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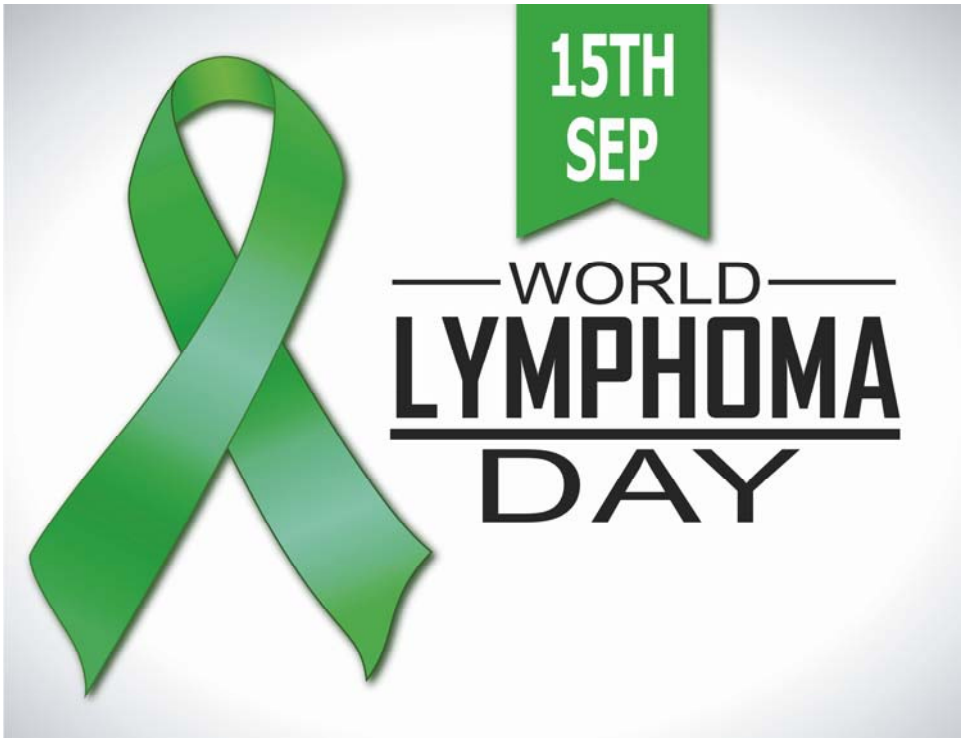

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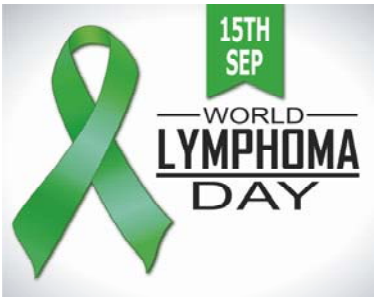

World Lymphoma Day is held on September 15 every year with goal to raise public awareness of lymphoma, an increasingly common form of cancer, in terms of early symptom recognition, and timely diagnosis and treatment.
It is established in 2004 by the Lymphoma Coalition, a non-profit network organisation of lymphoma patient groups from numerous countries around the world.
In Serbia, the Association of patients with lymphoma was established in 2006 in Belgrade.

Svetski dan limfoma održava se svake godine 15. septembra sa ciljem podizanja svesti o tom sve češćem obliku karcinoma, u smislu što ranijeg prepoznavanja simptoma i pravovremene dijagnoze i lečenja. Taj dan je ustanovljen 2004. godine od strane Koalicije za limfom, mreže neprofitnih organizacija bolesnika sa limfomom iz velikog broja zemalja širom sveta.
U Srbiji je Udruženje pacijenata sa limfomom osnovano 2006. godine u Beogradu.

ORIGINAL ARTICLES

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Factors associated with early treatment failure in adult hospitalized patients with community-acquired pneumonia

Faktori udruženi sa ranim neuspehom u lečenju odraslih hospitalizovanih bolesnika sa vanbolnički stečenom pneumonijom

Dubravka Vukadinović*, Natalija Samardžić†, Slobodan Janković*, Marijana Tomić Smiljanić‡, Radiša Pavlović*, Srdjan Stefanović*

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Abstract

Background/Aim. Early treatment failure (ETF) in patients hospitalized for community-acquired pneumonia (CAP) is associated with prolonged hospitalization, increased risk of mortality and high treatment costs. The aim of this study was to analyze the relative importance of factors influencing ETF in hospitalized adult patients with CAP that are still insufficiently explored. **Methods.** A retrospective case-control study was carried out on a sample of 126 adult patients treated for serious CAP at the Clinic for Pulmonary Diseases, Clinical Center of Serbia, Belgrade, Serbia, during the 5-year period (2007–2011). The cases (n = 63) were consecutive patients with ETF, observed within the three days upon the admission to hospital, while the control group consisted of the equal number of randomly selected patients without such an outcome. The association between potential risk/protective factors and ETF was estimated using logistic regression analysis. **Results.** The coexistence of gastrointestinal disorders [adjusted odds ratio (OR) 18.83, 95% confidence interval (CI) 1.15–309.04], higher CURB-65 (C – confusion; U – urea 7 mmol/L; R – respiratory rate ≥ 30 breaths/min; B – systolic blood pressure < 90 mmHg or diastolic blood pressure ≤ 60 mmHg; 65 – age ≥ 65 years) score on admission (adjusted OR 2.57, 95%CI 1.05–6.25), initial use of nonsteroidal anti-inflammatory drugs (NSAIDs) in hospital (adjusted OR 38.19, 95%CI 3.61–404.51) and previous outpatient use of inhaled corticosteroids (adjusted OR 22.41, 95%CI 1.03–489.06) were found to be significant risk factors for ETF. On the other hand, older age and use of antibiotics before the hospitalization were associated with a significantly lower chance of experiencing ETF, reducing the odds for 98% and almost 90%, respectively. **Conclusion.** The avoidance of the routine in-hospital use of NSAIDs as well as the outpatient use of appropriate antibiotics may be beneficial for patients hospitalized for CAP in terms of reducing the risk of ETF. The CURB-65 score could be a better predictor of ETF than Pneumonia Severity Index. Further prospective studies are required to confirm these findings.

Key words:
pneumonia; hospitalization; treatment outcome; risk factors; comorbidity.

Apstrakt

Uvod/Cilj. Rani neuspeh u lečenju (*early treatment failure* – ETF) hospitalizovanih bolesnika sa vanbolnički stečenom pneumonijom (*community-acquired pneumonia* – CAP) udružen je sa produženom hospitalizacijom, većim rizikom od smrtnog ishoda i visokim troškovima lečenja. Cilj ove studije bio je da analizira relativni značaj faktora koji utiču na pojavu ETF kod odraslih hospitalno lečenih bolesnika zbog CAP, a koji još uvek nisu dovoljno istraženi. **Metode.** Sprovedena je retrospektivna studija tipa slučaj-kontrola na uzorku od 126 odraslih bolesnika lečenih zbog težih oblika CAP na Klinici za plućne bolesti Kliničkog centra Srbije u Beogradu, u periodu 01.01.2007–31.12.2011. godine. „Slučajeve" su činila 63 uzastopno odabrana bolesnika sa uočenim neuspehom u lečenju u toku prva tri dana nakon prijema u bolnicu, dok se kontrolna grupa sastojala od identičnog broja nasumično izabranih bolesnika kod kojih takav ishod nije zabeležen. Povezanost između potencijalnih faktora rizika, odnosno protektivnih faktora i ETF procenjena je logističkom regresionom analizom. **Rezultati.** Udružena gastrointestinalna oboljenja [korigovani *odds ration* (OR) 18,83, 95% interval poverenja (CI) 1,15–309,04], viši CURB-65 (C – konfuzija; U – urea 7 mmol/L; *R* – frekvencija disanja ≥ 30 udisaja/min; B – sistolni krvni pritisak < 90 mmHg ili dijastolni krvni pritisak ≤ 60 mmHg; 65 – životno doba ≥ 65 godine) skor na prijemu (korigovani OR 2,57, 95% CI 1.05–6.25), inicijalna primena nesteroidnih antiinflamatornih lekova (NSAIL) u bolnici (korigovani OR 38,19, 95% CI 3,61–404,51) i prethodno ambulantno lečenje inhalacionim kortikosteroidima (korigovani OR 22,41, 95% CI 1,03–489,06), predstavljali su značajne faktore rizika za pojavu ETF. S druge strane, starije životno doba i upotreba antibiotika

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zbog iste infekcije pre prijema u bolnicu bili su povezani sa znatno nižim rizikom od razvoja ETF, smanjujući pritom šansu za 98%, odnosno za blizu 90%. **Zaključak.** Izbegavanje rutinske primene NSAIL u bolničkim uslovima i upotreba odgovarajućih antibiotika pre hospitalizacije mogu biti korisni za bolesnike obolele od CAP kod kojih je indikovano bolničko lečenje u smislu smanjenja rizika za nastanak ETF. CURB-65 skor na prijemu u bolnicu može biti bolji prediktor ETF od indeksa težine pneumonije. Dodatne prospektivne studije su potrebne kako bi se potvrdili ovi nalazi.

Ključne reči:
pneumonija; hospitalizacija; lečenje, ishod; faktori rizika; komorbiditet.

Introduction

In spite of recent progress in prevention, diagnosis and therapy of community-acquired pneumonia (CAP) remain serious public health problem worldwide. It is due to the relatively high incidence of CAP and its significant association with morbidity, mortality, reduced quality of life as well as increased healthcare costs, mainly in hospitalized older patients suffering from substantial comorbidities [1–8]. However, serious CAP requiring hospitalization and/or admission to the intensive care unit (ICU) also increases the risk of the aforementioned worse health outcomes even in younger adults without underlying chronic disorders [2, 4, 5, 7, 8].

While the majority of hospitalized patients with CAP achieve an adequate clinical response to initial empiric antibiotic and supportive therapy, some of them experience an early treatment failure (ETF) within 72 hours after initiation of the treatment, developing progression of underlying infection [9–15]. ETF inevitably leads to the more extensive use of microbiological and diagnostic tests, change in antimicrobial treatment and use of invasive therapeutic procedures, followed by prolonged hospitalization and much higher treatment costs [9, 11, 13, 14]. Moreover, there is a piece of evidence supporting a strong association between ETF and increased mortality [9, 10, 12–16]. As a consequence of considerable diversity in criteria used to define early CAP non-responders in hospitalized patients, there is a marked heterogeneity in reported prevalence of ETF with an average rate of 10–15% [9–13, 15, 16]. These criteria were mainly based on the deterioration of nonspecific clinical and radiological parameters previously used to diagnose CAP or the necessity to change antibiotic(s) [9–16]. However, previous studies showed that risk of death was significantly higher, up to five times, in patients experiencing ETF in comparison to satisfactory responders, regardless of criteria that were used to distinguish these two populations of inpatients with CAP [9, 10, 12, 13, 15–17].

Pursuant to the foregoing, adequate response to initial therapy is being regarded as essential for successful treatment of severe CAP. Therefore, a number of prior observational studies were particularly focused on determination of factors contributing to ETF [9–18]. According to their results, the occurrence of ETF can be influenced by factors related to characteristics of the pathogen, host and applied antimicrobial therapy, including also their mutual interactions. Many of these factors are well recognized, such as high-risk CAP according to Pneumonia Severity Index (PSI) scoring system (i.e., risk class IV or V) [12, 15, 16], multilobar lung involvement, pleural effusion, presence of pulmonary cavitations on radiological examination, CAP caused by *Legionella* or by Gram-negative bacteria, aspiration pneumonia, leucopenia, hyponatremia, high blood level of C-reactive protein (CRP), procalcitonin (PCT) and interleukin-6 (IL-6) in the first 24 hours upon admission to hospital, then coexisting malnourishment, malignant disease, neurological disorders and renal failure as well as initial use of inappropriate antibiotics (which is not aligned to susceptibility testing of causative pathogens) [12–18]. On the other hand, older age (≥ 65 years) [16], previous vaccination against influenza [12] and initial empiric use of moxifloxacin or combination of beta-lactam and macrolide antibiotics [11], may significantly reduce the risk of ETF. However, the literature data confirming consistency of clinical relevance of all aforementioned factors as well as the studies determining the relevance of the other potential risk and protective factors for ETF in hospitalized adults with CAP are lacking. Moreover, only a handful of previous studies [12, 15, 18] additionally addressed the factors contributing to late (after 72 hours) and/or any (early or late) treatment failure. These studies revealed not only the different factors associated with the observed outcomes (e.g., liver failure as a significant risk factor for late and any type of failure, and chronic obstructive pulmonary disease and initial use of levofloxacin as the important protective factors for any type of failure [12]), but also the differences in the strength of association for factors found to have a significant influence on the occurrence of ETF.

Thus, the purpose of this study was to analyze relative importance and potential synergistic effects of factors affecting the phenomenon of ETF in hospitalized adult patients with CAP that are still insufficiently explored.

Methods

Study settings

This study was conducted among adult patients who were admitted to the Clinic for Pulmonary Diseases (CPD), Belgrade, Serbia, due to serious CAP during the 5-year period (from January the 1st, 2007 to December the 31st, 2011). CPD, as a reference institution for lung disease operating within Clinical Center of Serbia (CCS), provides tertiary care to all adult inhabitants of city of Belgrade, and also to entire adult population of the Republic of Serbia as needed (patients referred to no CPD due to serious pulmonary disorders that can't be adequately treated in local hospitals). The diagnosis of CAP requiring hospitalization was based on typical clinical, laboratory and radiological signs according to internationally established standards [19], and left at the discretion of the attending physicians. All relevant data referring to demographic and clinical characteristics of patients, results of performed diagnostic exams as well as information on applied treatments and observed outcomes were gathered from

the patients' files. The study protocol had been approved by Ethics Committee of the CCS before the investigation begun.

Study design

A case-control design was chosen for this retrospective observational study. Based on outcome of interest, i.e. ETF in hospitalized patients with CAP, all participants were separated into two groups, as following: the cases were all patients from the study population in whom ETF was observed within the three days upon the admission to hospital, whereas the control group comprised of the equal number of patients treated in the same facility, but without such an outcome. Cases and controls were individually matched by age (± 1 year), gender and time of hospitalization (± one month), and then compared regarding the differences in the prevalence of exposure to putative risk factors. In addition, two groups were also compared in terms of differences in the death rate and the length of hospital stay. For each case, one of the matched controls was randomly chosen to participate in the study.

Pursuant to the recent study by Akram et al. [20], the identification of patients with ETF included the clinical instability according to standardized and validated Halm's criteria [21] in conjunction with any increase of CRP levels or decrease by less than 50% on third or fourth hospital day in relation to the values measured on admission to hospital, given that combined set of diagnostic parameters was found to be of greatest accuracy in predicting certain worse health outcomes [mortality, use of mechanical ventilation (MV) or vasopressor therapy, complicated pneumonia as well as their combination] in adult inpatients with CAP. In the present study, clinical instability was ascertained if any of the Halm's criteria had been "de novo" persistently abnormal for up to 72 hours upon the admission, i.e., temperature level above 37.8°C, pulse rate of over 100 beats *per* minute, systolic blood pressure of less than 90 mmHg, respiratory rate above 24 breaths *per* minute, oxygen saturation level of less than or equal to 90% or arterial partial pressure of oxygen below 8 kPa, altered mental function or inappetence [21].

Study population

A total of 126 consecutive adult patients (18 years of age and older) who required the hospital treatment of CAP during the observational period and underwent empiric antibiotic therapy within the 24 hours of admission lasting for at least two days, were enrolled in this study. As mentioned above, the allocation ratio of participants considering their outcome status was 1:1 (i.e. 63 patients were cases and 63 patients were randomly selected for the control group). All patients were referred to the CPD by their respective general practitioners and other medical doctors working at the primary health care facilities in the city of Belgrade and treated according to local CAP protocol of the CPD.

In order to distinguish the patients with high risk of nosocomial or health care-associated pneumonia due to the significant differences in their etiology, therapeutic approach and the prevalence of poor health outcomes compared to CAP [22], those who met any of the following criteria were not considered eligible for the study: transfer of patients from other hospitals or other wards who developed pneumonia after more than two days of admission, previous hospitalization for two or more days within the 90 days of pneumonia onset, use of intravenous antibiotics, chemotherapy or invasive procedures in the preceding 30 days, referral from nursing homes, actual hemodialysis treatment in health facilities, and underlying immunodeficiency due to any reason (e.g. malignant disease undergoing chemo- and/or radiotherapy, acquired immunodeficiency syndrome (AIDS), terminal stage of chronic progressive illness, use of long-term systemic corticosteroid therapy or other immunosuppressant drugs, etc.). Exclusion criteria also included patients who died due to any reason within the first two days of admission (that could be related to extremely severe, progressive infection with high risk of mortality despite the use of antibiotics), those with documented swine flu (influenza A H1N1 virus infection), those with tuberculosis, then pregnant and breastfeeding women as well as patients with lack of relevant information in their medical files.

The sample size calculation

A calculation of the adequate sample size was made by G*Power software [23], and based on the following input parameters: predicted differences between compared groups in the level of exposure to the most important risk factors for ETF in hospitalized CAP patients of 25% (which was assumed as a clinically relevant effect according to previous studies [11, 12, 16–18]), with its prevalence in the control group of 20%; statistical power of 90%; equal number of cases and controls; and a significance level (alpha) of 5% when using one-tailed z-test to compare two independent proportions of patients. Under these assumptions, a total of 118 participants (i.e., 59 *per* compared groups) were needed to provide for minimum sample size for this study.

Potential risk factors and other variables measured in the study

In the present study the following factors were investigated in terms of their association with ETF in adult hospitalized patients with CAP for each participant: body mass index of patients (kg/m^2); active smoking; alcohol consumption; associated chronic obstructive pulmonary disease (COPD); regular use of inhaled corticosteroids (ICS) for at least six months prior to the date of hospital admission; all patients were receiving ICS due to COPD; cardiovascular (CVS) comorbidity (e.g. arterial hypertension, angina pectoris, arrhythmias, chronic heart failure, cerebrovascular disorders and peripheral artery disease or combination of these disorders); coexisting diabetes mellitus (DM) type 1 or type 2; coexisting chronic renal failure (CRF) with exception of the patients on current dialysis treatment (as mentioned previously); it was defined as persistent decrease in creatinine clearance below 60 milliliters *per* minute *per* 1.73 square meters of body surface at least for three months [24] before the onset of the CAP; associated gastrointestinal disorders (GID) responsive to acid-suppressing medications, i.e., histamine H_2 receptor antagonists (H_2 blockers) and/or proton pump inhibitors (PPIs), such as chronic gastritis, peptic ulcer disease, gastroe-

sophageal reflux disease, etc.; all study subjects were treated with PPIs or H2 blockers before the hospitalization; use of PPIs in the preceding one month; use of H2 blockers in the preceding one month; pneumonia severity index (PSI) score calculated on admission to hospital based on retrospective data extracted from patients' files; it was set as an ordinal variable, distinguishing the patients into five classes (I–V) according to predicted risk of death [25]; CURB-65 (C – confusion, U – urea > 7 mmol/L, R – respiratory rate ≥ 30 breaths/min, B – systolic blood pressure < 90 or diastolic blood pressure ≤ 60 mmHg and 65 – age ≥ 65 years) score [26], which was also computed on admission to hospital based on retrospective data; presence of the multilobar involvement of lung parenchyma on initial radiographic exam; evidence of the pleural effusion based on initial radiographic exam; level of CRP on admission; leucopenia on admission, defined as the total white blood count below 4×10^9/L; hyponatremia on admission, defined as the sodium concentration below 130 mmol/L; initial admission to intensive care unit (ICU); initial use of mechanical ventilation; time interval in days from the onset of CAP symptoms to hospital admission; pre-hospital antibiotics therapy for the same infection; beta-lactams and macrolides were the only drugs from this group that were used before the admission to hospital; pre-hospital use of beta-lactam antibiotic (benzylpenicillin or amoxicillin ± clavulanic acid or oral cephalosporin); pre-hospital use of macrolides (azithromycin or clarithromycin); initial use of beta-lactams (amoxicillin + clavulanic acid or ceftriaxone or ertapenem); initial use of macrolides (azithromycin or clarithromycin); initial use fluoroquinolones (ciprofloxacin); ciprofloxacin was the only antibiotic from this group available as intravenous formulation and approved for the empiric treatment during the entire observational period; initial antimicrobial treatment with the combination of ceftriaxone and azithromycin or ceftriaxone and ciprofloxacin; these were the only combinations of antibiotics that had been used in our study patients; initial use of nonsteroidal antiinflammatory drugs (NSAIDs); only parenteral diclofenac 75 mg or ketorolac 30 mg were used in all patients treated with NSAIDs; initial use of paracetamol; initial use of H_2 blockers; intravenous ranitidine 50 mg was the only drug used in all patients treated with acid-suppressive medications; initial use of systemic corticosteroids; CAP of suspected pneumococcal origin based on detection of *Streptococcus pneumoniae* in the routine sputum culture; another tests that would increase the probability of confirming the diagnosis of pneumococcal CAP, such as isolation of pathogen from lung aspirate or pleural fluid or blood culture or urinary antigen assay, etc. were not performed in any of the patients with positive sputum culture; in addition, none of the patients was identified with penicillin resistant *Streptococcus pneumoniae* based on the usual antibiogram results, but E-test methods measuring minimal inhibitory concentrations as a gold standard for detection of resistant strains were not carried out; atypical CAP caused by *Mycoplasma pneumoniae* based on positive results of specific IgM and IgG antibodies testing; the other atypical pathogens, such as *Chlamydia pneumonia*, *Legionella pneumophila*, etc. were not detected in any of our study patients.

The age and gender of patients, as well as the date of admission to hospital, were recorded as the strong potential confounding variables used for matching cases and controls, as mentioned above. At that, the age was dichotomized in order to distinguish elderly patients (65 years and older) from younger participants. Finally, the mortality and the length of hospitalization were also observed as secondary outcomes in both compared groups.

Data analysis

All the collected data were summarized with descriptive statistics, as following: means and standard deviations were used for continuous data if they had followed normal distribution based on Kolmogorov-Smirnov test for normality ($p > 0.05$), while medians and interquartile ranges (IQR 25–75) were calculated when presenting non-normally distributed continuous data as well as ordinal variables; on the other hand, categorical variables were expressed as frequencies and percentages. Depending on the normality of actual data distribution the significance of differences in continuous variables between cases and controls was estimated using Independent Student t-test or alternate, non-parametric Mann-Whitney U test which was also used to compare ordinal data, while categorical data were analyzed using χ^2 tests for frequencies with Yates continuity correction only in 2*2 contingency tables. In order to determine factors influencing the observed dichotomous outcome (ETF) significantly as well as their possible additive effects, crude and adjusted odds ratios (OR) with corresponding 95% confidence intervals (95% CI) were calculated using univariate and a stepwise backwards conditional multivariate logistic regression analysis (with respect to matched pairs study design, as mentioned above). The alpha level of 5% was set in all analyses, while stepwise regression model removed all variables with an additional probability (p value) of 0.1 and above. The association between observed risk/protective factors and ETF was considered significant if 95% CI of adjusted OR did not include the value of 1. All statistical tests were performed using commercial software SPSS version 19.0 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics of cases and controls and differences among them in the level of exposure to putative risk factors are presented in Tables 1, 2 and 3. In both groups, there were twice as many women than men and almost an equal number of elderly without significant differences regarding the both confounders observed. Two compared groups were also very similar in terms of majority of other demographic characteristics, clinical features and unhealthy behaviors as well as regarding the frequency of identified causative agent of CAP and initial therapy they were receiving, with exception of considerably higher prevalence of CRF (Table 2), initial admission to ICU and initial use of mechanical ventilation (MV) (Table 3) in the group of cases. Relative to controls, the cases were also found to have a significantly higher CURB-65 score on admission (Table 2). Significant differences between compared groups in the

frequency of any of modalities of empirical initial antibiotic treatment were not observed (Table 3).

The results of both univariate and well-strengthened multivariate logistic regression analysis (the last step of stepwise backward elimination method: Cox & Snell R square 0.440, Nagelkerke R square 0.578, Hosmer-Lemeshow χ^2 3.725, df = 8, $p = 0.881$, overall model accuracy of 80.8%) are shown in Tables 1, 2, 3 and 4. Statistically significant but also clinically important (according to large effect size) association with the occurrence of ETF in hospitalized patients with CAP was found only for the coexistence of GID, higher CURB-65 score on admission, initial use of NSAIDs and use of ICS on a daily basis for at least six months prior to admission to hospital (see adjusted OR with 95%CI in Table 4). Albeit the crude OR for the coexistence of CRF, initial admission to ICU and initial ap-

Table 1

Demographic characteristics of the study population

Variable	Cases (n = 63)	Controls (n = 63)	Test value and significance of null hypothesis	Crude odds ratio with confidence intervals (1.96*SE)
Gender (male/female), n (%)	42 (66.7)/21 (33.3)	44 (69.8)/30.2	$\chi^2 = 0.035$ $p = 0.852$	0.87 (0.42, 1.81)
Age (years), mean ± SD	57.8 ± 17.6	57.8 ± 18.1	T = 0.00 $p = 1.00$	1.00 (0.98, 1.02)
Elderly (≥ 65 years age), n (%)	26 (41.3)	27 (42.9)	$\chi^2 = 0.00$	0.94 (0.46, 1.90)
BMI (kg/m^2), mean ± SD	28.3 ± 3.1	24.5 ± 7.6	T = -1.846 $p = 0.070$	1.09 (0.97, 1.21)

χ^2 – Chi-squared test; T – Independent samples *t*-test; SD – standard deviation; SE – standard error; BMI – body mass index.

Table 2

Lifestyle habits, comorbidities and therapy of the study subjects before the hospitalization

Variable	Cases (n = 63)	Controls (n = 63)	Test value and significance of null hypothesis	Crude odds ratios with confidence intervals (1.96*SE)
Active smokers, n (%)	40 (63.5)	31 (49.2)	$\chi^2 = 2.614$ $p = 0.106$	1.80 (0.88, 3.66)
Alcohol consumers, n (%)	4 (6.3)	6 (9.5)	$\chi^2 = 0.434$ $p = 0.510$	0.64 (0.17, 2.40)
Coexisting COPD, n (%)	10 (15.9)	8 (12.7)	$\chi^2 = 0.065$ $p = 0.799$	1.30 (0.48, 3.54)
Prior regular use of ICS, n (%)	8 (12.7)	6 (9.5)	$\chi^2 = 0.321$ $p = 0.571$	1.38 (0.45, 4.24)
Coexisting CVS diseases, n (%)	39 (61.9)	34 (54.0)	$\chi^2 = 0.521$ $p = 0.470$	1.39 (0.68, 2.82)
Coexisting DM, n (%)	11 (17.5)	10 (15.9)	$\chi^2 = 0.057$ $p = 0.811$	1.12 (0.44, 2.86)
Coexisting CRF, n (%)	8 (12.7)	1 (1.6)	$\chi^2 = 4.308$ $p = 0.038$*	9.02 (1.09, 74.41)*
Coexisting GID, n (%)	7 (11.1)	4 (6.3)	$\chi^2 = 0.151$ $p = 0.697$	1.84 (0.51, 6.64)
Prior use of PPIs, n (%)	6 (9.5)	2 (3.2)	$\chi^2 = 0.640$ $p = 0.424$	3.21 (0.62, 15.56)
Prior use of H_2 blockers, n (%)	5 (7.9)	3 (4.8)	$\chi^2 = 0.896$ $p = 0.344$	1.72 (0.39, 7.55)
Duration of symptoms onset to hospital admission (days), mean ± SD	6.2 ± 3.8	6.8 ± 4.5	T = 0.855 $p = 0.394$	0.99 (0.95, 1.04)
Pre-hospital use of antibiotics, n (%)	32 (50.8)	39 (61.9)	$\chi^2 = 1.581$ $p = 0.209$	0.64 (0.31, 1.29)
Pre-hospital use of beta-lactams, n (%)	20 (31.7)	24 (38.1)	$\chi^2 = 0.559$ $p = 0.455$	0.76 (0.36, 1.58)
Pre-hospital use of macrolides, n (%)	9 (14.3)	8 (12.7)	$\chi^2 = 0.068$ $p = 0.794$	1.15 (0.41, 3.19)

χ^2 – Chi-squared test; T – Independent samples *t*-test; * – significant association; SD – standard deviation; COPD – chronic obstructive pulmonary disease; ICS – inhaled corticosteroids; CVS – cardiovascular comorbidity; DM – diabetes mellitus; CRF – chronic renal failure; GID – gastrointestinal disorders; PPIs – proton pump inhibitors; H_2 blockers – histamine H_2 receptor antagonists.

Table 3

Etiology of CAP, clinical and laboratory parameters on admission and initial in-hospital therapy of the study subjects

Variable	Cases (n = 63)	Controls (n = 63)	Test value and significance of null hypothesis	Crude odds ratios with confidence intervals (1.96*SE)
Suspected pneumococcal CAP, n (%)	17 (27.0)	18 (28.6)	$\chi^2 = 0.040$ $p = 0.842$	0.92 (0.42, 2.02)
Mycoplasma pneumoniae CAP, n (%)	5 (7.9)	6 (9.5)	$\chi^2 = 0.00$ $p = 1.00$	0.82 (0.24, 2.84)
PSI risk class (I–V), median (IQR)	3 (2.5–4)	3 (2–4)	U = 1759.500 Z = -1.143 $p = 0.253$	1.24 (0.89, 1.74)
CURB-65 score (0–4)[§], median (IQR)	2 (1–2)	1 (0–2)	U = 1510.500 Z = -2.408 $p = 0.016$*	1.53 (1.08, 2.15)*
Multilobar pneumonia, n (%)	21 (35.0)	29 (46.8)	$\chi^2 = 1.748$ $p = 0.186$	0.61 (0.30, 1.27)
Pleural effusion, n (%)	10 (16.9)	4 (6.7)	$\chi^2 = 3.030$ $p = 0.082$	2.86 (0.84, 9.69)
CRP (mg/L) mean ± SD	128.1 ± 127.8	155.0 ± 118.3	T = 0.359 $p = 0.722$	0.99 (0.99, 1.01)
Leucopenia, n (%)	3 (4.8)	4 (6.3)	$\chi^2 = 0.151$ $p = 0.697$	0.74 (0.16, 3.44)
Hyponatremia, n (%)	2 (3.2)	3 (4.8)	$\chi^2 = 0.208$ $p = 0.648$	0.66 (0.11, 4.07)
Initial ICU admission, n (%)	20 (31.7)	8 (12.7)	$\chi^2 = 5.556$ $p = 0.018$*	3.20 (1.29, 7.96)*
Use of MV, n (%)	11 (17.5)	1 (1.6)	$\chi^2 = 7.309$ $p = 0.007$*	12.90 (1.61, 103.32)*
Empirical use of beta-lactams, n (%)	53 (84.1)	50 (79.4)	$\chi^2 = 0.479$ $p = 0.489$	1.38 (0.55, 3.43)
Empirical use of macrolides, n (%)	9 (14.3)	15 (23.8)	$\chi^2 = 1.853$ $p = 0.173$	0.53 (0.21, 1.33)
Empirical use of ciprofloxacin, n (%)	44 (69.8)	37 (58.7)	$\chi^2 = 1.694$ $p = 0.193$	1.63 (0.78, 3.40)
Empirical use of ceftriaxone plus azithromycin, n (%)	8 (12.7)	12 (19.0)	$\chi^2 = 0.535$ $p = 0.465$	0.62 (0.23, 1.64)
Empirical use of ceftriaxone plus ciprofloxacin, n (%)	24 (38.1)	30 (47.6)	$\chi^2 = 0.810$ $p = 0.368$	0.68 (0.33, 1.38)
Use of NSAIDs, n (%)	24 (38.1)	14 (22.2)	$\chi^2 = 3.768$ $p = 0.081$	2.15 (0.99, 4.71)
Use of paracetamol, n (%)	50 (79.4)	47 (74.6)	$\chi^2 = 0.672$ $p = 0.179$	1.31 (0.57, 3.01)
Use of H_2 blockers, n (%)	30 (47.6)	22 (34.9)	$\chi^2 = 2.096$ $p = 0.148$	1.69 (0.83, 3.47)
Use of systemic corticosteroids, n (%)	10 (15.9)	5 (7.9)	$\chi^2 = 1.892$ $p = 0.169$	2.19 (0.70, 6.82)

χ^2 – Chi-squared test; T – Independent samples *t*-test; [§]CURB-65 – CURB-65 (C – confusion, U – urea > 7 mmol/L, R – respiratory rate ≥ 30 breaths/min, B – systolic blood pressure < 90 or diastolic blood pressure ≤ 60 mmHg and 65 – age ≥ 65 years) score of 5 was not calculated in any of study patients; * – statistically significant association; SD – standard deviation; SE – standard error; CAP – community-acquired pneumonia; PSI – pneumonia severity index; CRP – C-reactive protein; MV – mechanical ventilation; NSAID – nonsteroidal anti-inflammatory drugs; H_2 blockers – histamine H_2 receptor antagonists; ICV – intensive care unit.

plication of MV hinted at potentially significant contribution to the observed outcome after adjustment for other variables such effects disappeared. Contrary to the above mentioned, the older age and outpatient use of antibiotics before the hospitalization were associated with a significantly lower likelihood of experiencing ETF, decreasing the chance for 98% and almost 90%, respectively (Table 4).

By exploring the mutual interactions between observed predictors of ETF in a clinically meaningful manner, with the inclusion of the factors for which the possible additive effects had been anticipated, clearly evident synergism was demonstrated for a higher CURB-65 score on admission and initial use of NSAIDs (adjusted OR 5.169; 95% CI 1.466, 18.222). The joint effects on observed outcome between the initial use of NSAIDs and GID comorbidity, then between initial use of NSAIDs and prior regular use of ICS as well as between older age and use of antibiotics before admission were found not to be statistically significant.

Ultimately, 11 of total 63 patients (11.7%) in the group of subjects who experienced ETF died in contrast with only one death (1.6%) in the control group, yielding a high level

Table 4

Crude and adjusted odds ratios of the risk and protective factors for early treatment failure (ETF) in hospitalized patients with community-acquired pneumonia (CAP)[#]

Risk/protective factors	Crude OR (95% CI)	Adjusted OR (95%CI)
Older age (≥ 65 years)	0.94 (0.46, 1.90)	0.02 (0.01, 0.36)*
Coexisting CVS disorders	1.39 (0.68, 2.82)	5.462 (0.70, 42.45)
Coexisting GID	1.84 (0.51, 6.64)	18.83 (1.15, 309.04)*
CURB-65 score on admission	1.53 (1.08, 2.15)*	2.57 (1.05, 6.25)*
Pre-hospital use of antibiotics	0.64 (0.31, 1.29)	0.12 (0.02, 0.86)*
Pre-hospital use of macrolides	1.15 (0.41, 3.19)	0.17 (0.02, 1.46)
Initial use of NSAIDs	2.15 (0.99, 4.71)	38.19 (3.61, 404.51)*
Prior regular use of ICS	1.38 (0.45, 4.24)	22.41 (1.03, 489.06)*

[#] Results are obtained at the final step of multivariate logistic regression analysis; * Statistically significant association; OR – odds ratio; CI – confidence interval; CVS – cardiovascular comorbidity; GID – gastrointestinal disorders; CURB-65: C – confusion; U – urea > 7 mmol/L; R – respiratory rate ≥ 30 breaths/min; B – systolic blood pressure < 90 mmHg or diastolic blood pressure ≤ 60 mmHg; 65 – age ≥ 65 years; NSAID – nonsteroidal anti-inflammatory drugs; ICS – inhaled corticosteroids.

of significance of observed difference: $\chi^2 = 7.461$, df = 1, $p = 0.006$. On the other hand, in terms of the length of hospital stay the cases (median 13 days, IQR 7.5–18.5) did not significantly differ from controls (median 12 days, IQR 9–14): Mann-Whitney test – U = 1869,00, Z = -0.565, $p = 0.572$.

Discussion

In view of the relatively frequent occurrence of ETF in hospitalized patients with CAP and its high risk of producing poor health, humanistic and economic outcomes, identification of the factors contributing to this phenomenon is particularly important for planning appropriate preventive measures and improving the care of such patients [9–19, 20, 22]. According to our knowledge, this is the first study investigating the risk factors for objectively assessed ETF in CAP on the population of hospitalized patients in Serbia, the country passing through the period of socio-economic transition with the exceptional specificities of the functional organization and financing of the health system. The most important results point out that patients admitted to hospital due to CAP who suffer from chronic GID responsive to acid-suppressing drugs, those with increased CURB-65 score on admission, then patients initially treated with NSAIDs or who were regularly treated with ICS prior to hospitalization, have higher chances of having ETF compared to patients without such characteristics, while the elderly persons or those who received antibiotics before the hospitalization are less likely to experience the observed outcome.

An interesting finding of this study is distinction of PSI and CURB-65 scores on admission in terms of relevant influence on the appearance of ETF, as these reliable severity assessment criteria were both recommended to be incorporated in individual clinical judgment concerning the decision for hospitalization or outpatient care based on the predicted risk of mortality [19, 22, 24–26]; additionally, several prior studies reported that increased initial scores by both tools may also be linked to other important bad clinical and economic outcomes, such as prolonged time to achieve clinical stability, longer duration of hospital stay and increased costs of treatment [11, 14, 20, 27]. When it comes to contribution to the development of ETF, there are inconsistent data on the relevance of these criteria, from those that show significant association (CURB-65 [11], PSI [12, 15, 16]) up to those who indicate the absence of any connection with the aforementioned outcome [14, 17, 18]. Our study found the initial CURB-65 score to be a better predictor of ETF in inpatients with CAP than PSI, indicating that any increase by 1 in this score increases the odds of having ETF for approximately 2.6 times. This discriminative effect of CURB-65 is consistent with the study by Ott et al. [11], but distinct to the findings of Martin-Loeches et al. [18] who found the difference between CURB-65 and PSI in favor of CURB-65 only regarding the positive relationship with the late treatment failure, not with ETF, in hospitalized CAP patients [18]. Our findings may be partially explained by imprecisions in the retrospective calculation of PSI score, which includes 20 variables and a quite complex scoring algorithm. Some relevant elements of PSI scoring system, such as certain chronic comorbid conditions and referral of patients from nursing homes, could not be taken into account when calculating the total PSI score in our study, because they were either among the exclusion criteria (such as presence of coexisting malignant diseases and admission of nursing home residents) or were not registered in any of our patients (such as presence of liver failure); in addition, as previously mentioned, the presence of cancer [17] or liver failure [12] have already been linked with the treatment failure in hospitalized patients with CAP. Therefore, any incorrectness or incompleteness of retrospective data in the patients' files relating to these criteria could possibly lead to an underestimation of total PSI score implying reduced generalizability of obtained results. On the other hand, based on the results of our study and the results of recent study by Wesemann et al. [28] who found CURB-65 to be significantly associated with the long-term mortality, it is easily measurable score that could be assessed in each patient hospitalized due to CAP, and it may help recognizing the patients not only at risk of in-hospital death but also the risk of death after discharge from hospital.

NSAIDs are widely used for symptomatic treatment in patients with CAP, even as self-medication at early phases of

disease in outpatient settings [29]. However, the recent observational studies [30, 31] suggest that early use of these drugs in patients suffering from CAP may put them at risk of postponing hospital admission and effective antimicrobial treatment due to initial alleviation of symptoms, thus leading to progression of the infection with higher incidence of local complications, particularly pleural empyema and pulmonary cavitations; on the other hand, significant association between the NSAIDs and distant organ dysfunction [30] or death rate [31] in these studies was not observed. Similarly, the multicenter case-control study by Legras et al. [32] did not show any connection between the use of NSAIDs and development of severe sepsis or septic shock in various community-acquired infections of bacterial origin of whom pneumonia was the most frequent, but it demonstrated an important contribution of these drugs to the delay in receiving appropriate antibiotics. Such distinct effect of NSAIDs in terms of increasing local while blunting systemic inflammatory response [30], may be explained by possible imbalance in inhibition of prostaglandin and leukotriene action, the mediators who were found to be among the major factors influencing the complex immune response of lungs to acute infection [33], but whose role in the pathogenesis and evolution of CAP was not fully elucidated in humans. Additionally, various experimental models indicate either augmented or reduced phagocytic and bactericidal activities of leukocytes and alveolar macrophages after applying different cyclo-oxygenase inhibitors [34–36]. Anyway, a causal association between use of NSAIDs and bad clinical outcomes could not be ascertained, so current treatment guidelines do not advise against their use in patients with CAP [29]. Yet we are concerned with the finding that initial use of NSAIDs in the hospital could largely increase the risk of developing ETF in CAP patients. Despite the lack of complete retrospective data on pre-hospital use of NSAIDs and their dosage regimens when they were applied in the hospital, taking into account the fact that clinically relevant association between use of NSAIDs and ETF was evident after adjustment for the effects of other confounders, we believe that these drugs should be used with extreme caution in hospitalized patients with CAP, especially in patients with coexisting CRF or cardiovascular diseases and those with severe infection as assessed by CURB-65 score on admission, because these conditions may also predispose patients to the serious adverse effects of NSAIDs. Therefore, in these patients, paracetamol may be antipyretic of choice due to much better safety profile while not affecting the ETF as it was shown in our study.

Strong positive association between prior regular use of ICS for COPD and ETF in patients hospitalized for CAP is a novel and particularly exciting finding of this study, given that the previous observational studies examining the impact of these medications use on other relevant clinical outcomes have yielded inconsistent results. Namely, there are reports suggesting potentially beneficial effects of ICS used in ambulatory settings for COPD on the short-term risk of death [37, 38] and the need for use of MV [38] in patients who developed CAP and required hospitalization, while in contrast, some studies did not find any significant influence of these drugs on both short- and long-term mortality [39, 40] as well as on the occurrence of complicated CAP [40]. Regardless of the absence of impact on mortality, Ferrer et al. [39] demonstrated a favorable effect of the previous use of ICS in terms of mitigating the systemic inflammatory response, which is similar to the above-mentioned effect of NSAIDs [30]. But opposite to NSAIDs, there is evidence about the protective role of prior treatment with ICS on the development of pleuropulmonary complications in patients with CAP suffering from various coexisting chronic respiratory diseases [41]. The patients with CAP experiencing ETF in our study had less pronounced systemic inflammatory response on admission based on average baseline level of CRP (< 150 mg/L) than the subjects without such an outcome (155 mg/L); interestingly, utilization of both NSAIDs and ICS was higher in group of cases compared to controls, but without significant differences. In addition, despite well-known favorable effects of ICS in the treatment of COPD, there are also conflicting data regarding their association with increased risk of CAP in such patients [42, 43]. The mechanisms of these controversial effects of ICS in CAP patients with co-existing COPD have not yet been fully clarified, and it may be strongly influenced by medication type, dosage regimen and duration of outpatients use, then by the severity of COPD as well as by concomitant use of systemic corticosteroids [37–43]. As we were unable to adjust the observed association between regular use of ICS in outpatient settings and ETF in patients hospitalized for CAP for the most of the confounders listed here due to incompleteness of retrospectively obtained data, this our finding require further confirmation in prospective studies with appropriate design and conducted on larger sample of inpatients with CAP and coexisting COPD.

Although coexistence of chronic GID observed as potential risk factor for ETF in CAP in our study (e.g. peptic ulcer disease or gastroesophageal reflux), was not identified as independent predictor of CAP previously [44], there is a lot of data indicating that use of PPIs and/or H2 blockers (which are considered as a gold standard for pharmacological treatment of these disorders) may increase risk of developing CAP significantly [45–47]. Such effects of acid-suppressive drugs could be explained by increased bacterial growth and colonization of the upper gastrointestinal tract due to suppression of gastric acid secretion, followed by secondary aspiration and translocation of pathogens to lungs [45, 47]. Additionally, PPIs may lead to bacterial overgrowth directly in the lungs by affecting pH of seromucinous secretion due to inhibition of proton pump in the mucous cells and ducts of respiratory tract [48]. Finally, there is also evidence from *in vitro* studies suggesting inhibitory effects of both PPIs and H2 blockers on function of leukocyte and natural killer cells [49–51]. As all our patients were treated with acid-suppressive medications before the hospitalization, we believe that aforementioned mechanisms of the effects of these medications regarding host-pathogen interaction, could be also involved to some extent in explanation of the significant association between GID and ETF in CAP as observed in this study.

Nowadays, the outpatient antibiotic use for CAP requiring subsequent hospitalization is relatively common, with a prevalence of more than 20% [52]. This phenomenon is particularly important since it can strongly influence clinical picture and severity of disease at initial presentation, inflammatory response of the patient, selection of diagnostic tests to identify a causative agent of infection as well as a choice of empirical antibiotic treatment [52, 53]. However, there are scarce and contradictory data regarding the relevance of its association with the in-hospital mortality and other relevant outcomes. Simonetti et al. [53] did not find significant differences in 30-day mortality and ICU admission rate among the patients with and without pre-hospital antibiotic use, respectively, while bacteraemia was less prevalent, and the occurrence of lung cavitations was more frequent in patients who had received antibiotics before the hospitalization. Moreover, Johnson et al. [54] showed that patients who had been pre-treated with antibiotics were significantly less likely to die, especially if they received suitable antibiotics in line with the applicable guidelines. On the other hand, van de Garde EM et al. [55] found pre-hospital antibiotic use to be a significant risk factor for death only in patients with coexisting chronic heart failure, while the significant difference in the length of hospital stay between compared groups was not observed. Protective role of the outpatient use of antibiotics before the hospitalization on the development of ETF in patients with CAP, as shown in our study, has reaffirmed the importance of prompt and appropriate empirical antibacterial treatment when serious lower respiratory tract infection is suspected.

Older age is another significant factor associated with the reduced likelihood of experiencing ETF in hospitalized patients with CAP in our study. This finding is similar to the previous study by Rosón et al. [16], but still remains unclear. Additionally, authors did not find the more prevalent initial use of broad spectrum antibiotics in a group of patients aged 65 years and over, which also was not observed in our study. As the vast majority of our elderly patients had more severe CAP on initial presentation at hospital according to PSI and CURB-65 score (i.e. 42/53 or 79% of them), we believe that these patients were probably receiving more appropriate symptomatic and supportive therapy due to a greater awareness of attending physicians regarding the increased risk of potential complications.

At last, this study also hinted at the potential contribution of ETF to mortality of hospitalized patients with CAP, but it was neither designed nor powered to examine the magnitude of such effect (due to small number of deceased patients), as were the previous studies who did find a clear association between these two phenomena [9, 10, 12, 13, 15, 16]. In contrast to many prior investigations [9, 11, 13, 14], the difference in duration of hospital stay between patients with and without ETF, respectively, in this study was not observed.

There are certain limitations of this study that deserve to be mentioned mainly lying in its retrospective nature. Due to incompleteness of data extracted from the inpatient medical records we could not assess all relevant factors that are likely to be associated with the observed primary outcome of interest correctly, such as physicians' consultations before hospitalization for CAP, dose regimens and duration of pre-hospital drug therapy (antibiotics, NSAIDs, ISC, systemic steroids, PPIs, H2 blockers) as well as compliance with these treatments, severity of coexisting chronic disorders, extent of smoking and alcohol consumption and baseline serum levels of procalcitonin. Therefore, these variables were not analyzed at all. Furthermore, any erroneous or contradictory information taken from the patients' files could have led to an inaccurate estimation of both the risk factors and the outcomes that we have observed. Eventually, the generali-zability of the results could be questionable given that this study was carried out in a single hospital and in a small country such as Serbia.

Conclusion

With the aim of reducing the risk of early treatment failure, this study advises against the routine use of non-steroidal anti-inflammatory drugs in adult patients hospitalized for community-acquired pneumonia, particularly in those with increased severity of disease as assessed by increased CURB-65 score on admission. The CURB-65 score on admission is not only easy to calculate but also better predicts the development of early treatment failure in patients with community-acquired pneumonia compared to Pneumonia Severity Index. Previous regular use of inhaled corticosteroids as well as gastrointestinal disorders responsive to acid-suppressive medications may be also associated with the increased risk of early treatment failure in this population of patients. On the other hand, prompt empirical use of appropriate antibiotics in outpatient settings could be beneficial for patients with community-acquired pneumonia requiring hospitalization as it may reduce the risk of early treatment failure. However, due to possible selection and measurement bias, these findings should be further confirmed in larger prospective studies.

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Conflict of interest

The authors declare that they have no conflict of interest in this study.

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Analgesic efficacy and safety of four different anesthesia/postoperative analgesia protocols in patients following total hip arthroplasty

Analgetska efikasnost i bezbednost četiri različita protokola anestezije/postoperativne analgezije kod pacijenata nakon aloartroplastike kuka

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Abstract

Background/Aim. Hip replacement surgery can initiate significant postoperative pain caused by bone alterations, implant, and soft tissue or nerve injuries. Postoperative analgesia using regional techniques has been shown to have numerous advantages over the intravenous use of morphine. However, numerous side effects and complications of postoperative continuous epidural analgesia have been reported recently. The aim of this prospective, randomized study was to investigate whether continuous lumbar plexus block can be a safe and efficacious alternative for postoperative analgesia in comparison with epidural analgesia and patient-controlled analgesia with morphine (PCA morphine) for hip arthroplasty. **Methods.** This prospective study included 60 patients, scheduled for total hip arthroplasty. Patients were randomized into 4 groups: the group central nerve block – epidural (CNB), the group peripheral nerve block – lumbar plexus block (PNB), the group spinal anesthesia-PCA morphine (SAM) and the group general anesthesia-PCA morphine (GAM). The quality of analgesia and side effects (hypotension, nausea, vomiting, urinary retention) were recorded in all groups at 4 h, 12 h, and 24 h after surgery. Pain scores were assessed using Visual Analogue Scale (VAS), both at rest and on moving. **Results.** Our findings demonstrated that the use of a continuous lumbar plexus block provides effective analgesia at rest and on moving, during 24 h after hip arthroplasty. Pain scores varied significantly among the groups 4 h postoperatively ($F = 21.827$; $p < 0.01$), 12 h postoperatively ($F = 41.925$; $p < 0.01$) and 24 h postoperatively ($F = 33.768$; $p < 0.01$) with the highest scores ≥ 3 in the GAM group. Patients from the PNB group had satisfactory analgesia, comparable with patients from the CNB group. The incidence of nausea was significantly lower in the PNB group 12 h after the operation ($\chi^2 = 9.712$; $p < 0.01$). The incidence of urine retention was significantly different 12 h after the operation, with a presence only in the CNB group, with the incidence of 33.3% ($\chi^2 = 16.365$; $p < 0.01$). In all studied groups, the incidence of hypotension was not significantly different postoperatively. **Conclusion.** Administration of postoperative analgesia using continuous lumbar plexus block produces satisfactory analgesia with a low incidence of side effects when compared to epidural analgesia or parenteral opioids following hip arthroplasty.

Key words:
arthroplasty, replacement, hip; pain, postoperative; nerve block; lumbosacral plexus; analgesia; methods; analgesia, epidural; pain measurement.

Apstrakt

Uvod/ Cilj. Zamena totalne proteze kuka može prouzrokovati značajne bolove nakon operacije, kako zbog hirurške traume kosti i prisustva implanta, tako i zbog oštećenja mekih tkiva i živaca. Metode postoperativne analgezije koje uključuju neku od tehnika regionalne anestezije imaju mnogobrojne prednosti u odnosu na intravensku primenu morfina postoperativno. Međutim, u skorije vreme su objavljeni mnogobrojni neželjeni efekti i komplikacije kontinuirane epiduralne analgezije. Cilj ove prospektivne, randomizovane studije bio je da se utvrdi da li kontinuirani blok lumbalnog pleksusa predstavlja sigurnu i efikasnu alternativu za postoperativnu analgeziju u poređenju sa epiduralnom analgezijom i pacijent kontrolisanom analgezijom (PKA) morfinom kod aloartroplastike kuka. **Metode.** U ovu prospektivnu studiju bilo je uključeno 60 pacijenata, pred-

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viđenih za aloartroplastiku kuka. Pacijenti su bili raspoređeni u četiri grupe: grupa centralni neuroblok – epidural (CNB), grupa periferni neuroblok – blok lumbalnog pleksusa (PNB), grupa spinalna anestezija – PKA morfin (SAM), i opšta anestezija – PKA morfin (OAM). Kvalitet analgezije i učestalost neželjenih efekata (hipotenzije, muke, povraćanja, retencije urina) praćeni su u svim grupama 4 h, 12 h i 24 h nakon operacije. Jačina bola procenjivana je pomoću vizuelne analogne skale (VAS), tokom mirovanja i pri pokretima. **Rezultati.** Ovo istraživanje pokazalo je da se primenom kontinuiranog bloka lumbalnog pleksusa obezbeđuje efikasna analgezija u mirovanju i pri pokretima tokom 24 h nakon aloartroplastike kuka. Jačina postoperativnog bola varirala je značajno među grupama 4 h nakon operacije ($F = 21,827$; $p < 0,01$), 12 h nakon operacije ($F = 41,925$; $p < 0,01$) kao i nakon 24 h ($F = 33,768$; $p < 0,01$), sa najvećim zabeleženim skorom ≥ 3 u grupi OAM. Pacijenti iz grupe PNB imali su zadovoljavajuću analgeziju, sličnu pacijentima iz CNB grupe. Incidencija muke bila je značajno niža u PNB grupi 12 h nakon operacije ($\chi^2 = 9,712$; $p < 0,01$). Incidencija retencije urina značajno se razlikovala 12 h nakon operacije i pojavljivala se jedino u grupi CNB, sa učestalošću od 33,3% ($\chi^2 = 16,365$; $p < 0,01$). Nije bilo statistički značajnih razlika u učestalosti hipotenzije u ispitivanim grupama. **Zaključak.** Postoperativna analgezija nakon aloartroplastike kuka kontinuiranom blokadom lumbalnog pleksusa obezbeđuje zadovoljavajuću analgeziju sa malim brojem neželjenih efekata, u poređenju sa epiduralnom analgezijom i parenteralnom primenom opioida.

Ključne reči:
artroplastika kuka; bol, postoperativni; blokada živca; lumbosakralni predeo; analgezija; metode; analgezija, epiduralna; bol, merenje.

Introduction

Hip replacement surgery can cause significant postoperative pain and surgical stress response. The ideal method of pain relief management for this type of surgery has not been established yet [1]. Inadequate pain relief leads to a higher risk of respiratory and cardiovascular complications, such as pneumonia caused by hypoventilation, tachycardia, hypertension, myocardial ischemia and myocardial infarction. Untreated acute pain has been found to be one of the risk factors for poor wound healing and transition into the chronic pain. Furthermore, early postoperative mobilization of the patient and hospital discharge can be delayed in these patients. More severe pain often requires opioid therapy. Increased opioid use may result in a subsequent increase in nausea and vomiting by stimulation of the chemoreceptor trigger zone in the floor of the fourth ventricle [2].

Pain related to the hip surgery itself can be associated with an implant, bone alterations and soft tissue or nerve injuries. Apart from surgical injury, patient's functional status, age, comorbidity, body mass index (BMI) and anxiety may influence the postoperative pain scores.

The hip joint is innervated by three nerves: femoral nerve (L2, L3, L4), sciatic nerve (L4, L5, S1, S2, S3) and obturator nerve directly from its anterior division (L2, L3, L4). Cutaneous innervation of the lateral aspect of the thigh is predominantly supplied by the lateral cutaneous nerve of the thigh. It is a sensory branch of the lumbar plexus, arising from the posterior divisions of the anterior rami of L2 and L3 spinal nerves sometimes with variable innervation proximally from the sub-costal nerve (T12) [3].

The origin of postoperative pain has been linked to nociceptive stimuli, by releasing of primary mediators such as prostaglandins, leukotrienes, bradykinins, and 5-hydroxytryptamine. Consequently, the release of secondary mediators – peptides [calcitonin gene-related protein (CGRP), substance P and cholecytokinin] at the site of injury stimulate nociceptors. Impulses from the peripheral nociceptors travel *via* A delta and C fibers to synapse in the spinal cord [4]. Greater understanding of pain mechanisms has led recently to the concept of preemptive analgesia. Analgesics have been given before hip surgery starts. Central sensitization and worsening of postoperative pain have been prevented by administration of analgesics prior to painful stimuli [5].

For this type of hip surgery, both general and regional anesthesia can be performed successfully. Regional anesthesia has been shown to have numerous advantages over general anesthesia for hip replacement. Reduction in intraoperative blood loss due to lowering mean arterial pressure and venodilatation and diminution of risk for deep vein thrombosis by almost 50% have been the main benefits associated with regional anesthetic techniques. Initiation of postoperative analgesia can be achieved using catheter techniques [6].

Epidural analgesia

Epidural analgesia provides superior analgesia even using low dose continuous infusions. The combination of an opioid and a local anesthetic has been advocated due to synergistic analgesic effect. Therefore, the concentration of each component in the solution can be reduced [7]. On the other hand, numerous side effects and complications of this method have been reported recently. Epidural analgesia has been associated with technical failures, hypotension, urinary retention, a bilateral motor block which may postpone ambulation and unrecognized compartment syndromes. Especially in the population of orthopedic patients, spinal hematomas are found to be more frequent due to higher doses of thromboprophylactic agents and platelet antiaggregation therapy [8].

Intravenous morphine

Postoperative analgesia using morphine hydrochloride *via* patient controlled analgesia (PCA) pumps presents the most sophisticated method for delivering intravenous (*iv*) opioids. Narrow therapeutic index of these drugs can be overcome using PCA pumps. On the contrary, most of patients scheduled for hip replacement are elderly people and their education for this method of analgesic administration may be difficult and time-consuming. *Iv* morphine can have numerous side effects - nausea, vomiting and sedation of the patient, which can delay rehabilitation [9].

Peripheral nerve blocks (lumbar plexus block)

Peripheral nerve blocks (PNBs) have become an increasingly popular alternative to epidural analgesia for postoperative pain relief after hip replacement. Insufficient evidence supports the efficacy of PNBs, suggesting that analgesia can be comparable to epidural analgesia, with a minimal motor block that enables early mobilization. Usage of PNBs has been associated with fewer side effects, such as hypotension, urinary retention. Morphine side effects – sedation, nausea, and vomiting, appear less frequently [10]. Innervation of the hip joint is complex, including all three main nerves which originate from the lumbar plexus – femoral (L2, L3, L4), obturator (L2, L3, L4) and cutaneous lateral femoral nerve of the thigh (L2/L3). Therefore, only lumbar plexus block can provide satisfactory blockade and efficacious postoperative analgesia [11]. Lumbar plexus block is considered to be an advanced method for the peripheral blockade. Lumbar plexus is formed from spinal roots which arise from L1-L4 intervertebral foramina with contribution of T12. Their anterior branches compose the lumbar plexus within the psoas muscle, and after division, emerge from the muscle into pelvis as individual nerves.

The aim of the study was to investigate whether continuous lumbar plexus block can be a safe and efficacious alternative for postoperative analgesia in comparison with epidural analgesia and PCA morphine for total hip replacement. Therefore, quality of postoperative analgesia, using visual analogue scale (VAS) and a number of side effects (nausea, urinary retention, and episodes of hypotension) were recorded in four groups of patients (lumbar plexus analgesia, epidural analgesia, and PCA morphine following general and spinal anesthesia).

Methods

After obtaining ethical committee approval, 60 patients, aged 59.61 ± 9.92 years, American Society of Anesthesiologist [(ASA) physical status II-III], scheduled for unilateral total hip arthroplasty were included in this prospective, randomized study. Type of implant prosthesis was influenced by the age of the patient (cementless prostheses – 73.3%, cemented prostheses – 18.3% and hybrid prostheses – 8.3%). Among the groups, there were no significant differences, regarding the age, gender, BMI, type of implant prosthesis, duration of surgery and postoperative blood loss in 24 h. The values are shown in Table 1.

Before inclusion, written informed consent was obtained from each patient. Exclusion criteria were: known allergy to local anesthetics and opioids, chronic pain, chronic opioid medication, contraindications to central or peripheral nerve block (local skin infections, coagulation disorders). Patients were randomized into 4 groups of 15 patients: the group central nerve block – epidural (CNB), the group peripheral nerve block – lumbar plexus block (PNB), the group spinal anesthesia + *iv* morphine (SAM) and the group general anesthesia + *iv* morphine (GAM).

All patients received midazolam 0.03 mg/kg *iv*, 20 min before planned surgery. Preoperatively, in the group CNB, epidural space was identified with normal saline, using 18 G epidural needle. Thereafter, epidural catheter 20 G (Braun, Meslungen, Germany) was inserted. A bolus of 3 mL levobupivacaine 0.5% and fentanyl 50 μg was administered *via* epidural catheter before anesthesia induction. Intraoperatively, boluses of 5 mL levobupivacaine 0.5% were added on a regular basis, every 30 min. Anesthetic induction was performed using propofol 2 mg/kg, fentanyl 100 μg and rocuronium 0.6 mg/kg. Following endotracheal intubation, anesthesia was maintained using sevoflurane 1–2% in a 50%/50% mixture of oxygen and N_2O. Postoperative analgesia was maintained via epidural catheter during the first 24 h, by continuous infusion of a mixture – levobupivacaine 0.1% and fentanyl 2 μg/mL, 8–15 mL/h.

In the group PNB, lumbar plexus was identified by nerve stimulator according to Capdevila's approach [12], using 15 cm long needle for the peripheral block. Contractions of the quadriceps muscle ("dancing patella sign") were obtained using an initial current of 1–2 mA. After twitches were observed, the current was reduced to 0.5 mA. A peripheral catheter (Braun, Meslungen, Germany) was inserted into psoas compartment where lumbar plexus is situated. A total of 20 mL levobupivacaine 0.25% was administered. Following catheter insertion, general anesthesia was performed in the same way as in the group CNB. Postoperative analgesia was maintained *via* the peripheral catheter during the first 24 h, by continuous infusion of levobupivacaine 0.25%, 5–10

Table 1

Patient baseline and surgical characteristics in the first 24 h after total hip arthroplasty

Variable	CNB	PNB	SAM	GAM	Significance
Age (years), $\bar{x}$ ± SD	59.60 ± 11.14	59.40 ± 8.87	58.00 ± 11.81	61.46 ± 8.13	F = 0.298 (ns)
Gender (M/F), n	8/7	7/8	8/7	7/8	$p > 0.05$
BMI (kg/m^2), $\bar{x}$	27.57	28.03	28.29	27.86	$p > 0.05$
Type of implant prosthesis (U/C/ H), n	11/3/1	12/2/1	11/3/1	10/3/2	$\chi^2 = 0.349$ (ns)
Duration of surgery (min), $\bar{x}$ ± SD	91 ± 38	99 ± 52	88 ± 43	93 ± 47	$p > 0.05$
Postoperative blood loss in 24 h (mL), $\bar{x}$ ± SD	926.66 ± 295.11	800.00 ± 280.30	810.66 ± 237.50	756.66 ± 274.42	F = 1.064 (ns)

M – male/F – female; BMI – body mass index; U – uncemented/C – cemented /H – hybrid; ns – non significant; CNB – central nerve block (epidural); PNB – peripheral nerve block (lumbar plexus block); SAM – spinal anesthesia + intravenous (*iv*) morphine; GAM – general anesthesia + *iv* morphine; $\bar{x}$ – arithmetic mean; SD – standard deviation; n – number of patients.

mL/h. Initial titration was performed postoperatively, using a 10 cm VAS and providing pain score lower than 3 cm.

In the group GAM, all patients received general anesthesia, using the same protocol as it was used for the patients from the CNB and the PNB group. The average duration of general anesthesia was 135 ± 52 min and did not differ significantly among the groups CNB, PNB and GAM. Preoperatively, patients included into the SAM and the GAM group were informed about postoperative pain management using PCA devices. In the recovery room, patients from the SAM and the GAM group received initial *iv* boluses of morphine hydrochloride (5 mg doses at 5 min intervals), titrated manually until their pain score was lower than 3 on a 10 cm VAS. Thereafter, PCA analgesia was initiated. PCA pump (μSP 6000, Arcomed ag, Switzerland) was connected, delivering 1 mg doses of morphine *iv*, with a 7 min lockout period and a maximum dose of 20 mg over 4 h.

After the surgery, the patients from all groups were transferred to the post-anesthesia care unit (PACU) and after a 2 h observation period, to the orthopedic ward.

In the SAM group, all the patients received spinal anesthesia in sitting position, using 25 G, 88 mm Quincke tip needles (Braun, Meslungen, Germany). A total of 12.5–17.5 mg of hyperbaric bupivacaine 0.5% was administered into subarachnoid space at the L3-4 spinous level. Postoperatively, morphine was administered *iv*, using PCA pump.

Statistics

The methods of descriptive statistics were applied. The numerical variables were presented as mean value, minimum, maximum, standard deviation, while the categorical ones as proportions (percentages). Dependence of the parameters in order to check the differences was analyzed using Pearson's χ^2 test and Fisher's exact test. The differences were considered to be significant when $p < 0.05$.

The quality of analgesia and side effects (hypotension, nausea, vomiting, urinary retention) were recorded in all groups at 4 h, 12 h, and 24 h after surgery. Pain scores were assessed using VAS at rest (VAS1) and on moving (VAS2). Patients were asked to report on episodes of nausea and/or vomiting at 4 h, 12 h, and 24 h after surgery. The non-invasive arterial pressure was measured hourly, on regular basis and recorded at 4 h, 12 h, and 24 h after surgery. Values lower than 100/70 mmHg, were considered as hypotension. Inability to urinate spontaneously with a presence of bladder distention and the need for urinary catheterization was recorded at 4 h, 12 h, and 24 h after the surgery.

Results

Quality of analgesia

Data analysis showed that postoperative pain scores at rest and on moving were significantly different among the groups (Table 2). Four hours after the operation, the lowest average level of pain was recorded in the CNB group, both at rest (00.7 ± 0.25) and on moving (0.4 ± 0.73). In the PNB and the SAM group, the level of pain was moderate with the highest score at rest 3 *vs* 4, and on moving up to 6. In the GAM group, pain at rest varied significantly, with a wide range at VAS from 1–9 ($F = 21.827$; $p < 0.01$).

Twelve hours after the operation, average scores in VAS1 and VAS 2 were significantly different ($F = 41.925$; $p < 0.01$). The GAM group had significantly higher pain scores, both at rest and on moving, when compared to other groups. The lowest pain scores at VAS were recorded in the CNB group, with no patient suffered from any pain at rest (Table 2).

Finally, 24 h after the operation, the average level of pain in all groups was reduced. Pain scores varied still significantly among the groups ($F = 33.768$; $p < 0.01$). The highest scores were recorded in the GAM group, both at rest and on moving. On the contrary, the lowest average pain scores were noticed in the CNB group.

While reduction of pain during 24 h was similar in the PNB and the GAM group, with the highest scores 4 h after the operation, the SAM group showed the highest levels of pain scores 12 h after the operation.

Table 2

Pain scores on visual analogue scale (VAS) at rest (VAS1) and on moving (VAS2) in the CNB, the PNB, the SAM and the GAM group 4 h, 12 h and 24 h after total hip arthroplasty

VAS	Time after operation (h)			Avarage VAS score
	4	12	24	
VAS1				
CNB	0.07 ± 0.25 (0/1)	0.00 ± 0.00 (0/0)	0.00 ± 0.00 (0/0)	0.02 ± 0.09 (0/1)
PNB	1.47 ± 1.24 (0/4)	0.93 ± 0.96 (0/3)	0.47 ± 0.51 (0/1)	0.95 ± 0.87 (0/4)
SAM	1.27 ± 0.88 (0/3)	2.47 ± 0.99 (1/4)	1.67 ± 0.72 (0/3)	1.80 ± 0.85 (0/4)
GAM	3.93 ± 2.12 (1/9)	2.73 ± 1.22 (1/6)	2.73 ± 1.22 (1/3)	3.13 ± 1.48 (1/9)
VAS2				
CNB	0.40 ± 0.73 (0/2)	0.13 ± 0.35 (0/1)	0.27 ± 0.45 (0/1)	0.27 ± 0.59 (0/2)
PNB	3.20 ± 1.97 (0/6)	2.80 ± 1.61 (1/6)	1.53 ± 1.06 (0/4)	2.56 ± 1.47 (0/6)
SAM	3.07 ± 1.54 (1/6)	5.07±1.14 (4/7)	3.57 ± 0.93 (2/5)	3.90 ± 1.21 (1/7)
GAM	6.80 ± 1.93 (2/9)	5.47 ± 1.88 (2/8)	4.86 ± 1.84 (2/8)	5.71 ± 1.89 (2/9)

Results are given as arithmetic mean ± standard deviation (minimal/maximal score);
CNB – central nerve block (epidural); PNB – peripheral nerve block (lumbar plexus block);
SAM – spinal anesthesia + intravenous (*iv*) morphine; GAM – general anesthesia + *iv* morphine.

Average consumption of *iv* morphine *via* PCA pump was 47.7 mg in the SAM group, and 48.8 mg in the GAM group. The amount of morphine used did not differ significantly between groups.

Side effects

Side effects, such as – nausea, urine retention, and episodes of hypotension were recorded in all groups. Data analysis showed the statistically high difference in the incidence of nausea among the groups, 4 h after the operation. In the GAM group, 60% of patients were found to have nausea, whereas the incidence of nausea in the CNB group was 46.7%. The lowest incidence of nausea was in the SAM group – 6.7%. ($\chi^2 = 10.769$; $p < 0.01$) (Table 3).

The incidence of nausea was also significantly different among the groups, 12 h after the operation. Episodes of nausea have been recorded in 46.7% of patients in the SAM group, while the incidence in other groups was lower, especially in the PNB group, where none of the patients had nausea ($\chi^2 = 9.712$; $p < 0.01$) (Table 3).

Table 3

Incidence of nausea in the patients 4 h and 12 h after the hip replacement

Group	After 4 h		After 12 h	
	no	yes	no	yes
CNB	8	7	12	3
PNB	11	4	15	0
SAM	14	1	8	7
GAM	6	9	12	3
Total	39	21	47	13

CNB – central nerve block (epidural); PNB – peripheral nerve block (lumbar plexus block); SAM – spinal anesthesia + intravenous (*iv*) morphine; GAM – general anesthesia + *iv* morphine.

The incidence of nausea was around 5% in all groups 24 h after the operation and did not differ among them ($\chi^2 = 1.053$; $p > 0.05$).

In all studied groups, incidence of hypotension was not significantly different postoperatively, 4 h ($\chi^2 = 3.057$; $p > 0.05$), 12 h, ($\chi^2 = 2.243$; $p > 0.05$) and 24 h after the operation (0% in all groups). The incidence of hypotension varied from 6.7% (the SAM and the GAM group) to 33% (the CNB group) 4 h after the operation. The highest percentage of episodes of hypotension (20%) was also recorded in the CNB group 12 h after the operation. None of the patients from any group was hypotensive 24 h postoperatively. All these episodes of hypotension were successfully treated with boluses of phenylephrine 0.1 mg iv and iv administration of crystalloids.

The incidence of urine retention was signifficantly different 12 h after the ooperation. Urine retention was present only in the CNB group with incidence of 33.3%. ($\chi^2 = 16.365$; $p < 0.01$). However, incidence of urine retention did not differ significantly 4 h ($\chi^2 = 2.342$; $p > 0.05$) and 24 h ($\chi^2 = 2.061$; $p > 0.05$) after the operation, among the groups.

Discussion

Numerous clinical data suggest that acute pain after hip replacement is undermanaged and not treated adequately [13]. Most of the techniques for postoperative analgesia are advanced and need educated staff and expensive equipment. Due to the fact that hip replacement can cause moderate to severe pain relief, regional anesthesia techniques such as epidural and lumbar plexus block should be continued during the early postoperative period [14, 15].

Numerous types of peripheral blocks have been investigated as a tool for postoperative analgesia after hip replacement. A possible analgesic alternative to lumbar plexus block is continuous femoral nerve block (FNB). Marino et al. [16] compared patients after hip replacement with lumbar plexus catheters and continuous FNB. The lumbar plexus block group showed overall higher patient satisfaction scores during 24 h after the operation when compared to femoral nerve catheter group. Sensory block in the area of femoral nerve and lateral femoral cutaneous nerve appeared to be better in the lumbar plexus group, but the main difference was in the area of the obturator nerve. Our findings confirmed the results of Marino et al. [16], and showed that the use of a continuous lumbar plexus block provided effective analgesia at rest and on moving during 24 h after primary hip arthroplasty.

On the other hand, Ilfeld et al.[10] demonstrated that, according to their results, continuous FNB may be a possible analgesic alternative to lumbar plexus block. The average pain scores for patients scheduled for total hip arthroplasty receiving a femoral infusion were 3.6 (1–8) *vs* patients who were administered a posterior lumbar plexus infusion, 3.5 (1–8). Patients with femoral infusion had a more dense motor block, and could not ambulate 24 h after surgery. Our data showed lower overall scores in the PNB group (lumbar plexus group), at rest 4 h postoperatively 1.47 (0-4), 12 h postoperatively 0.93 (0-3) and 24 h postoperatively 0.47 (0-1). A possible explanation is that lumbar plexus analgesia was started preoperatively, and a standard amount of local anesthetic was administered preemptively (20 mL levobupivacaine 0.25%). Therefore, immediately after the operation, all the patients had satisfactory analgesia (lower than 5 at VAS). Furthermore, the psoas compartment approach to the lumbar plexus is preferable for a surgery to the hip because it is the most proximal lumbar plexus technique, provides a complete block of the lumbar plexus, and the needle or catheter insertion site is distant from the surgical incision [11].

In the study of Voloshin et al. [17] who observed the efficacy of acute pain service after joint arthroplasty, 1,343 patients subjected to hip arthroplasty with epidural postoperative analgesia were studied. Average pain intensity was 20 mm according to VAS. Meta-analysis of Choi et al. [18] compared postoperative lumbar epidural analgesia to other methods for postoperative pain relief. Results showed standardized mean differences (SMD) – 0.77 [95%, confidence interval (CI) 0.31–1.24], suggesting that during the first four to six hours after surgery, patients receiving epidural analgesia had less pain at rest, when compared to systemic analgesia. However, benefits may be limited to the early postoperative period. Our study also confirmed the superiority of epidural

analgesia following hip replacement in all studied groups. The epidural group showed minimal pain scores at rest 4 h after the operation (0.07 ± 0.25) and even no pain 12 h and 24 h after the hip replacement.

Some authors, e.g. Tetsunaga et al. [19] and Singelyn et al. [20] have not found a significant difference among different types of analgesia for hip replacement. According to these studies, quality of pain relief, postoperative hip rehabilitation, and duration of hospital stay were comparable in all groups (*iv* morphine, epidural analgesia and peripheral block). Our results have not been consistent with these studies when postoperative analgesia is in question. Both groups with systemic analgesia using morphine (the GAM and the SAM group), had the highest level of pain according to VAS. In the present study, epidural analgesia and peripheral block were started preoperatively, providing good quality of analgesia immediately after the operation. Because of the fact that that preemptive analgesia decreases pain by preventing sensitization of pain pathways activated by operative trauma, the GAM group without any kind of intraoperative or postoperative regional technique had significantly higher pain scores.

Peripheral blocks have been shown to provide superior analgesia and fewer side effects when compared with parenteral opioids. Single shot peripheral blocks have limited duration of their analgesic effects. The introduction of continuous techniques using peripheral catheters enabled prolonged postoperative pain relief and reduction of opioid administration.

Marino et al. [16] investigated the mean pain scores on VAS, using lumbar plexus block or hydromorphone, at rest and during physical therapy (at 2 h, 24 h, and 48 h). According to their findings, pain scores were significantly lower in the continuous lumbar plexus block group [3.5 (95% CI, 3.0–4.0) and 2.6 (95% CI, 2.0–3.2), respectively] in comparison to the group that had opioid patient-controlled analgesia alone [6.4 (95% CI, 5.9-6.9) and 4.8 (95% CI, 4.3–5.3)] ($p < 0.05$). The present study showed comparable results. Average pain scores at rest, 4 h and 24 h after the operation in the PNB group were 1.47 ± 1.24 (95% CI; 0–4.0) and 0.47 ± 0.51 (95% CI; 0–1.0), respectively. In the GAM group, where postoperative analgesia was maintained using PCA morphine, average scores recorded 4 h and 24 h after the operation were significantly higher (3.93 ± 2.12 and 2.73 ± 1.22, respectively).

Although hydromorphone is a morphine derivative, it is much more potent than morphine. The estimated relative potency of hydromorphone to morphine is 7.5 : 1. Overall consumption of hydromorphone in Marino's et al. [16] study was 9.4 (7.7–11.1) mg which could be equivalent to 70.5 mg of morphine. Our data showed average consumption of 48.8 (23–78) mg morphine, up to 24 h postoperatively in the GAM group and 47.7 (28–72) mg of morphine in the SAM group and confirmed that in the absence of adequate central or peripheral blockade, opioid use is significant for hip arthroplasty.

Meta-analysis of Chan et al. [21], which included 45 eligible randomized controlled trials (RCT) compared FNB *vs* PCA opioid, epidural, local infiltration or oral analgesia for total knee arthroplasty. FNB group demonstrated a lower risk of nausea/vomiting [RR (Relative risk ratios) 0.63] and higher patient satisfaction (SMD 0.60), when compared with epidural analgesia group. Our study showed results consistent with the study of Chan et al. [21], confirming that epidural group (the CNB group) and the GAM group had a higher incidence of nausea when compared to the PNB group. Our results are also similar to study conducted by Jules-Elysee et al. [22] who found out significantly higher scores for nausea, vomiting, and itchiness in the epidural analgesia group ($p < 0.05$).

A systematic review of Choi et al.[18] analyzed the differences between epidural analgesia and systemic analgesia after hip and knee replacement due to the frequency of nausea and vomiting. They did not find statistically significant difference between groups [odets ratio (OR) 0.95; 95% CI: 0.60–1.49]. However, retention of urine [OR 3.50, 95% CI: 1.63 to 7.51; number needed to harm (NNH) 4.5; 95% CI: 2.3 to 12.2] and low blood pressure (OR 2.78; 95% CI: 1.15–6.72; NNH 6.7; 95% CI: 3.5–103) were more frequent in epidural analgesia group, when compared to systemic analgesia. It is important to add that if low concentration epidural analgesia has been used likewise in our study, negative effects have been occurring less frequently. Different authors use different concentrations of local anesthetics with or without opioids for epidural analgesia. Study of Misiran et al.[23] has not found a significant number of complications such as hypotension, pruritus, sedation or motor block when a low dose concentration of ropivacaine or levobupivacaine has been used for postoperative epidural analgesia after major orthopedic surgery.

Tetsunaga et al. [19] compared side effects of continuous epidural analgesia, PCA with morphine and continuous three-in-one FNB on postoperative outcomes after total hip arthroplasty. They found the significantly lower incidence of nausea/vomiting in the peripheral block group ($p < 0.05$). Our study showed the extremely low incidence of nausea in the PNB group. Twelve hours following surgery and later on, none of the patients had nausea in the PNB group. The difference was significant ($\chi^2 = 9.712$; $p < 0.01$), especially when compared with groups with postoperative use of opioids (SAM and GAM groups). Despite using opioids, the SAM group did not have a high incidence of nausea 4 h after the operation because of prolonged sensitive and motor block after spinal anesthesia. Therefore, patients did not use opioids *via* PCA device in early postoperative period.

Meta analysis of Richman et al. [24] included 19 studies with continuous peripheral nerve block or systemic opioids for pain control after extremity surgery without major complications. Patients who received opioid analgesia had opioid side effects more frequently, such as nausea/vomiting, sedation, and pruritus and the difference between groups was significantly higher ($p < 0.001$). Our data also showed the superiority of peripheral block over other groups in the incidence of nausea, hypotension and urine retention.

Limitations of the study were a relatively low number of patients in each group, different types of intraoperative anesthesia and superposing of the medications given intraoperatively with postoperative analgesia few hours after the operation.

Conclusion

Administration of postoperative analgesia using lumbar plexus block provides satisfactory analgesia with the low incidence of side effects when compared to epidural analgesia or parenteral opioids following hip arthroplasty.

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Clinical and laboratory parameters associated with death in acute pancreatitis

Klinički i laboratorijski parametri povezani sa smrtnim ishodom kod akutnog pankreatitisa

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Abstract

Background/Aim. Acute pancreatitis is an inflammatory condition having the significant mortality rate in the case of severe forms of the disease. The aim of this study was to investigate putative factors of increased mortality in patients with acute pancreatitis with contradictory prior evidence, and to reveal factors that were insufficiently explored previously. **Methods.** This prospective cohort study with nested case/control design included all adult patients treated for acute pancreatitis in the Clinical Center of Kragujevac, Serbia, during the 3-year period (from October 2011 to December 2014). The cases (n = 19) were patients who died, while the controls (n = 113) were patients who survived. The associations between putative risk factors and the study outcomes were tested by univariate and multivariate logistic regressions, and expressed as crude and adjusted odds ratios (OR) with corresponding 95% confidence intervals (CI). **Results.** Significant association with the lethal outcome in acute pancreatitis was found for advanced age (adjusted OR 1.12, 95%CI 1.02–1.23), presence of significant comorbidities (adjusted OR 10.62, 95%CI 1.01–111.39), higher interleukin-8 (IL-8) value on third day from onset of symptoms (adjusted OR 1.05, 95%CI 1.02–1.08), use of tramadol and/or morphine (adjusted OR 47.34, 95%CI 3.21–699.08), the Bedside index for severity in acute pancreatitis (BISAP) score ≥ 3 in the first 24 hours (adjusted OR 48.11, 95%CI 3.14–736.29), and prophylactic use of antibiotics (adjusted OR 0.07, 95%CI 0.01–0.85). **Conclusion.** Advanced age, significant comorbidities, use of tramadol and/or morphine and more severe disease as assessed by BISAP score can increase the risk of death in acute pancreatitis, while prophylactic use of antibiotics may have a protective role.

Key words:
pancreatitis; mortality; age factors; comorbidity; analgetics, opioid; severity of illness index; antibiotic prophylaxis.

Apstrakt

Uvod/Cilj. Akutni pankreatitis je zapaljenska bolest koja je u slučaju ispoljavanja teških oblika bolesti povezana sa visokom stopom smrtnosti. Cilj ove studije bio je da ispita faktore za koje postoje oprečni literaturni podaci o povezanosti sa povećanom smrtnošću kod bolelsnika sa akutnim pankreatitisom, kao i one faktore koji prethodno nisu dovoljno ispitivani. **Metode.** Ova prospektivna kohortna studija sa usađenom studijom tipa slučaj/kontrola, obuhvatila je sve bolesnike lečene zbog akutnog pankreatitisa u Kliničkom centru Kragujevac, Srbija, tokom trogodišnjeg perioda (od oktobra 2011. do decembra 2014. godine). Slučajevi (n = 19) bili su bolesnici koji su umrli, dok su kontrolnu grupu (n = 113) činili bolesnici kod kojih nije zabeležen smrtni ishod. Povezanost između pretpostavljenih faktora rizika i opserviranog ishoda ispitivana je pomoću univarijante i multivarijantne logističke regresione analize, a rezultati su prikazani vrednostima sirovog i korigovanog unakrsnog odnosa šansi (*odds ratio* – OR) sa pripadajućim 95% intervalom poverenja (*confidence interval* – CI). **Rezultati.** Značajna povezanost sa smrtnim ishodom kod akutnog pankreatitisa nađena je za starije životno doba bolesnika (korigovani OR 1,12, 95%CI 1,02–1,23), prisustvo značajnog komorbiditeta (korigovani OR 10,62, 95%CI

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1,01–111,39), povišene vrednosti interleukina (IL)-8 trećeg dana od početka bolesti (korigovani OR 1,05 95%CI 1,02, 1,08), primenu tramadola i/ili morfina (korigovani OR 47,34, 95%CI 3,21–699,08), *Bedside index for severity in acute pancreatitis* (BISAP) skor ≥ 3 u prvih 24 sata (korigovani OR 48,11, 95%CI 3,14–736,29), kao i za profilaktičku primenu antibiotika (korigovani OR 0,07, 95%CI 0,01–0,85). **Zaključak.** Starije životno doba, značajan komorbiditet, primena tramadola i/ili morfina i teži oblik bolesti procenjen BISAP skorom mogu povećati rizik od nastanka smrtnog ishoda kod bolesnika sa akutnim pankreatitisom, dok profilaktička primena antibiotika može imati zaštitnu ulogu.

Ključne reči:
pankreatitis; mortalitet; životno doba, faktori; komorbiditet; analgetici, opioidni; bolest, indeks težine; antaibiotici, profilaksa.

Introduction

Acute pancreatitis (AP) is an inflammatory condition with various clinical presentations ranging from mild to severe forms of the disease. The dominant pathological substrate of this disease is an acute inflammation which is usually not followed by fibrosis [1]. The incidence of AP all over the world ranges from 5 to 80 cases *per* 100,000 [2] and it is growing, e.g. in the United Kingdom with 2.7% yearly rate. The largest increase in incidence was noted in women younger than 35, and in men between 35 and 44 years of age [3].

Mortality in acute pancreatitis depends on the severity of the disease, being less than 1% in a mild form and 10–30% in severe forms [4]. Factors previously associated with greater severity of the disease and/or higher mortality rate are: levels of C-reactive protein (CRP), procalcitonin (PCT) and cytokines [interleukin (IL)-8, tumor necrosis factor-alpha (TNF-α), IL-6], acute phase proteins [5], and acute kidney injury [6]. Among the 27 possible risk factors investigated in one study, arterial pH, acute physiology and chronic health evaluation II (APACHE II) [7] scores, early shock, and multiple organ failures were associated with mortality [8]. Patients with the bedside index of severity in acute pancreatitis (BISAP) [9] and Ranson's [10] scores equal or higher than 3 had a significantly higher likelihood of mortality [11], as well as early surgery, advanced age, and sterility of tissue cultures [12]. On the other side, lower mortality was observed in patients with: higher values of serum calcium [13], in those who received fewer antibiotics and less amount of parenteral fluid [14], then in patients who started early enteral nutrition within the first 72 hours of the onset of symptoms [15].

Despite relatively large number of studies that have examined risk factors for mortality in AP, there are still disagreements in terms of the following factors: use of antibiotic prophylaxis [16–18], type of nutritional support and beginning of nutritional support since the onset of symptoms [15], amount and type of fluids administered for resuscitation [14], serum level of IL-8 and IL-6 during the first day after onset of symptoms [19–21], the accuracy of prognostic scores in predicting severity and/or death in patients with AP [22, 23], as well as age of a patient [24]. These controversies arise from the heterogeneity of methodological approaches in prior studies that consequently led to inconsistent results [14–24].

The aim of our study was to investigate putative factors of increased mortality in patients with AP contradictory prior evidence and to reveal factors that were insufficiently explored previously.

Methods

This study was of prospective cohort type, with nested case/control design. The cohort was composed of all patients with acute pancreatitis who were admitted to the Intensive Care Unit (ICU) of the Clinical Center Kragujevac Serbia from October 2011 to December 2014, providing that they fulfilled inclusion criteria: all patients with diagnosis of AP based on two of the three following criteria: abdominal pain characteristic of AP, serum amylase and/or lipase ≥ 3 times the upper limit of normal, and characteristic findings of AP on Computed tomography (CT) scan. The exclusion criteria were: patients with acute postoperative pancreatitis, pregnant women with AP, patients transferred from other hospitals or other wards to the ICU of the Clinical Center Kragujevac more than 48 hours after the admission, as well as those under 18 years of age. The cases were patients who died and controls all the other patients who were enrolled in the study.

After admission, the patients signed an informed consent and then were treated according to preferences of the responsible physician. Blood samples for measurements of laboratory parameters were taken within the first 24 hours of admission at our department and on the 3rd day of hospitalization, and then according to the requests of responsible physicians. There were no patients who were diagnosed with AP between 24 and 48 hours after the admission. The following laboratory parameters were measured: glucose, urea, creatinine, bilirubin, aminotransferase, alkaline phosphatase, amylase, lipase, lactate dehydrogenase, total protein, albumin, sodium, potassium, calcium, chloride, CRP, PCT, fibrinogen, bicarbonates, pH levels, base excess, the partial pressure of oxygen and carbon dioxide in the arterial blood, erythrocyte sedimentation rate, hematocrit, erythrocyte, leukocyte and platelet counts, leukocyte formula, triglycerides and cholesterol levels. All measurements except that of cytokines were made in the Central Laboratory of the Clinical Center Kragujevac, by competent specialists of biochemistry, independent from the study investigators. Serum levels of cytokines were measured in the following way: blood was collected following patient enrollment in the study within 24 hours from the onset of pain (1st day of admission) and on the third day of the disease course.

The blood clot was centrifuged for separating the serum and then all serum samples were kept at -20°C before measurement. Serum levels of cytokines TNF-alfa, epidermal growth factor (EGF), IL-6, IL-8, and IL-10 were measured using sensitive enzyme-linked immunosorbent assay (ELISA) kits specific for humans (R&D Systems, Minneapolis, MN) in the Center for Molecular Medicine and Stem Cell Research, the Faculty of Medical Sciences, the University of Kragujevac. We determined serum levels of cytokines using appropriate DuoSets (R&D Systems, Minneapolis, MN, USA): TNF-alfa (TNF-alfa: catalog number DY210; range of detection 15.6–1.000 pg/mL), EGF (EGF: catalog number DY236; range of detection 3.91–250 pg/mL), IL-6 (IL-6: catalog number DY206; range of detection 9.38–600 pg/mL, IL-8 (IL-8: catalog number DY208; range of detection 31.2–2,000 pg/mL) and IL-10 (IL-10: catalog number DY217B; range of detection 31.2–2,000 pg/mL). All samples with the cytokine levels above the range of detection of the assay used were diluted five times by adding phosphate buffer saline (PBS). Any of invasive diagnostic or therapeutic procedure which could affect the serum levels of these cytokines was not performed during the period when they had been measured. These cytokines were chosen given that they were strong mediators of a complex immune response having the crucial role in the pathophysiology of systemic pro- and anti-inflammatory response in AP.

The following demographic and clinical characteristics of the study patients were recorded: age and gender of the patient, body mass index, etiology of AP, alcohol consumption, smoking, prophylactic use of antibiotics, use of nonsteroidal anti-inflammatory drugs, use of other drugs, nutrition, artificial ventilation, severity of AP, systemic inflammatory response syndrome (SIRS), multiorgan dysfunction, the values of the vital parameters (blood pressure, heart and respiratory rate, blood oxygen saturation, body temperature), the values of Sepsis-related Organ Failure Assessment (SOFA) [25] score, APACHE II score, Ranson's score, modified Glasgow score, and BISAP score, evaluation of disease severity based on the findings of computed tomography – Balthazar's [26] score, radiological examinations of the chest, and comorbidities.

In order to define the severity of the disease course in this study, the original 1992 Atlanta classification of AP was used since the study started before the revision of these criteria in 2012 [27]. The severity of AP was defined according to criteria referring to the development of organ failure and/or local complications such as acute fluid collections, pancreatic necrosis, or pancreatic abscess, as well as initial Ranson's score 3 or higher or an APACHE II score 8 or higher. Organ failure and systemic complications were diagnosed if they occurred during the first 7 days from the onset of abdominal pain, lasted for more than 48 hours and included at least one of the following: hypovolemic shock (systolic blood pressure < 90 mmHg after fluid replacement), respiratory insufficiency (PaO_2 < 8 kPa), renal failure [blood creatinine level > 177 μmol/mL (2 mg/dL)], disseminated intravascular coagulation, or gastrointestinal bleeding (> 500 mL/24 hours). We used organ failure lasting for more than 48 hours as a strong confounding variable in order to assess the influence of other factors on the observed outcome.

The study was approved by the Ethics Committee of the Clinical Center Kragujevac, on September 1, 2011, (No 01-9024).

Statistics

The data were at first described by descriptive statistics, using measures of central tendency (median), variability (interquartile range, minimum and maximum values) and relative numbers. The significance of differences in values of continuous variables between the study groups was tested by Mann-Whitney test. The significance of difference in categorical variables between the study groups was tested by χ^2 test or Fisher's test (when values in some cells of contingency tables were lower than 5 or zero). The differences were considered significant if the probability of null hypothesis was below 0.05. Associations between putative risk factors and the study outcomes were tested by univariate and multivariate logistic regressions (using the stepwise approach with backward deletion, with removing all variables with $p \geq 0.1$), and expressed as crude and adjusted odds ratios. As we focused on various factors with inconsistent relevance according to prior studies or which were insufficiently examined previously, in multivariate logistic regression we also included those which had been found to have an insignificant association with death from AP in univariate analysis. All calculations were performed by the SPSS (Statistical Package for Social Science for Windows) software, version 20.

Results

A total of 132 patients with AP were enrolled in the study, of whom 19 (14.4%) died. Eight (42.1%) of them died within the first two weeks of admission to the ICU. From a total number of patients, 41 (31.1%) developed pancreatic necrosis, and in ten (7.6%) the necrosis was infected. In 22 (16.7%) patients the pancreatic pseudocyst was formed spontaneously. Regarding etiology of AP gallstone was found in 51.4%, alcohol consumption in 25.0%, and other causes in 23.6% of patients. The youngest patient was 23, and the oldest was 86 years old (59.61 ± 14.83). There were 84 (63.6%) men and 48 (36.4%) women. Average body mass index was 27.5 ± 4.5 kg/m^2 which puts our patients in a category of pre-obese.

Tables 1 and 2 show baseline characteristics of patients according to demographic and the majority of the examined clinical characteristics and the majority of the examined laboratory parameters. The differences between the cases and controls were significant in terms of age, severity of AP, organ failure, significant comorbidity, pleural effusion or consolidation of lung parenchyma, cardiovascular disease, blood glucose, urea, creatinine, alkaline phosphatase, LDL cholesterol, total proteins, potassium and albumins, as well as in all scores for predicting severity (SOFA, APACHE II, BISAP, Ranson's, Modified Glasgow and Balthazar's) (Table 3).

Table 1

Demographic characteristics and comorbidities in cases (deceased) and controls (surviving) patients with acute pancreatitis

Variable	Patients		Test value	Crude odds ratios
	cases (n = 19)	controls (n = 113)	*p*	(95%CI)
Age (years), median (IQR), range	75 (67–77) 41–84	60 (48.5–66) 23–86	U = 474.0[1] *p* < 0.001	1.07 (1.01, 1.12)
Body mass index (kg/m^2), median (IQR), range	26.17 (24.6–31) 21.6–35	27.21 (23.9–30) 19.16–42.50	U = 531.5[1] *p* = 0.878	1.02 (0.88, 1.17)
Gender, n (%)				
male	12 (63.2)	72 (63.7)	χ^2 = 0.002	0.96
female	7 (36.8)	41 (36.3)	*p* = 0.963	(0.35, 2.67)
Significant comorbidity, n (%)				
without	3 (15.8)	57 (51.4)*	χ^2 = 8.255	5.63
with	16 (84.2)	54 (48.6)*	*p* = 0.004	(1.55, 20.41)

[1]Mann-Whitney test; χ^2 – Chi-Square test; IQR – interquartile range; CI – confidence interval.
***the number is smaller than 113 for this calculation, since data for some patients were missing.**

Table 2

Clinical and laboratory parameters on admission in cases (deceased) and controls (surviving) patients with acute pancreatitis (AP)

Clinical parameters	Patients		Test value	Crude odds ratios
	cases (n = 19)	controls (n = 113)	*p*	(95%CI)
Severity of AP, n (%)				
mild form	2 (10.5)	76 (67.3)	χ^2 = 21.66	17.46
severe form	17 (89.5)	37 (32.7)	*p* < 0.001	(3.83, 79.58)
Cardiovascular disease, n (%)				
no	7 (36.8)	70 (61.9)		
yes, mild form	9 (47.4)	39 (34.5)	χ^2 = 7.10 *p* = 0.029	2.30 (0.80, 6.68)
yes, severe form	3 (15.8)	4 (3.5)		7.5 (1.39, 40.51)
Pulmonary disease, n (%)				
no	15 (78.9)	106 (93.8)	χ^2 = 4.70	4.03
yes	4 (21.1)	7 (6.2)	*p* = 0.300	(1.05, 15.45)
Pleural effusion or consolidation of lung parenchyma, n (%)				
no	6 (35.3)	67 (70.5)*	χ^2 = 7.89	4.38
yes	11 (64.7)	28 (29.5)*	*p* = 0.005	(1.47, 13.02)
Organ failure, n (%)				
without failure	3 (15.8)	101 (89.4)		
one organ/organ system	8 (42.1)	11 (9.7)	χ^2 = 63.55 *p* < 0.001	24.48 (5.65, 106.02)
more organ/organ system	8 (42.1)	1 (0.9)		269.33 (25.05, 2,895.34)
Laboratory parameters, median (IQR) range				
blood glucose (mmol/L)	9.1 (7.7–11.1) 5.8–17.7	7.4 (6.3–9.6) 3.2–33.6	U = 761.0[1] *p* = 0.043	1.07 (0.96–1.19)
urea (mmol/L)	9.9 (6.8–17.1) 5.6–91.2	5.3 (4.1–7.7) 5–70	U = 368.5[1] *p* < 0.001	1.07 (1.01, 1.13)
creatinine (umol/L)	116 (85–180) 73–1607	83 (70–100) 33–523	U = 499.5[1] *p* < 0.001	1.01 (1.00, 1.02)
alkaline phosphatase (U/L)	61.5 (35.25–87.75) 23–101	77 (56.63–146.13) 26–520	U = 380.5[1] *p* = 0.039	0.97 (0.95, 1.00)
LDL cholesterol (mmol/L)	2.29 (1.62–2.72) 1.33–3.68	2.92 (2.26–3.69) 0.74–6.61	U = 369.0[1] *p* = 0.018	0.51 (0.27, 0.94)
albumines (g/L)	29 (26–36.25) 21–41	35 (31–39) 18–52	U = 537.0[1] *p* = 0.002	0.88 (0.80, 0.96)
potassium (mmol/L)	4.0 (3.67–4.92) 3.3–6.1	3.8 (3.6–4.1) 2.8–5.0	U = 701.5[1] *p* = 0.038	4.42 (1.75, 11.16)
C - reactive protein (mg/L)	220.6 (54.5–310.1) 0.8–460	124.9 (50.1–213.7) 4.6–488	U = 723.0[1] *p* = 0.101	1.00 (1.00, 1.01)
procalcitonin (mg/L)	0.58(0.13–2.43) 0.12–118	0.25(0.12–0.73) 0.05–17.98	U = 588.0[1] *p* = 0.090	1.07 (0.99, 1.11)
hematocrit (%)	40.7 (37–47) 26–51	42.7 (38.1–45.5) 16.9–92.2	U = 975.5[1] *p* = 0.525	0.97 (0.89, 1.04)

[1]Mann-Whitney test; χ^2 – Chi-Square test; IQR – interquartile range; CI – confidence interval; LDL – low-density lipoprotein.
***the number is smaller than 113 for this calculation, since data for some patients were missing.**

Measured levels of cytokines were also associated with fatal outcome (Table 4). The greatest differences between the groups were observed in IL-6 value on the first and third day, IL-8 value on the first and third day, and IL-10 value on the first and third day. Values of TNF-α and EGF were not significantly different among those who died and survived.

Table 3

Scores used to predict the outcome in cases (deceased) and controls (surviving) patients with acute pancreatitis

Score	Patients		Test value	Crude odds ratios
	cases (n = 19)	controls (n = 113)	*p*	(95%CI)
SOFA, median (IQR), range	10.5 (8.75–13) 4–15	8 (7–9) 3–13	U = 469.0[1] *p* = 0.001	1.55 (1.20, 1.99)
SIRS, n (%)	14 (73.7)	27 (23.9)	χ^2 = 18.83 *p* < 0.001	8.92 (2.94, 27.03)
APACHE II, n (%)				
< 8	5 (26.3)	65 (62.5)*	χ^2 = 8.577	4.67
≥ 8	14 (73.7)	39 (37.5)*	*p* = 0.003	(1.56, 13.96)
BISAP, n (%)				
< 3	10 (55.6)	100 (90.9)*	χ^2 = 15.99	8.00
≥ 3	9 (44.4)	10 (9.1)*	*p* < 0.001	(2.57, 24.87)
Ranson's, n (%)				
< 3	7 (36.8)	80 (74.1)*	χ^2 = 10.38	4.89
≥ 3	12 (63.2)	28 (25.9)*	*p* = 0.001	(1.75, 13.67)
Modified Glasgow, n (%)				
< 3	2 (11.8)	65 (60.7)*	χ^2 = 14.17	11.60
≥ 3	15 (88.2)	42 (39.3)*	*p* < 0.001	(2.52, 53.37)
Balthazar's, n (%)				
< 3	5 (27.8)	57 (54.8)*	χ^2 = 4.49	3.15
≥ 3	14 (72.2)	47 (45.2)*	*p* = 0.034	(1.05, 9.48)

[1] Mann-Whitney test; χ^2 – Chi-Square test; CI – confidence interval; LDL – low-density lipoprotein; SOFA – sepsis ffelated organ failure assessment; SIRS – systemic inflammatory response syndrome; APACHE – acute physiology and chronic health evaluation; BISAP – bedside index of severity in acute pancreatitis.

***the number is smaller than 113 for this calculation, since data for some patients were missing.**

Table 4

Serum cytokines concentrations in cases (deceased) and controls (surviving) patients with acute pancreatitis

Cytokines (pg/mL)	Patients		Test value	Crude odds ratios
	cases (n = 19)	controls (n = 113)	*p*	(95%CI)
IL-6, median (IQR), range				
1 day	105.3 (37.5–164.3) 2.1–300	36.9 (13.4–87.9) 0–300	U = 605.0[1] *p* = 0.003	1.01 (1.00, 1.02)
3 day	68.7 (28.8–128.9) 1.3–203.0	26.29 (7.34–64.0) 0–221.2	U = 572.0[1] *p* = 0.003	1.01 (1.00, 1.02)
IL-8, median (IQR), range				
1 day	38.5 (22.1–75.5) 0–380.65	15.98 (0.4–38.4) 0–167.22	U = 607.0[1] *p* = 0.003	1.02 (1.01, 1.03)
3 day	36.0 (0.8–60.8) 0–287.2	0.17 (0–16.3) 0–120.7	U = 490.0[1] *p* < 0.001	1.03 (1.01, 1.04)
IL-10, median (IQR), range				
1 day	32.3 (15.3–76.5) 0–220.50	11.6 (0–2.6) 0–515.7	U = 661.0[1] *p* = 0.008	1.01 (0.99, 1.02)
3 day	17.2 (0–43.10) 0–116.45	0 (0–11.5) 0–258.11	U = 671.0[1] *p* = 0.016	1.01 (0.99, 1.02)
TNF-α, median (IQR), range				
1 day	0.3 (0–6.24) 0–28.0	0 (0–1.2) 0–250	U = 845.0[1] *p* = 0.093	0.99 (0.97, 1.02)
3 day	0 (0–6.4) 0–42.7	0 (0–0) 0–116.7	U = 847.0[1] *p* = 0.156	1.00 (0.97, 1.03)
EGF, median (IQR), range				
1 day	76.1 (52.2–105.7) 14.3–164.1	68.8 (35.7–107.8) 0–550.7	U = 997.5[1] *p* = 0.664	0.99 (0.99, 1.01)
3 day	51.9 (22.6–70.0) 0–104.7	64.8 (29.5–101.4) 0–467.4	U = 775.0[1] *p* = 0.116	0.99 (0.98, 1.00)

[1] Mann-Whitney test; CI – confidence interval; IQR – interquartile range; IL – interleukin; TNF-α – tumor necrosis factor alpha; EGF – epidermal growth factor.

Regarding parameters related to the treatment of patients with AP, Table 5 shows baseline characteristics of cases and controls. It could be seen from Table 5 that there was a significant correlation of certain parameters with the occurrence of fatal outcome, such as: type of solution used for intravenous fluid replacement, type of nutritional support, use of blood and blood derivatives, use of 20% albumin, use of opioid drugs, especially tramadol and/or morphine, and other invasive treatments (drainage, thoracocentesis), as well as the surgical procedure in AP.

Table 5

Treatment options used in cases (deceased) and controls (surviving) patients with acute pancreatitis

Treatment	Patients, n (%)		Test value	Crude odds ratios
	cases (n = 19)	controls (n = 113)	*p*	(95%CI)
Solution used for IV fluid replacement				
crystalloids	6 (31.6)	95 (84.1)	$\chi^2 = 24.94$	11.44
crystalloids and colloids	13 (68.4)	18 (15.9)	$p < 0.001$	(3.84, 34.03)
Amount of solution used for IV fluid replacement				
> 2,000 mL	18 (94.7)	102 (90.3)	$\chi^2 = 0.39$	0.51
< 2,000 mL	1 (5.3)	11 (9.7)	$p = 0.53$	(0.06, 4.24)
Nutritional support				
not required (regular oral food intake restored)	1 (5.3)	40 (35.4)		
total enteral nutrition through nasojejunal tube	1 (5.3)	9 (8.0)		4.45 (0.25, 77.96)
total enteral nutrition through nasogastric tube	5 (26.3)	8 (7.1)	$\chi^2 = 20.40$	25.00 (2.56, 243.75)
combined enteral and parenteral nutrition	6 (31.6)	17 (15.0)	$p = 0.001$	14.12 (1.58, 126.36)
total parenteral nutrition	6 (31.6)	16 (14.2)		15.00 (1.67, 134.70)
without nutritional support although it was indicated	0 (0)	23 (20.4)		0
Use of opioid drugs				
no	2 (10.5)	64 (56.6)		
yes, meperidin	1 (5.3)	8 (7.1)	$\chi^2 = 15.65$	4.00 (0.33, 49.24)
yes, other (tramadol and/or morphine)	16 (84.2)	41 (36.3)	$p < 0.001$	12.49 (2.73, 57.18)
Use of heparine				
no	6 (31.6)	77 (68.1)		
yes, LMWH	12 (63.2)	35 (31.0)	$\chi^2 = 10.25$ $p = 0.006$	4.40 (1.53, 12.68)
yes, standard	1 (5.3)	1 (0.9)		12.83 (0.71, 231.75)
Use of blood and blood derivatives	12 (63.2)	27 (23.9)	$\chi^2 = 12.05$ $p = 0.001$	0.18 (0.06, 0.51)
Use of 20% albumine	16 (84.2)	32 (28.3)	$\chi^2 = 21.96$ $p < 0.001$	0.07 (0.02, 0.27)
Other invasive treatment (drainage, thoracocenthesis)	6 (31.6)	8 (7.1)	$\chi^2 = 10.30$ $p = 0.001$	6.06 (1.81, 20.21)
Use of NSAID	12 (63.2)	91 (80.5)	$\chi^2 = 2.86$ $p = 0.090$	0.41 (0.14, 1.17)
Prophylactic use of antibiotics	11 (57.9)	66 (58.4)	$\chi^2 = 0.002$ $p = 0.967$	0.98 (0.37, 2.62)
Surgical treatment (operation)	7 (36.8)	6 (5.4)	$\chi^2 = 18.02$ $p < 0.001$	3.21 (1.72, 5.98)

χ^2 – Chi-Square test; CI – confidence interval; iv – intravenous; LMWH – low moleuclar weight heparin; NSAID – nonsteroidal anti-inflamatory drugs.

Baseline characteristics of the study patients (cases and controls) according to the occurrence of complications are shown in Table 6. One can see that the occurrence of any of local or systemic complication (such as necrosis of the pancreas and infection of necrosis) was associated with the fatal outcome ($p < 0.001$). The occurrence of pseudocyst of the pancreas was not higher in patients who died ($p = 0.912$).

The results of both univariate and multivariate logistic regression analysis (Cox and Snell R^2 0.401, Nagelkerke R^2 0.714, Hosmer-Lemeshow χ^2 1.836, df = 8, $p = 0.986$, overall model accuracy of 93.7%) presented in Table 7 suggest that the age, use of tramadol and/or morphine, BISAP score, comorbidity and IL-8 values on the third day were significantly associated with the occurrence of death in patients with AP. On the other hand, prophylactic use of antibiotics could have a protective role since it reduces the odds of the fatal outcome for slightly more than 93%. On the contrary, prophylactic use of antibiotics reduced the risk of death in our study.

The advanced age of a patient with AP can be a significant risk factor for adverse outcomes including death. Murata et al. [28], showed that patients with the advanced age (≥ 70 years) accompanied with severe comorbidities had an approximately double risk of death [23]. In the study of Kong et al. [29] there were significant differences in age between survivors and deceased patients with the severe AP (49.7 *vs* 62.8 years of age, respectively). The age difference between survivors and deceased in our study was even greater (60 *vs* 75 years, respectively). The advanced age is associated with fibrotic changes within the pancreatic tissue, which cause strictures and consequent dilatations of main pancreatic duct [30]; such abnormalities may contribute to the more severe course of the disease, and ultimately to death.

There is a variety of scoring systems for early detection of the severity of AP. The most commonly used in a daily practice are Ranson's score, APACHE II score, Balthasar CT score [26] and BISAP score [9, 31, 32]. BISAP scoring system in a simple manner can predict the clinical severity of AP within the first 24 hours after admission taking into account the following criteria: blood urea nitrogen > 8.92 mmol/L, impaired mental status, age > 60, ≥ 2 SIRS criteria, the presence of pleural effusion. In a recent study, Yang et al. [33] concluded that BISAP score was not an ideal single method for assessing the severity of AP, because the sensitivity was low. In another study BISAP score was a reliable tool for identification of pa-

Table 6

Occurrence of complications in cases (deceased) and controls (surviving) patients with acute pancreatitis

Complications	Patients, n (%)		Test value
	cases (n = 19)	controls (n = 113)	*p*
Any of local or systemic complications	19 (100)	42 (37.2)	$\chi^2 = 25.84$ $p < 0.001$
Necrosis of pancreas	12 (63.2)	29 (25.7)	$\chi^2 = 10.68$ $p = 0.001$
Infection of necrosis of pancreas	5 (26.3)	5 (4.4)	$\chi^2 = 11.13$ $p = 0.001$
Presence of pseudocyst of pancreas	3 (15.8)	19 (16.8)	$\chi^2 = 0.012$ $p = 0.912$
Presence of systemic complications	17 (89.5)	13 (13.5)	$\chi^2 = 47.43$ $p < 0.001$

χ^2 – Chi-Square test.

Table 7

Crude and adjusted odds ratios of factors associated with death in patients with acute pancreatitis

Risk factors	Crude OR (95% CI)	Adjusted OR (95%CI)
Age	1.07 (1.01, 1.12)	1.12 (1.02, 1.23)
IL-8 value on third day	1.03 (1.01, 1.04)	1.05 (1.02, 1.08)
EGF value on third day	0.99 (0.98, 1.001)	0.98 (0.95, 1.01)
TNF-α value on third day	1.00 (0.98, 1.03)	0.93 (0.85, 1.02)
Use of tramadol and/or morphine	12.49 (2.73, 57.18)	47.34 (3.21, 699.08)
Comorbidity	5.63 (1.55, 20.41)	10.62 (1.01, 111.39)
BISAP score	8.0 (2.57, 24.87)	48.11 (3.14, 736.29)
Prophylactic use of antibiotics	0.98 (0.37, 2.62)	0.07 (0.01, 0.85)

OR – odds ratio.
For other abbreviations see under previous tables.

Discussion

The mortality rate of AP in our cohort was 14.4% which is mostly in agreement with previously established rates. Some factors that we investigated may have an impact on the disease course or be associated with a fatal outcome such as: advanced age, presence of significant comorbidities, elevated IL-8 values on the third day from onset of symptoms, use of tramadol and/or morphine for pain relief and BISAP score equal or higher than 3 in the first 24 hours.

tients with high risk for adverse outcomes, although sensitivity for mortality was suboptimal [11]. However, both, our study and several other studies showed a strong relation between BISAP ≥ 3 and death of patients with AP [34–37]. Besides, BISAP score had better predictive power in comparison to Ranson's [10] score in our study, perhaps because it uses higher cut-off value for the age of patients (60 *vs* 55), as our patients who died were much older than those who survived.

There is widespread controversy about the effects of prophylactic antibiotics in AP. Although some studies did not show beneficial effects of antibiotic prophylaxis [38], the majority of published data favors the prophylactic use of antibiotics in patients with AP who develop necrosis [39], since necrotic tissue greatly increases the risk of infection [40]. In our study, even 64% of patients who died had necrosis of pancreatic tissue, while only 63% of patients in this group received antibiotic prophylaxis. Therefore, one of the reasons why some of the patients within this group died could have been a lack of necrotic tissue protection from infection. On the other hand, among the survivors, even 58% of patients received antibiotic prophylaxis, which surely helped to almost 26% of patients with necrosis to avoid infection and death. Rada and Pena [41] confirmed our results showing that prophylactic antibiotics may reduce mortality and length of hospitalization in patients with AP [42]. Surely routine antibiotic prophylaxis in all patients with AP is not justified [43], but physicians should be alert not to miss cases with necrosis, who definitely will benefit from antibiotic prophylaxis.

Since AP is an inflammatory disease, pro-inflammatory mediators are being released from leukocytes and the neutrophils in the beginning, but also during the disease course. IL-6 is being released from macrophage as a reaction to tissue injury and is responsible for the synthesis of the proteins in the acute phase of the inflammation. In the first 24 hours from the admission, it significantly correlates with the severity of the clinical picture and fatal outcome. Combined with lipase, IL-6 is a good diagnostic marker, and it may predict the outcome of AP. In our study, the most pronounced increase of IL-6 was registered in the first 24 hours from the admission, unlike CRP whose concentration increased later on, between 24 and 48 hours. IL-8, a chemokine which attracts neutrophils to the point of inflammation increases when the patients have a severe form of pancreatitis. When measured in the first 24 hours, IL-8, is a better predictor of the severity and adverse outcomes of AP [19–21], as it was shown in our study. Serum concentrations of IL-10, the cytokine which inhibits the release of the pro-inflammatory interleukins from macrophages, were much higher in our patients with AP who died. Pezzilli et al. [44] showed something in the opposite direction: plasma levels of IL-10 were lower in patients with more severe forms of pancreatitis. However, several previous studies in patients with other diseases showed that IL-10 reached higher

levels in those who died (e.g. in abdominal sepsis or brain injury) [45, 46]. So far investigations of IL-10 roles in immune response gave diverse results, and effective therapeutic strategies which target this cytokine were not developed in the area of inflammatory diseases [47]. The role of the EFG was so far mostly investigated in animal models in relation to its role in the prevention of intestinal permeability and bacteria translocation [48], as well as in prevention of septic complications in patients with AP [49]. However, in our study, we did not find a correlation between serum levels of EGF and fatal outcome. Likewise, levels of TNF-alpha, pro-inflammatory cytokine with multitudes of actions (activation of prostaglandin and leukotriene pathways, induction of apoptosis, expression of integrins, promotion of platelet aggregation, etc.) were not different among our patients with AP who died or survived. We are still far away from a complete understanding of immune and inflammatory responses in AP, and further studies focused on causal relationships and mechanisms of action of numerous mediators are necessary [50].

Comorbidity has been recognized as an important factor in patients with AP. In our study from 19 deceased patients, there were 3 (15.8%) without and 16 (84.2%) with comorbidities. Our study showed that patients with significant comorbidities have an increased risk from death compared to patients without. Several recently published studies came to the same conclusion [28, 51, 52]. Comorbidities decrease the capacity of vital organs to compensate for increased needs of tissues induced by inflammation and infection, resulting in lower chances of survival. However, there are some dissonant voices: in a study of Uomo et al. [53] the comorbidity had only the limited influence on the course and outcome of AP and did not correlate with mortality.

Pain is one of the major symptoms of AP. It spreads in a belt-like fashion and patients may experience it as very intensive. In the treatment of AP analgesics have an important role in mitigating stress and decreasing chances of shock. However, choice of analgesics is extremely important. In our study, the patients who received the opioid analgesics for pain relief, especially tramadol and/or morphine, had increased risk of death in comparison to the patients who received some other analgesics. Opioids may lead to spasm of the sphincter of Oddi and decrease the outflow of bile and pancreatic juice, aggravating the course of AP. Not all studies found the harmful effect of opioids in patients with AP, which could be explained by high variability of dosing regimens of opioids among the studies [54, 55].

Our study has several limitations which should be taken into account when interpreting its results. First, the study was uni-centric, which increased the possibility of study site personnel bias. Second, we were not able to measure cytokine levels beyond the third post-admission day, so full profiles of secretion could not have been established. And finally, our study had sufficient, yet modest statistical power, due to relatively small number of available patients with AP. Having regarded the aforementioned, and also the fact that we have only identified the significant association between some factors and mortality, but not independent risk factors for such outcome, this study should be considered as hypothesis-generating for further interventional investigations dealing with the causality of fatal outcome in AP.

Conclusion

Results of this study suggest that advanced age, the presence of significant comorbidities, the higher IL-8 value on

the third day from the onset of symptoms, use of tramadol and/or morphine, and BISAP score ≥ 3 in the first 24 hours are associated with lethal outcome in acute pancreatitis. On the other hand, prophylactic use of antibiotics may have the protective role and can reduce mortality in patients with severe acute pancreatitis.

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Conflict of interests

The authors declare that have no conflict of interest in this study.

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Estimation of the influence of hypoglycemia and body mass index on health-related quality of life, in patients with type 2 diabetes mellitus

Procena uticaja hipoglikemije i indeksa telesne mase na kvalitet života kod obolelih od šećerne bolesti tipa 2

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Abstract

Background/Aim. Recent data have shown that obese patients with type 2 diabetes mellitus (T2DM) have more commonly impaired glycemic control and complication such as neuropathy as well as a higher mortality rate than normal body weight patients with diabetes. The aim of this study was to quantify patient-reported rates of hypoglycemia, determine body mass index (BMI) and evaluate their association with health-related quality of life (HRQL) estimated by EuroQoL-EQ 5-dimensional (EQ-5D) questionnaire in individuals with T2DM after adjusting for age, duration of diabetes mellitus and presence of late diabetes complications. **Methods.** This clinical-epidemiological cross sectional study involved 269 consecutively selected adult patients of both sexes with T2DM duration longer than one year. The standardized Serbian version of the EQ-5D 3 level version – EQ-5D-3L questionnaire with a visual analogue scale-VAS (EQ-VAS), was used to assess respondents' HRQL and utility values. **Results.** The average age of participants was 65.1 ± 9.3 years; hypoglycemia was registered in 71.0%, obesity in 43.9% and chronic complications in 88.8% of the patients. Half of the patients did not reach the target value of glycated hemoglobin (HbA1c) < 7.0%. Patients that experienced episodes of hypoglycemia years compared to those with good glycemic control were significantly younger at diagnosis (54.19 *vs* 57.59 years, respectively) and less likely to be graduated at tertiary school (7.3% *vs* 35.1%, respectively). They had higher mean EQ-5D subscale score (pain/discomfort: 1.86 *vs* 1.68, respectively), lower VAS (55.99 *vs* 62.44) and EQ-5D index (0.85 *vs* 0.87, respectively). Obese patients were more likely to be female with higher systolic and diastolic pressure, triglycerides, waist circumference, subscale score (anxiety/depression 2.01 *vs* 1.80) and rates of hypoglycemia *per* year. After adjusting for confounders, the presence of hypoglycemia had 2,035 greater odds for experienced problems related to pain/discomfort. The unit increase of BMI had 1.05 greater odds for experienced problems related to mobility and anxiety/depression. Hypoglycemia was associated with significantly lower levels of the VAS score (b = -0.13). Increasing of BMI was associated with significantly lower EQ-5D index value (b = -1.78). The rates of hypoglycemia were not associated with VAS score and EQ-5D index values. **Conclusion.** Results demonstrated a significant association of hypoglycemia and obesity with impaired HRQL determined by the EQ-5D questionnaire. Lifestyle measures aimed to reduce obesity and hypoglycemia together with optimal diabetic therapy regiment can markedly improve patients' HRQL.

Key words:
diabetes mellitus, type 2; quality of life; hypoglycemia; body mass index.

Apstrakt

Uvod/Cilj. Noviji podaci sugerišu da gojazne osobe sa dijabetesom tip 2 (DMT2) češće imaju lošiju kontrolu glikemije i češće komplikacije poput neuropatije, kao i višu stopu mortaliteta u odnosu na obolele od dijabetesa sa normalnom telesnom masom. Cilj istraživanja bio je da se utvrdi učestalost hipoglikemijskih epizoda, odredi vrednost indeksa telesne mase (ITM) i proceni njihov uticaj na kvalitet života (QL) kod obolelih od DMT2 korišćenjem upitnika *EuroQoL-EQ 5-dimensional* (EQ-5D) nakon korekcije za starost, trajanje dijabetesa melitusa i prisustvo kasnih komplikacija bolesti. **Metode.** Ovom kliničko-epidemiološkom studijom preseka obuhvaćena su 269 uzastopno selektovana bolesnika oba pola, sa postavljenom dijagnozom DMT2 trajanja dužeg od jedne godine. Standardizovana verzija upitnika EQ-5D trećeg nivoa – EQ-5D-3L na srpskom jeziku i EQ vizuelna analogna skala (EQ-VAS) korišćeni su za procenu kvaliteta života u vezi sa zdravljem. **Rezultati.** Prosečna starost ispitanika iznosila je 65,1 ± 9,3 godina. Hipoglikemija je zabeležena kod 71%, gojaznost kod 43.9% i hronične komplikacije kod 88.8% isptanika. Polovina bolesnika nije dostigla ciljne vrednosti glikoziliranog hemoglobina (HbA1c) (HbA1c < 7%). Hipoglikemija je bila značajno češća kod mlađih bolesnika u trenutku postavljanja dijagnoze (54,19 *vs* 57,59) a ređa kod visokobrazovanih

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ispitanika (7,3% *vs* 35,1%). Bolesnici sa hipoglikemijom imali su veću ocenu za bol i nelagodnost (1,86 *vs* 1,68) u EQ-5D upitniku, niže vrednosti VAS skora (55,99 *vs* 62,44) i EQ-5D indeksa (0,85 *vs* 0,87) u odnosu na one sa dobrom kontrolom glikemije. Gojazni ispitanici su uglavnom bili ženskog pola, sa povišenim vrednostima sistolnog i dijastolnog pritiska, povišenim trigliceridima, obimom struka, ocenom za anksioznost i depresiju (2,01 *vs* 1,80) i većim brojem epizoda hipoglikemija na godišnjem nivou. Nakon korekcije za doprinoseće faktore, ispitanici sa hipoglikemijom imali su 2 035 puta veću šansu za teži stepen bola i nelagodnosti. Sa povećanjem ITM, šansa za probleme mobilnosti, anksioznost/depresiju povećala se 1,05 puta. Hipoglikemija je bila značajno povezana sa nižim vrednostima VAS skora (b = -0.13). Povećanje vrednosti ITM bilo je statistički značajno povezano sa nižim vrednostima EQ 5D indeksa (b = -1,78). Učestalost hipoglikemije nije bila povezana sa vrednostima VAS skorom i EQ 5D indeksa. **Zaključak.** Rezultati su pokazali značajnu povezanost hipoglikemije i gojaznosti sa smanjenim QL, procenjenim na osnovu EQ-5D upitnika. Korekcija životnih stilova usmerenih ka smanjenju gojaznosti i hipoglikemije uz optimalnu terapiju, može značajno poboljšati QL vezan za zdravlje kod bolesnika sa DMT2.

Ključne reči:
dijabetes melitus, insulin-nezavisni; kvalitet života; hipoglikemija; telesna masa, indeks.

Introduction

Diabetes mellitus is a metabolic disease with increasing incidence and prevalence in adults worldwide. The greatest increase of diabetes mellitus (DM) prevalence is registered in developing countries. The literature data showed 382 million people had diabetes mellitus worldwide in 2013 and this number will continue to increase up to 592 million by 2035 [1]. In 2013, the number of people with diabetes was estimated to be 56 million in Europe with an overall estimated prevalence of 8.5% [2].

It was estimated that there were about 600,000 patients with diabetes in Serbia (8.2% of total population) according to the Serbian registry of diabetes. There were about 15,400 newly diagnosed cases of type 2 DM (T2DM) in Serbia during 2011 [3].

The recent data from the United States and Europe have demonstrated that obese patients with T2DM have more commonly impaired glycemic control [4], and complications such as neuropathy, and have a higher mortality rate compared with normal weight individuals with diabetes [5].

Overweight and obesity, represent important risk factors for the development of T2DM. Obesity-induced insulin resistance lies in the basis of this association [6]. Since overweight and obesity are associated with metabolic disorders their presence could worsen diabetes symptoms and disease expression. Thus, reduction of body weight and obesity by lifestyle interventions (diet and increased physical activity) combined with oral antidiabetics is the first step in the therapy of DM [7]. Obesity plays an important role not only in clinical presentations and diabetes therapy choice but also had a great impact on health-related quality of life – QL (HRQL) which could be important determinator when selecting between therapies or evaluating the cost effectiveness of different diabetes treatment options [8].

Almost all diabetic patients share the same opinion that diabetes impairs their QL by making many restrictions and schedules in usual daily activities, by the development of chronic diabetic complications or by spontaneous/therapy induced hypoglycemic episodes. DMT2 decreases QL and together with obesity-associated depression could reduce patients motivation to properly control the disease.

It has been shown that hypoglycemia caused by oral antidiabetic drugs or insulin treatments impaired QL (QoL) and it is associated with increased mortality in diabetics [9–11]. However, diabetes therapy is very complex and hypoglycemia represents important factor limiting therapy effectiveness and optimal glycoregulation in patients with T2DM. It is estimated that about 12–30% T2DM patients on antidiabetic therapy experienced hypoglycemia [12]. But, there is still limited data about potential effects of hypoglycemia on QL in T2DM patients in real life settings.

HRQL can be estimated by using different tools. These tools include direct valuation methods such as visual analogue scale (VAS) and time trade-off (TTO) [13], as well as indirect questionnaire-based measures such as the World Health Organization (WHO)-5 index for QL [14], the EuroQoL – EQ 5-dimensional (EQ-5D) questionnaire [13], and the Short Form Health Survey (SF 36) [15].

Determining the prevalence of hypoglycemia and measuring its impact on QLcould be of great importance because they have a significant impact on optimal diabetic treatment and prevention of diabetic late complications development [16].

The aim of this study was to provide the frequency of self-reported hypoglycemia in real life settings, to determine body mass index (BMI) and to determine the influence of hypoglycemia and obesity on HRQL and its subscale scores in EQ-5D questionnaire in patients with T2DM after adjusting for age and presence of diabetes-related late complications.

Methods

This clinical-epidemiological cross sectional study involved 269 T2DM patients, treated and examined in the Department for Diabetes, Internal Ward of the Health Center in Leskovac, Serbia. All patients were selected in a consecutive manner if they satisfied inclusion criteria. The inclusion criteria were: adult patients with T2DM older than 30 years and duration of DM longer than one year. All the participants have signed an informed consent form and anonymously filled EQ-5D and EQ-VAS questionnaires. The clinical data (presence of late diabetic complications, hypoglycemia, blood pressure and BMI) were collected by physicians from the di-

rect interview and physical examination and by checking medical documentations.

The study protocol was approved by the institutional review board and the informed consent was obtained from all patients' prior to enrolment in the study.

The standardized Serbian version of the EQ-5D 3 level version (EQ-5D-3L) questionnaire was used to determine HRQL in patients with T2DM. The five health dimensions from the EQ-5D-3L questionnaire (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression) were used to determine the overall index. Each health dimension could be classified by three response levels (no, moderate, or extreme problems), which represents a matrix of 243 different health states. The patients also rated their health state using VAS (EQ-VAS) as a part of the EQ-5D-3L questionnaire. This scale had values ranging from 0-100 where 0 represented worst and 100 best health state.

Five health dimensions are converted into a weighted overall health index by applying scores from the EQ-5D preference weights derived from representative general population samples. These weights lie on a scale on which full health has a value of 1 and dead a value of 0. In this paper, we used the German TTO because this European population is similar to investigated population in Serbia [17].

According to the American Diabetes Association (ADA), we defined hypoglycemia as the presence of symptoms (hunger, shakiness, sweating, headache, dizziness, etc), and self measured blood glucose concentration ≤ 70 mg/dL (≤ 3.9 mmol/L) at the time symptoms were experienced (confirmed hypoglycemia) [18]. Unconfirmed hypoglycemia was defined as the presence of at least one symptom without a self measured blood glucose concentration ≤ 3.9 mmol/L. Patients without symptoms of hypoglycemia are marked as non hipoglycemic patients. Episodes of asymptomatic hypoglycemia (blood sugar reading of ≤ 3.9 mmol/L without symptoms) were also collected. The presence and frequency of hypoglycemia were also obtained from medical records.

The presence of DM was confirmed through anamnestic data and medical records. The patients with confirmed type 1 DM were excluded from the study. The presence of late microvascular and macrovascular diabetic complications was confirmed through medical documentation or patients' records.

The demographic data included age, sex, duration of DM, marital status (living with a partner or alone) and education (primary, secondary and tertiary school).

BMI was calculated from the most recent measurements (under one month) of weight and height and expressed as (kg/m^2). According to WHO classification, patients were divided into three BMI categories: normal (< 25 underweight and normal weight); overweight $25 \leq BMI < 30$ kg/m^2 and obese – $BMI \geq 35$ kg/m^2 [19]. These categories correspond to WHO classification and are widely employed by clinicians.

The blood pressure was measured by sphygmomanometer and the Korotkoff sound technique on the left upper arm in the sitting position after 5 min of resting [20].

The creatinine clearance was calculated by Cockcroft-Gault formula: {[(140 - age) × weight (kg) × F] / [serum creatinine (μmol/L) × 0.8136]} where F = 1 if male, and 0.85 if female, and expressed in mL/min.

Standard laboratory parameters [total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides (TG), fasting glycemia] were obtained from the medical documentation and they were accepted if not older than one month.

Statistical analyses

The data were analysed using descriptive statistical methods: frequency, percentage, mean ± standard deviation and 95% confidence interval (CI) for mean, depending on type and variable distribution.

The difference between groups of patients who experienced hypoglycemia and group without hypoglycemia was examined using χ^2 tests or Mann Whitney *U* test and *t*-tests for categorical and continuous dependent variables, respectively. Differences between patients according to BMI classification of obesity were examined using ANOVA and post-hoc test (Tukey or Dunnett's T3) depending on Levene Statistic or Kruskal–Wallis.

We used ordered logistic regressions to determine predictive importance of the BMI and hypoglycemia on EQ-5D subscale. Hierarchical multiple linear regression (Enter model) controlling for age, duration of DM and presence of diabetic late complications was applied to determine the association of the BMI and hypoglycemia on EQ-VAS score and EQ-5D index.

All analyses were conducted with SPSS version 17.0 (SPSS INC, NC) and statistical significance was set at $p < 0.05$. Where appropriate, for ordered logistic regressions beta-coefficients (b) and odds ratios (OR) were provided in the results section; for all other multivariate regressions, beta-coefficients were provided.

Results

A total of 269 diabetics aged 65.1 ± 9.3 years were analyzed. Hypoglycemia was registered in 71.0%, obesity in 43.9% and chronic complications of diabetes mellitus in 88.8% of the patients (microvascular in 85.1% and macrovascular in 46.5%) Almost half of the patients did not reach target HbA1c values under 7.0% (Table 1).

Patients experienced hypoglycemia compared to those who did not have significantly higher mean EQ-5D subscale score (pain/discomfort: 1.86 *vs* 1.68 respectively; $p < 0.01$) and significantly lower mean levels on the VAS (55.99 *vs* 62.44 respectively; $p < 0.05$) and EQ-5D index value (0.85 *vs* 0.87; $p < 0.05$) indicating poorer quality of life. Compared to those who did not report hypoglycemia, patients who did, were significantly younger at diagnosis (54.19 *vs* 57.59 respectively; $p < 0.05$) and less likely to be graduated at tertiary school (7.3% *vs* 35.1% respectively; $p = 0.01$) (Table 2).

Obese patients were more likely to be female with higher systolic and diastolic blood pressure (BP) and waist

Table 1

Demographic and clinical characteristics and complications in type 2 diabetic patients

Variable	Value
Total number of patients, n (%)	269 (100)
Males/females, n (%)	100/169 (37.2/62.8)
Age (years), $\bar{x} \pm SD$	65.1 ± 9.3
BMI (kg/m^2), $\bar{x} \pm SD$	29.7 ± 5.7
BMI WHO categories, n (%)	
normal	53 (19.7)
overweight	98 (36.4)
obese	118 (43.9)
Hypoglycemia, n (%)	191 (71.0)
Rates of hypoglycemia (event *per* patient-year)	10.8
EQ-5D index, $\bar{x} \pm SD$	0.86 ± 0.09
EQ-5D VAS, $\bar{x} \pm SD$	57.9 ± 19.3
Hypertension, n (%)	187 (69.5)
Chronic complications, n (%)	239 (88.8)
Macrovascular complications, n (%)	125 (46.5)
coronary artery disease	79 (29.4)
cerebrovascular disease	29 (10.8)
peripheral arterial disease	54 (20.1)
Microvascular complications, n (%)	229 (85.1)
retinopathy	174 (64.7)
nephropathy	37 (13.8)
polyneuropathy	200 (74.3)
Dialysis, n (%)	2 (0.8)
Renal transplantation, n (%)	0 (0.0)
Amputation, n (%)	11 (4.1)
HbA1c at target (< 7.0%), n (%)	131 (48.7)

BMI – body mass index; WHO – World Health Organization; EQ-5D – EuroQoL 5-dimensional; VAS – visual analogue scale; HbA1c – glycated hemoglobin; $\bar{x}$ – mean value; SD – standard deviation.

Table 2

Characteristics of patients experiencing hypoglycemia and those without hypoglycemia

Variable	Hypoglycemia symptoms (low blood sugar), n = 191	No hypoglycemia symptoms (no low blood sugar), n = 78	*p* – value
Males, n (%)	73 (38.2)	27 (35.1)	NS
Rural, n (%)	101 (52.9)	42 (53.8)	NS
Education			
no education, n (%)	54 (28.3)	23 (29.5)	NS
primary, n (%)	44 (23.0)	15 (19.5)	NS
secondary, n (%)	66 (34.6)	26 (33.8)	NS
tertiary, n (%)	14 (7.3)	27 (35.1)	< 0.01
Married/living with partner, n (%)	139 (72.8)	60 (76.9)	NS
Age (years), $\bar{x} \pm SD$	65.62 ± 8.99	64.06 ± 10.15	NS
Age at diagnosis (years), ($\bar{x} \pm SD$)	54.19 ± 10.39	57.59 ± 10.07	< 0.05
Quality of life, (95% CI)			
mobility	1.63 (1.55–1.71)	1.57 (1.44–1.71)	NS
self care	1.35 (1.27–1.44)	1.31 (1.18–1.45)	NS
usual activities	1.78 (1.68–1.88)	1.77 (1.59–1.94)	NS
pain/discomfort	1.86 (1.79–1.93)	1.68 (1.56–1.79)	< 0.01
anxiety/depression	1.93 (1.83–2.03)	1.84 (1.70–1.99)	NS
EQ-5D index value, $\bar{x}$ (95% CI)	0.85 (0.84–0.86)	0.87 (0.85–0.89)	< 0.01
Visual analogue scale, $\bar{x} \pm SD$	55.99 ± 18.75	62.44 ± 20.23	< 0.01
Laboratory			
creatinine (μmol/L), $\bar{x} \pm SD$	91.71 ± 68.55	91.99 ± 49.49	NS
creatinine clearance (mL/min), $\bar{x} \pm SD$	86.05 ± 36.33	89.91 ± 45.97	NS
waist circumference (cm), $\bar{x} \pm SD$	104.04 ± 14.51	101.83 ± 14.02	NS
body mass index (kg/m^2), $\bar{x} \pm SD$	29.65 ± 5.27	30.22 ± 6.70	NS

CI – confidence interval; $\bar{x}$ – mean value; SD – standard deviation; NS – no significant; EQ-5D – EuroQoL 5-dimensional.

circumference. The obese patients compared to those with normal BMI had significantly higher mean EQ-5D subscale score (anxiety/depression: 2.01 *vs* 1.82 and 1.80 respectively, $p < 0.05$) while there was no significant difference in VAS and EQ-5D index value. The proportion of patients who experienced hypoglycemic episodes was similar between BMI groups, but the rates of hypoglycemia *per* year were significantly higher in obese patients (Table 3).

In multivariate models, adjusting for confounders, patients who experienced hypoglycemic events had a 0.71 increase in the log odds of being in a higher level of pain/discomfort EQ-5D subscale. The one unit increase in BMI was accompanied with 0.05 increases in the log odds being in a higher level of mobility and anxiety/depression EQ-5D subscale; all of the other variables in the model were held constant. In presence of hypoglycemia there were 2.035 greater odds for experienced extreme problems *vs* no or moderate problems as well as experienced extreme and moderate problems *vs* no problems related to pain/discomfort ($p < 0.05$). Also, the unit increase of BMI had 1.05 greater odds for experienced extreme problems *vs* no or moderate problems as well as experienced extreme and moderate problems *vs* no problems related to mobility and anxiety/depression ($p < 0.05$) (Table 4).

Hypoglycemia was associated with significantly lower levels on the VAS score (b = -0.13, $p < 0.05$). The increase of BMI was associated with significantly lower EQ-5D index value (b = -1.78, $p < 0.05$). The rates of hypoglycemia *per* year were not significantly associated with EQ-5D VAS and EQ-5D index scores (Table 5).

Discussion

In this cross-sectional observational study, we measured HRQL using the EQ-5D, questionary which is widely used in Europe and Asian T2DM patients [21]. We used VAS scale direct methods to capture values that patients assign to their own health state [22]. We analyzed demographic characteris-

Table 3

Characteristics of patients according to body mass index (BMI) classification of obesity

Variables	Normal (BMI 20–24.9 kg/m^2) n = 53	Overweight (BMI 25–29.9 kg/m^2) n = 98	Obese (BMI > 30 kg/m^2) n = 118	
Males, n (%)	28 (52.8)	38 (38.8)	34 (28.8)[b]	< 0.01
Rural, n (%)	30 (56.6)	48 (49.0)	65 (55.1)	NS
Education, n (%)				
no education	14 (26.4)	29 (29.6)	34 (28.8)	NS
primary	10 (18.9)	23 (23.5)	26 (22.0)	NS
secondary	18 (34.0)	29 (29.3)	45 (38.1)	NS
tertiary	11 (20.8)	17 (17.3)	13 (11.0)	NS
Married/living with partner, n (%)	35 (66.0)	75 (76.5)	89 (75.4)	NS
Age (years), $\bar{x} \pm SD$	66.91 ± 11.54	65.63 ± 8.73	64.06 ± 8.63	NS
Age at diagnosis (years), $\bar{x} \pm SD$	56.30 ± 13.14	55.39 ± 9.75	54.60 ± 9.61	NS
Quality of life, mean (95% CI)				
mobility	1.55 (1.37–1.73)	1.60 (1.48–1.72)	1.65 (1.56–1.74)	NS
self care	1.43 (1.23–1.63)	1.34 (1.21–1.47)	1.30 (1.21–1.39)	NS
usual activities	1.75 (1.52–1.97)	1.76 (1.61–1.92)	1.79 (1.68–1.91)	NS
pain/discomfort	1.88 (1.73–2.04)	1.76 (1.65–1.87)	1.79 (1.72–1.87)	NS
anxiety/depression	1.80 (1.61–2.00)	1.82 (1.70–1.95)	2.01 (1.88–2.14)[a]	< 0.05
EQ-5D index value, mean (95% CI)	0.86 (0.83–0.88)	0.86 (0.85–0.88)	0.85 (0.84–0.87)	NS
Visual analogue scale, $\bar{x} \pm SD$	54.63 ± 22.19	60.35 ± 18.40	57.39 ± 18.75	NS
Waist circumference (cm), $\bar{x} \pm SD$	87.32 ± 8.56	100.09 ± 10.47[a]	113.28 ± 11.28[a]	< 0.01
sBP (mmHg), $\bar{x} \pm SD$	133.30 ± 16.11	138.37 ± 13.99	141.95 ± 16.33[b]	< 0.01
dBP (mmHg), $\bar{x} \pm SD$	82.08 ± 9.16	84.29 ± 8.21	85.51 ± 9.85[b]	< 0.01
Hypoglycemia, n (%)	34 (64.1)	75 (76.5)	82 (69.4)	NS
Rates of hypoglycemia *per* year, mean (95% CI)	17.23 (11.3–23.5)	17.1 (13.1–21.1)	31.7 (21.6–41.8)[a]	< 0.05
Late diabetes complication, n (%)	45 (84.9)	89 (90.8)	105 (88.9)	NS

[a]$p < 0.05$ or 0.01 *vs* other groups, [b]$p < 0.01$ *vs* group with normal BMI; NS – no significant; CI – confidence interval; sBP – systolic blood pressure; dBP – diastolic blood pressure. For other abbreviations see under previous tables.

Table 4

Adjusted effects of hypoglycemia, rates of hypoglycemia and body mass index (BMI) on health outcomes

Quality of life: ordered logit	b	95% CI (lower–upper)	OR	X^2	*p**
Presence of hypoglycemia					
EQ-5D: mobility	0.08	-0.50–0.66	1.084	0.07	NS
EQ-5D: self-care	0.04	-0.61–0.70	1.048	0.01	NS
EQ-5D: usual activities	0.07	-0.47–0.62	1.075	0.06	NS
EQ-5D: pain/discomfort	0.71	0.08–1.34	2.035	4.89	0.026
EQ-5D: anxiety/depression	0.187	-0.365–0.740	1.206	0.44	NS
Rates of hypoglycemia					
EQ-5D: mobility	0.003	-0.005–0.01	1.004	0.58	NS
EQ-5D: self-care	0.004	-0.003–0.01	1.004	1.17	NS
EQ-5D: usual activities	-0.003	-0.012–0.004	0.991	0.75	NS
EQ-5D: pain/discomfort	0.002	-0.008–0.012	1.002	0.16	NS
EQ-5D: anxiety/depression	0.018	-0.008–0.08	1.001	0.017	NS
BMI					
EQ-5D: mobility	0.05	0.006–0.09	1.054	5.04	0.024
EQ-5D: self-care	0.003	-0.04–0.05	1.003	0.01	NS
EQ-5D: usual activities	0.028	-0.01–0.07	1.028	1.71	NS
EQ-5D: pain/discomfort	0.017	-0.030–0.065	1.017	0.530	NS
EQ-5D: anxiety/depression	0.05	0.015–0.100	1.059	7.17	0.007

***p* adjusted for age, duration of diabetes mellitus and presence of diabetic late complications; OR – odds ratio. For other abbreviations see under previous tables.**

Table 5

Adjusted effects of hypoglycemia, rates of hypoglycemia and body mass index (BMI) on health outcomes

Quality of life: multiple regression	b	95% CI (lower-upper)	t	*p**
Presence of hypoglycemia				
EQ-5D: VAS	-0.13	-10.975 / -0.246	2.05	0.040
EQ-5D: index value	-0.993	-0.037 / 0.012	-0.99	NS
Rates of hypoglycemia				
EQ-5D: VAS	-0.005	-0.08 / 0.08	-0.085	NS
EQ-5D: index value	-1.572	-1.76 / 0.901	-1.57	NS
BMI				
EQ-5D: VAS	-0.042	-0.549 / 0.265	-0.68	NS
EQ-5D: index value	-1.787	-0.04 / -0.01	-1.98	0.045

***p* adjusted for age, duration of diabetes mellitus and presence of diabetic late complications. For other abbreviations see under previous tables.**

tics, duration of DM, the presence of late diabetes complication, obesity and hypoglycemic episodes and investigated their impact on HRQL.

A total of 269 diabetics aged 65.1 ± 9.3 years were analyzed. Hypoglycemia was registered in 71.0%, obesity in 43.9% and chronic complications of DM in 88.8% of the patients (microvascular in 85.1% and macrovascular in 46.5%). The gender distribution and age of examined patients reported in large SHIELD study [23] are similar to those reported in our study. The values of EQ-5D index 0.77 were lower while VAS score of 66.8 was larger in SHIELD study compared to our cohort of patients with T2DM. Almost half of the patients did not reach target HbA1c under 7.0%.

This study shows that QoL measured by ED-5D index and VAS scale was lower among T2DM that had experienced hypoglycemia. Significantly more patients among those reporting hypoglycemia indicated problems in the EQ-5D dimensions ‘‘pain/discomfort’’ which is in line with results observed by other authors. The Exhype study [24] also found problems in the EQ-5D dimension ‘‘anxiety/depression’’ which is not registered in our cohort of patients.

A significant proportion of diabetics experience hypoglycemic episodes regardless of HbA1c value and reaching HbA1c less then 7.0%. It must be mentioned that almost half of the examined diabetics did not reach target HbA1c under 7.0% which indicates poor management of

diabetics at the primary and secondary level of health care. The proportion of the patients in this study who reported hypoglycemia was somewhat larger than in other studies that examined diabetics on different types of hypoglycemic therapy. The prevalence of all hypoglycemia (mild and severe) in the group with insulin-treated type 2 diabetes was 45%[25] and 31% in patients treated with glibenclamide in UK prospective study of therapies for maturity onset diabetes (UKPDS)[26]. Patients who experienced hypoglycemia were significantly younger at diagnosis, and less likely to be with higher education which is often seen result in other similar studies[24, 27]. This is not in concordance with usual belief that low blood sugar was more prevalent among older diabetics and patients with long duration of DM, though older patients are generally considered to be at greater risk for hypoglycemia[28]. This could be explained in part that the older patients often may not recognize these symptoms or may attribute them to other chronic conditions. Duration of diabetes is proved as an important factor for hypoglycemia occurrence in this paper and previous studies. Having in mind these results, the prepared regression analysis was adjusted for age, duration of diabetes and presence of comorbidities.

The current study compared outcomes between BMI subgroups (normal, overweight and obese). Obesity was registered in 43.9% of the patients and obese patients were more likely to be female with a higher systolic and diastolic BP, a higher level of triglycerides and waist circumference. Similar data were published by other authors who analyzed obese patients with T2DM. Compared with the normal and overweight group, patients in the obese group were younger and significantly more were female. They were more often presented with dyslipidemia, hypertension, or both conditions compared to patients with normal BMI range[29]. The prevalence of micro and macrovascular complications was similar between BMI categories and no significant differences were noted for these conditions.

Patients in the obese group reported worse EQ-5D subscale score "anxiety/depression" compared with patients in the normal and overweight BMI groups. There was no significant difference in VAS score and EQ-5D index. The study by Ji et al.[29] showed obese patients significantly more reported having difficulty with mobility, difficulty completing usual activities, experienced moderate or extreme pain/discomfort or experienced moderate or extreme anxiety or depression. Obese patients with T2DM had a significantly greater disability and worse HRQL compared with non-obese patients with T2DM. Similarly, results from the Study to Help Improve Early Evaluation and Management of Risk Factors Leading to Diabetes, showed that patients with comorbid T2DM, obesity, and hypertension had a lower quality of physical and mental health, as well as moderate-to-severe depression compared to patients with diabetes only[30].

The proportion of patients' experienced hypoglycemic episodes was similar between BMI groups, but the rates of hypoglycemia *per* year were significantly higher in obese patients.

The prevalence of macrovascular and microvascular complications was very high with no significant differences between BMI categories which are results also observed by other authors[29]. The overall proportion of late diabetes complications in multinational A1chieve study[31] was 26%, but it was much higher in Russia (72.4%), which is similar to data obtained in our cohort of patients. This indicates great comorbidity load of examined patients with a high rate of hypoglycemia and prevalence of obesity which had a huge impact on HRQL.

In multivariate regression, the hypoglycemia and increased BMI, but not rates of hypoglycemia, have a significant association with HRQL, even after adjusting for age, duration of DM and presence of late diabetic complications. Those who experienced hypoglycemia were about 2.03 times more likely to experience extreme or moderate problems related to pain and discomfort compared with those who do not experience hypoglycemia. Hypoglycemia was associated with significantly lower levels of the VAS score. Similarly, those with a unit increase of BMI were 1.05 times more likely to experience extreme and moderate problems related to mobility and anxiety or depression. Also, increasing of BMI was associated with the significantly lower EQ-5D index value.

The similar study evaluating relationship between BMI and HRQL suggests that the functional relationship between BMI and utilities was nonlinear, with optimal health near 26 kg/m^2 and significantly decreasing health utilities with increasing levels of overweight and obesity[32] which could be explanation for lacking relationship between EQ-5D utilities – VAS scale with BMI in our study.

Estimation of the association between BMI and hypoglycemia with health utilities is important in economic evaluations of diabetes treatment options and obesity prevention programmes. BMI and hypoglycemia were significantly associated with EQ-5D health utilities even after adjustment for macro- and microvascular complications, duration of diabetes and age. This suggests that increased BMI and hypoglycemia symptoms in patients with T2DM are predictors of impaired health-related QL *per se*, and not only through its association with higher rates of comorbidities.

Conclusion

The results demonstrated a significant relationship between hypoglycemia and obesity with impaired HRQL determined by the EQ-5D utilities (subscale, index and VAS score), even after adjusting for an age, duration of diabetes and diabetic late complications. This indicates that lifestyle measures to reduce obesity and hypoglycemia together with optimal diabetic therapy regimen can markedly improve patients' health-related quality of life.

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Erythrocyte antioxidative enzymes activities in patients with chronic hepatitis C treated with pegylated interferon alpha-2a and ribavirin

Aktivnost antioksidativnih enzima eritrocita kod obolelih od hroničnog hepatitisa C, lečenih pegilovanim interferonom alfa-2a i ribavirinom

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Abstract

Background/Aim. In hepatitis C virus infection, oxidative stress and antioxidant imbalance are major triggers for the disease occurrence and its progression. The aim of the research was to determine the erythrocyte antioxidative enzymes activities, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), before and after therapy with pegylated interferon alpha-2a and ribavirin and to evaluate their clinical significance as potential diagnostic markers of sustained virological response (SVR). **Methods.** The study included 53 patients with chronic hepatitis C (CHC) and 56 healthy controls. SOD, GPx, CAT, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured in patients both before and after the treatment. **Results.** SOD, GPx and CAT activities prior to the treatment were significantly lower in CHC patients compared to the controls ($p < 0.001$), and they were significantly higher after the treatment ($p < 0.001$). A significant positive correlation existed between SOD, GPx, and CAT activites, before and after the treatment ($p < 0.001$) and with those of aminotransferases prior to the treatment ($p < 0.001$). After the treatment, only GPx activity showed significant negative correlation with that of aminotransferases ($p < 0.001$). Receiver operating characteristic curve analysis for SOD, GPx and CAT showed following values: area under the curve of 0.975, 0.988, and 0.817 respectively; sensitivity of 93.5%, 71.7%, 100% respectively and specificity of 100% for all, respectively. Forty six SVR achievers had significant increase of SOD, GPx and CAT activities ($p < 0.001$ for all), unlike 7 SVR non-achievers ($p = 0.31$, $p = 0.717$, $p = 0.85$, respectively). **Conclusion.** Oxidative stress is the initiator of onset and progression of CHC. The combined antiviral therapy leads to the restoration of antioxidant balance. GPx, SOD and CAT may be diagnostic markers of CHC treatment outcome.

Key words:
hepatitis c; erythrocytes; oxidative stress; antioxidants; superoxide dismutase; glutathione peroxidase; catalase; interferon-alpha; ribavirin.

Apstrakt

Uvod/Cilj. Oksidativni stres i narušena antioksidantna ravnoteža su važni pokretači nastanka i progresije hepatitis C virusne infekcije. Cilj ovog istraživanja bio je određivanje aktivnosti antioksidativnih enzima, superoksid dizmutaze (SOD), glutation peroksidaze (GPx) i katalaze (CAT), pre i posle terapije pegilovanim interferonom alfa-2a i ribavirinom i evaluacija njihovog kliničkog značaja kao potencijalnih dijagnostičkih markera stabilnog virusološkog odgovora (SVR). **Metode.** Istraživanjem je bilo obuhvaćeno 53 bolesnika sa hroničnim hepatitisom C (HHC) i 56 zdravih ispitanika. Aktivnost SOD, GPx, CAT, aspartat aminotransferaze (AST) i alanin aminotransferaze (ALT) određivane su kod bolesnika pre i posle terapije. **Rezultati.** Preterapijske aktivnosti SOD, GPx i CAT bile su statistički značajno niže kod bolesnika sa HHC u poređenju sa zdravim ispitanicima ($p < 0,001$), a posle terapije bile su statistički značajno više ($p < 0,001$). Utvrđena je statistički značajna pozitivna korelacija između aktivnosti SOD, GPx, CAT, pre i posle terapije ($p < 0,001$) i sa aktivnošću aminotransferaza posle terapije ($p < 0,001$). Posle terapije, jedino je aktivnost GPx statistički značajno negativno korelirala sa aktivnošću aminotransferaza ($p < 0,001$). Kriva operativnih karakteristika primaoca za SOD, GPx i CAT prikazala je sledeće vrednosti: površinu ispod krive redom

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od 0,975; 0,988; 0,817; senzitivnost redom 93,5%, 71,7%, 100% i specifičnost 100% za sva tri enzima. Četrdeset šestoro bolesnika sa postignutim SVR imali su značajno povećanje aktivnosti SOD, GPx i CAT ($p < 0.001$ za sva tri enzima) za razliku od sedam bolesnika bez postignutog SVR ($p = 0.31$, $p = 0.717$, $p = 0.85$ redom). **Zaključak.** Oksidativni stres je inicijator nastanka i progresije HHC. Kombinovanom antivirusnom terapijom postiže se uspostavljanje antioksidantne ravnoteže. Enzimi GPx, SOD i CAT mogu biti dijagnostički markeri ishoda terapije, kod bolesnika sa HHC.

Ključne reči:
hepatitis c; eritrociti; stres, oksidativni; antioksidansi; peroksid dismutaza; glutation peroksidaze; katalaza; interferon-alfa; ribavirin.

Introduction

In addition to the immune liver damage and direct cytotoxic damage mediated by a variety of the viral products, oxidative stress is also directly associated with the pathogenesis of chronic hepatitis C (CHC) [1, 2]. It is believed that antioxidant barrier impairment by viral hepatitis A and viral hepatitis B represents a consequential condition. On the contrary, oxidative stress present in hepatitis C virus (HCV) infection is the main trigger for the disease occurrence and progression [3–5]. The occurrence of oxidative stress in HCV infection is explained by chronic inflammation, lipid peroxidation, iron deposition in the liver parenchyma, glutathione (GSH) level alteration, and the effect of non-structural and structural proteins of HCV (core protein, NS3, NS5A) on enzyme activity, mitochondria and hepatocyte genes. Continuous accumulation of reactive oxygen species (ROS) occurs due to nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase activity of the Kupffer cells and polymorphonuclear cells of the liver [6, 7]. Protein NS3 activates NAD(P)H oxidase and Nox 2 protein of the phagocytes, thus increasing the generation of ROS [7–9]. Deposition of excess iron causes the formation of free radicals. Expression of HCV core gene is associated with ROS increasing and reduction of intracellular and / or the mitochondrial content of GSH [6]. It is thought that the antioxidative enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), play a decisive role in the development and progression of CHC [10].

Oxidative damage in CHC is characterized by decreased activity of antioxidant enzymes such as CAT, GPx, and SOD. It is believed that combined treatment with pegylated interferon-alpha (PEG-IFN-α) and ribavirin (RBV) may be responsible for establishing antioxidant response in patients with CHC [11].

Pegylated interferon-alpha has a potent anti-viral, anti-inflammatory and immunomodulatory effect on HCV. Its antiviral activity is the result of the ability to induce interferon stimulated genes, responsible for the inhibition of various stages of viral replication. Pegylated interferon-alpha, also expresses an immunomodulatory effect which initiates the differentiation of T-helper cells referring to the Th2 cells consequently leading to the increased production of interleukin-2 and interferon gamma. Its anti-inflammatory effect is presented through inhibition of various cytokine syntheses such as tumor necrosis factor and interleukin-1 [12]. The effect of therapy is significantly higher when IFN is combined with RBV in chronic HCV infection. Hemolysis, which universally occurs with RBV therapy and which is considered a limiting side effect, is precisely the mechanism by which the anti-HCV effect is exerted. Passive hemolysis results in anti-inflammatory/antiviral actions within the liver that disrupt the innate immune tolerance, leading to the synergy of RBV with IFN-α [13].

The aim of this study was to determine activities of erythrocyte antioxidative enzymes in patients with CHC before and after PEG-IFN-α-2a and RBV therapy. Evaluation of the clinical significance of SOD, CAT, and GPx as potential diagnostic markers of treatment success was set up as another goal of the research.

Methods

The prospective study included 53 patients with CHC and 56 healthy subjects. The selection of participants who were included in the study was performed based on the criteria for implementation of standard PEG-IFN-α-2a and RBV therapy, and additional criteria in line with current research: they were stringently matched for age (average 38.2 ± 9.8 years), sex and disease duration. Additional, wider criteria for the involvement, designed in line with the topic of the research, include absence of all acute or chronic medical conditions that would further disturb the oxidative-antioxidative status, in addition to HCV infection, (patients with CHC on dialysis, hemophiliacs, former opiate addicts on withdrawal pharmacotherapy, as well as vitamin supplemented patients were not included in the study). The control group consisted of healthy volunteers of both sexes, matched for age (average 41.2 ± 11.9 years), who were also not supplemented by vitamins in near past. They were taken only blood samples so as to determine the activity of antioxidant enzymes. Written consent to participate in the research and implementation of treatment was obtained from all the patients and volunteers. Prior to the start of the treatment, blood samples were taken from the patients in order to determine antioxidant enzymes basal activities, as well as other parameters of routine hematological and biochemical analyses within the standard treatment procedures. According to the already established guidelines for the treatment, patients with HCV genotype 2 and 3 received PEG-IFN-α-2a by once a week, by subcutaneous injection, and RBV orally, on daily basis for 24 weeks of therapy. Patients with HCV genotype 1 and 4 received PEG-IFN-α-2a subcutaneous injection once a week, and RBV *per os*, daily, for 48 weeks of treatment. Treatment dosage was the same in both groups of genotypes – PEG-IFN-α-2a in a dose of 180 mg weekly, RBV 1,000 to 1,200 mg *per* day. According to the guidelines for the

evaluation of treatment of PEG-IFN-α-2a and RBV, blood was also taken for the analysis 24 weeks after the end of the treatment, in order to determine the achievement of the so-called curing, i.e., a stable virological response (SVR), and determining the activity of antioxidant enzymes. Accordingly, we set another category of patients to compare, those who achieved and those who did not achieve SVR. In addition to "after treatment evaluation", we retrospectively analyzed their baseline antioxidant enzyme activities.

Liver function test parameters, as well as determination of the activities of antioxidative enzymes – SOD, CAT, and GPx, were conducted at the Center for Medical Biochemistry, Clinical Center Niš.

Standard biochemical parameters including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the Olympus analyzer (Olympus System Tokyo, Japan). Enzyme activity in plasma was expressed in units *per* liter (U/L).

Determination of the enzyme activities was performed in red blood cells haemolysate that was made from the red blood cells suspension of the heparinized blood by adding cold redestillated water and stored at -20°C until determination of the enzyme activity. CAT activity was determined by a kinetic method of Beutler [14]. Measurement of CAT activity was carried out on a Beckman spectrophotometer DU 650 (Beckman, USA). The enzyme activity was expressed in units *per* gram of hemoglobin $\times 10^4$ (U/gHb $\times 10^4$). SOD activity was determined by a commercial Randox Ransel assey, (Randox Laboratories Ltd, Crumlin, Co.Autrium, UK) on the automatic analyzer Olympus AU 400 (Olympus, Tokyo, Japan). SOD activity was expressed in international units *per* gram of hemoglobin (U/gHb), and a reference interval was from 1,092 to 1,817 U/gHb. GPx activity was determined by a commercial Randox Ransel assay (Randox Laboratories Ltd, Crumlin, Co.Autrium, UK). Enzyme activity was expressed in units *per* gram of hemoglobin (U/gHb). Reference values were from 29.6 to 82.9 U/gHb.

Determination of HCV ribonucleic acid (RNA) was carried out in the reference laboratory of the Institute for Infectious and Tropical Diseases in Belgrade, Serbia. We performed a qualitative Polymerase Chain Reaction (PCR) determination of HCV RNA by the COBAS Amplicor Hepatitis C Virus Test version 2.0, the sensitivity of the test is 50 IU/1 mL of plasma. Quantitative real-time PCR determination of HCV RNA was carried out using the Cobas TaqMan HCV Test, the sensitivity of the test is 15 IU/mL.

All statistical analyses were performed with SPSS statistical analysis software, version 10.0 (SPSS, Chicago, IL, United States), a significance level was set at $p < 0.001$. Characteristics of the study group were expressed as the mean ± standard deviation (SD) for normal distribution or median (interquartile range) for non-normal distribution, or with frequency and percentage for categorical data. Clinical and biochemical data of the CHC patients and the control group were compared by using Student *t*-test for normally distributed data and Mann-Whitney U test for data that were not normally distributed. The relationship between two variables was determined by Pearson's correlation coefficient (r). Receiver operating characteristic (ROC) curves were constructed to establish a sensitivity–specificity relationship. Cut-off values that provided the best combination of sensitivity and specificity were determined by ROC curve analysis.

Results

Comparing the values of the studied parameters in patients with hepatitis C and the results in the control group, it was found that there was a statistically significant difference in SOD activity between these two groups before treatment (913.41 ± 322.02 U/gHb *vs* 1,783.90 ± 189.69 U/gHb; $p < 0.001$), as it is shown in Figure 1.

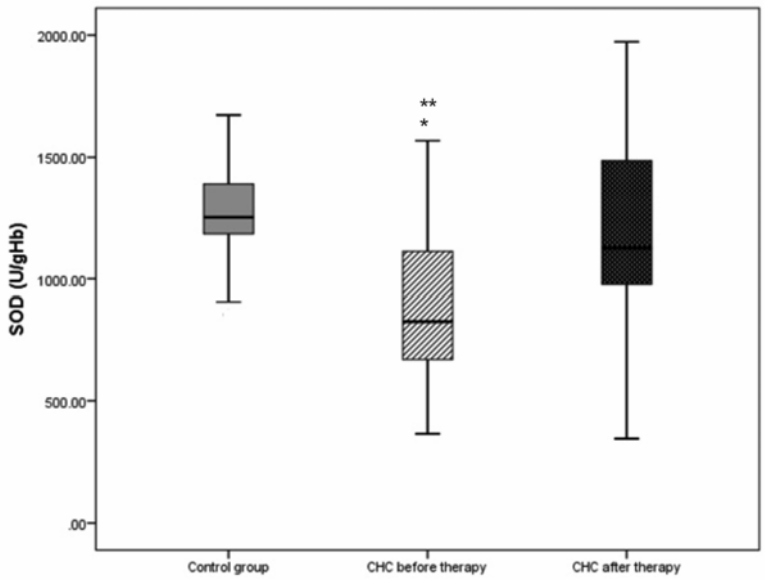

Fig. 1 – SOD activity of the control group and CHC patients before and after the treatment.
***p* < 0.001 – compared to the control group, ****p* < 0.001 – compared to the CHC patients after therapy;**
SOD – superoxide dismutase; CHC – chronic hepatitis C.

GPx activity in CHC patients before treatment was statistically significantly lower compared to healthy controls (22.49 ± 4.88 U/gHb *vs* 45.46 ± 7.77 U/gHb; $p < 0.001$) (Figure 2). CAT activity was also significantly lower in CHC patients before treatment compared to healthy subjects (5.12 ± 0.83 U/gHb × 10^4 *vs* 13.22 ± 2.44 U/gHb × 10^4; $p < 0.001$) (Figure 3). AST activity in patients with CHC before treatment was significantly higher compared to the activity of this enzyme in healthy subjects ($p < 0.001$) and compared to values in patients with CHC after the treatment ($p < 0.001$). ALT activity was also statistically significantly higher in patients compared to healthy subjects ($p < 0.001$) and compared to values in patients with CHC after the treatment ($p < 0.001$) (Table 1).

AST activity was statistically significantly reduced during the treatment ($p < 0.001$). ALT activity was also statistically significantly reduced during the application of therapy ($p < 0.001$) (Table 1).

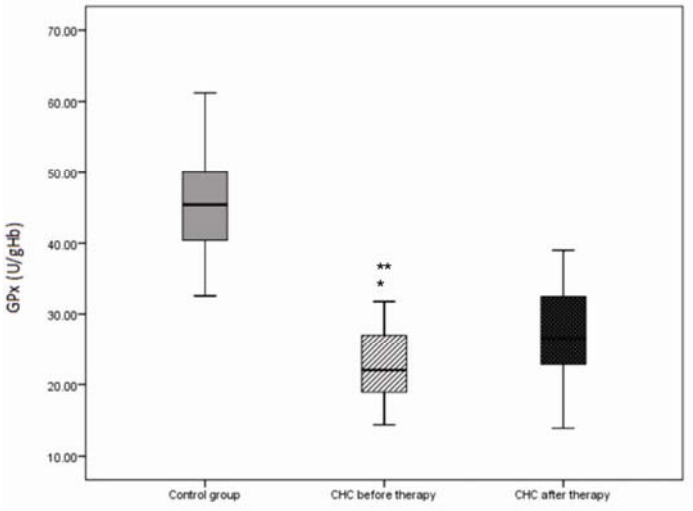

Fig. 2 – GPx activity of the control group and CHC patients before and after the treatment. *$p < 0.001$ – compared to the control group; **$p < 0.001$ – compared to the CHC patients after therapy; GPx – glutathione peroxidase; CHC – chronic hepatitis C.

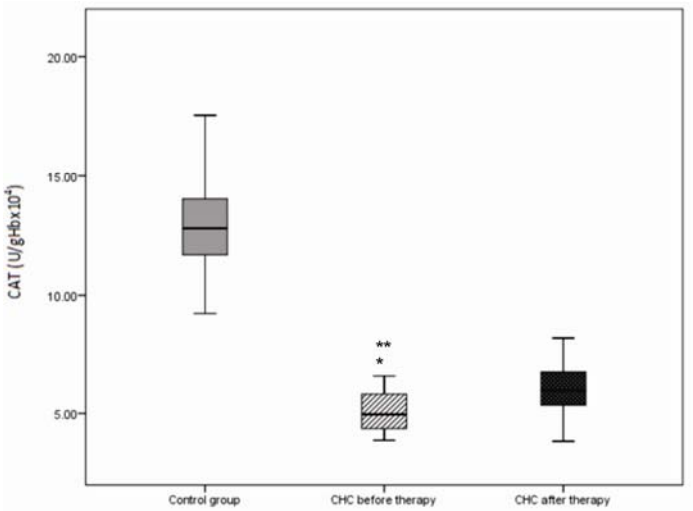

Fig. 3 – CAT activity of the control group and CHC patients before and after the treatment. *$p < 0.001$ – compared to the control group; **$p < 0.001$ – compared to the CHC patients after therapy; CAT – catalase; CHC – chronic hepatitis C.

Table 1

Aminotransferases activity of the control group and chronic hepatitis C (CHC) patients before and after the treatment

Aminotransferases	Control group (n = 56) $\bar{x} \pm SD$	CHC patients (n = 53) $\bar{x} \pm SD$	
		before therapy	after therapy
AST (U/L)	22.52 ± 7.43	66.58 ± 44.30 *, **	28.64 ± 40.76
ALT (U/L)	27.80 ± 19.35	85.72 ± 41.74 *,**	32.45 ± 37.82

$\bar{x} \pm SD$ – arithmetic mean ± standard deviation; p – significance of difference between groups; *$p < 0.001$ – compared to the control group, **$p < 0.001$ – compared to the CHC patients after therapy; AST – aspartate aminotransferase; ALT – alanine aminotransferase.

There was a statistically significant difference in SOD activity before compared to the after the treatment (913.41 ± 322.02 U/gHb *vs* 1172.83 ± 415.67 U/gHb; $p < 0.001$) (Figure 1).

Correlation analysis showed that there was a statistically significant correlation among all the parameters examined before the treatment. Statistically significant positive correlation ($p < 0.001$ for all) existed between SOD and GPx, SOD and CAT, GPx and CAT, AST and ALT. Statistically significant negative correlation ($p < 0.001$ for all) existed between the following parameters: SOD and AST, SOD and ALT, GPx and AST, GPx and ALT, CAT and AST, CAT and ALT (Table 2).

There was a statistically significant positive correlation ($p < 0.001$ for all) between activities of SOD and GPx, SOD and CAT, GPx and CAT, AST and ALT and ALT after the therapy, and statistically significant negative correlation ($p < 0.05$) between activities of GPx and aminotransferases (Table 2).

ROC curve analysis showed that GPx was the best diagnostic marker in monitoring the success of therapy. SOD had slightly lower values, while CAT had the weakest discriminative ability. The cut-off value, specificity and sensitivity of the studied parameters were also determined by this statistical methodology (Table 3). SOD and GPx have high specificity and sensitivity, and CAT had a slightly lower sensitivity (Figure 4).

Table 2

Correlation analysis between SOD, GPx, CAT and aminotransferases before and after the therapy

Enzyme	GPx	CAT	AST	ALT
SOD				
before therapy	0.722*	0.740*	-0.317*	-0.314*
after therapy	0.507*	0.429*	-0.169	-0.174
GPx				
before therapy	-	0.873*	-0.504	-0.603
after therapy		0.823*	-0.193†	-0.215†
CAT				
before therapy		-	-0.530*	-0.610*
after therapy		-	-0.144	-0.129
AST				
before therapy			-	0.901*
after therapy			-	0.912*

***p < 0.001; †p < 0.005; SOD – superoxid dismutase; GPx – glutathione peroxidase; CAT – catalase; AST – aspartate aminotransferase; ALT – alanine aminotransferase. The numerical values represent Pearson's correlation coefficient (r).**

Table 3

Analysis of the ROC curve of SOD, GPx and CAT in relation to the outcome of the therapy

Enzymes	Cut-off value	Sensitivity (%)	Specificity (%)	AUC	95% CI	*p*
SOD	627.58	93.5	100	0.975	0.938–1.013	< 0.001
GPx	17.10	97.8	100	0.988	0.961–1.014	< 0.001
CAT	4.66	71.7	100	0.817	0.706–0.928	0.007

***p* – significance of difference between groups; SOD – superoxid dismutase; GPx – glutathione peroxidase; CAT – catalase; ROC – receiver operating characteristics; AUC – area under the curve; CI – confidence interval.**

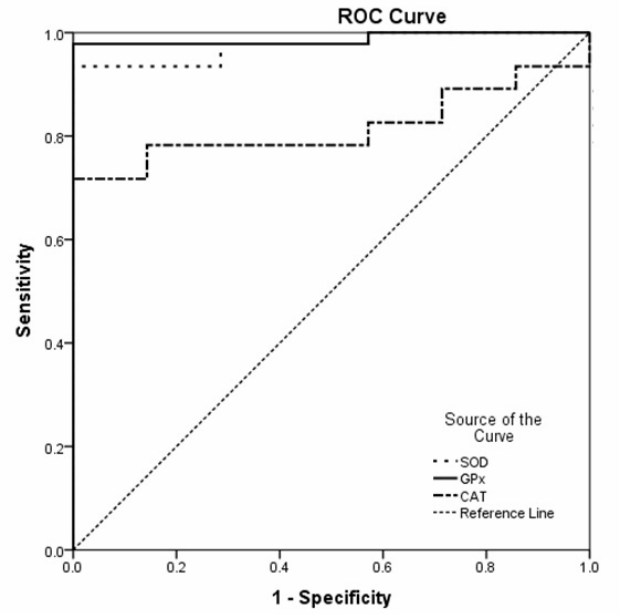

Fig. 4 – ROC curve for the activities of SOD, GPx and CAT in relation to the outcome of the treatment. SOD – superoxid dismutase; GPx – glutathione peroxidase; CAT – catalase; ROC – receiver operating characteristics.

In Table 4 we reported that examined patients who did not achieve SVR had significantly lower values of SOD, GPx and CAT measured before therapy initiation compared to subjects who achieved SVR ($p < 0.001$ for all). Also, it was shown significantly lower values of SOD, CAT and GPx after the therapy in patients who did not achieve SVR compared to subjects who achieved SVR ($p < 0.001$ for all three enzymes). No significant changes in activities of SOD, GPx and CAT were recorded in the group of patients who did not achieve SVR after completion of the therapy ($p = 0.310$, $p = 0.717$, $p = 0.850$, respectively), in contrast to significant changes of SOD, GPx and CAT activities after the treatment in patients who achieved SVR ($p < 0.001$ for all) (Table 4).

Analysis of the genotypes with respect to achievement of SVR is demonstrated in Table 5. Genotypes 1, 2, 3 and 4 consisted of 27, 2, 20 and 4 patients, respectively. The number, as well as the percentage of patients who achieved SVR with genotypes 1, 2, 3 and 4, was 22 (81.5%), 2 (100%), 20 (100%), 4 (50%), respectively. On the basis of the genotypes, patients were divided into two groups, according to the duration of the therapy and the presumed response to the therapy. The first group consisted of genotypes 1 and 4, while the second group consisted of genotypes 2 and 3. Insufficient patient numbers for statistical analysis within genotypes 2 and 4 also favored this mode of division.

Discussion

Results of researches related to the antioxidant status of patients with CHC in general, and the ability to predict the outcome of therapy are very contradictory. There is little data in the scientific literature concerning the levels of activity of all three antioxidative enzymes (SOD, CAT, GPx) in patients with CHC. The results of measurements of these enzymes

Table 4

Antioxidant enzymes activities in regard to the chronic hepatitis C (CHC) therapy outcome

Enzyme	Therapy outcome HCV positivity (n = 7) (SVR not achieved) $\bar{x} \pm SD$	p*	HCV negativity (n = 46) (SVR achieved) $\bar{x} \pm SD$	p*	$p^{\dagger}$
SOD (U/gHb)					
baseline	503.89 ± 87.10	0.310	975.73 ± 297.92	< 0.001	< 0.001
after therapy	475.90 ± 99.33		1.288 ± 337.91		< 0.001
GPx (U/gHb)					
baseline	15.44 ± 0.89	2.717	23.56 ± 4.31	< 0.001	< 0.001
after therapy	15.52 ± 1.32		28.72 ± 5.45		< 0.001
CAT (U/gHbx10^4)					
baseline	4.34 ± 0.21	0.850	5.24 ± 0.82	< 0.001	< 0.001
after therapy	4.36 ± 0.29		6.27 ± 0.81		< 0.001

$\bar{x} \pm SD$ – arithmetic mean ± standard deviation.
***p – significance of difference within the group (baseline *vs* after therapy); $^{\dagger}p$ – significance of difference between groups (SVR not acthived *vs* achived); HCV – hepatitis C virus; SVR – sustained virological response; SOD – superoxide dismutase; GPx – glutathione peroxidase; CAT – catalase.**

Table 5

Genotypes and therapy outcome

Genotypes	Before therapy, ($\bar{x} \pm SD$)		After therapy, ($\bar{x} \pm SD$)	
	SVR achieved	SVR not achieved	SVR achieved	SVR not achieved
1 and 4	n = 24	n = 7	n = 24	n = 7
SOD (U/gHb)	887.96 ± 250.47*,**	503.89 ± 87.10	1,212.80 ± 326.39	608.76 ± 140.17
GPx (U/gHb)	22.51 ± 4.15*,**	15.44 ± 0.89	27.31 ± 5.15	15.52 ± 1.32
CAT (U/gHbx10^4)	5.13 ± 0.79*,**	4.34 ± 0.21	6.11 ± 0.75	4.36 ± 0.30
2 and 3	n = 22	n = 0	n = 22	n = 0
SOD (U/gHb)	1,071.49 ± 321.06**,***		1,393.20 ± 340.83****	
GPx (U/gHb)	24.72 ± 4.27**,***		30.06 ± 5.30****	
CAT (U/gHbx104)	5.37 ± 0.85**		6.40 ± 0.86	

$\bar{x} \pm SD$ – arithmetic mean ± standard deviation; $^{*}p < 0.05$ – difference between SVR achievers and SVR non-achievers before therapy among one group of genotypes; $^{**}p < 0.05$ – difference between SVR achievers before and after therapy among one group of genotypes; $^{***}p < 0.05$ – difference between SVR achievers before therapy comparing two groups of genotypes; $^{****}p < 0.05$ – difference between SVR achievers after therapy comparing two groups of genotypes; SVR – sustained virological response; SOD – superoxide dismutase; GPx – glutathione peroxidase; CAT - catalase.

activities before and after the treatment with PEG-IFN α-2a and RBV are even scarcer. There could be found extremely different research results, some indicating reduced and some revealing increased activities, with different explanations [15–17]. Patients with CHC have lower activities of antioxidative defense enzymes such as SOD, GPx, and CAT in erythrocytes and peripheral mononuclear cells in CHC patients [17–23], although increased activities of these enzymes are also described [24]. Some studies proved that HCV may impair antioxidant body systems and reduce the activities of antioxidative enzymes along with an observation that manganese superoxide dismutase (MnSOD) is one of the first therapy candidates for the reversion of fibrosis process. It is considered that this role is performed through the activation of nuclear factor kappa-B [25, 26].

According to some authors, during the replication of HCV, the amounts of MnSOD, heme oxygenase 1, CAT and GSH are increasing as an adaptive response to non-structural proteins of HCV. HCV multifunctional non-structural protein NS5A, essential for replication of HCV, induces an increase of MnSOD activity, which is mediated by the activation of activating protein-1 transcription factor by p38 mitogen-activated protein kinase and Jun N-terminal kinase signaling pathways [27, 28]. In contrast, there are researchers who claim that intracellular GSH status does not change during replication, and that the level of ROS is not high enough to initiate an adaptive increase in GSH [4]. However, oxidative damage is characterized by reduced levels of GSH, and upregulation of antioxidative enzymes such as CAT, GPx and SOD, according to most authors [11].

Direct measurement of the liver tissue revealed increased concentrations of ROS, and two- to five times decrease in the activity of antioxidative enzymes. The significant increase is also described in lymphocytes of patients with CHC [29]. It is important to note that the intensity of decrease of antioxidative enzymes activities in the liver tissue and leukocytes (above- mentioned study) and erythrocytes (our results) is equal. It is considered that treatment with PEG-IFN α-2a and RBV is directly responsible for the establishment of antioxidant response in patients with CHC [11]. It is considered that change in the oxidative-antioxidative balance plays a crucial role in the progression of liver injury, and interferon-alpha can be effective in the treatment of liver damage and improvement of oxidative system [17]. During *in vivo* studies on rats exposed to oxidative stress, Japanese scientists found that the IFN-α dose-dependently increases the levels of SOD and GPx and reduces levels of lipid peroxidation products in the liver of experimental animals [30].

A small number of researches that focus on the pathogenesis of CHC and oxidative stress show the results which directly indicate that disturbed oxidative balance initiates liver damage, and not *vice versa* [3]. Correlation analysis in our study showed that there was a mutual significant negative correlation between all three antioxidant enzyme activities to aminotransferases levels before the therapy where one can not conclude what the primary trigger of the disease is, and what is the consequence and intermediary progression element. Correlation analysis of the above- mentioned parameters after the treatment showed that there was no significant negative correlation between antioxidative enzymes and AST and ALT except GPx.

It speaks to the fact that disturbed antioxidant status may be representative of oxidative stress as the initiator of the pathogenesis CHC, and not the result of liver damage. Only GPx significantly negatively correlated with AST and ALT after the treatment ($p < 0.05$). On the other hand, the analysis of ROC curves singled this enzyme out as the most sensitive diagnostic marker (97.8%) in monitoring the success of therapy, perhaps because its reduced activity is a consequence of liver damage. As well, SOD and CAT could be considered as good diagnostic markers, albeit with lower specificity (93.5% and 71.7%, respectively).

Our findings also point to a positive relationship between the SVR and antioxidant status. Antioxidant enzyme activities were not significantly changed in patients who did not achieve SVR. For the time being, there are no published results of other studies to show whether there are differences in SOD, CAT and GPx levels in patients who did and who did not achieve SVR. However, there are results that did not find any change in the oxidative stress level among the mentioned groups of patients, though, antioxidant status was determined by the total antioxidant capacity rather than enzyme activities [31]. Our study participants who achieved SVR showed a statistically significant increase in GPx and SOD, CAT (activities $p < 0.001$). This fact shows that the baseline antioxidant status in patients with CHC could serve as a predictor of success or failure of combination antiviral therapy [32]. However, the difference in enzyme activities before and after the treatment showed a statistically significant increase between the patients who achieved SVR and the ones who did not achieve SVR. Since we singled out a group of patients who did not achieve SVR, we compared the activity of the antioxidant enzymes. It could be observed that SOD, CAT, and GPx activities were not significantly changed. In contrast, patients who achieved SVR, presented with a statistically significant change in the activity of antioxidant enzymes i.e. normalization of the activities. This speaks in favor of basic antioxidant enzyme activities as a possible factor predicting SVR [33, 34].

The most important prognostic factor for SVR achievement prior to the start of the therapy was the genotype of the virus. Genotypes 1 and 4 exhibited much lower SVR rates compared to genotypes 2 and 3. Examination of the relation between oxidative stress and HCV genotypes showed that more serious disease in HCV genetic subtype 1a/1b might be associated with more severe oxidative stress. Milder damage in subtypes 4, 2a/c, 2b and 3a could be related to lower oxidative response, respectively [35]. In our study, patients with genotypes 1 and 4 also had lower rates of SVR. Group of patients with genotypes 2 and 3 showed a statistically significant increase in both "baseline" and "after the therapy" SOD and GPx activities comparing to genotypes 1 and 4, as was expected ($p < 0.05$). Unexpectedly, there was no statistically significant difference in CAT activities between aforementioned groups, neither before nor after the treatment.

Conclusion

Oxidative stress present in patients with CHC is manifested by reduced activities of antioxidative enzymes. The combined antiviral therapy, which is lately considered to have antioxidant potential, leads to the restoration of antioxidant balance. Oxidative stress is the cause and not the consequence of the occurrence and development of CHC. Antioxidative enzymes (SOD, CAT, GPx) are good diagnostic markers of CHC treatment success. The results of this study, simultaneously considered together with the results of other authors may be the basis for the proposal of adding the antioxidative enzymes to the antiviral therapy, in order to achieve a better therapeutic response.

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Correlation between visual acuity, external limiting membrane and photoreceptor status in patients with neovascular age-related macular degeneration treated with bevacizumab

Korelacija između vidne oštrine, spoljašnje granične membrane i fotoreceptora kod bolesnika sa neovaskularnom senilnom degeneracijom žute mrlje lečenih bevacizumabom

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Abstract

Background/Aim. The integrity of outer retinal structures, primarily the photoreceptor layer, is important because of its direct correlation with visual acuity. The aim of this study was to investigate the correlation between best-corrected visual acuity (BCVA), the foveal photoreceptor-inner segment/outer segment (IS/OS) junction and external limiting membrane (ELM) in patients with neovascular age-related macular degeneration (NVAMD) after the treatment with bevacizumab, as well as the correlation between the above-mentioned parameters and different types of neovascular membrane, classified by fluorescein angiography (FA). **Methods.** The study included 82 patients with NVAMD, treated with intravitreal bevacizumab. All patients underwent a basic ophthalmological examination, FA and optical coherence tomography (OCT). Based on the results of FA, all the patients were divided into two main groups – type I (the occult and minimally classic) and type II (classic and predominantly classic) of the choroidal neovascular membrane (CNV). The OCT images revealed either the presence or the absence of IS/OS and ELM. **Results.** After the treatment, the mean best corrected visual acuity improved significantly in both groups ($p < 0.01$). Preserved IS/OS and ELM were registered in a smaller number of patients as compared to the condition before the treatment ($p < 0.01$). After the treatment, the mean BCVA was significantly better in patients with preserved IS/OS and ELM ($p < 0.01$). In addition, we registered a higher number of patients with preserved ELM in the first group than in the second group ($p < 0.01$), whereas there was no significant difference in the integrity of IS/OS between the groups ($p > 0.05$). **Conclusion.** The patients with preserved IS/OS and ELM achieved better final visual acuity as compared to the patients without preserved IS/OS and ELM. In our patients, the absence of IS/OS and ELM were more frequent in type II (classic and predominantly classic) CNV than in type I (the occult and minimally classic) CNV.

Key words:
retina; macular degeneration; neovascularization, pathologic; bevacizumab; tomography, optical coherence; fluorescein angiography.

Apstrakt

Uvod/Cilj. Integritet spoljašnjih struktura mrežnjače, primarno sloja fotoreceptora, važan je zbog njihove direktne povezanosti sa oštrinom vida. Cilj rada bio je da se ispita povezanost između najbolje korigovane vidne oštrine, fovealnih fotoreceptora i spoljašnje granične membrane kod bolesnika sa neovaskularnom senilnom degeneracijom žute mrlje nakon lečenja bevacizumabom, kao i odnos navedenih parametara sa različitim tipovima neovaskularne membrane klasifikovane metodom fluoresceinske angiografije. **Metode.** Istraživanjem su obuhvaćena 82 bolesnika sa neovaskular-

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nom senilnom degeneracijom žute mrlje lečena intravitealnom primenom bevacizumaba. Svim ispitanicima urađen je osnovni oftalmološki pregled, fluoresceinska angiografija i optička koherentna tomografija. Na osnovu nalaza fluoresceinske angiografije svi bolesnici podeljeni su u dve osnovne grupe, tip I (okultna i minimalno klasična) i tip II (klasična i predominantno klasična) horoidalne neovaskularne membrane. Pomoću optičke koherentne tomografije defisano je prisustvo ili odsustvo fotoreceptora i spoljašnje granične membrane. **Rezultati.** Prosečna najbolje korigovana vidna oštrina značajno se popravila po završetku lečenja u obe grupe ($p < 0,01$). Očuvan kontinuitet fotoreceptora i spoljašnje granične membrane registrovan je kod manjeg broja ispitanika u odnosu na stanje pre tretmana ($p < 0,01$). Prosečna najbolje korigovana vidna oštrina po završetku lečenja bila je značajno bolja kod ispitanika sa očuvanim fotoreceptorima i spoljašnjom graničnom membranom ($p < 0,01$). Takođe, registrovali smo više ispitanika sa očuvanim kontinuitetom spoljašnje granične membrane u prvoj u odnosu na drugu grupu ($p < 0,01$), dok se broj ispitanika sa očuvanim kontinuitetom fotoreceptora po završetku lečenja nije značajno razlikovao između grupa ($p > 0,05$). **Zaključak.** Kod bolesnika sa očuvanim fotoreceptorima i spoljašnjom graničnom membranom vidna oštrina nakon završetka lečenja bila je značajno bolja u odnosu na ispitanike kod kojih ove strukture nisu bile prisutne. Kod naših ispitanika odsustvo fotoreceptora i spoljašnje granične membrane bilo je češće kod tipa II (klasična i predominantno klasična) u odnosu na tip I (okultna i minimalno klasična) neovaskularne membrane.

Ključne reči:
retina; žuta mrlja, degeneracija; neovaskularizacija, patološka; bevacizumab; tomografija, optička, koherentna; angiografija, fluoresceinska.

Introduction

Choroidal neovascular membrane (CNV) is the main cause of severe visual impairment in patients with wet age-related macular degeneration (AMD). Vascular endothelial growth factor (VEGF) is one of the main factors responsible for the development of CNV. The drugs that block its activity, anti-VEGF drugs, have improved considerably the course of this disease and the patients' quality of life [1–3]. Today, they represent standard therapy for the treatment of neovascular AMD (NVAMD) [4, 5].

Bevacizumab (trade name Avastin®) is a monoclonal VEGF antibody, approved for intravenous use in the management of colorectal carcinoma. In ophthalmology, it is used off-label. The first papers related to the intravitreal administration of this drug in the treatment of NVAMD were published as early as in 2006 [6–8].

Optical coherence tomography (OCT) is a useful tool in the diagnosis and monitoring of AMD. After the introduction of a spectral-domain OCT (SD-OCT), which provides image resolution of up to 5 μm, it becomes possible to see clearly all retinal structures [9, 10]. The monitoring of CNV [presence of intraretinal fluid, subretinal fluid or fluid under the retinal pigment epithelium (RPE)] is important for the assessment of its activity. After the treatment, i.e. after the fluid has retreated, a subretinal fibrosis or atrophy can occur, which consequently influences the status of inner segment/outer segment (IS/OS) and external limiting membrane [11].

The monitoring of the integrity of external retinal layers, primarily the photoreceptor layer, is important because of its direct correlation with visual acuity [12–15]. In some studies, the IS/OS line on the SD-OCT images was reportedly a good indicator for predicting best-corrected visual acuity (BCVA) in NVAMD patients treated with anti-VEGF therapy [16]. ELM status is another useful parameter during the evaluation of retinal morphology and function in patients with NVAMD [17].

The aim of this study was to investigate the correlation between BCVA, IS/OS and ELM in patients with NVAMD after the treatment with bevacizumab, as well as the correlation between the above-mentioned parameters and different types of neovascular membrane, classified by fluorescein angiography (FA).

Methods

This clinical, cohort, prospective, non-randomized study was conducted at the Military Medical Academy, Belgrade, Serbia between February 2013 and February 2015. The protocol of this study was approved by the Ethical Committee of the Military Medical Academy. The patients were informed about the off-label use of bevacizumab.

The study involved 105 patients in total. Out of that number, 23 patients were excluded due to insufficient documentation. Here we present 82 patients, in whom the fluid retreated after the sixth dose of the medication, i.e. the CNV activity decreased. CNV was considered active if contrast leakage was seen on FA and if OCT detected an increased and/or persistent presence of macular fluid. As there is no precise dosing protocol for this medication, based on our experience and after consulting literature, we opted for six-monthly doses.

All patients were over the age of 65 and were suffering from AMD-related subfoveolar CNV which had not been treated previously. To qualify for the study, the patients had to have the mean BCVA of 0.05 or higher (Snellen chart). The patients did not have acute or chronic eye inflammations, other fundus changes or decompensated glaucoma. The presence of early-stage/cataract or pseudophakia were not a reason for the exclusion from the study.

At the beginning, each patient underwent the following: complete ophthalmological examination, BCVA assessment, FA on Topcon Trc-NW7SF fundus camera and OCT on Topcon 1000-SD OCTTOp 1000-T3D3. Based on the FA results, the patients were divided into two main groups, depending on whether they had Type I CNV (the occult and minimally classic) or Type II CNV (classic and predominantly classic) membrane. Each group consisted of

41 patients. Using OCT scans we detected the presence or the absence of ELM and IS/OS. This analysis was conducted by two ophthalmologists independently and in the case of inconsistencies in the findings, it was supervised by the mentor. Structural changes of CNV seen on OCT were analyzed in the subfoveal area of 1 mm. The OCT findings were analyzed immediately before the administration of each dose of the medication. In cases where macular fluid disappeared completely before the sixth dose of the medication was given, we interrupted the treatment and such patients were excluded from the study.

All the patients received 1.25 mg of bevacizumab (0.05 mL of the commercial phial of Avastin®) intravitreally. The control assessments were carried out on the first, seventh and thirtieth days following the intervention. One month after administration of the first dose, the next dose was administered. A total of six doses were administered in one-month intervals (+-2).

Statistical analysis was performed before and after the therapy, using SPSS version 19.0 (SPSS., Chicago, IL, USA).

Distribution of variables was assessed by Kolmogorov-Smirnov test and it was concluded that BCVA-related data should be analyzed using non-parametric statistics. The values within a group were analyzed by Friedman's test. For the comparison of BCVAs between the groups, we used the Mann-Whitney U-test.

Results

The patients [82 patients (eyes), 41 in each group] were between 65 and 92 years old. The average age was 77.2 years in the first group and 77.8 years in the second, one. The mean initial BCVA was 0.19 in the first group, and 0.14 in the second one. After the therapy, the mean BCVA was 0.42 in the first, and 0.30 in the second group ($p < 0.01$) (Table 1).

Before the therapy, preserved ELM was registered in all patients (100%), in both groups. In the first group, after the sixth dose, ELM was preserved in 26 patients (63.4%) and in the second one in 11 (26.8%) patients. Before the treatment, the IS/OS was preserved in 39 (95.2%) patients from the first group, and in 35 (85.4%) patients in the second group. After the treatment, the number of patients with preserved IS/OS in the first group decreased to 28 (68.3%) and to 22 (53.4%) in the second group (Tables 2).

In the first group, in patients in whom ELM was preserved, the mean BCVA was 0.19. After the treatment, ELM was preserved in 26 patients and their mean BCVA was 0.53 ($p < 0.01$). After the treatment, in 15 patients in whom ELM was not preserved, the mean BCVA was 0.23 (0.19–0.23) ($p < 0.05$). In the first group, IS/OS were preserved in 39 patients before the treatment, and their mean BCVA was 0.15. After the treatment, IS/OS was preserved in 28 patients, with mean BCVA of 0.39 ($p < 0.01$). In two patients without preserved IS/OS before the therapy the mean BCVA was 0.10, and after the therapy, IS/OS were absent in 13 patients with mean BCVA 0.18 ($p > 0.05$) (Table 3).

In the second group, in patients in whom ELM was preserved, the mean BCVA was 0.14. After the treatment, ELM was preserved in 11 patients and their mean BCVA was 0.46 ($p < 0.01$). In 30 patients in whom ELM was not preserved, after the treatment the mean BCVA was 0.24 ($p < 0.05$). In the second group, 35 patients had complete IS/OS before the treatment, and their mean BCVA was 0.22. After the treatment, IS/OS were preserved in 22 patients, with mean BCVA of 0.33 ($p < 0.05$). In six patients without preserved IS/OS before the therapy the mean BCVA was 0.15, and after the therapy IS/OS were absent in 19 patients with mean BCVA 0.22 ($p < 0.05$) (Table 3).

Table 1

Best-corrected visual acuity (BCVA) before and after the administration of bevacizumab

Group	BCVA		*p*-value
	pre-treatment	post-treatment	
I	0.19	0.42	< 0.01
II	0.14	0.30	

Group I – occult and minimally classic membrane; Group II – classic and predominantly classic membrane.

Table 2

Integrity of external limiting membrane (ELM) and inner segment/outer segment (IS/OS) in bevacizumab-treated patients according to groups

Parameter	Group I, n (%)		Group II, n (%)	
	pre-treatment	post-treatment	pre-treatment	post-treatment
ELM				
present	41 (100)	26 (63.4)	41 (100)	11 (26.8)
absent	–	15 (36.5)	–	30 (73.2)
IS/OS				
present	39 (95.2)	28 (68.3)	35 (85.4)	22 (53.4)
absent	2 (4.9)	13 (31.7)	6 (14.6)	19 (46.6)

Group I – occult and minimally classic membrane; Group II – classic and predominantly classic membrane.

Table 3
Best-corrected visual acuity (BCVA) according to external limiting membrane (ELM) and inner segment/outer segment (IS/OS) in the group I and the group II

Groups	BCVA	
	pre-treatment	post-treatment
Group I		
ELM		
present	0.19	0.53
absent	/	0.23
IS/OS		
present	0.15	0.39
absent	0.10	0.18
Group II		
ELM		
present	0.14	0.46
absent	/	0.24
IS/OS		
present	0.22	0.33
absent	0.15	0.22

Group I – occult and minimally classic membrane;
Group II – classic and predominantly classic membrane.

The results of our study showed that after the treatment there were 26 patients with preserved ELM in the first group (the occult and minimally classic CNV), as compared to 11 patients in the second group (classic and predominantly classic) ($p < 0.01$). After the treatment, IS/OS were preserved in 28 patients in the first group and in 22 patients in the second group ($p > 0.05$) (Table 1).

Discussion

The results of our study confirmed that the visual acuity in patients with wet AMD was significantly improved after the intravitreal administration of bevacizumab, as previous studies had demonstrated [1–3, 6–8].

There are several types of anti-VEGF medications, registered for intravitreal administration. Since the study by Martin al. [3] CATT showed that ranibizumab is not superior to bevacizumab in terms of their effects, and, as bevacizumab is significantly cheaper, we decided to use bevacizumab.

The protocol for the administration of anti-VEGF drugs has not been precisely established yet. In the studies such as ones by Brown et al. [1] (ANCHOR) and Rosenfeld et al. [2] (Marina), this medication was administered monthly. Some studies dealt with treat-and-extend dosing regimens, whereas in other studies, the dosing regimen was *pro re nata* (PRN) – treat and observe [5]. Based on our previous experience, we decided to apply a monthly dosing regimen.

The correlation between the type of neovascular membrane (identified by FA), and its structural characteristics (identified by OCT) was described by Freund et al [18]. The final visual acuity in patients with NVAMD is influenced by various changes in the macula (subretinal, intraretinal or fluid under the RPE) [11, 19]. The drug itself has no effect either on ELM or on IS/OS, but after the treatment is over, and after the fluid retreats, the integrity of these parameters influences the final visual acuity, which was described by many authors [14, 15, 20].

A correlation between visual acuity, ELM and IS/OS integrity in the treatment of neovascular AMD with photodynamic therapy was described as early as in 2010 by Oishi et al. [15], and was also mentioned by Sayanagi et al. [16], but subsequent to anti-VEGF therapy. The importance of these structures in the preservation of vision in patients with uveitic macular oedema was described by Tortorela et al. [14].

Sayanagi et al. [16] confirm the importance of IS/OS status in patients who were given anti-VEGF therapy and conclude that IS/OS is a good indicator in terms of prognosis for visual acuity following an anti-VEGF therapy.

Shin et al. [20] conclude that the integrity of foveal photoreceptors is strongly correlated with final visual acuity after the treatment in patients with NVAMD.

Kwon et al. [13] conclude that IS/OS and ELM can be good predictors of visual acuity after anti-VEGF therapy.

Upon the analysis of our data, we noted that the presence of ELM and IS/OS correlate with better visual acuity, both before and after the therapy. A significant role of ELM and IS/OS, as important prognostic factors for final BCVA, was also confirmed by Mathew et al [19].

Our study showed the significant improvement of BCVA in patients with preserved ELM and IS/OS in both groups, whereas in patients with not preserved ELM and IS/OS, the BCVA was lower (there was an improvement, but the difference was not statistically significant).

The number of patients with preserved IS/OS and ELM decreased after the treatment, especially in the second group, where the number of patients with preserved ELM after the therapy was considerably lower. This means that there were more patients with preserved ELM and IS/OS in the first group (the occult and minimally classic CNV) than in the second group (classic and predominantly classic CNV). The literature offers diverse findings regarding this issue. Bloch et al. [21] in their study obtained the findings similar to ours, while Freund et al. [18] in their study actually argue that the response to anti-VEGF therapy is better in patients from the second group (classic and predominantly classic CNV).

The studies, such as ANCHOR [1] and MARINA [2], showed that, regardless of the type of neovascular membra-

ne, classified by FA method, the vision would improve after the anti-VEGF therapy. We confirmed such findings, as well as that visual acuity is considerably better if IS/OS and ELM are preserved at the end of the treatment.

Conclusion

The presence of ELM and IS/OS is an important prognostic factor for final visual acuity in patients with NVAMD. The absence of ELM and IS/OS is more frequent in type II CNV (classic and predominantly classic) than in type I CNV (the occult and minimally classic) and this is the reason why these patients can be expected to have somewhat lower final visual acuity after the anti-VEGF therapy.

The development of modern technology provides an even more precise insight into the structure of the neovascular membrane, so future analyses will be able to define even more precisely its various structural parameters and open new approaches to treating these patients.

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Calculation of electromagnetic field from mobile phone induced in the pituitary gland of children head model

Izračunavanje elektromagnetnog polja mobilnog telefona unutar hipofize na modelu glave deteta

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Abstract

Background/Aim. A mobile phone is a source of electromagnetic radiation located close to the head and consequently its intense use may cause harmful effects particularly in younger population. The aim of this study was to investigate the influence of electromagnetic field of the mobile phone on the pituitary gland of the child. **Methods**. In order to obtain the more accurate results for this research 3D realistic model of child's head whose size corresponds to an average child (7 years old) was created. Electric field distribution in child head model and values of Specific Absorption Rate (SAR) at the region of pituitary gland were determined. This study was performed for the frequencies of 900 MHz, 1800 MHz, and 2100 MHz, as the most commonly used in mobile communications. The special attention was dedicated to the values of the electric field and the values of the SAR in the pituitary gland. For all frequencies over 10 g and 1 g of tissue average SAR was calculated. The electric field distribution and values of average SAR for 10 g and 1 g trough the model of child's head were obtained by the using numerical calculation based on the Finite Integration Technique (FIT). **Results**. The largest value of electric field in the region of the pituitary gland was at the frequency of 900 MHz, as a consequence of the highest penetration depth. Lower values of the electric field in the region of the pituitary gland were at frequencies of 1,800 MHz and 2,100 MHz. The SAR in the pituitary gland decreased as the frequency increased as a direct consequence of lower penetration depth. **Conclusion**. The electric field strength from a mobile phone is higher than the value specified by standards for the maximum allowable exposure limits. The high values of the electric field are not only in the vicinity of a mobile phone but also in tissues and organs of the human head. Particular attention should be paid to the exposure of children to radiation of mobile phones. Smaller dimensions of children's head and smaller thickness of tissues and organs have as a consequence greater penetration of electromagnetic waves.

Key words:
cellular phone; electromagnetic fields; child; models, theoretical; pituitary hormones.

Apstrakt

Uvod/Cilj. Mobilni telefon je izvor elektromagnetnog zračenja u blizini glave i zbog toga njegova preterana upotreba može prouzrokovati štetne efekte osobito kod mlađe populacije. Cilj ovog rada bio je da istraži uticaj električnog polja mobilnog telefona kao izvora elektromagnetnog zračenja na hipofizu deteta. **Metode**. U cilju dobijanja što tačnijih rezultata napravljen je realan 3D model glave deteta čije dimenzije odgovaraju dimenzijama deteta od 7 godina. Određena je raspodela električnog polja unutar modela glave deteta i vrednosti specifične količine apsorbovane energije *Specific Absorption Rate* (SAR) u predelu hipofize. Ovo istraživanje izvršeno je za frekvencije od 900 MHz, 1 800 MHz i 2 100 MHz kao najčešće korišćene frekvencije u mobilnom komunikacionom sistemu. Posebna pažnja bila je posvećena vrednosti električnog polja i vrednosti SAR u hipofizi. Za sve pomenute frekvencije proračunat je usrednjeni SAR za 10 g i 1 g tkiva. Raspodela električnog polja i vrednosti usrednjenog SAR za 10 g i 1 g dobijene su korišćenjem numeričkog metoda koji je zasnovan na tehnici konačnih integrala (FIT). **Rezultati**. Najveća vrednost električnog polja u hipofizi bila je na frekvenciji od 900 MHz zbog veće dubine prodiranja. Za frekvencije od 1 800 MHz i 2 100 MHz vrednosti električnog polja u hipofizi bile su manje. Vrednosti SAR u hipofizi su se smanjivale kako je frekvencija rasla što je direktna posledica manje dubine prodiranja. **Zaključak**. Vrednosti električnog polja koje su posledica zračenja mobilnog telefona veće su od maksimalnih graničnih vrednosti koje su propisane standardima. Velike vrednosti električnog polja nisu samo u okolini telefona, već i u organima i tkivima ljudske glave. Posebnu pažnju treba obratiti na izlaganje dece zračenju mobilnih telefona. Manje dimenzije dečije glave kao i manja debljina tkiva i organa za posledicu ima veću dubinu prodiranja elektromagnetnih talasa.

Ključne reči:
mobilni telefon; elektromagnetna polja; deca; modeli, teorijski; hipofiza, hormoni.

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Introduction

The wide variety of available options of mobile phones such as games, the internet, calls and video calls, as well as their accessible prices, have led to the daily use of mobile phones mostly in the younger population (children) which can be measured in hours. The mobile phone is a source of electromagnetic radiation which is located close to the head and because of that, the intense use of mobile phones in the younger population causes concern for health effects.

The influence of electromagnetic field from a source of electromagnetic radiation such as mobile phone close to child's head is bigger than an influence on the adult head. This is due to smaller dimension of child's head and consequently thinner pinnae and skulls. Because of that in the case of child's head, the source of electromagnetic radiation is closer to the brain and pituitary gland than in the case of adults head. Relevant data show that the exposure of children to electromagnetic radiation is higher than adult exposure [1, 2].

Because of ethical considerations, human exposure to electromagnetic fields in experimental purposes is limited. Due to this, it is much more convenient to develop a realistic model of the human head by using numerical simulation [3]. Numerical analyses of the human head exposed to electromagnetic radiation of mobile phones provide useful information about absorbed electromagnetic energy under different conditions of exposure. The International Agency for Research on Cancer (IARC) has classified the radiation of electromagnetic fields in 2B group as possibly carcinogenic to human, based on an increased risk of a malignant type of brain cancer. In this category, there is a limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals [4].

The most important indicator when considering the health risk as a result of the effect of electromagnetic fields is the Specific Absorption Rate (SAR). SAR is directly dependent on the electromagnetic properties of biological tissues exposed to the effects of electromagnetic waves and can be defined as (Equation 1):

$$\mathrm{SAR} = \frac{\sigma |E|^2}{\rho}$$

where E is the maximum value of the internal electric field, σ is the conductivity of the tissue and ρ is the density of the tissue. Maximum values of SAR which must not exceed, are defined in Regulation of the limits of exposure to non-ionizing radiation, Official Gazette of the Republic of Serbia, no. 36/09. This regulation defines the limits of exposure to non-ionizing radiation, or the basic restrictions and reference boundary levels of the population exposure to electric, magnetic and electromagnetic fields of different frequencies [5].

In this research, the level of electric field strength due to mobile phone and SAR values at the region of pituitary gland were determined.

The pituitary is a small gland which has a diameter of around 1cm and a weight of about 0.5–1 g, and it is located at the base of the brain. Physiologically it can be divided into two parts: the front part called the adenohypophysis and the rear part called neurohypophysis. Adenohypophysis secretes six very important hormones, which have a very significant role in the control of metabolic functions of the whole organism such as prolactin, growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH). The main role of rear part or neurohypophysis is to store antidiuretic hormone (ADH) and oxytocin. Hormones from the hypothalamus almost completely regulate the secretion of pituitary hormones which are delivered through the bloodstream to the pituitary gland.

As shown in one study [6], exposure to 900 MHz of global system for mobile communication radio frequency (GSM RF) on pituitary hormone levels in healthy males such as: TSH, GH, prolactin and ACTA, led to significant decreases of concentrations of GH and cortisol for about 28% and 12%, respectively.

Due to a higher level of electromagnetic radiation within the child's head compared to that found inside adult's head, the electric field can be one of the causes of serious biological effects on the pituitary gland. Because of harmful effect previously mentioned on the pituitary gland and consequently on the concentration of GH which is essential for normal growth of the child, in this paper special attention is devoted to numerical calculation and distribution of electrical field and SAR in the region of this gland for 7-years old child. Also in this study, an overview of the possible biological effects that may occur in pituitary gland due to exposure to electromagnetic fields is presented.

Methods

In order to obtain more accurate results for this research 3D realistic model of child's head whose size corresponds to an average child (7 years old) had to be created [7, 8]. This model of child's head consisted of following tissues and organs: skin, fat, muscle, skull, jaw with teeth, tongue, eyes, vertebrae, cartilage, spinal cord, cerebrospinal fluid, brain, and pituitary gland.

All of these tissues and organs had to be described by adequate electromagnetic parameters such as electric conductivity, permittivity, heat capacity, density and thermal conductivity [9]. These electromagnetic characteristics vary with frequency and their values for frequencies of 900 MHz, 1,800 MHz, and 2,100 MHz), as the most often used in mobile communication, are shown in Table 1.

Modeling of 3D child's head model was performed in two stages. First external look for every tissues and organs was created in 3D Max Studio [10]. The second step was creating a full model with actual tissues and organs and connecting certain electromagnetic properties with corresponding tissues and organs by using software package CST Microwave Studio [11]. The same software was used for simulation of the electromagnetic field and its influence on child's head. Numerical calculation method used in this software was based on the Finite Integration Technique [12].

External look, horizontal and vertical cross-sections of actual tissues and organs are shown in Figures 1 and 2 with organs numbered according to their numbers in Table 1.

Table 1

Average values of electromagnetic properties of tissues and organs at frequencies of 900 MHz, 1,800 MHz and 2,000 MHz

Tissue/Organ	ε_r	σ (S/m)	ρ* (kg/m^3)	Heat* capacity (kJ/kgK)	Thermal conductivity (W/m°C)
1 – Cortical Bones	12.45[a]	0.143[a]	1,908	1.313	0.32
	11.8[b]	0.275[b]	1,908	1.313	0,32
	11.6[c]	0.328[c]	1,908	1.313	0.32
2 – Brain	45.805[a]	0.7665[a]	1,046	3.630	0.51
	46.1[b]	1.710[b]	1,046	3.630	0.51
	45.50[c]	1.880[c]	1,046	3.630	0.51
3 – Cerebrospinal Fluid	68.60[a]	2.410[a]	1,007	4.096	0.57
	67.2[b]	2.920[b]	1,007	4.096	0.57
	66.80[c]	3.150[c]	1,007	4.096	0.57
4 – Fat	11.30[a]	0.109[a]	911	2.348	0.21
	11.0[b]	0.190[b]	911	2.348	0.21
	10.90[c]	0.224[c]	911	2.348	0.21
5 – Cartilage	42.70[a]	0.782[a]	1,100	3.568	0.49
	40.2[b]	1.290[b]	1,100	3.568	0.49
	39.50[c]	1.490[c]	1,100	3.568	0.49
6 – Pituitary Gland	59.70[a]	1.040[a]	1,053	3.687	0.51
	58.1[b]	1.500[b]	1,053	3.687	0.51
	57.70[c]	1.700[c]	1,053	3,687	0,51
7 – Spinal Cord	32.50[a]	0.574[a]	1,075	3.630	0.51
	30.9[b]	0.843[b]	1,075	3.630	0.51
	30.50[c]	0.951[c]	1,075	3.630	0.51
8 – Muscle	55.00[a]	0.943[a]	1,090	3.421	0.49
	53.5[b]	1.340[b]	1,090	3.421	0.49
	53,20	1.510	1,090	3,421	0,49
9 – Eyes*	49.60[a]	0.994[a]	1,052	3.615	0.53
	46.3[b]	1.369[b]	1,052	3.100	0.50
	47.88[c]	1.530[c]	1,052	3.043	0.50
10 – Skin	41.40[a]	0.867[a]	1,109	3.391	0.37
	38.9[b]	1.180[b]	1,109	3.391	0.37
	38.40[c]	1.310[c]	1,109	3.391	0.37
11 – Tongue	55.30[a]	0.936[a]	1,090	3.421	0.49
	53.6[b]	1.370[b]	1,090	3.421	0.49
	53.10[c]	1.560[c]	1,090	3.421	0.49
12 – Teeth	12.50[a]	0.143[a]	2,180	1.255	0.59
	11.8[b]	0.275[b]	2,180	1.255	0.59
	11.60[c]	0.328[c]	2,180	1.255	0.59

***values are the same for all frequencies.**

ε_r – permittivity, σ – electric conductivity, ρ – density

[a] – value at frequency of 900 Hz

[b] – value at frequency of 1,800 Hz

[c] – value at frequency of 2,100 Hz

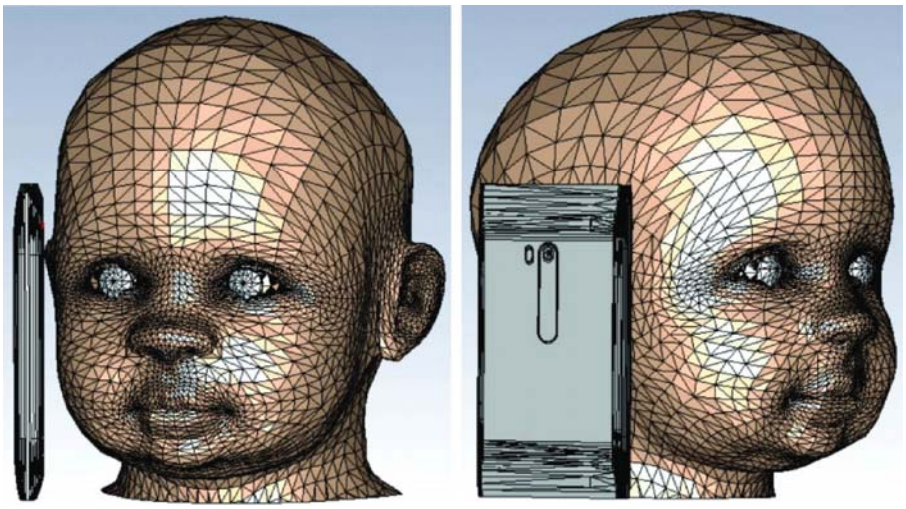

Fig. 1 – External look of the child's head model.

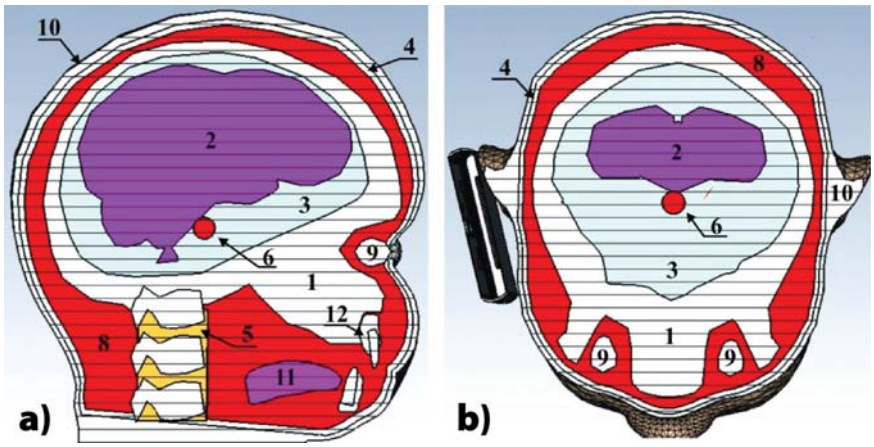

Fig. 2 – a) Vertical and b) Horizontal cross-section of the child's head model. 1 – cortical bones, 2 – brain, 3 – cerebrospinal fluid, 4 – fat, 5 – cartilage, 6 - pituitary gland, 8 – muscle, 9 – eyes, 10 – skin, 11 – tongue, 12 – teeth (the numbers are the same as in Table 1).

In this study actual smartphone (Figure 1) was used as a source of electromagnetic radiation. The mobile phone consisted of following parts: planar inverted F antenna (PIFA), display and mobile housing. The planar inverted F antenna (PIFA) as a source of electromagnetic radiation was modeled for three different frequencies: 900 MHz, 1,800 MHz, and 2,100 MHz, with reference power of $P = 1$W [13] and impedance of $Z = 50\ \Omega$.

The numerical calculation was performed for open space (reflected electromagnetic waves and the other sources of electromagnetic radiation were not taken into consideration). The only source of electromagnetic radiation in this simulation was a mobile phone with an output power of 1W, defined according to the Standard of the Institute of Electrical and Electronics Engineers (IEEE) C.95.3 [13].

Results

The penetration depth of the electric field was the largest in the case of the electric field at a frequency of 900 MHz (Figure 3a). On the other hand, the penetration depth of the electric field at the higher frequencies was smaller resulting in a stronger electric field in tissues that are close to the source of electromagnetic radiation such as mobile phone (Figures 3b and 3c). The peak of the electric field in the pituitary gland at the frequency of 2,100 MHz was less than those at the frequencies of 1,800 MHz and 900 MHz (0.3045 V/m, 0.8643 V/m, and 5.6615 V/m, respectively) (Figure 4).

SAR values averaged over 1 g and 10 g of the tissue for all three frequencies used in this study are presented in Figures 5 and 6, respectively.

Because the penetration depth of electric field was the largest in case of the frequency of 900 MHz (Figure 6), SAR values in the region of the pituitary gland was the highest for this frequency and amounted SAR1g = 0.08521 W/kg (Figure 5) and SAR10g = 0.10325 W/kg (Figure 6).

Discussion

In this study, the electric field distribution within the child`s head model was investigated for different frequencies

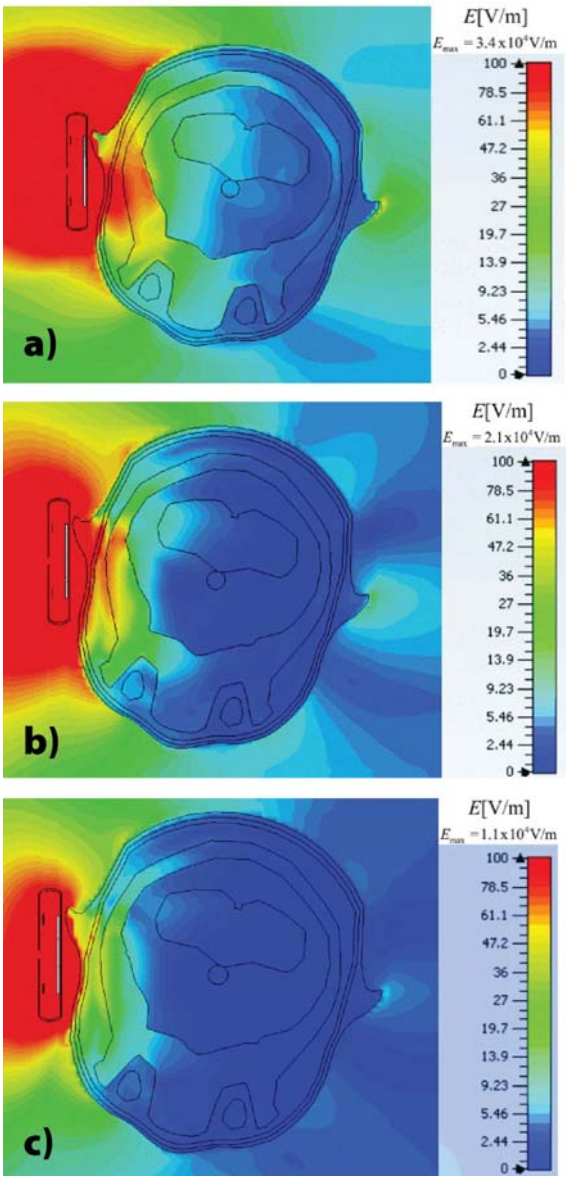

Fig. 3 – Electric field distribution within the child's head model for frequency of a) 900 MHz, b) 1,800 MHz, and c) 2,100 MHz.

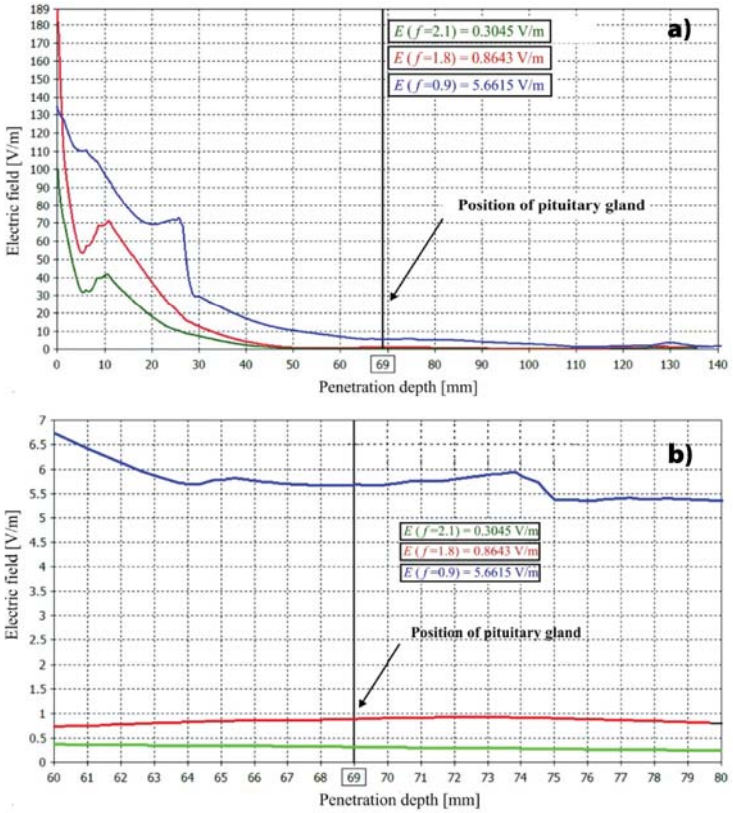

Fig. 4 – Penetration depth of electric field through the child's head model for three different frequencies (f). a) from 0 mm to 140 mm; b) from 60 mm to 80 mm.

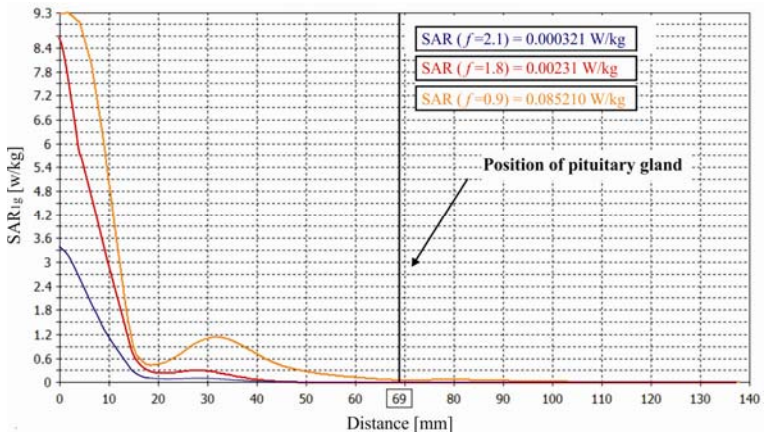

Fig. 5 – Comparative analysis of specific absorption rate (SAR_{1g}) for different frequencies (f) through the child's head model.

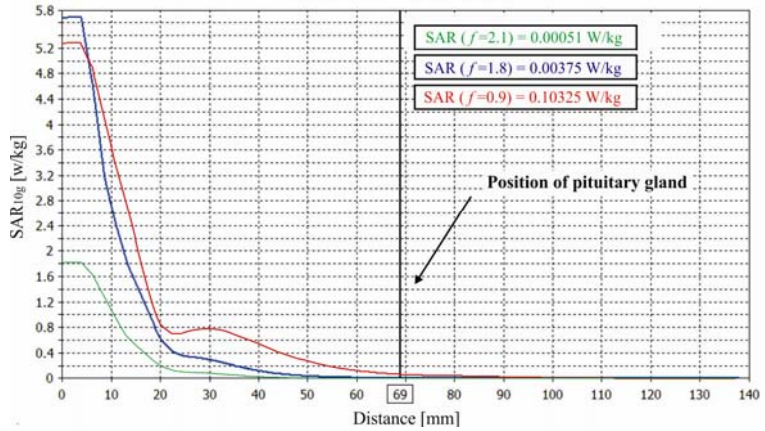

Fig. 6 – Comparative analysis of specific absorption rate (SAR_{10g}) for different frequencies (f) through the child's head model.

of a mobile phone. Our results showed that the penetration depth of the electric field was the largest at a frequency of 900 MHz and decreased at higher frequencies resulting in a stronger electric field in tissue that was close to the source of electromagnetic radiation like a mobile phone. The wavelength of electromagnetic waves has an impact on penetration depth because it varies for different frequencies and it has to be taken into consideration that electromagnetic properties are different for different biological tissues [14].

Generally, the one part of the energy due to the propagation of electromagnetic waves penetrates into certain biological object and it is being absorbed. Differences of wave energy at the boundaries of a biological object (the input energy and output energy) represent the absorbed energy. Because of the need to precisely introduce this absorbed energy it was defined as the term Specific Absorption Rate - SAR. The values of the SAR are different for different tissues and electromagnetic waves of different characteristics, in this case, different frequencies. It can be said that SAR characterizes the interaction of electromagnetic fields with biological tissue. SAR is related to a certain point as an extremely small area in a biological tissue in which the electromagnetic field can be considered as homogeneous one. More practical value is the average SAR as the ratio of the absorbed power in the body and the body mass of biological entity.

For this research, values for the average SAR over 10 g and 1 g of tissue were calculated. Because of different frequencies values of SAR varied. Accordingly, the penetration depth of the electric field was the largest in case of a frequency of 900 MHz, and SAR values in the region of the pituitary gland were the highest for this frequency

The results obtained in this paper are compared with those obtained in the study of Krstić et al. [15]. After comparison, it can be concluded that the value of SAR in the region of pituitary gland for child`s head model used in this research, is greater than SAR in a model of adult person`s head with few layers in that study [15]. SAR in a case of child`s head model is almost five times greater than results in a case of adult`s head model. This is expected due to different characteristics of the model: size, different thickness of layers and therefore the pituitary gland was at greater distances from the source in case of adult person`s head model.

There are many differences among different countries in Europe in terms of upper limits for RF radiation from GSM mobile telephony. Based on Recommendation 1999/519/EC, the limit values which are prescribed for the electric field strength for the following frequencies: 900 MHz, 1,800 MHz, and 2,100 MHz, are 41 V/m, 58 V/m i 61 V/m, respectively[16]. The certain European governments have adopted lower values such as for example Greece (32 V/m, 45 V/m and 47 V/m), Belgium (21 V/m, 29 V/m and 31 V/m), Serbia (16.5 V/m, 23.3 V/m and 24.4 V/m), Slovenia (13 V/m, 18 V/m and 19 V/m), Poland (7 V/m, 7 V/m and 7 V/m), Italy (6 V/m, 6 V/m and 6 V/m), Switzerland (4 V/m, 6 V/m and 6 V/m), etc. for all three frequencies, respectively [17].

The values for the maximum field strength that are prescribed by the standard are given for free space in the absence of people. We have to keep in mind that the values for field strength inside the biological tissues or organs are lower because of the propagation through the material environment and due to the increasing distance from the radiation source.

If the value of the field is known inside biological tissue then, based on the boundary conditions at the surface of the two separate areas, the value of the incident field can be evaluated. Since the value of the electric field in the pituitary gland is known, based on the relationship that is valid for the normal vector components of the electric field at the separate area, the value of the field before the penetration of EM waves in the pituitary gland can be determined.

Based on the value of the dielectric constant and conductivity of air and pituitary gland for different frequencies, the ratio of electric fields strengths at the separate surface of these two areas can be approximately determined. This is certainly the worst case, from the standpoint of the electric field strength, because in this case it does not take into consideration the impact of other layers on the weakening of electromagnetic wave that spreads from the radiation source. However, this approach can give us information about the minimum field strength to which man is exposed, and if it is greater than allowed.

The ratio of normal vector components of electric field at the crossover semiconductor environment is determined from the expression

$$\frac{\underline{E}_1}{\underline{E}_2} = \frac{\sigma_2 + j\omega\varepsilon_2}{\sigma_1 + j\omega\varepsilon_1} \quad (2)$$

For different frequencies and the corresponding values for the dielectric constant and conductivity of the air and the pituitary gland, the ratio of the normal components of the electric field for these two environments is calculated by using previously formula (2) and results are:

$$\left|\underline{E}_{\text{air}} / \underline{E}_{\text{p. gland}}\right\|_{f=0.9\text{GHz}} \approx 63 \text{ V/m},$$

$$\left|\underline{E}_{\text{air}} / \underline{E}_{\text{p. gland}}\right\|_{f=1.8\text{GHz}} \approx 60 \text{ V/m, and}$$

$$\left|\underline{E}_{\text{air}} / \underline{E}_{\text{p. gland}}\right\|_{f=2.1\text{GHz}} \approx 59.5 \text{ V/m.}$$

Based on the previously obtained values for the electric field strength in the pituitary gland, the values of the electric field strength in the air are:

$$\underline{E}_{\text{air}}\big|_{f=0.9\text{GHz}} \approx 356.67\,\text{V/m}$$

$$\underline{E}_{\text{air}}\big|_{f=1.8\text{GHz}} \approx 51.86\,\text{V/m} \text{ , and}$$

$$\underline{E}_{\text{air}}\big|_{f=2.1\text{GHz}} \approx 18.12\,\text{V/m} .$$

If we compare this value with the maximum permissible values specified in the above mentioned countries, we can conclude that they are considerably higher or in range with the maximum allowable ones. Of course, if we take into account the impact of all other layers between the radiation source and pituitary gland obtained values would have been far greater.

Pituitary gland as one of the most important glands of the endocrine system, *via* ACTH has an impact on the cortex

of the adrenal gland. In this way, it stimulates the secretion of steroid hormones.

Some investigations revealed that stimulation of the adrenal axis by electromagnetic radiation from a mobile phone in rats has as a consequence general hyperthermia. In animals exposed to high levels of electric fields, stimulation of the hypothalamic-hypophysial-adrenocortical (HHA) axis was found, mediated by the central nervous system (CNS) [18].

Another very important function of the pituitary gland is that it secretes gonadotropins FSH and LH that regulate testicular spermatogenesis and steroidogenesis. The impact of a mobile phone radiation on gonadotropins level has been considered in man and animals. The results of some studies have shown that a mobile phone radiation cannot cause significant biological effects. But there is a possibility that the time of exposure to radiation from a mobile phone in these studies was not long enough to show some significant biological effects [19–20].

Research conducted by Fang et al. [19] showed progressive histological derangement in rat pituitary glands. These derangements were manifested in the form of swollen mitochondria as well as dilatation of Golgi complex and diffusive lysosomes. Also, this research revealed that with increasing duration of exposure and electromagnetic wave energy this disorder increased. For instance, it has been observed also and mitochondrial vacuolization, the formation of myelin figures, distinct dilatation of endoplasmic reticulum, the occurrence of numerous secondary lysosomes, and clustering of heterochromatin under the nuclear membranes[19].

In the study of Eskander et al. [21], it was shown that people living a long period of time in the vicinity of base stations have a significant reduction of the release into the blood of a number of hormones, including ACTH which is produced and secreted by the anterior pituitary gland [21]. The highly significant decrease of serum cortisol levels in people exposed to electromagnetic radiation was also found.

Conclusion

The penetration depth of the electric field is the largest at the frequencies of 900 MHz and decreases, at the higher frequencies, resulting in a stronger electric field in the tissues that are close to the source of electromagnetic radiation (mobile phone).

Results obtained by numerical analysis show that the electric field at the frequency of 900 MHz has the greatest impact on the pituitary gland, which is a consequence of the highest penetration depth as mentioned before.

This level of radiation may cause substantial harmful health effect in children having in mind our study results that the level of electric field strength inside pituitary gland is higher than the values for the maximum field strength specified by the standard.

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Endothelin-1 and nitric oxide in 3-year prognosis after acute myocardial infarction

Endotelin-1 i azot-monoksid u trogodišnjoj prognozi nakon akutnog infarkta miokarda

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Abstract

Background/Aim. Acute myocardial infarction (AMI) is an important cause of mortality/morbidity worldwide. Biomarkers improve diagnostic and prognostic accuracy in AMI. The aim of this study was to investigate an increase of markers of endothelial dysfunction in AMI, measured on the 3rd day after the initial event and to investigate their association with short- and long-term (3-year) prognosis (outcome). **Methods**. The prospective study included 108 patients with AMI in the experimental group and 50 apparently healthy subjects in the control group. Endothelin-1 (ET-1) and nitric oxide degradation products (NOx) were determined. **Results.** The average age of the participants in the experimental group was 62 ± 10 years and 59 ± 9 years in the control group; 74.1% of the patients in experimental group were males and 68.8% in the control group. In 74.1% of the patients, ST-elevation myocardial infarction (STEMI) was diagnosed, and 25.9% of the patients presented with non-ST-elevation myocardial infarction (NSTEMI). Thirteen (5.6%) patients died during 3 years and they had significantly higher ET-1 levels compared to survivors [4.02 (2.72–5.93) *vs* 3.06 (2.23–3.58) pg/mL; $p = 0.015$]. Endothelin-1 in 46 (42.6%) patients with composite endpoint (3-year mortality and rehospitalization) was significantly increased compared to other patients [3.14 (2.54–4.41) *vs* 3.05 (2.18–3.56) pg/mL; $p = 0.035$]. Intrahospital complications were found in 41 (48%) patients. Participants with echocardiographically detected complications (ventricular dyskinesia, left ventricular thrombus and papillary muscle rupture) had higher ET-1 levels compared to other patients [4.02 (2.78–5.57) *vs* 3.06 (2.29–3.66) pg/mL; $p = 0.012$]. Endothelin-1 concentration above the 75th percentile (> 3.77 pg/mL) was associated with the increased risk for composite endpoint [Log Rank ($\chi^2 = 13.44$; $p < 0.001$)]. Patients who were rehospitalized had significantly lower NOx concentration [125.5 (111.4–143.6) *vs* 139.3 (116.79–165.2) μmol/L; $p = 0.04$]. Endothelin-1 positively correlated with high sensitivity troponin I (hsTnI), brain natriuretic peptide (BNP) and a number of leukocytes. **Conclusion.** Endothelin-1 and NOx were increased on the 3rd day after AMI, and they were predictors of worse short- and long-term (3-year) prognosis (outcome). Endothelin-1 positively correlated with conventional prognostic markers in AMI.

Key words:
myocardial infarction; biological markers; ultrasonography; endothelins; nitric oxide; prognosis; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Akutni infarkt miokarda (AIM) je značajan uzrok obolevanja i umiranja u svetu. Biomarkeri imaju značaj u postavljanju dijagnoze i u prognozi kod bolesnika sa AIM. Cilj studije bio je utvrđivanje porasta markera endotelne disfunkcije kod bolesnika sa AIM, merenih trećeg dana, poređuju sa vrednostima u kontrolnoj grupi zdravih ispitanika i značaja pomenutih markera u kratkoročnoj i dugoročnoj (trogodišnjoj) prognozi (ishodu). **Metode.** U prospektivnu studiju bilo je uključeno 108 bolesnika sa AIM koji su činili eksperimentalnu grupu i 50 zdravih dobrovoljaca kontrolne grupe. Kod svih ispitanika određivani su endotelin-1 (ET-1) i degradacioni produkti azot-monoksida (NOx). **Rezultati.** Prosečna starost ispitanika u eksperimentalnoj grupi bila je 62 ± 10 godina, a 59 ± 9 godina ispitanika u kontrolnoj grupi. U eksperimentalnoj grupi 74.1% ispitanika bilo je muškog pola a 68.8% u kontrolnoj grupi. Kod 74.1% bolesnika iz eksperimentalne grupe postavljena je dijagnoza infarkta miokarda sa elevacijom ST segmenta (STEMI), dok je 25.9% imalo infarkt miokarda bez delovanja ST segmenta (NSTEMI). Tokom 3 godine praćenja umrlo je 13 (5.6%) bolesnika. Oni su imali više koncentracije ET-1 u poređenju

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sa preživelim bolesnicima [4,02 (2,72–5,93) *vs* 3,06 (2,23–3,58) pg/mL; $p = 0,015$]. Zajedno, mortalitet i rehospitalizacija bili su prisutni kod 46 (42,6%) bolesnika, koji su takođe imali više koncentracije ET-1 [3,14 (2,54–4,41) *vs* 3,05 (2,18–3,56) pg/mL; $p = 0,035$]. Intrahospitalne komplikacije bile su prisutne kod 41 (48%) bolesnika, a oni sa ehokardiografski uočenim komplikacijama (ventrikularna diskinezija, tromb u levoj komori i ruptura papilarnog mišića) imali su značajno viši ET-1 [4,02 (2,78–5,57) *vs* 3,06 (2,29–3,66) pg/mL; $p = 0,012$]. Vrednosti ET- 1 iznad 75-og percentila (> 3,77 pg/mL) bile su udružene sa povećanim rizikom od lošeg ishoda [Log Rank (χ^2=13,44; $p < 0,001$)]. Bolesnici koji su rehospitalizovani imali su niže vrednosti NOx [125,5 (111,4–143,6) *vs* 139,3 (116,79–165,2) μmol/L; $p = 0,04$]. Vrednosti endotelina-1 bile su u pozitivnoj korelaciji sa visoko-senzitivnim troponinom I (hsTnI), moždanim natriuretskim peptidom (BNP) i brojem leukocita. **Zaključak.** Endotelin-1 i NOx su bili povišeni trećeg dana od AIM i bili su pokazatelji loše kratkotrajne (intrahospitalne) i trogodišnje prognoze ishoda. Vrednosti endotelina-1 bile su u korelaciji sa tradicionalnim prognostičkim markerima u AIM.

Ključne reči:
infarkt miokarda; biološki pokazatelji; ultrasonografija; endotelini; azot, oksidi; prognoza; osetljivost i specifičnost.

Introduction

Almost seven million people or 12.8% of the whole human population die due to coronary artery disease (CAD) during one year [1].

The endothelium is included in the development of atherosclerosis since it has a role in the maintenance of the vascular tonus, regulation of platelet and leukocyte activity and thrombosis/thrombolysis. Increased oxidative stress is closely related to endothelial dysfunction during the process of atherogenesis and development of complications such as acute myocardial infarction (AMI) [2–4].

Vascular smooth muscle tonus and leukocyte and platelet activity are controlled by the endothelium through a release of different mediators such as nitric oxide (NO) or endothelin-1 (ET-1) [5]. Nitric oxide and ET-1 are in physiological equilibrium in normal vasculature and low NO production or increases of ET-1 levels initiate endothelial dysfunction [6].

Endothelin-1 is a small peptide and it originates from the precursor called preproendothelin-1 (212 amino-acids). After removing its signaling portion and after cleavage action of endothelin converting enzyme, preproendothelin-1 forms two molecules: active ET-1 (with 21 amino acids) and inactive C-terminal part. Endothelin-1 is normally present at very small concentrations in the blood due to its quick reaction with receptors and due to the action of plasma neutral endopeptidases. Its half-life is estimated to be only 2 minutes [7, 8]. Endothelin-1 was first isolated from the porcine aortic endothelium. It is the most powerful vasoactive biomolecule isolated so far [9]. In AMI level of ET-1 in the blood rapidly increases. In patients without complications, the peak of ET-1 concentration is achieved approximately six hours after chest pain onset and afterwards, ET-1 starts to decrease to its normal values [10].

Nitric oxide (NO) is a free radical biosynthesized by the various forms of NO synthase (NOS) using L-arginine as a substrate. Nitric oxide has a short half-life *in vivo* and its degradation forms: nitrites and nitrates (NOx) that are used for its indirect measurement. Under normal physiological conditions, NO mediates many actions of the endothelium. Nitric oxide promotes relaxation in the vascular smooth muscle cells and has antithrombotic and antiatherogenic properties [11, 12]. In patients with stable CAD, NOx levels are decreased, however during the AMI inducible NOS (iNOS) from leukocytes is stimulated and therefore NOx concentrations are higher [11].

It seems that in AMI, endothelin-1 and NO exert the best predictive role when they are measured 48h after MI onset [11, 13].

The aim of our study was to determine is there an increase in markers of endothelial dysfunction (ET-1 and NOx) on the 3rd day after AMI. The second aim was to investigate the association of those biomarkers with short and long-term (3-year) prognosis (outcome).

Methods

In the prospective study 108 patients admitted to the Coronary Care Unit (CCU) at Clinic for Cardiovascular Diseases, Clinical Center Nis, during April–June 2012, without previously diagnosed diabetes mellitus, chronic kidney disease, and connective tissue disorders or other severe chronic diseases requiring treatment were included in the experimental (AMI) group. Control group comprised of 50 apparently healthy, age and gender matching, volunteer blood donors from the Blood Transfusion Institute, Niš. Subjects who were willing to participate in the study were scheduled for ambulatory physical and resting electrocardiographic (ECG) examination when detailed medical history was taken and blood samples were drawn. Volunteers with detected abnormalities were sent to primary care institutions for further diagnostic and therapeutic interventions. Diagnosis of AMI was made according to the European Society of Cardiology Guidelines [14].

The study was conducted in accordance with the Helsinki Declaration and Regional Ethics Committee approval. Prior to the study inclusion, all participants signed the informed consent.

For biomarkers measurement, venous blood samples from patients with AMI were collected on the 3rd day after admission to the CCU. In the control group, sampling was done in the morning, after 12 hours of fasting and no nicotine use. Plasma samples were stored at -20ºC. Endotelin-1 was measured by ELISA method with commercial Quantikine Endothelin-1 Immunoassay test from R&D Systems (USAR&D Company Minneapolis). Assay sensitivity is 0.207 pg/mL, and the assay range is 0.390–25 pg/mL. The

coefficient of variation for intra-assay precision in three different samples was 4%, 2.3%, and 1.9%. Concentrations of NOx**:** nitrites/nitrates (NO_2^- /NO_3^-) were measured using the modified cadmium-reduction method of Navaro-Gonzalez et al. [15] based on the Griss reaction. The within-day (control plasma sample, n = 7) and between-days coefficient of variation (the same sample for 5 consecutive days) were 7% and 8.6%, respectively. Recoveries of both nitrites and nitrates in our samples were greater than 95% and the detection limit of the assay was 2.5 μmol/L.

Blood samples for routine biochemical and hematology analysis, including brain natriuretic peptide (BNP) and the high sensitivity C-reactive protein (hsCRP) were taken at the admission according to the protocol of the Clinic. Blood samples for the high sensitivity troponin I (hsTnI) were taken at least 6 hours after the symptom onset. Levels of BNP, hsCRP, and hsTnI were measured according to the description of the manufacturer [BNP (ARCHITECT assay, Abbott, USA), hsCRP (BECKMAN COULTER, USA), hsTnI (ARCHITECT STAT High sensitive Troponin-I assay, Abbott Diagnostics, USA)] at the Central Biochemical Laboratory, Clinical Center Niš.

All patients underwent a complete echocardiographic examination during 48 h after admission.

Ambulatory follow-up visits were scheduled on every 6 months. Subjects from the control group were contacted by phone. For those who died during the follow-up period, data were obtained from the family physician and death certificate.

All statistical calculations were performed using appropriate (non)parametric tests after verification of parameter distribution in each group. All comparisons between subgroups were performed using the Mann Whitney test, or ANOVA when appropriate. Spearman's rank correlation coefficient and Pearson's bivariate correlation analysis were used to investigate the relationship between two comparable variables. Variables without normal distribution (ET-1, NOx, hsTnI, BNP, hsCRP) were transformed to their natural logarithms for logistic regression analysis. Odds ratios refer to 1 standard deviation (SD) in the natural logarithmic scale. All data were presented as means ± SD or medians with range. The $p < 0.05$ was considered as significant. All statistical calculations were done using "SPSS 17.0 for Windows" (SPSS Inc., USA).

Results

The average age of participants in the study was 62 ± 10 years in the experimental and 59 ± 9 in the control group, 74.1% being males in the experimental and 68.8% in the control group.

In 74.1% of patients, ST-elevation myocardial infarction (STEMI) was diagnosed, and 25.9% of patients presented with non-ST-elevation myocardial infarction (NSTEMI). In 68.7% of patients with STEMI, the primary percutaneous coronary intervention (PCI) was performed, 27.9% received thrombolytic therapy (rt-PA), and in 3.4% rescue PCI was performed. Left ventricular ejection fraction (LVEF) was 52.59 ± 10.54% in the experimental and 64.33 ± 18.63% in the control group. Clinical and laboratory characteristics of participants are shown in Tables 1 and 2.

Plasma levels of ET-1 ranged from 1.86 to 6.27 pg/mL in the experimental, and from 1.18 to 4.63 pg/mL in the control group, with a median and inter-quartile range (IQR) presented in Table 2. Nitric oxide degradation products were significantly higher in AMI compared to the control group ($p < 0.001$) (Table 2 and Figure 1).

Forty-one (48%) patients had intrahospital complications. Echocardiographically detected complications: ventricular dyskinesia, left ventricular thrombus and papillary muscle rupture were found in 9 (8.3%) patients and 5 (4.6%) had myocardial re-infarction. Arrhythmias and conductance disturbances were found in 24 (22%) patients. They included ventricular fibrillation, ventricular tachycardia, paroxysmal atrial fibrillation and other forms of supraventricular

Table 1

Clinical characteristics of participants

Parameters	Experimental group n (%)	Control group n (%)
Age (years), x̄ ± SD	62.44 ± 10.42	59.37 ± 9.46
Gender (males)	80 (74.1)	34 (68.8)
Arterial hypertension	82 (75.9)	/
Dyslipidemia		
no	51 (47.2)	50 (100)
yes and treated	45 (41.7)	/
yes and not treated	12 (11.1)	/
Smoking		
never	29 (26.9)	18 (66)
current	51 (47.2)	15 (30)
stopped	28 (25.9)	2 (4)
Diabetes mellitus *de novo*	10 (9.25)	/
Impaired fasting glucose	6 (5.55)	/
Atrial fibrillation	13 (12.03)	/
Angina pectoris	42 (38.9)	/
Previous myocardial infarction	21 (19.4)	/
CABG	3 (1.3)	/

CAGB – coronary artery bypass graft surgery; x̄– arithmetic mean; SD – standard deviation.

tachycardia, and second and third-degree atrioventricular block requiring a temporary or permanent pacemaker. Differences between ET-1 and NOx levels in patients with and without intrahospital complications are presented in Figures 2 and 3. A significant difference was found in ET-1 levels in patients with in-hospital echocardiographically detected complications (ventricular dyskinesia, left ventricular thrombus and papillary muscle rupture) compared to patients without those complications (Table 3). Similar results were not obtained regarding the NOx levels (Table 4). Higher incidence of intrahospital complications was associated with higher levels of ET-1 in a simple logistic analysis [OR = 1.440, 95% confidence interval (CI) (1.050–1.975); $p = 0.024$]. During 3 years, 29 (26.8%) patients were re-hospitalized due to cardiovascular causes and 13 (5.6%) patients died, of whom 3 (1.3%) died during the initial hospitalization. Except for one patient who died due to major gastrointestinal bleeding associated with dual antiplatelet therapy, all others died due to cardiovascular causes (re-infarction, sudden cardiac death and stroke). Survival curves for composite endpoint (3-year mortality and re-hospitalization) in patients with ET-1 and NOx concentrations below and above the 75th percentile are presented in Figure 4. Cox regression analysis showed that patients with ET-1 concentrations above the 75th percentile (≥ 3.79 pg/mL) had higher risk for mortality and/or rehospitalization during 3 years [hazard ratio (HR) = 2.893, 95%CI (1.595–5.246); $p < 0.001$]. A significant difference was not found between percentiles of NOx for a composite endpoint in Cox regression analysis.

Endothelin-1 was predictive for mortality in univariate analysis and kept independence in the multivariate logistic model adjusted for hsTnI, BNP, hs CRP, NOx, LVEF, gender, and type of AMI (STEMI *vs* NSTEMI). Though, ET-1 was predictor for 3-year mortality, as well as age (Table 5). In a simple logistic analysis, higher ET-1 levels [odds ratio (OR) = 1.398, 95%CI (1.054–1.854); $p = 0.02$] were associated with higher intrahospital mortality risk. Lower NOx levels [OR = 0.971, 95%CI (0.955–0.999); $p = 0.028$] and lower LVEF [OR = 0.959, 95%CI (0.918–0.999); $p = 0.037$] were associated with increased risk for re-hospitalization in a simple logistic analysis.

Interestingly, there were no significant differences in levels of ET-1 and NOx, among patients with STEMI and

Table 2

Laboratory parameters in study participants

Parameters	Experimental group	Control group	*p*
WBC (10^9/L), mean ± SD	11.25 ± 3.85	6.72 ± 1.35	$p < 0.001$
hs CRP (mg/L), median (IQR)	9.29 (4.52–35.65)	1.28 (0.56–2.81)	$p < 0.001$
CK-MB (U/L), median (IQR)	378.35 (161.45–715.57)	48.75 (21.90–97.80)	$p < 0.001$
hs TnI (ng/mL), median (IQR)	12.35 (3.02–41.27)	/	/
ET-1 (pg/mL), median (IQR)	3.08 (2.41–3.77)	3.04 (2.38–3.78)	n.s.
NOx (μmol/L), median (IQR)	131.80 (115.65–161.10)	89.3 (81.8–96.8)	$p < 0.001$

Data are presented as mean ± standard deviation or median with interquartile range (IQR) in parenthesis; WBC – white blood cell; CK-MB – creatine kinase MB isoenzyme; hs CRP- high sensitivity C-reactive protein; BNP – brain natriuretic peptide; hs TnI – high sensitivity troponin I; ET-1 – endothelin-1; NOx – nitric oxide degradation products (nitrates/nitrites).

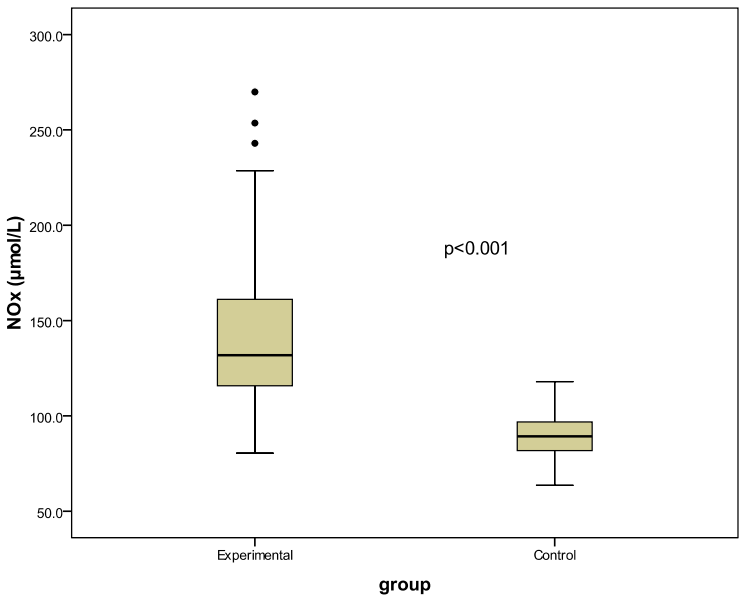

Fig. 1 – Nitric oxide degradation products (NOx) in the experimental and control groups. NOx are shown as median with interquartile range between 25th and 75th percentile. Values higher than 75th percentile are presented as dots.

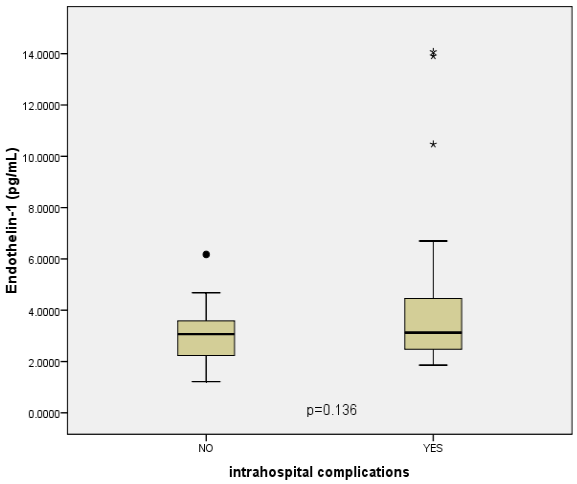

Fig. 2 – Endothelin-1 (ET-1) in patients with and without intrahospital complications (composite of arrhythmias and conduction disturbances, re-infarction, echocardiographycally detected complications and mortality). ET-1 concentrations are shown as median with interquartile range between 25th and 75th percentile. Values higher than 75th percentile are presented as dots and asterisks.

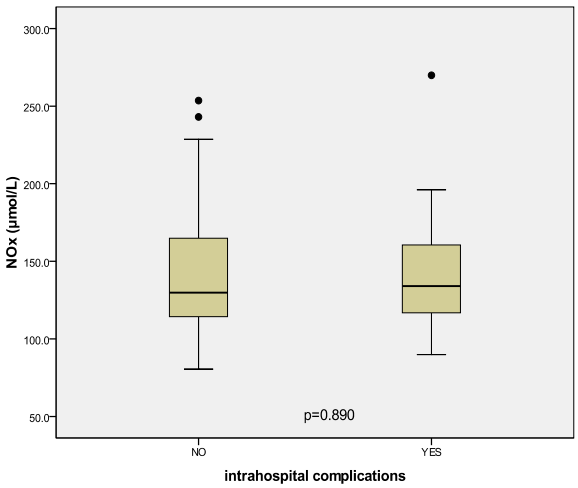

Fig. 3 – Nitric oxide degradation products (NOx) in patients with and without intrahospital complications (composite of arrhythmias and conduction disturbances, re-infarction, echocardiographycally detected complications and mortality). NOx concentrations are shown as median with interquartile range between 25th and 75th percentile. Values higher than 75th percentile are presented as dots and asterisks.

Table 3

Endothelin-1 (ET-1) levels in acute myocardial infarction patients with or without complications during 3 years

Complications	Patients n (%)	ET-1 level in patients without complication (pg/mL)	ET-1 level in patients with complication (pg/mL)	*p*
3-year mortality	13 (5.6)	3.06 (2.23–3.58)	4.02 (2.72–5.93)	0.015
In-hospital mortality	3 (1.3)	3.07 (2.34–3.71)	4.08 (3.28–8.99)	0.002
Arrhythmias and conductance disturbances	24 (22)	3.08 (2.47–3.72)	2.95 (2.21–4.11)	0.642
Re-infarction during initial hospitalization	5 (4.6)	3.08 (2.31–3.69)	4.45 (2.53–9.69)	0.160
Ventricular dyskinesia, ventricular thrombosis, papillary muscle rupture	9 (8.3)	3.06 (2.29–3.66)	4.02 (2.78–5.57)	0.012
Re-hospitalizations	29 (26.8)	3.06 (2.38–3.63)	3.11 (2.38–4.02)	0.585
Composite endpoint	46 (42.60)	3.05 (2.18–3.56)	3.14 (2.54–4.41)	0.035

Data are shown as median with interquartile range in parenthesis.

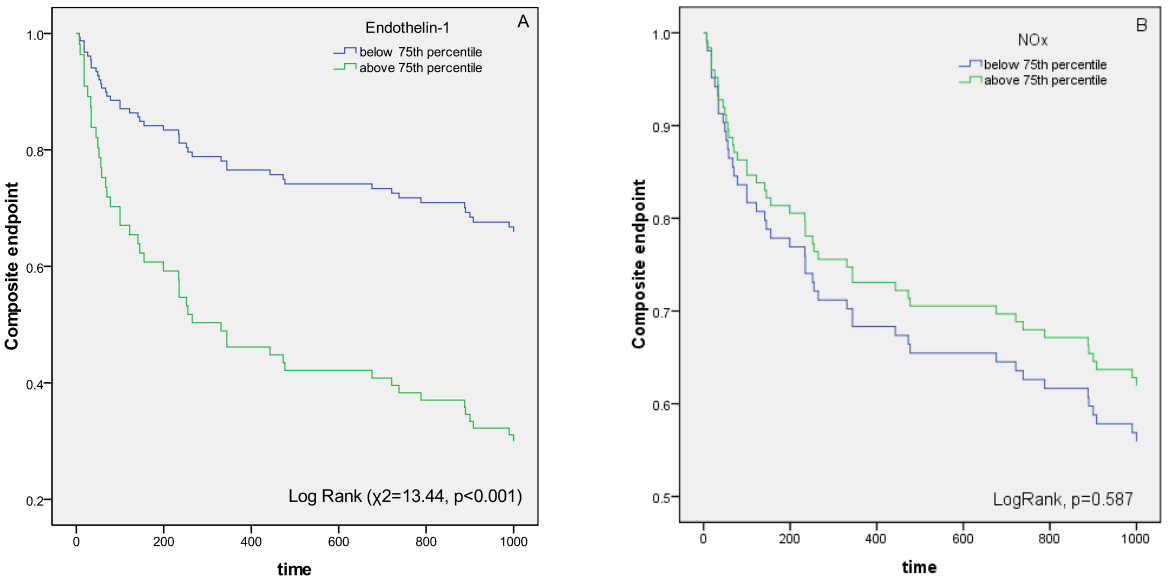

Fig. 4 – Kaplan-Meier curves for time until composite endpoint (mortality or rehospitalization) for ET-1 (A) and NOx (B) below and above 75th percentile.
NOx – Nitric oxide degradation products (nitrates/nitrites).

Table 4

Nitric oxide degradation products (NOx) in acute myocardial infarction patients with or without complications during 3 years

Complications	Patients n (%)	NOx levels in patients without complication (μmol/L)	NOx levels in patients with complication (μmol/L)	*p*
3-year mortality	13 (5.6)	131.8 (115.5–161.1)	139.3 (109.89–163.3)	0.962
In-hospital mortality	3 (1.3)	131.1 (114.9–160.8)	172.4 (155.85–178.65)	0.100
Arrhythmias and conductance disturbances	24 (22)	131.45 (114.6–164.275)	132.5 (116.8–158.775)	0.790
Re-infarction during initial hospitalization	5 (4.6)	131.8(116.1–161.1)	121.1 (105.35–175.2)	0.787
Ventricular dyskinesia, ventricular thrombosis, papillary muscle rupture	9 (8.3)	131.8 (115.5–161.1)	134.9 (113.95–170.2)	0.929
Re-hospitalizations	29 (26.8)	139.3 (116.79–165.2)	125.5 (111.4–143.6)	0.04
Composite endpoint	46 (42.6)	133.05 (115.2–164.9)	129.9 (115.44–160.5)	0.460

Data are shown as median with interquartile range in parenthesis.

Table 5

Logistic regression analyses for 3-year mortality

Variable	Simple model OR (95% CI)	*p*	Multiple model OR (95% CI)	*p*
Age	1,194 (1.022–1.272)	0.010	1.240 (1.047–1.468)	0.013
Male gender	0.761 (0.215–2.696)	0.761	not shown	ns
Ln ET-1	2.380 (1.073–5.774)	0.012	2.218 (1.171–4.202)	0.015
Ln NOx	0.997 (0.979–1.014)	0.692		ns
Ln TnI	1.006 (0.999–1.013)	0.119		ns
Ln hsCRP	0.873 (0.300–2.539)	0.803	not shown	ns
Ln BNP	3.378 (0.777–14.686)	0.105		ns
LVEF	0.988 (0.934–1.046)	0.682		ns
STEMI vs. NSTEMI	0.352 (0.107–1.156)	0.055		ns

STEMI – myocardial infarction with ST segment elevation; NSTEMI – myocardial infarction without ST segment elevation; LVEF – left ventricular ejection fraction; Ln – natural logarithm; OR – odds ratio; CI – confidence interval; ET-1 – endothelin-1; BNP – brain natriuretic peptide; hsCRP – high sensitivity C-reactive protein; NOx – nitric oxide degradation products (nitrates/nitrites); TnI – troponin; ns – non-significant.

NSTEMI [(ET-1: 3.14 (2.41–3.91) *vs* 2.76 (2.33–3.47) pg/mL; NOx: 131.80 (114.3–161.75) *vs* 131.45 (116.79–160.5) μmol/L], nor among those with STEMI treated with primary PCI or with thrombolytic therapy [(ET-1: 3.21 (2.41–3.98) *vs* 2.95 (2.31–3.68) pg/mL]; NOx: 128.6 (114.9–161.1) *vs* 165.5 (114.6–173.35) μmol/L].

Endothelin-1 positively correlated with hsTnI ($\rho = 0.291$; $p < 0.01$) and BNP levels ($\rho = 0.315$; $p < 0.05$) (Figure 5) and white blood cell (WBC) count ($\rho = 0.198$, $p < 0.05$).

Also, hsCRP correlated with hsTnI ($\rho = 0.399$; $p < 0.01$) and BNP levels ($\rho = 0.460$, $p < 0.01$). There were no correlations between NOx and other investigated biomarkers.

Discussion

Myocardial infarction continues to be a significant cause of mortality and morbidity in the western world. Biomarkers improve diagnostic and prognostic accuracy in AMI [16].

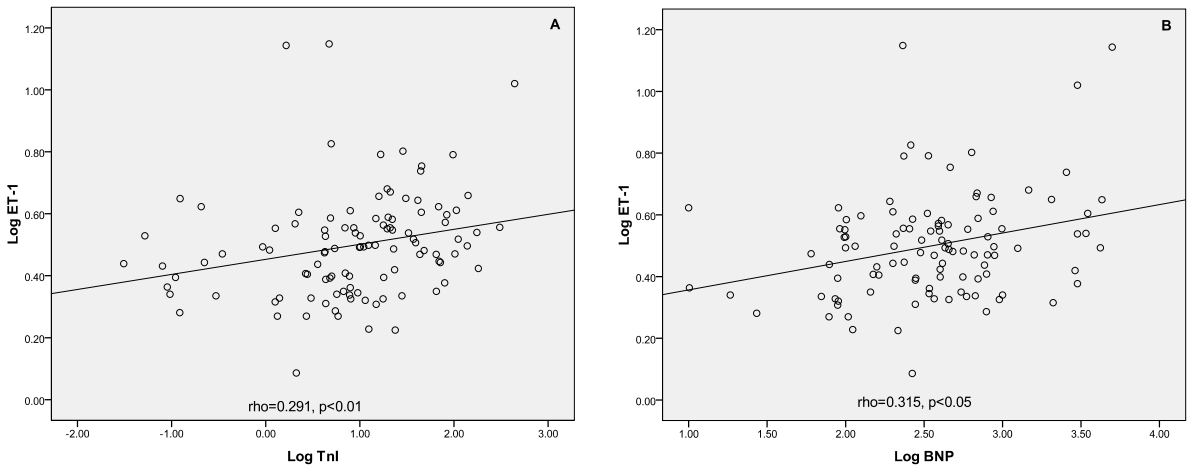

Fig. 5 – Linear correlation: A) between logarithmically transformed endothelin-1 (ET-1) and tropinin I (TnI) level values, and B) between logarithmically transformed ET-1 and brain natriuretic peptide (BNP) level values.

The clinical combination of hsCRP, troponins, and BNP has been used as a universal tool for risk stratification [17]. In our study, those biomarkers were not associated with the short or long-term cardiovascular mortality and morbidity. This could be the consequence of sample timing. Troponin I was measured only at admission. New studies emphasize the prognostic importance of TnI levels not only during the acute phase of NSTEMI, but also within the following 72 h. In large STEMI, the release of TnI over time is markedly different than in small NSTEMI or in micro-infarctions as a consequence of fluctuations in myocardial perfusion which has an impact on repeated measurements of TnI and its predictive role in different acute coronary syndromes [18, 19].

Endothelin-1 is synthesized in the vasculature and myocardium by various cell types [20]. Stewart et al. [21] showed that in uncomplicated AMI, ET-1 is elevated during a short period (reaches a peak after few hours), whereas in patients who develop heart failure, pulmonary oedema, shock or reinfarction, plasma ET-1 concentrations rise sharply and remain high during few days [21]. Therefore, to evaluate the relationship between plasma ET-1 and prognosis in AMI, it appears mandatory to measure plasma ET-1 after its early increase. That was the rationale for measurement of ET-1 on the 3rd day after AMI in the present study. Omland et al. [22] were first to describe a relation between ET-1 levels obtained in the subacute phase of AMI and 1-year mortality. In line with previous studies, our experimental group had higher levels of ET-1 than the control group, although insignificantly for those without complications. Also, elevated ET-1 levels were associated with higher risk for in-hospital and 3-year cardiovascular mortality. In particular, values above the 75th percentile were associated with a higher incidence of the composite endpoint (3-year mortality and rehospitalization).

In AMI patients with hemodynamic complications, the significant inverse relation was demonstrated between the highest plasma ET-l levels and LVEF [21]. Our patients with higher percentiles of ET-1 had the lowest LVEF, and we also found a significant positive relationship between ET-1 levels and BNP, which confirms the role of ET-1 in the hemodynamic complications, as an innocent bystander or involved in its pathogenesis, not known so far.

In experiments on animals, it was demonstrated that the rise of ET-1 has a significant impact on the size of the infarcted myocardial area. However, the correlation between ET-1 and creatine kinase MB Isoenzyme (CK-MB) was not found in previous studies [21, 23]. In the present study, ET-1 positively correlated with hsTnI. Accordingly, elevated ET-1 levels were associated with higher risk for intrahospital complications. Endothelin-1 was significantly higher in patients with echocardiographiocally detected complications (ventricular dyskinesia, left ventricular thrombus) and papillary muscle rupture compared to other AMI patients. Endothelin-1 is an arrhythmogenic substance, and that property is not associated with its other ability to induce myocardial ischemia in animal models [24]. However, we did not find a significant difference in ET-1 levels in patients with or without arrhythmias.

Endothelin-1 is a signaling molecule which transmits the signals to the leukocytes which are then activated and attracted to the myocardial tissue where they release reactive oxygen species (ROS) and cause injury of both myocardium and endothelium [25]. In our patients, ET-1 correlated with white blood cells (WBC) count.

Nitric oxide has a controversial role in AMI. Emerging evidence indicates that iNOS that produces NO is a hypoxia-inducible protein. Upregulation of iNOS is an important protective reaction of the heart in response to ischemia. Pre-existing endothelial (e)NOS is activated as a rapid response to myocardial ischemia but upregulation of iNOS leads to the continuous release of NO in this setting which is important for the protection of myocardial viability. Therefore, NO has a beneficial role in AMI [26]. It was shown that elevated ET-1 levels reduce NO bioavailability [27]. In our study however, ET-1 and NOx were not in correlation. It seems that in AMI activation of iNOS has a crucial role in regulation of NO production.

Experiments on animals demonstrated that after coronary artery occlusion NOx level is increased with a peak after 3 days. In patients with AMI, serial measurements of NOx after 24, 48, and 72 hours showed its significant increase compared to the control group. Nitric-oxide degradation products reached a peak after 2 or 3 days when iNOS was maximally stimulated by inflammatory cytokines [11]. This fact was the rationale for measurement of NOx on the 3rd day after AMI in the present study.

Accordingly, in our patients, we found an increase in NOx levels, which was more pronounced than ET-1 increase, on the 3rd day after AMI. Together with lower LVEF, lower NOx concentrations were predictors for re-hospitalization due to cardiovascular causes during 3 years. This could be the consequence of lower bioavailability of NO and endothelial dysfunction in extensive coronary artery diseases with a worse prognosis.

Mayyas et al. [28] found that ET-1 was significantly higher in patients with STEMI than in those with NSTEMI one week after an acute event. Reperfusion therapy in AMI increases oxidative stress which leads to decrease of NO and its protective effects during ischemia [29]. Eitel et al. [30] found that ET-1 measured prior to primary PCI was a marker of no-reflow and mortality in STEMI patients. Small sample studies showed long-term benefits of endothelin A receptor antagonists in STEMI patients given during primary PCI (less frequent no-reflow and higher LVEF). It seems that ET-1 is a prognostic indicator which is closely involved in the pathology of acute coronary syndrome and its complications [31, 32]. However, no difference was found in NOx and ET-1 levels between our patients with STEMI treated with a different type of reperfusion therapy, and those with NSTEMI. The timing of the assessment of biomarkers, with respect to the timing of the reperfusion therapy, could explain this finding.

Conclusion

Endothelin-1 and nitric-oxide degradation products were increased on the 3rd day after acute myocardial infarction, compared to healthy matching control. Endothelin-1 was significantly increased in patients with in-hospital papillary muscle rupture, echocardiographically detected complications (ven-

tricular dyskinesia, left ventricular thrombus) and in those who died during 3 years of follow-up. Endothelin-1 levels above the 75th percentile were associated with higher risk of 3-year mortality and rehospitalization. Decreased concentrations of nitric-oxide degradation products were associated with higher risk for rehospitalization during 3 years.

New biomarkers are emerging and it remains to be seen if consideration of endothelial dysfunction markers can add clinically important information to patient care. Compared to invasive or noninvasive imaging modalities, they offer the advantage of being relatively risk-free, less expensive, and applicable to a wide range of populations at risk.

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Organizational identification, commitment and orientations of professional military personnel

Organizacionа identifikacija, predanost i orijentacije profesionalnih vojnih lica

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Abstract

Background/Aim. All military organizations seek such employees who will advocate for the organization's mission and act responsibly in the direction of achieving the objectives of operational and working groups to which they belong. Accordingly, the primary task of the military organization management is not only the cultivation of the members who would be committed to the organization, but also the officers and soldiers who identify with the organizational mission. The aim of this study was to examine differences in organizational identification, commitment to the organization and organizational orientations of the professional military personnel and employees in service and administrative activities. **Methods**. The research sample consisted of 450 respondents, of whom 150 were professional soldiers, 150 civilian employees in the service sector and 150 employees in the civil sector in administration. For statistical analysis of the data, the analysis of variance and canonical discriminant analysis were used. **Results**. Professional military personnel was characterized by a high degree of both organizational commitment and organizational identification, compared with employees in the civil sector - service and administrative activities. Through the process of canonical discriminant analysis, it was found that the professional military personnel are different from the other personnel in the sense that they identify with their colleagues and they feel a high degree of loyalty to the military organization, as key aspects of organizational identification. In addition, professional military personnel have pronounced affective commitment to the organization. **Conclusion**. Human resources are the key and the essential factor of advantage in the context of strong competitiveness in the field of military defense's reality. Given that they are more adaptable and flexible, compared with the technological and structural resources, a high degree of experienced similarity with the other members of the organization, pronounced loyalty and affective commitment to the organization, to a large extent guarantee new successes and the progress of the military organization.

Key words:
military personnel; psychology, military; administrative personnel; organization and administration; surveys and questionnaires; psychology; job satisfaction.

Apstrakt

Uvod/Cilj. Sve vojne organizacije traže takve zaposlene koji će se zalagati za misiju organizacije i delovati odgovorno u smeru postizanja ciljeva operativnih i radnih grupa kojima pripadaju. U skladu s tim, primarni zadatak menadžmenta vojnih organizacija nije samo formiranje pripadnika koji bi bili posvećeni organizaciji već i oficira i vojnika koji se identifikuju sa organizacionom misijom. Cilj istraživanja bio je da se ispita postojanje razlika u organizacionoj identifikaciji, predanosti organizaciji i organizacionim orijentacijama kod profesionalnih vojnih lica i lica zaposlenih u uslužnim i administrativnim delatnostima. **Metode**. Uzorak istraživanja činilo je ukupno 450 ispitanika – 150 profesionalnih pripadnika vojske, 150 zaposlenih u civilnom sektoru na uslužnim poslovima i 150 zaposlenih u civilnom sektoru na administrativnim poslovima. Za statističku obradu podataka korišćene su analiza varijanse i kanonička diskriminativna analiza. **Rezultati**. Profesionalna vojna lica odlikovao je visok stepen kako organizacione predanosti tako i organizacione identifikacije u poređenju sa zaposlenima u civilnom sektoru na uslužnim i administrativnim delatnostima. Kroz postupak kanoničke diskriminacione analize utvrđeno je da je profesionalna vojna lica od ostalih razlikovao visok stepen doživljaja sličnosti sa kolegama i visok stepen lojalnosti vojnoj organizaciji, kao ključnim aspektima organizacione identifikacije. Uz to, profesionalna vojna lica imala

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su izraženiju afektivnu privrženost organizaciji. **Zaključak**. Ljudski resursi predstavljaju ključ i glavni faktor konkurentske prednosti u kontekstu snažne konkurentnosti na polju vojno-odbrambene realnosti. S obzirom na to da su oni adaptibilniji i fleksibilniji, u poređenju sa tehnološko-strukturalnim resursima, visok stepen doživljaja sličnosti sa ostalim pripadnicima organizacije, izražena lojalnost i afektivna predanost organizaciji, u dobroj meri garantuju nove uspehe i napredak vojne organizacije.

Ključne reči:
vojni kolektiv; psihologija, vojna; kadar, administrativni; organizacija i rukovođenje; ankete i upitnici; psihologija; posao, zadovoljstvo.

Introduction

Human resources and their potentials are becoming the main factor of competitiveness and organizational performance, and they have to be addressed with great attention and interest [1].

The works of some authors stress the importance of the selection of the employees who are a part of the army of a country. These employees would have a high degree of organizational identification because that is certainly a way to act in the direction of strengthening the commitment of military professionals [2].

According to the theory of social identity, organizational identification is a form of social identification where employees see themselves as a member of a certain social entity – in this case, the organization in which one is employed. Identification of the employees with the organization is important for the more one identifies with the organization, the more they think and act in accordance with the perspective and objectives of the organization [3]. This point of view in supported by numerous studies is which the need of the organization to be, in some way, supported by the employees' organizational identification is emphasized [4–9] because their positive or negative effects may affect the performance of the organization.

Organizational identification has long been recognized as a critical construct which is of a great importance to the organizational behaviour, because it can have an impact on both the satisfaction of employees and their efficiency at work [10–14]. Moreover, organizational identity has been proven to be a vital factor of organizational life.

The findings of Mael and Ashforth [2] indicate that the highlighted organizational identification with the members of an army is not necessarily related to the length of their service, the success in their career or to general satisfaction with the assigned position. As theorists of social identity [15] have pointed out, simply a "deployment" in the sector of the given organization can lead to the almost immediate appearance of strengthening the expression of identification with the group in which one is being allotted. The results of the research by Mael and Ashfort [2] suggest that one of the most important factors of a prominent organizational identification is actually the existence of congruence between personal interests and organizational activities.

A military unit is an organized and unified social group with a specific social function, with strict subordination in relationships, where members of the collective feel a social and psychological connection with the unit when the collective interests regard as their own interests [16, 17]. All military organizations seek such employees who will advocate for the organization's mission and act responsibly in the direction of achieving the objectives of operational and working groups to which they belong. Accordingly, the primary task of the management of a military organization is not only the cultivation of the members who would be committed to the organization, but also of the officers and soldiers who identify with the organizational mission. Employees who are committed to the organization believe that the organization is a good place to work, do not search another workplace in a new organization, they have developed positive effects towards the organization, and believe that there are no better alternatives in other working organizations that would meet their needs [18, 19]. In comparison with that, the individuals who "identify" with their organizations build self-images that are in harmony with the image of the organization and its values [20–23]. In accordance with this is also the fact that when individuals adopt values and goals of the organization, they develop a premise in the process of decision-making that is complementary to the goals and values-based premises that the organization constructs [22, 24]. From the perspective of a commander as a kind of manager, identification represents some sort of advantage for the organization since it ensures that the employees make decisions which are in the best possible interest for the organization, even in the absence of supervision [25]. Mael and Alderks [26] examined the organizational identification with regard to the military effectiveness during a combat of the military personnel. Their findings indicate that organizational identification, cohesion and motivation with a task are directly related to the combat performances and success in a battle.

That the concept of organizational identification is of importance for the functioning of military structures is pointed out through a wide range of literature. Thus, Little et al. [27] state that organizational identification is similar to team spirit, and it refers to the sense of creating a tactical unit. In this direction, Wilkes and Krebs [28] indicate that it is of a cardinal importance for the military organization to form some kind of awareness about the pride that derives from belonging to a given group, or a sense of common (shared) purpose and destiny.

Organizational identification occurs when, in the process of decision making, a person in one or more of his or her organizational roles perceives and understands that the organizational values or interests are relevant in the evaluation of alternatives in the decision-making process [22]. These authors distinguish three aspects in organizational identification: a sense of belonging (a feeling of solidarity or membership) – a strong sense of attachment or emotional attracting

related to organizational membership, but also pride in being a part of the organization; loyalty to the organization and enthusiasm tied to organizational goals; and identification with colleagues – observed similarity related to the common characteristics, and also having respect for the common (shared) values or goals. According to Simon [25], when individuals adopt the values and goals of an organization, they develop a premise in decision-making processes that are complementary to the goals and values which are based on the premise that sets the organization.

Organizational commitment can be determined by a certain degree of wishes and needs and obligations that an individual feels towards the organization he/she works for. Allen and Meyer [29–31] distinguish 3 components of organizational commitment: affective commitment exists when an employee wants to remain in the organization because of the emotional attachment; normative commitment stems from feelings of obligation of the employee to remain in the organization because of the incentives given or favors done (salaries and training); commitment through staying in the organization refers to the notion that there are accumulated benefits that could be lost if one leaves the organization (friends in the workplace, benefits specific to a particular organization). Researches show that people who are committed to the organization generate positive contributions to the organization, and above all manifest lower expression of absenteeism (absence from work) and through more pronounced working performance than the people who are not committed [32–36].

The organizational orientation of employees – Presthus [37] explicated the theory of organizational orientation as a form of explaining fundamental differences in the way the employees of organizations approach their jobs. The organization not only affects the behavior of its members, it also affects the formation of their permanent attitudes, values and interests. Presthus [37] assumes that this orientation can result in employees having different orientations towards their work as such, work motivation, job satisfaction and in the ways in which employees interact with their colleagues, superiors and subordinates [37]. Presthus [37] came to the conclusion that there are 3 main "personal styles" (orientations of the employees) in an average organization, but this orientation can be applied to almost any organization. At the top of the organizational pyramids are those who want to rise in the hierarchy. They react positively to the bureaucratic structure and succeed in such an environment. The second group consists of the majority of the non-aligned "indifferent" for whom the work of just a means to achieve goals outside of work. The third group is a minority consisting of ambivalent people. They neither give up their demands for progress, nor accept a disciplined role in order to achieve those goals.

The aim of this study is to examine whether there are differences in the intensity of organizational identification and its aspects between the professional military personnel and employees in the civil service sector and administrative sector. The second aim was to examine whether professional military personnel may be discriminated against individuals employed in the civilian sector on the administrative service positions, based on the intensity of organizational identification, commitment, and organizational orientation.

Methods

The research sample

The research sample consisted of 450 respondents, 150 were employed in the military service – professional members of the military, 150 employed in the civilian service sector and 150 employed in the civil sector in administration. A sample from military service includes professional military personnel in the category of officers, noncommissioned officers and professional soldiers and it is not proportionally distributed because the method of appropriate choices was opted for.

The instruments of research and statistical analysis

Organizational identification of employees was measured using the scale of organizational identification (Organizational Identification Questionnaire [20–22]). The scale had the following subscales, that measured the following aspects of organizational identification: the feeling of belonging, loyalty and identification.

To measure the intensity of organizational commitment Alen-Meyer's organizational commitment questionnaire was used (Organizational Commitment Scale Allen and Mayer [29–31]), which had the following subscales, for measuring the following aspects of organizational commitment: affective commitment, staying in the organization commitment and normative commitment.

To measure the intensity of organizational orientations an organizational orientation questionnaire was used [38].

The reliability of the instruments used has been proven through the research process: the value of Cronbach alpha for the questionnaire by which organizational identification was measured was 0.860; for the questionnaire by which organizational commitment was measured it was 0.865; while the Cronbach alpha for the questionnaires measuring organizational orientation was ranging from 0.876 for a questionnaire by which the expression of ambivalent orientation was measured to 0.803 for the questionnaire which measured indifferent organizational orientation, and Cronbach alpha for the questionnaire which measured the orientation towards the advancement in the hierarchy of the organization was 0.818. From these findings, it can be concluded that all the questionnaires used had satisfactory reliability.

All statistical analyses were performed in SPSS 20.0 (SPSS Inc, Chicago, Illinois) statistical package. The results are presented as frequency, percent and mean. The ANOVA (F-test) was used to compare the groups of respondents. Discriminant analysis was used to discriminate different groups of respondents based on their level of organizational identification, commitment, and organizational orientations. All *p*-values of 0.05 or less were considered significant.

Results

The findings indicate that there were differences in the intensity of organizational identification and its aspects

between professional soldiers and the people who were employed on service and administrative positions in civil sector (Table 1).

Professional soldiers had the highest scores on the scale of organizational identification - the total score (M = 48.00; $p < 0.01$), as well as on the scale that measures identification with colleagues (M = 18.98; $p < 0.01$) in comparison to the employees in civil service and administrative sectors, thus making this difference statistically significant. It should be remarked that professional military personnel had a more prominent aspect of organizational identification related to loyalty (M = 15.37; $p > 0.05$) in relation to the employees in the service and administrative sectors, although this difference has not at the level of statistical significance (Table 1).

The results of the analysis (Table 1) showed that professional military personnel had not only more pronounced affective aspect (M = 12.67; $p < 0.01$) of organizational commitment, but also the normative aspect (M = 20.40; $p < 0.01$) compared to the employees in civil service and administrative sectors. It is evident that the professional members of the military had the most emphasized organizational commitment – total score, compared to those employed in civil service and administrative sectors, but this difference was not a statistically significant.

With a view to checking whether the professional military personnel may be discriminated against the employees of the civil sector on the basis of components of organizational identification, commitment, and organizational orientation, we applied the method of canonical discriminant analysis (Table 2).

Applying canonical discriminant analysis 2 functions that discriminated different groups of employees were signed out. The results showed that on the basis of organizational identification, organizational commitment and organizational orientation of employees was possible to discriminate well against members of the military profession with a canonical correlation of 0.393 forklifts. Specifically, in Table 3 it can be seen that the first allocated function was a characteristic of professional military personnel. In particular, on the negative side of the discriminant functions were the employed in the civil sector, while the positive pole was characterized by professional members of the army.

Table 1

Organizational identification, commitment and orientation among professional army staff and workforce in service/administrative jobs

Characteristics	Type of employement (job)			F	*p*
	Professional army staff	Workforce in service jobs	Workforce in administrative jobs		
Organizational identification					
feeling of membership	13.6267	13.9333	13.1000	2.640	0.072
lojality	15.3867	14.9067	14.5733	2.067	0.128
perceptions of shared characteristics	18.9867	18.7067	17.0067	17.512	0.000
general score	48.0000	47.5467	44.6800	6.407	0.003
Organizational commitment					
affective	12.6667	12.1400	11.4467	6.275	0.002
continuance	14.5133	14.3600	15.0600	1.536	0.216
normative	20.4067	19.1333	18.0400	7.384	0.001
general score	47.0600	46.1600	44.5467	2.395	0.092
Organizational orientation					
upward mobile	39.4333	40.120	38.4867	3.198	0.042
ambivalent	18.8867	20.0800	21.8400	6.612	0.001
indifferent	23.4200	24.8000	25.4400	3.605	0.028

F – test; $p < 0.05$ considered significant.

Table 2

χ^2 of canonical discriminant functions

Function	Eigenvalue	Canonical R	Wilks	χ^2	Df	*p*
1	0.182	0.393	0.760	121.57	18	0.000
2	0.132	0.318	0.899	47.32	8	0.000

Df – degrees of freedom.

Table 3

Functions at group centroids of canonical discriminant functions

Type of employment (job)	Function I	Function II
Professional army staff	0.579	- 0.131
Workforce engaged in service jobs	- 0.145	0.459
Workforce engaged in administrative jobs	- 0.433	- 0.329

*** Unstandardized canonical discriminant functions evaluated at group means.**

Table 4 shows the matrix structure of isolated discriminatory functions. As it can be seen from the Table 4 belonging to professional military service was best defined by the experience of similarity (identification) and loyalty, as 2 aspects of organizational identification, a high score on the affective commitment and low expression of commitment staying-in-organization commitment, as 2 dimensions of organizational commitment and the absence of ambivalent and indifferent organizational identification.

The second discriminatory function discriminated well the employees in the service sector against the rest of respondents, whereby the pronounced normative commitment and organizational orientation towards the advancement in the hierarchy are the key variables on the basis of whose intensity discrimination can be made.

Especially appealing was the finding (Table 5) that with the given model belonging to and engagement in the professional military service can be accurately predicted with 55%.

In other words, the presence of high identification with colleagues from a military organization, high loyalty, and high affective commitment to the military organization, as well as low intensity of staying-in-the-organization commitment and the lack of ambiguous and indifferent organizational orientation, all of which were highly desirable attributes of the employees, provided a very good basis on which, in more than a half of the cases, can be accurately estimated that an individual is a member of professional military service.

Discussion

The military organization of each country, especially its structure, organization, and functioning, is undoubtedly important for stability, security and prosperity of the entire society. Technological equipment and structural components of the military organization are certainly important in terms of defining its strength and power, but human resources themselves are undoubtedly the key of competitive advantages in the light of the contemporary trends, not only in the environment and the region but also in the global sociocultural trends. These trends are supported by the statements

Table 4

Structure matrix of canonical discriminant functions

Variables	I function	*p*
Perceptions of shared characteristics (aspect of OI)	0. 527*	< 0.05
Continuance commitment	-0.387*	< 0.05
Organizational orientation_ambivalent	-0.387*	< 0.05
Organizational orientation _indifferent	-0.297*	< 0.05
Loyalty (aspect of OI)	0.223*	< 0.05
Affecitve commitment	0.194*	< 0.05
	II function	
Normative commitment	0.406*	< 0.05
Organizational orientation_upward mobile	0.332*	< 0.05

***Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions; OI – organizational identification.**

Table 5

Classification results – canonical discriminat analysis

Type of job	Predicted group membership			Total
	Workforce in service jobs	Workforce in administrative jobs	Professional army staff	
Original count (%)				
workforce in service jobs	79	42	29	150
workforce in administrative jobs	43	81	26	150
professional army staff	36	27	87	150
workforce in service jobs	52.7	28.0	19.3	100.0
workforce in administrative jobs	28.7	54.0	17.3	100.0
professional army staff	24.0	18.0	58.0	100.0
Cross-validated count (%)				
workforce in service jobs	74	47	29	150
workforce in administrative jobs	44	76	30	150
professional army staff	37	30	83	150
workforce in service jobs	49.3	31.3	19.3	100.0
workforce in administrative jobs	29.3	50.7	20.0	100.0
professional army staff	24.7	20.0	55.3	100.0

of different foreign, but as well as, national authors and researchers [1, 2].

Through the research conducted and presented in this article, it was tested to what extent the organizational identification, organizational commitment and organizational orientations present in professional military personnel in comparison to the personnel employed in the civil sector, as well as whether it is possible, the basis of these variables, to discriminate professional military personnel against the employees in civil service and administrative sectors.

The results showed that professional military personnel are characterized by a high degree of both organizational commitment and organizational identification, compared to the employees in the civil sector - service and administrative activities. Especially through the process of canonical discriminatory analysis, it was concluded that professional military personnel are characterized by a high level of identification with colleagues and a high degree of loyalty to the military organization, as key aspects of organizational identification. In addition, professional military personnel have a strong affective commitment to the organization, and on the other hand, not so prominent commitment to staying in the organization. These findings are clearly intriguing if we bear in mind that it is a precisely affective commitment that represents the desire of an individual to remain in the organization because of his/her emotional attachment and identification with organizational goals and values. On the other hand, by the commitment to stay in the organization actually explicates the employee's awareness of the price of leaving the organization, that is, the perception that there are accumulated investments on the side of the organization that could be lost if one leaves the organization (benefits – salaries, promotion, social networks, and contacts, etc, specific to a particular organization). Exactly this kind of results underpin each other, bearing in mind that identification with colleagues – as an aspect of organizational identification – is actually a perceived similarity related to the common characteristics with the other members of the military organization, and in addition to, respect for the common (shared, or military organization's) values or goals [14, 20, 21] and expressed loyalty to the organization and enthusiasm tied to the organizational goals. In line with this is also the fact that professional military personnel have low ambivalent commitment to the organization – which in fact is characterized by a lack of orientation of the organizational system as such, so the need "not to play the role for the organization" but "to play the role for the personal preferences and goals", which frequently leads to the discrepancy between an individual employee and the organization. In addition, it is extremely suitable that the indifferent organizational orientation is not a characteristic of the professional military personnel, because it is characterized by a low level of identification and low expectations of an employee from the organization, and thus frequent "avoiding" to participate in the achievement of organizational goals, values, and norms.

Conclusion

The high degree of affective organizational commitment, but also pronounced organizational identification in terms of identifying with other members of the army and loyalty to the military organizational values and goals are an apparent basis upon which, in the forthcoming period, a stable, and above all, powerful military organization can be built and developed, considering that exactly the human resources are the key and essential factor of the competitive advantage in context of strong competitiveness in the field of military defense reality. If taken into account that human resources are more adaptable and flexible, compared to technological structural resources, exactly they can be a crucial factor of the success and progress of the military organization in the conditions of more frequent necessity for changes, innovations and adapting to turbulent and intense global social circumstances.

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GENERAL REVIEW

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Surgical treatment of osteoporotic fractures

Hirurško lečenje osteoporotičnih preloma

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Keywords:
osteoporosis; fractures, bone; orthopedic procedures; spinal injuries; hip fractures; radius fractures; reoperation.

Ključne reči:
osteoporoza; prelomi; ortopedske procedure; kičma, povrede; kuk, prelomi; radijus, prelomi; reoperacija.

Introduction

The world's older population continues to grow at an extraordinary rate. Thus orthopedic and traumatology surgeons are faced with an ascending rate of osteoporotic fractures. Mechanism of injury in osteoporotic fractures is presented as low energy trauma or as a spontaneous occurrence. Fixation of these fractures is considered as a challenge and there are several implant types used for this purpose – endoprosthetic implants, locking plates with screws and redesigned intramedullary nails for different types of fractures [1]. External fixation also has a role in the treatment of osteoporotic fractures. Authors of this paper have a good experience with the use of Mitkovic type Selfdynamysable Internal Fixator, especially in the treatment of osteoporotic proximal, diaphyseal and distal femoral fractures and of periprosthetic femoral fractures after hip or knee arthroplasty [2–5].

Osteoporosis

Osteoporosis is defined as an asymptomatic disease and it is the main risk factor in the fracture occurrence. Osteoporosis is described as a change of bone tissue structure quality and as a reduction of bone mass. There is a reduction of the bone tissue volume (bone mass) relative to the total bone volume, under the level required for normal bone function. The reduction of mineralized tissue is presented as a bone cortex thinning, decreasing number and volume of cancellous bone trabeculae and as the quantitative osteoblasts reduction. More than 10 million people from the United States have osteoporosis with T-score of -2.5 standard deviations (SD). About 34 million people have the osteopenia with T-score between -1 SD and -2.5 SD. More than 1.5 million fractures are caused by osteoporosis every year and more than 70% cases of osteoporosis are female patients. The basic mechanism of the bone homeostasis control is still unknown, but it is supposed that several factors are taking a part there: genetic, hormonal, nutritional and other factors. From pathophysiological aspect, osteoporosis is presented as a dysregulated osteoblast-osteoclast interaction, resulting in a relative increase of bone resorption or in bone formation reduction.

Primary (idiopathic) osteoporosis is classified into two groups: Type 1 (postmenopausal) osteoporosis associated with estrogen deficiency and Type 2 (senile) osteoporosis occurring both in male and in female patients after the age of 70 years. Secondary osteoporosis is presented as a consequence of some drugs, endocrinopathies, chronic illness, poor diet etc. Fracture occurred spontaneously or after low energy trauma is the main clinical sign of osteoporosis. Typical osteoporotic fractures are presented as hip fractures (femoral neck fractures and transtrochanteric fractures), vertebral compression fractures and fractures of the distal radius. There is another group of fractures resulting from the action of low energy trauma in elderly patients with osteoporosis: proximal humeral fractures, subtrochanteric fractures, pelvic fractures, fractures of the distal femur, tibial plateau fractures, distal tibial and ankle fractures. Treatment of the osteoporosis is of great importance for the prevention of above-mentioned fractures. In most clinical cases, patients who have osteoporosis are firstly observed by an orthopedic surgeon due to the fracture presence. Parallel with improvements in drugs treatment of osteoporosis, new implants are being developed for the fixation of osteoporotic fractures. Hence there are locking plates,

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intramedullary implants, and artificial joints. The gold standard in the biological stimulation of bone healing is cancellous autograft application. Autograft has an osteoconductive and osteoinductive effect and it is a source of pluripotent osteoprogenitor cells. Poly-methylmethacrylate (PMMA) cement may be useful if the fixation of osteoporotic bone has been compromised. Fixation failure before fracture healing, due to a poor bone quality, is the main problem in surgical treatment (osteosynthesis) of osteoporotic fractures. This situation requires a reosteosynthesis having a risk for later fracture nonunion and permanent functional problems [1, 6–9].

Vertebral compression fractures

American Academy of Orthopaedic Surgeons reports over 700,000 of compression fractures annually. The incidence is increasing from 26% in female patients older than 50 years to 80% in female patients older than 80 years. Osteoporosis is reported as the most common cause of compression fracture occurrence (85%), but a malignant disease with bone metastasis also can cause these fractures (15%). There is the clinical presence of back pain, limited mobility of the spine, weight loss, spine deformity (kyphosis) and functional disability. Non-operative treatment is based on non-steroid analgesics, bed-rest, and physical therapy. Many authors recommend orthosis (orthopedic corsets) in patients with severe posttraumatic deformity (kyphosis). Surgical treatment includes percutaneous vertebroplasty using bone cement, kyphoplasty and vertebroplasty combined with posterior instrumentation (transpedicular vertebral fixation above and below the fracture site) (Figure 1). Transpedicular fixation with minimally invasive percutaneous technique is performed in recent years. Spinal cord compressions should be treated by posterior decompression (laminectomy). To avoid fixation loss, transpedicular screws are attached to the vertebral body by the cement augmentation. To stabilize the fracture in patients who have a neurological deficit (paraparesis or potential paraplegia) caused by spinal cord injury in compression fractures corpectomy of the fractured vertebral body using anterior instrumentation, spinal cord decompression, and special titanium meshes or artificial vertebral bodies implantation can be performed [10, 11]. Several dozen patients with osteoporotic fracture of the vertebral body are hospitalized every year at the Clinic for Ortopedics and Traumatology in Clinical Center of Niš. Their treatment is usually nonoperative – bed rest, analgesics, osteoporosis treatment and physical therapy.

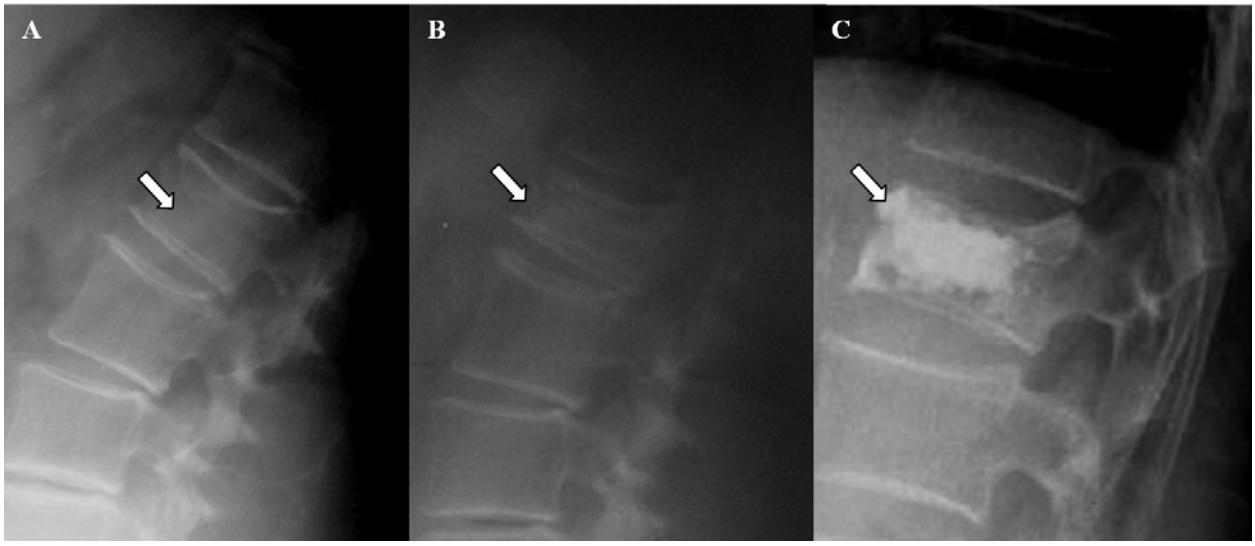

Fig. 1 – A) and B) Radiography of spontaneous vertebral compression fracture, without trauma; C) Radiography after vertebroplasty.

Hip fractures

Hip fractures are encountered in everyday orthopedic practice. These fractures are often present in the elder population, older than 65 years, as the consequence of osteoporosis and it more often occurs in female patients. Age distribution of hip fractures is also referred to a fault of motor coordination, eyesight quality, standing balance and protective reflexes, neurological disorders and other factors unrelated to the osteoporosis but related to the aging process. There is also a disbalance referred to the relation between hip load during the gate and maximal force that a bone may be applied on and not to be broken. When a bone is weak by osteoporosis process the fracture can be induced by an indirect force as the result of uncoordinated and excessive muscles contraction. Protective factors are slower and more careful gait and weak muscle tone, in elders, and stronger bones, in the younger population. With a longer average human life, today hip fractures have become a major problem in modern civilization. Patients with this type of trauma occupy about 30% of hospital beds in orthopedic institutions and their treatment is spending a significant amount of economic resources. Hip fractures are much more than a medical problem and they affect both family and the whole society. Around 340 thousands of hip fractures are treated annually in the United States. It is supposed that there would be around 650 thousands of annual hip fractures by the year of 2050. About 20 billion dollars (40 thousand dollars *per* fracture) are spent for the

treatment of these fractures annually. At the world level, there are around 1.6 million of annual hip fractures and it is supposed that it will be around 6.3 million of annual hip fractures by the year 2050. More than 90% of patients with hip fractures are older than 65 years. The average age of the population in Serbia is 42 years and 17.3% of the population is older than 65 years. Our country is considered as the country with a very old nation. Several thousand patients with hip fracture are hospitalized every year in Serbia with a mean annual incidence of 51.7 *per* 100,000 adults [12]. A significant amount of Serbian Health Fund resources is spent for the treatment of patients with hip fracture. In the period from the year 2005 to 2010, there were 1,806 patients hospitalized with hip fracture at the Clinic for Orthopedics and Traumatology in the Clinical Center Niš. The average age of patients was 73.5 years and the osteoporosis was found to be the cause of 90% of these fractures. Hip fractures are considered as injuries at risk for life. It can also decrease the quality of life if it is not properly treated. Most of these patients are in very old age and two-thirds of them are suffering from cardio-vascular, respiratory, cerebral, endocrine, genitourinary and other diseases. This type of trauma is often followed by an acute exacerbation of existing disease resulting in a high rate of mortality (15–35%). More than 4% of patients with a hip fracture die during their initial hospitalization, while 10–35% die within the first year of the fracture occurrence. Many patients with hip fracture are not able for an independent life and they need the help from family or they go to an institution specialized for elders help. These patients can not return activity levels they had before the injury. There are also dementia presence or depression signs in many patients with hip fracture, worsening their condition and affecting their quality of life. Hip fracture treatment depends on the type of the fracture. Displaced femoral neck fractures are treated surgically – arthroplasty, implanting a partial or total hip endoprosthesis (Figures 2 and 3). Transtrochanteric fractures are usually treated surgically by intramedullary nailing (Figure 4) or by other implants for proximal femoral fractures fixation (Dynamic hip screw, Selfdinamysable Internal Fixator by

Fig. 2 – A) Bipolar hip endoprosthesis; B) Total hip cemented endoprothesis.

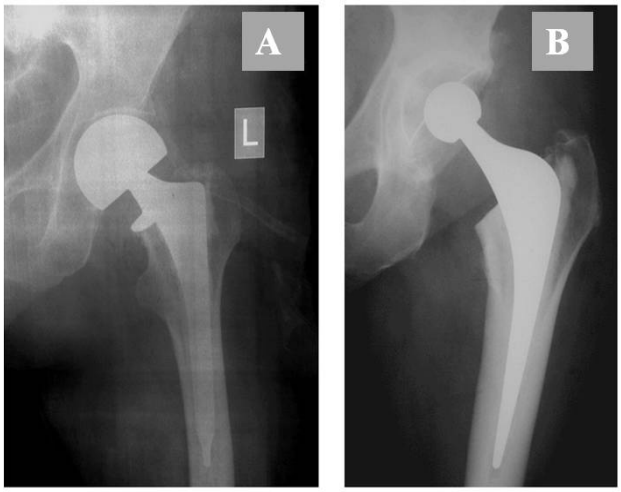

Fig. 3 – A) Radiography of bipolar hip endoprothesis; B) Radiography of total cemented hip endoprothesis.

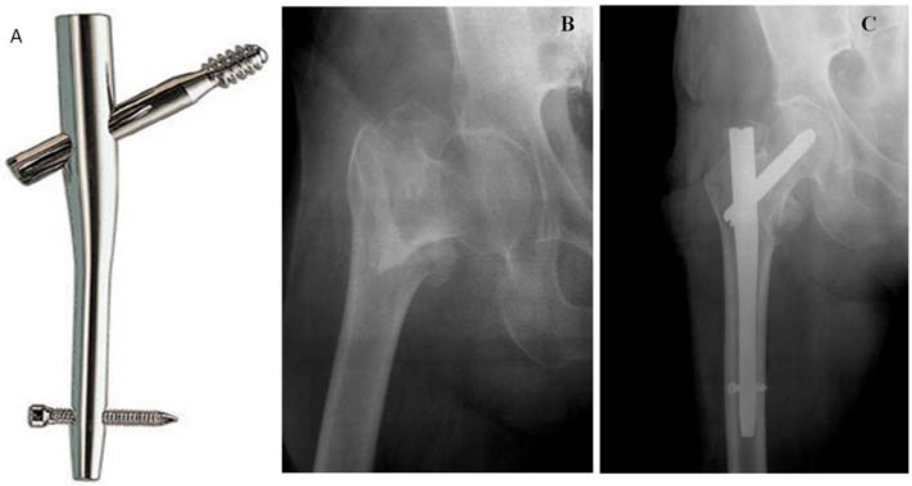

Fig. 4 – A) Gamma interlockin nail; B) Radiography of transtrochanteric fractures; C) Radiography after transtrochanteric intramedular osteosynthesis.

Mitkovic). Patients with transtrochanteric fractures are rarely treated by hemiarthroplasty or by total arthroplasty. In addition to surgical treatment of hip fractures, it is necessary to provide medical treatment of osteoporosis to avoid refracture in the contralateral hip or in another part of the body. Such cases are not rare in orthopedic practice. There is a group of patients with periprosthetic femoral fractures at the upper part of the femur, around the top of endoprosthetic stem or below in supracondylar part of the femur. Periprosthetic femoral fractures occur most often by an effect of low energy trauma, in elderly patients with osteoporosis who have had a hip fracture and afterward hip arthroplasty (Figures 5 and 6). Treatment of these fractures can be very complex and there are different options, from a simple fracture fixation to the replacement of endoprosthesis followed by fracture fixation. Osteoporotic femoral fractures may also occur after a knee arthroplasty as periprosthetic fractures. The treatment of these fractures can also be very complex and it consists of osteosynthesis or revision knee arthroplasty with or without osteosynthesis [8, 9, 13–19].

Fractures of the distal radius

Fractures of distal radius account for 20% of all fractures observed in emergency departments. This is the most common fracture type in our daily orthopedic practice. Distal radial fractures occur primarily after falling on the wrist of the stretched arm, usually as the effect of low energy trauma on osteoporotic bone. These fractures are most often in women at middle age, with an increasing incidence just after menopause. These fractures are for men most common under 70 years of age. The main clinical signs are a pain, swelling, and characteristic deformity. These fractures are treated surgically or nonsurgically. Nonsurgical treatment consists of orthopedic reduction and plaster cast immobilization and it is recommended for stable fractures. Unstable fractures are treated surgically and it is performed by open surgical reduction and fixation (osteosynthesis) with locking plates and screws (Figure 7). Closed reduction – ligamentotaxis and external fixation with or without minimal internal fixation with Kirschner wires is also a solution and gives good results (Figure 8) [20–23]. Several hundred patients with fracture of the

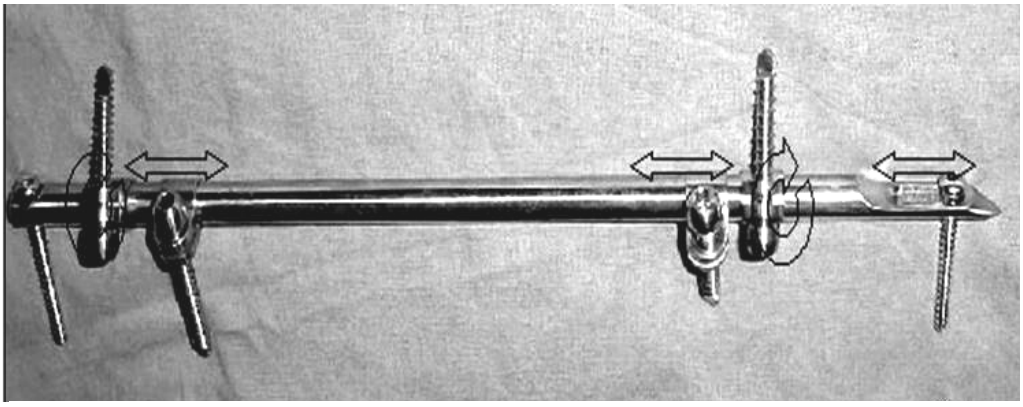

Fig. 5 – Selfdinamisable internal fixator according to Mitkovic.

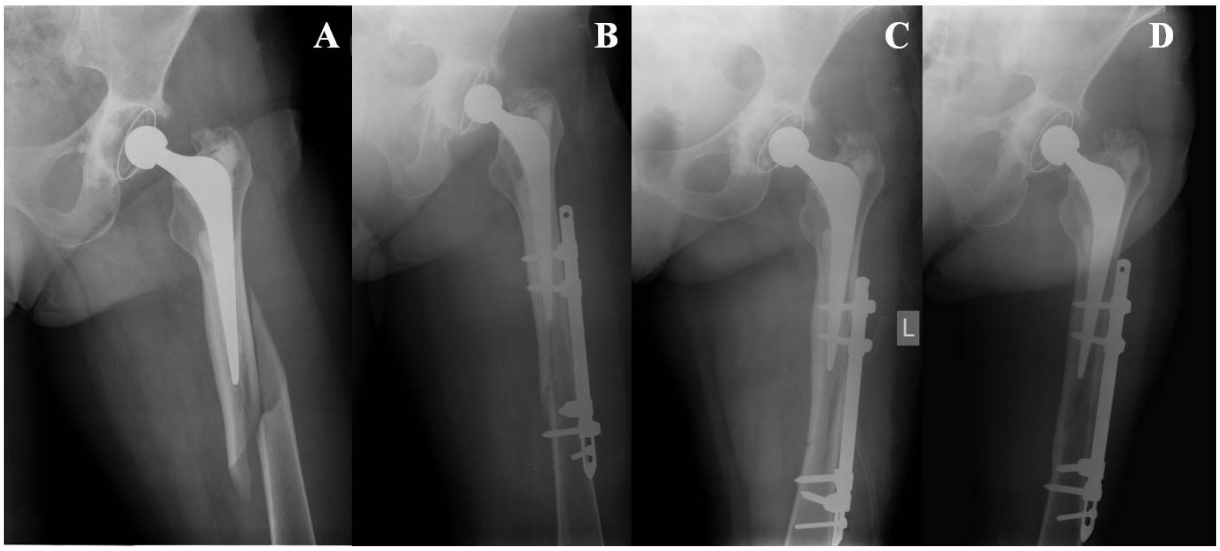

Fig. 6 – A) Radiography of periprosthetic femoral fractures; B) Radiography after periprosthetic femoral fracture osteosynthesis; C) Radiography three months after osteosynthesis; D) Radiography one year after osteosynthesis.

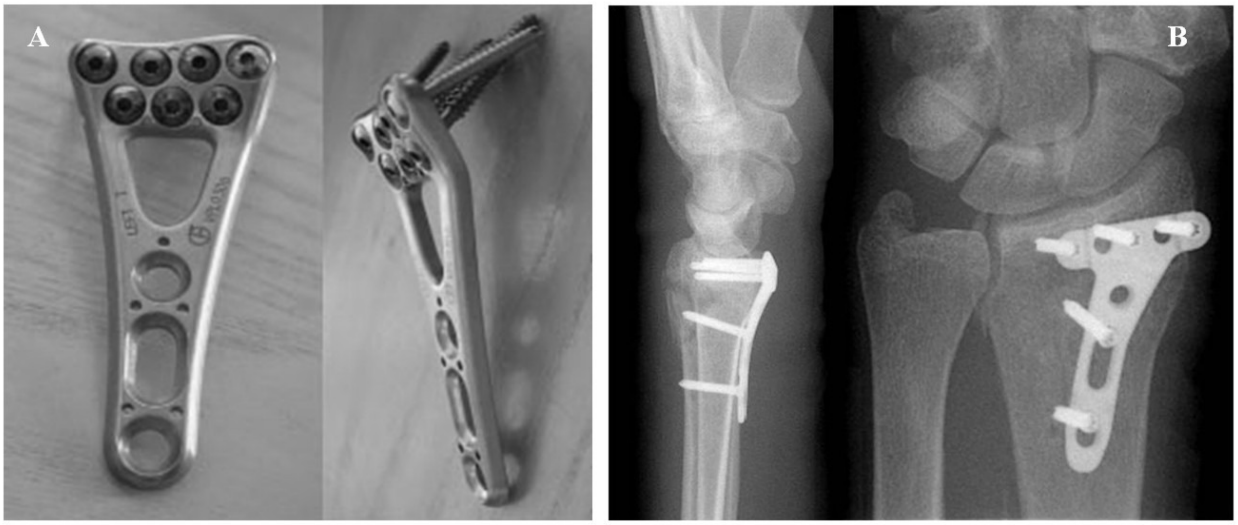

Fig. 7 – A) Locking compression plate for distal radius osteosynthesis; B) Radiography after distal radius osteosynthesis.

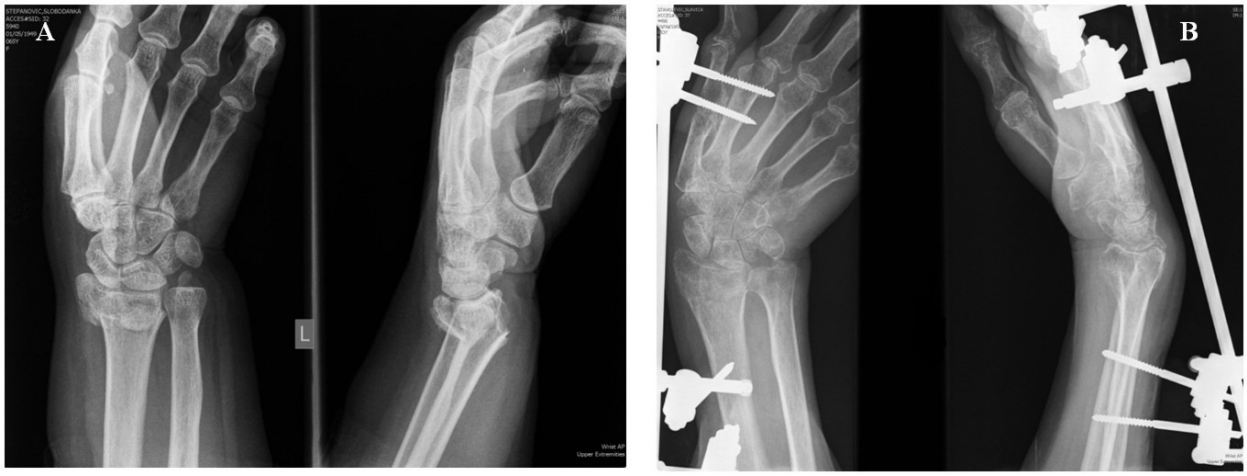

Fig. 8 – A) Radiography of distal radius fracture; B) Radiography after external fixation of distal radius fracture.

distal radius are treated every year at Clinic for Orthopedics and Traumatology in Clinical Center Niš. About 30% of patients are treated surgically – open reduction and internal fixation or closed reduction (ligamentotaxis) and bridging external fixation with or without additional K-wires**.**

Conclusion

The main clinical presence of osteoporosis is a bone fracture. Despite actual pharmacotherapy of osteoporosis, more and more of osteoporotic fractures occur every year and it becomes a major orthopedic problem. Contemporary orthopedic implants used in surgical treatment of osteoporotic fractures provide high-quality fixation with lower risk for mechanical complications or nonunion.

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CASE REPORTS

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A case of eclampsia complicated by cerebral haemorrhage and iatrogenic hypopharyngeal oedema and haemathoma

Eklampsija komplikovana cerebralnom hemoragijom, jatrogenim hipofaringealnim edemom i hematomom

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Abstract

Introduction. Eclampsia, serious complication of preeclampsia, can further be complicated by intracranial haemorrhage. Cesarean section under general anesthesia represents an additional risk factor. **Case report.** We present a case of 22 years old primipara in the 38th gestational week who after a sudden occurrence of a headache, within one hour developed eclampsia. Emergent Caesarean section was undertaken; she was intubated after several attempts. Severe tongue and hypopharyngeal edema and haemathoma made the extubation impossible; she remained intubated, sedated, mechanically ventilated, on anti-oedematous, anticonvulsive, antihypertensive therapy. On the third postoperative day, tracheostomy was performed. On the sixth day, she complained of a headache and visual disturbances. Neurological examination revealed left-sided hemiparesis. Multislice computed tomography showed intracranial hemorrhage. It was not until the closure of tracheostoma (eleventh day) that her blood pressure normalized and the headache ceased. Four days later she was dismissed from the hospital with improved clinical state. **Conclusion.** In order to avoid sudden and unexpected, but serious complications of preeclampsia/eclampsia, we emphasize the need of searching for more subtle signs of the disease, of prompt radiologic diagnosis and aggressive blood pressure control, with a prepared strategy for difficult airway management.

Key words:
eclampsia; intubation, intratracheal; treatment outcome; intracranial hemorrhages; iatrogenic disease.

Apstrakt

Uvod. Eklampsija, teška komplikacija preeklampsije, i sama može imati komplikacije; intrakranijalna hemoragija je jedna od najozbiljnijih. Carski rez je dodatni faktor rizika. **Prikaz bolesnika.** Prikazali smo dvadesetdvogodišnju prvorotku, u 38. nedelji trudnoće, kod koje je, nakon iznenadne pojave glavobolje, unutar jednog sata došlo do razvoja eklampsije. Urađen je hitan carski rez. Pacijentkinja je intubirana nakon više pokušaja. Zbog izraženog traumatskog edema i hematoma hipofarinksa i jezika, po završetku operacije ekstubacija je bila nemoguća; ostala je intubirana, sedirana, na mehaničkoj ventilaciji, uz antiedematoznu, antikonvulzivnu i antihipertenzivnu terapiju. Trećeg postoperativnog dana načinjena je traheostoma. Šestog dana pacijentkinja se požalila na glavobolju i smetnje vida. Utvrđena je levostrana hemipareza. Multislajsnom kompjuterizovanom tomografijom utvrđena je intrakranijalna hemoragija. Tek po zatvaranju traheostome, jedanaestog dana, arterijski pritisak se normalizovao i glavobolja je prestala. Četiri dana kasnije otpuštena je sa Klinike u poboljšanom opštem stanju. **Zaključak.** Da bismo predupredili iznenadni nastanak teških komplikacija preeklampsije/eklampsije, potrebno je tragati za suptilnijim znacima bolesti, obaviti brzu neurološku dijagnostiku, agresivno lečiti hipertenziju i pripremiti strategiju za slučajeve otežane intubacije.

Ključne reči:
eklampsija; intubacija, endotrahejna; lečenje, ishod; krvarenje, intrakranijalno; jatrogena bolest.

Introduction

Eclampsia, defined as the presence of new-onset grand mal seizures in a women with preeclampsia, that cannot be explained by another cause [1, 2], is one of the most serious complications of hypertensive disorders of pregnancy [3–5]. It complicates 0.05–0.3% of all pregnancies [6] and 2–3% of cases of preeclampsia, with the maternal mortality of 1.8–14% [2, 7, 8]. Eclampsia itself could be complicated by several serious conditions, like ischemic and hemorrhagic stroke, with estimated maternal mortality of 10–13% [9]. It has been shown that intracerebral haemorrhage occurs in 9 of

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100,000 deliveries; 25–45% of that number is associated with preeclampsia/eclampsia, due to hypertension, endothelial damage and disturbed cerebral autoregulation [9, 10]. The delivery by Caesarean section is also associated with 2–12 times increased risk of postpartum stroke [9], partly because of intracranial pressure elevation, due to exaggerated neuroendocrine and cardiovascular stress response to endotracheal intubation and surgical incision [11–13].

We presented a case of eclampsia complicated by intracranial hemorrhage and hypopharyngeal edema and hematoma, as a consequence of intubation attempts during induction to anesthesia for the urgent Caesarean section.

Case report

A 22-year old primipara was admitted to our intensive care unit (ICU) sedated, intubated, mechanically ventilated, under the diagnosis: *Graviditas m.l.* IX1/2. *Status eclampticus. Haemorrhagio regio hypopharyngis traumatica. Status post sectionem caesaream isthmicotransversalis sec* Dörfler. Her family members claimed that she was healthy until the day of the delivery. She regularly visited her gynaecologist and her pregnancy passed uneventfully. At the beginning of pregnancy, her blood pressure (BP), was 90/65 mmHg, at the second trimester it was 100/60 mmHg and BP of 120/80 mmHg was measured two weeks before the delivery. During the pregnancy, she gained 20 kg. This morning she complained about a headache that was not taken seriously, so she was left alone in the house. After 30 min she was found unconscious on the floor and was immediately transferred to her hometown hospital. At the admission, she was in *status eclampticus*. Emergent Caesarean section was indicated and within 10 min she was on the operating table. BP measured at that moment was 140/100 mmHg. Because of large facial, tongue and neck edema, endotracheal intubation was extremely difficult – she was intubated after five attempts. The rest of the operation passed uneventfully. The parturient gave birth to live female child, 3,500 g /55 cm. At the end of the operation the extubation was impossible, due to hypopharyngeal edema and hemorrhage (the consequence of intubation attempts), so she remained intubated, on controlled mechanical ventilation. Her state was additionally complicated by the rise in BP to 220/130 mmHg and it was decided to transfer the patient to our Clinic.

At the admission to our ICU the patient, although sedated, reacted to painful stimuli and responded to call. Physical examination showed no pathological signs other than large facial, tongue and neck edema. Neurological and ophthalmological examinations were also normal. The laryngological examination was impossible. BP was 140/100 mmHg. After those initial examinations, we enhanced the sedation and continued with pressure controlled mechanical ventilation, FiO_2 – 0.4, that provided satisfactory oxygen saturation (94–96%). The patient was continuously monitored: BP, heart rate, oxygen saturation, capnography, temperature, diuresis. Venous blood samples were immediately taken for hematological and biochemical analyses and were repeated every day during her stay at our hospital (Table 1).

Initial laboratory results out of reference ranges were as follows: white blood cells (WBC) 19 × 10^9/L, [94.1% neut-

Table 1

Laboratory results during hospitalization.

Analyses	day 1	day 2	day 3	day 4	day 6	day 15
Glycaemia (mmol/L)	7.3	4.5	3.6	5.3	5.1	4.1
BUN (mmol/L)	1.5	1.9	2.5	2.5	3.4	2.4
Creatinine (μmol/L)	55.5	71.2	79.3	65.7	52.6	51.0
Urate (μmol/L)	461	420	315	126	155	135
Bilirubin total (μmol/L)	8.0	7.0	5.2	7.4	8.1	6.0
Bilirubin direct (μmol/L)	1.7	1.3	1.2	1.4	1.7	0.8
Proteinaemia (g/L)	51.7	52.0	44.2	54.4	59.3	63.0
Albuminaemia (g/L)	26.9	26.2	24.2	31.5	31.8	32.0
AST (U/L)	43.8	58.6	25.2	27.7	30.1	29.2
ALT (U/L)	15.7	24.3	15.6	14.4	14.3	13.5
LDH (U/L)	866.2	990	811	833	1012	595
Fe (μmol/L)	18.8	17.2	10.2	10.9	7.66	18.6
Na (mmol/L)	136	136	141	140	141	140
WBC (x 10^9/L)	19.0	20.2	15.7	16.4	14.8	9.9
Ne (%)	94.9	89	86	87	82	73.3
RBC (x 10^{12}/L)	4.39	4.08	2.65	3.57	3.99	4.18
Hb (g/L)	137	126	81	106	115	126
Ht (%)	38	36	24	33	35	37
PLT (x 10^9/L)	155	184	152	167	225	228
PT (s)	12.8	13.5	12.3	12.2	12.3	12.5
INR	0.913	0.968	0.897	0.865	0.916	0.899
APTT (s)	24.2	25.1	25.9	26.6	24.7	24.9
Fibrinogen (g/L)	7.2	6.8	5.9	6.6	7.3	5.9
D-dimer (ng/mL)	3,929	3,000	2,500	2,143	1,751	870
Proteinuria	ns	ns				

BUN – blood urea nitrogen; AST – aspartate transaminase; ALT – alanine transaminase; LDH – lactate dehydrogenase; Fe – ferrum; Na – sodium; WBC – white blood cells; Ne – neutrophils; RBC – red blood cells; Hb – hemoglobin; Ht – hematocrit; PLT – platelets; PT – prothrombin time; INR – international normalized ratio; APTT – activated partial tromboplastin time; ns – non significant.

roplies (Ne)]; lactate dehydrogenase (LDH) 866 U/L; proteinemia 51.7 g/L; albuminemia 26.9 g/L; fibrinogenemia 7.2 g/L; D dimmer 3,929 ng/mL.

We immediately started: antiedematous therapy (solution of Mannitol 20% 125 mL/6 h, Dexamethasone 4 mg/8h); anticonvulsive, sedative, analgesic therapy (solution $MgSO_4$ 1 g/h, slow midazolam infusion if needed, remifentanil 100–200 μg/h); antihypertensive (urapidil 25 mg boluses and 10–100 mg/h infusion; followed by captopril 12.5 mg/8 h and Nifedipine 20 mg/12 h, when peroral application became possible); antibiotic, uterotonic, anticoagulant and substitutional therapy according to laboratory results (crystalloid and amino-acid solutions, 20% albumin solution, solutions for parenteral and enteral nutrition).

During the hospitalization, BP was 130/70 to 160/110 mmHg.

After partial reduction of the oedema, on the third day of hospitalization, laryngeal examination became possible. *Haemathoma et oedema radicis linguae et hypopharyngis* was diagnosed and tracheostomy was indicated and performed. After that intervention we started weaning the patient from the ventilator, so the next morning she was conscious, oriented, breathed spontaneously. Neurologic examination showed no pathological signs. During next three days we mobilized the patient and started enteral nutrition.

On the 6th postoperative day, the patient complained of a headache and visual disturbance, but ophthalmologic examination did not reveal any pathological changes. Since the symptoms persisted, the examination was repeated the next day. The diagnosis was *Oedema papillae nervi optici bill*. *Papilla stagnans*. Discrete left-sided hemiparesis was found on neurological examination. Endocranial multislice computed tomography (MSCT) was indicated and showed intracranial hemorrhage (46 × 23 mm) in right parietooccipital cortical brain area (Figure 1). Digital subtraction angiography (DSA) of the right carotid artery in anterior-posterior (AP) (Figure 2a) and latero-lateral (LL) (Figure 2b) projection revealed normal angiographic findings. A neurosurgeon advised the conservative treatment. During next two days, we continued with antiedematous, sedative and antihypertensive therapy. Our patient was very cooperative, but at the same time very tense, so it was not easy to control her BP. A headache persisted. On the

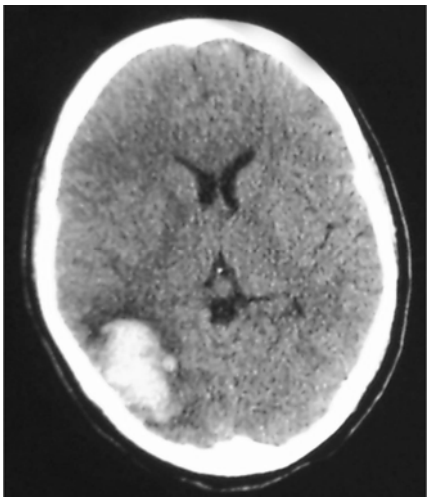

Fig. 1 – Computed tomography (CT) examination in axial cross-section showing intracerebral haematoma in the occipital lobe of the right cerebral hemisphere. Present oedema of the right cerebral hemisphere.

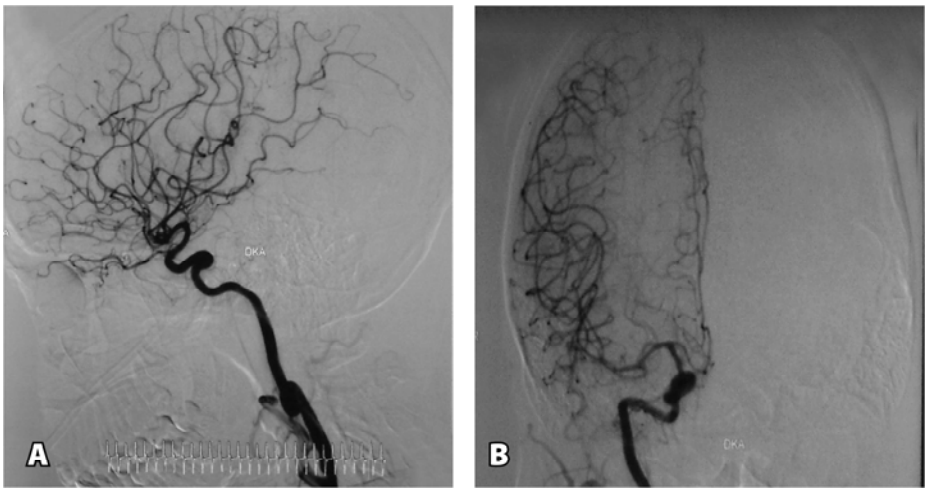

Fig. 2 – A) Digital subtraction angiography (DSA) of the right carotid artery in arterior-posterior (AP) and B) latero-lateral (LL) projection reveal normal angiographic findings.

8th postoperative day, after a termination of gynecological treatment and normalization of laboratory parameters, she was transferred to the Clinic for the Neurological Diseases. The same therapeutic regime was applied, but it was not until the closure of tracheostoma (11th postoperative day) that her BP returned to normal values and headache ceased. On the 15th postoperative day, she was dismissed from the hospital with improved clinical state but with persistent discrete left-sided hemiparesis.

Discussion

Besides the cases of eclampsia with the typical clinical course (occurring between the 20th gestational week and 48 h after delivery, and progressing from mild to severe preeclampsia, culminating in eclamptic seizures), there is growing evidence of atypical forms of the disease regarding the time of onset and clinical course [5, 14, 15]. Hypertension is the hallmark of the disease (systolic BP more than 140 mmHg or diastolic BP more than 90 mmHg on two occasions at least 4 hours apart after 20 weeks of gestational age, or systolic BP more than 160 mmHg or diastolic BP more than 110 mmHg confirmed within a short interval [1, 13]), but BP elevation over 160/110 mmHg was registered in only 25–54% of eclampsia cases; proteinuria was absent in 16–40% of cases; there were cases without both hypertension and proteinuria, where the diagnosis was made based on symptoms of increased capillary leak (ascites, pulmonary oedema, pleural effusions, large facial and hand edema) or disturbed hemostasis [4, 7, 16–19]. In 2013 the American Congress of Obstetricians and Gynecologists (ACOG) Task Force Report proteinuria was excluded as necessary sign for the diagnosis of preeclampsia; the diagnosis can be made based on a presence of high BP and any of following severe features: thrombocytopenia, renal insufficiency, impaired liver function, pulmonary oedema or cerebral/visual symptoms [1, 13]. Persistent occipital /frontal headache, sudden BP elevation, visual disturbances, nausea, vomiting, restlessness, hyperreflexia, altered mental status, sudden development of facial and hand edema, right upper quadrant and epigastric pain are typical symptoms and signs that precede the occurrence of eclampsia, but in 20% of cases eclampsia starts suddenly, without prodromal signs [4, 14, 17, 18, 20–22].

According to anamnesis, our patient was always healthy; her pregnancy was controlled and passed uneventfully. The highest BP value, measured two weeks before the delivery, was 120/85 mmHg, which is below the threshold value for the diagnosis of a hypertensive disorder of pregnancy [1]. The only thing that could arouse suspicion was the elevation of systolic and diastolic pressure from the baseline value measured before the 20th week of gestation of 30 and 15 mmHg respectively, which are also the criteria that could refer to a hypertensive disorder of pregnancy [23, 24]. There was a weight gain of 20 kg, much greater than recommended in pregnancy (12 kg). It seems that we have to be aware, in our clinical practice, of the existence of those rather subtle changes and signs; searching for them could help us avoid more serious complications. In case of our patient, progression from first complain of a headache to eclampsia was indeed very fast. It is emphasized in the literature that the rapidity of BP elevation could be of great clinical relevance. A headache, a typical prodromal symptom of eclampsia as well as a symptom of stroke, should not be neglected. Transcranial Doppler ultrasound examinations of the mild cerebral artery revealed a strong association between a headache and abnormal cerebral perfusion pressure [2].

In eclamptic patients, according to the treatment protocol, we immediately started antioedematous therapy (solution Mannitol, dexamethasone). For the treatment and prevention of new eclamptic seizures, we used $MgSO_4$, as it is the first choice medication, useful also as sedative and antihypertensive agent [7, 14, 16, 17, 25–32]. Our main problem was BP regulation, complicated by the need for mechanical ventilation and, later, by fear, anxiety in awaken patient with tracheostomal cannula.

BP control is crucial in the treatment of eclamptic patients, not in removing the cause of the disease, but in preventing numerous complications: cardiac decompensation, pulmonary oedema, retinal ablation, ischemic or haemorrhagic stroke with subsequent neurologic and cognitive disturbances [14, 16, 22, 27, 33, 34]. In pregnancy, during BP elevation, there is reduced vascular resistance in small cerebral vessels, that raises blood-brain barrier permeability and enables the development of oedema and seizures; this is even more pronounced in cases of generalized endothelial injury, as in preeclampsia [14, 18, 35, 36]. This is why it is believed that in pregnancy, the acute BP elevation over 160/110 mmHg, persisting more than 15 min, represents hypertensive emergency [37], which demands a prompt intravenous antihypertensive treatment. Since the sudden reduction in BP could compromise fetoplacental perfusion, only 10% of BP reduction is recommended during the first hour and another 15% gradually over next 2–3 h [8, 14, 17, 38–42]. The long-term goal should be to keep BP at 140–150/85–90 mmHg.

In our hospital we do not have first choice medications for the treatment of hypertensive disorders of pregnancy (labetalol, hydralazine, nicardipine) and since we wanted to avoid nitroglycerine because of supposed elevation of intracranial pressure after the eclamptic attack, we chose urapidil. This arteriolar and venous α_1-receptor blocking agent reduces systemic vascular resistance and preload without reflex tachycardia and elevation of intracranial pressure [8, 29, 42]. When our patient woke up and was able to swallow, we started using nifedipine and captopril (as all angiotensin-converting enzyme (ACE) inhibitors, it is contraindicated in pregnancy, but allowed during lactation period) [8, 33, 43–47]. BP was not easy to control; in spite of our patient's patience and cooperation, together with sedative and analgesic support (although reduced, because of fear of respiratory depression), every manipulation (i.e. aspiration through tracheostomic cannula) led to significant BP elevation.

One explanation for the failure of antihypertensive therapy in certain patients with preeclampsia/eclampsia (that goes up to 42%), might be the existence of polymorphisms in genes that influence pharmacokinetics and pharmacodynamics of antihypertensive medications and lead to the resistance to conventional therapy. Individualized pharmacogenomic approach to the therapy could be the solution [48]. The understan-

ding of underlying pathophysiological mechanisms of preeclampsia should help to identify novel therapeutic targets. For example, it is well known that placental dysfunction provokes excessive production of antiangiogenic proteins, soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin, and a decrease of proangiogenic placental growth factor (PlGF). This imbalance, that leads to endothelial dysfunction, precedes the clinical manifestation of preeclampsia by weeks or even months, so measurement of serum (and urinary) concentrations of these proteins or, even more accurate, circulating sFlt-1/PlGF ratio or PlGF/soluble endoglin ratio in early pregnancy could predict the development of preeclampsia and adverse maternal and fetal outcome. sFlt-1/PlGF ratio could serve as a useful biomarker for diagnosis and differential diagnosis of preeclampsia in uncertain and atypical cases, aid the management of such pregnancies and monitor the new options for the treatment (administration of proangiogenic or removal of antiangiogenic factors) [1, 49, 50]. However, because of limited clinical experience, these biomarkers are still not included in most guidelines [1, 49].

Such clinical course raises another question: did intracranial haemorrhage occur during patient's hospitalization at our clinic, or was it a consequence of initial events? If we had done MSCT initially, at the day of the admission, we would perhaps have known the answer, but on the basis of initial examination, the neurologist did not think there was the reason to indicate radiologic imaging. Having in mind that therapeutic approach (based on clinical experience) is usually the same with and without radiologic examination (in majority of cases it turns to be posterior reversible encephalopathy syndrome with vasogenic oedema [14, 36], we have a dilemma – should every eclamptic patient undergo MSCT or magnetic resonance imaging (MRI), or should it be reserved for atypical forms of eclampsia, or for differential diagnosis in dubious cases, where it could help choosing appropriate therapeutic regime [4].

According to the recommendations in the literature, cerebral imaging should be performed in patients with sensory or motor deficits or prolonged coma [14, 51]. Hemiparesis and visual disturbance in our patient might have been masked by deep sedation during the period on mechanical ventilation (although neurologic examination did not reveal any pathological sign at that time). We reacted as soon as she was able to complain. The finding on MSCT posed diagnostic dilemma: was this initially haemorrhagic stroke or intracranial haemorrhage superimposed on eclampsia. Eclampsia and stroke share numerous risk factors (hypertension, coagulopathy, tobacco abuse, maternal age over 35) as well as signs and symptoms (a headache altered consciousness, seizures, focal neurologic or visual disturbances) [2, 52, 53]. Having hypertension and *grand mal* seizures (preceded by a headache), our patient met the criteria for preeclampsia with severe features [1], although her laboratory results revealed only mild elevation in serum concentrations of lactate dehydrogenase (LDH) and uric acid, without proteinuria and coagulation screen disturbances (except elevated D-dimer concentration). Our patient's clinical symptoms overlap with many clinical conditions, like idiopathic seizure disorder, cerebral venous or arterial thrombosis, cerebral vasculitis, angiomas, previously undiagnosed brain tumors, metabolic/toxic encephalopathy, thrombophillia, hemolysis, elevated liver enzyme levels, low platelet levels (HELLP) syndrome, thrombotic thrombocytopenic purpura [1, 51, 52, 54]. On a basis of anamnestic, laboratory and cerebral imaging findings we excluded these possibilities. On the other hand, cerebral aneurysms and artherio-venous malformations, as the most common causes of intracranial haemorrhage [1, 2, 53, 54], were not confirmed in MSCT. Having in mind that eclampsia represents the most common cause of intracerebral haemorrhage in pregnancy (89% of eclampsia related strokes were hemorrhagic [2, 55]), we presume that our case was the case of eclampsia complicated by haemorrhagic stroke. Since intracranial haemorrhage is one of the most important causes of eclampsia related maternal mortality [2, 10, 11, 54] and that uncontrolled acute hypertension is usually the trigger of disease; we emphasize again the importance of prompt and aggressive BP control [2, 9, 52, 53].

Presented case also illustrates already well known fact that pregnancy enhances the risk of difficult (1–6%) and failed (0.13–0.6%) intubation. Large airway edema, weight gain, enlarged breasts, changed Mallampati score, should always be kept in mind [56]. The situation is additionally complicated by reduced tolerance to apnea – a consequence of pregnancy induced reduction in functional residual capacity on the one hand, and raised metabolic rate and oxygen consumption on the other [56–60]. Besides that, there is exaggerated neuroendocrine stress response to endotracheal intubation and surgical incision under relatively light anesthesia during induction to delivery period of Caesarean section; BP could rise by 40–50% even in otherwise healthy patients, so it is obvious how dramatic can induction to anesthesia be in eclamptic patient [60–63]. Unstable hemodynamic during the induction to anesthesia is one of the reasons that made Caesarean Section an independent risk factor for stroke [10, 11, 64–66]. That is why it seems reasonable in vulnerable cases to pharmacologically attenuate hypertensive response to surgical stress (antihypertensives, magnesium sulphate, lidocaine, low-dose opioids) and to have protocols for situations of difficult endotracheal intubation [13, 59, 62, 67–74].

Conclusion

We emphasize the need of more careful examination and search for more subtle signs and symptoms of preeclampsia in pregnant patients, in order to avoid sudden, unexpected, but serious complications. Initial radiologic examination of severe cases, especially those transferred from other hospitals, should help us make the right diagnosis and choose adequate therapeutic regime. In cases of severe preeclampsia, the need for endotracheal intubation should always be anticipated and difficulties expected, so we need to have protocols for cases of difficult intubation and to pharmacologically attenuate cardiovascular stress response in such situations.

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CASE REPORT

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Our experience in the treatment of botulism

Naše iskustvo u lečenju botulizma

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Abstract

Introduction. Botulism is a neuro -intoxication caused by a toxin secreted by *Clostridium botulinum*. Due to extremely high toxicity and lethality, this toxin can be used as an agent in a biological warfare. **Case report**. We presented six patients, mean age 28.8 years, who ate canned food and in whom the diagnosis of disease was made based on the typical clinical picture. Predominant symptoms were blurred vision, double vision (diplopia), dry mouth and constipation which were present in all patients. The patient whose disease was recognized only after 23 days and who did not receive the anti botulinum serum underwent the longest hospital treatment. All the patients received antibiotics and 4 patients received antitoxin. Neostigmine and enemas were used for the treatment of the disorder of intestinal motility and constipation. **Conclusion**. The diagnosis of botulinum was made based on afebrility, preserved states of consciousness, double vision, dry mouth and history data on consumption of suspicious food. Polyvalent serum anti botulinum should be applied as soon as possible because it reduces the occurrence of complications, length of hospital stay and mortality rate.

Key words:
botulism; decontamination; botulinum antitoxin; treatment outcome; bioterrorism.

Apstrakt

Uvod. Botulizam predstavlja neurointoksikaciju izazivanu toksinom koji luči *Clostridium botulinum*. Zbog visoke toksičnosti i smrtnosti, ovaj otrov se može koristiti kao biološko oružje. **Prikaz bolesnika**. Prikazano je šest bolesnika, srednje starosti 28,8 godina, koji su konzumirali konzervisanu hranu i kod kojih je dijagnoza postavljena na osnovu tipične kliničke slike. Najčešći simptomi, prisutni kod svih bolesnika bili su: zamagljen vid, dupla slika (diplopija), suva usta i opstipacija. Najduže lečenje primenjeno je kod bolesnika kod kojeg je bolest prepoznata tek posle 23 dana i koji nije primio serum antibotulinum. Svi bolesnici su primili antibiotike, a četvoro je primilo antitoksin. Neostigmin i klistir su korišćeni za lečenje poremećaja pokretljivosti creva i opstipacije. **Zaključak**. Dijagnoza botulizma bila je postavljena se na osnovu afebrilnosti, očuvanog stanja svesti, duple slike, suvih usta i podataka o konzumiranju sumnjive hrane. Polivalentni antibotulinski serum treba da se primeni što pre, jer smanjuje pojavu komplikacija, dužinu bolničkog lečenja i stopu smrtnosti.

Ključne reči:
botulizam; dekontaminacija; botulin, antitoksin; lečenje, ishod; bioterorizam.

Introduction

Botulism is a neuro -intoxication which is characterized by gastrointestinal, nervous and secretory disorders induced by a toxin secreted by *Clostridium botulinum– C. botulinum* (rarely C. Baratii F, and C. butyricum E) [1], a Gram-positive, anaerobic bacterium. There are 8 known antigenic types: A, B, C alpha, C beta, D, E, F and G. As a disease cause, the most frequently isolated are serotypes A, B and E [2] while serotype F is rarely isolated as a disease cause. Spores of *C. botulinum* can be found in the environment, they are resistant to heat, so they can survive a few hours at 100°C. If they are exposed to moist heat at a temperature of 120°C, they can be destroyed within 30 min. On the other hand, toxins are easily destroyed by heat, therefore, heat treatment of food at 80°C for 30 min can protect from botulism. Toxin production (in particular of type E) is possible at lower temperatures, even at 3°C [3], which is the temperature inside the refrigerator.

Spores of *C. botulinum* are everywhere in the environment and the disease can be caused by the ingestion or inhalation of dust, by absorption through the eyes and through the damaged skin. Botulism usually occurs in three forms: botulism transmitted by food, botulism entered through a wound and infant botulism (usually infants younger than 6 months). Some authors mention the inhalation botulism as the fourth type of botulism [4].

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Botulism transmitted by food occurs after consuming contaminated food in which the toxin is produced; in the other two forms, *C. botulinum* produces a neurotoxin *in vivo*, in the infected tissue or in the colon. Today, it is known that the botulinum neurotoxin on peripheral nerve endings binds to two receptors on neurons by its synaptic vesicle R subdomain with a low affinity for the ganglioside and high for a synaptic vesicle protein known as a synaptic vesicle (SV2A) and SV2B and synaptotagmin II (Syt II). After that, a toxin that is bound to the receptor by endocytosis enters the cell, [5, 6] and leads to the blockage of acetylcholine release at the level of neuromuscular synapse, which results in the appearance of paralysis.

Infant botulism occurs most often in infants younger than 6 months [3]. Since botulinum is one of the strongest toxins, it is considered that 1 ng / kg is lethal to humans, while 200 g in the form of crystalline is enough to kill all of the humanity. It can be used in bio-warfare [7, 8] and it belongs to class A of biological agents [9, 10]. It can be used by spraying into the air or injecting into food.

Since botulism is a rare disease, early recognition of clinical symptoms is highly important for serum anti botulinum application. Furthermore, it is also necessary to report the disease and epidemiological treatment of each case.

Case report

We reported 6 patients diagnosed with botulism, who were treated during the period of ten years (from January 2004 to January 2014) in the Clinic for Infectious Diseases, the Clinical Center Niš, Serbia. The diagnosis of botulism was made based on the clinical picture and the epidemiological surveys, and according to the disease evolution and further paraclinical tests, differential diagnostic capabilities (CDC recommendations - USA) were excluded [11].

Case 1

A 44-year-old patient, an electrical engineer, presented with nausea, vomiting and watery excrement. The disease occurred in November 2007. Two days after the initial symptoms, in the evening hours, diplopia, dryness of the mouth, fatigue, difficulty in swallowing and speaking occurred. He gave the epidemiological data that he had eaten a can of squid seven days before the onset of symptoms and smoked meat the day before the onset of the symptoms. He was hospitalized three days after the illness had occurred, with signs of dryness of mucous membranes and mydriasis. Lung X-ray showed vague contours of the left dome of the diaphragm, which may correspond to the left, basal pulmonary infiltrate. The control lung X-ray done five days later was normal. The test of neuromuscular transmission was also performed and it showed a slight increase, up to 10%, on the system *nervus medianus- musculus abducens* poll left of evoked muscular responses I-IV, while there was no increase in the amplitude of evoked muscular responses in other tested systems. During the hospitalization for constipation, deep enema with sodium chloride solution 0.9% (NaCl) was also applied to the patient on two occasions. He was treated with anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E.), neostigmine (0.5 mg im for 7 days) and metronidazole 500 mg/8 h for 10 days. The patient was cured and discharged after fourteen days.

Case 2

A 26-year-old male patient, a student, presented with vomiting and constipation in mid-July 2008. He gave an indication that the previous day he had eaten "a can of meat". Diplopia, dryness of mucous membranes in the mouth and difficulties swallowing and speaking occurred two days after consuming the suspected food. On admission, four days after the onset of symptoms, mydriatic pupils, non-responsive to light, dehydration signs, left tonsil arch elevation and speech difficulty were noticed. He was treated with neostigmine 0.5 mg im for 7 days, lactulose and penicillin G, 10 million IU daily for 10 days. During hospitalization, despite the application of lactulose and deep enemas with 0.9% NaCl, the constipation persisted for few days after discharge.

Case 3

A 19-year-old female patient, a student, presented with nausea and vomiting in October 2009, the day after she consumed pate of unusual taste and sardines. The next day, blurred vision, dryness of oral mucous membranes, sore throat and difficult swallowing occurred. She was referred to a neurologist who prescribed her medication for "circulation". She was hospitalized four days after the onset of clinical symptoms, with signs of adynamia, dehydration, mydriatic pupils which poorly responded to light and accommodation, as well as with decreased strength. On admission, laboratory analysis of blood verified leukocytosis 11.6×10^9/L [normal range (nr) 4–10.5 $\times$ 10^9/L], with dominance of polymorphonuclears 80% (nr 50–70%), C–reactive protein (CRP) 45.3 (nr less than 3 my/L, fibrinogen 7.4 g/L (nr 1.5–3 g/L (she was also diagnosed with concomitant urinary tract infection for which she was treated with ceftriaxone). During hospitalization, the enema was applied on several occasions and stool became regular 15 days after the treatment. She was treated with neostigmine 0.5 mg im for 7 days, penicillin G, 10 million IU daily for 10 days, and received the anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E). Clinically rehabilitated, she was discharged from the clinic after 17 days.

Case 4

An 11-year-old patient, a pupil, presented with nausea and vomiting in June 2010, the same day he consumed pate. The next day, he felt pain in the lower leg and forearm muscles. The disease showed further clinical progression and after 48 h of onset of symptoms he presented with diplopia and vision difficulties, watery excrement, sore throat, difficult

swallowing, hoarseness. He was afebrile during the whole course of illness. He was hospitalized on the fifth day of the illness and on admission he had eyelid ptosis, mydriasis, dryness of mucous membranes. The patient was adynamic and arrived in a wheelchair. The patient had elevated sedimentation 80 mm/h (nr 0–22 mm/h), with leukopenia 4.2 × 10^9/L, normal findings of leukocytes formulas and CRP 17.3.g/L. He was treated with neostigmine 0.5 mg im for 7 days, ceftriaxone 2.0 g daily for 10 days, and has received the anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E). The problem of constipation was solved by enema (0.9% NaCl) which was performed on the fourth day of hospitalization, and thereafter the patient had regular excretion of feces. The patient was cured and released after eleven days of hospital treatment.

Case 5

A 45-year-old patient, a journalist, presented with nausea, pain in bowels, vomiting and mild diarrhea in mid-July 2010, a day after he had eaten pate in the casing of dubious quality. Dryness of the palms and soles, difficulties in swallowing, "blurred vision", hoarseness and constipation occurred after two days. His chosen doctor treated him with metoclopramide hydrochloride and he was also treated by a neurologist and ophthalmologist. He was admitted six days after the first symptoms appeared and on admission, he had anisocoria, dysphagia, dysphonia, adynamia, dehydration, and tachycardia 98 beats/ min. He was treated with neostigmine 0.5 mg im for 7 days, metronidazole 500 mg/8 h for 10 days and the anti botulinum serum (one dose of commercial serum, consisting of 750 IU of antitoxin A, 500 IU antitoxin B and 50 IU antitoxin E.). Due to constipation, enema with 0.9% NaCl was done only on the day of admission and later he had regular stools. Clinically cured, he was released after fourteen days.

Case 6

A 31-year-old patient, an electrical engineer, presented with nausea, belching, vomiting and 5-6 watery excrements in February 2013. After 72 h he developed blurry vision regardless of distance, difficult swallowing and constipation, tiredness and was unstable in standing position. During the treatment, he passed stool on the third day and after that his stool was regular so there was no need for using enemas. He was treated with penicillin G, 10 million IU daily for 10 days, neostigmine 0.5 mg im for 7 days and infusion solutions with vitamins B and C. The patient was cured and released after thirteen days of treatment. Table 1 summarizes the different symptoms and signs in patients with botulism.

Discussion

Botulism can occur regardless of age. Sobel et al. [12] showed that in a sample of 263 patients, the average age was 48 years. Leclair et al. [13] tested a sample of 205 patients from Canada during a period 1985–2005 and showed that mean age was 45 years (3–83 years), with 48.4%. They also stated that the prevalence of type E botulinum toxin was 86.2%. These findings are consistent with our case series. The patients hospitalized in our Clinic were aged from 11 to 45 years, with a mean age of 28.8 years. Four patients were male and two were female.

Table 1

Review of clinical symptoms and signs of botulism

Symptoms	Patients, n (%)
Gastrointestinal tract	
nausea	6 (100)
vomiting	6 (100)
diarrhoea	4 (50)
Autonomic nervous system	
constipation	6 (100)
dryness of mouth	6 (100)
dryness of palms\ soles	3 (50)
Neurological system	
mydriasis	3 (50)
blurred vision	6 (100)
diplopia	2 (33.3)
eyelid ptosis	1 (16,6)
hoarsness	2 (33.3)
difficulty swallowing	6 (100)
general weakness	4 (66,6)

Based on epidemiological surveys, the most likely route of infection in four patients was the pate, in one patient the luncheon meat, and in one it was most likely dried meat. Therefore, the route of infection in all cases was alimentary. It was not possible to prove the presence of *C. botulinum* toxins in suspicious food samples, considering that they had not been preserved. Apart from the patients, no one else had consumed the suspected food so there was no control group.

Besides the most frequently mentioned antigenic types that cause botulism, Bouvet et al. [14], described the case of an 83 year-old-person with clinical signs of severe sepsis in whom *C. botulinum* type III, which had a "mosaic structure" of genetic parts of C and D types, was isolated from the blood cultures.

The incubation period ranged from 24 h to four days. Arnon et al. [15] showed that the first symptoms, in the form of descending symmetric paralysis, bulbar paralysis, diplopia, dysarthria, dysphagia and dysphonia occur 12 to 72 h after exposure. The youngest patient was 11 years old and had the shortest incubation period because he stated that 30 min after eating pate, he felt nauseated and vomited abundantly, and the next day he got the neurological symptoms.

The most predominant symptom, which aroused general practitioner's suspicion of botulism, was a disturbed vision. All patients had blurred vision, double vision and could not read. In all cases the dryness of mouth, difficulties in swallowing and speaking were recorded. With the youngest patient, in addition to mydriasis, the ptosis of the eyelids was registered. Some authors state that the clinical picture is characterized by the triad – symmetric descendent paralysis and bulbar paralysis, afebrility and preserved sensorium [16, 17]. Aurora et al. [18] give an overview of the case of a 35-year-old patient from India, who, 4 days after consuming food, deve-

loped eyelid ptosis, blurred vision, difficult swallowing, progressive weakness of the lower limbs and fasciculation of the muscles of the whole body, which is rarely seen.

Describing the occurrence of 30 cases of patients suffering from botulism poisoning after eating in a restaurant in El Paso, Texas, Angulo et al [19] said that in 18 of them the diagnosis was confirmed, with 5 it was likely, and in 2 cases it was suspicious. The most common symptoms were fatigue, diplopia, blurred vision and dizziness. They specifically point out two cases with the asymptomatic clinical picture. With one, the diagnosis was made on the basis of positive coprocultures, and in the other the findings of electromyography were specific. Sobel [20] pointed out that in a case of an epidemic, all patients did not have the same clinical picture because the toxin was not equally distributed in all contaminated groceries.

Khakshoor et al. [21] described the case of a 37-year-old Iraqi who had a binocular horizontal diplopia as the only sign of the disease. Three days later, the patient began to vomit, and two days later he developed other symptomatology of botulism.

As it can be seen from the mentioned cases, the diagnostic wanderings are frequent, whether the patients are treated with suspicion of other diseases or whether the referral to virologist was belated, even three weeks after the onset of the symptoms.

Vossen et al. [22] pointed out the rare frequency of the disease and the importance of early recognition of the clinical picture indicates. They described two cases of botulism in Vienna that were diagnosed after a period of 21 years in which there was no botulism. Besides the selected physician, an ophthalmologist, laryngologist, and gastroenterologist were consulted and on the eighth day of the disease, when a neuro-ophthalmologist was consulted, an infectologist was included and the disease was diagnosed. Early diagnosis is needed for timely application of serum which is the first 36 h. Sobel [20] points out that it would be ideal to apply the anti botulinum serum in the first 24 h of the symptom onset.

Kotan et al. [23] state that in addition to the characteristic clinical picture, it is also possible to use electromyoneurography (EMNG) for the purpose of diagnosis. They presented the case of a 43-year-old female patient who developed neurological disorders and indicated the specific findings of EMNG in botulism. The response to repeated stimulation of the ulnar nerve with low-frequency (3 Hz) is lowered, while at the repeated stimulation with high frequencies (10–50 Hz), the response is facilitated [23, 24].

In two patients the therapy did not include the serum anti botulinum, because one of them appeared 23 days from the onset of symptoms and the other after 5 days. Both patients were treated by antibiotics, neostigmine, lactulose, and infusion of electrolytes and vitamins. Lonati et al. [25] presented the case of a nine–month- old infant with a severe clinical picture of botulism who, in addition to serum anti botulinum 250 mL / 25 mL / h, was also orally given 5 g activated charcoal through a nasogastric tube and prostigmine 0.05 i.v. mg/kg. The same group of authors believes that the application of prostigmine inhibits the enzymatic degradation of acetylcholine and therefore may be useful in preventing ileus [26].

Other four patients were given trivalent serum anti botulinum - Botulism Antitoxin Behring® from the company Novartis. The serum contains horse proteins but since the patients were not previously treated with serums of animal origin, the premedication was not carried out. Antibotulinum serum was applied in the form of a slow i.v. infusion of 250 mL, and then sequenced with another 250 mL. After 4–6 h another 250 mL of the serum was infused. There was no reaction in any of the patients. With the use of neostigmine, the patients were also given antibiotics, lactulose, and other symptomatic therapy. Robinson et al. [27] believe that early use of serum anti botulinum prevents the progression of paralysis but it does not cure it. A group of authors believes that there are certain doubts about the application of serum anti botulinum since it can cause anaphylaxis, and as additional treatment options, they listed corticosteroids, plasmapheresis, and implementation of immunoglobulins [18]. Tacket et al. [28] describe the evaluation of the effect of using trivalent horse serum in 132 patients with clinical signs of botulism and note that it has a positive effect on the survival and shortens the course of the disease.

Some authors believe that passively applied animal serum only leads to neutralization of toxins in circulation by binding antitoxins to receptors of neurons, where it cannot directly inhibit the proteolytic activity of toxin which entered the cell. Humanized monoclonal antibodies bind and block the action of zinc metalloprotease intracellularly. In that case, botulinum neurotoxin cannot cause specific and direct adverse effects. Accordingly, humanized monoclonal antibodies should be immunotherapeutic medicine for botulism [29].

Lonati et al. [26] say that Dr. Zamani recommends the sterilization of hose by using sorbitol and does not recommend the use of magnesium salts as they can lead to a deterioration of neuromuscular blockades.

A group of Japanese authors described the case of a 69-year-old married couple suffering from severe forms of botulism after eating vacuum packed food. They tested the presence of toxin and *Clostridium* in the feces and toxin in the serum. For epidemiological reasons, it is essential that, at the beginning of their disease, Clostridium in feces was isolated in both patients and toxin was detected in serum and feces. After three months, the toxin was not detected in the serum, in one patient it was detected in the feces, and in both patients *Clostridium* was isolated in the feces [30].

The role of antibiotics in the treatment of botulism remains unclear but penicillin G has been recommended to treat wound botulism. Any other antibiotics that treat *Clostridium* species, such as cefalosporins or metronidazole could also be administrated. Antibiotics are useful in wound botulism, but they have no role in foodborne botulism. Antibiotics are also useful in the treatment of secondary infections. In infant botulism, antibiotics are used only to treat secondary infections because lysis of intraluminal *C. botulinum* may increase the amount of toxin available for absorption [31].

The length of hospitalization ranged from 11 to 20 days, noting that two patients were treated for 14 days, and one 13

and 17 days. The longest treatment had the patient who was admitted 21 days after the first symptoms since the disease was not recognized on time and he had not received anti botulinum serum. All patients were cured without sequelae.

Conclusion

Botulism is a neurointoxication which is characterized by afebrility, the appearance of visual disturbances, difficult swallowing, dryness of mucous membranes in the mouth, constipation and preserved sensorium. The main clinical symptoms that aroused the doctor's suspicion of the disease are diplopia and visual disturbances. *Clostridium botulinum* can be used in bioterrorism as a biological poison and, therefore, there is an imperative need to report every case of suspicion of the disease. Treatment of patients must be carried out in intensive care units. Anti botulinum serum should be applied as soon as possible because it reduces the occurrence of complications, length of hospital stay and mortality rate, but the problem is the fact that, in any case when the serum was required it could not have been obtained in the country and had to be commissioned from abroad.

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CASE REPORT

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Intratendinous ganglion cyst of peroneus brevis tendon and its reconstruction with semitendinosus graft

Intratendinozna ganglijska cista tetive kratkog peronealnog mišića i njena rekonstrukcija sa semitendinoznim graftom

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Abstract

Introduction. A ganglion cyst is a common benign tumor, mainly found in the wrist and ankle. The ones originating from a tendon, such as in our case, are the most rare. The ganglion cyst presents with a variety of symptoms, offering a wide range of possible differential diagnoses. Physical examination is crucial to make an accurate diagnosis, but magnetic resonance imaging (MRI) can help in identifying the ganglion cyst. The treatment is mostly conservative, but in cases when the ganglion cyst disables the patient's ability for normal life functioning, due to pain and decreased mobility, surgery is necessary. **Case report**. A 38-year-old female with persistent ankle pain and edema was clinically diagnosed with luxation of peroneal tendons. Further investigation with MRI showed tenosynovitis of peroneal tendons and rupture of the superior peroneal *retinaculum* with an intratendinous ganglion cyst of *peroneus brevis* tendon. Surgical treatment with the reconstruction of *peroneus brevis* and peroneal *retinaculum* was performed with semitendinosus graft and anchor sutures. Histology confirmed the diagnosis of the intratendinous ganglion. After four months of rehabilitation, the patient returned to normal daily and sports activities and was pain-free on the follow-up. No recurrence of the ganglion cyst was acknowledged. **Conclusion**. Surgery is crucial for patients with intratendinous ganglion cyst and symptomatic instability of the peroneal tendons with chronic subluxation.

Key words:
leg injuries; ganglion cysts; tendons; orthopedic procedures; transplants; treatment outcome.

Apstrakt

Uvod. Ganglijska cista je čest benigni tumor, uglavnom pronađen u zglobovima, nasuprot našem primeru, gde ganglijska cista poreklom iz tetive, po svim klasifikacijama predstavlja najređi izvor. Ganglijska cista se ispoljava različitim simptomima i nudi širok raspon mogućih diferencijalnih dijagnoza. Fizikalni pregled je od ključne važnosti za stvaranje tačne dijagnoze, ali magnetna rezonanca (MR) može pomoći u identifikaciji ganglijskih cisti. Lečenje je uglavnom konzervativno, ali u slučajevima kada ganglijska cista uzrukuje bolove i smanjuje funkciju i onemogućuje sposobnost pacijenta za normalan život, potrebna je operacija. **Prikaz bolesnka**. Žena stara 38 godina sa upornim bolovima u skočnom zglobu, sa edemom u obrnutoj everziji stopala, obavila je pregled kod specijaliste traumatologa, koji je indikovao dalju dijagnostiku. Snimak magnetne rezonance (MR) pokazao je da se radilo o tendosinovitisu peronealne tetive i rupturi gornjeg peronealnog retinakuluma sa intratendinoznom ganglijskom cistom u tetivi *musculus peroneus brevis-a.* Pacijentkinja je hirurški lečena, napravljena je adhezioliza, resekcija tetive *musculus peroneus brevis-a* u kojoj je bila ganglijska cista. Posle toga, rekonstruisana je resecirana tetiva sa semitendinoznim graftom i sidro šavovima. Histološki je potvrđena dijagnoza intratendinozne ganglijske ciste. Postoperativno, noga je bila imobilizirana u gipsu, sa 5 stadijuma everzije i neutralnim položajem, a posle toga bila je podvrgnuta krioterapiji i kasnije fizioterapiji. **Zaključak**. Hirurški zahvat je odlučujući za simptomatske pacijente sa intratendinoznom ganglijskom cistom, sa znakovima nestabilnosti peronealne tetive i hroničnom luksacijom.

Ključne reči:
noga, povrede; ciste, ganglijske; tetive; ortopedske procedure; graftovi; lečenje, ishod.

Introduction

Ganglion cysts are most commonly found benign masses in the area of hand, wrist, shoulder, knee or ankle [1]. They vary in size, are mainly solitary and occur in younger people, aged from 15 to 40, more frequently in women [1, 2]. Their classification is based not only on the location of the ganglion cyst but also on its origin. They can arise from bones, joints, soft tissues and tendon sheaths. Although they are common, those originating from a tendon, the so-called intratendinous ganglion cysts, are the rarest, especially in the area of the ankle, or more precisely in the area of peroneal tendon [1, 3].

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The etiology of the formation of the ganglion cyst is not well understood, but many agree with the hernial hypothesis, which correlates its formation with the distention of a weakened area of the tendon, where consequently ganglion forms [1]. In one-third of the cases conditions as local trauma or increased burden and stress to the part where the ganglion forms are associated with its development. Researchers associate the ganglion cyst formation with degenerative and inflammatory conditions of soft tissue or bone [1, 3].

When identifying the mass of an unknown origin, many differential diagnoses are considered. Patient's history and status should be taken thoroughly, focusing on the accompanying trauma of the area, onset of symptoms and possible relation to increased activity [1–3]. Physical examination is crucial in making the accurate diagnosis by palpation of the mass. To exclude possible bone lesions X-ray should be performed. Ultrasound of soft tissue can be beneficial. The most reliable method to determine the accurate location, the involvement of adjacent structures and the size of the ganglion cyst is magnetic resonance imaging (MRI) [1–4].

Treatment decision depends on the presence of pain and impaired ankle eversion. In most cases, conservative measures are taken, such as immobilization of the involved joint. Semi-invasive treatment includes aspiration of the cyst or injection of anti-inflammatory agents (for example corticosteroids) into the cyst. This method has a high recurrence rate. The third option is surgery, which is the method of choice in case of severe, chronic and symptomatic ganglion cyst [1–3].

Case report

A 38-year-old female was examined at the emergency department where she reported a crack and later pain in the lateral side of the ankle during walking. Ankle sprain was diagnosed and functional treatment was advised. After one month she complained of increasing pain and edema on the lateral side of the ankle. Clinical examination revealed edema at the lateral side, inversion position of the foot with painful palpation, especially on the anterior talofibular ligament and fibular malleolus from the lateral and posterior side with palpable formation retromalleolarly, in the course of *peroneus brevis* tendon (Figure 1). X and ultrasound revealed no pathology. Clinical diagnosis of luxative peroneal tendons with superior peroneal retinaculum rupture was established. MRI was performed to confirm the clinical diagnosis and showed tendinosis of peroneal tendons with severe synovitis, intratendinous ganglion cyst of the peroneus brevis tendon and rupture of the superior peroneal *retinaculum*. Due to clinical findings and MRI results, the surgical treatment decision was made. Adhesiolysis was performed followed by reconstruction of peroneus brevis tendon defect and superior peroneal *retinaculum* with semitendinosus graft (Figures 2 and 3) that was proximally fixated by anchor suturing the

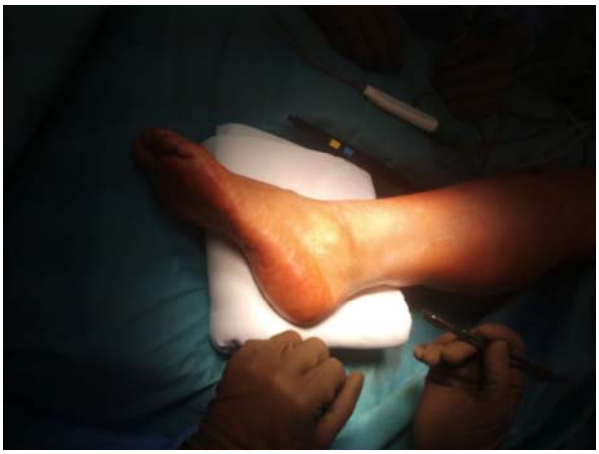

Fig. 1 – Left ankle with lateral edema.

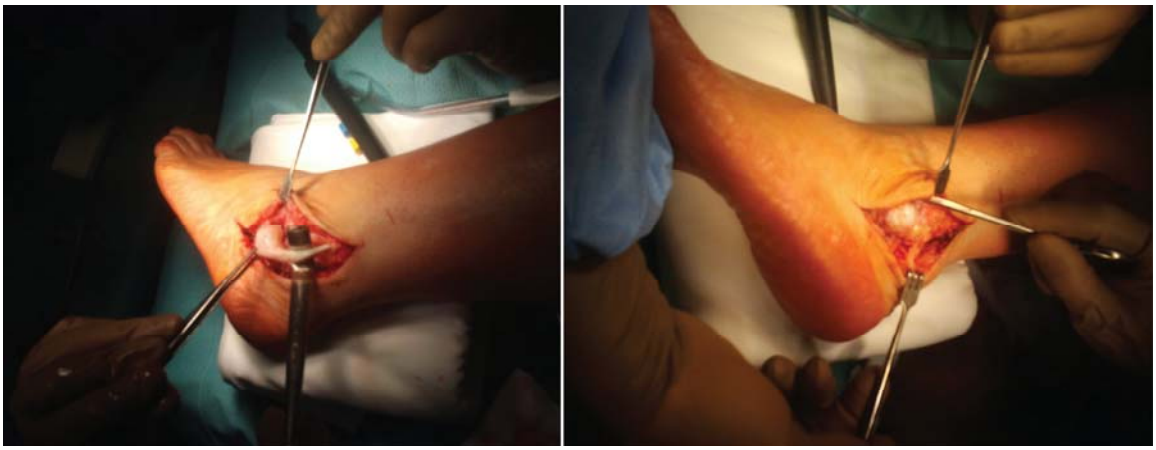

Fig. 2 – *Peroneus brevis* ganglion in surgery.

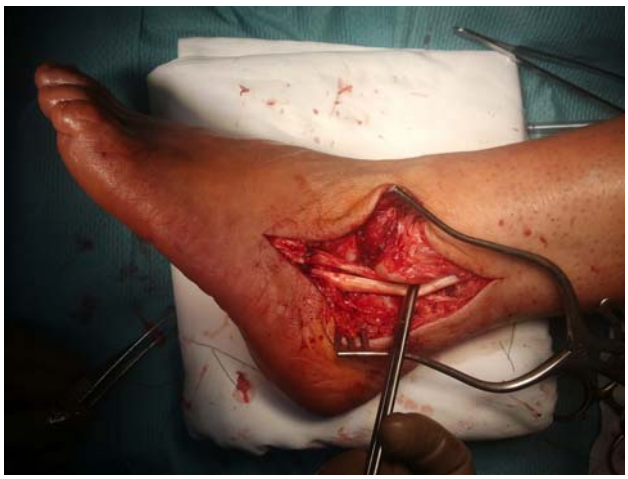

Fig. 3 – Reconstruction of *peroneus brevis* tendon defect and superior peroneal *retinaculum* with semitendinosus graft.

graft to the reminder of retinaculum (Figure 4). At operation, peroneal sulcus appeared too shallow and it was deepened to avoid reoccurrence of the peroneal tendon luxation. Histology confirmed the intratendinous ganglion cyst.

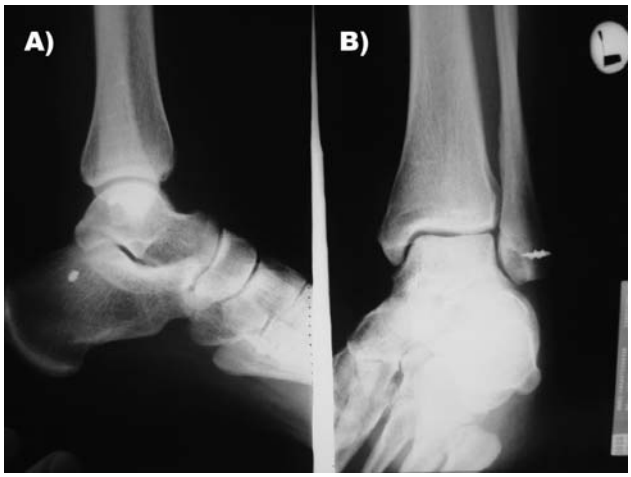

Fig. 4 – A) Fixation of semitendinosous graft with anchor sutures, where anchor is seen on the X-ray, and B) distally suturing the graft to the remainder of retinaculum.

Postoperatively she received a below-knee non-weight-bearing cast in 5 degrees eversion and neutral position for six weeks (Figure 5). After one year follow-up, there was no recurrence of symptoms. Cryotherapy with immediate verticalisation was indicated after the operation. Control X-ray showed the stable position of the operated foot. Afterwards, the cast was replaced by a weight-bearing one for another 2 weeks. When the cast was removed, she was referred to physiotherapy. After four months she was able to go back to work and her after daily activities.

Discussion

Although ganglion cysts are the most common soft tissue pathology, they are quite rare in the peroneal tendons [1]. Peroneal tendon tears are a common cause of chronic lateral ankle pain, but in many cases remain untreated due to mis- or undiagnosis [5–7]. Peroneal tendon pathology is common but rarely mentioned in the literature. Reports found in the literature are all from a distant history, no new cases have been described [1–12]. Therefore we presented a case where we reconstructed a retinaculum as well as the defect of *peroneus brevis* tendon after excision of the ganglion cyst with semitendinosus graft.

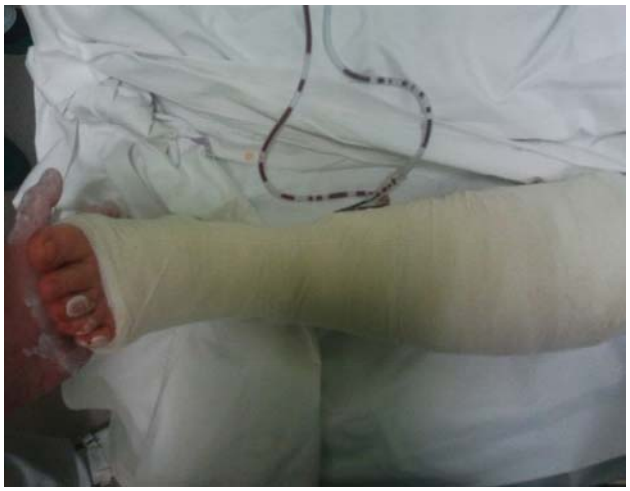

Fig. 5 – Below-knee non weight bearing cast in 5 degree eversion and neutral position.

Since symptoms of the ganglion cyst are unspecific, palpable and/or visible swelling and pain, and because the differential diagnosis is wide, the ganglion cyst of the peroneal tendon is commonly overlooked or wrongly diagnosed [1].

In making the diagnosis we can perform an X-ray, which mainly helps in narrowing the possible differential diagnoses [1, 3]. As in our case, X-ray of such patients is usually normal. It is not uncommon that osteophyte formations are present in the adjacent surfaces of the bone or joint [1]. The best technique to confirm the diagnosis is MRI, which in our case showed severe tenosynovitis, formed intratendinous cyst and undefined upper *retinaculum* of the peroneus [1, 4]. Tenosynovitis and tendon tears are commonly present to a tendinous ganglion cyst and therefore represent a major differential diagnosis based on MRI [4]. Excluding differentials it is very important to first exclude those that are endangering the patient, such as synovial sarcoma, myxoid chondrosarcoma or liposarcoma and distant metastasis to a primary tumor, if such is known [1, 4].

However, Costa et al. [4] suggest that MRI findings of a lobulated mass within the tendon with rich enhancement suggest the diagnosis of an intratendinous ganglion cyst. To absolutely confirm the diagnosis, histology is crucial.

Once the diagnosis is confirmed, treatment can be performed by aspiration and injection with cortisone, operation by removal of the ganglion cyst or conservatively with or without a cast [1, 3]. Every method has its side effects and success rate is variable, but common to all is the recurrence of the ganglion cyst with varying probability, the highest being in semi-invasive method [1].

The main criterion by which we decided what treatment method will be used is the duration of symptoms, whether it is the acute or chronic course of signs and symptoms such as swelling, pain.

In case of chronic ganglion cyst with symptomatic instability of the peroneal tendons, surgery is preferred. There are many different surgical techniques described in the literature [8]. In our case we performed adhesiolysis, that suggested on the ganglion formation after trauma, infection or inflammation, we already discussed as a cause for the formation of ganglion cyst and was noted in the MRI report. Afterwards, revision of peroneal tendon and removal of the ganglion cyst took place. We needed to reconstruct the ruptured the peroneus brevis tendon and peroneal *retinaculum*, by using semi-tendinosous graft. Such procedure was described by Paterson et al. [9] who reported that by performing semi-tendinosous graft reconstruction of the peroneal tendons, its function was obtained.

Additionally, we performed deepening of peroneal sulcus due to the shallowness of the sulcus, in order to prevent peroneal tendon further luxation and to increase its long-term stability. Peroneal sulcus is important since it allows peroneal tendons to move along it [8].

Possibilities for surgical repair and reconstruction of peroneal tendon tears are with or without the fibular osteotomy, by transposition of the tendon, by anchor sutures of the tendon or with the use of a graft, as used in our case [7, 9–11]. The method of fibular osteotomy is the application of the *retinaculum* and augmentation with the calcaneofibular ligament, deepening on the depth of peroneal sulcus, which is shallow in most of the reported cases [9–11]. The shallow peroneal sulcus is the second most common cause of peroneal subluxation, right after the ankle sprain [8, 9].

Our patient was postoperatively immobilized and later reported being satisfied by the outcome. We can not correlate the success rate with the immobilization since there are studies reporting that time of immobilization does not affect the outcome [10, 12].

At the emergency department, where such patients mostly receive the first check-up, such conditions should be somewhere in the subconsciousness of the doctor, especially in patients, that report prolonged ankle pain, lateral swelling, ankle instability and chronic ankle sprains [2, 3, 6, 12]. Since imaging available at the emergency department is not suitable for making a diagnosis of peroneal tendon subluxation or dislocation due to the ganglion cyst, when suspecting such pathology, these patients should be referred to a foot and ankle specialist, for further investigations and management. This painful condition requires surgery [8].

Conclusion

This case report presents a rare chronic intratendinous ganglion cyst arising from *peroneus brevis* tendon that was surgically treated by its excision and by reconstruction of tendon brevis and superior peroneal *retinaculum*, using the semitendinosus graft.

It is important to diagnose these conditions that are causing chronic pain to the patient and to allow the patient to have a normal quality of life. Four months post surgery our patient returned to normal daily and sports activities and reported having no problems. No recurrence of the ganglion cyst was acknowledged.

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CASE REPORT

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"Tails-up" capnogram: the role in detecting anaesthetic equipment malfunction

Kapnogram sa "uzdignutim repom": uloga izmenjene kapnografske krivulje u otkrivanju poremećaja funkcionisanja anesteziološke opreme

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Abstract

Introduction. Capnography is an essential part and standard monitoring tool during the perioperative period, which can be invaluable in detecting anaesthetic equipment malfunction. **Case report.** The atypical, "tails- up" capnographic waveform was noticed during routine surgical operation. Comprehensive physico- mathematical and graphical explanation of this complex capnographic pattern has been given, together with in- depth analysis of possible differential diagnosis and clinical significance for routine clinical practice. **Conclusion.** "Tails-up" capnographic trace gives early clue to diagnosing and fixing the problem of cracks in sampling line, before leading to an inadequate course of action. The understanding of the physics and physiology behind capnography is of vital importance for the analysis of capnographic waveforms, for early detection of anaesthetic equipment malfunction and for safe clinical practice.

Key words:
capnography; monitoring, intraoperative; equipment and supplies; equipment failure; physics; physiology.

Apstrakt

Uvod. Kapnografija predstavlja standardni uređaj za monitoring tokom perioperativnog perioda, koji može biti od neprocenjivog značaja u otkrivanju problema u funkcionisanju anesteziološke opreme. **Prikaz slučaja.** Atipičan oblik kapnograma sa "uzdignutim repom" zabeležen je tokom rutinske operacije. Prikazano je detaljno fiziko-matematičko i grafičko objašnjenje ovog kompleksnog kapnografskog oblika, zajedno sa analizom mogućih diferencijalnih dijagnoza i kliničkim značajem za svakodnevnu praksu. **Zaključak.** Oblik kapnograma sa "uzdignutim repom" omogućava ranu dijagnozu i rešavanje problema pukotina u sistemu za uzorkovanje izdahnutog gasa, pre nego što dovedu do daljih neadekvatnih postupaka. Detaljno razumevanje fizike i fiziologije nastanka kapnografske krivulje značajno je za uspešnu analizu kapnografskih talasa, za ranu detekciju kvara anesteziološke opreme i za bezbednu kliničku praksu.

Ključne reči:
kapnografija; fiziološke funkcije, intraoperativno praćenje; oprema i pribor; oprema, malfunkcija; fizika; fiziologija.

Introduction

Capnography is a standard monitoring equipment used during perioperative period, and it can be utilized to monitor several physiologic parameters, body metabolism, systemic and pulmonary circulation and ventilation. The integrity of anaesthetic machine and equipment can be monitored and misconnections and faults diagnosed, which could be lifesaving [1, 2]. First of all, one can easily misdiagnose type of capnographic trace with similar ones seen in pregnant, obese patients and in patients with low compliance states of the lungs [1–3]. Low CO_2 levels could easily be misinterpreted as a hyperventilation, hypoventilation, or hypometabolism. Moreover, falsely low levels of anaesthetic gases can lead to inadequate depth of anaesthesia.

We present unusual and atypical capnographic trace known as the "tails-up" capnogram [4, 5], with its in-depth analysis and clinical importance, by using the basic fundamental laws of physics.

Case report

A 65 years old male patient was admitted to the emergency department with acute abdominal pain located in

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the epigastric area. The diagnosis of perforated gastric ulcer was made, and the patient underwent emergent laparotomy. All vital signs were within normal limits during surgery, including the values of electrocardiography, pulse oximetry, intrathoracic pressures and ventilatory parameters. However, after two hours of surgery, the change in capnographic trace was recorded with anesthetic gas monitor (Draeger Vamos, Lübeck Germany) (Figure 1). After careful examination of the sidestream sampling line, the small slit-like hole was found approximately in the middle of the line (Figure 2). The sampling line was exchanged, the waveform returned to normal and the surgical procedure was finished uneventfully.

Discussion

The atypical capnographic waveform was seen during uneventful anesthesia, which led to the diagnosis of the broken sidestream sampling line. The sampling line is a thin, long and frail plastic tube, vulnerable to cracks and brakes during machine movement [3].

The prominent features of changed capnographic waveform are the dip, or the "valley" part, and the "tail" part of capnogram. In order to understand this capnographic pattern, we need to briefly review the basics of capnography. The normal capnogram consists of four segments and two angles, corresponding to various phases of respiratory cycle (Figure 3): the first phase represents the elimination of a CO_2 – free gas from anatomical dead space, following with expiration of a mixture of gases from anatomical and alveolar dead space –phase two; the third phase, or alveolar plateau represents the elimination of the CO_2-rich gas from alveoli, followed by the beginning of inspiration of CO_2-free gas and sudden drop to the baseline. The alveolar plateau phase is especially important for the explanation of the "tails-up" capnogram, and it can be conceptualized as the proportion between the volume of the CO_2 gas in the mixture of gases, or the CO_2 content within the volume of all exhaled gases from the patient and inside the sampling tube.

Sidestream sampling line is a long, rigid and thin plastic tube, 100–200 cm in length and with 1–1.5 mm inside diameter, which aspirates the gas from the patient's end of the breathing system, at a rate between 100–250 mL *per* minute. Since it is rigid and non-collapsible, we can apply the modified Ohm's law for laminar flow of gas (or fluid) through the rigid tube (Equation 1):

$$Q = \frac{\Delta P}{R}$$

which states that the flow (Q) is directly proportional to the pressure drop (ΔP) along the tube and indirectly proportional to the resistance (R) of the tube.

The Hagen-Poiseuille Law (Equation 2):

$$R = \frac{8 \times \mu \times l}{\pi \times r^4}$$

states that the resistance of the tube is directly proportional to the length (*l*) of the tube and viscosity of the gas (μ), and indirectly proportional to the fourth power of the radius of the tube (r^4) [4].

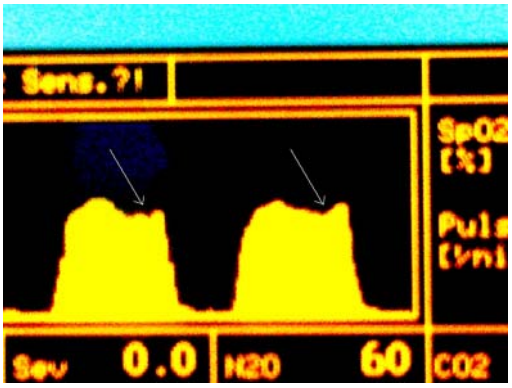

Fig. 1 – The "Tails-up" capnogram. Note the valley part (arrow) and the "tail" part of the capnographic trace.

Fig. 2 – The hole (arrow) in the sidestream sampling line.

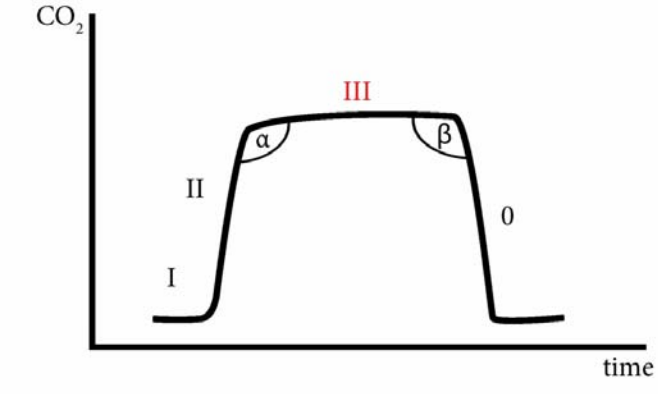

Fig. 3 – The model of the sidestream sampling line.

Rearranging these equations (Equation 3):

$$\Delta P = Q \times \frac{8 \times \mu \times l}{\pi \times r^4}$$

we can see that the drop in pressure along the tube is directly proportional to the length of the tube (all other parameters held constant). This means, the longer the tube, the greater the drop in pressure, and *vice versa*.

Based on this theoretical consideration, a model of the sidestream sampling line was built (Figure 4). P1 represents the pressure at the patient (breathing system) end of the tube, P2 is further away along the tube, inside the tube is aspirated gas from the breathing system (P_{ins}) under some amount of pressure, and outside the tube is atmospheric air with atmospheric pressure (P_{atm}). The resistance is directly proportional to the length of the tube, and there is a drop in pressure from P1 to P2. At some point of the sampling tube, there has to be equalization of the outside and inside pressures, or an equal pressure point (EPP). After passing this point, the pressure outside the tube will be greater than the pressure of the aspirated gas inside, i.e. $P_{atm} > P_{ins}$.

We can add to this consideration the Law of conservation of energy, which states that "energy cannot be created or destroyed, but can only change from one form to another" [4]. This means that the total energy (i.e. the pressure – P_0) of the system, in this case the rigid sampling tube, must always be constant, i.e. the sum of the kinetic energy (dynamic pressure – *q*), which is a function of the velocity of the gas, and the potential energy (static pressure – *p*), is always constant (Equation 4).

$$P_0 = p + q$$

So, if the velocity of flow increases, the pressure within the tube must decrease which is, actually, the practical application of the Law of conservation of energy, known as The Bernoulli's principle [4].

The Law of conservation of flow relates the velocity of gas through the rigid tube with a cross-sectional area of the tube and states that the flow through any part of the tube must remain constant. This means that the smaller the cross-sectional area of the tube (or, the greater the resistance), the velocity along that part of the tube has to increase (Figure 5).

Combining the principle of conservation of flow, with the previously explained Hagen-Poiseuille Law and Bernoulli's principle, it can be stated that the smaller the radius, i.e. the cross-section of the tube, the greater the velocity inside the tube and the smaller the pressure that is exerted on the wall of the sampling tube. A longer tube, higher resistance of the tube and higher gas velocity is needed in order to conserve the constant flow inside the tube. This leads to a greater drop in pressures, the gas flows further away from the patient end of the sampling tube, finally reaching the EPP.

If a crack or a breach appears in the sidestream sampling line as in our case the following changes are present (Figure 6). During the expiratory phase of positive pressure mechanical ventilation (IPPV), the pressure inside the line will progressively decline along the length of the line, until it equalizes with the atmospheric pressure, i.e. until it reaches the EPP. When the pressure inside the tube becomes lower than the EPP, the suctioning of atmospheric air occurs across

Fig. 4 – The model of the sidestream sampling line.

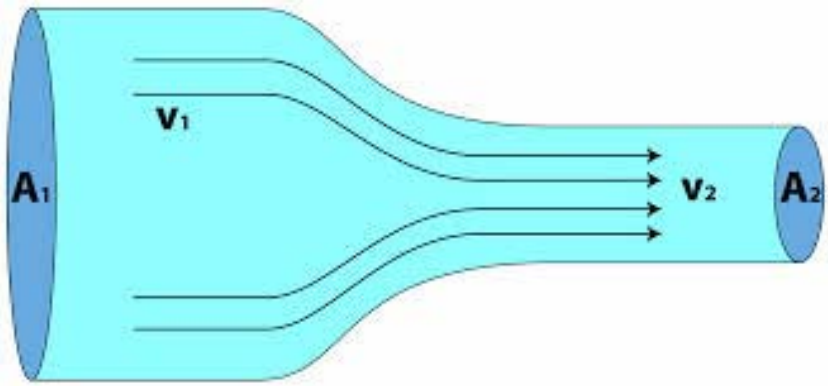

Fig. 5 – The conservation of flow: the velocity (V2) of the gas inside the tube increases with decreasing diameter, i.e. the cross- sectional area (A2) of the tube. The pressure at A2 will be lower than pressure at A1 (p2 < p1).

Fig. 6 – The model of the sidestream sampling line, with the breach in wall. When the Pins < Patm during expiration, the suc-tioning of the atmospheric air occurs.

the pressure gradient (the Venturi effect) [3]. This leads to the dilution of the in-line CO_2, which gives rise to the "valley" part of the capnographic trace (Figure 7). However, during the beginning of the inspiratory part of IPPV (Figure 8), just before the opening of the inspiratory valve of the breathing circuit, there is a building-up of pressure inside the breathing system.

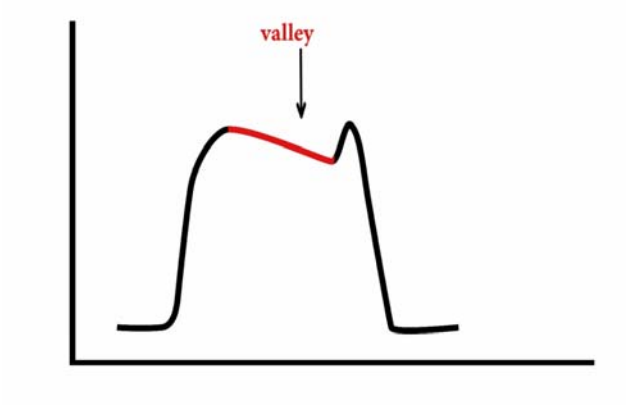

Fig. 7 – The "valley" part of the capnographic trace.

Fig. 8 – During inspiratory part of the positive pressure ventilation, the $P_{ins} > P_{atm}$, there is a functional sealing of the hole, which prevents the suctioning of the atmospheric air.

This positive pressure transmits to the sampling line, which leads to the constant positive pressure exerted all along the line. Since the pressure inside is higher than the atmospheric pressure, this leads to the functional sealing of the hole, which prevents suctioning of atmospheric air and dilution of in-line CO_2. The end-tidal CO_2 value instantaneously returns toward normal, which gives rise to the "tail" part of the capnographic trace (Figure 9).

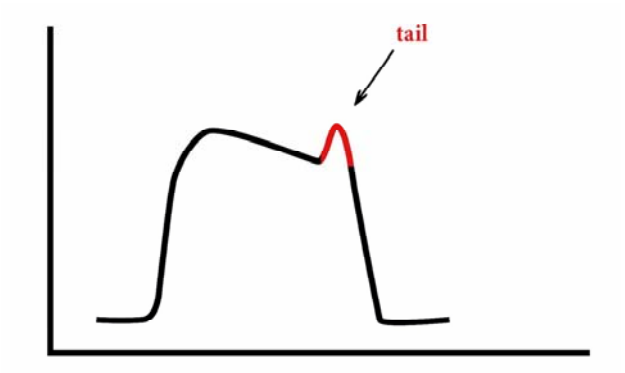

Fig. 9 – The "tail" part of the capnographic trace.

Tripathi et al. [5] confirmed experimentally the theoretical background and predictions stated in this article, that during the expiratory phase of IPPV, lower levels of CO_2 and anaesthetic gases exist inside the breached sampling tube, and that atypical capnographic trace can be seen. On the other hand, during spontaneous breathing, there is a drop of pressure inside the sampling tube in both phases of the respiratory cycle, the dilution and lower levels of CO_2 and anaesthetic gases occur in both inspiration and expiration, and the pseudo-normalization of capnographic trace can be seen.

Conclusion

"Tails – up" capnographic trace gives early clue to diagnosing and fixing the problem of cracks in sampling line, before leading to an inadequate course of action[5]. Regular inspection of the sampling lines should be performed as a routine and mandatory part of the pre-anesthetic machine check-up protocol.

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LETTER TO THE EDITOR
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Informed consent in elective gynecological surgery

Informisani pristanak u elektivnoj ginekološkoj hirurgiji

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To the Editor:

According to the regulations of the Law of patients' rights, consent to a surgery is an act of sanity, demanding a patient to be mature, with full mental capacity and with information available providing the possibility of valid decision making [1]. A patient has the right to decide with free will on everything regarding his/her life and health; not a single medical treatment can be undertaken without his/her approval [2]. A patient has the right to information necessary to make the decision on giving consent to the medical measure (risks of both the undertaken and not undertaken procedure, possible consequences, alternative treatments). The information ought to consist of a diagnose, prognosis, a brief medical measure description and its duration. The consent can be given in an oral, written or silent way. For an invasive procedure, a written consent must be given. The notification language needs to be clear and the procedure well-explained [2]. The signature needs to be willing, signed under no pressure. The consent can come out of a communication implying an active involvement of both sides, or the doctor is allowed to make the decisions. The communication ought to be initiated by the doctor who is supposed to give information on the character of the disease, the surgery plan, prognosis and existing alternatives. It is necessary to inform the patient about the consequences (usual consequences: inability to work and perform everyday activities) and also about the possible complications (in spite of the degree of probability of their occurrence).

There has been an active question on how much information a patient needs. Most doctors share an opinion that little should be said. A patient needs to understand the risks of an intervention/non intervention and to possess the capacity of decision making [3]. Doubting patient's competency imposes the need to consult her parents, guardians, a social worker or a psychiatrist for an evaluation of her mental status and the capacity of decision making. The exceptions are some urgent situations which suppose the doctor to react for the patient's best benefit [3]. Indecisive patients are advised to read some literature, brochures, and even some Internet sites, to gather more information [4]. Modern means of communication (the Internet, forums) can be both useful and harmful in the process of getting information; so, relevant sites ought to be recommended. Articles advising non-standard new surgical techniques and procedures represent a special issue if a patient requires them to be included in the treatment. In this case, only through a conversation about the benefits and the risks of such procedures, based on scientific proofs, the doctor and the patient should reach a mutual agreement. Otherwise, the patient may ask for another opinion [5]. Consent to the intervention and the risk does not make the doctor free from responsibility.

Many patients often don't read the details of the informed consent. The reason for that mostly lies in the doctor-patient relationship, in which the doctors find themselves in the position of the ones possessing knowledge, information and even a certain amount of power. This kind of paternalistic physician-patient model was predominant in the past. The opposite way is a doctor to help the patients discover their best interests after evaluation of their own needs, expectations and beliefs. Sometimes, through such conversations, the patient's need for a certain intervention may disappear, and also, the counseling to initiate a surgical intervention instead [3]. Another model is a tutoring doctor-patient relationship, in which the doctor (the one who knows what the best is) stands opposed to the passive, partially informed, almost believing patient. This kind of a relationship is a relic of the past and considered to be unacceptable in the modern medicine [6, 7].

We tend to achieve a balance in this unequal doctor-patient relationship and to create a partnership. In that case, the possible complications would rarely become an issue at court trials and requests for material and non material damages. In the era of serious debates on human rights, the rights of patients, the right to quality information and the right to free choices are genuinely some of the crucial ones in medicine.

In our prospective study of the informed consent, we included 100 randomly chosen patients who underwent the elective gynecological surgery and who filled an anonymous

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questionnaire conducted on the day of their release from the hospital. The subject of our survey was to find out if it was their formal consent or it was a decision made as a consequence of doctor-patient communication.

The results of the questionnaire showed that 68% of the patients were satisfied with the full quantity and quality of the information they were provided with prior the surgery; almost 1/3 of the patients was dissatisfied both with the quantity and quality of the information (32%). The consent was read only by 36% of the patients. The decision on the operation plan and their active involvement was applied on 12% of the patients, whereas 38% of them claimed that they did not have any influence on deciding about the treatment plan. Half (50%) of the patients share the opinion that doctors would make better decisions than themselves about what options are the most beneficial for them. The surgeon who performed the intervention dedicated the most time and information to the patients (43%); 12% of the patients got informed by unauthorized individuals and 1/5 of the patients did not get any information (Table 1).

Our survey confirmed the fact that patients, in most of the case, are well informed about their medical condition and who will take care of them. A huge number of patients gather a lot of information about their chosen doctor, but after being admitted to the hospital, their attitude becomes passive and they let the surgeon decide for them. However, a significant number of patients never read the written consent (64%) which is a fact other authors also confirmed [8]. An impression is formed that surgeons have too little time to spend with patients after their admittance, which leads to a conclusion that handing a written document to a patient, without communication and talk, is worthless and it is only a formal legal and

Table 1

Information on diagnosis, prognosis and treatment method of the studied patients

Question	Answer	Patients (%)
Who refers the patient for surgery?	Doctor in primary healthcare	23
	Doctor in private practice	25
	Clinical doctor	33
	Consilium	19
Confirmation of awareness about the disease and diagnosis in preparation for surgery	Yes	92
	No	8
Does the patient know who will operate?	Yes	82
	No	18
Are patients informed about the diagnosis before hospitalization?	Yes	92
	No	8
Surgical treatment indicated by	Primary healthcare	23
	Oncology Consilium	25
	Surgeons themselves	19
	Government sector	33
	Private sector	25
Did the patient choose the surgeon?	Yes	82
	No	18
Manner of selection	Recommendation from friend or relative	26
	Recommendation from medical staff	30
	Acquaintance with the surgeon	14
	Do not specify the reason	12
Assessment of patient satisfaction with the quantity and quality of time and information	Satisfied	68
	Dissatisfied	32
Time of obtaining information	Before admission	52
	After admission	24
	Without sufficient information	24
Did they have notice of the plan of operation	Yes	62
	No	38
What impact did the patients have on the plan of operation	Agreement – consensus	12
	No impact	38
	Decision left to the doctor	50
Prior knowledge on the approach (abdominal, vaginal, laparoscopic)	Yes	83
	No	17
Prior knowledge on possible complications	Yes	92
	No	8
Reaction to possible complications	Expecting none	68
	Blaming none	30
	Will complain	2
Assessment of elements related to written consent	Read	36
	Unread	64
Memory of the details of consent at the time of discharge	Total	49
	Partial	16
	No recollection	35
Information best given by	Operator	43
	Departmental doctor	25
	Unauthorized personnel	12
	No one	20

ethical obligation. We consider that a surgeon needs to take part in the last stage of giving consent and signing it and to take that time to talk and respond to the patient's final dilemmas. By doing this, it would become an act of ethics, not just pure formality [9]. But, there is still a question of how much and what to say. How much info does a patient need? Is there time for busy surgeons to tell everything, enough and in the best possible way? Apart from having great surgical skills, a surgeon needs to be well educated for giving information, as well as delivering bad news and informing the patients about possible undesirable situations [10]. Doctors most likely find it much easier to communicate with patients who have a high level of trust and an acceptable attitude towards complications or even non expectance of complications. However, it is a fact that doctors must make patients aware of their own responsibility to make relevant decisions about their lives, despite all medical knowledge, capabilities and good intentions of doctors [11].

Adopted models change slowly and the implementation of new models of behavior and acting demands education of the medical staff. Besides acquiring surgical skills and techniques, surgeons need also to be trained in communicational skills with patients. It is necessary the medical staff understand the rules and regulations of the healthcare laws. The communication training process should be in accordance with the existing standards of the countries where the informed consent has been stable and long present. We should also learn from the mistakes of the countries which are a step ahead of us in their attempts to adopt the desired values. Patients should obtain due attention and respect at every stage of the treatment. Eventually, the informed consent should be considered as a final document in the doctor-patient communication process, and after the surgery, that communication should continue, as well as the counseling and care of the patient, in order to have more successful process.

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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ*, editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

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

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