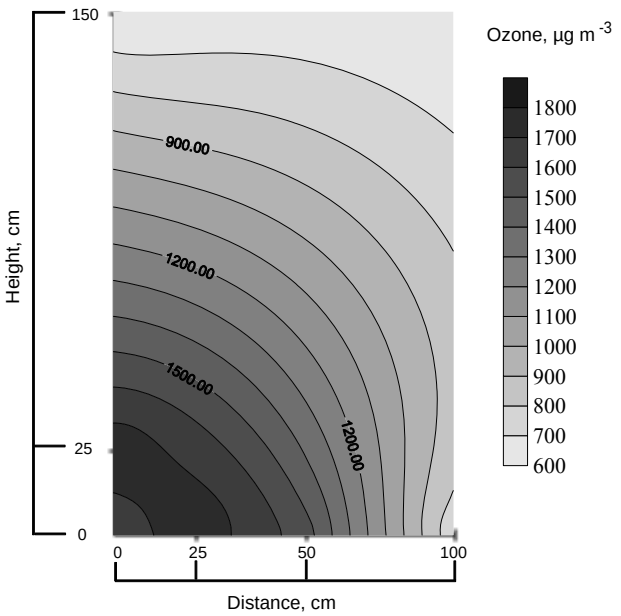

Fig. 4 – Spatial dispersion of ozone concentration in a welding room.

to intensive turbulent flow, the ozone concentration near the welding instrument just after its formation is heterogeneous, therefore large variations of concentrations were observed. The obtained results showed that the ozone concentration decreases with increase of the distance from the electrode. The

maximum ozone concentration was found at the distance of 25 cm from the electrode where the ozone concentration varied in the range of 380−1,850 μg/m3, and at the distance of 1 m from the source, the ozone concentration decreased 2.5 times.

The upward trend of the annual ozone mean concentration was established in Lithuania during the last 25 years. Mann-Kendall trend analysis showed that the annual mean ozone concentration increased (0.73 μg/m3, $p < 0.001$). The increasing trend (0.69 μg/m^3, $p < 0.001$) was also found during a warm (April-September) period. Nevertheless, the ozone level (> 180 μg/m^3) danger to human health practically was not observed in Lithuania. A total of 6% of the data exceed the new WHO guideline of 100 μg/m^3, i.e. they were observed over more than 10,000 hours. The number of days when high ozone concentration was observed varied no significantly year by year (Figure 2) except in 2005 and 2006. The warm seasons (April – September) of this period were hot, especially in 2006 and the favorable conditions for formation of high ozone concentration were established. It should be noted that such concentrations are observed mostly during spring and summer months when people spend more time outside. Another situation is observed by analyzing the variation of the indicator SOMO 35. The similar ground-level ozone concentrations are observed in Latvia and higher ones in Poland. Ambient ground-level ozone is created from ozone precursors, the largest source of which are cars and other vehicles. Ozone pollution is usually a problem in southern cities of Europe and not in northern cities. In northern countries, ozone causes a larger problem in rural areas. However, the obtained results of ozone level analysis allow us to stay that the ambient ozone level in Lithuania is not dangerous for people to date. Another situation was determined with indoor ozone concentrations. The obtained results suggest that the outdoor ozone concentration under Lithuanian conditions does not cause risks to human health in door. Only when an additional source of indoor ozone exists, the total level of ozone in separate cases may exceed the permissible concentration level.

As was revealed from measurement results in workplaces, the ozone concentration sometimes can exceed the level dangerous for workers. Our proposition is somewhat contradictory to the results published by other authors, for instance, Kowalska and Zajusz-Zubek [14] and coincides with that of Zhou et al. [15]. The ozone concentration is strongly dependent on the distance of measurement places from sources (Figure 3). Measurements made in the subject's breathing zone are often considered to be the most accurate estimate of a person's "true" exposure. Therefore, the operator can be in a different situation than other people in the same room, who can be affected by the lower ozone concentration. Today, newer models use a different system to reduce the amount of ozone produced by the copying machine. Our investigations were performed with an old machine. As early as 1978, Allen et al. [16] investigated photocopying machines and found that ozone concentrations up to 490 μg/m^3 could be generated in poorly ventilated rooms.

Conclusion

The danger of the outdoor ozone level to human health practically was not observed in Lithuania during 1995−2011. However, 6% and 1% of all data exceed the World Health Organization guidelines of 100 μg/m^3 and 120 μg/m^3, respectively.

The indicator of the human health impact, the sum of means over 35 ppb (SOMO 35) varied year from year and reached a maximum of 6,000 μg/m^3 *h value in 2006.

The maximum ozone concentration during welding varied from 380 to 1,850 μg/m^3, and in the copying room it was in the range of 50–330 μg/m³ and can be dangerous for human health.

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Impact of the combined presence of left ventricular systolic and renal dysfunction on the 5-year outcome after ST-elevation myocardial infarction

Uticaj istovremenog prisustva sistolne disfunkcije leve komore i renalne disfunkcije na 5-godišnji ishod nakon akutnog infarkta miokarda sa elevacijom ST segmenta

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Abstract

Background/Aim. The coincidence of left ventricular systolic dysfunction (LVSD) and renal dysfunction (RD) is a strong independent predictor of adverse events in the short-term and mid-term follow-ups of patients with ST-elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI). The aim of this study was primarily to assess the prognostic impact of the LVSD-RD combination on the 5-year all-cause mortality in patients with STEMI treated with pPCI, as well as to assess the prognostic impact of the LVSD-RD combination on the occurrence of major adverse cardiovascular events (MACEs: cardiovascular death, reinfarction, stroke and target vessel revascularization) in these patients. **Methods.** We analyzed 951 patients divided into 4 groups according to the presence of LVSD (ejection fraction < 40%) and/or baseline RD (creatinine clearance < 60 mL/min): group I (no LVSD, no RD); group II (LVSD, no RD); group III (RD, no LVSD); group IV (LVSD+RD). **Results.** The 5-year mortality rates were 2.3%, 17.6%, 11.7% and 38.3%, while the 5-year MACE rates were 8.8%, 28.4%, 18.3% and 44.4% in the groups I, II, III and IV, respectively ($p < 0.001$). The highest percentage of lethal outcomes and MACE was registered in the first year of follow-up in all the groups. The 1-year landmark analysis confirmed that the patients with LVSD-RD combination had the highest percentage of lethal outcomes in the period of 1 to 5 years ($p = 0.028$). There was a strong trend toward the significance in the occurrence of MACE among the analyzed groups in the period of 1 to 5 years ($p = 0.085$). In the Cox regression model the LVSD-RD combination was a strong independent predictor of 5-year mortality and the occurrence of MACE: mortality hazard ratio (HR) 4.5 (95%CI 1.9–10.8); MACE HR 2.5 (95%CI 1.4–4.5). **Conclusion.** The strong negative independent prognostic impact of the LVSD-RD combination persisted in the long-term follow-up of the patients with STEMI treated with pPCI.

Key words:
myocardial infarction; comorbidity; ventricular dysfunction, left; angioplasty, baloon; mortality; prognosis.

Apstrakt

Uvod/Cilj. Istovremeno prisustvo sistolne disfunkcije leve komore (LVSD) i renalne disfunkcije (RD) je snažan nezavisni prediktor pojave neželjenih događaja u kratkoročnom i srednjoročnom praćenju bolesnika sa akutnim infarktom miokarda sa elevacijom ST segmenta (STEMI) koji su lečeni primarnom perkutanom koronarnom intervencijom (pPCI). Cilj ovog rada bio je da se utvrdi, pre svega, prognostički uticaj istovremenog prisustva LVSD i RD na ukupni 5-godišnji mortalitet kod bolesnika sa STEMI koji su lečeni pPCI, kao i da se utvrdi prognostički uticaj LVSD i RD na pojavu značajnih neželjenih kardiovaskularnih događaja [*major adverse cardiovascular events* (MACEs): kardiovaskularni mortalitet, reinfarkt, šlog i hitna revaskularizacija zbog ishemije] kod ovih bolesnika. **Metode.** Analiziran je 951 bolesnik. Bolesnici su bili podeljeni u četiri grupe u zavisnosti od prisustva LVSD (ejekciona frakcija < 40%) i/ili bazalne RD (klirens kreatinina < 60 ml/min): grupa I (bez LVSD, bez RD), grupa II (LVSD, bez RD), grupa III (RD, bez LVSD), grupa IV (LVSD+RD). **Rezultati.** Dobijeni rezultati pokazuju da je 5-godišnji mortalitet iznosio 2,3%, 17,6%, 11,7% i 38.3%, a 5-godišnja pojava MACE 8,8%,

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28,4%, 18,3% i 44, 4% u grupama I, II, III i IV ($p < 0.001$). Najviši procenat smrtnih ishoda i pojave MACE registrovan je u prvoj godini praćenja u svim grupama. Korišćenjem jednogodišnje *landmark* analize potvrđeno je da su bolesnici sa istovremenim prisustvom LVSD i RD imali najviši procenat smrtnih ishoda u periodu između jedne i pet godina ($p = 0,028$). Postojala je snažna tendencija statističke značajnosti za pojavu MACEa između analiziranih grupa u periodu između jedne do pet godina ($p = 0.085$). U Cox regresionom modelu, istovremeno prisustvo LVSD-RD bilo je snažan nezavisni prediktor mortaliteta i pojave MACE u 5-godišnjem praćenju: mortalitet *hazard ratio* (HR) 4,5 [95% interval poverenja (CI) 1,9–10,8]; MACE, HR 2,5 (95% CI 1,4–4,5). **Zaključak.** Snažan negativan, nezavisan prognostički uticaj istovremenog prisustva LVSD-RD održava se u dugoročnom praćenju bolesnika sa STEMI koji su lečeni primarnom PCI.

Ključne reči:
infarkt miokarda; komorbiditet; srce, disfukncija leve komore; angioplastika, balonska; mortalitet; prognoza.

Introduction

Left ventricular systolic dysfunction (LVSD) and renal dysfunction (RD) are strong predictors of short-term and long-term mortality and of major adverse cardiovascular events (MACE) after acute myocardial infarction (AMI) [1–10]. RD is frequently accompanied by coronary disease and cardiac insufficiency. One of the reasons for this lies in the fact that the risk factors are identical, e.g. hypertension or diabetes mellitus [11]. On the other hand, introducing modern therapeutic procedures in AMI treatment, primarily the introduction of primary percutaneous coronary intervention (pPCI) in treatening infarction with ST segment elevation (STEMI), has reduced but not eliminated the risk of LVSD development [12]. This is why even in the pPCI era the group of patients with substantial damage to the myocardium of the left ventricle stands out as the one with a high risk of future adverse events [1, 2]. The coincidence of LVSD and RD (the cardio-renal syndrome) over a longer period of time may lead to further deterioration of the renal and cardiac function *via* complex and as yet insufficiently understood neurohumoral mechanisms [11–18]. The prognostic significance of the combined presence of RD and LVSD for long-term follow-up was analyzed in patients with AMI (STEMI and non-STEMI) in the era before routine pPCI [17, 19]. In the pPCI era, the prognostic significance of the combined presence of LVSD and RD was analyzed during intrahospital and 1-year follow-up and proven that the LVSD-RD combination is the strongest independent predictor of the intrahospital and 1-year mortality and morbidity [20, 21]. Taking into consideration these findings, the authors of this study feel that it would be important to ascertain whether and to what extent does the negative prognostic influence of the LVSD-RD combination persist in long-term follow-up of patients with STEMI treated with pPCI.

The primary aim of this study was to assess the prognostic significance of the combined presence of LVSD and RD on 5-year all-cause mortality in patients with STEMI treated with pPCI, as well as to assess the prognostic significance of the combined presence of LVSD and RD on the occurrence of MACEs (including cardiovascular mortality, non-fatal myocardial reinfarction, non-fatal stroke and target vessel revascularization – TVR) during a 5-year follow-up period.

Methods

In the present study data were used from the prospective Clinical Center of Serbia STEMI Register for a subgroup of 951 consecutive patients, hospitalized between February 2006 and April 2008. The purpose of the prospective Clinical Center of Serbia STEMI Register was published elsewhere [22]. In brief, the objective of the register is to gather complete and representative data on the management and short- and long-term outcome of patients with STEMI undergoing primary PCI in our center. The study protocol was approved by a local research ethics committee. All consecutive patients with STEMI, aged 18 or older, who were admitted to the Coronary Care Unit after undergoing pPCI in our center were included in the Register. For the purpose of this study, patients with cardiogenic shock at admission were excluded. Coronary angiography was performed *via* the femoral approach. Primary PCI and stenting of the infarct-related artery was performed according to the standard technique. Aspirin, 300 mg and clopidogrel, 600 mg, were administered to all eligible patients before pPCI. The selected patients, with visible intracoronary thrombi, were also given the glycoprotein (GP) IIb/IIIa receptor inhibitor, tirofiban during pPCI. Flow grades were assessed according to thrombolysis in myocardial infarction (TIMI) criteria. After pPCI the patients were treated according to current guidelines.

Demographic, baseline clinical, angiographic and procedural data were collected and analyzed. The baseline RD was defined as creatinine clearance (CrCl) < 60 mL/min/m^2 at admission. Creatinine clearance was calculated using the Cockrof-Gault formula:

CrCl = [(140-years)*body weight]/(72*creatinine in mg/dL). The value was multiplied by 0.85 in females.

Echocardiographic examination was performed within the first 3 days after pPCI. The left ventricular ejection fraction (LVEF) was assessed according to the biplane Simpson's method in classical two- and four-chamber apical projections. LVEF < 40% was considered as LVSD. LVEF was missing in 10% of patients. The missing data were imputed *via* the single imputation method.

Based on LVEF and estimated glomerular filtration rate (rGFR) values the patients were divided into four groups: group I included patients with no LVSD and no RD; the group II those with LVSD, but no RD; the group III those with RD, but no LVSD, and the group IV those with LVSD and RD.

The primary end-point was all-cause mortality. The secondary end-point was the occurrence of MACEs (including cardiovascular mortality, non-fatal myocardial reinfarction, non-fatal stroke and target vessel revascularization – TVR) [22, 23]. The patients were followed-up up to 5 years after enrolment.

Continuous variables were expressed as the mean ± standard deviation (SD), if the distribution was normal, or median (med) with 25th and 75th quartiles (IQR), if the distribution was skewed, while categorical variables were expressed as frequency and percentage. Analysis for the normality of data was performed using the Kolmogorov-Smirnov test. Baseline differences between the groups were analyzed using the one-way ANOVA test for continuous variables and the Pearson χ^2 test for categorical variables. The Cox regression model (backward method, with $p < 0.1$ for entrance into the model) was used to identify independent risk factors for the occurrence of 5-year all- cause mortality and 5-year MACE. The Kaplan-Meier method was used for constructing the probability curves for 5-year survival and the free interval until the occurrence of the first event in the composite MACE event, while the difference between the groups was tested with the Log- Rank test. One year landmark analysis was performed. The patients who were event-free (no death, no MACE) at the 1-year check-up and had completed the 1-year follow-up were assigned into 4 groups according to the presence of LVSD and/or RD, as previously described. Outcomes for these patients were evaluated up to 5 years after the initial pPCI procedure. The SPSS statistical software version 14.0 was applied (SPSS Inc, Chicago, IL).

Results

Out of a total of 951 patients, 255 (26.8%) were women. The average age of the examined patients was 59.3 ± 11.2 years. A total of 574 (60.3%) patients had the preserved renal and left ventricular systolic function (group I); 81 patients (8.5%) had both LVSD and RD (group IV); 120 (18.5%) patients had only LVSD (group II); and 176 (12.6%) patients had RD (group III). There were no patients on dialysis. The demographic characteristics, risk factors, previous cardiovascular diseases or procedures, characteristics on admission, as well as angiographic and procedural characteristics in relation to the analyzed patient groups are shown in Table 1.

Table 1

Baseline characteristics of the patients according to the presence of left ventricular systlic dysfunctions (LVSD) and /or renal dysfunction (RD)

Characteristics	Group I No LVSD, no RD (n = 574)	Group II LVSD, no RD (n = 120)	Group III RD, no LVSD (n = 176)	Group IV LVSD+RD (n = 81)	*p* value
Age (years), med (IQR)	56 (49–62)	57 (51–64)	71 (65-76)	74 (69–78)	< 0.001
Women, n (%)	120 (20.9)	33 (18.7)	60 (50)	42 (51.8)	< 0.001
Previous MI, n (%)	62 (10.8)	34 (19.3)	19 (15.8)	13 (16)	0.012
Previous AP, n (%)	24 (4.2)	13 (7.4)	7 (5.8)	11 (13.6)	0.004
Previous PCI, n (%)	4 (0.6)	5 (2.8)	1 (0.8)	1 (1.2)	0.127
Previous stroke, n (%)	15 (2.6)	10 (5.7)	7 (5.8)	98 (11.1)	0.001
Diabetes, n (%)	77 (13.4)	41 (23.2)	21 (17.5)	20 (24.7)	0.002
Hypertension, n (%)	350 (60.9)	105 (5.9)	92 (76.7)	61 (75.3)	< 0.001
HLP, n (%)	401 (69.8)	110 (62.5)	77 (64.2)	42 (51.8)	0.031
Smoking, n (%)	375 (65.3)	105 (59.6)	47 (39.2)	22 (27.1)	< 0.001
Pain duration (hours), med (IQR)	2.5 (2–4)	3 (2–5)	3.2 (2–5)	3.5 (2–5)	0.020
Anterior MI, n (%)	194 (33.8)	130 (73.8)	36 (30)	44 (54.3)	< 0.001
HF at admission, n (%)	4 (0.7)	51 (28.9)	2 (1.7)	36 (44.4)	< 0.001
Systolic BP (mmHg) at admission, $\bar{x}$ ± SD	136.4 ± 26.3	133.8 ± 29.9	147.9 ± 26.1	130.4 ± 36.2	< 0.001
HR at admission, $\bar{x}$ ± SD	76.8 ± 15.9	83.1 ± 17.4	74.8 ± 15.3	80.5 ± 21.8	< 0.001
Door to balloon time, minutes, $\bar{x}$ ± SD	160.7 ± 68.5	157.2 ± 74.3	162.3 ± 54.7	167.1 ± 73.6	0.939
3-vessel disease, n (%)	123 (21.4)	56 (31.8)	40 (33.3)	36 (44.4)	< 0.001
Preprocedural flow TIMI 0, n (%)	473 (82.5)	148 (84.1)	100 (83.3)	67 (82.7	0.431
LM stenosis, n (%)	25 (4.4)	13 (7.4)	6 (0.5)	9 (11.1)	0.038
IIb/IIIa blockers, n (%)	259 (45.2)	102 (57.9)	48 (40)	43 (53.1)	0.003
Stent implantation, n (%)	555 (96.7)	161 (91.4	115 (95.8)	70 (86.4)	< 0.001
Postprocedural flow TIMI < 3, n (%)	11 (1.9)	14 (7.9)	4 (3.3)	12 (14.8)	< 0.001
Acute stent thrombosis, n (%)	2 (0.3)	2 (1.1)	0	4 (4.9)	0.358
CK max (U/L), med (IQR)	1795 (968–3185)	2861 (1441–4718)	1800 (917–2968)	1810 (1162–3467)	< 0.001
Troponin I (μg/L), med (IQR)	30.1 (7.4–74.6)	34.1 (6.4–104.7)	31.8 (6.2–113.1)	31.5 (2.2–85.1)	< 0.001
Baseline CrCl (mL/min), $\bar{x}$ ± SD	96.5 ± 21.1	89.4 ± 20.7	49.4 ± 10.1	45.8 ± 12.7	< 0.001
Hgb (g/L), $\bar{x}$ ± SD	144.3 ± 16.6	143.4 ± 19.3	134.4 ± 15.9	129.4 ± 19.6	< 0.001
LVEF (%), $\bar{x}$ ± SD	53.8 ± 6.9	37.5 ± 3.1	52.9 ± 7.3	34.3 ± 5.6	< 0.001

IQR – interquartile range; AP – angina pectoris; HLP – hyperlipidemia; MI – myocardial infarction; HF – heart failure; BP – arterial blood pressure; HR – heart rate; TIMI – Thrombolysis in Myocardial Infarction; LM – left main coronary artery; CK – creatinine kinase; CrCl – creatinine clearance; Hgb – hemoglobin; LVEF – left ventricular ejection fraction; $\bar{x}$ – mean value; SD – standard deviation.

Table 2 shows the adverse events, planned revascularization and therapy in relation to LVSD and/or RD during the entire 5-year follow-up period. The highest percentage of adverse events was registered in the group with LVSD and RD.

The causes of mortality were: cardiovascular causes (fatal reinfarction, progression of heart failure, sudden death, ischemic stroke) in 82 patients and other noncardiovascular causes (cancer, ileus, pneumonia) in 7 patients.

During the follow-up period there was no significant difference between the analyzed groups as far as the referral for planned myocardial revascularization was concerned (patients with multivesel disease). As to the therapy, the only registered difference was in the administration of angiotensin converting enzyme (ACE) inhibitors (group I 76.4%, group II 73.8%, group III 77.5% and group IV 62.9%, $p = 0.032$).

Figure 1a) shows the Kaplan-Meier probability curves for 5-year survival while Figure 1b) shows probability curves for the free interval until the occurrence of MACE in relation to the presence of LVSD and/or RD.

Using 1-year landmark analysis we analyzed 822 patients (group I, n = 539; group II, n = 101; group III, n = 101 and group IV, n = 48) who were event-free (no death, no MACE) at the 1-year check-up after the initial pPCI procedure. In the Figure 2a) the Kaplan Meier probability curves of 5-year survival is shown. The MACE free probability curves according to the presence of LVSD and/or RD using 1-year landmark analysis are shown in Figure 2b).

One-year landmark analysis confirmed that the highest percentage of the patients with a fatal outcome was in the LVSD+RD group. The patients with LVSD+RD combination had also the highest MACE rate in the period between 1 and 5 years of follow-up. There was no statistically significant difference in the occurrence of MACE among the examined groups in the period between one and five years, but there was a strong trend toward significance.

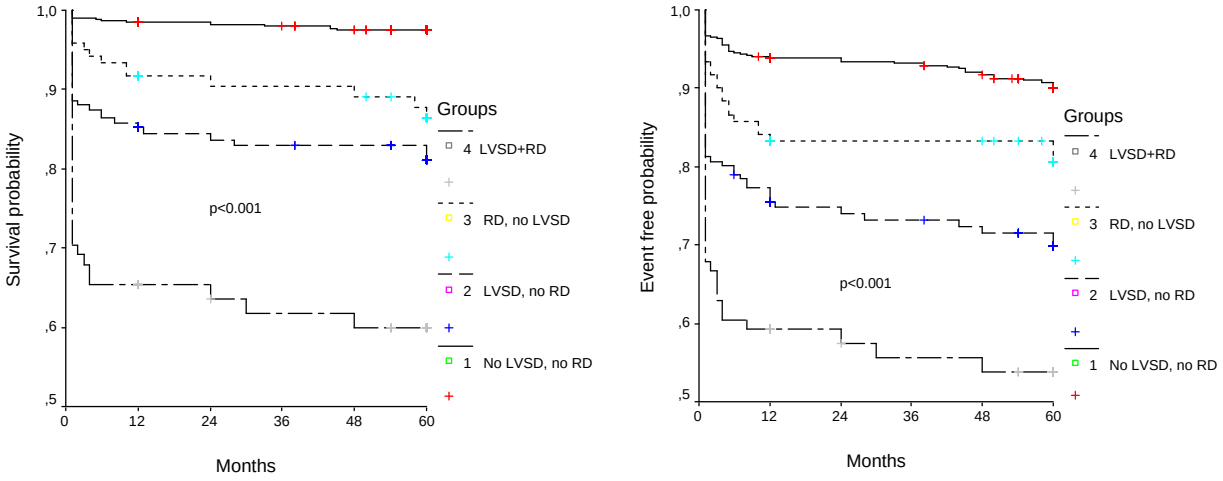
The causes of a lethal outcome in the period between one and five years of follow-up were: malignant diseases – lung cancer and gastric cancer (n = 5); myocardial reinfarction (n = 3); sudden cardiac death (n = 3); ischemic stroke (n = 1); unknown causes n = 2.

Table 2

Adverse events during the entire 5-year follow-up period

Event	Group I No LVSD, no RD (n = 574)	Group II LVSD, no RD (n = 120)	Group III RD, no LVSD (n = 176)	Group IV LVSD + RD (n = 81)	*p* value
Overall mortality, n (%)	13 (2.3)	31 (17.6)	14 (11.7)	31 (38.3)	< 0.001
MACE, n (%)	51 (8.8)	50 (28.4)	22 (18.3)	36 (44.4)	< 0.001
Cardiovascular death, n (%)	11 (1.9)	28 (15.9)	12 (10)	31 (38.3)	< 0.001
Nonfatal reinfarction, n (%)	21 (3.6)	17 (9.6)	4 (33.3)	6 (7.4)	< 0.001
Nonfatal stroke, n (%)	4 (0.7)	3 (1.7)	6 (5)	3 (3.7)	< 0.001
TVR, n (%)	23 (4.1)	12 (6.8)	5 (4.2)	5 (6.2)	0.161
Subacute stent thrombosis, n (%)	14 (2.4)	20 (11.4)	3 (2.5)	6 (7.4)	< 0.001
Late stent thrombosis, n (%)	5 (0.9)	3 (1.7)	1 (0.8)	1 (1.2)	0.943

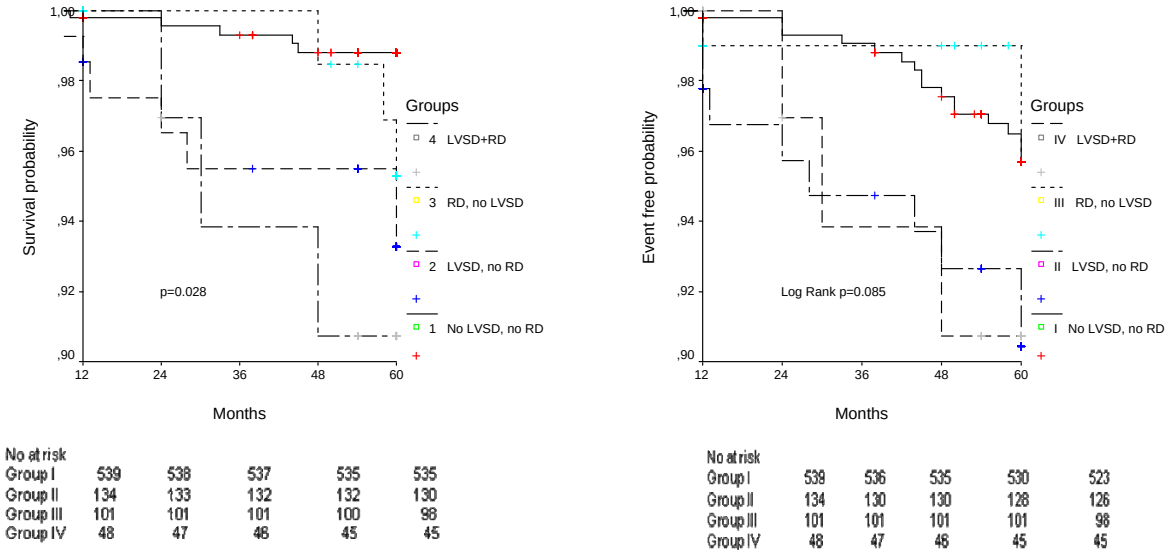
LVSD – left ventricular systolic dysfunction; RD – renal dysfunction; MACE – major adverse cardiovascular events (including cardiovascular death, nonfatal reinfarction, nonfatal stroke and TVR); TVR – target vessel revascularization.



a) **b)**

Fig. 1 – a) Kaplan Meier estimating the 5-year all-cause mortality, and b) MACE free probability according to the presence of LVSD and/or RD.

MACE – major adverse cardiovascular events; LVSD – left ventricular systolic dysfunction; RD – renal dysfunction.



a) **b)**

Fig. 2 – a) Kaplan Meier estimating the 5-year all-cause mortality, and b) MACE free probability according to the presence of LVSD and/or RD using 1-year landmark analysis.
For abbreviations see under Figure 1.

Table 3 shows the unadjusted hazard ratios (95% confidence interval) for the occurrence of all-cause mortality and MACE during follow-up (Cox regression model).

After adjustment to variables defined in the univariate analysis, as predictors of mortality and MACE, LVSD, RD and LVSD+RD remain independent predictors of all-cause mortality and MACE during a 5-year follow-up, as shown in Table 4. In the Cox regression model for 5-year all-cause mortality, adjustment was made for the following variables: age, prior myocardial infarction, diabetes, heart failure on admission, systolic blood pressure on admission, heart rate on admission, anterior infarction, peak creatinine kinase (CK), 3-vessel disea-

Table 3

Unadjusted hazard ratios (HR) [(95% confidence interval (95% CI)] for all-cause mortality and major adverse cardiovascular events (MACE)

All cause mortality	30 days	1 year	5 years
No LVSD, no RD	1.0	1.0	1.0
LVSD, no RD	11.4 (4.5–28.3)	9.7 (4.6–20.8)	8.3 (4.4–15.4)
RD, no LVSD	4.9 (1.6–15.1)	5.4 (2.2–13.2)	5.4 (2.5–11.2)
LVSD+RD	33.5 (13.7–43.3)	24.3 (11.4–51.5)	20.2 (10.6–38.7)
MACE			
No LVSD, no RD	1.0	1.0	1.0
LVSD, no RD	4.9 (2.9–8.1)	4.1 (2.6–6.5)	3.5 (2.5–5.2)
RD, no LVSD	2.1 (1.1–4.2)	2.7 (1.6–4.8)	2.2 (1.3–3.6)
LVSD+RD	9.6 (5.7–16.4)	7.8 (4.8–12.7)	6.2 (4.1–9.6)

LVSD – left ventricular systolic dysfunction; RD – renal dysfunction.

Table 4

Multivariable Cox regression model for 5-year overall mortality and major adverse cardiovascular events (MACE)

Variable	5-year overall mortality		5-year MACE	
	HR (95% CI)	*p*	HR (95% CI)	*p*
LVSD + RD	4.5 (1.9–10.8)	< 0.001	2.5 (1.4–4.5)	0.003
LVSD	4.4 (2.1–9.2)	< 0.001	2.2 (1.4–3.4)	0.003
RD	3.4 (1.5–7.8)	0.004	1.7 (1.1–2.9)	0.050
HF at admission	3.5 (2.0–6.0)	< 0.001	2.5 (1.6–3.8)	< 0.001
Postprocedural flow TIMI < 3	2.8 (1.6–4.9)	< 0.001	2.5 (1.6–4.0)	< 0.001
Age (years)	1.03 (1.01–1.06)	< 0.001	1.01 (1,0–1.03)	0.040
3-vessel disease	1.5 (1.1–2.4)	0.050	ns	
Previous infarction	ns		1.8 (1.2–2.6)	0.002

LVSD – left ventricular systolic dysfunction; RD – renal dysfunction; HF – heart failure; HR – hazard ratio; CI – confidence interval; TIMI – Thrombolysis in Myocardial Infarction.

se, left main stenosis, and post-procedural TIMI flow grade < 3. In the Cox regression model for the 5-year occurrence of MACE, adjustment was made for the following variables: age, previous infarction, previous stroke, diabetes, heart failure on admission, anterior infarction, 3-vessel disease, and post-procedural TIMI flow grade < 3.

Discussion

The results of this study show that the patients with STEMI treated with pPCI, who had a combined presence of LVSD and RD, had a significantly greater all-cause mortality and occurrence of MACE during a 5-year follow-up period as compared to patients with preserved left ventricular systolic function and preserved renal function, and in comparison with patients with only LSVD or RD. The highest percentage of lethal outcomes and MACE was registered in the first year of follow-up in all the groups. One-year landmark analysis confirmed that the patients with LVSD+RD had the highest percentage of lethal outcomes in comparison with all the other examined groups in the period 1 and 5 years. A statistically significant difference in the occurrence of MACE between the analyzed groups in the period 1–5 years of follow-up was not found, but there was a strong trend toward a significance. The LVSD+RD combination was the strongest independent predictor of all-cause 5-year mortality and the occurrence of MACE – the patients with the LVSD+RD combination had a 4.5 times greater risk of 5-year mortality and a 2.5 times greater risk of the occurrence of MACE in the 5-year follow-up period as compared to patients with preserved left ventricular systolic function and with preserved renal function.

It has previously been published that the LVSD+RD combination is the strongest independent predictor of mortality and the progression of cardiac and renal insufficiency after pPCI during hospitalization [20]. During a 1-year follow-up period of patients with a first time myocardial infarction treated with pPCI, the risk of mortality and MACE was increased by approximately 5 times in the group with the LSVD+RD combination in comparison with the patients with preserved cardiac and renal function, i.e. the LVSD+RD combination was also the strongest independent predictor for the occurrence of adverse events during the 1-year follow-up period [21]. The results of the present study confirm these findings and show that the LVSD+RD combination remains the strongest independent predictor of mortality and the occurrence of MACE in a longer period of time, at least 5 years after pPCI.

The results of the present study are consistent with the results of the study by Palmer et al. [19] who analyze the prognostic significance of the renal and cardiac function in over 1,000 consecutive patients who suffered myocardial infarction, over a 10-year follow-up period. In this study, the coincidence of VRD and cardiac dysfunction increased the risk of lethal outcome and the occurrence of cardiac insufficiency by 5 to 10 times in the 10-year follow-up period as compared to patients with intact renal and cardiac function. As opposed to the present study, where only the patients with STEMI were analyzed, in the study by Palmer et al. [19] non-STEMI patients were also included (around 18% of the studied patients), there were no patients treated with pPCI, thrombolytic therapy was applied in 58% of the patients, while intrahospital PTCA was performed on 20% of the patients. In this study the left ventricular systolic (dys)function was analyzed in two ways: as the N-terminal–probrain natriuretic peptide (NT-pro BNP) value and as the LVEF (LVSD was considered to be LVEF < 50%). The results obtained when the decreased eGFR < 60 mL/min) was analyzed in combination with an increased NT-proBNP (10-year mortality was 60%) did not differ from the results obtained by combining eGFR < 60 mL/min and LVEF < 50% (10-year mortality was 58%) [19].

In the study by Schou et al. [17] the influence of the eGFR and the wall motion score index (WMI) on the prognosis of patients with myocardial infarction or cardiac insufficiency during a 10-year follow-up period was analyzed. This study show a significant interaction between the values for eGFR and WMI. The highest percentage of 10-year mortality was registered in patients with eGFR < 60 mL/min/m^2 and LVSD low (WMI < 1.4), while the lowest one in patients with eGFR > 60 mL/min/m^2 and a preserved left ventricular systolic function, which coincides with the results of the present study. As opposed to the presented study, however, in the study by Schou et al. [17], patients with decreased eGFR (< 60 mL/min/m^2) and a preserved left ventricular systolic function had higher mortality than patients with LVSD and a preserved renal function. Also, the study by Schou et al. [17] examined a different population of patients, i.e. in addition to patients with myocardial infarction, patients with cardiac insufficiency were also included. All of the studied patients were treated in 1990s and early 2000s, when the treatment of myocardial infarction (and cardiac insufficiency) differed from the treatment of today, which the authors themselves stated as one of the limitations of the study [17].

The finding related to the highest percentage of analyzed adverse events in the first year of follow-up in the present study is not unusual. In a study by Kümler et al. [1], which followed up patients with LVSD after myocardial infarction for a period of up to 17 years, it was also found that the highest percentage of lethal outcomes occurred in the first year after myocardial infarction and that it then became stabilized and much lower.

Clinically speaking, taking into consideration the results of the present study and the aforementioned studies as well as the well-known fact that neurohormonal mechanisms contribute to the development and progression of chronic cardiorenal syndrome [11, 24], it is important to make a note of the fact that as of the moment when the patient is hospitalized onwards, all patients with LVSD and RD should be advised to take medication which can, to a certain extent, modify and block these mechanisms, and this primarily relates to ACE inhibitors and beta blockers [11, 17, 25]. But, at this moment there is no ideal therapeutic strategy that would improve the prognosis of patients with LVSD and RD [24, 26, 27]. The reason for this lies not only in the complex interactions between the heart and the kidneys but also in the fact that some of the risk factors for coronary disease and a decreased CrCl are un-

changeable, primarily the age of the patient [27]. In some studies, the glomerular filtration rate was not an independent predictor of mortality when age was also included into the regression model [27, 28], while in others this was not the case, and both the patient's age and the renal function were independent predictors of mortality and other adverse events [19–21]. In the present study the LVSD+RD combination proves to be the strongest independent predictor of the occurrence of all-cause mortality and MACE after adjusting other variables, including age.

Study limitations

The study is observational, prospective, including consecutive patients, and limiting a possible selection bias. There are no data on follow-up echocardiographic examinations during the follow up of the patients with LVSD that would have shown whether there was a certain degree of recovery of the myocardial contractility. On the other hand, the coincidence of RD may influence the deterioration of the systolic function and the remodeling of the left ventricle [18]. Basal renal function can be the indicator of the chronic state or acute deterioration. As the present study excluded patients with cardiogenic shock at admission, the authors considered the risk of acute renal function deterioration minimized. The renal function during follow-up of the patients with LVSD+RD, to determine the progression of RD, was not evaluated, however, during a 5-year follow-up the development of terminal renal insufficiency did not occur and none of the patients was started on hemodialysis. The renal function was assessed with the use of the Cockroft-Gault formula [28], which has its limitations. As far as antiplatelet therapy is concerned, all of the patients in the study received clopidogrel (loading dose including the follow-up period), there were no patients receiving new antiplatelet drugs (prasugrel or ticagrelor), which may have a more potent antiplatelet effect and can therefore reduce the occurrence of MACE [8]. The study was not designed to evaluate whether changing pharmacological treatment would have impact on the long-term outcome in patients with LVSD+RD.

Conclusion

Patients with the combined presence of left ventricular systolic dysfunction and renal dysfunction have a significantly higher mortality upon percutaneous coronary intervention during a 5-year follow-up period, as well as during a follow-up period of 1 and 5 years as compared to patients with normal renal and cardiac function, patients with only left ventricular systolic dysfunction or those with only renal dysfunction. The combined presence of left ventricular systolic dysfunction and renal dysfunction is the strongest independent predictor of all-cause mortality and the occurrence of major adverse cardiovascular events in a 5-year follow-up period. The results of the present study confirms the strong negative independent prognostic impact of the left ventricular systolic dysfunction and renal dysfunction combination, and shows that this negative impact persisted in the long-term follow up of patients with ST-elevation myocardial infarction treated with percutaneous coronary intervention.

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Migraine with aura and TCD bubble-test: The significance of positive result

Migrena sa aurom i TKD *bubble* test: značaj pozitivnog rezultata

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Abstract

Background/Aim. The association between the right-to left shunt (RLS) and migraine with aura (MA) has been proven so far. The aim of this study was to determine if the presence of RLS detected as a result of transcranial doppler (TCD) bubble-test, makes any difference in clinical presentation, aura and headache in patients with MA. **Methods.** A single-group descriptive study was conducted on 153 patients diagnosed with MA. TCD bubble-test was performed on 135 of them. The recorded demographic and clinical features of patients were analyzed and compared with the results of the TCD bubble test. **Results.** In the group of 135 patients, 88 (65.2%) had positive TCD bubble-test. The difference in the investigated clinical features of patients of the patients and aura between the patients with and without RLS, was not found. **Conclusion.** The results of our study confirm a high prevalence of right-to-left shunt in patients with MA, but the clinical relevance of this association was not shown.

Key words:
migraine with aura; ultrasonography, doppler, transcranial; diagnosis.

Apstrakt

Uvod/Cilj. Udruženost desno-levog šanta (*right-to-left shunt* – RLS) i migrene sa aurom (MA) je poznata. Cilj ove studije bio je da se odredi da li prisustvo RLS otkriveno transkranijalnim doplerom (*transcranial doppler* – TCD) utiče na kliničku sliku, auru i glavobolju bolesnika sa MA. **Metode.** Ova deskriptivna studija sprovedena je na grupi od 153 bolesnika sa MA. Test TCD *bubble* test urađen je kod njih 135. Demografske i kliničke karakteristike bolesnika analizirane su i upoređene sa rezultatima TCD *bubble* testa. **Rezutalti.** Od 135 bolesnika, kod 88 (65,2%) TCD *bubble* test bio je pozitivan. Nije utvrđena razlika u ispitivanim kliničkim osobinama bolesnika i aure kod bolesnika sa i bez RLS. **Zaključak.** Rezultatima ove studije potvrđena je visoka prevalencija RLS kod bolesnika sa MA. Klinička značajnost ove udruženosti, međutim, nije utvrđena.

Ključne reči:
migrena sa aurom; ultrasonografija, dopler transkranijumska; dijagnoza.

Introduction

The association between the cardiac right-to left shunt (RLS) and migraine with aura (MA) has been widely examined [1–6] and shown that there is a relationship between migraine with aura and patent foramen oval (PFO). The prevalence of PFO in the MA patients is around 50% according to results of different studies, in contrast to approximately 20% in the migraine without aura patients and even less in healthy control subjects. The tendency of paradoxical emboli for the posterior circulation [7, 8] was the basis for the hypothesis about causality of RLS and the cortical spreading depression originating in occipital cortex with subsequent, the most common, visual aura. According to the results of some authors, patients with migraine who have RLS tend to recognize activities that can cause Valsalva's manoeuvre, increasing the extent of shunt as a trigger of their migraine attacks [9]. Nevertheless, a prospective, multicenter, randomized, double blind, placebo-controlled trial revealed no casual relationship between PFO closure and reduction in the number and severity of headache attacks in patients with MA [10]. The pulmonary RLSs, with arteriovenous malformations background, are associated with a high migraine prevalence, as well [11–13].

The aim of this study was to determine if the presence of RLS detected as a result of transcranial Doppler (TCD)

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bubble-test is related to demographic features of patients with MA or the clinical presentation of aura in them.

Methods

A single-group, descriptive study was conducted over 153 patients diagnosed with MA, selected from specific outpatient visits for headaches during the period of four years, from 2008 to 2012. Written informed patient consent was obtained from all the examined subjects. Research protocol of the study was approved by the review board of the Neurology Clinic, Clinical Center of Serbia, Belgrade.

All the patients included in the study met the International Headache Society (IHS) criteria for typical aura with migraine headache (diagnostic criteria 1.2.1) [14]. The demographic features (age at the time of examination, age at the time of headache onset, gender) data about family history of migraine and cerebrovascular disorders were recorded together with the duration and symptoms of aura reported by the patients. According to diagnostic IHS criteria aura was diagnosed as visual or sensory, and aura with both, visual and sensory symptoms was labeled as complex aura. Other transitory cortical dysfunctions experiencing regularly during aura, meaning disturbances in color naming, object and face recognition, memory, speech, calculation, spatial orientation and praxia, were also recorded.

The RIMED Digi-Lite (RIMED, Israel), dual channel TCD system was used in the present study. The insonation was performed through the pre-auricular acoustic bone windows according to a standard approach using 2 MHz transducers to visualize the middle cerebral artery (MCA). Bilateral monitoring was performed with each probe held in place over a temporal bone by the head frame. The MCA gate was selected for each spectrogram and the continuous recording and counting of bubble embolic signals was provided by a computer hard disk. All embolic tracks were counted in the bilaterally insonated MCA from a depth of 40 mm to 75 mm. In all the patients, the TCD bubble-test was performed in the supine position with the head inclined forward for the 30 degrees. At least 2 contrast bolus injections with 9 mL of saline, 1 mL of air were agitated and mixed with the small amount of the patient's blood, administered into an antecubital vein. The first injection was performed during normal respiration, the second injection was preformed immediately prior to non-calibrated Valsalva and if no embolic tracks were detected, additional injections were made while the patient performed the second non-calibrated Valsalva. TCD bubble-test was considered to be positive if at least 1 microbubble was detected during the first 12 seconds after injection [15]. To grade RLS, a 6-level logarithmic scale was used for both resting and Valsalva injections [16].

The recorded demographic and clinical features were analyzed and compared with results of TCD bubble-test.

The data are presented as arithmetic mean or as percentages. The Kolmogorov–Smirnov test was applied to assess the normality of the studied continuous data. Independent-samples *t*-test and χ^2-test were used to compare data between the two groups. The significance level for the analysis was set at 5% ($p < 0.05$).

Results

TCD bubble-test was performed in the 135 of 153 studied patients diagnosed with MA. The group of 18 patients did not undergo this test due to inadequacy of the bone windows. Positive TCD bubble-test was recorded in 88 (65.2%) of the patients. On a 6-level logarithmic scale that was used to grade RLS, 34 (25.2%) of the patients had RLS grade I, 17 (12.6%) grade II, 27 (20.0%) grade III, 9 (6.7%) grade IV and only 1 (0.7%) patient grade V.

The patients with positive TCD bubble-test, comparing to those with a negative test result, did not show any difference in gender, age at the time of examination and in age at the time of migraine onset (Table 1). Migraine without aura was present in approximately one quarter of the patients and family history of migraine in half of them in both groups (Table 1).

There were no differences between the two groups of the patients in regard to duration of aura, as well as aura symptoms (Table 2). In both groups, the most common type of aura was visual reported by more than 90% of patients, followed by sensory symptoms. More than half of the patients in both groups had complex aura, and the other cortical dysfunctions during aura were reported by one third of the patients in both groups (Table 2).

Also, there were no differences in demographic features, personal and family history nor characteristisc of aura, between the groups of the patients with different grade of RLS, based on a 6-level logarithmic scale.

Table 1

Demographic features, personal and family history of migraine with aura (MA) patients with positive and negative transcranial Doppler (TCD) bubble-test

Demographic features	MA patients with positive TCD bubble-test, n = 88	MA patients with negative TCD bubble-test, n = 47	*p*-value
Gender – female, n (%)	68 (77.3)	30 (63.8)	0.108
Actual age (years), $\bar{x} \pm SD$	33.6 ± 11.1	36.2 ± 14.6	0.241
Age at the onset of migraine (years), $\bar{x} \pm SD$	19.0 ± 8.3	20.9 ± 10.9	0.259
Migraine without aura in personal history, n (%)	22 (25.0)	13 (27.7)	0.837
Family history of migraine, n (%)	48 (54.5)	22 (46.8)	0.470
Family history of cerebrovascular disorders, n (%)	12 (13.6)	5 (10.6)	0.787

Table 2

Characteristics of aura in the migraine with aura (MA) patients with positive and negative transcranial Doppler (TCD) bubble-test

Aura features	MA patients with positive TCD bubble-test, n = 88	MA patients with negative TCD bubble-test, n = 47	*p*-value
Duration (min), $\bar{x} \pm SD$	30.7 ± 18.6	28.9 ± 18.2	0.495
Somatosensory aura, n (%)	54 (61.4)	23 (48.9)	0.202
Visual aura, n (%)	80 (90.9)	44 (93.6)	0.747
Other cortical dysfunctions during aura, n (%)	25 (28.4)	17 (36.2)	0.435
Complex aura, n (%)	52 (59.1%)	25 (53.2)	0.585

Discussion

In contrast to a large number of studies examining the potential causal relationship between MA and RLS, there is a few number of papers giving us data about the clinical characteristics of aura types in migraine patients with RLS [17]. The results of our study, completed on 135 patients, confirmed a high prevalence of RLS in the patients with MA, detected in 65.2% of our patients. The obtained results are in accordance with the results of the other authors [1–4]. In a line with results of these authors [17], the significant difference of investigated demographic features of patients and clinical characteristics of aura between patients with and without RLS, was not found.

The proposed explanation of the comorbidity between MA and RLS is shared genetic inheritance [18]. According to the results of our study, migraine in the family history was equally present in the patients with and without RLS.

A substantial number of studies point to the increased risk of stroke in patients with MA compared to the patients with migraine without aura [19]. The association between MA and RLS could contribute to this increased risk. The mechanism that underlies this association remains unclear, and according to the results of our study is not related to patient's demographic features or aura presentation.

Conclusion

The results of our study confirm a high prevalence of right-to-left shunt in patients with migraine with aura, but the clinical relevance of this association has not been shown yet.

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Motivation and job satisfaction of health workers in a specialized health institution in Serbia

Motivacija i zadovoljstvo poslom zdravstvenih radnika u specijalizovanoj zdravstvenoj ustanovi u Srbiji

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Abstract

Background/Aim. Health care system is specific for each country, and therefore involves different motivation and job satisfaction factors. The aim of this study was to confirm the validity and applicability of the General Nordic Questionnaire translated into Serbian for analyzing motivation and job satisfaction, as well as to analyze the motivation factors and job satisfaction of health workers at the Institute for Treatment and Rehabilitation "Niška Banja". **Methods.** The General Nordic Questionnaire was translated into Serbian. It included 73 questions (5 answers by the Likert scale), which formed 24 scales. In May, 2012, 196 (82.3%) health workers filled in the questionnaire. **Results.** A correlation of Ceronbach's alpha values between the Scandinavian study results and the results of Serbian study was statistically significant ($r = 0.424$; $p < 0.05$). The lowest Cronbach' alfa coefficient was for the scale "learning demand in job demands" (alpha 0.28). The other scale with no satisfactory reliability was "control of work pacing" (alpha 0.46). Intrinsic motivation positively correlated with all the scales of organizational module, apart from inequality. Within this module, the degree of extrinsic motivation correlated with the support from the superior and coworkers, with fair leadership and human resources primacy. A negative correlation appeared with inequality. **Conclusion.** The general Nordic Questionnaire can be applied to a great extent to the conditions in our country. Our study shows a low consistency of the scales learning job demands, control of decision, control of work pacing, so the questions in them should be adjusted to the conditions in our country. Extrinsic motivation resulted in higher values than the values of intrinsic motivation in our workers, which is opposite to the results of the original questionnaire.

Key words:
job satisfaction; medical staff; questionnaires; serbia.

Apstrakt

Uvod/Cilj. Sistem zdravstvene zaštite ima svoje specifičnosti u različitim zemljama, a samim tim i različite motivacione faktore i faktore zadovoljstva poslom. Cilj ovog rada bio je da se ispita validnost i primenljivost upitnika za ispitivanje motivacije i zadovoljstvo poslom *Genereal Nordic Questionnaire*, prevedenog na srpski jezik, kao i da se ispitaju motivacioni faktori i zadovoljstvo poslom zdravstvenih radnika u Institutu za lečenje i rehabilitaciju „Niška Banja". **Metode.** *General Nordic Questionnaire* preveden je na srpski jezik. Izdvojena su 73 pitanja (5 odgovora po Likertovoj skali) sadržana u 24 skale. Upitnik je popunilo 196 (82,3%) zdravstvenih radnika tokom maja 2012. godine. **Rezultati.** Korelacija Cronbach-ove alfa vrednosti između rezultata istraživanja u skandinavskim zemljama i u Srbiji bila je statistični značajna ($r = 0,424$; $p < 0,05$). Najniži koeficijent alfa imala je skala „zahtev radnog zadatka za učenjem" (alfa 0,28). Druga skala koja nije imala zadovoljavajuću pouzdanost bila je skala „kontrola nad radnim vremenom" (alfa 0,46). Unutrašnja motivacija pozitivno je korelisala sa svim skalama koje se odnose na organizaciju radnog procesa, osim sa nejednakim odnosom prema zaposlenim. U okviru ovog modula, stepen spoljašnje motivacije korelisao je sa podrškom nadređenog i saradnika, sa fer liderstvom i primarnošću ljudskih resursa. Nađena je negativna korelacija sa nejednakim odnosom prema zaposlenim. **Zaključak.** Opšti nordijski upitnik u najvećoj meri primenljiv je na naše uslove. Testiranjem upitnika nađena je niska konzistentost za skale zahtev radnog zadatka za učenjem, kontrola nad radnim vremenom i kontrola odlučivanja, za koje bi trebalo prilagoditi pitanja u anketi prema našim uslovima. Spoljašnja motivacija za rad kod naših ispitanika bila je jače izražena od unutrašnje, što je u suprotnosti sa rezultatima originalnog upitnika.

Ključne reči:
posao, zadovoljstvo; kadar, medicinski; upitnici; srbija.

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Introduction

Motivation of workers is closely connected with the work process and represents a significant job satisfaction determinant. Motivation is multidimensional and the importance of certain motivation factors changes in accordance with the living conditions, market and personal characteristics of workers. Health care system is specific in different countries; therefore developing individual instruments for analysis of motivational factors are necessary, especially in developing countries [1]. Furthermore, every health organization is specific and there is always the need for analysis of motivation factors and considering possibilities for improving motivation and job satisfaction [2]. Low motivation of workers can greatly influence health outcomes and patient safety. Motivation system can increase work performance if properly implemented [3]. In developing countries with significantly limited financial possibilities, managers and health care policy creators must develop and apply other motivation factors from working environment in order to enable higher degree of job satisfaction of workers [4, 5].

Having in mind the effect of motivation and job satisfaction on quality and quantity of provided health care services, the aim of this study was to confirm the validity and applicability of the General Nordic Questionnaire for Psychological and Social Factors at Work [QPS (Nordic)] in Serbian for analyzing motivation and job satisfaction, to analyze motivation factors and job satisfaction of health workers at the Institute for Treatment and Rehabilitation "Niška Banja", and to suggest measures for improving management by increasing motivation and job satisfaction.

Methods

This study included health workers from the Institute for Treatment and Rehabilitation "Niška Banja", a health care institution with cardiology, rheumatology and orthopedic departments (40 beds for each department). Primary activity of this Institute is rehabilitation of cardiovascular patients (350 beds); therefore it is included in the network of health care institutions of the Republic of Serbia (with the total number of 470 beds), financed by the Republic Institute for Health Insurance [6]. The remaining 90 beds are used for recreation of pensioners, workers, and citizens. The Institute has national importance since it is the only institution which provides extended rehabilitation of cardiovascular patients in Serbia in its entire scope. The study participations were 60 doctors, 99 medical nurses, technicians and laboratory technicians, as well as 60 physiotherapists. They represented 92% of total 238 health workers empoloyed in the Institute. Only health workers who were not at the place of residence at that moment did not participate in this study. The participants voluntarily filled in the QPS (Nordic) questionnaire translated from English to Serbian, which they put in the box at work after having it filled in [7]. Properly filled in questionnaires were handed in by 47 (78%) doctors, 92 (92.9%) medical nurses/ technicians and 57 (95.0%) physiotherapists. Survey was conducted during May, 2012, and it was approved by local Ethics Board of the Institute for Treatment and Rehabilitation "Niška Banja".

Out of a great number of questionnaires available in the literature, one adequate questionnaire was to be chosen [8–10]. Important condition for choosing the questionnaire was long tradition in testing and monitoring working environment and job satisfaction. Apart from having a good design, the questionnaire had to be noteworthy and applicable to different professions, to enable research as well as to have a well generated database for further studies and monitoring [11]. QPS (Nordic) is one of such questionnaires [6]. Main features of this questionnaire are applicability and importance for health and well-being. Furthermore, as a frequently used research instrument, it is based on theories and conceptual models of organizational working environment, motivation and satisfaction, on theories of work-related stress, well-being and health in general [12]. The questionnaire encompasses a number of dimensions regarding job demands, such as quantitative demands, decision demands, role clarity, and the existence of role conflict, positive challenge at work, control of decisions, control of work pace, and predictability during the following month. Organization is visible through support from superiors, coworkers, friends and relatives, analysis of leadership, social climate, innovative climate, inequality and human resource primacy. Individual worker characteristics are observable in predictability during the next two years, the individual's preference for challenges, mastery of work, commitment and motivation to work related to both intrinsic and extrinsic motives.

The original QPS (Nordic) consists of 118 questions [13], out of which individual and questions inapplicable in this study were excluded. Finally, 73 questions were selected and classified in 24 scales. Each scale contained 2–4 questions. All questions were of multiple choice questions with 5 offered answers by Likert scale. For the first group of questions, the offered answers were: e.g.: "Do you work overtime?", offered answers: "Very seldom or never", "Rather seldom", "Sometimes", "Rather often", "Very often or always". For the second group, for example: "Do you prefer the challenge presented by working in different places?" with the answers: "Very little or not at all", "Rather little", "Somewhat", "Rather much", and "Very much". The third group of questions referred to the degree of agreement with certain statement. For example, for the statement "To my friends I praise this organization a great place to work", given answers were: "Disagree totally", "Disagree to some extent", "Indifferent", "Agree to some extent", and "Agree totally". The fourth group referred to the level of importance, e.g. "How important are the following considerations in relation to your ideal job? To have a peaceful and orderly job?" Only one of the offered answers was to be chosen: "Unimportant", "Not so important", "Rather important", "Very important", and "Absolutely necessary".

The internal consistency is analyzed by Cronbach's alpha coefficient. Its values below 0.60 are defined as unacceptable for further analysis. Coefficient measured ≥ 0.70 was considered valid. Correlation between scales measuring more than 0.40 was acceptable for this research. Scales

which did not meet the given criterion were excluded from further analysis and final conclusion. Survey results were presented as distribution of frequencies or as a mean total value of scales with standard deviation. Difference testing was performed using Student's *t*-test (ANOVA test) and χ^2 test. Bivariate correlation between scales and related to demographic parameters was presented by Pearson's coefficient with statistical significance. Statistical processing was done in statistical software SPSS version 14.0.

Results

Distribution of questionnaires which have to be filled according to type of health workers is shown in Table 1. The number of employees at the Institute for Treatment and Rehabilitation "Niška Banja" has significantly changed throughout the years, depending on the workload. On the average, 340 people are employed full-time, and in summer months, the number of workers increases up to 420 on account of part-time employed workers. The total average number of patients treated during a year amounts to 14,000 people. Approximately 4,000 patients are treated in the hospitals. Around 6,000 patients from the entire Serbia come to rehabilitation to Niška Banja. Out of the total number of treated people, around 4,000 are pensioners and employed people, and significantly less number of people come to treatment at their own expense. During the year, over 40,000 of specialistic consultative services are provided and the same number of various diagnostic procedures for stationary patients and outpatients. However, the capacity of the Institute is not fully used. Workload, as well as the quality of services can be increased by improving worker motivation in all the areas of service, especially in the area of commercial services.

The average number of patients (or procedures) *per* doctor, nurse/technician or physiotherapist during the year is shown in Table 2.

In terms of gender distribution, the majority of participants were women (75%), regarding age predominant were participants younger than 50 (77%) (Table 1). The average age of the participants was 40.1 ± 11.4 years. The average age of doctors was 45.7 ± 10.2, which was statistically higher value than the age of medical nurses/technicians (37.5 ± 11.7 years of age; $p < 0.0001$) and physiotherapists (39.4 ± 10.2 years old; $p < 0.01$). There were no statistically significant differences regarding the age of medical nurses/technicians and physiotherapists. Distribution of participants according to gender, age and profession is shown in Table 3.

Out of the total number of participants, 25 (12.8%) were at managing positions. The majority of participants work in the morning shift, 123 (62.8%) of them, 48 (24.5%) participants work in two shifts and 25 (12.8%) of them work in 12-hour shifts.

The modified QPS (Nordic) questionnaire which was created in Scandinavian countries (Sweden, Norway,

Table 1

Distribution of properly filled in questionnaires

Profession	Handed out questionnaires (n)	Filled out questionnaires (n)	Valid questionnaires (%)
Doctor	60	47	78
Nurse/laboratory technician	99	92	92
Physiotherapist	60	57	95
Total	219	196	89.5

Table 2

Average number of patients (procedures) *per* doctor, nurse/technician or physiotherapist during the year

Type of service	Average number	
	per doctor	*per* nurse/technician or physiotherapist
Rehabilitation (patients)	9,896	329.7
Hospital (patients)	5,643	235.1
Outpatients department (patients)	37,659	4,707.4
Diagnostics (patients)	430.5	/
Physical therapy (procedure)	332,944	5,549.1

Table 3

Distribution of participants according to gender, age and profession

Variables	Nurses/technicians	Physiotherapists	Doctors	Total
	n (%)	n (%)	n (%)	n (%)
Gender				
male	9 (9.8)	19 (33.3)	21 (44.7)	49 (25)
female	83 (90.2)	38 (66.7)	26 (55.3)	147 (75)
Age, (years)				
≤ 35	43 (46.7)	26 (45.6)	9 (19.1)	78 (39.8)
36–49	31 (33.7)	20 (35.1)	22 (46.8)	73 (37.2)
≥ 50	18 (19.6)	11 (19.3)	16 (34.1)	45 (23)

Finland, and Denmark) was used in this analysis. The correlation of alpha value between our questionnaire and the modified QPS (Nordic) questionnaire was statistically significant ($r = 0.424$; $p < 0.05$). The reliability of job demands analysis, that is, consistency of the answers between the scales is presented by alpha coefficient. The lowest coefficient was for the scale "learning demand in job demands" (alpha 0.28); therefore, this scale was not considered further. The second scale which did not have satisfactory reliability was "control of work pacing" (alpha 0.46), which was also excluded from further analysis. Differences in middle offered answers between scales in the job demand module are presented in Table 4.

In the organizational module, the scale with the lowest value was "support from friends and relatives" (alpha 0.65), and approximately the same value had the scale for "inequality" (Table 4). The scales testing "support from coworkers" and "fair leadership" had the highest mean value, 4.2. The differences in middle answers between scales in the area of organizational module are given in Table 4.

Regarding individual module, the highest mean value was of the scale which tested "extrinsic motivation" (mean value 4.34), but reliability of this scale was the lowest in this module (Table 4). Differences in middle answers are presented in Table 4.

Table 5 shows coefficients of correlation between motivation aspects (intrinsic and extrinsic) and other scales. Only statistically significant correlations are shown.

Two scales in job demands module correlated positively with motivation: role clarity and preference for challenges (Table 5).

Table 4

Mean values, standard deviations and consistency for specific scales

Scales	Average of answers ($\bar{x} \pm SD$)	Alpha (Cronbach's)
Job demands module		
Quantitative demands	2.67 ± 0.71	0.68
Decision demands	3.67 ± 0.81	0.66
Learning demands	2.74 ± 0.77	0.28
Role clarity	4.50 ± 0.66	0.74
Role conflict	2.21 ± 0.83	0.67
Positive challenge at work	4.28 ± 0.76	0.72
Control of decision	2.27 ± 0.74	0.62
Control of work pacing	2.30 ± 0.72	0.46
Predictability of the next month	3.09 ± 1.2	0.72
Organizational module		
Support from superior	4.03 ± 0.82	0.76
Support from coworkers	4.19 ± 0.85	0.77
Support from friends and relatives	3.75 ± 0.94	0.65
Empowering leadership	3.06 ± 0.97	0.78
Fair leadership	4.16 ± 0.9	0.83
Social climate	3.37 ± 0.84	0.73
Innovative climate	3.39 ± 0.97	0.80
Inequality	2.13 ± 0.88	0.66
Human resource primacy	2.99 ± 0.91	0.72
Individual level module		
Predictability of next two years	2.56 ± 1.12	0.67
Preference for challenge	2.59 ± 0.96	0.70
Perception of mastery	4.10 ± 0.60	0.70
Commitment to organization	4.05 ± 0.78	0.74
Intrinsic motivation to work	3.78 ± 0.75	0.70
Extrinsic motivation to work	4.34 ± 0.57	0.65

$\bar{x}$ – mean value; SD – standard deviation.

Table 5

Correlation between motivation to work and other examined scales

Scales	Intrinsic motivation to work (r)	Extrinsic motivation to work (r)
Role clarity	0.184**	0.241**
Positive challenge at work	0.293**	0.271**
Support from superior	0.302**	0.257**
Support from coworkers	0.252**	0.203**
Support from friends and relatives	0.239**	0.095
Empowering leadership	0.277**	0.073
Fair leadership	0.243**	0.241**
Social climate	0.234**	0.149*
Innovative climate	0.321**	0.124
Inequality	-0.110	-0.241**
Human resource primacy	0.301**	0.169*
Predictability of next two years	0.216**	0.176*
Preference for challenge	0.232**	0.135
Perception of mastery	0.357**	0.321**
Commitment to organization	0.397**	0.313**

r – coeficient of correlation;*$p < 0.05$; **$p < 0.01$.

Intrinsic motivation positively correlated with all scales of organizational module, apart from inequality. Within this module, the degree of extrinsic motivation correlated with the support from superior and coworkers, with fair leadership and human resources primacy. Negative correlation appeared with inequality. Scales: control of individual work pacing, commitment to organization, and predictability of the following two years correlated well with both intrinsic and extrinsic motivation, while preference for challenges correlated only with intrinsic motivation.

The module of age positively correlated with decision demands ($r = 0.191$; $p < 0.05$), and negatively with preference for challenges ($r = -0.245$; $p < 0.01$) and with the support from coworkers ($r = -0.158$; $p < 0.05$) as well as with human resources primacy ($r = 0.169$; $p < 0.05$).

Discussion

Internal consistency

In our questionnaire, the scale "learning demands" had a very low consistency (alpha 0.28), as well as "control of work pacing" (alpha 0.46), and "decision control" (alpha 0.62). The question "Are your work tasks too difficult for you?" had the lowest correlation with other questions from the scale and different formulation of this question, that is, "Are your work tasks too complex for you?" should be tested in future analyses. This points out that every country, according to its own specific circumstances, should develop individual questionnaire. However, this comparison is limited, since this research was performed on small sample (196 participants) in one organization, compared to the Wännström et al. [7] study which included 3,976 participants.

Demographic structure of the participants

Studies show that there is no difference in the degree of job satisfaction between genders, which was also confirmed in our study [14]. It is widely accepted that the nature of job is a factor which determines the degree of job satisfaction, and not gender. Contrary to this statement, Guppy and Rick [15], point out numerous differences between genders. Higher satisfaction with job in men was expressed in promotional aspects, while good relations with coworkers and patients were more important for women. Considering gender distribution and professional structure of our participants, there were no differences in job satisfaction between genders, since the majority of medical staff were women (stress was in relation with coworkers and patients) and men were predominant in the group of doctors and with a higher level of intrinsic motivation.

The majority of data in the literature point out to interdependency between personal characteristics, motivation and job satisfaction of workers [16]. People of older age are not motivated by the need to prove themselves through their achievements; therefore, they prefer to perform more sensible work [17]. Apart from this, the desire for learning and acquiring new skills decreases over time. Thus, they are less motivated by salary, and more by praises [14]. This study also shows that the preference for challenges decreases over time.

Managers are satisfied with their job to a higher degree than other participants. Lipińska-Grobelny and Wasiak [18] point out in their study that female managers of androgenic type are most satisfied with job, while women of feminine type without managing position are most dissatisfied with their job. In this study, managing position positively correlates with job challenges, expectations, excessive workload, decision control and extrinsic motivation.

Job demands module

Organizations based on quality, certainty for workers, and culture of innovation and creativity, have higher degree of workers' job satisfaction [19]. This study shows that job demands for learning, cannot be estimated globally due to low consistency of the result. Job demands influence not only motivation and job satisfaction, but also mental health of health care workers [20]. In their two-factor model Demerouti et al. [21] argue that excessive workload and lack of resources lead to burnout. Bennett et al. [22] discovered high occurrence of burnout and high level of stress in Canadian nurturing children and youth professionals, contrary to the high degree of their job satisfaction.

Compared to the original Nordic questionnaire, our participants consider that workload is not excessive, but that it is more demanding in the aspect of deciding. Organizations which emphasize performance usually have low degree of job satisfaction. This was confirmed by Agho et al. [23], who pointed out the negative correlation between high performance and job satisfaction.

The greater role clarity, the less conflict demands, that is, the person knows what is expected at work. Compared to the original questionnaire, this study resulted in fewer occurrences of conflicts and higher degree of role clarity. This indicates good leadership and support from superiors in the Institute "Niška Banja".

In organizational cultures where decisions are made and put into practice without considering opinion of subordinates, job satisfaction is at lower degree, as well as commitment to the organization [24]. Participating style of making decisions results in higher degree of job satisfaction provided that management includes workers in decision-making and problem-solving process and enables certain degree of control of their work [25]. In the original QPS (Nordic) questionnaire, control of work pace correlates with decision control, control of working hours, and positive work challenge. Studies show that motivation increases with challenging work tasks [26]. Such motivation is intrinsic, that is, it refers to satisfying internal needs for achieving challenging goals. A greater number of challenging tasks, flexible working hours, and participating style of making decisions (control of work pacing) significantly improve job satisfaction.

Comparison of scales with the mean values from the original questionnaire and scales with our results points out that the mean values of decision control and control of work pace were lower in the original questionnaire than in our. However, our health care workers experienced their work statistically more challenging than workers in Scandinavian countries. This indicates that higher level of autonomy in work should be introduced in the organization at the Institute "Niška Banja".

Organization module

Support from superiors and coworkers are significant components of job satisfaction. A number of authors argue that job satisfaction is higher if there are positive horizontal and vertical connections among workers, as well as effective and efficient cooperation [19, 25, 27]. This aspect of organizational culture module is greatly beyond management control. Sterud et al. [28], point out that the absence of support from coworkers was one of the strongest and most common stressors in survey which included dispensary medical staff in Norway.

Encouraging, empowering and safe environment increase the degree of job satisfaction [29]. In our organization, empowering and fair leadership was predominant, with values above the average mean values of scales compared to Scandinavian organizations.

Equality, which is expressed in fair relations and equal chances for every worker, contributes to improvement of job satisfaction [30]. Studies show that organizations which encourage and support their employees to suggest and develop new ideas have more satisfied workers. Additionally, workers are more committed to the organization [31]. Comparing results of the two questionnaires shows unequal treatment of workers in our organization. Social climate was more favorable in Scandinavian countries, while the scale of human resources primacy had higher value in our survey. In the case of the scale of innovation climate there was no difference in mean values in both questionnaires.

Individual model and work motives

Positive correlation between job satisfaction and preference for challenges, which included variability, was presented in literature [32, 33]. Jobs which include repetition of activities have influence on the decrease in job satisfaction [34]. Autonomy, control of work pace, flexible procedures and structures result in higher degree of satisfaction and intrinsic motivation. A connection between motivation and satisfaction greatly depends on subjective perception of control of individual work pacing. In this study, health care workers experienced a high level of control of work pace than Scandinavian workers. These differences are the result of different payment systems and organization of health care system. For example, changes in health market in California significantly influenced job satisfaction of doctors. Burdi and Baker [35] presented that lower autonomy of doctors regarding work performance significantly decreased the degree job satisfaction of doctors in the period from 1991 to 1996.

Extrinsic motivation is expressed in the need for financial rewards, promotion, and higher status. Intrinsic motivation was "rather important" of "very important" to participants, while extrinsic motivation was "very important" to "absolutely necessary". This is contrary to the results of Kontodimopoulos et al. [36] study which is perhaps the consequence of unfavorable financial situation of health care workers in Serbia.

Comparison of mean values of scales in the two questionnaires presented higher extrinsic motivation in Serbian participants, while their intrinsic motivation was lower than in Scandinavian participants. In the study conducted in Pakistan, main motivation factors for doctors were intrinsic and sociocultural factors (serving people, career advancement, gaining respect), and demotivators were organizational problems in current work such as: low salaries, insecurity for women and the absence of possibility of acquiring higher qualifications [37]. Study in Mali, West Africa, with salary of 240 $ *per* citizen, main motivators for health workers were related to responsibility, training and rewards. Salary was also a significant motivator [38].

Conclusion

The General Nordic Questionnaire can be applied to a great extent to conditions in our country. The questionnaire resulted in low consistency of scales: learning job demands, control of decision, control of work pacing, in which the questions should be adjusted in accordance with our conditions. Extrinsic motivation resulted in higher values than the values of intrinsic motivation in our workers, which is opposite to the results of the original questionnaire. According to the low average sum of the scales, specific measures were suggested, and expected results consequently included decrease of morbidity and mortality of patients with the increase in their satisfaction and quality of life.

Based on presented problems, measures for improvement of management by increasing the degree of motivation and job satisfaction are suggested. Implementation of measures and activities, as well as more effective utilization of working hours and resources, is expected to result in improvement of quality and quantity of service, and consequently decrease morbidity and mortality of patients with the increase in their satisfaction and quality of life. The suggested measures and activities include: reorganization of human resources, which will enable employees with less years of service to perform challenging tasks (new tasks, coworkers, and new work positions), and simultaneously increase quantity of workload to moderate level; proposing incentives, adjusted to specific features of the institution and organizational units; to draw greater attention to financial stimuli in a subgroup of medical nurses/technicians and physiotherapists, and to nonfinancial stimuli (connected to stronger intrinsic motivation) in the group of doctors and management; improving work organization in its units by increasing the degree of autonomy, general planning for few months in advance, introducing tasks to workers and including their suggestions for plan realization; analyzing job positions regarding workload and demands for additional qualifications. Defining the necessary skills for performing certain tasks and thus to transparently securing the need for additional education. Enabling both internal and external education of the staff; empowering social interactions through stimulation of forming associations, organizing sports activities, celebration of important dates, etc; reducing inequality to the lowest level possible, and define criteria for equal treatment of workers in all work situations; increasing care for human resources, especially for people with longer time of service, enabling additional health and

personal security through various conveniences, improving social interactions and reducing negative aspect of support from coworkers by introducing programs of education and transfer of knowledge and experience; motivating health workers to nurture humanity and to aspire to higher quality of health services, introducing periodical promotion of best worker in the field of humanity and quality at work with patients.

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Treatment of recurrent aphthous stomatitis by laser therapy: A systematic review of the literature

Lečenje recidivirajućeg aftoznog stomatitisa primenom lasera: sistematski pregled literature

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Abstract

Background/Aim. Recurrent aphthous stomatitis (RAS) is defined as multifactor immunologic inflammatory lesions in the oral cavity, characterized by painful, recurrent single/multiple, shallow, round or ovoid ulcerations of mucosal tissues. To date, a considerable number of RAS treatment protocols have been suggested, but since the etiology of RAS is idiopathic, these treatment options have symptomatic rather than curative or preventive effect. Recently, it has been suggested that laser therapy could be successfully used as an efficient treatment approach in therapy of RAS. Therefore, the aim of this review was to estimate the effects of laser therapy in treatment of RAS analyzing results of clinical studies published in peer reviewed journals. **Methods.** The studies published until 31 December 2013 were obtained from the Medline/PubMed, Science Direct and Cochrane Library of the Cochrane Collaboration (CENTRAL) online databases, using following search terms and key words: "laser" AND "recurrent aphthous stomatitis", "laser" AND "aphthous", and "laser" AND "aphthae". In total 4 original research articles met the all required inclusion/exclusion criteria, and were used for this review. The main outcome measures assessed were: a reduction of pain associated with RAS and a reduction in episode duration (faster RAS healing). **Results.** The assessed literature demonstrates the benefits of laser therapy mainly due to immediate analgesia and ability to speed up a RAS healing process. **Conclusion.** Even though the assessed literature suggests beneficial outcomes of laser therapy in treatment of RAS, these results should be interpreted with caution. The issues related to the study designs and different sets of laser irradiation parameters of a limited number of available studies with the same treatment outcomes prevent us from making definite conclusions.

Key words:
laser therapy, low level; stomatitis aphthous; recurrence; treatment outcome.

Apstrakt

Uvod/cilj. Rekurentni aftozni stomatitis (RAS) definisan je multifaktorskim imunološkim zapaljenskim lezijama u usnoj duplji, koje se karakterišu bolnim, recidivirajućim pojedinačnim/multiplim, plitkim, okruglim/ovoidnim ulceracijama sluzokože. Do danas je predstavljen niz protokola o terapijskom zbrinjavanju RAS, ali kako je oboljenje idiopatske etiologije, predložene terapijske opcije su simptomatske, a ne preventivne ili kurativne. Nedavno je laseroterapija predstavljena kao veoma efikasna opcija terapijskog zbrinjavanja RAS. Cilj ovog rada bio je procena efekata laseroterapije u terapijskom zbrinjavanju RAS na osnovu rezultata kliničkih studija objavljenih u recenziranim časopisima. **Metode.** Analizirani su radovi objavljeni do 31. decembra 2013, dobijeni pretraživanjem Medline/PubMed, *Science Direct* i *Cochrane Library* of the *Cochrane Collaboration* (CENTRAL) *online* baza podataka, ukucavanjem ključnih riječi: *laser* i *recurrent aphthous stomatitis*, *laser* i *aphthous* i *laser* i *aphthae*. Ukupno 4 originalna naučna rada, koji su zadovoljili sve kriterijume uključenja/isključenja u ovu studiju podvrgnuta su daljoj analizi. Poseban akcent stavljen je na efeke laseroterapije na ublažavanje bola (analgeziju), te na skraćenje dužine trajanja RAS epizode, odnosno na ubrzano zarastanje lezija. **Rezultati.** Dostupna literatura ukazuje na prednosti korišćenja laseroterapije u zbrinjavanju RAS, i to u neposrednoj analgeziji, te sposobnosti ubrzanja zarastanja rana. **Zaključak.** Iako podaci

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iz dostupne literature ukazuju na prednosti upotrebe laseroterapije u zbrinjavanju RAS, ove rezultate trebalo bi interpretirati uz oprez. Naime, različit dizajn kliničkih studija, različiti iradijacijski parametari lasera korišćeni u tim studijama, te veoma mali broj dostupnih studija u kojima su praćeni isti terapijski ishodi, sprečavaju nas da donesemo konačne zaključke o prednostima laseroterapije u lečenju RAS.

Ključne reči:
laseri male snage; stomatitis aftozni, recidiv; lečenje, ishod.

Introduction

Recurrent aphthous stomatitis (RAS) is one of the most frequent pathologic conditions in the oral cavity in an otherwise healthy individual. It is defined as multifactor immunologic inflammatory lesions that affect 10–20% of the population, mainly in developed parts of the world [1–4]. RAS has three main clinical subtypes – minor (miRAS), major (maRAS) and herpetiform ulcers [1–4]. Minor ulcers (Mikulicz ulcers) are the most common subtype, which comprise over 80–90 % of cases. They are usually less than 1 cm in diameter, last up to 7–14 days, and heal without scar. Major ulcers (Sutton's ulcers) are over 1 cm in diameter, their healing may take 20–30 days at a time, and often with scaring. Herpetiform ulcers (HU) are multiple, clustered, 1–3 mm lesions that may integrate into larger ulcers. They typically heal with scar within 15 days [1–4]. Although the majority of cases are benign and heal in less than two weeks, these ulcerations may be indicative of underlying systemic diseases ranging from vitamin deficiency to autoimmunity, especially pointing on immunodeficiency [2, 3]. Awareness of these correlations can help the dentist make the diagnosis of potentially serious conditions. RAS can also have clinical and histological aspects in common with Behcet's, Sweet's, Stevens-Johnson and Reiter's syndrome [3].

Even though exact underlying etiology of RAS is unknown, many etiologic, predisposing factors have been suggested. Several microbial agents such as *Herpes Simplex*, *Varicella Zoster*, *Coxsackie A* and other viruses, *Toxoplasma gondii* and organisms which cause Vincent's angina, *Mycobacterium tuberculosis, Helicobacter pylori, Actinomyces, Neisseria* and other bacterial agents including the pleomorphic, transitional L-forms of *Streptococcus sanguis* have been mentioned [1–5]. The current literature also suggests correlation of RAS with fungal agents, such as *Coccidioides immitis*, *Cryptococcus neoformans* and protozoans such as *Entamoeba histolytica*. Also trauma, physical or psychical stress, hematological deficiencies, chemical injuries, hormonal changes (mostly in women), allergy, vitamin C, B_1 and B_{12}, iron and folic acid deficit and smoking are potentially related to RAS [2, 3]. Moreover, there is considerable evidence that aphthous ulcers are related to a focal immune system dysfunction in which T-lymphocytes have very important role [2, 3].

A considerable number of treatment protocols for RAS have been described, but since the etiology of RAS is unknown, none of these treatment options have curative or preventive effect [6]. The basic of the treatment is focused on pain relief and promotion of the healing in order to reduce the duration of the disease and its recurrence [3, 6–8]. Therapy of RAS includes topical (e.g. triamcinolone acetonide) or systemic corticosteroids (e.g. prednisolone), systemic immunomodulators (e.g. thalidomide), antibacterial (e.g. tetracycline), nonsteroidal anti-inflammatory drugs (e.g. pentoxifylline-PTX, colchicine, 5% amlexanox), antimicrobials (e.g. chlorhexidine gluconate), chemical cauterizers and/or cryotherapy. Further, nonprescription options, such as vitamin supplements, herbal supplements and/or local anesthetics gels or pastilles can be often used to reduce discomfort [5, 7–13]. Recently, it has been suggested that statins – cholesterol lowering drugs, whose immuno-modulatory and anti-inflammatory actions have been proven in local [14] and systemic inflammation, particularly by inhibition of pro-inflammatory cytokines production [15] could be successfully used in several inflammatory diseases. Auto-inflammatory disease, such as RAS, is predominantly mediated by pro-inflammatory cytokines of the innate immune system, particularly IL-1β and TNF-α [16], suggesting potential therapeutic benefits of blocking these cytokines. Although, it has been showed that simvastatin exerts anti-inflammatory properties in experimental periodontitis [17], and reduces IL-1α-induced production of inflammatory cytokines by human oral epithelial cells [18], the therapeutic effectiveness of statins in the treatment of RAS remains to be established. Even though widely accepted, conservative/pharmacological therapeutics is often disappointing and palliative, and recurrences of the lesions are common after the therapy is ceased. The need for better treatment alternatives was obvious, especially for patients unresponsive to conservative therapy of RAS.

Low-level laser therapy (LLLT) is nondestructive amount of energy that occurs at the periphery of the target tissue, simultaneously along high-level laser irradiation ("simultaneous LLLT"), or as independent ("pure LLLT") amount of power and energy density below the destructive level [19]. It has bioactivating effects, such as increase of cell metabolism and/or tissue regeneration, thereby accelerating healing of the tissue [20–24], anti-inflammatory effects on the targeting tissues and cells, as well as reduction of pain of various etiologies [25, 26]. Since it has been recently reported that LLLT can be successfully used as an advanced treatment modality in therapy of RAS, the aim of this study was to determine the clinical effectiveness of laser therapy in treatment of RAS lesions.

Methods

Search strategy

The studies published until 31 December 2013 were obtained from the Medline/PubMed, Science Direct and Cochrane Library of the Cochrane Collaboration (CENTRAL)

online databases, using following search terms and key words: "laser" and "recurrent aphthous stomatitis", "laser" and "aphthous" and "laser" and "aphthae". Screening and study selecting process was performed independently by two authors to avoid the potential for reviewer bias. Further, the references of all selected articles were scanned. The corresponding authors were contacted in case of missing and insufficient data reported originally in studies. The online databases' search initially yielded a total of 228 publications. On the basis of title and abstract evaluation, authors agreed by discussion to exclude 204 publications. Remaining 24 publications in full-text format (relevant or possibly-relevant) were retrieved for more detailed analysis.

Study inclusion and exclusion criteria

The selected publications were further analyzed according to the following inclusion criteria: 1) publication in an international peer reviewed literature; 2) English language publications; 3) randomized controlled clinical trials (RCTs) and/or comparative clinical studies; 4) studies on RAS lesions, regardless clinical subtype (miRAS, maRAS, HU); 5) any type of low-level laser therapy, as an intervention to at least one of the treatment groups; and 6) presence of at least 5 patients in test and/or control group.

The studies that fulfilled the inclusion criteria were further analyzed according to the following exclusion criteria: 1) no definition of inclusion and/or exclusion criteria; 2) no sufficient information on laser parameters' settings; 3) no outcome of interest.

In total, 4 original research articles were identified as appropriate (met the required inclusion/exclusion criteria) for this review. The main outcome measures assessed were a reduction of pain associated with RAS and a reduction in episode duration (faster RAS healing).

Quality assessment

After establishing the scores of quality assessment (Table 1), the overall estimation of risk of bias (low – all of criteria met, moderate – one or more criteria partly met and high – one or more criteria not met) was determined [27] for each selected study (Table 2). A quality assessment of all the selected studies was performed independently by two authors (V. P. and V. VA.).

Table 1

Categories used to assess the quality of the selected studies

Category	Category description	Grading
A	Sample size calculation (minimal number of participants, in order to achieve a stat. significant difference among compared groups)	0 = not mentioned 1 = reported, but not confirmed 2 = reported and confirmed
B	Randomization and allocation concealment methods	0 = clearly inadequate 1 = possibly adequate 2 = clearly adequate
C	Clear definition of inclusion and/or exclusion criteria	0 = no 1 = yes
D	Completeness of follow-up	0 = no 1 = yes
E	Experimental and control group comparable at study baseline	0 = no 1 = unclear 2 = clearly adequate
F	Presence of masking	0 = no 1 = unclear 2 = yes
G	Appropriate statistical analysis	0 = no 1 = unclear 2 = yes

Table 2

Quality assessment of the selected studies

Author and the year of the publication (reference)	A (0–2)*	B (0–2)*	C (0–1)*	D (0–1)*	E (0–2)*	F (0–2)*	G (0–2)*	Estimated risk of bias
Zand et al. 2009. [5]	2	2	1	1	2	0	2	Low
de Souza et al. 2010. [6]	2	2	1	1	2	0	2	Low
Zand et al. 2012. [28]	2	2	1	1	2	0	2	Low
Prasad et al. 2013. [29]	2	2	1	1	2	0	2	Low

***For explanation see Table 1.**

Results

De Souza et al. [6] compared InGaAlP (670 nm) diode laser therapy to conventional therapy (topical corticosteroids-triamcinolone acetonide). Both treatments were applied until the complete resolution of RAS lesions. The study evaluated 20 miRAS patients (12 females and 8 males), with the mean age of 23.65 (Table 3). Laser parameters employed were: power output 50 mW; energy density 3 J/cm^2 per point; and exposure time 1 min. Irradiation was applied daily (once *per* day) on consecutive days. The distance between a laser beam and the mucosa was constant (laser pen touching the surface of the lesion). As the result, a reduction of pain (75% of the patients) in the same session after laser treatment was demonstrated. Further, a total regression of the lesion was achieved 4 days following laser irradiation (40% of the patients) compared to prolonged time (5–7 days) required to obtain the same results in the corticosteroid group. The authors concluded that under the conditions administered in the study, InGaAlP laser therapy can be safely used as the advanced RAS treatment technique in order to achieve immediate analgesia and faster healing of RAS lesions [6].

Other selected studies reported the effects of non-contact, non-ablative CO_2 laser (10,600 nm) therapy on pain relief and faster tissue healing of RAS lesions compared to placebo in patients with 2 miRAS lesions present at the same time (Table 3). Randomly allocated miRAS lesion was treated with laser, while another served as placebo [5, 28, 29]. As a precaution to prevent thermal damage to the mucosa due to the heat produced by CO_2 laser, a thick layer of high water content transparent non-anesthetic gel (3–4 mm thickness) on RAS lesion prior to CO_2 laser irradiation was applied [5, 28, 29]. As the result, authors claimed that no side effects, such as warmth on laser targeted spot, erythema, carbonization or vaporization had been reported [5, 28, 29]. Further, these procedures were pain-free and did not require anesthesia prior to irradiation.

The selected studies used defocused (angulated) handpiece for scanning over RAS lesion at the distance of 5–7 mm (circular motion) for about 5–10 seconds [5, 28, 29]. The study sample size and its characteristics varied from study to study: Zand et al. [5] analyzed 15 patients (13 female and 2 males), with their mean age of 37.9 years, while in another study 10 patients (9 females and one male) with the mean age of 35.6 years were enrolled [28]. Prasad et al. [29] conducted a study on 25 patients, mean age 27.48 years (Table 3).

Regarding the effects of CO_2 laser therapy on pain relief, Zand et al. [5] operated at power of 1 W, while Prasad et al. [29] employed even lower power settings of 0.7 W (Table 4). As the result, Zand et al. [5] reported pain levels of 6.2 ± 1.3 preoperatively and 0.07 ± 0.3 postoperatively (immediately after 4 h, 8 h, 12 h, 24 h, 48 h, 72 h and 96 h following irradiation), which was similar to Prasad et al. [29] pain reports of 8.48 ± 0.71 before treatment and 0.68 ± 0.63 24 h following irradiation. In total, both studies [5, 29] demonstrated similar results measured by the visual analog scale (VAS) in the statistically significant difference in pre- and postoperative pain levels in CO_2 laser group, whereas no such difference was observed in the placebo groups. As the result, the authors suggested the use of CO_2 laser therapy in significant immediate and dramatic reduction of idiopathic and contact RAS pain, with sustained analgesic effect [5, 29]. As for enhanced RAS wound/tissue healing following CO_2 laser therapy, Zand et al. [28] assessed healing every day after irradiation until complete re-epithelization of RAS lesion, while Prasad et al. [29] assessed healing at days 3 and 4 following CO_2 laser irradiation, and every 2 days until 14 days. Zand et al. [28] reported main healing score of 4.8 ± 2.4 days in the laser group compared to 7.6 ± 2.5 days in the placebo group. These results were similar to what Prasad et al. [29] reported: the mean healing score was 4.08 ± 0.81 and 7.84 ± 0.90 days for the laser and placebo treatment groups, respectively. Both authors [28, 29] confirmed statistically significant differences in RAS faster wound healing following CO_2 laser therapy (Table 4).

Table 3

General information on the selected studies

Author and the year of the publication (reference)	Study design/ Number of patients	RAS clinical subtype	Exp.group	Control group
Zand et al. 2009. [5]	RCT (15 patients)	miRAS	Laser	Placebo
de Souza et al. 2010. [6]	Comparative study (20 patients)	miRAS	Laser	Topical corticosteroids (triamcinolone acetonide)
Zand et al. 2012. [28]	RCT (10 patients)	miRAS	Laser	Placebo
Prasad et al. 2013. [29]	Prospective clinical study (25 patients)	miRAS	Laser	Placebo

RCT – randomized controlled clinical trial; miRAS – mirror recurrent aphthous stomatitis.

Discussion

Combined problems of unknown etiology, lack of specific therapy and the frequency of recurrence of RAS have made the management of these patients a difficult problem to general dentist. All the three clinical types of RAS are as-

Table 4

Effects of low-level laser therapy (LLLT) treatment of recurrent aphthous stomatitis (RAS)

Author and the year of the publication (reference)	Laser device (wavelength, emission mode)	Laser parameters	Anaesthesia prior to irradiation	Oral gel prior to irradiation	Laser distance (between laser and RAS lesion)	Laser application	Observation period and follow-up	Treatment outcome
Zand et al. 2009. [5]	CO_2 laser (10.600 nm) continuous emission mode	Power: 1W Irradiation time: 5-10 s	No	Yes	5–6 mm (circular motion)	Single	Before Immediately after, and 4h, 8h, 12h, 24h, 48h, 72h and 96h after irradiation	Immediate pain relief
de Souza et al. 2010. [6]	InGaAlP diode laser (670 nm) continuous emission mode	Power: 50 mW Energy density: 3 J/cm^2 Irradition time: 60 s	No	No	Touching the surface of RAS	Daily (once *per* day) on consecutive days	Before Immediately after irradiation and every day up to 10 days	Immediate pain relief Enhanced healing
Zand et al. 2012. [28]	CO_2 laser (10.600 nm) continuous emission mode	Power: 1W Irradiation time: 5-10 s	No	Yes	5–6 mm (circular motion)	Single	Before Immediately after irradiation and every day until the resolution of signs	Enhanced healing
Prasad et al. 2013. [29]	CO_2 laser (10.600 nm) continuous emission mode	Power: 0.7 W Irradiation time: 5-8 s	No	Yes	5–7 mm (spiral motion)	Single	Pain: Before Immediately after and 24h after irradaition Healing: Before-3-4 days after irradiation and up to 14 days	Immediate pain relief Enhanced healing

sociated with painful, recurrent, single or multiple, shallow necrotizing ulcerations of mucosal tissues. Although patients in most of the cases have spontaneous healing within 14 days, treatment is often indicated to mainly control pain and to reduce the duration and severity of symptomatic outbreaks, especially during the periods of quiescence and exacerbation (period of increased pain and sensitivity) of RAS lesions [30]. Pain usually reduces after 4–5 days, but during this period it can cause discomfort to a patient during eating, swallowing, speaking and wearing dental prostheses [1–5]. Pain control is also very important in order to maintain patient physical and mental condition, further improving effectiveness of the therapy. To date, it is widely accepted that the first-line therapy for patients with RAS are topical corticosteroids, even though the evidence of their efficiency is not overwhelming. Recently, laser as a new treatment modality has been introduced. To date, lasers are widely used in dentistry, namely due to beneficial clinical outcome achieved in shorter time and with a better patient acceptance. Lasers are successfully used in surgical (ablative) and non-ablative (LLLT) manner to treat painful RAS lesions. In ablative manner lasers are used to remove as much of necrotic RAS tissue as possible, including the inflamed halo around the aphthae [31, 32]. Patients feel mild warmth on the targeted place; therefore anesthesia is required prior to irradiation, as a part of surgical procedure. One of the biggest concerns in ablative manner is laser-related hazard-plume (having a potential for carrying viral particles). Also, a potential for pseudoisomorphic (Köebner) phenomenon in susceptible persons with ulcerations (seen in Behcet's disease) after laser irradiation, as trigger, has been mentioned [33]. In contrast to ablative lasers, LLLT is non-destructive, non-thermal and pain free procedure, which usually does not require anesthesia and do not carry any potential of plume hazard to the surgeon and personnel [5]. Further, it does not produce visual effects of thermal damage to the oral mucosa such as ablation, coagulation, vaporization or erythema [5]. Therefore, LLLT is described as more convenient to use, with a fewer possible adverse events and it became a treatment of choice, when it comes to the use of lasers in therapy of RAS.

It is well-known that LLLT causes immediate analgesia in various painful oral lesions [34–36]. For that indication [37] LLLT have been approved for marketing by the U.S. Food and Drug Administration through the premarket notification/510(k). To date, there are several suggested mechanisms for pain reduction following LLLT application, such as effect in modulating key factors of inflammation, reduction of the prostaglandin E_2 level, inhibition of cyclo-oxygenase, and/or lymphocyte metabolism that could lead to reducing of edema, and further reduction of inflammatory processes [38, 39].

Also, release of endogenous pain relievers – endorphins and enkephalins [6], the increase in production of serotonin and suppression of bradikinin activity [5, 40, 41] has been suggested. It has been also shown that laser therapy increases systemic microcirculation by nitric oxide synthesis, causing the reduction in swelling and pain [40]. Even though, there are several potential mechanisms proposed, the real underlying mechanism following laser therapy for pain reduction is yet to be determined. It is believed that not just one, but two or more coexisting mechanisms or their combination are responsible for the beneficial outcome of LLLT in achieving analgesia. Apart from documented analgesic effect, LLLT is successfully applied for tissue healing, mainly due to successful hemostasis, decontamination (sterilization) and anti-inflammatory effect [38]. Further, a potential biostimulation of underlying and surrounding cells, increased collagen organization and promoting of growth factors and cytokines in response to laser irradiation have been demonstrated [19–24].

Although the assessed literature demonstrated significant analgesia and enhanced RAS tissue healing following laser therapy without any reported side effects (Table 4), the results should be interpreted with caution due to insufficient evidence (small number of studies available for evaluation). Firstly, selected studies employed different sample size (number of patients enrolled) with further varieties, such as female/male ratio and patients' main age. Secondly, beneficial results of the laser therapy were only reported on miRAS lesions. The miRAS could be considered as a prototype of painful RAS lesions, but it would be of a great interest to report laser therapy effects in the treatment of the maRAS and HU, too. Thirdly, laser devices employed were different (InGaAlP and CO_2), with different wavelengths and completely different characteristics and biological effects on targeted tissues. Further, laser irradiation protocol (power, dose, observation period/follow up) was also inconsistent. Since there is no firm proof-backed framework for treatment of RAS, it is really difficult to support the effectiveness of any specific laser therapy approach presented, as being superior. In order to determine the real efficacy of laser therapy in treatment of RAS lesions, further carefully designed clinical studies with precise sample standardization (number of patients, gender and age) as well as the type of laser and clinical subtype of RAS should be rigorously studied in order to further evaluate the obtained results.

Conclusion

Low-level laser therapy of selected wavelengths used at the documented energy settings seems to be the appropriate procedure in therapy of recurrent aphthous stomatitis. This fact was evidenced by significant analgesia and enhanced wound healing, without any major adverse effects reported. However, issues related to the design and laser irradiation parameters of a limited number of studies prevent us from making definite conclusions. Therefore, further research, especially long-term follow up randomized control trials with a larger number of patients are required in order to determine the optimal laser therapy protocol in treatment of recurrent aphthous stomatitis.

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Medicinska veštačenja u krivičnom i parničnom postupku

Medical expertise in criminal and civil proceedings

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Uvod

Veštačenja su u svim sudskim postupcima, a posebno u krivičnom i parničnom, veoma važna procesna radnja. Preduzimaju se u istom cilju i pod sličnim uslovima kada je neophodno obezbediti sudu pomoć stručnjaka, koji će svojim znanjem i stručnim autoritetom omogućiti utvrđivanje određenih činjenica koje nisu pravne prirode, ali su važne za razjašnjenje predmeta spora. Zbog toga se u kontinentalnom pravnom sistemu (pa tako i u Republici Srbiji), u posebnoj procesnoj ulozi veštaka, u postupak uključuje stručnjak koji je procesno nezainteresovan za ishod spora sa zadatkom da, u skladu sa pravilima svoje naučne oblasti, stečenim tehničkim znanjima, veštinom kojom vlada ili na osnovu vlastite umetničke orijentacije, ispita objekte veštačenja, zapazi činjenice važne za postupak i nakon toga dâ svoj stručni nalaz i mišljenje [1]. Uloga veštaka u postupku je utoliko značajnija pošto sud ne raspolaže znanjima koje ima veštak (što je i osnovni razlog da ga uključi u postupak), a činjenice utvrđene veštačenjem i dati nalaz i mišljenje veštaka neophodne su za pravilno sudsko odlučivanje u konkretnom sudskom postupku. U anglosaksonskom pravu procesna funkcija veštaka je ista, ali se procesni status menja i približava položaju veštaka u kontinentalnom pravu. Nekada je veštak imao u svemu status svedoka (na šta asocira i sam naziv za veštaka – *expert witness*) koji zahvaljući svom vanpravnom znanju i veštini, određene činjenice koje mu budu predočene, treba da protumači i objasni sudu, strankama i poroti, kako u svom svedočkom iskazu, tako i u izveštaju. Za razliku od ostalih svedoka, on danas više ne podleže tzv. unakrsnom ispitivanju (*cross-exemination*) u sudskoj raspravi, niti je obavezan da joj prisustvuje, nego samo ima dužnost da posle izvršenog veštačenja strankama koje ga angažuju ili sudu podnese svoj izveštaj [2, 3].

Za razliku od ranijih vremena, kada se smatralo da sud raspolaže dovoljnim znanjem da bez pomoći stručnjaka koji se bave drugim profesijama može razjasniti sve odlučne činjenice, danas se različita veštačenja masovno i redovno primenjuju u sudskim postupcima. Tome je doprinelo širenje granica ljudskog saznanja i naučnotehnološki napredak, naročito intenzivan od sredine XX veka. Zbog toga se može reći da su veštaci u savremenom sudskom postupku jednako neophodni kao i pravnici [4].

Medicinska veštačenja su među najčešće zastupljenim u sudskim postupcima. Ona imaju poseban značaj u krivičnim postupcima, pa su tako i u Zakoniku o krivičnom postupku Republike Srbije istaknuta kao posebna vrsta veštačenja [3]. Zakonik u dvadeset članova reguliše veštačenje (čl. 113–126), a posebno se propisuju veštačenja telesnih povreda, veštačenje leša i psihijatrijsko veštačenje (čl. 127–132). U parničnim postupcima se raspravljaju sporovi u vezi sa imovinskim interesima, pa zato medicinska veštačenja kao posebna vrsta veštačenja nisu posebno istaknuta, nego se i na njih primenjuju opšta pravila iz odeljka o veštacima (čl. 259–275) Zakona o parničnom postupku [5, 6]. U parničnim postupcima medicinska veštačenja često se određuju kada se postupak vodi radi naknade nematerijalne štete, ponekad i u bračnim, porodičnim i naslednim sporovima.

Bez obzira na to da li se preduzimaju u krivičnom ili parničnom postupku, veštačenja podležu istim procesnim pravilima, koja s jedne strane određuju procesnu ulogu veštaka, uslove za određivanje veštaka, njegova prava i dužnosti, a sa druge strane procesne faze u kojima se veštaci mogu uključiti u postupak. Zato, potrebno je ukratko izložiti formalne uslove koje stručnjaci moraju ispuniti da bi mogli učestvovati kao veštaci u postupku i dati pregled njihovih prava i obaveza, pre nego što se izlože specifičnosti medicinskih veštačenja u krivičnom i parničnom postupku.

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Formalni uslovi

Veštak je poseban, nepristrasni učesnik u sudskom postupku koji se u postupak uključuje po sudskoj naredbi, kada sud oceni da je radi utvrđivanja ili razjašnjavanja neke činjenice potrebno stručno znanje kojim ne raspolaže. Veštak treba da se izjasni o činjenicama koje postoje u vreme vođenja postupka i koje je zapazio u postupku na osnovu svog stručnog znanja.

Sud određuje veštačenje na zahtev stranaka ili po službenoj dužnosti, s tim što se u parničnom postupku češće dešava da stranka predlaže izvođenje dokaza veštačenjem i konkretnog veštaka. Ako se druga strana saglasi sa tim predlogom, izvrši se veštačenje, a stranka koja je predložila veštaka prilaže njegov pisani nalaz i mišljenje (čl. 260 i 261 Zakona o parničnom postupku). Troškove veštačenja snosi stranka koja je predložila veštačenje i veštaka. Izuzetno, sud će po službenoj dužnosti odrediti veštačenje. Zbog toga što se u krivičnom postupku pojedincu sudi u javnom interesu za krivično delo koje mu se optužbom stavlja na teret, sud (ili organ koji vodi tzv. pretkrivični postupak) češće određuje veštačenje po službenoj dužnosti, ali to može učiniti i na predlog stranke, dok se troškovi veštačenja nadoknađuju iz budžetskih sredstava (a kasnije mogu pasti na teret okrivljenog, ako bude osuđen za krivično delo). Novina predviđena u članovima 125 i 126 Zakonika o krivičnom postupku jeste da stranka može angažovati vlastitog stručnog savetnika. To je osoba koja raspolaže odgovarajućim stručnim znanjem, a stranka ga opunomoćuje da prisustvuje veštačenju koje veštak preduzima, razgleda predmet veštačenja, predlaže veštaku da izvede određene radnje, daje primedbe na nalaz veštaka i potom da u ime stranke postavlja pitanja veštaku. Stručni savetnik se tako javlja u ulozi posebnog veštaka koga imenuje jedna od stranaka i svojim procesnim angažovanjem doprinosi kontradiktornosti sudskog postupka [7, 8].

Procesna uloga veštaka zahteva da se pravilima postupka obezbede njegov nezavisan položaj i nepristrasnost. Prvi uslov za obezbeđivanje takve pozicije veštaka jeste njegova stručna sposobnost, jer mora raspolagati stručnim znanjem potrebnim za utvrđivanje činjenica i davanje mišljenja o njima. Prema Zakonu o sudskim veštacima (čl. 6) imenovanje veštaka je uslovljeno visokim obrazovanjem i radnim iskustvom u struci od najmanje pet godina, a pored toga se zahteva stručno znanje, praktično iskustvo i dostojnost [9].

Dodatno, stručnjak mora ispunjavati pravne uslove da se kao veštak uključi u sudski postupak. Ti uslovi treba da otklone svaku sumnju u moguću pristrasnost stručnjaka koji bi veštačio u konkretnom slučaju. Zbog toga je veštak zamenljiv, tj. ako konkretni stručnjak iz zakonom predviđenih razloga ne bi smeo da bude određen za veštaka, sud će veštačenje poveriti drugom stručnjaku. Pravni uslovi su negativno određeni Zakonikom o krivičnom postupku. Tako je isključena mogućnost da se kao veštak u konkretnom predmetu odredi: lice koje po zakonu ne može biti ispitano kao svedok (a tu spada i lekar, ako bi svedočenjem povredio profesionalnu tajnu, sem ako te obaveze odlukom nadležnog tela ne bi bio oslobođen), lice koje je oslobođeno dužnosti svedočenja (ako je lekar bračni drug okrivljenog ili sa njim živi u vanbračnoj ili drugoj trajnoj zajednici ili je njegov bliski srodnik, usvojenik i usvojilac) i lice prema kome je krivično delo učinjeno. Više se izričito ne precizira da lekar koji je lečio umrlog ne sme obavljati pregled i obdukciju leša, pošto je ta mogućnost isključena članom 129 stav 1 Zakonika o krivičnom postupku u kome je propisano da se veštačenja ove vrste poveravaju isključivo specijalistima sudske medicine.

Češće se u praksi događa da se neki od razloga zbog kojih se može posumnjati u nepristrasnost konkretnog veštaka naknadno sazna, pošto je već određen da veštači. U ovom slučaju stranke mogu da traže izuzeće tog veštaka pozivajući se na zakonom predviđene razloge. Radi se o situacijama u kojima se stručnjak koga je sud odredio da veštači u konkretnom slučaju nalazi u sukobu interesa pa se može steći utisak da će lično biti zainteresovan za ishod postupka. Na primer, ako je u bračnom, vanbračnom odnosu ili u srodstvu sa okrivljenim ili oštećenim, ako je zaposlen kod njih ili je zajedno sa njima ili nekim od njih zaposlen kod istog poslodavca. Takođe, veštak po pravilu ne može biti svedok u tom istom suđenju.

Sa svoje strane i veštak je u obavezi da sudiji iznese razloge zbog kojih bi se morao izuzeti od veštačenja u konkretnom slučaju, čim sazna za takve razloge. Potrebno je da ukaže čak i na one posredne razloge koji bi možda mogli uticati na njegov nepristrasan sud (na primer, zbog tazbinskog srodstva sa okrivljenim ili oštećenim, prijateljstva i slično), jer bi kada saznaju za takve razloge stranke mogle tražiti izuzeće veštaka ili kasnije osporavati nalaz i mišljenje veštaka i samu sudsku presudu ako bude zasnovana na nalazu veštaka koji se morao izuzeti. Slični uslovi za oslobođenje od dužnosti veštačenja i izuzeće veštaka kao u krivičnom postupku, predviđeni su u članovima 265 i 266 Zakona o parničnom postupku.

Kako je osnovno pravo veštaka da se upozna sa podacima koji su potrebni za davanje tačnog i potpunog nalaza i mišljenja, to se zakonima predviđaju posebne obaveze suda da veštaku pruži potrebna razjašnjenja, omogući da razgleda predmet veštačenja, prisustvuje izvođenju pojedinih radnji i postavlja pitanja strankama ili drugim učesnicima u postupku. Pored ovih procesnih prava, veštak ostvaruje pravo na naknadu troškova, izgubljene zarade i nagradu.

Dužnosti veštaka su da se odazove pozivu suda i podnese nalaz i mišljenje (ponekad se može tražiti i samo jedno od toga). U krivičnom postupku veštak koji prvi put u tom svojstvu učestvuje dužan je da položi zakletvu. Veštak je uvek u obavezi da se odazove pozivu suda (čak i kada će potom izneti razloge zbog kojih se mora izuzeti), a ako prihvati veštačenje, treba da rezultate veštačenja na vreme dostavi sudu, kako ne bi doveo u pitanje efikasnost sudskog postupka. U skladu sa Preporukom Saveta Evrope broj R (84) 4 o načelima građanskog postupka za unapređivanje ostvarivanja pravde od 28. februara 1984. godine u Zakonu o parničnom postupku je predviđeno da veštak može biti obavezan da naknadi troškove koje je prouzrokovao svojim neopravdanim nedolaskom ili neopravdanim odbijanjem da vrši veštačenje [10]. Ako veštak u (naknadnom) roku koji mu sud odredi ne dostavi rezultate veštačenja, mogao bi da bude kažnjen procesnom novčanom kaznom, a sud može obavestiti ministarstvo nadležno za pravosuđe i tražiti brisanje imena te osobe iz registra sudskih veštaka.

Zbog specifičnosti krivičnog postupka, prilikom obavljanja veštačenja Zakonik o krivičnom postupku po članu 114 nalaže sudiji da prednost da stručnjacima iz stručne ustanove za veštačenja ili državnog organa koji raspolaže uslovima za vršenje određenih veštačenja. Ako nema tih mogućnosti, sud angažuje stalne sudske veštake odgovarajuće struke, upisane na listu stalnih sudskih veštaka. Tek ako za tu vrstu veštačenja kod suda ne postoje stalno određeni veštaci, može sud odrediti i druge stručnjake kao veštake, ali samo ako postoji opasnost od odlaganja, ili su stalni sudski veštaci sprečeni, ili ako to zahtevaju neke druge okolnosti. U parničnom postupku važe blaža pravila: po članu 264 Zakona o parničnom postupku sud određuje veštaka iz registra sudskih veštaka, a ako nema takvog upisanog veštaka, veštačenje se može poveriti licu odgovarajuće struke.

Postupak veštačenja

Zadatak da obavi veštačenje stavlja lekara u drugačiju poziciju u odnosu na njegov redovni posao, jer činjenica da se veštačenje obavlja za potrebe sudskog postupka čini bitnu razliku od uobičajene medicinske delatnosti. U stranoj literaturi jasno se ukazuje na to da lekar koji bude angažovan kao veštak, u suštini obavlja svoj posao prilikom veštačenja, ali je razlika u tome što svaka njegova dijagnoza, nalaz i mišljenje (koji imaju medicinsku sadržinu) sada dobijaju pravnu funkciju i postaju dokumenti pravno relevantni u sudskom sporu i to, kako se naglašava u nemačkoj literaturi, u naročitim formama priznatog zdravstvenog sertifikata u krivičnom postupku ili privatne isprave u građanskom postupku [11, 12]. Prema tome, specifičnost se ogleda u tome što medicinski veštak određene činjenice sagledava, sintetiše i izlaže u formi prilagođenoj potrebama suda i baš zbog toga podleže posebnoj ekspertiznoj etici kojom se štite čovek i društvo, a koja je vezana za stručnu istinu [13]. Zato se i u zakonskim pravilima koja regulišu izbor veštaka i postupak veštačenja toliko insistira na uslovima kojima se obezbeđuje nezavisan položaj i nepristrasnost veštaka, kako bi bio u stanju da dâ što objektivniji sud, prema vlastitom znanju i iskustvu. Pri tome, zakoni regulišu samo organizaciju veštačenja, dok se veštačenje sprovodi po pravilima medicinske nauke i struke.

Sud treba da označi predmet koji veštaci treba da opaze i ocene i postavi tačna pitanja na koja treba da odgovore. Veštak je ovlašćen da od suda traži preciziranje svog zadatka, jer se često u parničnoj praksi dešava da sudije ne odrede dobro predmet veštačenja ili ne izvrše nužnu pripremu predmeta za veštačenje (utvrđivanje određenih činjenica, pribavljanje medicinske dokumentacije i slično), što dovodi do lošeg veštačenja. Uprkos procesnom pravilu da se veštačenje preduzima u postupku u prisustvu suda i zapisničara, medicinska veštačenja se po pravilu odvijaju van prostorija suda i često se, u krivičnim postupcima, poveravaju stručnjacima iz određene ustanove (što se po samom zakonu smatra pouzdanijim veštačenjem nego kad ga obavlja pojedinac). Zbog toga što neka veštačenja (na primer, psihijatrijska posmatranja) mogu da dovedu do znatnih ograničenja prava okrivljenog, u Zakoniku o krivičnom postupku, u članu 122, određeno da je se prema okrivljenom može odrediti mera smeštaja u zdravstvenu ustanovu radi neophodnih medicinskih ispitivanja, koja može trajati najduže petnaest dana (sa mogućnošću naknadnog produženja za još petnaest dana). Ispitivanje veštaka je poslednja faza u kojoj, po dozvoli suda, mogu učestvovati okrivljeni i oštećeni sa svojim pitanjima.

Unutrašnji postupak veštačenja odvija se u svemu autonomno, po pravilima medicinske nauke i struke. Rezultate izvršenog veštačenja veštak predočava sudu u pisanoj formi nalaza i mišljenja. Kada veštak dostavi sudu nalaz i mišljenje koji su nejasni, nepotpuni i protivrečni sami sebi ili utvrđenim okolnostima, sud određuje veštaku da ih dopuni ili ispravi. Sud nije vezan ocenom i mišljenjem veštaka (iako u praksi preovladava stav da se sud priklanja mišljenju veštaka, pa se ponekad stiče utisak da veštak „sudi“). Moguće je da se pokaže da veštak nije razjasnio sva sporna pitanja ili se posumnja u rezultate veštačenja. U tom slučaju sud može odrediti novo veštačenje koje se poverava drugom veštaku ili ustanovi.

Specifičnosti medicinskih veštačenja u krivičnom i građanskom postupku

Zakonik o krivičnom postupku sadrži posebne odredbe koje se odnose na veštačenja leša, telesnih povreda i veštačenje psihijatrijskim pregledom okrivljenog (čl. 127– 132). Osim toga, lekar može biti angažovan da izvrši telesni pregled osumnjičenog lica, svedoka ili nekog drugog lica (član 134).

Veštačenje leša obavlja lekar specijalista sudske medicine. Veštačenje podrazumeva pregled leša i obdukciju, a po potrebi veštačenju može prethoditi ekshumacija. Kada se obdukcija vrši van stručne ustanove, leš će pregledati i obducirati po potrebi dva ili više lekara. Na osnovu člana 129. Zakonika ovakvo veštačenje se obavezno preduzima u slučaju sumnje da je smrt posredno ili neposredno posledica krivičnog dela, ako je u trenutku smrti lice bilo lišeno slobode ili identitet leša nije poznat. U svim takvim zakonom predviđenim slučajevima obdukcija je obavezna, pa se zato sa pravom ukazuje na propuste u dosadašnjoj sudskoj praksi kada sud prihvati predlog veštaka koji svoj nalaz zasnuje samo na osnovu pregleda leša, bez obdukcije ili bez potpune obdukcije [14].

Zbog značaja utvrđivanja uzroka smrti za procenu postojanja radnje krivičnog dela, u Zakoniku o krivičnom postupku se precizira sadržina mišljenja koje veštak treba da dâ na osnovu pregleda i obdukcije leša. Zahteva se izjašnjavanje o neposrednom uzroku smrti, vremenu nastupanja smrti, objašnjenjima u vezi sa uočenim telesnim povredama na lešu (kojim su sredstvom i na koji način mogle nastati, da li je i koja od više mogućih povreda dovela do smrti ili je do toga došlo njihovim ukupnim delovanjem), kao i da li je do smrti došlo zbog ličnog svojstva, naročitog stanja organizma povređenog ili zbog slučajnih okolnosti ili okolnosti pod kojima je povreda naneta i da li je blagovremeno pružena pomoć mogla sprečiti smrtni ishod. Zakonikom o krivičnom postupku predviđena je dužnost veštaka da prilikom pregleda i obdukcije leša obrati pažnju na pronađeni biološki materijal, da ga opiše i sačuva za kasnije biološko veštačenje, poput forenzičko-genetičkog (DNK) veštačenja. Zakonik, takođe, detaljno precizira koje je činjenice neophodno utvrditi prilikom pregleda i obdukcije začetka ili leša novorođenčeta ili u slučaju sumnje da je smrt nastupila trovanjem.

Veštačenje telesnih povreda obavlja se po pravilu telesnim pregledom povređenog, a ako to nije moguće ili nije potrebno, na osnovu medicinske dokumentacije ili drugih podataka u spisima. Veštak treba tačno da opiše povrede i da svoje mišljenje o vrsti i težini svake pojedine i o njihovom ukupnom dejstvu s obzirom na njihovu prirodu ili posebne okolnosti slučaja. Potrebno je da veštak ukaže na to kakvo dejstvo takve telesne povrede obično proizvode, a kakvo su imale u konkretnom slučaju. Osim toga, veštak treba da opiše sredstvo kojim su povrede nanete i način na koji su nastale. Uprkos čestom insistiranju u sudskoj praksi, veštak nije dužan da se izjašnjava da li povreda predstavlja laku ili tešku (prema zakonu), jer medicinskih kriterijuma za ovakvo razgraničenje nema. Zakonik predviđa obavezu veštaka da od povređenog izuzme biološki materijal neophodan radi kasnijih veštačenja.

Veštačenje psihijatrijskim pregledom okrivljenog sprovodi se u slučaju sumnje u neuračunljivost učinioca (zbog toga što su prilikom izvršenja krivičnog dela bile isključene njegove mogućnosti da shvati šta čini ili da upravlja svojim postupcima zbog duševne bolesti, privremene duševne poremećenosti, zaostalog duševnog razvoja ili druge teže duševne poremećenosti). Ovakvo veštačenje se po pravilu obavlja u odgovarajućoj zdravstvenoj ustanovi u koju se okrivljeni u toku postupka upućuje na određeno vreme radi posmatranja. Veštak treba da ustanovi da li je duševno stanje okrivljenog poremećeno ili ne. U slučaju da se utvrdi postojanje te poremećenosti, veštak treba da se izjasni o prirodi, vrsti, stepenu i trajanju poremećenosti i u svome mišljenju da odredi u kojoj meri je poremećaj postojao u vreme izvršenja krivičnog dela i uticao na smanjenje psiholoških funkcija okrivljenog. Konstatovani duševni poremećaj koji isključuje intelektualne ili voluntativne sposobnosti učinioca omogućava procenu suda da zbog neuračunljivosti učinilac nije kriv za učinjeno delo, a tada se smatra da krivičnog dela nema i da se prema učiniocu ne može primeniti kazna (nego mere bezbednosti obaveznog psihijatrijskog lečenja), što se smatra povoljnijim po okrivljenog. Osim kod procene neuračunljivosti, psihijatrijska veštačenja se u krivičnom postupku često koriste kada je potrebno ustanoviti da li je krivično delo učinjeno na mah. Radi se o izvršenju krivičnog dela u afektu, stanju povećane emotivne razdraženosti izazvane stimulusom u vidu prethodnog fizičkog napada ili verbalne provokacije koje je preduzeo oštećeni prema učiniocu. U takvim slučajevima je psihijatrijskim veštačenjem neophodno odrediti da li je kod osobe postojao tzv. normalni ili patološki afekt, koji isključuje uračunljivost. Moguće je da psihijatrijsko veštačenje bude određeno radi provere sposobnosti okrivljenog da učestvuje u postupku ili svedoka da svedoči, ali su to ređe situacije u praksi.

Pokazuje se da od rezultata psihijatrijskog veštačenja zavisi da li će učinilac biti kažnjen za izvršeno krivično delo ili će se ostvariti uslovi za primenu blaže kazne ili mera bezbednosti prema njemu. Iz toga proizilazi praktični značaj psihijatrijskih veštačenja čiji rezultati mogu biti zloupotrebljeni, kako pokazuju primeri neetičnih (a ponekad moralno opravdanih) postupaka psihijatara koji su svojim „uslužnim dijagnozama“ pomagali učiniocima optuženima za ratne zločine na prostoru bivše Jugoslavije da izbegnu krivičnu odgovornost [15]. Istini za volju mora se naglasiti da je uloga psihijatrije u pravu kontroverzna kako zbog bliske povezanosti kliničke i sudske psihijatrije koja se istorijski iz nje izdvaja (ali zadržava sa njom znatne sličnosti), tako i zbog različitog shvatanja pojmova duševnog zdravlja i duševnog poremećaja u psihološkoj, psihijatrijskoj literaturi i pravu [16]. Tako je, na primer, brojna prelazna stanja između stanja duševnog zdravlja i duševnog poremećaja koja su dobro poznata u kliničkoj praksi neophodno u pravu svrstati samo u jednu jedinu kategoriju bitno smanjene uračunljivosti. Otuda veštaci ponekad imaju potrebu da se upuste u pravnu ocenu i često se izjašnjavaju da je kod učinioca postojalo stanje „smanjene uračunljivosti, ali ne bitno“, što je zakonski nepostojeća kategorija uračunljivosti. Drugi problem se tiče profesionalne etike. Veštak psihijatar se često nalazi u protivrečnoj ulozi „dvostrukog agenta“, naročito ako je angažovan u pretkrivičnom postupku kada treba otkriti učinioca po profilu ličnosti. Tada je, s jedne strane, upućen na to da izgradi odnos poverenja sa pacijentom koji je objekt veštačenja kako bi dao tačnu dijagnozu, a sa druge strane, taj odnos iskorišćava da bi svojim nalazom i mišljenjem pomogao sudu, pred kojim je njegov pacijent najčešće u ulozi osumnjičenog ili okrivljenog [17].

U Zakoniku o krivičnom postupku, član 134, preciziraju se uslovi pod kojima se može izvršiti tzv. uviđaj lica (veštačenje na osnovu telesnog pregleda). Telesni pregled okrivljenog može se preduzeti i bez njegovog pristanka, ako je potrebno da se utvrde činjenice važne za krivični postupak. I druga lica mogu biti bez svog pristanka podvrgnuta telesnom pregledu, ali pod uslovom da se mora utvrditi da li se na njihovom telu nalazi određeni trag ili posledica krivičnog dela. Ta druga lica, po pravilu, su oštećeni ili svedoci.

Prilikom pregleda tela okrivljenog ili drugih lica zdravstveni radnik može i bez njihovog pristanka uzeti uzorke biološkog porekla, pod uslovom da time ne bi bila prouzrokovana šteta po njihovo zdravlje (član 141, stavovi 1 i 2). Takođe, po članu 142 Zakonika o krivičnom postupku, javni tužilac ili sud mogu odrediti uzimanje uzorka za forenzičko-genetičku analizu sa tela okrivljenog, oštećenog ili drugih lica povezanih sa krivičnim delom.

Prisilni telesni pregled, a pogotovu uzimanje uzoraka radi analize, spada u postupke kojima se zadire u tuđ telesni integritet, zbog čega se može otvoriti pitanje odgovornosti kako onoga ko takve mere neosnovano određuje, tako i onoga ko ih preduzima. U našoj sudskoj praksi nije bilo takvih primera, ali u nemačkoj upravno-sudskoj praksi utvrđeni su kriterijumi kada se smatraju opravdanim ovakvi zahvati u tuđ telesni integritet. Ističe se da takve mere mogu biti određene samo onda kada su usmerene na ostvarenje zakonom predviđenih ciljeva, potrebne i opravdane jakim stepenom sumnje u okrivljenog i značajem stvari (BverfGNJW 1970, 505). Primera radi, dopušta se prinudno uzimanje uzoraka krvi, urina, kose ili ispljuvka ali se nedopuštenim smatraju postupci koji su opasni po zdravlje poput: angiografije, uzimanja likvora ili uzimanja urina uz upotrebu katetera [12].

U parničnim postupcima od najvećeg značaja su medicinska veštačenja nematerijalne štete koja bude pretrpljena telesnom povredom. Medicinski supstrat nematerijalne štete čine fizički bol, strah, duševni bolovi (psihičke patnje), kao propratne pojave uz povredu ili oboljenje koje dovode do prava na

naknadu nematerijalne štete koja se priznaje u našem pravu (za slučajeve povreda prava na život, na telesni integritet ili slobodu) [18]. Medicinsko veštačenje obavlja se u parnici pregledom oštećenog, pregledom prateće medicinske dokumentacije, na osnovu podataka u sudskim spisima, podataka koji se saznaju od oštećenog i drugih. Predmet veštačenja su najčešće postojanje, intenzitet i trajanje posledica koje je oštećeni trpeo zbog nastale povrede ili bolesti. To pretpostavlja utvrđivanje uzročne veze između povrede i štetnih posledica koje su pravno relevantne. Vrste nastalih posledica zavise od karaktera same povrede, a na njihov intenzitet mogu uticati dodatne komplikacije tokom lečenja, kao i lična svojstva povređenog i ranija oboljenja i degenerativne promene. Potrebno je veliko stručno iskustvo veštaka da opredeli vrstu štetnih posledica u saglasnosti sa zakonskom terminologijom i da što je moguće objektivnije dâ svoj nalaz, jer se susreće sa fenomenom da svaka oštećena osoba subjektivno doživljava promene svog zdravstvenog stanja uz odnosu na stanje pre povrede, a neretko su, iz lukrativnih razloga, prisutne pojave simulacije i agravacije [19, 20]. Čak i da toga nema, obim štetnih posledica u vidu nematerijalne štete kod sličnih povreda različitih osoba varira u zavisnosti i od njihovog životnog doba, psihičkog i društvenog statusa, obrazovnog nivoa i profesije, životnih navika i slično.

Kada su u pitanju veštačenja duševnih bolova zbog umanjene životne aktivnosti, potrebno je posledice povrede ili obolevanja individualno proceniti u odnosu na ranije psihofizičke karakteristike i životnu aktivnost oštećenog. Zbog toga se preduzimaju detaljni klinički pregledi oštećenog kako bi se u meri u kojoj je to moguće postigla objektivizacija posledica povrede (naročito na kostima, zglobovima, mišićima), a potom ustanovilo postojanje umanjenja životne aktivnosti i intenzitet drugih oblika nematerijalne štete (pretrpljenih fizičkih i duševnih bolova ili straha).

Poneki oblici nematerijalne štete mogu nastati nezavisno od somatske povrede. Sa druge strane, psihosomatska oboljenja u kasnijoj fazi mogu dovesti do značajnih psihičkih promena (šećerna bolest, angina pektoris, infarkt miokarda, moždani insulti itd.).

Drugo područje aktivnosti medicinskih veštaka u parničnim postupcima tiče se procene testamentarne sposobnosti ostavioca u naslednim sporovima. Veštačenje koje se obavlja na osnovu medicinske dokumentacije i drugih raspoloživih podataka ima za cilj da se naknadno utvrdi da li je u momentu izjavljivanja poslednje volje testator bio sposoban za rasuđivanje.

Odgovornost veštaka

U davanju svog stručnog nalaza i mišljenja veštak je ograničen samo svojom strukom, znanjem i principima profesionalne etike. Da bi sa uspehom mogao da odgovori svojim obavezama u sudskom postupku, zaštićen je imunitetom, pa tako ne može biti tužen zbog svojih zaključaka i mišljenja. Imunitet veštaka se tradicionalno prihvata kako u kontinentalnom pravu, tako i u anglosaksonskom, ali u novije vreme trpi izvesne korekcije.

Da bi izbegli vlastitu odgovornost u engleskom i američkom pravu veštaci se mogu pozivati na princip imuniteta svedoka, koji zbog svog svedočenja ne treba da bude izložen krivičnom gonjenju, niti tužen u parničnom postupku. Smatralo se da bi uvođenje ovakve odgovornosti dovelo do autocenzure kod svedoka (a veštaci su svedoci-stručnjaci), pa bi oklevali da daju iskaz, čak i kada su na to zakletvom obavezani ili bi davali pristrasan ili neodređen iskaz i tako onemogućili da se utvrde istinite činjenice. U sudskoj praksi dešava se, međutim, da veštaci koji daju pogrešni nalaz budu gonjeni za krivokletstvo ili tuženi za pričinjenu štetu. Vrhovni sud Sjedinjenih Američkih Država izjasnio se da nehotično dat pogrešni nalaz, a posebno razlika u mišljenjima veštaka i drugih stručnjaka nije dovoljna da se uspostavi odgovornost veštaka zbog kršenja standarda profesionalne dužne pažnje. Ipak, u novijoj američkoj sudskoj praksi od 1999. važi pravilo Wintoll koje promoviše obavezu veštaka da iznese pouzdano svedočenje, pri čemu se odgovornost veštaka za štetu temelji na propustima u vezi sa vršenjem svog zanimanja, a ne na davanju lažnog iskaza. Pravilo nazvano po prezimenu tužioca postavljeno je u sudskom slučaju LLMD of Michigan et al. v. Jackson-Cross et al. u kome se sudilo zbog propusta kod finansijskog veštačenja, ali se kao precedent odnosi i na sve druge vrste veštačenja. Odgovornost veštaka zavisi od toga da li ga angažuje stranka po ugovoru kao neku vrstu stručnog savetnika ili ga određuje sud. Ako prekrši obaveze iz ugovora i pričini stranci koja ga je angažovala štetu, medicinski veštak odgovara prema pravilima koja inače važe pri proceni odgovornosti za štete zbog lekarske greške [21]. Međutim, stranke često nemaju uspeha sa tužbom istaknutim protiv veštaka zbog lekarske greške koju je učinio pri veštačenju jer kod *ad hoc* veštačenja nedostaje prethodni poverljiv odnos pacijenta i lekara (što je uslov za odgovornost zbog pogrešnog lečenja), pa tako ne može biti obavezan da naknadi štetu psihijatar ako pogrešno proceni opasno stanje psihotičnog pacijenta koji nakon kratkog vremena ubije nekoga [11]. Podsetićemo da najnoviji sličan primer iz francuske sudske prakse ukazuje na suprotan stav: decembra 2012. godine u Tuluzu osuđena za ubistvo iz nehata na jednogodišnju uslovnu kaznu zatvora Danijel Kanareli, psihijatar sa tridesetogodišnjim iskustvom, čiji je pacijent Žoel Gajar 2004. godine sekirom ubio čoveka u Gornjim Alpima u Francuskoj. Sudije su zaključile da je u svom radu učinila „tešku grešku“ jer nije prepoznala da njen pacijent može da predstavlja pretnju za okolinu. Za razliku od ugovorne odgovornosti medicinskog veštaka koga stranka privatno angažuje, odgovornost sudskog veštaka u angloameričkom pravu temelji se na krivici i procenjuje prema pravilima deliktnog prava. On bi mogao da bude obavezan na naknadu štete svakom onom licu koga je svojim nalazom oštetio, ali samo ako je postupao namerno ili sa krajnjom nepažnjom [22, 23].

Kao u angloameričkom pravu i u kontinentalnom pravu germanske varijante (sa kojom srpsko pravo ima velikih sličnosti) veštak je u principu zaštićen imunitetom koji ga štiti od klevete ili odmazde stranke po koju su rezutati veštačenja bili nepovoljni. Ipak, medicinski veštak može odgovarati za štetu koju drugome pričine pogrešnim i neblagovremenim veštačenjem, ali samo ako je postupao sa namerom i krajnjom nepažnjom. Ako je angažovan na zahtev stranke, nalogodavcu veštak odgovara zbog povrede ugovora. Drugima

odgovara po deliktnom pravu (uključujući tu i situacije u kojima štetu pričini odugovlačenjem sa podnošenjem izveštaja o veštačenju) [24].

Krivičnopravna odgovornost veštaka mogla bi se uspostaviti zbog očigledno nezakonitog postupanja medicinskog veštaka, recimo, u slučajevima krivokletstva, kada izdaje ispravu sa neistinitim podacima, kada primi mito da bi dao pristrasan nalaz, narušava privatnost stranke ili povređuje dužnost čuvanja tajne koju sazna tokom veštačenja i slično. Takvi postupci redovno predstavljaju teška kršenja pravila sadržanih u etičkim kodeksima stručnih udruženja veštaka i dovode i do disciplinske odgovornosti veštaka.

Zaključak

I u krivičnom i u građanskom postupku medicinska veštačenja predstavljaju važna dokazna sredstva. Pod sličnim zakonskim uslovima veštaci se uključuju u oba postupka, a rezultat svoga rada na isti način prezentuju sudu u pisanoj formi. Uspešno veštačenje pretpostavlja da lekar, osim stručnih znanja i iskustva, dobro poznaje zakonsku regulativu iz ove oblasti. S obzirom na specifičnost predmeta sudskog postupka, u Zakoniku o krivičnom postupku regulisane su pojedine vrste medicinskih veštačenja, uključujući i obavezni sadržaj nalaza i mišljenja. U građanskim postupcima medicinska veštačenja se najčešće preduzimaju u sporovima radi naknade nematerijalne štete, kada je potrebno proceniti postojanje fizičkih ili duševnih bolova ili straha i „izmeriti" njihov intenzitet u skladu sa okolnostima pod kojima je došlo do povređivanja i ličnim svojstvima oštećenog. Zbog značaja svoje procesne funkcije kojom doprinose utvrđivanju istine, veštacima se formalno garantuje nepristrasan položaj u postupku i zaštićeni su imunitetom u pogledu mišljenja i nalaza koji daju. Imunitet se, međutim, ne proteže na namerno saopštavanje pogrešnih podataka, namerno davanje pogrešnog nalaza, primanje mita i druge nezakonite postupke kojima veštak može učiniti krivično delo. Za štetu koju učini drugom veštak može odgovarati po ugovoru ili na osnovu pravila deliktnog prava pod uslovom da je namerno pogrešio u veštačenju ili da se krajnje neobazrivo odnosio prema tom poslu (što može da uključi kašnjenje sa dostavljanjem rezultata veštačenja).

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CASE REPORTS

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Posterior reversible encephalopathy syndrome – A case report

Sindrom posteriorne reverzibilne encefalopatije

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Abstract

Introduction. Posterior reversible encephalopathy syndrome (PRES) is characterized by the following symptoms: seizures, impaired consciousness and/or vision, vomiting, nausea, and focal neurological signs. Diagnostic imaging includes examination by magnetic resonance (MR) and computed tomography (CT), where brain edema is visualized bilaterally and symmetrically, predominantly posteriorly, parietally, and occipitally. **Case report.** We presented a 73-year-old patient with the years-long medical history of hipertension and renal insufficiency, who developed PRES with the symptomatology of the rear cranium. CT and MR verified changes in the white matter involving all lobes on both sides of the brain. After a two-week treatment (antihypertensive, hypolipemic and rehydration therapy) clinical improvement with no complications occurred, with complete resolution of changes in the white matter observed on CT and MR. **Conclusion.** PRES is a reversible syndrome in which the symptoms withdraw after several days to several weeks if early diagnosis is made and appropriate treatment started without delay.

Key words:
brain diseases; syndrome; diagnosis; treatment outcome.

Apstrakt

Uvod. Sindrom posteriorne reverzibilne encefalopatije (PRES) karakterišu sledeći simptomi: epi napadi, poremećaji svesti i/ili vida, povraćanje, mučnina i fokalni neurološki znaci. Dijagnostičko snimanje obuhvata pregled magnetnom rezonancijom (MR) i kompjuterizovanom tomografijom (CT) gde se moždani edem vizuelizuje bilateralno i simetrično, preovlađuje posteriorno, parijetalno i okcipitalno. **Prikaz bolesnika.** Prikazali smo bolesnika starog 73 godine, dugogodišnjeg hipertoničara i bubrežnog bolesnika kod koga se razvio PRES sa simptomatologijom zadnje lobanjaske jame i verifikovanim promenama moždane bele mase na CT i MR, obostrano na svim režnjevima mozga. Nakon dve nedelje lečenja (antihipertenzivi, hipolipemici i rehidraciona terapija) nastupilo je kliničko poboljšanje bez ikakvih komplikacija i sa kompletnom rezolucijom promena bele moždane mase viđenim na CT i MR. **Zaključak.** PRES je reverzibilni sindrom čiji simptomi se povlače za nekoliko dana do nekoliko nedelja, ukoliko se rano postavi dijagnoza i odgovarajuće lečenje počne bez odlaganja.

Ključne reči:
mozak, bolesti; sindrom; dijagnoza; lečenje, ishod.

Introduction

Posterior reversible encephalopathy syndrome (PRES) is characterized by the following symptoms: epileptic seizures, consciousness impairment, visual abnormalities, nausea, vomiting and focal neurological signs. It was first described by Hinchey et al. [1] in 1996. based on the study of 15 cases. Since then this syndrome has been also designated by reversible posterior leukoencephalopathy syndrome, reversible posterior cerebral edema syndrome and reversible occipital parietal encephalopathy [2–5].

Studies have shown that various conditions can lead to development of PRES, hypertension, autoimmune diseases, toxic agents, sepsis, preeclampsia/eclampsia, kidney diseases being among them [1, 6]. Regardless to this heterogeneity main pathophysiological mechanism which leads to development of this syndrome is cerebral vasogenic edema which occurs as a result of abnormality in blood flow through the brain – cerebral blood flow (CBF) [7].

Diagnostical imaging includes magnetic resonance imaging (MR) and, less commonly, computed tomography (CT), where cerebral edema is visualized bilaterally and symetrically

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predominantly in posterior parietally and occipitally, but frontal and temporal lobes can also be affected, as well as basal ganglia, brainstem and cerebellum. Also, alongside with the white matter, cortical gray matter can be affected as well [8].

The diagnosis of PRES is complicated, since CT results are often normal or non-specific, and MR scanners not available in many centers. Standard MR sequences, which include T1-weighted images (T1W), T2-weighted images (T2W), fluid attenuated inversion recovery (FLAIR) and diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) map, as well as contrast T1W, are sufficient for the diagnosis.

The treatment should primarily consist of correction of the underlaying causes which led to neurological symptomatology, and then symptomatic measures should be taken. Some patients may develop severe manifestations of PRES, such as coma or status epilepticus, which require intensive care unit (ICU) admission [9, 10].

PRES is a reversible syndrome, but in a small number of patients neurological deficit is permanent.

Death occurs in up to 15% of cases due to acute hemorrhage and ischemia [7, 11–14].

Case report

A 73-year-old patient was complaining of headache, instability while walking and loss of balance which lasted for a few days. The patient had a history of hypertension and chronic renal insufficiency. Arterial blood pressure reached 160/90 mmHg. Clinical presentation at admittance was dominated by symptomatology of posterior fossa with discreet right faciobrachial hemiparesis. Initially, CT scanning without application of contrast was performed showing the presence of both-sided hypodensic zones, frontally more prominent on the left as well as parietally right subcortically, which resembled mostly vasogenic edema (Figure 1).

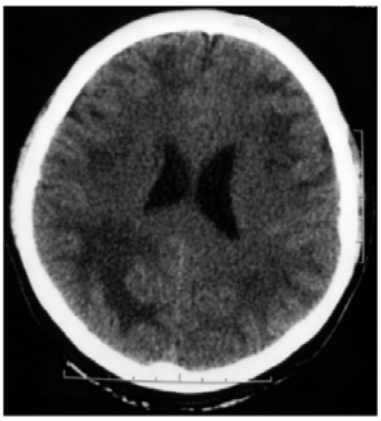

Fig. 1 – Native computed tomography (CT) scan showing hypodense zones on both sides of the frontal and parietal lobe, more pronounced on the right.

After CT, MR scanning was also performed in T1W, T2W, FLAIR, T2*, DWI with ADC map, as well as postcontrast T1W and 3D T1 FSPGR.

MR spectroscopy using 2D multivoxel SE 144 and single voxel SE 144 and SE 35 sequences with positioning of the volume of interest occipitoparietally was also performed.

This scanning showed cortico-subcortical temporopolar on both sides, parieto-occipital and posterior parietal both sidedly more dominant on the right, pachy, unsharply bordered, partly confluent lesions hyperintense in T2W and FLAIR sequence with mild compressive effect on occipitial horn of the right lateral lobe, without postcontrastive enhancment of signal intensity (Figure 2).

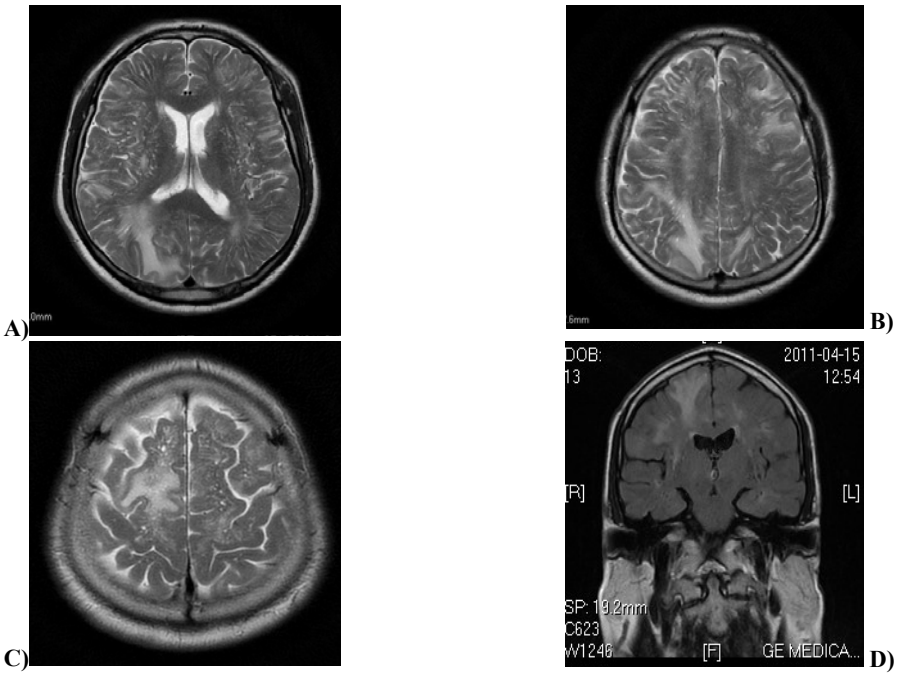

Fig. 2 – A) T2W axial tomogram showing the zones of vasogenic edema of cortical/subcortical localization in the parietal lobes, more pronounced on the right; B) T2W axial tomogram showing the asymmetric changes frontally on the left, parietally bilaterally, more pronounced on the right; C) T2W axial tomogram showing the changes frontally on the right; D) Coronal T2W axial tomogram showing vasogenic edema bilaterally frontally, pronounced on the right.

There were no signs of diffusion restriction. In DWI sequence occipitoparietally on the right there were no changes in signal intensity, but on ADC map hypointensity of the signal was observed, implicating the existence of vasogenic edema.

Based on the CT and MR scanning results the patient was referred to MR spectroscopy. Using 2D multivoxel spectroscopy the obtained spectres showed normal or slightly higher values of choline for the area in question (Cho/Cr), and NAA was slightly lower. By single voxel spectroscopy spectres of low apsolute concentrations were obtained, ratio Cho/Cr did not significantly deviate from normal values for the area in question. NAA was lower. Resonance lines of lipides and lactates were not noticed. Diffusion Tensor Imaging (DTI) reveled that in the area of lesion occipitoparietally on the right fractional anizotrophy was lower.

After diagnosing the patient was regularly treated with antihypertensive, antilypemic and rehydration therapy and was discharged two weeks later with arterial tension 140/80 mmHg and normalized neurological findings.

Control MR scanning, two months after the initial one, showed complete regression of T2W and FLAIR hyperintense changes which had previously affected cortex and subcortical white matter (Figure 3).

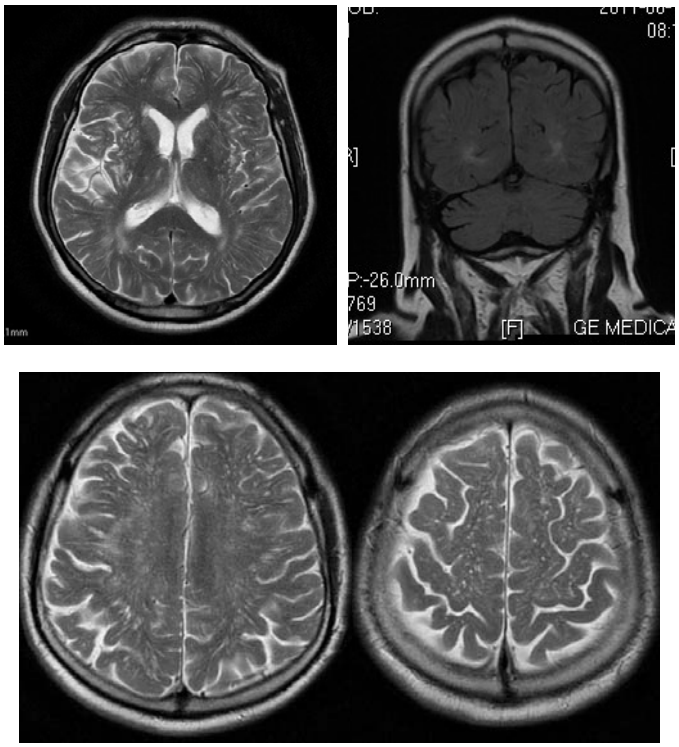

Fig. 3 – Control magnetic resonance imaging (MRI) after two month. T2W axial tomogram and coronal T2W tomogram showed almost complete regression of the lesion.

Discussion

PRES has been reported in patients aged 4 to 90 years, although most cases occur in young to middle-aged adults. Mechanical ventilation is required in 35% to 40% of patients [12, 13]. The average hospital stay is 20 days, and the mortality rate up to 15% [12, 13] . Numerous conditions may lead to the syndrome: acute hypertension, renal function disorder, immunosuppressive therapies being some of them [1]. Other possible causes are eclampsia, transplantation, chemotherapy [15], systemic infection, shock [16] and insect bites [17, 18].

Until recently it was thought that PRES tipically affects the white matter, simetrically, predominantly in occipital and posterior parietal areas. Sporadically, changes were described in frontal and temporal lobes, basal ganglia, brain stem and cerebellum, as well as in cortical grey matter [6].

Recent cohort studies showed that changes are asymmetric in 3–15% of cases, that occipital lobes are affected in 99%, and parietal lobes in 67% to 99% of cases. Changes are less often detected in frontal (68–89%) and temporal (40–83%) [13, 16, 19, 20] lobes of cerebral parenchyma. The brainstem is affected in 13–58%, cerebellum in 30–58%, and basal ganglia in 12–34% of cases [16, 19, 20]. Till now changes are least often detected in the grey matter, i.e. in 10–44 % of cases [11, 16, 19, 20]. In the presented case the changes were asymmetrical and affecting frontal, temporal, parietal and occipital lobes, cortical grey matter, as well as subcortical and deep cerebellar white matter.

The main pathophysiological mechanism which leads to this syndrome is cerebral vasogenic edema. Some authors think that the occurence of edema is the consequence of a disorder in cerebral autoregulation of blood flow through the brain. Other authors think that it is caused by endothelium dysfunction with cerebral hypoperfusion [7].

CT scan is often normal or non-specific, as in our case. Topographic regions suggest the diagnosis of PRES [20].

T2W sequence shows lesions of higher intensity signal, which indicates the existence of edema, and T1W shows low

intensity signal. Changes in PRES are best seen in FLAIR sequence, as hyperintense zones cortically and/or subcortically [8] and such changes are more often frontally localized compared to the posterior presentation using this technique [21].

Signal intensity on the DWI sequence is normal, but it is higher on ADC [22].

Fractional isotrophy shows zones of decrease, which indicates a mild damage of brain paths which can be reversible and it is in accordance with a mild decrease of the values of N-acetylaspartate (NAA) obtained by MR spectroscopy [23, 24].

MR spectroscopy is not superior to conventional MR sequences, but it helps us to rule out other etiology of changes. Signal intensity increase after application of contrast agents is seen in about one half of cases [19].

In recent years susceptibility-weighted imaging (SWI) is used for detecting microhemorrhagies. This examination shows a higher occurrence rate of microhemorrhage in this syndrome which is associated with vasculopathy [25].

All those characteristics of lesions were also seen in our case.

Many conditions may resemble PRES. The differential diagnosis of findings obtained by the examination of the brain using MR imaging in patients with abnormalities of cerebellar white matter includes the following [5, 26]: acute disseminated encephalomyelitis (ADEM) in which, unlike the PRES, lesions in T1 can be hypo- to isointense, in DWI the signal is without changes, and on ADC map an increase of signal intensity is seen, changes being contrast uptaken [27]; in progressive multifocal leukoencephalopathy (PML) in DWI new and active lesions show hyperintensity of signal, the old ones hypointensity, while in ADC map new and active lesions are hypointense and the old ones are hyperintense [28]; in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) in DWI and ADC sequences the restriction of diffusion always occurs, and the changes after *iv* application of contrast do not show signs of signal hyperintensity, changes simetrically affect basal ganglia and white matter widening the perivascular space [29]; in acute ischemia a restriction of diffusion does exist (signal hyperintensity in DWI, signal hypointensity in ADC map) without contrast increase of signal intensity [30]; in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like (MELAS) episodes signal hyperintensity occurs in T1 W sequence, in T1 W and T2 FLAIR it is hypointense, and in DWI iso- or hypointense. In ADC it is iso or hyperintense. The increase in signal intensity may occur post-contrastivelly [31]; in CNS vasculitis lesions show a decrease in signal intensity in T1 W, an increase in signal intensity in T1 W and FLAIR, and in DWI and ADC. Signal hyperintensity occurs post-contrastivelly [32]; in creutzfeldt – jakob disease the changes are isointense in T1 W, hyperintense in T2 W, T2 FLAIR and DWI, and in ADC they are hypointense [33].

Conclusion

Posterior reversible encephalopathy syndrome has no specific clinical presentation and mortality rate is up to 15% of cases due to acute hemorrhage and ischemia. Studies show that magnetic resonance scanning is crucial for diagnosing, monitoring the course and assessing the treatment effectiveness of this syndrome.

Although the name of the syndrome implicates that posterior cerebral circulation is affected, the changes are often localized in frontal and temporal lobes, as noted in our case study, as well as in the structures of brain stem and cerebellum.

Because of the complications (hemorrhage, ischemia), as well as the lethal outcome, the acronym denoting this clinicoradiological entity has been challenged.

We consider that a suggestion to change the term into potentially reversible encephalopathy syndrome should be accepted.

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CASE REPORT

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Lymphoproliferative neoplasms and renal cell carcinoma of clear cell type – Where is the link?

Limfoproliferativne neoplazme i adenokarcinom bubrega – Da li postoji povezanost?

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Abstract

Introduction. The etiology of higher than expected occurrence of lymphoproliferative neoplasms (LPN) and renal cell carcinoma (RCC) in the same patient has not yet been clarified. Several explanations for this co-occurrence have been postulated: prior cytotoxic treatment, viral infections, immunomodulatory effects of tumor itself and shared genetic and/or environmental factors. **Case report.** Medical records of 680 consecutive patients with LPN and 570 consecutive patients with RCC diagnosed between January 1997 and December 2011 in two centers were retrospectively analyzed. Co-occurrence of both diseases was registered in five of the patients (3 males, 2 females) and their demographic, clinical and pathological characteristics were presented. **Conclusion.** Synchronous occurrence of LPN neoplasms and RCC or a short latent period between the diagnoses of these two malignancies in the same patient, as well as the lack of cytotoxic treatment for firstly occurring neoplasm implies a possible common pathobiology of both diseases.

Key words:
lymphoproliferative disorders; neoplasms; kidney noplasms; comorbidity; risk factors; prognosis.

Apstrakt

Uvod. Epidemiološkim studijama utvrđena je značajna povezanost limfoproliferativnih neoplazmi (LPN) i adenokarcinoma bubrega (*renal cell carcinoma* – CRC), ali uzrok ove povezanosti nije utvrđen. Kao mogući etiološki faktori navode se: efekat primenjene citotoksične terapije, infekcije virusima, imunomodulatorni efekat tumora, genetska predispozicija i uticaj faktora spoljašnje sredine. **Prikazi bolesnika.** Retrospektivnom studijom obuhvaćeno je 680 bolesnika sa LPN i 570 bolesnika sa adenokarcinomom bubrega dijagnostikovanih u dve ustanove u peridu januar 1997 – decembar 2011. godine. Udruženost oboljenja utvrđena je kod pet bolesnika (3 muškarca, 2 žene) čije su demografske i kliničkopatološke karakteristike prikazane. **Zaključak.** Istovremeno postavljanje dijagnoze ili suviše kratak latentni period između postavljanja dijagnoze LPN i adenokarcinoma bubrega kao i odsustvo primarne citotoksične terapije govore u prilog zajedničkoj patobiologiji ovih maligniteta.

Ključne reči:
limfoproliferativni poremećaji; neoplazme; bubreg, neoplazme; komorbiditet; faktori rizika; prognoza.

Introduction

Lymphoproliferative neoplasms (LPN) and renal cell carcinoma (RCC) account for about 4% [1] and 3% [2] of all adult malignancies, respectively. In addition to the trend of steady increase in the incidence of both type of malignancies [1, 3], several population-based epidemiological studies have also confirmed previously reported clinical observations of higher than expected co-occurrence of both type of malignancies in the same patient. Thus, patients with RCC have 1.51 [4] and 1.86 [5] higher overall relative risk respectively of multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) than general population. On the other hand, the relative risk for developing RCC in patients with MM and NHL is 1.89 [4] and 2.67 [5], respectively. Several explanations for this co-occurrence have been postulated: prior cytotoxic treatment, viral infections, immunomodulatory effects of tumor itself and shared genetic and/or environmental factors [2, 6–11], yet the etiology of this association has not been clarified.

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Case report

Medical records of 680 consecutive patients with LPN and 570 consecutive patients with RCC (82% with clear cell histological type) diagnosed between January 1997 and December 2011 in the Clical Hospital Center Zemun and the Clinic for Hematology, Clinical Center Serbia were retrospectivelly analyzed. Co-occurence of both diseases was registered in five of the patients (2.5%, 3 males and 2 females). Their median age at the LPN diagnosis was 58 years (range 44–70) and median age at the RCC diagnosis was 57.8 years (range 44–69 years) as shown on Table 1.

Case 1

A 69-year-old male underwent right nephrectomy due to 6.5 cm renal mass in January 2007. A pathological finding revealed early-stage RCC of clear cell type. Five months later he fell by accident and gained fractures of his right humerus and right shoulder blade. Since fractures had not been healed for the next three months, a bone biopsy was performed, which demonstrated plasmocytoma. He was referred to a hematologist who staged him as III B IgA kappa MM. At that time, he had multiple lytic bone lesions, renal failure (creatinine 912 μmol/L), monoclonal spike of 52.8 g/L IgA kappa and highly elevated beta-2 microglobulin (26.4 mg/L). The patient was initially treated with dexamethasone, hemodialysis and palliative radiation therapy. After normalization of renal function he received 6 cycles of vincristine-adriamycin-dexamethason (VAD) chemotherapy. As his MM progressed melphalan-prednisone-thalidomide (MPT) was introduced. He died 17 months after the MM diagnosis without clinical signs of RCC recurrence.

Case 2

A 44-year-old male presented in October 2004 with a large soft palate and tongue mass. Biopsy of the soft palate revealed diffuse large B-cell non-Hodgkin lymphoma (NHL) (DLBCL) and immunophenotyping showed the cells to be positive for CD19, CD20, CD79a, bcl-6 and negative for CD10, CD23. While staging, abdominal computed thomography (CT) scan revealed a large 7 × 5 cm right renal mass, with no hepatosplenomegaly and lymphadenopathy. Further evaluation by magnetic resonance imaging (MRI) excluded central nervous system or thoracic involvement. He had no peripheral lymphadenopathy, his hemogram and biochemistry were within normal ranges, his cerebrospinal fluid was acellular and bone marrow biopsy was unremarkable. It was decided to start with anti-lymphoma treatment. After 6 cycles of CHOP (cyclophosphamide-doxorubicin-vincristine-prednisone) regimen the lesion in the oral cavity completely resolved. However, abdominal CT scan showed further enlargement of the right kidney tumor mass. He underwent right nephrectomy, revealing early-stage clear cell RCC. At the tone of this report, the patient was still disease free from NHL and RCC.

Case 3

A 68-year-old female patient presented in November 1996 with abdominal pain. As her complete blood count (CBC) revealed mild lymphocytosis (11×10^9/L) and abdominal CT scan showed a 50 × 62 × 62 mm lobulated mass with well-defined margins at the lower pole of the left kidney she was referred to the hematologist and urologist. Hematological evaluation revealed chronic lymphocytic leukemia (CLL) clinical stage (CS) 0. Then the patient underwent left radical nephrectomy and splenectomy, which showed renal cell carcinoma RCC with no lymph node involvement. Splenectomy was performed due to iatrogenic injury. At the patient CLL remained stable without treatment, and there was no signs of RCC recurrence.

Case 4

A 48-year-old female patient was diagnosed with early stage RCC of clear cell type after right nephrectomy in June 2000. Eighteen months later she presented with back pain

Table 1

Summary of the patients' demographic, clinical and pathological characteristics

Patient	Gender	Age at RCC	RCC type/TNM stage	RCC treatment	LP	Age at LPN	LPN Type/ clinical stage	LPN treatment	Outcome & survival
1	Male	69	Clear cell type pT1N0M0	right N	8	70	MM IgA kappa IIIB	VAD (VI cy) MPT (VI cy)	17 months
2	Male	44	Clear cell type pT2N0M0	right N	0	44	NHL DBCL IE (oral cavity)	CHOP (VI cy)	CR ≥ 9* years
3	Female	68	Clear cell type pT2N0M0	left N	0	68	CLL A	∅	≥ 17* years
4	Female	48	Clear cell type pT2N0M0	right N	18	50	MM IgG kappa IIIB	VAD (VI cy) MP (X cy)	25 months
5	Male	60	Clear cell type pT1N0M0	right N	-28	58	CLL A	∅	≥ 7* years

N – nefrectomy; LPN – lymphoproliferative neoplasms; LP – latent period (in months); minus represents primary LPN occurence; TNM – tumor; nodes, metastasis; ∅ – "watch and wait"; * – alive; RCC – renal cell carcinoma; MM – multiple myeloma; NHLDBCL – non-Hodgkin lymphoma diffuse B-cell; CLL –chronic lymphocytic leukemia; VAD – vincristine, adriamycin dexamethasone; MP – melphalan, prednisone, thalidomide; CHOP – cyclophosphamide, doxorubicin, vincristine, prednisone; Cy – cycles.

with subsequent diagnosis of MM IgG kappa CS IIIB (monoclonal IgG kappa 72 g/L, hemoglobin 72 g/L, creatinine 139 mmol/L, multiple lytic bone lesions with compression fracture of the thoracic vertebra). The patient was given 6 cycles of VAD chemotherapy together with palliative spinal radiotherapy. As her MM progressed she received ten cycles of melphalan-prednisone regimen, and died 25 months after MM diagnosis without signs of RCC recurrence.

Case 5

A 60-year-old male patient was referred to the urologist in February 2006 because of hematuria and a 3 × 2.5 cm solid mass at lower pole of right kidney without lymph node enlargement seen on abdominal CT scans. The patient's past medical history revealed the diagnosis of CLL CS 0 established 28 months prior the RCC diagnosis. He underwent right nephrectomy which showed RCC, clear cell type. At present, 8 years from diagnosis of CLL and 6 years from the diagnosis of RCC he was without evidence of CLL progression or RCC recurrence.

Discussion

Based on data from the National Cancer Institute's Surveillance, Epidemiology and Results Program (SEER) database the median age at the diagnosis of cancer of the kidney and renal pelvis was 64 years while the age-adjusted incidence rate was 15.3 *per* 100,000 men and women per year with male preponderance (male:female ratio = 2 : 1). These rates are based on cases diagnosed in 2006–2010. Using statistical models for analysis, rates for new kidney and renal pelvis cancer cases have been rising on average 1.7% each year. In the same period the age-adjusted incidences of CLL, NHL and MM were 4.3, 19.7 and 5.9 *per* 100,000 people, respectively [12]. Rates for new leukemia, non-Hodgkin lymphoma and myeloma cases have been rising much slower (0.1%, 0.5% and 0.7%, respectively) [12]. A reciprocal increase in the risk of RCC and after LPN and *vice versa* have been implicated several decades ago by Travis et al. [13, 14]. Evaluating data from SEER database they reported that, comp ared with the general population, NHL patients were at significantly increased risk of developing RCC with the observed to expected ratio 1.47 in 10-year survivors, and 2.07 after 15 years [14]. Two large recently published population based studies – one based on data of the Swedish Family cancer Database [3] and another based on data of the Cancer Registry of Norway [15] confirmed a higher standardized incidence ratio (SIR) for NHL in RCC patients, i.e. 2.09 and 2.47, respectively. Higher SIR for concomitant occurrence of NHL and RCC in the same patient was also found in large hospital-based studies [2, 5]. Based on data registered in the SEER between 1973 and 2006 the risk of hematologic malignancies was highest in the first six months after the diagnosis of RCC, but declined thereafter [16]. Lossos et al. [17] reported the elevated risk of RCC after NHL persisting over 10 years after RCC diagnosis.

Concerning the association between MM and RCC, the largest so far reported population-based study revealed that the relative risk of MM occurrence was 51% higher among patients with RCC than in the general population and that the relative risk of RCC occurrence was 89% higher among MM patients than in the general population. The highest risk of second malignancy was within the first year after the diagnosis of first malignancy [4]. In reported case series based on single institution data, an increased association between MM and RCC was also observed [9, 10, 18].

Hitherto, only two reports with differing results had been published by Rabbani et al. [19, 20]. Notably, in both studies patients with RCC and antecedent or synchronous diagnosis of the second cancer were excluded from analysis of the observed and expected numbers of second cancers that may bias the data away from finding association.

In our series five patients with concomitant LPN and RCC were identified. Concerning RCC, the most common histological subtype is clear cell RCC representing 75–80% of RCC [21]. Our experience is similar – 82% of 570 evaluated consecutive patients with RCC had clear cell hystological subtype. Ohsawa et al. [6] reported that the distribution of histological subtypes of RCC in 42 patients with associated lymphoma is almost similar to that in sporadic RCC. Notably, clear cell RCC was the exclusive RCC histological subtype in our series and all the patients in our series had early-stage RCC requiring only nephrectomy.

Concerning the LPN type, two patients in our series had advanced stage multiple myeloma, two early stage CLL requiring no treatment, while the only patient with NHL and RCC had extranodal DLBCL. In a report of Kunthur et al. [7] four of six patients with NHL and RCC had extranodal lymphoma. In a case series reported by Serefhanoglu et al. [11] the only patient with NHL and RCC also had extranodal lymphoma presented as paravertebral mass. There are also several interesting case reports of concurrent extranodal NHL and RCC [22–24]. Ohsawa et al. [6] commented that the frequency of extranodal lymphoma was higher that would be expected. Reasons for eventual higher extranodal lymphoma occurrence in RCC patients are not yet clarified.

Of interest is that two patients in our series had synchronous occurrence of RCC and LPN (patients 2 and 3). One patient immediately underwent left nephrectomy (patient 3), but in another patient right kidney tumor mass was initially misdiagnosed as another extranodal localization of NHL (patient 2). Not until the cytotoxic treatment for NHL had been completed the patient underwent nephrectomy, and fortunately his RCC didn't spread for that period.

Such disease timing and lack of prior cytotoxic treatment suggests common etiologic factors as an explanation for common relationship between LPD and RCC. The increased incidence of dual malignancy may be explained by common genetic mutations [25, 26]. Structural abnormalities of chromosomes 17 and 8 involving the p53 and c-myc genes are present in MM patients and carry a poor prognosis [25], and an over-expression of c-myc located in 8q24 has been observed in up to 20% of clear cell RCC resulting in cell cycle promotion and renal oncogenesis [26]. Documented alterations of immune system in LPN and RCC may be caused by first malignancy or predispose to both malignanci-

es [27–29]. Interleukin-6 (IL-6) is known for its ability to support cell growth and prevent apoptosis of multiple myeloma, lymphoma and leukemia cells [27], Vascular endothelial growth factor (VEGF) is one of the important endogenous factors that promote angiogenesis in hematological malignancies [28, 29]. An increase in IL-6 and VEGF was also observed in the serum of patients with RCC [30]. So far, several anti-IL-6/IL-6 receptor monoclonal antibodies and five drugs targeting VEGF or its receptors (bevacizumab, sunitinib, sorafenib, pazopanib and axitinib) have been developed for targeted therapy in cancer patients, including LPN i RCC and have demonstrated promising results in both preclinical studies and clinical trials [31–33]. Obesity and smoking are the most consistently established epidemiological predisposing factor for RCC and LPD [34, 35], but none of our patients was obese, and only two (patients 1 and 5) were smokers.

Conclusion

Even though the number of patients in our series is small, our results suggest that association of lymphoproliferative neoplasms and renal cell carcinoma cannot be explained by chance alone. Synchronous appearance and lack of chemo-, imuno- or radiation therapy for first malignancy in our series favors the common biology of these malignancies. From the clinical perspective, this emphasizes the need for more careful follow-up of patients with localized renal cell carcinoma within the first years following nephrectomy. On the other hand, for the patients with non-Hodgkin lymphoma the histological confirmation of solid tumor masses must be provided to distinguish the spread of initial neoplasm from the occurrence of another cancer. This may enhance the chances for accurate diagnosis, better treatment timing and favorable prognosis.

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CASE REPORT

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Severe form of streptococcal necrotizing fasciitis of the upper limb – diagnostic and therapeutic challenge: A case report

Teška forma streptokoknog nekrotizujućeg fasciitisa ruke – dijagnostički i terapijski izazov

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Abstract

Introduction. Since delay in recognition and effective treatment of necrotizing fasciitis (NF) caused by invasive group A *streptococcus* increases the mortality and disability, the early diagnosis and management of this disease are essential for a better outcome. We presented a patient with a severe form of streptococcal NF of the left upper limb in whom amputation was performed as a life saving procedure. **Case report.** A 65-year-old man, previously healthy, suffered an injury to his left hand by sting on a fish bone. Two days after that the patient got fever, redness, swelling and pain in his left hand. Clinical examination of the patient after admission indicated NF that spread quickly to the entire left upper limb, left armpit, and the left side of the chest and abdomen. Despite the use of aggressive antibiotic and surgical therapy severe destruction of the skin and subcutaneous tissues developed with the development of gangrene of the left upper limb. In this situation, the team of specialists decided that the patient must be operated on submited to amputation of the left arm, at the shoulder. After amputation and aggressive debridement of soft tissue on the left side of the trunk, the patient completely recovered. β-hemolytic streptococcus group A was isolated from the skin and tissue obtained during the surgery. **Conclusion**. In the most severe forms of streptococcal NF of the extremities, adequate multidisciplinary treatment, including limb amputation, can save the life of a patient.

Key words:
fasciitis, necrotizing; streptococcus; arm; amputation; anti-bacterial agents.

Apstrakt

Uvod. S obzirom na to da kašnjenje sa postavljanjem dijagnoze i lečenjem nekrotizujućeg fasciitisa (NF) uzrokovanog invazivnim streptokokom grupe A povećava smrtnost i invaliditet, rana dijagnoza i lečenje ove bolesti esencijalni su za bolji ishod. Prikazali smo bolesnika sa teškom formom streptokoknog NF leve ruke kod koga je urađena amputacija, kako bi se spasio život. **Prikaz bolesnika.** Muškarac, star 65 godina i prethodno zdrav, zadobio je povredu leve šake ubodom na riblju kost. Dva dana kasnije on je dobio visoku temperaturu, crvenilo, otok i bol u levoj ruci. Klinički pregled bolesnika nakon prijema ukazao je da se radi o NF koji se brzo širio na celu levu ruku, levu pazušnu jamu i levu stranu trupa. Uprkos agresivnoj primeni antibiotske terapije i hiruškog lečenja, kod bolesnika je došlo do razvoja teške destrukcije kože i potkožnih tkiva sa razvojem gangrene leve ruke. U ovoj situaciji konzilijum specijalista odlučio je da se kod bolesnika mora uraditi amputacija leve ruke u ramenu u cilju spašavanja života. Posle amputacije i debridmana mekih tkiva na levoj strani trupa, kod bolesnika je došlo do potpunog oporavka. Iz kože i mekih tkiva dobijenih tokom operacije izolovan je β hemolitički streptokok grupe A. **Zaključak**. U najtežim formama nekrotizujućeg fasciitisa ekstremiteta, adekvatan multidisciplinaran tretman, uključujući i amputaciju ekstremiteta, može spasiti život obolelog.

Ključne reči:
fasciitis, nekrotizujući; streptococcus; ruka; amputacija; antibiotici.

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Introduction

Necrotizing fasciitis (NF) is a severe, life-threatening bacterial infection that causes rapid destruction of skin, subcutaneous tissue, fascia and/or muscle [1–4]. NF caused by invasive group A streptococcus (GAS) may be one of the most dramatic conditions seen in medicine. This type of NF can develop over hours to days usually with a precipitating traumatic event and can deteriorate quickly. The illness is more commonly known as a "flesh-eating disease" that can kill a human in less than a day [5–10]. Since delay in recognition and effective treatment increase the mortality and disability, the early diagnosis and management of GAS NF are essential for a better outcome [10–13]. In all patients intravenous antibiotic therapy and aggressive surgical debridement should be urgently initiated. Other modes of surgery, such as amputation, may be required in streptococcal NF of the extremities [12–17]. However, the decision about amputation, although it should save the patient's life, may be difficult and often delayed because of the lack of knowledge of disease nature. Establishing the diagnosis of streptococcal NF can be challenging in treating these patients, and the knowledge of all available tools is the key for the early and accurate diagnosis [18–21].

We presented a patient with a serious form streptococcal NF of the upper limb and emphasized the importance of the early diagnosis and prompt decision about adequate surgical treatment.

Case report

A 65-year-old man, previously healthy, allergic to penicillin, suffered an injury to his left hand by sting on a fishbone on December 16, 2013. Two days after that he got a very strong pain, redness and swelling of the left forearm and upper arm, followed by chills, and high fever. So, he was urgently admitted to the local hospital. Under the assumption that the patient was a case of cellulitis of the left hand intravenous therapy with amikacin and ciprofloxacin was given. Because the clinical findings of the left hand were worsening, the patient underwent repeted incisions of the skin and subcutaneous tissue, and afterwards partial fasciotomy on the inside of the forearm and upper arm. Despite the applied treatment, the clinical findings and the patient's general condition were getting worse, and the patient was transferred to the Military Medical Academy on December 25, 2013. On admission to the Clinic for Infectious and Tropical Diseases the patient was in poor general condition, tachypneic, with reduced oxygen saturation, hypotensive, tachycardic. The development of severe destruction of the skin and subcutaneous tissues with the development of the gangrene of the left upper limb were present (Figure 1).

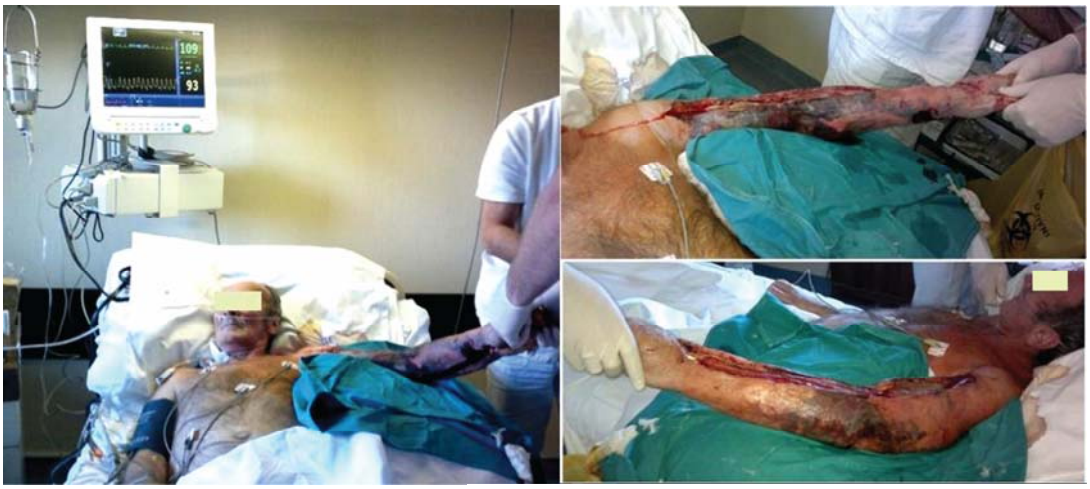

Fig. 1 – Streptococcal necrotizing faciitis of the left upper limb after repeated incisions and partial fasciotomy.

In laboratory findings erythrocyte sedimentation rate was 111mm/1st h, C-reactive protein (CRP) 275 mg/L, erythrocytes 3.09×10^{12}/L, hemoglobin 95 g/L, leukocytes 20.3×10^9 /L, neutrophils 85%, platelets 146×10^9/L, glycemia 13.7 mmol/L, sodium 130 mmol/L, potassium 3.1 mg/L, urea 25.5 mmol/L, creatinine 289 umol/L, protein 44 g/L, albumin 17 g/L, with the rapid increase of serum creatine kinase (CK) level to 3090 U/L, and with a slight increase of serum transaminases levels.

Thanks to our past experience, we made the prompt diagnosis of probable streptococcal NF. Right after admission we started with a combined intravenous antibiotic therapy (clindamycin at the dose of 2,400 mg *per* day plus vancomycin at the dose of 2 g *per* day), with the use of fluids, electrolytes, human albumin, fresh frozen plasma, and blood transfusion. At the same time we consulted the team of specialists, and also planned application of emergency hyperbaric oxygenation (HBO). On the second day of hospitalization, a wide incision was made in the area of the left upper limb, with partial excision of necrotic tissue. Clinical examination indicated NF which was instantly and quickly spreading to whole left upper limb, left armpit, and the left side of the chest and abdomen (Figure 2). The patient's Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) score [22, 23] quickly increased to 10, and clearly indicated that it was NF. Culture of smear from the wound and necrotic tissue which were taken during the surgery showed isolated β-hemolytic group A *streptococcus*.

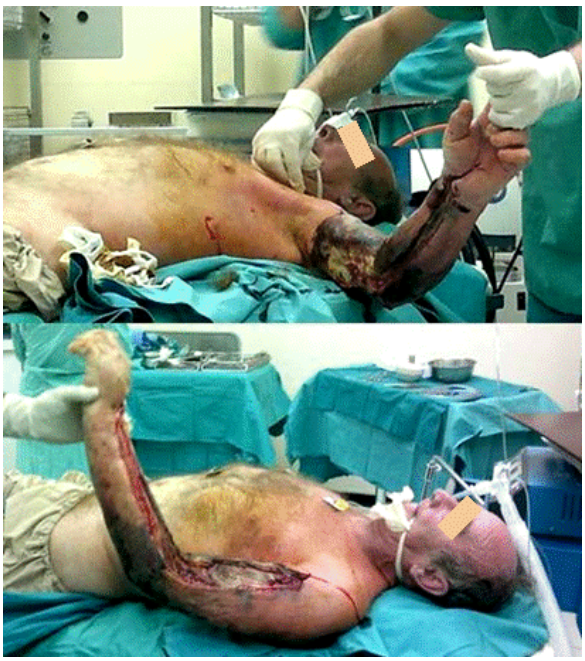

Fig. 2 – Streptococcal necrotizing faciitis of the left upper limb and chest before amputation of the arm at the shoulder.

Despite the implementation of very aggressive treatment the illness took a very serious course. In this situation, the team of specialists decided that the patient must be submitted to amputation of his left arm, at the shoulder, and on December 28, 2013, shoulder disarticulation was made. In addition, aggressive debridement of soft tissue up to healthy tissue of the left side of the chest was done. After amputation, we continued applying antibiotic therapy and HBO, and the general state of the patient was gradually improving, up to full recovery and the patient was released from the hospital few weeks later.

Discussion

According to the literature streptococcal NF can 'enter' through trauma to the skin, sometimes even minor. Besides others, fish fin injury is one of the predisposing factors for the development of NF, as it happened in the presented patient[1, 24–26]. The disease can involve any part of the body but primarily the extremities [24–29]. Anaya et al. [20] in their study of 150 patients with NF estimated that the extremities were the most common site of infection (in 57.8%) followed by the abdomen and perineum.

Although NF can cause a number of bacterial species, invasive types of GAS are the most important causes of this illness, because the speed at which it can induce local infection, streptococcal toxic shock syndrome (STSS), and death cannot be compared with any other infectious agents. In recent years, hyperacute and subacute variants of NF are increasingly reported in the literature [6, 24–29]. Mikić et al. [6] showed a 65-year-old man with STSS, without comorbidities, who died in less than 30 hours after admission, and recently, Kojić et al. [30] described a 46-year-old previously healthy patient with fulminant course of NF of the right leg, who developed rapid STSS and died for less than 40 hours after the first medical examination. In these, as in many other cases, there was no sufficient time to apply appropriate therapy, including the amputation of the limb.

Early diagnosis, and early therapy are the main predictors of NF outcome [2, 4, 10, 20]. Early diagnosis, extensive debridement and, in extreme cases, willingness to amputate the limbs at a very early stage may be the only intervention that can save the lives of patients. Otherwise, the disease has an almost 100% mortality [7, 9, 12]. The patient presented in this paper had a subacute form of NF. Therefore, despite the delayed diagnosis of NF, after amputation of his left arm the outcome was favorable. Unfortunately, after the treatment there remained a serious disability. In the literature, there is a consensus that the delay in treatment for more than 6–12 hours or inadequate primary surgical debridement are prerequisites of greater morbidity and mortality, as it happened in the presented patient [12–17].

The diagnosis of NF and its distinction from the other less fatal soft tissue infections is still notoriously difficult and challenging. The results of a retrospective study have shown that 35% of later NF are misinterpreted like cellulitis or severe non-necrotizing soft-tissue infection, while in another study the authors have found that only 14% of patients with NF are admitted with the right diagnosis. It is essential to accentuate that there are no mandatory “hard signs” for the diagnosis of NF, which leads to the missing early diagnosis in 85% to 100% of all cases [12–17]. This is exactly what happened in the presented patient in whom the initial diagnosis was cellulitis of the left hand.

Necrotizing fasciitis of one or more limbs was a medical emergency which requires prompt recognition and aggressive excision of affected tissues. The diagnosis, however, is a challenge, because the infection is usually confused with a milder condition such as cellulitis. Various tools for reaching an early diagnosis have been suggested but the key is a high level of suspicion [31–34]. Reliance upon adjunctive diagnostic tests may delay operative treatment. We believe that the accurate diagnosis of infection can be made clinically and that no investigation can replace meticulous and repeated physical examination. The diagnosis of NF is primarily clinical with corroborating operative findings, so it should be suspected in patients with high fever, symptoms of severe infection and unclear pain of soft tissue. The main sign of the disease is very strong pain.[1] However, thrombosis of the vessels of the skin will lead to necrosis and severe pain will fade as the nerves die, when it comes to the appearance of anesthesia in the most necrotic area [35].

Early diagnosis of NF is not always possible due to paucity of cutaneous findings early in the disease therefore high index of suspicion is important in infected cases that are refractory to antibiotics. In the laboratory findings there is elevated number of leukocytes, associated with the increase in CRP, and creatine kinase level. Biopsy of soft tissue and histopathologic verification of NF are useful only in the early stage of disease. Isolation of GAS is most important for making the diagnosis of streptococcal NF [1, 14, 17, 25]. LRINEC score is a new and very useful diagnostic scoring system for

distinguishing NF from other soft tissue infections. A score greater than 8 is very predictive of NF, which was confirmed by the case of the presented patient [22, 23].

The treatment of streptococcal NF may involve an interdisciplinary care team. For example, in the case of the presented patient, the team included the infectious disease specialist, orthopedic surgeons, the vascular surgeon, the plastic surgeon, the anesthesiologist, the radiologist and the microbiologist. Antibiotic therapy alone has been shown to have high mortality rates due to reduced antibiotic delivery in poorly vascularized/necrotic tissue at the infection site. Their application is essential for the full success of therapy. Surgical debridement is required to decrease necrotic tissue load and provide a viable tissue bed amenable to the delivery of antibiotics. [3, 12-15, 36] Other therapies such as HBO therapy and intravenous immune globulin may be useful as adjunctive treatment in patients with NF but remain controversial [37–40].

Antimicrobial therapy must be directed at pathogens and used in appropriate doses. After having been repeated, operative procedures are no longer needed when a patient demonstrates obvious clinical improvement, and no fever for 48–72 h. NF with or without STSS should be treated with clindamycin and penicillin. Unfortunately, the presented patient was allergic to penicillin. The rationale for clindamycin is based on *in vitro* studies demonstrating both toxin suppression and modulation of cytokine (i.e. TNF) production. On animal studies clindamycin demonstrated superior efficacy to penicillin. Penicillin should be added because of the increasing resistance of GAS to macrolides. Vancomycin, or linezolid should be considered until MRSA infection has been excluded. An acceptable empiric antibiotic regime includes linezolid, which covers MRSA with the additional benefit of inhibiting invasive GAS virulence proteins similar to clindamycin [3, 12–15, 36]. Taking into account the above mentioned we conduced antibiotic therapy in our patients with clindamycin, and vancomycin since we add suspicion of simultaneous infection with MRSA. The above treatment should be accompanied with supportive measures such as fluid replacement, blood pressure support, analgesia, nutritional support and intensive care involvement etc.

Early and radical surgical excision of necrotic tissue up to healthy tissue for limiting further necrosis of subcutaneous fascia and fat tissue significantly increases survival compared to patients with delayed debridement even for just a few hours [3, 7, 9, 12–17], McHenry et al. [41] reported that the average time from admission to operation was 90 hours in non-survivors *versus* 25 hours in survivors. Similar findings were also reported by Wong et al. [16]. Angoules et al. [19] reported that patients with NF required an average of 3 surgical debridements, with 22.3% of patients requiring amputation for failure of multiple surgical debridements to control infection. Other authors warn that limb amputation may be necessary to stop the spread of infection in nearly 50% of patients with GAS NF [17–21]. Some of these authors have not found that amputation correlates with survival, but it should be noted, that surgeons are more likely to amputate in more severely affected cases and therefore skew the mortality rate [21]. Amputation is usually a shorter procedure associated with less blood loss than radical debridement of the skin and fascia and requires less, if any, further reconstructive surgery. Nevertheless, the decision to amputate must be made with great caution in patients with a very proximal involvement, particularly when infection affects the trunk. In these cases, the mutilating procedure may not always control infection nor improve the chances of survival [17–21, 27]. However, it turned out that in our patient the decision to amputate the arm at the shoulder was correct and timely even though infection had already affected the trunk.

For a long time there were no clear guidelines on limb amputation because of the small number of cases NF. Since 1995, guidelines for undertaking an amputation have been adopted, but they are not a strict protocol [21]. Each team of experts has made the final decision for amputation after discussion between colleagues and the patient in the presence, when possible, of his or her relatives. Tang et al. [21] suggested some guidelines for decision regarding amputation of a limb with NF. These guidelines include concurrent medical disease with high anesthetic risk (e.g. poorly controled diabetes mellitus), extensive tissue necrosis with involvement of the underlying muscles, shock requiring more than one ionotropic agent, concurrent vascular insufficiency, rapidly progressing infection with large area of tissue necrosis. In the presented case there was extensive necrosis of the tissues of the left hand with the spread of infection into large areas of the trunk, but the team of specialists was sufficient for the decision on amputation, and that decision proved to be correct.

The worldwide mortality rate of NF reflects the severity of the desease. McHenry et al. [41] summarized the reported mortality rates from different studies. The cumulative mortality rate was 34% (6% to 76%). The mortality was related to age, the percentage of body surface involved, and the presence of hypotension, as well as the time delay between admission to hospital and surgical debridement. Some authors conclude that patients with the involvement of the proximal part of a limb at the time of admission have a high mortality rate, which may imply a longer period from the onset of the disease and therefore an increased delay in adequate treatment start [2, 4, 7, 9, 10, 25, 26, 28]. The presented 65-year-old patient in whom NF affected the entire left arm and a large part of the left side of the body survived, thanks mainly to adequate treatment.

Conclusion

Necrotizing fasciitis of the limbs caused by invasive group A *streptoccocus* is frequently fatal. This is particularly common among patients with the diagnosis established late and late initiated treatment. However, even in the most severe forms of the disease adequate multidisciplinary treatment, including limb amputation can save the life of a patient and reduce the mortality of streptococcal necrotizing fasciitis of the limbs.

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CASE REPORT

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Diagnostic approach to localised organising pneumonia – A case report

Dijagnostički pristup lokalizovanoj organizovanoj pneumoniji

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Abstract

Introduction. Localised organising pneumonia, radiologically presented with oval or round shadows mimicing lung cancer or metastases, is a major issue in differential diagnosis. **Case report.** A female patient was hospitalized to clarified the etiology of multiple nodular lung lesions. The chest X-ray and the chest computed tomography (CT) revealed bilateral patchy and nodular shadows, and round lung lesions, respectively. Neither sputum analyses, nor histology of bronchoscopy samples clarified the etiology of these lung lesions. As secondary deposits in the lungs were suspected, video-assisted thoracoscopy and anterolateral right minithoracotomy with atypical upper and lower lobe resection were performed. The frozen-section analysis suggested the benign nature of the lesion, and the definite histopathological finding of localised organising pneumonia was established. Due to bilateral lung lesions, corticosteroids were applied. Seven weeks later, the chest CT finding revealed a total regression of the lesions. **Conclusion.** A surgical resection was necessary to diagnose the localised organising pneumonia which mimiced secondary malignant lesions, thus establishing the definite etiology of lung lesions. Bronchoscopic cryobiopsy, recently introduced in order to obtain peripheral lung biopsy samples, has provided new possibilities in the diagnosis and treatment of neoplastic and non-neoplastic lung diseases.

Key words:
pneumonia; lung neoplasms; neoplasm metastasis; diagnosis; diagnosis differential; thoracic surgical procedures.

Apstrakt

Uvod. Lokalizovana organizovana pneumonija, predstavljena ovalnim ili okruglim senkama, zbog sumnje na karcinom bronha ili metastaze, čini diferencijalno dijagnostički problem. **Prikaz bolesnika.** Prikazana je bolesnica koja je hospitalizovana radi razjašnjenja etiologije multiplih nodularnih plućnih promena. Radiogram grudnog koša pokazao je obostrana mrljasta i nodularna zasenčenja, a kompjuterizovana tomografija obostrane okruglaste promene. Pregledi sputuma i patohistološke analize materijala uzetih tokom bronhoskopije nisu razjasnili etiologiju promena. Zbog sumnje na sekundarne depozite u plućima, urađena je videoasistirana torakoskopija i anterolateralna desna mini torakotomija sa atipičnom resekcijom gornjeg i donjeg režnja. *Ex tempore* nalaz ukazao je na benignost promene, a definitivni patohistološki nalaz na lokalizovanu organizovanu pneumoniju. Zbog obostranih plućnih promena primenjeni su kortikosteroidi. Nakon sedam nedelja nalaz kompjuterizovane tomografije ukazao je na potpunu regresiju promena. **Zaključak.** Hirurška resekcija bila je neophodna u dijagnostici lokalizovane organizovana pneumonija koja je ličila na sekundarne maligne lezije, a u cilju definitivnog razjašnjenja etiologije promena u plućima. Nedavnim uvođenjem bronhoskopske kriobiopsije bioptata otvorene su nove mogućnosti u dijagnostici i terapiji neoplazmi i drugih bolesti pluća.

Ključne reči:
pneumonija; pluća, neoplazme; neoplazme, metastaze; dijagnoza; dijagnoza, diferencijalna; hirurgija, torakalna, procedure.

Introduction

Acute bacterial pneumonias respond to antibiotic therapy by regression and inflammatory process resolution. However, an intraalveolar fibrin exudate, or organising pneumonia (OP) may develop in some cases. OP is a unique histopathological entity which represents an unresolved or delayed slow-regression pneumonia occurring in 5–10% of all pneumonia cases [1–4].

Depending on its inducing agent, three OP classes are differentiated: OP induced by a determined agent (bacteria, viruses, parasites, fungi, drugs, irradiation), OP induced by an undetermined inducing agent or associated with other diseases, and OP of unknown origin (cryptogenic organising pneumonia – COP) [1, 2].

COP is the OP induced by an unknown agent with specific radiological and clinical signs. It usually occurs in 50–60 years old subjects, taking a subacute course and developing unspecific clinical symptoms (fever, cough, fatigue, dyspnea, anorexia, weight loss). The radiological finding is presented with multiple, bilateral, patchy, peripheral alveolar shadows, ground

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glass or consolidation. More rarely, the radiological finding is presented with bilateral diffuse infiltrates and focal solitary lesions [1, 2, 5]. Focal COP is a rare entity, which may radiologically mimic malignant lung lesions and lung cancer. In most focal OP (FOP) cases, the diagnosis is established only after a surgical resection due to a suspected malignancy [1, 2, 5]. Tiny nodular shadows are more frequent, found in 10–50% of patients, while huge nodular lesions are rare [6]. The nodules may be around 8 mm in size and are defined as o (micro)nodules. Multiple OP nodules may suggest metastatic lesions [1, 2].

The diagnosis of COP is established on the basis of the histopathological OP finding, clinical and radiological characteristics, having excluded the known agents or comorbid conditions [7]. The sample for histopathology may be obtained by transbronchial biopsy (TBB), but video-assisted thoracoscopy (VATS) is not rarely indicated as the method providing a sufficient lung tissue sample [1, 2, 5].

Recently, a new technique for bronchoscopic lung biopsy has been developed, using flexible cryoprobes [8, 9]. In patients with diffuse lung diseases, transbronchial lung cryobiopsy (TBLC) is a safe procedure, less invasive than a surgical lung biopsy, providing large and well-preserved biopsy samples of the lung parenchyma, as compared to the standard forceps biopsy [8–11]. Constantly increasing the experience with bronchoscopic cryobiopsy, it has emerged to be the technique promising to become the first-line approach to lung biopsies in diagnosing diffuse lung diseases, enabling to avoid surgical lung biopsy [8]. The initial results of transbronchial cryobiopsy accompanied with endobronchial ultrasound (EBUS) in diagnosing peripheral lung lesions sizes < 4 cm show it is a feasible, safe and useful sampling technique which provides a larger sample than standard forceps biopsy [12]. Additional research is required to analyse and compare the cost-effectiveness of bronchoscopic cryobiopsy and surgical lung biopsy in the diagnosis of diffuse lung diseases and peripheral lung lesions [8–10, 12].

Pneumonias which do not regress, tending to organise instead, may resemble tumorous lesions. Focal pneumonic consolidations and focal malignant lesions may sometimes be radiologically presented as round or oval lesions. It is often difficult to differentiate between inflammatory and malignant nodules, and a surgical resection is the most reliable method to clarify their etiology [4, 13]. Nevertheless, bronchoscopic cryobiopsy offers new diagnostic prospects.

The aim of this work was to present a female patient with the localised organising pneumonia (LOP) which radiologically resembled malignant lung lesions, in whom the histopathological dilemma was resolved by surgical resection.

Case report

A 58-year-old female patient, a preschool teacher, was admitted to the Institute for Pulmonary Diseases of Vojvodina (IPDV), Sremska Kamenica, in November 2013, to clarify the etiology of multiple nodular lung lesions.

The patient went to the doctor because of fever (37.7°C) and dry cough, when she was submitted to chest X-ray, presented with bilateral merged patchy and nodular shadows (Figure 1). Computed tomography (CT) of the chest revealed the upper and lower lobes of both lungs predominantly involved by multiple, hyperdense lesions, patchy or roundish in shape, either isolated or merged, 9 mm in diameter, a few of which were localized in the subpleura (Figure 2).

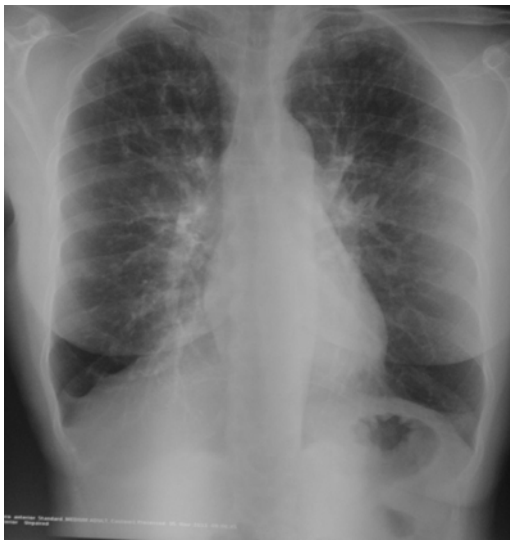

Fig. 1 – Chest X-ray on admission.

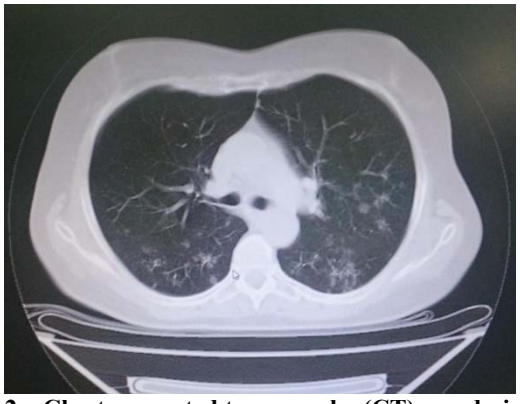

Fig. 2 – Chest computed tomography (CT) on admission

The patient was referred to the IPDV. On admission, the patient had no major respiratory symptoms, complaining of stabbing bellow the right rib arch. The patient was afebrile, eupneic, cardially compensated, normotensive, with no deviations registered on physical examination. Anamnestically, the patient reported a 20-year history of chronic bronchitis, stent implantation after the myocardial infarction six years ago, and long-term smoking.

Laboratory findings were: elevated erythrocyte sedimentation rate (66 mm/-), C reactive protein 6.9 mg/L and procalcitonine 0.08 ng/mL; blood cell count, fibrinogen and standard laboratory findings within reference values.

Lung function estimation revealed: moderate obstructive ventilation disorder, with moderate hyperinflation of the lungs (FVC 2.35–93.6%; FEV1 1.16–55%; ITGV 4.45–174.1%, Rt 0.28;). In acute test with a bronchodilator, the obstruction of the airways was partially reversible in character. Moderately decreased diffuse capacity (DLCO 3.88–54.1%) was registered. Gas analysis of the arterial blood

suggests mild hypoxemia (PaO_2 9.09 kPa; $PaCO_2$ 4.89 kPa; pH 7.464; SaO_2 94.4%). Echocardiography finding was normal (60% ejection fraction of the left ventricle).

The patient was submitted to bronchoscopy, providing a normal endoscopy finding. Histopathology of the obtained lung sample was negative.

Sputum cytology revealed catarrhal exudate. No bacteriological growth was present. The sputum smear and culture, as well as catheter biopsy samples were negative to *Mycobacterium tuberculosis*.

Due to the radiological finding which might be suggestive of secondary lung deposits, ultrasond of the upper abdomen and the pelvis, colonoscopy, thyroid examination, mammography and gynecological examination were performed, providing normal findings.

As the etiology of the lung lesions was not clarified, surgical diagnostics was indicated. Thoracotomy-inducing video-assisted thoracoscopy of the right lung was performed (having approached the pleural cavity, the exploration verified smooth and bright pleura free of carcinosis) at the Chest Surgery Clinic of the Institute. The right anterolateral mini thoracotomy was performed, and the exploration verified a few nodular lesions in the upper and lower right lung lobes. An atypical resection of the upper and lower lobe was performed, and the frozen section assay suggested a benign nature of the lesion. The material sent for histopathology included three lung edge samples sized 7 × 2 × 1 cm, 5.5 × 1.5 × 1 cm and 4 × 2 × 0.5 cm, presented with a tiny greyish capsule enclosing tumorous lesions sized 1 × 1 cm. On cross-section, they were relatively clearly defined, pinkish grey or light yellowish in colour, firm, with a relatively perserved pulmonary parenchyma between them. Histologically, the lung architecture was typicaly presereved (Figure 3). The nodular character of the lesion was well seen even at a low magnification (Figure 4). The terminal lumen of airways the neighbouring alvoli were involved with numerous buds of multiplied edematous loose granulation tissue (Figure 5). The interstitium was involved with infiltrates of lymphocytes, plasma cells and histiocytes in different quantities. Some alveoli were coated with atypical cuboid alveolar cells, and filled with alveolar macrophages (Figure 6). The definite histopathological finding was: localised organising pneumonia.

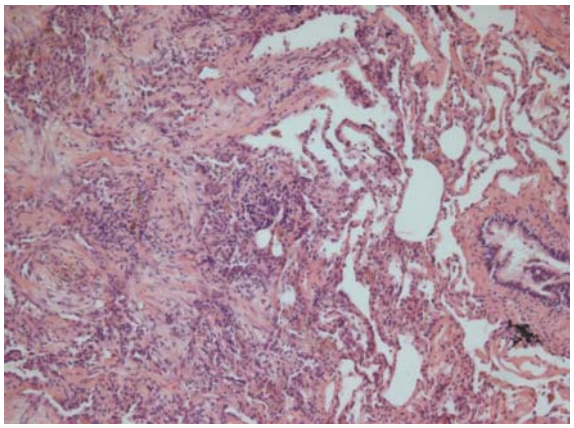

Fig. 3 – Preserved lung structure (HE, ×200).

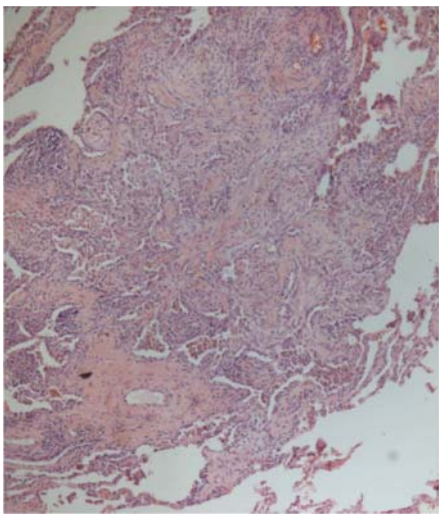

Fig. 4 – Nodular nature of the process at low magnification (HE, ×200).

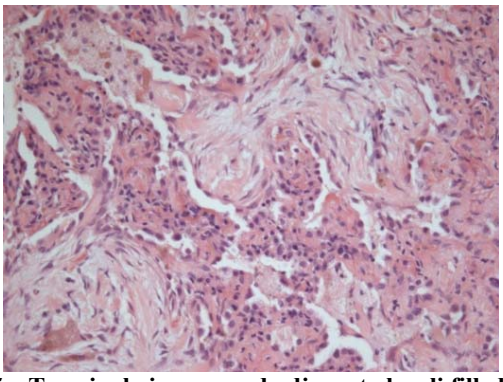

Fig. 5 – Terminal airways and adjacent alveoli filled with loose granulation tissue (HE, ×400).

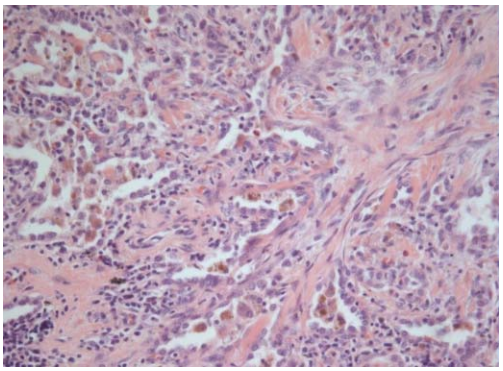

Fig. 6 – Fibrosis and interstitial inflammation with alveolar hyperplasia. Massive accumultaion of alveolar macrophages (HE, ×400).

During the hospital stay, the patient was receiving the inhalant desopstruction therapy and antihypertensives, and as soon as the diagnosis of organising pneumonia had been established, corticosteroids (prednisolon 0.75 mg/kg/day) were introduced, accompanied with a gastroprotective drug. The patient was discharged in a good general condition, instructed to gradually reduce the prescribed corticotreatment.

Seven weeks later, the patient was feeling well. The inflammation markers were normal, as well as the chest X-ray finding (Figure 7). The control CT of the chest revealed a to-

tal regression of the lesions in both lungs (formerly described lesions sized ≤ 9 mm in different segments of both lungs). Some sites of former lesions were now involved with striped pleuropulmonary sequels, and the sites of performed typical resections S1 and S10 with scar lesions. Vesiculous pulmonary emphysema persisted. No enlarged mediastinal or axillary lymph nodes were registered, nor uni- or bilateral pleural effusion signs (Figure 8).

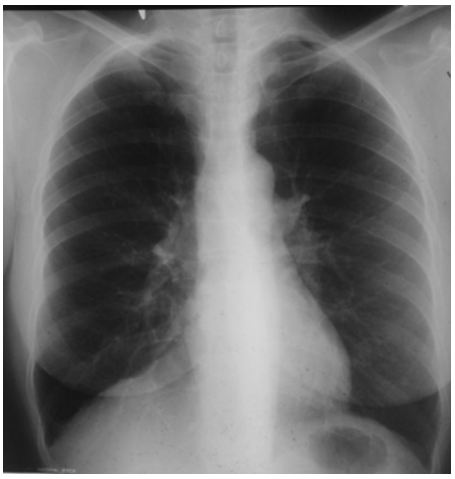

Fig. 7 – Control chest X-ray.

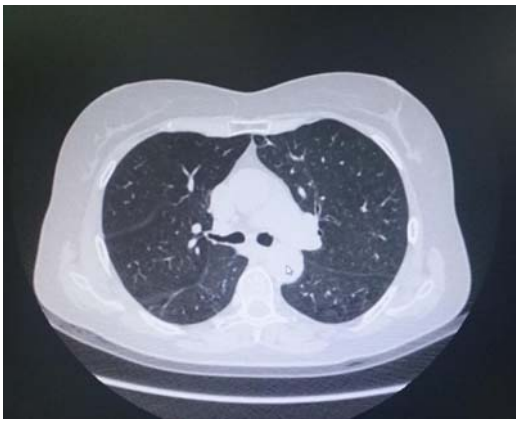

Fig. 8 – Control computed tomography (CT) of the chest.

Discussion

As OP is a localised process presented with oval or round shadows mimicking lung cancer or metastases, it is a major issue in differential diagnosis, investigated by many authors [13].

If chest CT in OP is characterized with focal lesions they are hardly differentiated from malignant ones, requiring bronchoscopy sampling of the lungs. In case it fails to clarify the etiology of lung lesins, a surgical resection (VATS, thoracotomy) is need [14–16]. There exist no specific clinical and radiological features enabling a reliable differentiation between focal OP and lung cancer, and therefore a surgical resection cannot be avoided in some cases. Zheng et al. [17] have come to a similar conclusion in his study of 20 FOP patients compared to 40 patients with lung cancer.

In the presented case, bronchoscopy with standard lung biopsy sampling failed to clarify the etiology of lung lesions. The experience in applying TBLC to diagnose diffuse parenchymal lung disease shows the following: the technique provides a larger tissue sample than the standard transbronchial forceps biopsy; the diagnosis is established in a large percentage of cases; intervention is safe in regard to pneumothorax and bleeding, and a surgical diagnosis is required in 5–12% of the cases [8–10]. Cryobiopsy samples are larger, with no artefacts, containing alveolar tissue more often. Cryobiopsy can therefore be said to have a diagnostic perspective [11]. The results of applying transbronchial cryobiopsy in diagnosing peripheral lung lesions also confirm it as safe and useful technique [12]. Still, additional, large-scale investigations are also needed.

In our patient, thoracotomy-introducing VATS was performed, suceeded by the atypical resection, correlating to other authors' reports: of 27 patients, 14 had wedge resection, and 13 lobectomy with or without VATS [13]; of 21 patients, wedge resection was performed in 16, and lobectomy in 5; wedge resection was therefore performed in 81%, segmentectomy in 11%, and lobectomy in 8% of the patients [17]. In our patient, the lesions were localized below the visceral pleura, the largest one over 2 cm away from the visceral pleura, in the right upper lobe. Close to this lesion, there was another, smaller one, which was extirpated. Due to the accidental mistake possible in randomly selecting the multiple lesions, the third lesion was also extirpated from the right lower lobe, thus reducing the accidental mistake to the minimum.

Many authors emphasize the role of surgical diagnostic procedures in clarifying the etiology of peripheral lung lesions. Wu et al. [13] applied surgical resection of tumorous lesion in patients thoracotomized due to undiagnosed peripheral focal lung lesions suspected for malignancy, establishing OP. Their etiology would not have been clarified if surgical resection had not been applied. As lung cancer was suspected due to unifocal shadowing unclarified by bronchoscopy, surgical resection was indicated, clarifying the diagnostic dilemmas of these cases and establishing the diagnosis of FOP [17]. Due to the suspected malignant nature of our patient's lesions presented with 9–10 mm nodules, surgical resection was also indicated, correlating to the reports of Yang et al. [3] who analysed the patients with FOP submitted to thoracotomy due to 9–60 mm sized nodules, mimicking lung cancer on CT.

Corticosteroid treatment is a standard for OP. In our patient, it resulted in a radiological regression of bilateral lung lesions. Bronchoscopy confirmed FOP is treated by corticotrherapy, resulting in elimination of the symptoms and radiological regression [5]. In a study on LOP, patients with bilateral lesions were operated to establish the diagnosis, and due to bilateral lesions they were prescribed for corticotherapy which improved their condition, correlating to our reported case. Resected solitary lesions require no additional corticotherapy, unlike bilateral localized OP lesions [16].

When COP is treated with corticosteroids, it has good prognosis, while the prognosis of secondary OP is less certain and depends on the underlying disease, as reported by many authors [18, 19].

As there exist no specific clinical and radiological features to enable a reliable differentiation between FOP and lung cancer or metastases, surgical resection is the method of choice to either establish or abandon malignancy in problematic cases.

Conclusion

Surgical resection is necessary in the diagnosis of localised organising pneumonia mimicking lung cancer or metastases, in order to clarify the etiology of these lesions. Bronchoscopic cryobiopsy, recently introduced in order to obtain peripheral lung biopsy samples, has provided new possibilities in diagnosis and treatment of neoplastic and non-neoplastic lung diseases.

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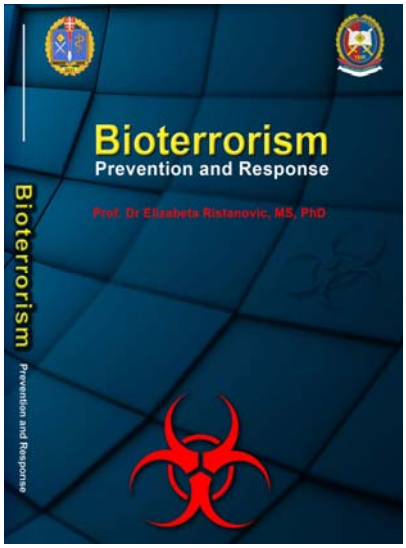
B O O K R E V I E W

Bioterrorism-Prevention and Response. 2nd, revised, English edition

Author: Elizabeta Ristanović
Publisher: Odbrana Media Center /University of Defence, Belgrade
Year: 2015.
ISBN: 978-86-335-0458-4



As a multi-dimensional political phenomenon, modern terrorism can be theoretically determined as a complex form of organization of a group, and a number of individuals or a sort of an institutional violence marked not only by frightening brachial-physical and psychological, but also sophisticated technological methods of political struggle aimed at achieving "great goals" in the morbidly spectacular manner.

Thus, there is no worse manner to do that than misusing pathogens, micro-organisms and their products, i.e. bioterrorism. Although this phenomenon is as old as civilization and society as a whole, we can say that by the development and misuse of science, bioterrorism is assuming monstrous proportions. Otherwise, at the end of the 20th century, the modern world has sailed into great contradictions, marked by strengthening the old and the emergence of new motives of terrorists, as well as new forms of terrorism, in particular those related to the use of weapons of mass destruction, especially biological.

Awareness of this phenomenon and its possible consequences is not at the highest level, and this problem has not been adequately treated in societies burdened by economic, political, ideological crises, poverty and many other problems and difficulties. On the other hand, threat and risk objectively exist and are not negligible, so this complex problem must be addressed seriously in our society and worldwide. Preparing our capacity to act in the case that bioterrorist attack may occur, we also become part of the global front combating terrorism. Thus we will further strengthen our international position contributing to the common good, as well as national and collective security.

For all this adequate knowledge is necessary. Prof. Dr. Elizabeta Ristanovic, an expert with extensive experience in this field, recognized beyond the borders of our country, identified this, starting a pioneering effort to educate students, the security sector, academia and the general public about the problem of bioterrorism.

About the manner in which she presents her knowledge and experience speaks the multimedia e-book in which are methodically and systematically, in an original and unconventional way, included the definition of bioterrorism and biological weapons, its classification and history of biological suffering, the use of biological weapons in war and terrorist activities, but also the subjects like agro-terrorism, misuse of genetic engineering and biotechnology with the aim of upgrading biological weapons and their application. The methods for detection and identification of biological agents, the epidemiology of infectious diseases and epidemiological surveillance in the event of a biological attack, the standards of biosafety and biosecurity, procedures for crisis management in the case of bioterrorist acts, as well as the psychological effects of such events are also analyzed.

Due to its content and unique approach, this book can be very interesting and important, not only within the University of Defence, students of political, medical-biological and other sciences, but also for professionals of different profiles from the security sector, diplomacy, media that each one of them, in their line of work, is an important factor within the national strategy for the protection against bioterrorism.

This work, as well, as a very significant engagement of Prof. Dr. Elizabeta Ristanovic represents a major contribu-

tion to what we want to create in Serbia, a complex and well-organized system of response and protection in emergency situations which also covers the topic of this electronic book.

I gladly recommend it for learning, analysis and acquisition of valuable information that we need in order to increase our knowledge, critical access to perceiving the problems and improving capabilities in the field of biological protection and biodefence. I especially emphasize and underline the international dimension, because if the know-how of the world join together, we can defeat such evil as terrorism, where a handful of extreme people bent on destroying property and billions of lives. With this book, we open a new chapter in international and regional cooperation so necessary for combating the dangers such as today's bioterrorism. I expect it will soon be translated in other languages, because as I have already mentioned, only through joint efforts, organization of capacities and resources, and above all, awareness and prevention we could win in this fight.

I want the author, Dr. Elizabeta Ristanovic, to realize her ambitious plans for further active engagement in order to mobilize domestic and international subjects in the field of fight against terrorism. This book published in English is a testimony to the right path.

Prof. Dr. Dragan Simeunovic,
The University of Belgrade

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Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

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Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

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Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

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2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure – **Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi – **Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

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 simboli

Koristiti samo standardne skraćenice, izuzev u naslovu i apstraktu. Pun naziv sa skraćenicom u zagradi treba dati kod prvog pominjanja u tekstu.

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