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CONTENTS / SADRŽAJ

The Belgrade Gate in Istanbul, constructed during the Ottoman Empire, and named after the capital of Serbia, is one of numerous historical monuments in this enchanting city.
This year, from 22 to 26 May, in Istanbul the 18th Congress of the Balkan Committee of Military Medicine was held (see p. 796–7).

Beogradska kapija u Istambulu, sazidana za vreme Otomanskog carstva i nazvana po glavnom gradu Srbije, jedan je od brojnih istorijskih spomenika kojima obiluje ovaj očaravajući grad.
U Istambulu je ove godine, od 22. do 26. maja, održan 18. kongres Balkanskog komiteta vojne medicine (vidi str. 796–7).

ORIGINAL ARTICLES

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Treatment of chronic hepatitis C in injecting drug users – A 5-year follow-up

Lečenje hroničnog hepatitisa C kod intravenskih zavisnika – 5-godišnje praćenje

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Abstract

Background/Aim. Hepatitic C infection (HCV) is a systemic, generalised disease with the prevalence of inflammation in the liver. The aim of this study was to determine the success of treatment for chronic hepatitis C with pegilated interferon alfa 2a and ribavirin in injecting drug users. **Methods.** This a 5-year follow-up study included 30 patients [63.3% men and 36.7% women, average age 30.2 years (SD 7.1 years)] injecting drug users in one-year abstinence, with chronic hepatitis C, treated with the pegilated interferon α 2a and ribavirin. Complete history with possible route of infection, the standard biochemical tests, liver biopsy, quantification of the viral genome in sera and HCV genotyping and subtyping were done prior to the therapy initiation. Depending on the HCV genotype, the therapy was conducted over a period of 48 weeks for genotype 1 and 24 weeks for genotype non 1. Five years later all 30 patients were invited on control examination; 22 of them appeared at the check-up and quantification of the viral genome in their sera were analized. **Results.** The established degree of liver fibrosis was: F0 in 40%, F1 in 23.33%, F2 in 26.67%, F3 in 3.33% and F4 in 6.67% of the patients. Genotype 3a was dominant (50.0%), 1b was registered in 40.0%, 1a in 6.66% and 2b in 3.33% of the patients. Sustained virologic response (SVR) was achieved in 86.7% of the patients, 10.0% of the patients were non-responders, while 3.33% of them revealed recurrence of HCV. Opiate abuse recurrence during antiviral therapy happened in 6.7% of the patients. Five years after the antiviral therapy 73.3% of the patients appeared at the check-up and all of them were in stable abstinence from opiate abuse. All of those, with a sustained viral response of five–year duration, had the negative PCR HCV RNA test (< 50 IU ml^{-1}). In the patients showing unsatisfactory therapy response 5 years before, antiviral therapy was repeated by the same therapeutic regimen, but without adequate therapeutic response. A total of 26.7% of the patients were lost from the records. **Conclusion.** In a 5-year follow-up period 73.3% of the patients used to come regularly to check-ups and among them neither the opiate abuse recurrence nor HCV infection recurrence were registered.

Key words:
hepatitis c, chronic; interferon alfa-2a; ribavirin; substance abuse, intravenous; treatment outcome.

Apstrakt

Uvod/Cilj. Infekcija virusom hepatitisa C (*hepatitis* C virus – HCV) spada u sistemske generalizovanje bolesti sa prevalencijom zapaljenja jetre. Cilj istraživanja bio je da se ustanovi uspeh kombinovane antivirusne terapije pegilovanim interferonom α 2a i ribavirinom u lečenju hroničnog hepatitisa C u grupi zavisnika od opijata u apstinenciji, definisan periodom od 5 godina. **Metode.** Ova retrospektovno-prospektivna studija obuhvatila je 30 obolelih od hroničnog hepatitisa C [63,3% muškog, 36,7% ženskog pola, prosečne starosti 30,2 godine (SD 7,1 godina)], zavisnika od opijata u jednogodišnjoj apstinenciji, lečenih pegilovanim interferonom α 2a i ribavirinom. Kod bolesnika su analizirani anamnestičko-epidemiološki podaci, klinički tok bolesti, patohistološki nalaz biopsije jetre, kao i serološki i virusološki parametri HCV infekcije (kvalitativni PCR HCV RNA i genotip HCV), pre uvođenja terapije. U zavisnosti od genotipa HCV sprovedena je terapija u trajanju od 48 za genotip 1, odnosno 24 nedelje za genotip *non* 1. Pet godina po završetku terapije svi bolesnici pozvani su na kontrolni pregled, 22 se odazvalo pozivu i kod njih je urađen kvalitativni PCR HCV RNA test. **Rezultati.** Prema klasifikaciji METAVIR utrvđen je stepen fibroze jetre: F0 kod 40%, F1 kod 23,33%, F2 kod 26,67%, F3 kod 3,33% i F4 kod 6,67% bolesnika. Genotip 3a bio je dominatan

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(50,0%), 1b registrovan je kod 40,0%, 1a kod 6,66% i 2b kod 3.33% bolesnika. Stabilni virusološki odgovor je postignut kod 86,7% bolesnika, 10,0% bolesnika nije odgovorilo na terapiju, dok je kod 3,3% bolesnika ustanovljena relaps HCV ($p > 0,05$). Do relapsa zloupotrebe opijata u toku antivirusne terapije došlo je kod 6,7% bolesnika. Pet godina po završetku antivirusne terapije na kontrolni pregled odazvalo se 73,3% bolesnika i svi su bili u stabilnoj apstinenciji od upotrebe opijata. Kod onih koji su postigli stabilni virusološki odgovor, 5 godina po završetku terapije, PCR HCV RNA test bio je negativan (< 50 IU ml -1). Ukupno 26,7% bolesnika bilo je izgubljeno iz evidencije. **Zaključak.** U 5-odišnjem periodu praćenja 73,3% bolesnika redovno se odazivala na kontrole i među njima nije zabeležen relaps zloupotrebe opijata, kao ni relaps HCV infekcije.

Ključne reči:
hepatitis c, hronični; interferon alfa-2a; ribavirin; zloupotreba supstanci, intravenska; lečenje, ishod.

Introduction

Hepatitis C virus (HCV) infection is a systemic, generalized disease with the prevalence of inflammation in the liver. "Silent epidemic" of HCV infection is spread around the world, with an overall prevalence of approximately 3% of the infected [1]. It is well-known that in 80% of the cases HCV infection leads to chronic hepatitis with the progression to liver cirrhosis in 20% of the patients, and the emergence of primary hepatocellular carcinoma in 10% of the patients with liver cirrhosis [2]. However, it is believed that we will face the real consequences of this infection only in 2020, when there will be the manifestation of the HCV infection progression, in the most endangered population today, addicts to psychoactive substances [3]. With the contemporary treatment of chronic hepatitis C (CHC), pegylated interferon α and ribavirin (peg IFN-α + rbv), eradication of the virus is achieved in 50%–80% of patients, the degree of fibrosis is reduced and the progression into liver cirrhosis is stopped [4]. There are conflicting views on the treatment of CHC injecting drug users in the world [5]. Today, the current antiviral therapy of peg IFN-α + rbv is extremely expensive – 30,000 to 40,000 dollars per patient [6], so the question of investment in the treatment of the population, which is often socially and economically unproductive, is raised. There is a widespread opinion that the population of injecting drug users is unsuitable for the therapy due to the compliance problem and the possibility of reinfection [7].

The aim of the research was to determine the success of the combined antiviral therapy (peg IFN-α 2a + rbv) in the treatment of chronic hepatitis C in a group of injecting drug users in 1-year abstinence, defined with a period of 5-year follow-up.

Methods

This 5-year follow up stady included 30 patients with chronic hepatitis C, injecting drug users in 1-year abstinence, treated with the combined therapy peg IFN-α 2a + rbv, (Pegasys®+Copegus®) in the Clinic for Infectious Diseases, Clinical Centre of Vojvodina, from January 1, 2004 to January 1, 2005. Complete history with a possible route of infection, physical examination, assessment of alcohol consumption, an abdominal ultrasound, standard biochemical liver functional tests, histological grades (activity) and stages (fibrosis) using the METAVIR scoring system, quantitative PCR HCV RNA, HCV genotyping and subtyping were analysed in the patients prior to the therapy initiation.

In the whole cohort of the patients, there were 19 males and 11 females, aged from 22–40 years (mean ± SD = 30.2 ± 7.1 years). All the patients provided informed written consent, and the study way carried out in accordance with the declaration of Helsinki (2000) of the World Medical Association.

Depending on the HCV genotype, the therapy was conducted over a period of 48 weeks for genotype 1 and 24 weeks for genotype non-1. PegIFN-α 2a (Pegasys^R) was administered s.c. at a dose of 180 mcg once a week with daily ribavirin (rbv) (Copegus^R) pill – taking at a dose of 800 mg a day for the patients with genotype non-1 HCV, regardless of body weight for 24 weeks, for genotype 1 HCV 1000 mg a day for the patients with body weight up to 75 kg, and 1200 mg a day for 48 weeks for the patients with body weight over 75 kg.

Data analysis was performed by the software SPSS version 10.0. Among the methods of descriptive statistics the measures of central tendency (mean, $\bar{X}$), standard deviation (SD), as well as variability measures, absolute and relative frequency were used. The parametric tests (Student's *t*-test) and non-parametric tests (Mann-Whithney U, Kruskal-Wallis test, χ^2-square test and Spearsman's rho correlation coefficient) were used for data analysis. The chosen levels of significance were statistically highly significant ($p < 0.01$) and not statistically significant ($p > 0.05$).

Results

Out of 30 injecting drug users in 1-year abstinence with chronic hepatitis C, 19 (63.3%) males and 11 (36.7%) were females, aged from 22 to 40 years ($\bar{X}$ ± SD, 30.2 ± 7.1 years). The assumed average duration of infection was 8.9 ± 7.4 years. All the infected patients had intravenuous opiate addiction as a way of HCV transmission, and all of them had a psychiatric confirmation of a stable 1-year opiate abuse abstinence.

Before the antiviral therapy was administered in all the patients, a blind aspirational liver biopsy was done. According to METAVIR classification a degree of liver fibrosis was established: F0 in 12/30 (40%), F1 in 7/30 (23.33%), F2 in 8/30 (26.67%), F3 in 1/30 (3.33%) and F4 in 2/30 (6.67%) of the patients.

Genotype 3a was dominant in 15/30 (50.0%), 1b was registered in 12/30 (40.0%), 1a in 2/30 (6.66%) and 2b in 1/30 (3.33%) of the patients (Table 1).

Table 1

Distribution of hepatitis C virus (HCV) genotype

HCV genotype	Patients n (%)
1a	2/30 (6.67)
1b	12/30 (40.00)
3a	15/30 (50.00)
2b	1/30 (3.33)
Total	30 (100)

A statistically significant difference between the degree of liver fibrosis and HCV genotype was not found ($p > 0.05$).

A sustained viral response (SVR) (HCV RNA PCR test negative 6 months after the therapy) was achieved in 26/30 (86.7%) patients, 3/30 (10.0%) patients did not respond to the treatment and were marked as "non-responders" (NR) (HCV RNA PCR positive at the end of the therapy, as well as at the end of follow-up period), while 1/30 (3.33%) patients revealed recurrence of HCV (HCV RNA PCR negative at the end of the therapy, but positive at the end of a follow-up period) (Table 2)

Opiate abuse recurrence during antiviral therapy appeared in 2/30 (6.7%) patients. In both cases the treatment continued with psychiatric support.

Five years after the antiviral therapy 22/30 (73.3%) patients appeared at the check-up and all of them were in stable abstinence from opiate abuse. A total of 8/30 (26.7%) patients were lost from the records.

Among 22/30 (73.3%) of the patients who appeared at the check-up, 20/22 (90.9%) of the patients had SVR 6 months after the therapy. All of them, 20/20 (100.0%) had a sustained viral response of a 5-year duration, defined by the negative PCR HCV RNA test (Table 2). In 2/22 (9.1%) patients who 5 years before had an unsatisfactory therapy response, the antiviral therapy was repeated by the same therapeutic regimen, but without adequate an therapeutic response.

Table 2

Sustained virological response (SVR)

Clinical outcome	Patients (after the treatment) n (%)	Patients (after a 5-year follow-up) n (%)
SVR	26/30 (86.7)	20/22 (90.9)
NR	3/30 (10.0)	2/22 (9.1)
Relaps	1/30 (3.3)	0
Lost from follow up		8/30 (26.7)
Total	30 (100)	30 (100)

NR – non responders.

Discussion

Out of 30 injecting drug users in 1-year abstinence, infected by HCV, included in the study, the ratio between men and women was equal, although recent studies have indicated that among newly diagnosed cases with chronic hepatitis in drug addicts, the percentage of women is greater [8]. It is obvious that the main route of transmission is sharing of needles, but it is estimated that the risk of HCV transmission increases with the duration of substance abuse, more frequent daily use of opiates, smoking "crack" and belonging to the so-called "shooting galleries" [9]. Among the male population, risk factors are primarily related to the careless use of opiates, while in women the risk of HCV infection transmission was increased by risky sexual behaviour [10].

It is believed that HCV infection in 50%–80% occurs in the first year of intravenous drug use [9]. We hypothesized that inoculation with HCV in our patients was in the first year of intravenous use of opiates, so the likely duration of infection was 8.9 years.

Most patients surveyed did not have, or had just a low degree of liver fibrosis: F0 in 12/30 (40%), F1 in 7/30 (23.33%), F2 in 8/30 (26.67%), F3 in 1/30 (3.33%) and F4 in 2/30 (6.67%) of the patients. It is well-known that most important factors of liver fibrosis within the chronic hepatitis C are patient's age at the time of initial infection and the duration of infection [11]. The low level of fibrosis in our study stems exactly from the fact that these were young people, of the average age of 30.2 years and relatively short infection duration of 8.9 years on average.

The dominant genotype in our study was genotype 3a (50.0%), followed by 1b (40.0%). The dominance of genotype 3 is consistent with the pattern of HCV genotypes distribution among risk groups in Western Europe, as in Serbia [12–14]. Clinical significance of determining HCV genotypes derived from the proved correlation between certain genotypes and the risk of developing severe degree of liver damage or the development of HCC [15]. A recent study conducted in Serbia confirmed that the patients infected with HCV genotype 1b had more frequently moderate or severe necroinflammatory activity of the disease, and a significantly higher grading score as compared with other genotypes [16]. However, we did not find a statistically significant difference between the degree of liver fibrosis and HCV genotype ($p > 0.05$). A probable reason for this discrepancy is that this study included younger patients with shorter duration of HCV infection.

The aim of antiviral therapy is to achieve SVR. With the combined therapy, peg IFN α 2a + rbv, SVR was achieved in 70%–80% of the patients with genotype 2 or 3 and in 40%–50% of the patients with genotype 1 or 4 [17–19]. SVR among the examined patinets was noted in a significantly higher percentage (86.7%), and it is explained by a younger age, milder degree of liver damage, higher frequency of genotype 3, but the main reason probably was the short duration of HCV infection

among the examined patinets. In fact, for every decade of the treatment postpone, the chance of obtaining SVR will decrese by approximately 10% [20].

Opiate abuse recurrence and the possibility of HCV reinfection is one of the most important arguments "against" the treatment of HHC of injecting drug users [21]. It is well known that about 50% of the treated injecting drug users return to drug use even to a much worse form of addiction [22]. In our study opiate abuse recurrence occured in 2 (6.7%) of the patients during the antiviral therapy. In both patients, with the psychiatric support the therapy was resumed, in one patient SVR was achieved, while the other patient did not respond to the antiviral therapy favourably.

The incidence of HCV reinfection after injecting drug abuse recurrence is 0–4 per 100 person-years of follow-up [23]. A lower incidence of HCV reinfection compared to the "primo" infection is explained by the prevention measures: intravenous addicts take care of their personal protection measured by using personal equipment for taking drugs [21]. Some authors believe that the lower infection incidence, and the recurrence of intravenuous abuse of opiates, indicate the exsistence of partial cell-mediated immunity after the successful treatment of chronic hepatitis C [24]. In our study, the recurrence of injecting drug abuse (during a 5-year follow-up) was not registered and neither was HCV infection.

Five years after the antiviral therapy 8/30 (27.3%) patients were lost from the register in the Clinic for Infectuous Diseases, so their abstinence from opiate abuse, as well as the status of chronic hepatitis C cannot be determined. A total of 22/30 (73.3%) patients came regularly for a check-up, and all of them were in stable abstinence from opiate abuse. All the patients who achieved SVR, maintained SVR 5 years after the therapy, so they can be considered cured.

Two patients (9.1%) with isolated genotype 1b, did not respond to the therapy adequately ("nonresponders"). Two years following the antiviral therapy peg IFN α 2a+rbv (Pegasys®+Copegus®) was reintroduced, but again with no adequate therapeutic response.

Conclusion

This study indicates that opiate addiction does not influence the course of CHC nor the outcome of CHC therapy. The patients with CHC from the group of former drug addicts achieved a good virological response (86.67%) to the therapy peg IFN α + rbv (Pegasys®+Copegus®). In a 5-year follow-up period 73.3% of the patients used to come regularly to check-ups and among them neither the opiate abuse recurrence nor HCV infection recurrence was registered.

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Correlation of N-terminal pro-B-type natriuretic peptide with clinical parameters in patients with hypertension

Korelacija N-terminalnog pro-B-tipa natriuretskog peptida sa kliničkim parametrima kod bolesnika sa hipertenzijom

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Abstract

Background/Aim. Identification of patients with arterial hypertension and a possible onset of heart failure by determining the concentration of N-terminal pro-B-type natriuretic peptide (NT-proBNP) enables timely intensification of treatment and allows clinicians to prescribe and implement optimal and appropriate care. The aim of this study was to evaluate NT-proBNP in patients with longstanding hypertension and in patients with signs of hypertensive cardiomyopathy. **Methods.** The study involved 3 groups, with 50 subjects each: "healthy" persons (control group), patients with hypertension and normal left ventricular systolic function (group 1) and patients with longstanding hypertension and signs of hypertensive cardiomyopathy with impaired left ventricular systolic function (group 2). We measured levels of NT-proBNP, C-reactive protein and creatinine according to the manufacturer's instructions. All the patients were clinically examined including physical examination of the heart with blood pressure, pulse rate, electrocardiogram (ECG) and echocardiogram. **Results.** Our results showed that the determined parameters generally differed significantly (Student's *t*-test) among the groups. The mean (± SD) values of NT-proBNP in the control group, group 1 and group 2 were: 2.794 (± 1.515) pmol/L, 9.575 (± 5.449) pmol/L and 204.60 (84,93) pmol/L, respectively. NT-proBNP correlated significantly with the determined parameters both in the group 1 and the group 2. In the group 1, the highest correlation was obtained with C-reactive protein (r = 0.8424). In the group 2, the highest correlation was obtained with ejection fraction (r = -0.9111). NT-proBNP showed progressive increase in proportion to the New York Heart Association (NYHA) classification. The patients in the-group 2 who belonged to the II and III NYHA class had significantly higher levels of NT-proBNP than those in the NYHA class I (ANOVA test, $p = 0.001$). **Conclusion.** The obtained results suggest that NT-proBNP is a useful biomarker in the treatment of patients with longstanding hypertension who are at risk for heart failure.

Key words:
natriuretic peptide; biological markers; hypertension; cardiomyopathy, hypertrophic; risk assessment.

Apstrakt

Uvod/Cilj. Identifikacija bolesnika sa arterijskom hipertenzijom kojima preti srčana slabost, pomoću određivanja koncentracije N-terminalnog pro-B-tipa natriuretskog peptida (NT-proBNP) omogućava kliničarima pravovremeno intenziviranje lečenja i propisivanje i sprovođenje optimalne i odgovarajuće nege, jer bolesnici sa hipertenzivnim srčanim oboljenjima imaju povišene koncentracije NT-proBNP. Cilj rada bio je da se izvrši procena razlike u NT-proBNP kod bolesnika sa hipertenzijom i normalnom sistolnom funkcijom i onih sa dugogodišnjom arterijskom hipertenzijom i znacima hipertenzivne kardiomiopatije. **Metode.** U ispitivanje su bile uključene tri grupe, svaka sa po 50 ispitanika: "zdrave" osobe (kontrolna grupa), bolesnici sa arterijskom hipertenzijom i normalnom sistolnom funkcijom leve komore (grupa 1) i bolesnici sa dugogodišnjom arterijskom hipertenzijom i znacima hipertenzivne kardiomiopatije sa oslabljenom sistolnom funkcijom leve komore (grupa 2). Nivoi NT-proBNP, C-reaktivnog proteina i kreatinina određivani su prema uputstvu proizvođača. Svi bolesnici bili su klinički pregledani uključujući fizički pregled srca sa merenjem krvnog pritiska i pulsa, elektrokardiogramom (EKG) i ehokardiogramom. **Rezultati.** Naši rezultati pokazuju da se određivani parametri generalno

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značajno razlikuju (Studentov *t*-test) među grupama. Srednje (± SD) vrednosti NT-proBNT u kontrolnoj grupi, grupi 1 i grupi 2 bili su: 2,794 (± 1,515) pmol/L, 9,575 (± 5,449) pmol/L i 204,60 (84,93) pmol/L, redom. NT-proBNT značajno koreliše sa određivanim parametrima u grupi 1 i u grupi 2. U grupi 1, najviša korelacija dobijena je sa C-reaktivnim proteinom (r = 0,8424). U grupi 2, najviša korelacija dobijena je sa ejekcionom frakcijom leve komore (r = -0,9111). Utvrđeno je progresivno povećanje NT-proBNT u odnosu na klasifikaciju *New York Heart Association* (NYHA). Bolesnici u grupi 2 koji su pripadali NYHA klasi II i III imali su značajno više vrednosti NT-proBNP od bolesnika u NYHA klasi I (ANOVA test, p = 0,001). **Zaključak.** Rezultati istraživanja ukazuju na to da je NT-proBNP koristan biomarker u lečenju bolesnika sa dugogodišnjom arterijskom hipertenzijom kojima preti zastojna srčana slabost.

Ključne reči:
natriuretski peptidi; biološki pokazatelji; hipertenzija; kardiomiopatija, hipertrofička; rizik, procena.

Introduction

Like other natriuretic peptides, N-terminal pro-B-type natriuretic peptide (NT-proBNP) is secreted from the heart in response to cardiac hemodynamic stress mediated by volume and/or pressure overload [1]. The identification of patients with arterial hypertension with the onset of heart failure by determining the concentration of NT-proBNP, enables timely intensification of treatment and allows physicians to prescribe and implement optimal and appropriate therapy [2–4]. The increase of NT-proBNP is related to the left ventricular mass index, left ventricular hypertrophy and diastolic left ventricular dysfunction. Concentrations of NT-proBNP in the serum were not significantly increased in hypertensive patients with the normal left ventricular geometry compared to normotensive individuals [5, 6].

Heart failure is characterized by a dysfunctional natriuretic peptide system. Natriuretic peptides are semiquantitative markers of cardiac stress and heart failure, and thus related to the extent of atrial, ventricular, and valvular dysfunction [1]. Diagnosis of heart failure in the primary stage using only clinical criteria in 50% of cases gives false positive results [7, 8]. Heart failure, especially in its early stages, is difficult to diagnose. The most commonly applied method of investigation to confirm the diagnosis of heart failure is echocardiography, which offers structural and functional information about the heart. However, assessment of cardiac function by echocardiography requires considerable time and is expensive to use in daily practice. For clinicians there is a legitimate medical requirement for a biomarker that would be a reliable and objective test to identify hypertensive patients with the onset of heart failure [9–15].

Inflammatory markers are increased in chronic heart failure, including C-reactive protein (CRP) [16]. Additionally, CRP predicts morbidity and mortality in patients with established heart failure [17–19].

NT-proBNP has a principal effect on the kidney, promoting tubular natriuresis and diuresis. As with heart failure evaluation, knowledge of the cardiac and noncardiac factors that influence the NT-proBNP concentration is necessary. Therefore, proper study of the evaluation of heart failure must include the estimation of glomerular filtration rate or determination of creatinine [20].

The aim of this study was to compare the concentrations of NT-proBNP with the parameters of clinical examination and biomarkers (systolic blood pressure, diastolic blood pressure, ejection fraction, CRP and creatinine) in patients with hypertension, and to determine the relationship between NT-proBNP and the New York Heart Association (NYHA) classification in patients with longstanding hypertension with signs of hypertensive cardiomyopathy.

Methods

We measured all the biomarkers according to the manufacturer's instructions. The analytical performance of the methods has been evaluated and described elsewhere [21]. The study included three groups, each with 50 subjects: "healthy" persons (control group), patients with hypertension and normal systolic left ventricular function (group 1) and patients with longstanding hypertension and signs of hypertensive cardiomyopathy with impaired systolic function of the left ventricle (group 2). The control group included healthy adults of both sexes subjected to the routine systematic health examination at the Institute of Occupational Medicine, Military Medical Academy in Belgrade, Serbia and voluntary blood donors at the Institute of Transfusion, Military Medical Academy in Belgrade, Serbia. The patients of the group 1 and group 2 were treated at the Clinic of Cardiology, Military Medical Academy and had the diagnosis of these diseases. These patients were clinically examined including physical examination of the heart with blood pressure, pulse rate, electrocardiogram (ECG) and echocardiogram. Left ventricular ejection fraction (EF) was derived from 2-dimensional echocardiography. Blood samples were taken from all the subjects and the serum was separated from cells within 60 minutes of collection and centrifuged at 2028g (4000 rpm) for 10 min. We measured all the biomarkers in a single batch at the Institute of Medical Biochemistry, Military Medical Academy. The study was approved by the institutional Ethics Committee, in compliance with the Helsinki criteria. All the study participants gave written informed consent.

All the biomarkers were measured according to the manufacturer's instructions. The analytical performance of the methods has been evaluated and described elsewhere [21]. Levels of NT-proBNP were measured by a one-step enzyme immunoassay based on electrochemiluminescence technology on the Elecsys® 2010 platform (Roche Diagnostics). The reference range, as reported by the manufacturer, was < 14.75 pmol/L. Levels of CRP were measured using the Behring BN II Nephelometer (Dade Behring/Siemens Medical Solutions

Diagnostics). The reference range, as reported by the manufacturer, was < 3 mg/L. Creatinine concentrations were measured by the kinetic alkaline picrate method (improved Jaffe reaction) on a Dimension RxL Max analyzer (Dade Behring/Siemens Medical Solutions Diagnostics). The reference range, as reported by the manufacturer, was 53–115 μmol/L (71–115 μmol/L for men and 53–88 μmol/L for women).

Adherence to Gaussian distributions was assessed using the Kolmogorov-Smirnov test. The mean, standard deviation (SD), Student's *t*-test and Pearson's test were used for statistical analysis. All the probabilities were two tailed and $p < 0.05$ was regarded as significant. The 95% confidence interval (CI) was also shown in reported data. The data were statistically analysed with the MedCalc®, Ver. 11.3.3.0 package (MedCalc Software, Mariakerke, Belgium).

Results

The control group included 25 women and 25 men aged 50–65 years ($\bar{x}$ ± SD, 56.3 ± 4.20 years). The group 1 included 19 women and 31 men aged 50–65 years ($\bar{x}$ ± SD, 57.7 ± 4.57 years), and in the group 2 there were 17 women and 33 men aged 50–65 years ($\bar{x}$ ± SD, 58.1 ± 4.82 years). Clinical and echocardiographic examinations were performed in all patients in order to evaluate the NYHA-class and the left ventricular EF.

The mean (SD) values of NT-proBNP, creatinine, CRP, systolic blood pressure, diastolic blood pressure and in the control group, group 1 and group 2 were presented in Tables 1–3. Comparison studies showed that levels of NT-proBNP and systolic blood pressure were significantly higher in the group 1 and the group 2 than in the control group, and also higher in the group 2 than in the group 1. EF values were significantly lower in the group 1 and in the group 2 than in the control group, and also lower in the group 2 than in the group 1. Levels of creatinine and diastolic blood pressure did not differ significantly between the groups 1 and 2, while CRP levels did not differ between the control group and the group 1 (Tables 1–3, Figure 1).

In the group 2, the patients were divided into three subgroups according to the NYHA classification. The number of patients in the group 2 in different NYHA classes was: I (n = 29.58%), II (n = 16.32%) and III (n = 5.10%). The group 2 subjects that belonged to the NYHA class II and III had significantly higher levels of NT-proBNP than those in the NYHA class I (ANOVA test, $p = 0.001$). The levels of NT-proBNP were also significantly higher in the NYHA class I than in the control group (Tables 4–5, Figure 2).

The distribution of data in all the groups was generally normal, so the Pearson's test was used for correlation analysis. The values for the correlation coefficient (r), the confidence interval for r (95% CI) and *p* are given in Tables 6 and 7.

Table 1

Comparison analysis data for the determined parameters between the control group and the group 1

Parameter	Control group	Group 1	*p* value
NT-proBNP (pmol/L)	2.794 (1.515)	9.575 (5.449)	< 0.0001*
Creatinine (μmol/L)	85.5 (12.4)	90.9 (13.7)	0.0414*
CRP (mg/L)	2.64 (1.02)	2.73 (1.07)	0.6609
Systolic blood pressure (mm/Hg)	126.9 (9.5)	146.3 (9.4)	< 0.0001*
Diastolic blood pressure (mm/Hg)	81.2 (4.9)	92.2 (5.0)	< 0.0001*
EF (%)	63.2 (4.5)	60.8 (5.3)	< 0.0001*

Data are presented as means (± SD); * statistically significant difference (Student's *t*-test); NT-proBNP–N–terminal pro B-type natriuretic peptide; CRP – C-reactive protein; EF – ejection fraction.

Table 2

Comparison analysis data for the determined parameters between the control group and the group 2

Parameter	Control group	Group 2	*p* value
NT-proBNP (pmol/L)	2.794 (1.515)	204.60 (84.93)	< 0.0001*
Creatinine (μmol/L)	85.5 (12.4)	90.9 (14.3)	0.0464*
CRP (mg/L)	2.64 (1.02)	4.17 (1.03)	< 0.0001*
Systolic blood pressure (mm/Hg)	126.9 (9.5)	150.5 (6.3)	< 0.0001*
Diastolic blood pressure (mm/Hg)	81.2 (4.9)	95.5 (11.0)	< 0.0001*
EF (%)	63.2 (4.5)	48.0 (6.0)	< 0.0001*

Data are presented as means (± SD); * statistically significant difference (Student's *t*-test); NT-proBNP–N–terminal pro B-type natriuretic peptide; CRP – C-reactive protein; EF – ejection fraction.

Table 3

Comparison analysis data for the determined parameters between the group 1 and the group 2

Parameter	Group 1	Group 2	*p* value
NT-proBNP (pmol/L)	9.575 (5.449)	204.60 (84.93)	< 0.0001
Creatinine (μmol/L)	90.9 (13.7)	90.9 (14.3)	1.000
CRP (mg/L)	2.73 (1.07)	4.17 (1.03)	< 0.0001
Systolic blood pressure (mm/Hg)	146.3 (9.4)	150.5 (6.3)	0.0099
Diastolic blood pressure (mm/Hg)	92.2 (5.0)	95.5 (11.0)	0.0558
EF (%)	60.8 (5.3)	48.0 (6.0)	< 0.0001

Data are presented as means (± SD); * statistically significant difference (Student's t-test); NT-proBNP–N–terminal pro B-type natriuretic hormone; CRP – C-reactive protein; EF – ejection fraction.

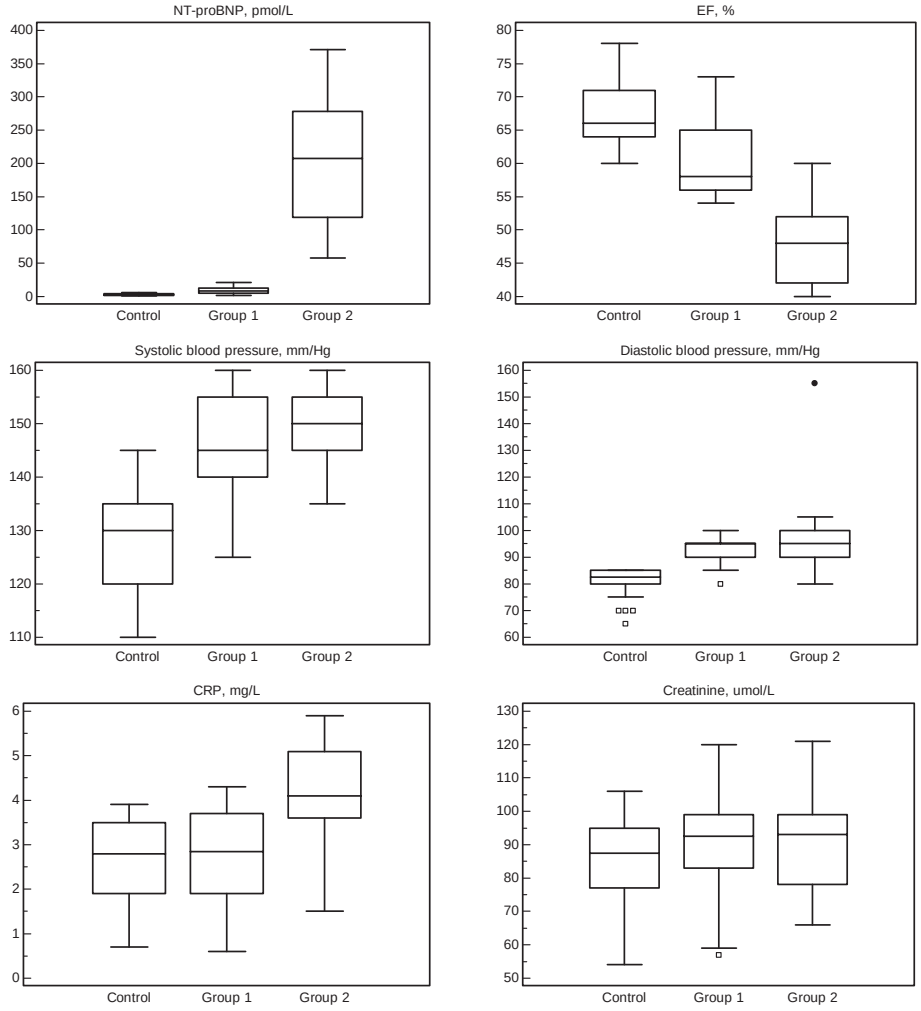

Fig. 1 – Distribution of N–terminal pro B-type natriuretic peptide (NT-proBNP), ejection fraction (EF), systolic and diastolic blood pressure, creatinine and C-reactive protein (CRP) value in the control group, the group 1 and the group 2

Table 4

Comparison analysis data for the determined parameters in the group 2 in regard to the New York Heart Association (NYHA) classes

Parameter	*p* values	NYHA	
		class	mean
NT-proBNP (pmol/L)	< 0.001*	I	147.4 (II) (III)
		II	367.2 (I)
		III	336.4 (I)
Creatinine (μmol/L)	0.447	I	89.5
		II	91.2
		III	98.4
CRP (mg/L)	< 0.001*	I	3.69 (II) (III)
		II	4.60 (I) (III)
		III	5.58 (I) (II)
Systolic blood pressure (mm/Hg)	0.004*	I	148.3 (II) (III)
		II	152.5 (I)
		III	157.0 (I)
Diastolic blood pressure (mm/Hg)	0.251	I	93.4
		II	97.5
		III	101.0
EF (%)	< 0.001*	I	52.1 (II) (III)
		II	43.1 (I)
		III	40.0 (I)

The numbers in brackets [(I), (II), (III)] indicate a NYHA class which was significantly different from the present NYHA class; * statistically significant difference (ANOVA test); NT-proBNP–N–terminal pro B-type natriuretic peptide; CRP – C-reactive protein; EF – ejection fraction.

Table 5

Comparison analysis data for the determined parameters between the control group and the NYHA I subgroup in the group 2

Parameter	Control group	NYHA I	*p* values
NT-proBNP (pmol/L)	2.794 (1.515)	147.30 (49.58)	< 0.0001*
Creatinine (μmol/L)	85.5 (12.4)	89.5 (15.9)	0.2197
CRP (mg/L)	2.64 (1.02)	3.69 (0.85)	< 0.0001*
Systolic blood pressure (mm/Hg)	126.9 (9.5)	148.3 (6.2)	< 0.0001*
Diastolic blood pressure (mm/Hg)	81.2 (4.9)	93.4 (6.3)	< 0.0001*
EF (%)	63.2 (4.5)	52.1 (4.0)	< 0.0001*

Data are presented as means (± SD); * statistically significant difference (Student's t-test); NT-proBNP–N–terminal pro B-type natriuretic peptide; CRP – C-reactive protein; EF – ejection fraction.

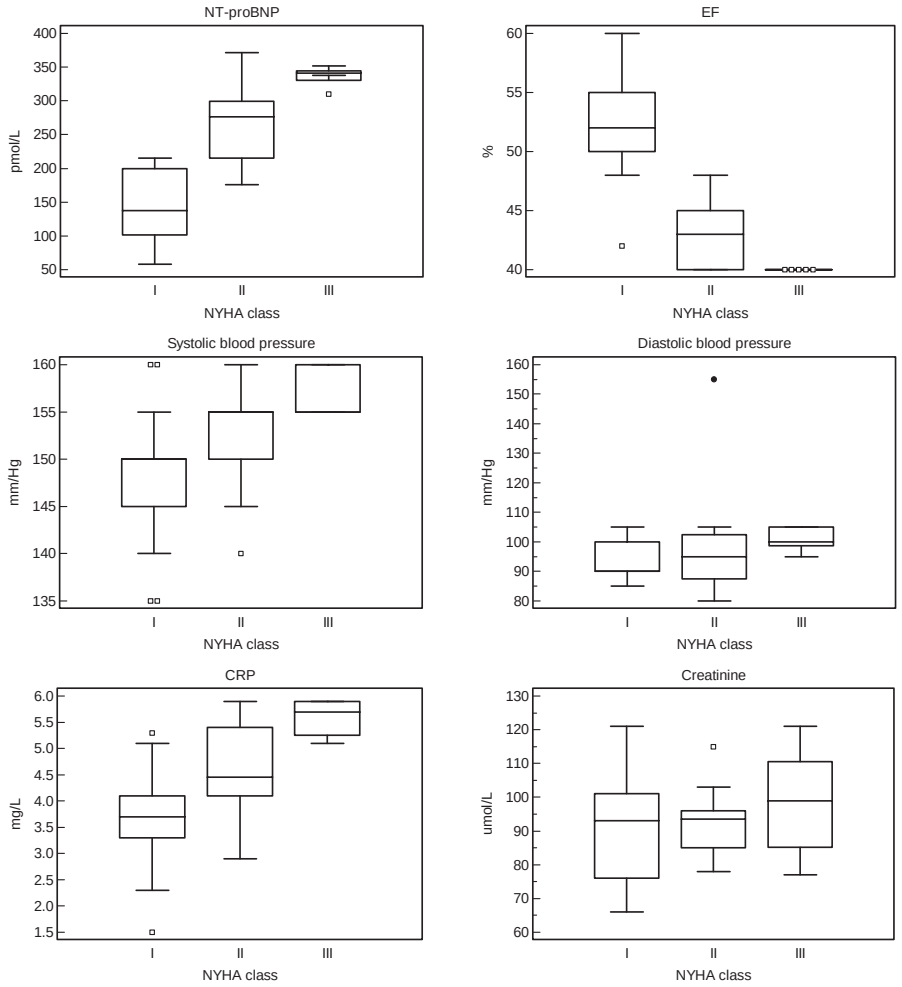

Fig. 2 – Distribution of N–terminal pro B-type natriuretic peptide (NT-proBNP), ejection fraction (EF), systolic and diastolic blood pressure, creatinine and C-reactive protein (CRP) value in the group 2 according to the NYHA classification

In the group 1, NT-proBNP correlated significantly with all the determined parameters (Table 6). The highest correlation was obtained between NT-proBNP and CRP (r = 0.8424, 95% CI 0.7369–0.9079). In the group 2, NT-proBNP also correlated significantly with all the parameters, except with creatinine (Table 7). The highest correlation was obtained between NT-proBNP and EF (r = -0.9111, 95% CI -0.9489 – -0.8478).

Table 6

The correlation of N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations and the values of determined parameters in the group 1 (n = 50)

Parameter	NT-proBNP (pmol/L)		
	r	95%CI	*p* values
Creatinine	0.3379	0.0657–0.5633	0.0164*
CRP	0.8424	0.7369–0.9079	< 0.0001*
Systolic blood pressure	0.7213	0.5542–0.8325	< 0.0001*
Diastolic blood pressure	0.4282	0.1701–0.6313	0.0019*
EF	-0.7390	-0.8438 – -0.5800	< 0.0001*

*** statistically significant correlation (Pearson's test); CRP – C-reactive protein; EF – ejection fraction.**

Table 7

The correlation of N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations and the values of determined parameters in the group 2 (n = 50)

Parameter	NT-proBNP, pmol/L		
	r	95%CI	*p* values
Creatinine	0.1737	-0.1100–0.4312	0.2276
CRP	0.6650	0.4745–0.7960	< 0.0001*
Systolic blood pressure	0.4856	0.2396–0.6730	0.0004*
Diastolic blood pressure	0.3989	0.1355–0.6095	0.0041*
EF	-0.9111	-0.9489 – -0.8478	< 0.0001*

*** statistically significant correlation (Pearson's test); CRP – C-reactive protein; EF – ejection fraction.**

Discussion

Elevated blood pressure causes left ventricular hypertrophy as an independent factor for the development of arrhythmias, heart failure and sudden death. NT-proBNP now appears to be an indicator of asymptomatic cardiac organ damage in patients who eventually develop left ventricular hypertrophy, left arterial dilation, atrial fibrillation, and left ventricular systolic dysfunction [22, 23].

This study aimed to assess the characteristics of NTproBNP, CRP, creatinine and the parameters of clinical examination (systolic blood pressure, diastolic blood pressure) in patients with longstanding hypertension and in patients with signs of hypertensive cardiomyopathy.

NT-proBNP showed good sensitivity in detecting heart failure in the group of patients with hypertension and cardiomyopathy. A single measurement of NT-proBNP at the time of hospital admission provides important information about left ventricular EF in patients with hypertension [1]. Comparison with the group of patients with hypertension showed that levels of this natriuretic peptide correlated equally ($p < 0.001$) with systolic blood pressure, EF, CRP and creatinine. Piechota et al. [24] reported that NT-proBNP correlated equally well with the clinical and echocardiographic parameters of chronic heart failure, which makes them equally adequate in the biochemical staging of chronic heart failure severity.

Several clinical trials have shown that NT-proBNP is a sensitive marker of cardiac function whose rise indicates the presence of heart failure, and on the other hand, when the level of NT-proBNP is within the normal limits, it excludes cardiac dysfunction. The level of NT-proBNP is directly correlated with the severity of disease (I–IV NYHA classification) [9, 19]. In our study patients in the group with NYHA class II and III had significantly higher plasma concentrations of NT-pro-BNP, hsCRP and lower EF ($p < 0.001$). Seino et al. [10] also evaluated NT-proBNP in 105 patients with chronic heart failure, and a progressive increase in NT-proBNP in proportion to the NYHA classification was confirmed in this study.

It was shown that CRP is elevated in patients with chronic heart failure and its prognostic value was established in these patients [16–18]. Our study confirms these findings. The mean CRP of patients in the group 2 (patients with hypertension and cardiomyopathy) was 4.17 ± 1.03 mg/L, and 2.73 ± 1.07 in the group 1 (patients with hypertension) ($p < 0.0001$). Because we had excluded patients with infections or inflammatory diseases, our data suggest that inflammation was related to heart failure and not to external factors.

Conclusion

This study supports the importance of NT-proBNP measurement in patients with longstanding hypertension with signs of hypertensive cardiomyopathy. It shows that NTproBNP correlates significantly with EF. NT-proBNP showed a progressive increase in proportion to the NYHA classification. These data suggest that NT-proBNP is a useful biomarker in the treatment of patients with longstanding hypertension who are at risk for heart failure.

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Radiotherapy treatment planning: benefits of CT-MR image registration and fusion in tumor volume delineation

Planiranje radioterapije: značaj registracija i fuzije CT-MR slike za određivanje zapremine tumora

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Abstract

Background/Aim. Development of imaging techniques, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET), made great impact on radiotherapy treatment planning by improving the localization of target volumes. Improved localization allows better local control of tumor volumes, but also minimizes geographical misses. Mutual information is obtained by registration and fusion of images achieved manually or automatically. The aim of this study was to validate the CT-MRI image fusion method and compare delineation obtained by CT versus CT-MRI image fusion. **Methods.** The image fusion software (XIO CMS 4.50.0) was applied to delineate 16 patients. The patients were scanned on CT and MRI in the treatment position within an immobilization device before the initial treatment. The gross tumor volume (GTV) and clinical target volume (CTV) were delineated on CT alone and on CT+MRI images consecutively and image fusion was obtained. **Results.** Image fusion showed that CTV delineated on a CT image study set is mainly inadequate for treatment planning, in comparison with CTV delineated on CT-MRI fused image study set. Fusion of different modalities enables the most accurate target volume delineation. **Conclusion.** This study shows that registration and image fusion allows precise target localization in terms of GTV and CTV and local disease control.

Key words:
radiotherapy; radiotherapy planning, computer-assisted; tomography, x-ray computed; magnetic resonance imaging.

Apstrakt

Uvod/Cilj. Brzi razvoj tehnika snimanja kao što su kompjuterska tomografija (CT), magnetska rezonanca (MR), i pozitronska emisiona tomografija (PET), ima veliki uticaj na kvalitet individualnih radioterapijskih planova, u smislu bolje lokalizacije tumora. Bolje lokalizovanje tumora podrazumeva i bolju lokalnu kontrolu bolesti. Spajanje CT, MR i PET slika u jednu (registracija i fuzija slike) može se izvesti automatski ili manuelno. Cilj ovog rada bio je da se proceni upotrebljivost fuzije i uporedi delineacija tumora sa CT preseka i sa slike dobijene fuzijom CT-MR. **Metode.** Softver za planiranje radioterapije i fuziju slike (XIO CMS 4.50.0) primenjen je kod 16 bolesnika. Bolesnici su snimljeni na CT i MR u istim pozicijama u kojima će se i zračiti. Ukupni (GTV) i klinički volumeni tumora (CTV) određeni su prema protokolu na kompletu CT slika, a zatim, nakon fuzije CT sa MR, i na novonastalom kompletu snimaka. **Rezultati.** Fuzija slike pokazala je da je CTV određen samo sa CT snimaka uglavnom neadekvatan za planiranje terapije, odnodno da fuzija informacije sa CT i MRI omogućuje mnogo preciznije i bolje određivanje kontura tumora. **Zaključak.** Registracija, a potom i fuzija slike CT-MR, omogućuje najprecizniju moguću lokalizaciju tumora (GTV) i volumena kliničkog širenja bolesti (CTV), kao i bolju lokalnu kontrolu bolesti.

Ključne reči:
radioterapija; radioterapija, kompjutersko planiranje; tomografija, kompjuterizovana, rendgenska; magnetska rezonanca, snimanje.

Introduction

Medical imaging techniques in the past decades became crucial for medical diagnosis or analyses. Techniques such as X-rays, computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) enabled different approaches and insights of human body, important for analysis and diagnosis. For example, CT scans provide high resolution information on bony structure while MRI scans provide detailed information on tissue types within the body [1].

Precise determination of target volumes is the most crucial and difficult part of radiotherapy (RT) planning process,

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especially because modern RT planning techniques such as conformal RT (CRT) and intensity-modulated RT (IMRT) are used. Otherwise, a geographical miss of a tumor or a systematic error will go on throughout the therapy. In order to avoid such problem, MRI is being increasingly used in oncology not only for staging, assessing tumor response and evaluating disease recurrence, but also for delineation of target volume in RT [2]. The improved characterization of soft tissues and visualization of tumour extent using MRI can be used to benefit the RT treatment planning (RTP) process from delineation of target volumes to determining planning margins and treatment response [3].

Standard RTP uses CT data that provide good distinguishing between structures that have substantially different X-ray attenuation properties or Hounsfield units, such as among air, tissue and bone. On the other hand, it is more difficult to discriminate between soft tissue structures with similar electron densities, including tumors [4].

Correct determination of tumor localization and extension is of major importance in radiation oncology. Modern RT techniques require the increased sophistication of different imaging modalities. With image fusion protocols it is possible to use both, the diagnostic superiority of MRI and the geometric superiority of CT [5, 6]. CT-MRI image fusion provides better tissue discrimination, and distinguishing between tumor with its boundaries of infiltration and the adjacent normal structures. In this manner, CT-MRI fusion provides improved target delineation for RTP. This approach applies not only to the initial RT treatment of tumors, but also potentially to re-treatments by being able to differentiate between changes due to recurrent cancer or that secondary to post-treatment fibrosis. It can also provide better delineation of organs at risk (OARs) for dose avoidance in RTP [4].

The aim of this research was to determine and present the most interesting cases chalenging for the delineation of target volumes using CT only, that were successfully solved and in which the delineation process was successfully finished using CT-MRI fusion. As we know, this method has not been used at clinics of radiotherapy in our country so far.

Methods

A total of 16 patients with intracranial tumors were prepared for standard RT treatment. The CT scanner used was SIEMENS Somatom plus. Each patient was scanned in treatment position, with an immobilization applied. CT data were then exported from CT and imported to the RT treatment planning system. The MR data sets were obtained by a Siemens Avanto or Siemens Magnetom Trio. The patients were scanned according to the standard diagnostic protocol, and stored to the hospital Picture Archiving and Communications System (PACS).

The first step correlating CT and MR images is image registration. Image registration is the process in which two image data sets are put into the common coordinate system. The most often is a combination of CT and MR images, where CT set is used as a reference, and MR set is reoriented and registered to the CT coordinate system. Finally, two images can be fused (blended into a single image), where one, or another image can be more or less weighted for visualization (Figure 1). Practically, it is done with the fusion license of a treatment planning software (XIO CMS v.4.50.0).

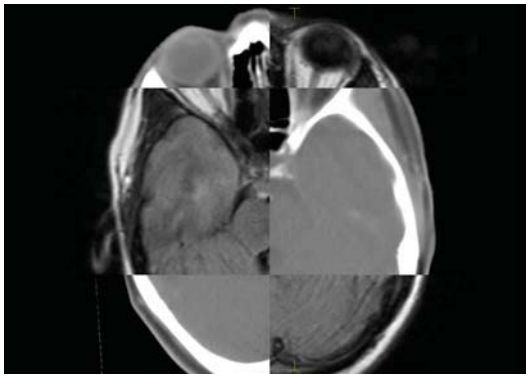


Fig. 1 – Computed tomography–magnetic resonance imaging (CT-MRI) fusion

An RTP system with fusion licence, enables registration and fusion of different DICOM modalities [CT, MR, PET, single-photon emission computed tomography (SPECT)....] The hospital's PACS archives all patients DICOM files, which could be used and retrieved at any time. The requested data set (MR) for the selected patient is obtained via the network, and imported into the treatment planning system XIO CMS. The patient's data (CT and MR) were loaded, one by one, and automatically registered and fused (Figure 2). Fusion software allows manual rotation and movement in all

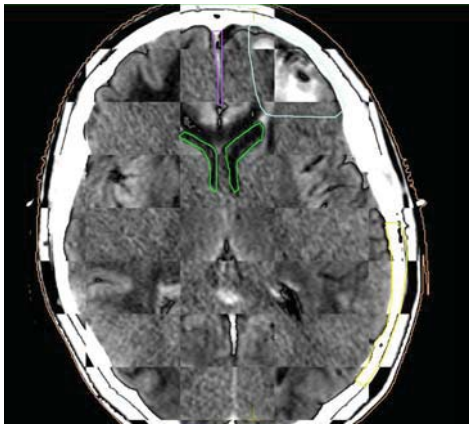


Fig. 2 – Computed tomography–magnetic resonance imaging (CT-MRI) fusion with delineated falx cerebri (violet line), *cornu anterius ventriculi lateralis* (green line) and astrocitoma gr. II (blue cyan line)

the three spatial directions, and enables corrections of a patient position, if it is changed between the two imaging sessions (CT and MR). Registration and fusion was immidiatelly visually evaluated. Visual inspection of all slices and cross-sections means that registration and fusion have actually passed individual quality control, i.e. verification of resulting image matching [7]. Manual correction of image registration was necessary in some cases, depending on the matching results, i.e. quality of CT and MR data in certain

areas of the brain. The next step was delineation of target volumes, gross tumor volume (GTV), clinical target volume (CTV), planing target volume (PTV) and the organs at risk, according to recommendations [8]. CTV was determined by adding 10 mm on the GTV [8, 9].

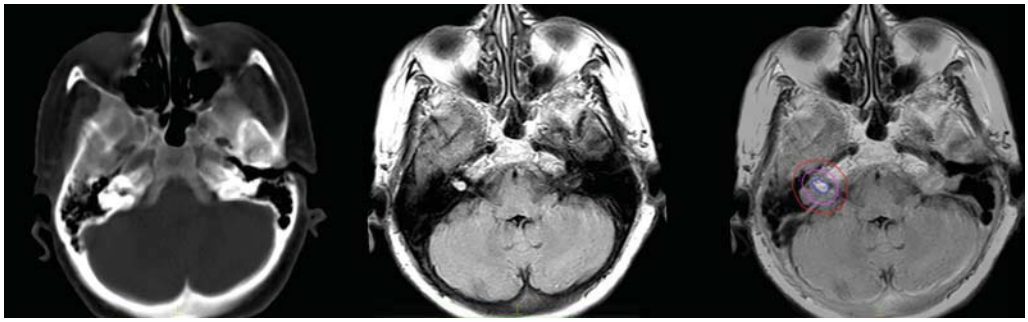
Results

The CT and MR images of all sixteen patients included were successfully fused. Of them, in 4 patients preoperative MRI was used and in the others MRI was performed before CT as a part of the planning process for RT treatment, for the patients with surgery treatment or those without surgery treatment. The results of completed delineation of target volumes using MRI-CT fusion are shown in three different cases, chosen as representative results. One of them belonged to the group with preooperative MRI, and the after two were the second group patients. The successful fusion and delineation process was obtained for all the patients, and the shown cases were randomly chosen.

The first patient had the diagnosis of vestibulocochlear nerve schwannoma on the right side. CT imaging (Figure 3a) failed to provide clear boundaries of schwannoma. MRI (Figure 3b) solely could not be used for the planning process, since the treatment planning system calculations are based on CT electron density data. The effectiveness of medical image fusion can be illustrated by this example (Figure 3c). CT-MRI fusion provided clear boundaries of schwannoma visualized by MRI T2 sequence together with CT image required for precise RT delineation and planning.

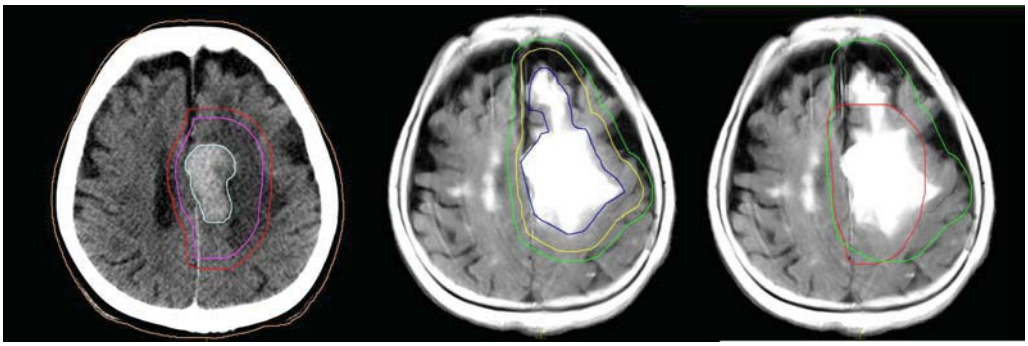
The second patient had the diagnosis of low-grade multicentric astrocytoma of the left cerebral hemisphere. The patient underwent stereotactic brain biopsy, which was positive for low-grade II astrocytoma. MRI (Figure 4b) revealed tumor expansive tissue with perifocal edema with clear boundaries. Edema boundaries revealed on MRI T2 sequence were GTV. CT image (Figure 4a) showed tumor with unclear edema boundaries insufficient and not enough sophisticated for the best RT delineation. Comparison between PTV according to CT (green line) and MRI (yellow line) is showed in Figure 4c.

The third patient had the diagnosis of low-grade astrocytoma. After the surgery a small part of tumor tissue was left due to localization. For this patient, RT planning process included preoperative MRI T2 sequence for CT-MRI fusion to encompass all micrometastases. Postoperative MRI was also done, as well as comparison of pre- and postoperative MRI revealing that the broader area should be included, which encouraged the usage of preoperative MRI for CT-MRI fusion in this case. Application of CT-MRI fusion revealed better boundaries of the tumor compared to CT only, especially due to the left part of the tumor (Figure 5.). CT-MRI fusion provided more precise localization of edema surrounding tumor tissue, which enables more precise determination of target volumes.



a) **b** **c)**

Fig. 3 – a) Computed tomography (CT) of intracanulicar schwannoma on the right side; b) Computed tomography – magnetic resonance imaging (CT-MRI) T2 sequence of the same section as given in 3a; c) CT-MRI fusion and delineation of target volumes.



a) **b)** **c)**

Fig. 4 – a) Computed tomography (CT) image with grass tumor volume (GTV), clinical target volume (CTV) and planning target volume (PTV); b) Magnetic resonance imaging (MRI) T2 sequence with GTV, CTV and PTV; c) Comparison between PTV according to CT (red line) and MRI (green line).

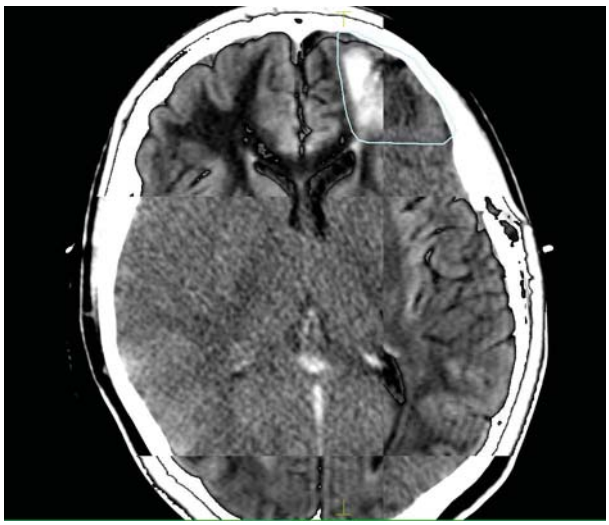


Fig. 5 – Astrocytoma gradus II located in the left frontal lobe.

Discussion

CT-MRI fusion at the Institute of Oncology Vojvodina was proved to be very important. In our relatively short time period experience, CT-MRI fusion was applied in 16 patients. Three patients with different CT-MRI benefit were chosen in order to present advantages of this method.

In all the showed cases, applied imaging techniques were followed by delineation of target volumes.

CT-MRI fusion in the patient with schwannoma diagnosis enabled more precise target volume delineation, due to advancements provided by MRI comparing to CT imaging in distinguishing between tissues with similar densities. Without MRI, neither GTV, nor CTV would be appropriate, due to unadequate visualization. There should be a possibility of either larger target volume in order to be sure that neccessary region is treated or unsufficient target volume to prevent large dose application to adjacent organs at risk. Subsequently, the organs at risk such as cohlea and inner ear, would be exposed to higher dose. A large number of authors stress advantages of MRI comparing to CT for the evaluation [10] and delineation of target volumes [11] for auditory and vestibulary systems pathology. Bartling et al. [12] advice using of CT-MRI fusion in temporal bone pathology RT treatment.

Maybe the more obvious example of problems that can arise without CT-MRI fusion is showed by the patient with low-grade multicentric astrocytoma (case 2). This example gives data of insufficient visualzation for low-grade and high-grade gliomas by CT in some cases. Usage of T2 sequence MRI or FLAIR [8, 9] for delineation, decreases chances for lower dose or geographical miss for tumor RT treatment, since tumor and edema could be preciselly veryfied. After delineation of CTV (GTV (T2 sequence)+10m), 5 mm are added for PTV. Usage of T2 sequence MRI or FLAIR for GTV and addition of 10 mm for CTV the most probably encounters micrometastatic extants. We decided to use these recommendations since there were studies reporting micrometastasis out of T2 sequences, especially in high-grade astrocytomas and gliomas [13].

The third patient in our study had the same diagnosis as the second one, but this patient had underwent surgery treatment. Even though postoperative MRI was done, we have decided to use preoperative MRI in order to include larger area to provide encountering of all micrometastasis possible after surgery. Usage of preoperative MRI in CT-MRI fusion enabled determination of initial tumor size and during delineation according to that size to decrease possibility for geographical miss.

Both CT and MRI provide very good, but not identical, information on macro- and microscopic tumor extension. In the ideal situation the target volume is delineated based on pre- and postoperative CT and MRI. This set of data is used for constructing composite target volumes. With image fusion protocols it is possible to use both the diagnostic superiority of MRI and the geometric superiority of CT in 3D RTP [6, 13, 14].

Conclusion

This paper illustrates the effectiveness of medical image fusion. It also proves that medical image fusion is a powerful technique for medical imaging analysis. Image fusion allows better visualization for RT delineation and planning of target volumes. CT-MRI fusion provides even better estimation of target volumes that may permit treatment individualization, organ sparing or functional avoidance. It is also clear, although not emphasized here, that CT-MRI fusion allows strategies of boosting and dose escalation. Future dynamic imaging modalities like, PET, SPECT and functional MRI should be included in our practice.

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Long-term graft occlusion in aortobifemoral position

Kasna okluzija grafta u aortobifemoralnoj poziciji

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Abstract

Background/Aim. Aortobifemoral (AFF) bypass is still the most common surgical procedure used in treatment of aortoiliac occlusive disease. One of the most common complications of AFF bypass procedure is long-term graft oclussion. The aim of this study was to determine the cause of long-term graft occlusion in AFF position, as well as the results of early treatment of this complication. **Methods.** This retrospective study, performed at the Clinic of Vascular and Endovascular Surgery, Clinical Center of Serbia in Belgrade, involved 100 patients treated for long-term occlusion of bifurcated Dacron graft which was ensued at least one year after the primary surgical procedure. **Results.** The most common cause of the long-term graft occlusion was the process at the level of distal anastomosis or below it ($Z = 3.8$, $p = 0.0001$). End-to-end type of proximal anastomosis has been associated with a significantly increased rate of long-term graft occlusion ($Z = 2.2$, $p = 0.0278$). Five different procedures were used for the treatment of long-term graft occlusion: thrombectomy and distal anastomosis patch plasty (46% of the cases); thrombectomy and elongation (26% of the cases); thrombectomy and femoropopliteal bypass (24% of the cases); crossover bypass (2% of the cases) and a new AFF bypass (2% of the cases). The primary early graft patency was 87%. All 13 early occlusions occurred after the thrombectomy associated with patch plasty of distal anastomosis. Thrombectomy with distal anastomosis patch plasty showed a statistically highest percentage of failures in comparison to thrombectomy with graft elongation, or thrombectomy with femoro-popliteal bypass ($Z = 2\,984$, $p = 0.0028$). Redo procedures were performed in all the cases of early occlusions. In a 30-day follow-up period after the secondary surgery, 90 (90%) patients had their limbs saved, and above knee amputation was made in 10 (10%) patients. **Conclusion**. Long-term AFF bypass patency can be obtained by proximal end-to-end anastomosis on the juxtarenal part of aorta and distal anastomosis on the bifurcation of the common femoral, or on the deep femoral artery.

Key words:
graft occlusion, vascular; risk assessment; diagnosis; comorbidity; aortic diseases; vascular surgical procedures.

Apstrakt

Uvod/Cilj. Aortobifemoralni (AFF) bajpas još uvek je najčešća hirurška procedura koja se koristi u lečenju aortolijačne okluzivne bolesti. Jedna od najčešćih komplikacija AFF bajpas procedure je kasna okluzija grafta. Cilj ovog rada bio je utvrđivanje uzroka kasnih okluzija grafta u poziciji AFF i ranih rezultata lečenja ove komplikacije. **Metode.** Retrospektivnom studijom u Klinici za vaskularnu i endovaskularnu hirurgiju Kliničkog Centra Srbije u Beogradu bilo je obuhvaćeno 100 bolesnika kod kojih je lečena kasna okluzija bifurkacionih Dacron graftova, nastala najmanje godinu dana posle primarne operacije. **Rezultati.** Najčešči uzrok kasne okluzije grafta bio je proces u predelu distalne anastomoze (njeno postavljanje na zajedničku butnu arteriju ili neointimalna hiperplazija) ili ispod nje (distalna progresija okluzivne bolesti) ($Z = 3{,}8$; $p = 0{,}0001$). Terminolateralna forma proksimalne anastomoze statistički je značajno povećavala stopu kasnih okluzija ($Z = 2{,}2$; $p = 0{,}0278$). U lečenju kasne okluzije primenjivani su: trombektomija grafta i *patch* plastika distalne anastomoze – 46% bolesnika; trombektomija grafta i njegova elongacija na duboku butnu arteriju – 26%; trombektomije grafta i dopunski femoropoplitealni bajpas – 24%; *cross over* bajpas – 2% i novi AFF bajpas – 2% bolesnika. Rana prohodnost iznosila je 87%. Svih 13 ranih okluzija nastale su nakon trombektomije grafta udružene sa *patch* plastikom distalne anastomoze. Statistička analiza je pokazala da je u odnosu na

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trombektomiju grafta sa elongacijom na duboku butnu arteriju, odnosno trombektomiju grafta sa femoropoplitealnim bajpasom, ova procedura opterećena statistički najvećim procentom neuspeha (Z = 2, 984; p = 0,0028). U svim slučajevima rane okluzije izvedena je ponovna intervencija. Ukupan broj spašenih ekstremiteta u prvih 30 dana bio je 90 (90%), dok je u istom periodu urađeno ukupno 10 natkolenih amputacija (10%). **Zaključak.** Udaljena prohodnost AFF bajpasa može se produžiti proksimalnom anastomozom terminoterminalnog tipa na jukstarenalnoj aorti, i distalnom anastomozom na bifurkaciji zajedničke femoralne ili dubokoj butnoj arteriji.

Ključne reči:
vaskularni graft, okluzija; rizik, procena; dijagnoza; komorbiditet; aorta, bolesti; hirurgija, vaskularna, procedure.

Introduction

Aortobifemoral (AFF) bypass is still the most common surgical procedure used in treatment of aortoiliac occlusive disease. Compared to other procedures, AFF reconstruction provides the best long-term results. From the middle of the last century, when AFF reconstruction was first performed [1], this surgical procedure has been continuously improving, but it is still very complex. One of the most common complications of AFF bypass procedure is long-term graft occlusion. The frequency of this complication is 0.9%–20% [2–13] in a 5-year period following surgery, and 20%–40% [13–15] in a 10-year period. The aim of this study was to determine the cause of long-term graft occlusion in AFF position, as well as the results of early treatment (within first 30 days) of this complication.

Methods

This retrospective study, performed at the Clinic of Vascular and Endovascular Surgery, Clinical Center of Serbia, Belgrade included 100 patients with long-term bifurcated Dacron graft occlusion ensued at least one year after AFF reconstruction. This study did not include patients with AFF reconstruction of abdominal aortic aneurysm treating, patients previously treated for occlusive disease of the abdominal aorta, patients with polytetrafluoroethylene (PTFE) graft AFF reconstruction and patients with surgical procedure on the iliac or femoral arteries.

Tabular and graphical presentation of the data and methods of descriptive statistics (mean value, standard deviation, Student t-test, χ2-test, test for a single proportion) were used for statistical analysis.

Results

Table 1 shows the demographic characteristics of patients; risk factors; cardiovascular comorbidity; clinical presentation; period from primary surgery to the occurrence of the long-term graft occlusion and type of graft occlusion.

The average age of patients was 59.88 ± 8.57 years. The youngest patient was 37, and the oldest one 75 years old. Most patients were male (82 or 82%). Arterial hypertension was present in 43% of the patients. Out of the patients, 35% were smokers, and nine (9%) patients were overweight. Diabetes mellitus was found in 13% of the patients. Nineteen patients had cerebrovascular disease [transient ischemick atack (TIA, stroke, previous carotid endarterectomy)], and 50 (50%) patients had ischemic heart disease (myocardial infarction, angina pectoris).

Table 1

The demographic characteristics of patients, risk factors, cardiovascular comorbidity, clinical presentation, and a period from primary surgery to the occurrence of long-term graft occlusion and graft occlusion type

Characteristics	Patients number (%)
Age (years), $\bar{x}$ ± SD (range)	59.88 ± 8.57 (37–75)
Gender	
male	82 (82.00)
female	18 (18.00)
Risk Factors	
arterial hypertension	43 (43.00)
obesity	9 (9.00)
smoking	35 (35.00)
diabetes mellitus	13 (13.00)
Cardiovascular Comorbidity	
cerebrovascular disease	19 (19.00)
ischemic heart disease	50 (50.00)
Clinical Presentation	
claudication discomfort	24 (24.00)
acute limb ischemia	65 (65.00)
gangraena	11 (11.00)
Occlusion type	
unilateral occlusion	76 (76.00)
bilateral occlusion	24 (24.00)
Time from primary surgery to the late graft occlusion	4.47 ± 3.94 (1–16) years

The majority (65%) of the patients had acute, while 35% chronic limb ischemia (24% of the patients had claudication discomfort and 11% gangrene). A total of 76% of the patients had unilateral and 24% bilateral graft occlusion. The average time period from AFF reconstruction to long-term graft occlusion was 4.47 (1–16) years.

The most common (50% of the cases) cause of long-term graft occlusion was neointimal hyperplasia. Proximal progression of the occlusive disease was the cause of long-term grafts occlusion in 4.72% of the cases. In 33.02% of the cases the cause was distal progression of occlusive disease, and graft failure in 12.26% of the cases. The causes of long-term occlusion after AFF bypass are shown in Table 2. Influence of proximal anastomosis type and site of distal anastomosis on long-term graft occlusion is shown in Table 3.

End-to-side type of proximal anastomisis was associated with long-term graft occlusion in 64.89% of the cases and end-to-end type in 35.11%. It was statistically significant ($Z = 2.2$, $p = 0.0278$). Distal anastomosis of AFF bypass was located on the common femoral artery in 70.41% of the cases with long-term graft occlusion. It was statistically significant ($Z = 3.8$, $p = 0.0001$).

Five surgical procedures in treatment of long-term AFF bypass occlusion were used (Table 4) graft thrombectomy and distal anastomosis patch plasty (46% of the patients) graft thrombectomy and its elongation on the deep femoral artery (26% of the patients), graft thrombectomy and femoropopliteal bypass (24% of cases), cross-over bypass (2% of cases) and a new AFF bypass (2% of the patients). In the first 30 postoperative days there was no mortality, while 13 (13%) cases with early graft occlusion were found. The primary early graft patency was 87%. All 13 cases with early occlusions occurred after graft thrombectomy associated with patch plasty of distal anastomosis. A graft thrombectomy associated with distal anastomosis patch plasty had a statistically highest percentage of failures ($Z = 2\ 984$, $p = 0.0028$).

Redo procedures were performed in all the cases of early graft occlusion after the secondary surgery. In five cases graft elongation on the deep femoral artery was performed. In other five cases we performed additional femoropopliteal bypass, while in three cases, due to disability of new revascularization, above knee amputations were necessary. In seven patients correction of long-term graft occlusion would not be able to provide adequate limb vascularization, and therefore, the above knee amputations were made. The limb salvage rate during a 30-day follow-up period after the secondary surgery was 90%. Above knee amputation was made in 10 (10%) patients. Several examples

Table 2

Causes of long-term graft occlusion after aortobifemoral bypass

Intraoperative finding	Occurence of intraoperative findings number (%)
Proximal progression of occlusive artery disease	5 (4.72)
Distal progression of occlusive artery disease	35 (33.02)
Neointimal hyperplasia	53 (50.00)
Graft failure	13 (12.26)
Total	106 (100.00)

Table 3

Influence of proximal anastomosis type and distal anastomosis site on long-term graft occlusion

Parameters	Number (%)	Z	*p*
Proximal anastomosis type			
end-to-end	33 (35.11)	2.2	0.0278
end-to-side	61 (64.89)		
Site of distal anastomosis			
common femoral artery	69 (70.41)	3.8	0.0001
deep femoral artery	29 (29.59)		

For six of the patients, data about proximal anastomosis type were not found; for two of the patients data about distal anastomosis site were not found, too.

Table 4

Procedures used in the treatment of long-term graft occlusion in aortobifemoral (AFF) position

Procedure	Patients number (%)	Early occlusion number (%)	*p*
Thrombectomy and distal anastomosis patch plasty	46 (46.00)	13 (28.2)	0.0028
Thrombectomy and graft elongation	26 (26.00)	0 (0.0)	
Thrombectomy and femoro-popliteal bypass	24 (24.00)	0 (0.0)	
Crossover bypass	2 (2.00)	0 (0.0)	
New AFF bypass	2 (2.00)	0 (0.0)	
Total	100 (100.0)	13 (13.0)	

of our documented experiences with long-term graft occlusion after AFF bypass reconstruction are shown in Figures 1–4. Recommended procedures in the treatment of long-term graft occlusion after AFF bypass reconstruction are shown in Figure 5.

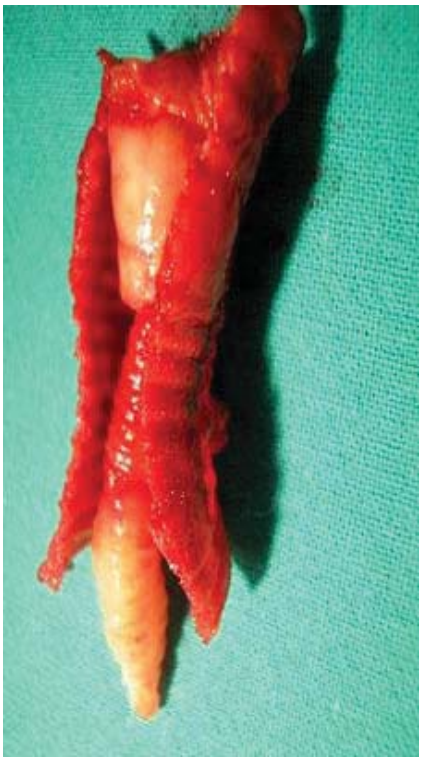


Fig. 1 – A resected anastomotic part of the graft with a secondary thrombus and neointimal hyperplasia.

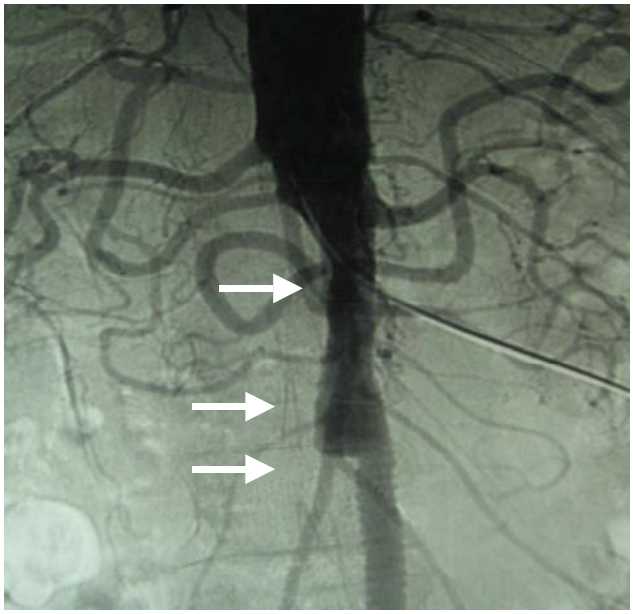


Fig. 2 – Control angiography after aortobifemoral reconstruction shows proximal progression of occlusive artery disease caused by low-set proximal anastomosis (arrow). The right limb of bifurcated graft is occluded (two arrows).

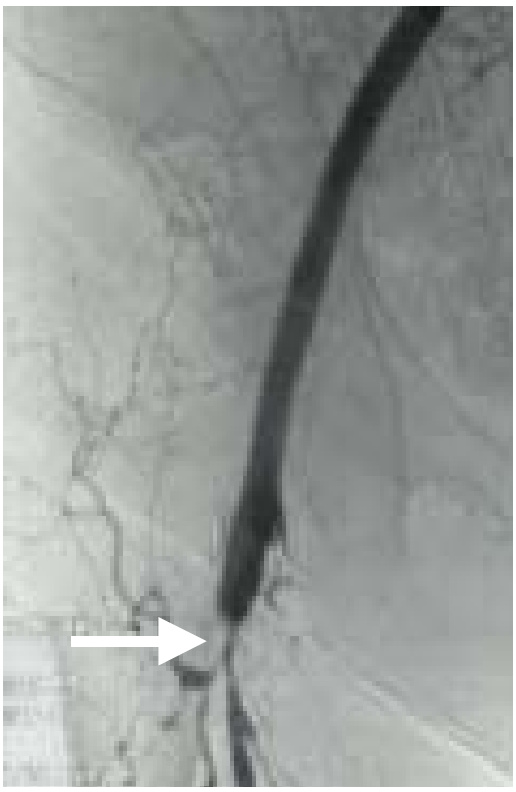


Fig. 3 – Control angiography shows stenosis at the level of the right distal anastomosis of aortobifemoral bypass (arrow) caused by distal progression of occlusive artery disease.

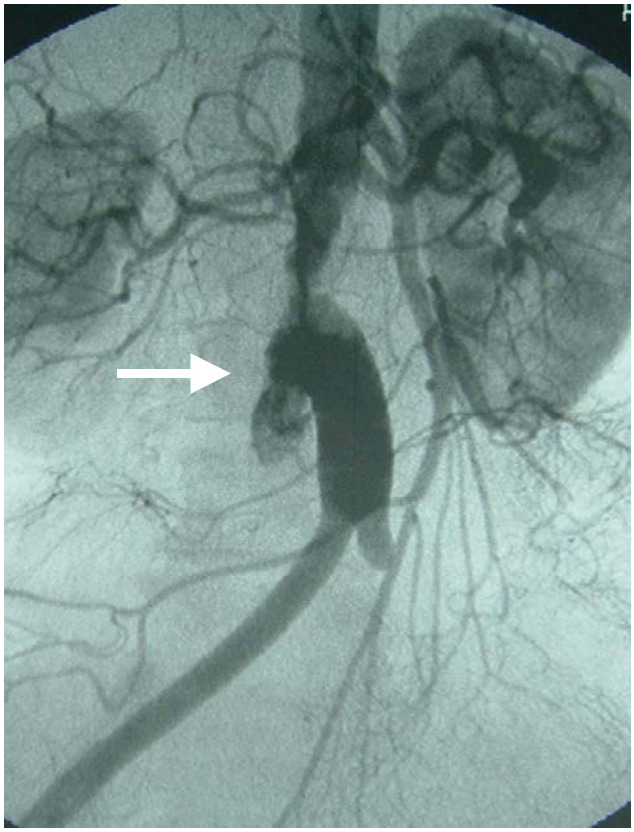
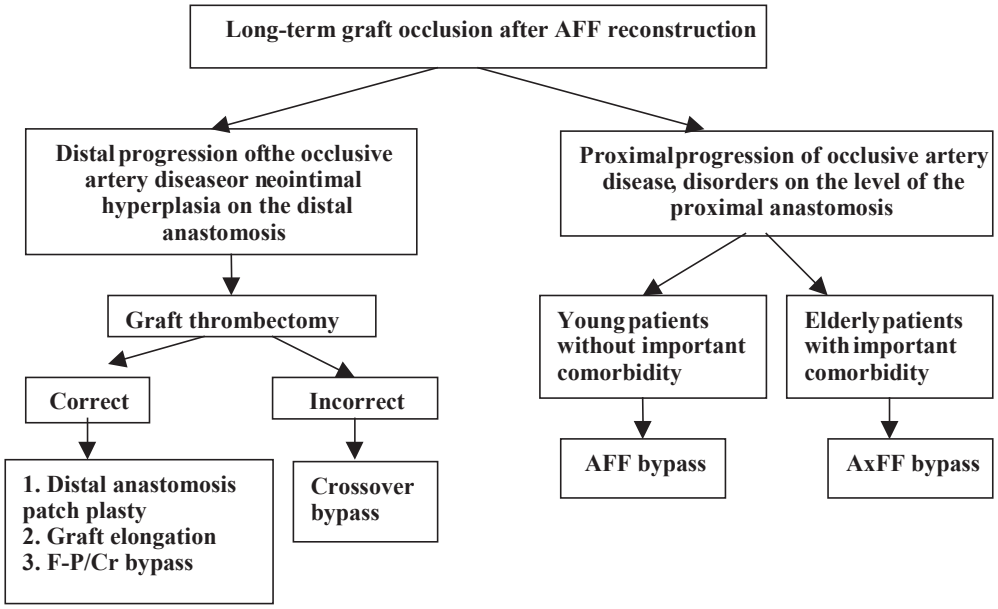


Fig. 4 – Control angiography after aortobifemoral bypass reconstruction with end-to-side type of proximal (arrow). The left limb of a bifurcated graft is occluded.



Legend: AFF – aortobifemoral bypass; DFA – deep femoral artery; F-P/Cr – femoro-popliteo/crural bypass; AxFF – axilobifemoral bypass.

Fig. 5 – Recommended procedures in treatment of long-term graft occlusion after AFF bypass reconstruction.

Discussion

There are several classifications of long-term graft occlusion causes after AFF reconstruction. Wesolowski [16] divided them into primary and secondary causes. Primary long-term graft occlusion is a consequence of graft failure, while secondary is caused by proximal or distal progression of occlusive artery disease. Causes of graft failure are kinking (22% by Szilagyi et al. [17]) and anastomotic stenosis caused by neointimal hyperplasia (2%–13%) [4, 9, 15, 17–20].

According to this classification, in our study 60 (60%) patients had primary, while 40 (40%) secondary long-term graft occlusion. If proximal anastomosis of AFF bypass is set just below the renal artery, proximal progression of occlusive artery disease is rarely the cause of long-term graft occlusion [4, 10]. In the early period of abdominal aortic surgery, low-set proximal anastomosis and proximal progression of occlusive artery disease, caused long-term graft occlusion in AFF position more frequently.

Crawford et al. [8, 9] find that the proximal progression of occlusive artery disease cause long-term graft occlusion in 13% of cases. Many other authors suggest that the distal progression of occlusive artery disease cause long-term graft occlusion most frequently [4, 9, 10, 20]. It especially often occurs in distal anastomoses created on the common femoral artery. Long-term AFF bypass patency can be improved by distal anastomosis on the femoral bifurcation, or on the deep femoral artery. Distal anastomosis of AFF bypass was made on the common femoral artery in 70.41% of our cases with long-term graft occlusion. It was statistically significant ($Z = 3.8$, $p = 0.0001$).

Becquemin´s et al. [20] classification of long-term graft occlusion cases after AFF bypass reconstruction is slightly different. There are neointimal hyperplasia, atherosclerosis, anastomotic process and others [20]. In his study, 36% of long-term graft occlusions were caused by neointimal hyperplasia, which was more common in younger patients with fewer risk factors. In this study 50% of the patients had femoral artery narrower than 5 mm, and 2% had large disproportion between the diameter of the graft and artery.

Atherosclerosis was the cause of long-term graft occlusion in 35% of Becquemin´s patients [20]. It was more frequent in elderly patients with multiple risk factors. Anastomotic process caused late graft occlusion in 6% of cases, and other reasons in 17% [20]. By Becquemin et al. [20], other reasons of long-term graft occlusion are hypercoagulability, infection, transient hypotension or unknown causes. Many different disorders are associated with hypercoagulability, such are obesity, malignancy, trauma, age, prolonged using of contraceptives, smoking, and reduced level of tissue plasminogen activator and antithrombin III [21]. Towne et al. [22] described seven cases of early or late thrombosis of synthetic grafts, caused by deficiency of antithrombin III. We did not find cases of long-term graft occlusion caused by hypercoagulability or by indeterminate causes. Other authors state that the causes of long-term graft occlusion remain indeterminated in 6%–24% of cases [8, 9, 18].

Having in mind all the classifications of long-term graft occlusion causes of AFF bypass, the simplest might be the one that includes the following four reasons: disorders on the level of proximal anastomosis or above it (low-set proximal anastomosis, end-to-side type of proximal anastomosis, proximal progression of occlusive artery disease), graft failure (untight graft, folded graft, graft torsion, external graft compression), disorders on the level of distal anastomosis or below it (neointimal hyperplasia, distal progression of occlusive artery disease). According to this classification, disorders on the level of proximal anastomosis or above it caused long-term graft occlusion in 5% of the cases in our study. In all the cases it was end-to-side type of proximal anastomosis.

In our study graft failure caused long-term graft occlusion in 13% of the cases, while disorders on the level of distal anastomosis or below it were the cause in 83% (neointimal hyperplasia was cause in 50% of the cases, and distal progression of occlusive artery disease in 33%).

If it is well indicated and technically correctly performed, bilateral graft occlusion of AFF bypass is relatively rare. In our study unilateral graft occlusion was in 76% of the cases, and bilateral in 24%. Bilateral graft occlusion usually ensues after a long follow-up period. The most likely reason of bilateral graft occlusion is low-set of proximal anastomosis [2–4]. End-to-side type of proximal anastomosis contributes to long-term graft occlusion because it has worse hemodynamic properties [2–4]. End-to-side type of proximal anastomosis was in all of our patients with complete graft occlusion, which was statistically significant ($Z = 2.2$, $p = 0.0278$).

In our study, the average time period from AFF reconstruction to graft occlusion was 4. 47 years. In Najafi´s et al.[10] study that period was 3.5 years. Le Grand et al. [23] say that 28% of unilateral graft occlusion occurs within the first months after surgery, and 72% within the first 30 months.

In 65% of our cases long-term graft occlusion of AFF bypass was manifested with acute, and 35% with chronic limb ischemia (claudication discomfort or gangrene). In Becquemin´s et al. [20] study, 63% of cases with long-term graft occlusion had claudication discomfort, 20% acute ischemia, while 2% of the patients were asymptomatic.

In the treatment of unilateral long-term graft occlusion, Crawford et al. [9] recommended complete graft replacement. They thought that previous graft, in graft preserving surgical procedure, is very thrombogenic. Therefore, that graft remains *locus minoris resistentiae*. We have done two new AFF bypasses. Most other authors consider that complete graft replacement should be avoided because it is associated with the morbidity (injury of the duodenum, injury of inferior caval vein, injury of ureter, extensive bleeding etc.) and mortality. Complete graft replacement is recommended in cases with bilateral occlusion, disorders on the level of proximal anastomosis or above it, elongated graft or folded graft [15, 17, 24]. These were our criteria for a complete graft replacement. Lower risk surgical procedures could be performed in treatment of unilateral graft occlusion. Bernhard et al. [18] think that the choice of procedure depends on the graft type, period from surgery to graft occlusion, period from graft occlusion to reintervention, clinical presentation, general condition of a patient, angiographic findings before primary surgery, angiographic findings after graft occlusion, and perioperative findings. They also state that only 60% of their patients with unilateral graft occlusion are treated within four weeks from the occlusion.

According to recommendations of the literature and the results from a different studies, we formulated the following preliminary conclusion about the type of long-term graft occlusion treatment [2–4, 18, 20, 23]. If late graft occlusion is caused by distal progression of occlusive artery disease or neointimalna hyperplasia on distal anastomosis, you should try the following procedures in the following order: correction of disorders at the level of distal anastomosis is necessary after successful graft thrombectomy; graft elongation on the deep femoral artery or on its lower part is the best treatment after thrombectomy; if deep femoral artery is not suitable for reconstruction, femoropopliteal/crural bypass should be done; "crossover" bypass should be performed in cases when graft thrombectomy is not possible. If AFF bypass occlusion is caused by graft failure or disorder on proximal anastomosis or above it, a new AFF (in younger patients with less perioperative risk) or axilo-bifemoral (in elderly patients with high perioperative risk) should be done.

All these procedures can be divided into three groups: procedures on the "inflow" tract ("crossover" bypass, AFF bypass and axillo-bifemoral bypass), procedures on the graft (thrombectomy), concomitant procedures on the graft (thrombectomy) and "outflow" tract (graft elongation or femoropopliteal/crural bypass).

Early graft occlusion after correction of AFF bypass occlusion ranges from 9.6% to 30% [18–20, 23, 25, 26]. We noticed 13 (13%) early graft occlusions after correction. All 13 early graft occlusions occurred after graft thrombectomy associated with patch plasty of distal anastomosis, which was statistically significant ($Z = 2, 984$, $p = 0.0028$).

Conclusion

Long-term AFF bypass patency can be obtained by proximal end-to-end anastomosis on the juxtarenal part of the aorta and distal anastomosis on the femoral bifurcation, or on the deep femoral artery. It is necessary to eliminate or control risk factors in a postoperative period. Long-term AFF bypass patency could be achieved by adequate secondary surgical procedure.

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The role of hysteroscopy in diagnosis and treatment of postmenopausal bleeding

Primena histeroskopije u dijagnostici i lečenju postmenopauznog krvarenja

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Abstract

Background/Aim. Abnormal uterine bleeding is the most common problem which brings woman to the gynecologist during the postmenopausal period. The aim of this study was to define the significance of hysteroscopy as a diagnostic procedure for the evaluation of patients with postmenopausal bleeding, as well as to define it as a surgical procedure by which the cause of bleeding can be treated in most cases in the same sitting. **Methods.** The study involved 148 female patients referred to the Clinic for Gynecology and Obstetrics in Niš for postmenopausal bleeding in the period of 12 months. Hysteroscopy with endometrial biopsy were performed in all the patients. Biopsy materials were directed to histological examination, and the hysteroscopic and histological findings were compared afterwards. Polyps and submucous miomas were hysteroscopically removed in the same sitting and also directed to histological examination. **Results.** The success rate of the method was 95.1%, while complications occurred in 1.37% of the cases. The hysteroscopic findings were normal in almost 30% of the cases, and the most common pathological finding was endometrial polyp. The sensitivity of hysteroscopy in the detection of intrauterine pathology was 100%, the specificity 81%, the positive predictive value 92% and the negative predictive value 100%. In 69.7% of the patients the cause of bleeding was hysteroscopically removed. Hysteroscopy was performed in 58.1% of the patients in the same sitting, and in 11.6% of the patients after obtaining histological findings. **Conclusion.** Hysteroscopy is a safe, highly sensitive diagnostic procedure, thus being an ideal method for evaluation of patients with postmenopausal bleeding. The application of hysteroscopy with endometrial biopsy leads to accurate diagnosis. An adequate diagnosis is crucial for the selection of relevant treatment of postmenopausal bleeding and avoidance of unnecessary major surgical procedures. Except for being a diagnostic method hysteroscopy, is also an outpatient minimally invasive surgical procedure for treating the cause of bleeding in the majority of cases in the same sitting.

Key words:
hysteroscopy; uterine hemorrhage; postmenopause; women; diagnosis; gynecologic surgical procedures.

Apstrakt

Uvod/Cilj. Patološko krvarenje iz materice je najčešće problem koji dovodi ženu na pregled tokom postmenopauznog perioda. Cilj ove studije bio je da se definiše značaj histeroskopije kao dijagnostičke procedure za detekciju uzroka postmenopauznog krvarenja, kao i operativne procedure kojom se u velikom broju slučajeva može ukloniti uzrok krvarenja u istom aktu. **Metode.** U studiju je bilo uključeno 148 bolesnica koje su se javile Ginekološko-akušerskoj klinici u Nišu zbog postmenopauznog krvarenja tokom perioda od 12 meseci. Kod svih bolesnica urađena je histeroskopija sa biopsijom endometrijuma. Bioptati su slati na patohistološki pregled, a zatim upoređivani histeroskopski i patohistološki. Polipi i submukozni miomi su histeroskopski uklanjani u istom aktu i, takođe, slati na patohistološki pregled. **Rezultati.** Procenat uspešnosti metode iznosio je 95,1%, a komplikacija 1,37%. Histeroskopski nalaz bio je kod oko 30% slučajeva normalan, a najčešći patološki nalaz bio je polip endometrijuma. Senzitivnost histeroskopije kod detekcije intrauterine patologije iznosila je 100%, specifičnost 81%, pozitivna prediktivna vrednost 92% i negativna prediktivna vrednost 100%. Kod 69,7% bolesnica histeroskopski je uklonjen uzrok krvarenja. Kod 58,1% bolesnica operativna histeroskopija rađena je u istom aktu kada i dijagnostička, a kod 11,6% nakon pristizanja patohistološkog nalaza. **Zaključak.** Histeroskopija je sigurna, visokosenzitivna dijagnostička procedura koja predstavlja idealnu metodu za ispitivanje uzroka postmenopauznog krvarenja. Primena histeroskopije sa biopsijom endometrijuma obezbeđuje preciznu dijagnozu. Adekvatna dijagnoza je preduslov za izbor odgovarajućeg lečenja postmenopauznog krvarenja i izbegavanje nepotrebnih velikih hirurških zahvata. Pored dijagnostičke, histeroskopija je i jednodnevna, operativna, minimalno invazivna metoda kojom se u velikom broju slučajeva u istom aktu može ukloniti uzrok krvarenja.

Ključne reči:
histeroskopija; materica, krvarenje; postmenopauza; žene; dijagnoza; hirurgija, ginekološka, procedure.

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Introduction

Abnormal uterine bleeding is the most common problem which brings the woman to a gynecologist during a postmenopausal period [1]. The cause of bleeding could be often discovered using simple methods, such as gynecological examination (injuries of vulva and vagina, vaginitis) and speculum examination (pathology of cervix). As a non-invasive method, the transvaginal ultrasound represents the following diagnostic tool which has low specificity and sensitivity in diagnosis of the cause of bleeding [2].

Dilatation and curettage (DC) have been the key diagnostic procedures in evaluation of patients with postmenopausal bleeding for decades. However, this method has a great number of false negative results, mainly for the fact of being a blind technique. Therefore, there is a great possibility that the pathologically altered place can be missed and hence the biopsy not being representative [3].

The introduction of hysteroscopy has open a new dimension in evaluation of patients with postmenopausal bleeding. The entire uterine cavity is directly visualized and it is possible to identify pathological changes and perform biopsy of the suspected lesion under visual inspection. An additional advantage is the fact that a great number of benign pathological changes may be treated in the same sitting by using the office hysteroscope or resectoscope [4, 5].

The aim of this study was to define the significance of hysteroscopy as a diagnostic procedure for evaluation of patients with postmenopausal bleeding, as well as to define hysteroscopy as a surgical procedure for treating the cause of bleeding in most cases in the same sitting.

Methods

The study involved 148 female patients with postmenopausal bleeding referred to the Clinic for Gynecology and Obstetrics in Niš from January to December 2010. All the patients had previously been processed anamnestically, clinically and by ultrasound, with the aim of excluding other possible causes of bleeding (injuries of the vulva and vagina, pathology of the cervix and the ovaries).

Hysteroscopy was performed in all the patients in the operation room, in the intravenous sedation, by using a rigid hysteroscope of 4.8 mm (Karl Storz) without grasping and dilatation of the cervix (free-hand technique). A normal saline was used as a distending medium. Endometrial biopsy was performed with scissors or a bipolar tweezle electrode, and the samples were referred to histological examination. The endometrium was described as atrophic when seemed to be thin and pale, hyperplastic when it was thickened and with multipolyp appearance. Endometrial carcinoma was pronounced in case of irregular growth of the endometrium with atypical vascularisation.

As focal intrauterine lesions, polyps and submucous miomas were removed in the same sitting. Dependent on the size of finding, polypectomy or myomectomy was performed using an office hysteroscope or resectoscope (Karl Storz 8mm) . Polyps and myomas were also directed to histological examination. All the patients, apart from the cases with complications, were discharged from the hospital two hours after the intervention.

Results

Out of 148 hysteroscopies 3 (0.49%) were unsuccessful due to cervical canal stenosis. Two patients (1.37%) had complications, in both cases it was uterine perforation. The treatment was conservative in both cases. The average age of the patients was 69 (ranging from 42 to 88).

Hysteroscopic and histological findings of 145 patients are shown in Table 1. The hysteroscopic findings were normal in 26.2% of the cases, and the most common intrauterine pathology was endometrial polyp. The sensitivity of hysteroscopy in the detection of intrauterine pathology was 100%, specificity 81%, positive predictive value was 92% and the negative predictive value was 100%. The sensitivity, specificity, positive predictive value and negative predictive value of hysteroscopy in diagnosing various endometrial pathologies are shown in Table 2.

Table 1

The hysteroscopic and hystological findings in the studied patients

Findings	Diagnostic method	
	Hysteroscopy	Histology
	n (%)	n (%)
Normal endometrium	38 (26.2)	43 (29.6)
Endometrial polyp	43 (29.6)	47 (32.4)
Cervical polyp	24 (16.5)	24 (16.5)
Submucous myoma	8 (5.51)	4 (2.75)
Endometrial hyperplasia	11 (7.58)	8 (5.51)
Endometrial atrophy	18 (12.4)	16 (11)
Endometrial cancer	3 (2.06)	3 (2.06)

Table 3 presents different options of management. Out of 129 patients indicated for treatment, 27.9% required only drug treatment (progesterone therapy, or hormonal intrauterine device Mirena®), 2.32% of the patients required the major surgical procedure such as hysterectomy, and in 69.7% of the patients endometrial pathology was removed hysteroscopically. In 75 (58.1%) patients, hysteroscopy was performed in the same sitting and in 15 (11.6%) after histological verification.

Table 2

Sensitivity, specificity, positive and negative predictive value of hysteroscopy in diagnosis of different intrauterine pathologies

Findings	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Endometrial polyp	91.5	100	100	96
Submucous myoma	100	96	50	100
Endometrial hyperplasia	100	98.5	73	100
Endometrial atrophy	100	98	89	100

PPV – positive predictive value, NPV – negative predictive value

Table 3

Management of the 129 studied patients

Treatment	Patients	
	n	(%)
Medical treatment	36	27.9
Polypectomy	71	55
Myoma resection	4	3
Enometrial ablation	15	11.6

Discussion

Postmenopausal bleeding is an important problem and the most common reason for female patients to be referred to the gynecologist after menopause. To solve this problem, a precise diagnostic is required [6]. Hysteroscopy is a superior method with high sensitivity and specificity in diagnosing the cause of postmenopausal bleeding thanks to the fact that the uterine cavity and intrauterine pathology are directly visualized [7].

The extremely low percentage of failures (0.49%) indicates the simplicity of the method. In all cases it was impossible to place a hysteroscope due to cervical canal stenosis, which is also the reason for complicated dilation of the cervical canal, thus making it impossible to perform even DC in these cases. The method success rate of 95.1% corresponds to the results of other studies (96.9% in the study of Van Dongen et al. [8] and 96% in the study of Nikolaou et al. [9]).

The complication rate of 1.37% was slightly higher than in the large study of Singhi et al. [10] (0.9%) which can be explained by the number of hysteroscopies and the experience of the surgeon. However, the rate was much lower than the percentage of uterine perforation during DC found in the literature, which was expected because of placing the hysteroscope into the cavity under direct view [11].

The pathology of the uterine cavity was present in 73.7% (107/145) of the patients. Such a high percentage justifies the use of hysteroscopy in cases of postmenopausal bleeding. The results of other studies also indicate a high percentage of abnormal hysteroscopic findings (Lasmar et al. [12] 80%, Sunitha et al. [13] 69%).

The most common finding was endometrial polyp (29.6%). The majority of other studies also state the highest incidence of endometrial polyp as the abnormal hysteroscopic finding but with a slightly higher percentage (32.5% Dibi et al. [14], 37.6% Cordeiro et al. [15]). Five patients were referred to hysteroscopy after DC. The histological findings were normal but the bleeding from the uterus continued. In all cases the diagnosis of small endometrial polyps in the fundus of the uterus, which were probably not affected by the curette during DC, was made hysteroscopically. In our study, 3 patients (2.06%) were diagnosed with endometrial cancer by histeroscopy, which was histological verified. The incidence of endometrial cancer that is seen in the literature is generally higher [16, 17]. Such a low incidence in our study may be explained by the fact that patients with postmenopausal bleeding are usually referred from ambulance for DC, which is still opted by a great number of physicians in our clinic.

The results of our study indicate a high sensitivity and specificity of hysteroscopy in detection of intrauterine pathology (100% and 81%). The study of Allameh et al. [18] presents the results 100% sensitivity of hysteroscopy and specificity of 80.5%, the study of Tandulwadkar et al. [19], 97% and 98%, and Barati et al. [20], 98.7% and 99%, respectively. Reviewing the majority of studies from the literature shows that sensitivity of hysteroscopy in the detection of intrauterine pathology exceeds 80%, so we can say that hysteroscopy is a valid diagnostic tool for detecting the cause of postmenopausal bleeding. Most studies also indicate the highest sensitivity and specificity in the detection of focal intrauterine pathology such as polyp and myoma [21]. In our study 4 cases of endometrial polyp were diagnosed as submucous myoma using hysteroscopy which can be explained by the difficulty to distinguish small submucous myoma type 2 from a large sessile polyp. There were also 5 cases of endometrial hyperplasia and atrophy diagnosed by hysteroscopy that proved to be normal findings after the histological examination, but there were no cases of intrauterine pathology that were missed by hysteroscopy and later appeared at histological finding. The majority of studies, as well as our study, show that endometrial cancer has specific hysteroscopic appearance, so it is difficult to hysteroscopically declare it normal and in combination with biopsy the possibility of error is 0%.

The treatment of postmenopausal bleeding depends on the cause of bleeding, thus, the proper diagnosis is crucial. No treatment was applied in 16 (11%) of patients considering the fact that the histological finding was either normal or the case of endometrial atrophy and that bleeding stopped within six months. Drug treatment was applied to 27.9% of the patients. In 58.1% of the women diagnosed with endometrial polyps, cervical polyps and submucous myomas, the polyp or myoma was removed in the same sitting. A total of 19 women were referred to hysteroscopy after DC. The rea-

son was bleeding continuation and the histological finding that showed an endometrial polyp or myoma. This, once again, confirms the fact that only in rare cases endometrial polyp and submucous myoma can be removed by a blind technique such as DC. A total of 11.6% of patients underwent hysteroscopic endometrial ablation after the histological verification, in cases of simple endometrial hyperplasia and normal findings that did not respond to drug treatment. Similar percentages of the share of hysteroscopy in the treatment of postmenopausal bleeding can be seen in the literature [22]. Classical hysterectomy with adnexectomy was performed in only 2.32% of patients.

Conclusion

Hysteroscopy is a safe, highly sensitive diagnostic procedure, thus being an ideal method in evaluation of patients with postmenopausal bleeding. The application of hysteroscopy with endometrial biopsy leads to an accurate diagnosis. An adequate diagnosis is crucial for the selection of relevant treatment of postmenopausal bleeding and avoidance of unnecessary major surgical procedures. Except for being a diagnostic method, hysteroscopy is also an outpatient minimally invasive surgical procedure by which the cause of bleeding may be treated the majority of cases in the same sitting in.

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Environmental lead pollution and its possible influence on tooth loss and hard dental tissue lesions

Zagađenje okoline olovom i njegov uticaj na gubitak zuba i lezije tvrdih zubnih tkiva

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Abstract

Bacground/Aim. Environmental lead (Pb) pollution is a global problem. Hard dental tissue is capable of accumulating lead and other hard metals from the environment. The aim of this study was to investigate any correlation between the concentration of lead in teeth extracted from inhabitants of Pančevo and Belgrade, Serbia, belonging to different age groups and occurrence of tooth loss, caries and non-carious lesions. **Methods.** A total of 160 volunteers were chosen consecutively from Pančevo (the experimental group) and Belgrade (the control group) and divided into 5 age subgroups of 32 subjects each. Clinical examination consisted of caries and hard dental tissue diagnostics. The Decayed Missing Filled Teeth (DMFT) Index and Significant Caries Index were calculated. Extracted teeth were freed of any organic residue by UV digestion and subjected to voltammetric analysis for the content of lead. **Results.** The average DMFT scores in Pančevo (20.41) were higher than in Belgrade (16.52); in the patients aged 31–40 and 41–50 years the difference was significant ($p < 0.05$) and highly significant in the patients aged 51–60 (23.69 *vs* 18.5, $p < 0.01$). Non-carious lesions were diagnosed in 71 (44%) patients from Pančevo and 39 (24%) patients from Belgrade. The concentrations of Pb in extracted teeth in all the groups from Pančevo were statistically significantly ($p < 0.05$) higher than in all the groups from Belgrade. In the patients from Pančevo correlations between Pb concentration in extracted teeth and the number of extracted teeth, the number of carious lesions and the number of non-carious lesions showed a statistical significance ($p < 0.001$, $p < 0.01$ and $p < 0.001$, respectively). **Conclusion.** According to correlations between lead concentration and the number of extracted teeth, number of carious lesions and non-carious lesions found in the patients living in Pančevo, one possible cause of tooth loss and hard dental tissue damage could be a long-term environmental exposure to lead.

Key words:
environmental pollution; lead; dmf index; serbia.

Apstrakt

Uvod/Cilj. Zagađenost životne sredine olovom predstavlja globalni problem. Čvrsto zubno tkivo ima sposobnost da akumulira olovo i ostale teške metale iz okruženja. Cilj ovog rada bio je da se ispitaju korelacije između koncentracije olova u izvađenim zubima stanovnika Pančeva i Beograda, pripadnika različitih starosnih grupa, i učestalosti gubitka zuba, karijesa i nekarijesnih oštećenja tvrdog zubnog tkiva. **Metode.** Za ovo istraživanje konsekutivno je odabrano 160 volontera iz Pančeva (eksperimentalna grupa) i Beograda (kontrolna grupa) podeljenih u pet starosnih podgrupa od po 32 ispitanika. Kliničkim pregledom dijagnostikovani su karijes i nekarijesna oštećenja. Izračunati su karijes ekstrahirani plombirani (KEP) indeks i značajni karijes indeks. Organski sadržaj ekstrahovanih zuba uklonjen je UV digestijom pre određivanja koncentracije olova voltametrijskom analizom. **Rezultati.** Prosečni KEP indeks bio je veći kod ispitanika iz Pančeva (20,41) nego kod ispitanika iz Beograda (16,52); kod ispitanika starih 31–40 i 41–50 godina razlika je bila značajna ($p < 0,05$), a kod ispitanika starih 51–60 godina veoma značajna (23,69 i 18,5, $p < 0,01$). Nekarijesna oštećenja dijagnostikovana su kod 71 (44%) ispitanika iz Pančeva i 39 (24%) iz Beograda. Koncentracija olova kod svih ispitanika iz Pančeva bila je značajno veća ($p < 0,05$) nego kod svih ispitanika iz Beograda. U eksperimentalnoj grupi (Pančevo) korelacije između koncentracije olova u ekstrahovanim zubima i broja ekstrahovanih zuba, broja karijesnih zuba i broja zuba sa nekarijesnim oštećenjima bile su statistički značajne ($p < 0,001$, $p < 0,01$ i $p < 0,001$, respektivno). **Zaključak.** Imajući u vidu pronađene korelacije između koncentracije olova u ekstrahovanim zubima i broja ekstrahovanih zuba, broja karijesnih zuba i broja zuba sa nekarijesnim oštećenjima kod ispitanika iz Pančeva, može se zaključiti da je jedan od mogućih uzroka gubitka zuba i nekarijesnih oštećenja dugotrajna izloženost životnoj sredini zagađenoj olovom.

Ključne reči:
životna sredina, zagađenje; olovo; dmf indeks; srbija.

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Introduction

The main causes of environmental pollution are production and use of energy, industrial chemicals and increased agricultural activity. As a result, all biological organisms, including humans, live in a chemically polluted environment. The city of Pančevo is a modern, small-sized city and one of the most powerful industrial centers in Serbia, and this inevitably brings with it the frequent occurrence of air pollution and contamination of water and soil. The existence of these risk factors in the environment has a negative impact on public health. An analysis of morbidity in the adult population of Pančevo showed that this population most often suffers from respiratory diseases. The Institute of Public Health in Pančevo has monitored concentrations of SO_2, NO_2, NH_3 and soot in the air in a 10-year period (1991–2001). The results showed that concentrations of soot and NH_3 were high above the legal limit during this period.

Environmental lead (Pb) pollution is a global problem. Pb is one of the most important and widely distributed pollutants in the environment [1–3] and a great part of this pollution comes from vehicle exhaust fumes through the combustion of leaded petrol. This, and other human activities such as the extensive use of Pb in industry, has resulted in its redistribution in the environment and, hence, the contamination of air, water and food. Consequently, the levels of Pb content in blood, saliva and other human organs [4] are significantly increased. The levels of Pb in blood and saliva reflect recent exposure. Long-term deposition of Pb is much greater in skeletal tissues than in soft tissues [5]. Heavy metals, *ie* Pb and cadmium (Cd), have no known physiological functions and are toxic even in low concentrations [6].

It has been demonstrated in a number of studies [7–15], that hard dental tissues have the capacity to accumulate Pb and other heavy metals from the environment.

Hard dental tissue lesions include caries and non-carious lesions (abrasion, erosion, attrition). The mechanism of caries development has been the subject of many studies during previous years and consequently general and local predisposing factors are now well-known [16]. In recent years, the subject of many studies has been the determination of the effects of heavy metals on the occurrence and incidence of caries. In addition, it has been speculated for some time that environmental pollution, especially by acid fumes, could also be one of the factors involved in the occurrence and incidence of non-carious lesions. A high incidence of tooth-structure damage significantly affects the functional ability of chewing, mental and work capacity of individuals, causes diseases of the digestive tract and other systems and organs and also represents a serious medical, social and economic problem of the global society.

The principal hypothesis of this study was that pollution of the environment by lead cause dlong-lasting changes in teeth through its deposition in dental hard tissues. Therefore, the aim of this study was to find a correlation between the concentration of lead in teeth extracted from inhabitants of Pančevo and Belgrade belonging to different age groups and occurrence of tooth loss, caries and non-carious lesions in the same groups.

Methods

This study was undertaken on 160 patients of both sexes from Pančevo (the experimental group) and 160 patients of both sexes from Belgrade (the control group). The volunteers were selected from patients who visited the Institute of Stomatology at the Faculty of Stomatology, Pančevo, Serbia. The primary criterion for inclusion and subsequent sample collection was that these patients had been living in either Pančevo or Belgrade for a period of at least 15 years prior to the study beginning. They had to be in good general health with no signs of disease or medication use. The volunteers were then divided into five separate age subgroups for each city, with 32 volunteers in each group.

The study proposal was submitted to the Research Ethics Committee (Approval Protocol No. 1323/1-2008, according to Resolution sections 3, 7, and 8 of the National Commission of Ethics in Research). Patients had to sign an informed consent form prior to the inclusion in the study. Additionally, signed permission for collection of samples of extracted teeth (only if extraction was necessary as a therapeutic procedure) had to be obtained from each participant in the study.

Clinical examination

The patients were clinically examined by the standardized procedure for dental examination, using a dental mirror, a straight or proximal dental probe. Dental caries lesions were diagnosed by standard criteria and marked in universal templates for dental status. The Klein-Palmer system Decayed Missing Filled Teeth (DMFT) was applied in assessing the prevalence of dental caries. Also, for each of the age groups of patients a Significant Caries Index (SiC) was calculated, which represents the mean values of the DMFT index for one third of respondents with the highest DMFT values, using tables recommended by the World Health Organisation (WHO) [17]. Information related to clinically diagnosed loss of enamel and dentin of a non-carious etiology, the so-called non-carious lesions, was recorded for each patients.

Collection of samples – extracted teeth

Following a detailed examination, all teeth to be extracted were carefully evaluated for the presence of fillings or caries. The final decision for tooth extraction was reached following careful consideration of periodontal status and restorative possibilities. In many cases the cause for extraction was either subsequent orthodontic therapy or progressive periodontal disease. The weight of each sample was at least 0.5 g which is a cut-off value for valid chemical analysis.

Chemical analysis

All chemical analyses were done in an independent laboratory, Department of Ecotoxicology at the Institute of Public Health, Pančevo.

The collected samples of extracted teeth were stored in ultrafiltered deionized water. Before analysis all soft tissue remnants and surface stains were removed. Drying of sam-

ples to a constant mass was done under laboratory conditions for 48 h at 80°C. Dried samples were then finely ground to grains under 1 mm in diameter. For further voltammetric analyses all organic substances had to be removed from the sample. This was done by UV digestion (MILESTONE SK-10, Milestone, Sorisole, Italy). Batches of 0.5 g of the samples were diluted for 30 minutes using 7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2 at 200°C. After cooling to room temperature, the digested samples were transferred directly to the appropriate vessel for further analysis. The concentrations of Pb in the final digested solution of samples were determined by the PS control system for voltammetry 797 VA Computrace (Metrohm, Herisau, Switzerland). This method was chosen because it can distinguish between different oxidation states of metal ions as well as between free and bound metal ions, which provides important information regarding the bioavailability and toxicity of Pb. Validation of the voltammeter was done using the GLP Wizard of the machine. Chemical analysis was done using a Pb-ion standard. The decisive parameters for the validation of the measuring instrument are the accuracy and the scatter of the result. Both values are calculated automatically by the internal software of the 797 VA Computrace. Electronic validation included: current at – or + 200 mV: measured values – or + 2 μA: tolerances from -1.6 μA to -2.4 μA. Peak voltage: measured values -497 mV; tolerances: from -450 mV to +450 mV. Chemical validation included: measuring: 20 mL H_2O + 0.5 mL KCl + 100 μL Pb standard. Electrolyte was c (KCl) = 3 mol/L, and standard: β (Pb) = 1 g/L. Accuracy was 0.95 – 1.05 g/L and scatter was ± 3%. Sensitivity of the method was achieved to the level of Pb of 0.0005–2.5 μg/m^3.

Reagents used for the voltammetric determination of lead were: suprapure NaOH, suprapure acetic acid, suprapure KCl and Pb stock solution-β (Pb) = 1g/L (commercially available). Solutions used were: KCl-acetate: c(KCl) = 1.5 mol/L, c(CH_3COONa) = 0.5 mol/L, 55.9 g KCl + 25 mL NaOH + 14.2 mL CH_3COOH filled up to 500 mL with high purity water, standard solution β (Pb) = 0.5 mg/L; diluted solution for Pb was prepared using c (HNO_3) = 0.014 mol/L.

The detection limit for Pb used in this study, as stated by the manufacturer, was 0.02 μg/g. The detection limit for atomic spectroscopy for the same metal was 0.2 μg/g.

The statistical significance was calculated by the Student's *t*-test and its modification by Bonferroni [18].The processor CORR from the SAS package, version 6.4 [19], was used to estimate correlations between trait pairs (the number of extracted teeth and concentration of lead; the number of carious lesions and concentration of lead; the number of non carious lesions and concentration of lead) within locality. Correlations were computed as Pearson product-moment correlations.

Results

All the results were statistically processed and shown in tables. Each investigated parameter is represented by mean value and statistical significance and separately marked for both sexes since no sex-related differences were found.

The mean values of the number of extracted teeth in all the five age groups, from Pančevo and from Belgrade are given in Table 1. It was clear that the number of extracted teeth was statistically significantly ($p < 0.001$) higher in groups III, IV and V from Pančevo than in groups III, IV and V from Belgrade. The number of extracted teeth in the group I from Pančevo was higher (no statistical significance) than in the group I from Belgrade, whereas the number of extracted teeth was higher (no statistical significance) in the group II from Belgrade than in the group II from Pančevo.

Table 2 shows the mean values of the DMFT index for each of the five groups of patients from the experimental and

Table 1

Total number and mean values of extracted teeth from Pančevo and Belgrade

Age subgroups (years)	Extracted teeth			
	Pančevo		Belgrade	
	total	mean ± SE	total	mean ± SE
I (20–30)	72	2.25 ± 0.75	44	1.37 ± 0.63
II (31–40)	166	5.18 ± 1.20	233	7.25 ± 1.42
III (41–50)	332*	10.37 ± 2.12	169	5.28 ± 1.80
IV (51–60)	428*	13.37 ± 3.45	68	2.12 ± 0.61
V (over 60)	452*	14.12 ± 3.85	158	4.93 ± 1.48

***Statistically significant; SE = standard error.**

Table 2

Mean values of the Decayed Missing Filled Teeth (DMFT) index and significant caries (SiC) index in the patients from Pančevo and Belgrade

Age subgroup (years)	Number of patients		DMFT (mean ± SE)		SiC	
	Pančevo	Belgrade	Pančevo	Belgrade	Pančevo	Belgrade
I (20–30)	32	32	12.12 ± 2.36	9.56 ± 2.08	19.09	14.27
II (31–40)	32	32	19.87 ± 2.30*	17.09 ± 2.31	25.09	22.91
III (41–50)	32	32	21.44 ± 1.90*	18.47 ± 2.10	24.63	24.54
IV (51–60)	32	32	23.69 ± 3.07§	18.50 ± 3.02	30.00	26.27
V (over 60)	32	32	24.94 ± 3.41§	19.00 ± 3.56	28.36	25.27
Total	160	160	20.41	16.52	25.43	22.65

SE = standard error; *statistically significant; § statistically highly significant.

control groups, as well as the SiC index values. The average value of the DMFT index in the experimental group and in the control group was 20.41 and 16.52, respectively. It is notable that the index value increased with the age of patients and that it was higher in each subgroup of patients from the experimental group, compared to the control group. In the group I the difference was not statistically significant ($p > 0.05$), while in the groups II and III the difference between the coefficient of DMFT patients from the experimental group and the control group was statistically significant ($p < 0.05$). The most striking differences were in the group IV, where the recorded value of the DMFT index in the experimental group was 23.69 *vs* 18.50 in the control group, which was a highly statistically significant difference ($p < 0.01$). Also in the group V the recorded value of the DMFT index in the experimental group was 24.94 *vs* 19.00 in the control group, which was an extremely statistically significant difference ($p < 0.001$). The SiC index values were also higher in all age subgroups from the experimental group than in the control group (Table 2).

Non-carious lesions were diagnosed in 71 patients (44%) from the experimental group and 39 patients (24%) from the control group (Table 3).

Table 3

Mean number of teeth affected with non-carious lesions

Age subgroup (years)	Number of patients		Teeth affected with noncarious lesions (mean ± SE)	
	Pančevo	Belgrade	Pančevo	Belgrade
I (20–30)	32	32	0.19 ± 1.28	0.06 ± 1.17
II (31–40)	32	32	2.16 ± 1.33	0.31 ± 1.06
III (41–50)	32	32	1.03 ± 1.75	1.03 ± 1.75
IV (51–60)	32	32	4.16 ± 2.24	2.44 ± 1.84
V (over 60)	32	32	8.09 ± 3.42	6.66 ± 2.78

SE = standard error.

The mean concentrations of Pb (presented in μg/g) in extracted teeth in all age subgroups from Pančevo and from Belgrade are given in Table 4. The concentrations of Pb in extracted teeth in all the groups from Pančevo were statistically significantly ($p < 0.05$) higher than in all the groups from Belgrade. When the measured levels of Pb did not reach the threshold values for the method used, they were marked as 'undetectable' in the corresponding tables.

Table 4

Mean values of lead concentrations in extracted teeth

Age subgruops	Pb concentrations (μg/g), mean ± SE	
	Pančevo	Belgrade
I (20–30)	1.57 ± 0.35	0.61 ± 0.14
II (31–40)	4.48 ± 1.12	0.39 ± 0.09
III (41–50)	4.60 ± 0.78	0.80 ± 0.22
IV (51–60)	11.10 ± 2.43	4.15 ± 1.76
V (over 60)	26.61 ± 3.89	4.86 ± 2.00

SE = standard error.

Correlation determination was done three times, separately for the patients from Pančevo and from Belgrade, with two variables. The first correlation was done between the number of extracted teeth from 32 patients in each of the 5 subgroups, and the concentration of Pb in 8 teeth from each of the five subgroups. The second correlation was done between the number of carious lesions from 32 patients in each of the 5 subgroups, and the concentration of Pb in 8 teeth from each of the 5 subgroups. The third correlation was done between the number of non-carious lesions from 32 patients in each of the 5 subgroups, and the concentration of Pb in 8 teeth from each of the 5 subgroups. For the patients from Belgrade all correlations were negative and without statistical significance. However, all the correlations for patients from Pančevo showed a statistical significance (the number of extracted teeth and the concentration of Pb in extracted teeth – $p < 0.001$, the number of carious lesions and the concentration of Pb in extracted teeth – $p < 0.01$ and the number of non-carious lesions and the concentration of Pb in extracted teeth – $p < 0.001$).

Discussion

In this study it was noticed that the patients from Pančevo had fewer teeth than those from Belgrade although the patients from both cities had similar oral hygiene habits and visited dentists at approximately the same intervals. Therefore, one possible cause of tooth loss in the patients from Pančevo aged over 40 years could be the long-term exposure to a polluted environment.

The value of the DMFT index and the SiC index values, both in Pančevo and Belgrade, must be considered as extremely high, given some of the values that WHO has defined as acceptable [20]. Namely, as expressed by DMFT index, 6 is the acceptable value of oral health for members of the group aged 35–44 years, while in the experimental group this value was as much as 20, and in the control group 17. These results are worse than in many well-developed countries (Turkey 12.62, Austria 14.7, Germany 16.1, UK 16.6, Denmark 16.7), however, they are similar to, for example, Norway (20.5) and Canada (20.0) [21, 22]. For people over 65 years of age, WHO considers acceptable DMFT to be 12, while in the experimental group this value was as much as 25 and in the control group it was 19. This low level of oral health in patients aged over 60 years resulted from the high DMFT index values in all age groups and also from a large number of extracted teeth.

An alarming fact is that the most frequent component of the DMFT index in the experimental group was extracted teeth, with the proportion of 44% *vs* 25% in the control

group (which is also a high percentage). A large number of people prefer to have teeth extracted rather than undergo some kind of conservative treatment, partly due to fear and partly because of low income. Besides caries, the reasons for the large number of extracted teeth can also be found in a low level of health education.

Abrasions of anterior teeth and premolars and wedge-shaped erosions where the loss of tooth substance extends to both the enamel and dentin, were the most frequently diagnosed non-carious lesions in the patients from the experimental group, while abrasions were the most frequently diagnosed non-carious lesions in the patients from the control group. In our sample of younger patients (up to 50 years of age) presented with less non-carious lesions, therefore, the standard error was higher. The explanation for the representation of abrasions, could be related to early loss of molar teeth and, therefore, over-loading of the remaining teeth during mastication [23]. The most frequently cited etiological factor in forming cervical erosions is chemical etching, more precisely, acid dissolution of hard dental tissues [24]. Studies have confirmed that cervical erosions are more frequent in people who are exposed to acids in the workplace or living environment, in competitive swimmers, in people who frequently consume acidic drinks, or use chemicals for oral hygiene which chelate calcium. Due to frequent vomiting, a significant frequency of dental erosive lesions was noticed in patients with gastrointestinal problems, bulimia and anorexia neurosa, in pregnant women and alcoholics [25–28]. Many different studies in recent years, have confirmed that "bending" or "flexing" of teeth caused by eccentric occlusal forces is one of the factors which could explain the occurrence of cervical lesions [29–35] while numerous epidemiological studies consider inappropriate teeth brushing technique as one of the reasons for cervical erosions etiology [31, 36]. One of the possible reasons for higher number of non-carious lesions in the older patients from this investigation could be heavy metals accumulation during the years.

This study showed that the concentration of Pb in extracted teeth in all the groups of patients from Pančevo was higher compared to the patients from Belgrade. Additionally, the concentration of Pb increased rapidly for the older patients from both cities, indicating that the concentration of Pb is age-dependent. Other studies also showed that concentrations of Pb are age-dependent. Baranowska et al. [37] and Nowak and Chmielnicka [38] found a positive correlation between age and Pb in human teeth.

Teeth are not a uniform mass of calcified tissues and it has been well-established that Pb is not homogeneously distributed within a fully developed tooth, with Pb levels in dentine being significantly higher than in enamel [39]. Furthermore, Arora et al. [40] presented the spatial distribution of Pb in the roots of human primary teeth while other authors measured the Pb content in whole teeth [41].

In this study a consistent relationship was demonstrated between a long-term environmental Pb exposure and its incorporation into hard dental tissues. This is similar to the results of de Almeida et al. [42], although they measured the content of Pb in the surface enamel of deciduous teeth sampled *in vivo* from children living in uncontaminated and in lead-contaminated areas of Brazil using different methods. It had been shown previously that heavy metals can be incorporated into dental tissues if there is exposure during the process of dentinogenesis [43]. Therefore, unlike bone, in which the mineral phase is subject to turnover, once formed, teeth provide a permanent, cumulative and relatively stable record of environmental exposure [44].

It should be noted that in the patients living in Pančevo statistically significant correlations between the concentration of Pb and the number of extracted teeth ($p < 0.001$), the number of carious lesions ($p < 0.01$) and the number of non-carious lesions ($p < 0.001$) were found, whereas the same correlations in the patients from Belgrade were of no statistical significance. This evidence suggests that the possible cause of tooth loss and hard dental tissue lesions (carious and non-carious) in the patients from Pančevo could be a long-term environmental exposure to lead.

Conclusion

Significantly higher values of the DMFT index and higher frequency of non-carious lesions were recorded in the patients from Pančevo. The concentration of lead in extracted teeth increased rapidly in the older patients from both Pančevo and Belgrade, indicating that the deposition of lead is age-dependent. According to the correlations between the concentration of lead in extracted teeth and the number of extracted teeth, the number of carious lesions and non-carious lesions found in the patients living in Pančevo, one possible cause of tooth loss and hard dental tissue damage could be a long-term environmental exposure to lead. That means that polluted environment is one of the factors that cannot be ignored, but also requires confirmation by further comprehensive basic research.

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ORIGINAL ARTICLE

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Blunt trauma of bone structures of the chest – computed tomography *vs* multidetector computed tomography

Tupa trauma koštanih struktura grudnog koša – kompjuterizovana tomografija *vs* multidetektorska kompjuterizovana tomografija

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Abstract

Background/Aim. Computerized tomography (CT), especially multidetector CT (MDCT), has had a revolutionary impact in diagnostic in traumatized patients. The aim of the study was to identify and compare the frequency of injuries to bone structures of the thorax displayed with 5-mm-thick axial CT slices and thin-slice (MDCT) examination with the use of 3D reconstructions, primarily multiplanar reformations (MPR). **Methods.** This prospective study included 61 patients with blunt trauma submitted to CT scan of the thorax as initial assessment. The two experienced radiologists independently and separately described the findings for 5-mm-thick axial CT slices (5 mm CT) as in monoslice CT examination; MPR and other 3D reconstructions along with thin-slice axial sections which were available in modern MDCT technologies. After describing thin-slice examination in case of disagreement in the findings, the examiners redescribed thin-slice examination together which was ultimately considered as a real, true finding. **Results.** No statistically significant difference in interobserver evaluation of 5 mm CT examination was recorded ($p > 0.05$). Evaluation of fractures of sternum with 5 mm CT and MDCT showed a statistically significant difference ($p < 0.05$) in favor of better display of injury by MDCT examination. **Conclusion.** MDCT is a powerful diagnostic tool that can describe higher number of bone fractures of the chest in traumatized patients compared to 5 mm CT, especially in the region of sternum for which a statistical significance was obtained using MPR. Moreover, the importance of MDCT is also set by easier and more accurate determination of the level of bone injury.

Key words:
thoracic injuries; wounds, nonpenetrating; diagnosis; tomography, x-ray computed; imaging, three-dimensional; diagnostic techniques and procedures.

Apstrakt

Uvod/Cilj. Kompjuterizovana tomografija (CT), naročito multidetektorska (MDCT), ima revolucionarni uticaj na dijagnostiku kod traumatizovanih bolesnika. Cilj istraživanja bio je da se utvrdi i uporedi učestalost povreda koštanih struktura grudnog koša prikazanih aksijalnim CT snimcima debljine 5 mm i tankoslojnim MDCT pregledom uz upotrebu 3D rekonstrukcija, prvenstveno multiplanarnih reformacija (MPR). **Metode.** Ova prospektivna studija obuhvatila je 61 bolesnika sa tupom traumom kojima je urađena CT grudnog koša kao deo inicijalne dijagnostike. Dva iskusna radiologa opisali su posebno (nezavisno) aksijalne CT preseke debljine 5 mm (5-mm CT) kao što se to čini na monoslajsnom skeneru; MPR i druge 3D rekonstrukcije zajedno sa tankoslojnim aksijalnim presecima koje omogućuje MDCT tehnologija. Nakon opisa tankoslojnog pregleda, u slučaju nesuglasica u ovom nalazu ponovo je zajednički opisivan tankoslojni pregled koji je na kraju smatran istinitim nalazom. **Rezultati.** Razlika pregleda preloma kostiju grudnog koša (5 mm CT) između istraživača nije bila statistički značajna ($p > 0,05$). Poređenje rezultata pregleda sternuma (5 mm CT i MDCT) pokazuje postojanje statistički značajne razlike ($p < 0,05$) u korist boljeg prikaza povrede od strane MDCT pregleda. **Zaključak.** Multidetektorski CT je moćno dijagnostičko sredstvo kojim se može prikazati veći broj preloma kostiju grudnog koša kod bolesnika sa tupom traumom u odnosu na 5 mm CT, naročito u području sternuma za koji je uočena statistički značajna razlika u korist MPR. Sem toga, važnost MDCT ogleda se u lakšem i preciznijem prikazu nivoa povrede kosti.

Ključne reči:
toraks, povrede; povrede, zatvorene; dijagnoza; tomografija, kompjuterizovana, rendgenska; snimanje, trodimenzionalno; dijagnostičke tehnike i procedure.

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Introduction

Trauma affects young people, and has a high rate of morbidity and mortality and major socioeconomic consequences. Studies on the incidence of thoracic trauma indicate that injuries occur in 12 out of a million people every day. Knowledge of the mechanism and time of injury, condition of the motor vehicle and speed during collision, and the presence of associated injuries are essential for better understanding and access to chest trauma [1].

Initial radiological diagnostic approach to chest trauma is classically based on the assessment of chest radiography in the moment of hospital admission. Therefore, it is necessary to be familiar with the possibilities and limitations of this method in displaying thoracic injuries. Nowadays it is known that the information provided by conventional chest radiography sometimes can be insufficient for diagnosing thoracic injuries [2-5]. Since the advent in clinical practice in the 1970s, computerized tomography (CT) has had a revolutionary impact on diagnostic in patients under emergency setting. Speed, precision, and the increasing availability make multidetector computerized tomography (MDCT) a valuable new tool in modern medicine. Present-day scanners have high-quality multiplanar reformatted (MPR) images with spatial resolution the same as that of images in the axial plane [5].

Although trauma is one of the most important topics in radiology, there is insufficient number of published papers on the contribution of MDCT technology to diagnosing blunt trauma of the chest. The fact that CT examination is more sensitive in displaying traumatic lesions of the chest in relation to the conventional radiography is generally known, however there are some questions about the contribution of multiplanar and other 3D reconstructions in traumatized patients compared to standard axial CT sections.

The aim of the study was to identify and compare the frequency of injuries of bone structures of the thorax displayed with 5-mm-thick axial CT slices and thin-slice (MDCT) examination using 3D reconstructions, primarily multiplnar reformations (MPR).

Methods

The study was a prospective review of 61 patients (mean age 43.9 years) with blunt trauma who were treated in our clinical center and submitted CT scan of the thorax in the initial assessment. It should be pointed out that the patients got their CT examination report within clinically reasonable time, independently on our study.

These CT examinations were saved in the computer and afterwards two experienced radiologists independently and unblinded to referring diagnosis, but with no insight into physical examination and laboratory results, described the findings. The same examination was interpreted using two ways: firstly, as in monoslice CT – 5-mm-thick axial CT slices (5 mm CT); and secondly, using MDCT technology performed at the workstation – 1-mm-thick axial sections, coronal and sagittal MPR and other 3D reconstructions (MDCT).

In describing, the data of each patient were entered separately in the protocol of the study regarding fractures of the ribs, vertebra, sternum, scapula and clavicle.

All the data were entered separately by the two examiners. After describing thin-slice examination, in case of disagreement in the findings the examiners redescribed thin-slice examination (MDCT) together that was ultimately considered as the "gold standard" since its findings made a definitive determination of the presence or the absence of bone fractures. The results of 5 mm CT were mutually compared.

Description of numeric variables was performed using classical methods of descriptive statistics (arithmetic mean, mode) and measures of variability (standard deviation, minimum and maximum values). Relative values were to show read values. The Pearson's chi-square (χ^2) and Mc Nemar's test (χ^2MCN) were used for comparison of a frequency difference in non-parametric data. The value of $p < 0.05$ was considered significant.

All CT studies were performed using Siemens 16- and 64-section MDCT (120 kV, 220 mAs/slice, 5 mm section thickness, pitch of 1.4). Approximately, 1.2 mL of iodinated contrast agent (Ultravist 370 or Omnipaque 350) per kilogram of body mass was injected intravenously using a mechanical power injector at 2 mL/s. The volumetric MDCT data were reconstructed into axial and MPR 1-mm-thick sections.

Results

The average age of the patients was 43.9 years (min 14.0, max 82.0, SD 17.7 years). The number of male patients was 45, and of female patients 16, the ratio 3.46 : 1, which was statistically significant (χ^2, $p < 0.01$).

Regarding interobserver evaluation of traumatized patients using 5 mm CT (Table 1), no statistically significant differences in interpreting injuries of the ribs, sternum, scapula, clavicle, vertebral bodies and posterior processes were recorded (χ^2MCN, $p > 0.05$). The same structures of the chest were further analyzed using MDCT (Table 1).

The number of fractures of the ribs on both sides evaluated by MDCT was higher than the number of fractures evaluated by 5 mm CT, but not statistically significant (for both sides: χ^2MCN, $p > 0.05$). On the right side, 23 patients suffered rib injury with the average number of almost 5 fractured ribs ($\bar{x} = 4.7$), whereas in 32 patients with rib injury on the opposite side the average number of fractured ribs was slightly above 4 ($\bar{x} = 4.2$). The fifth rib was the most often fractured rib on both sides (mode = 5).

Considering the number of fractures of the scapula (n = 11; 18%), there was no statistically significant difference between those evaluated by 5 mm CT and MDCT (χ^2MCN, $p > 0.05$).

Evaluation of sternum fractures (n = 10; 16.4%) with 5 mm CT and MDCT, showed a statistically significant difference (χ^2MCN, $p < 0.05$) in favor of better display of injury by MDCT examination (Figure 1).

Table 1

Number of patients with fractures of chest bones detected by both readers using 5-mm-thick axial CT slices (5 mm CT); 1-mm-thick axial sections, coronal and sagittal multiplanar reformations and other 3D reconstructions (MDCT)

Finding	5 mm CT – number of the patients, n (%)		MDCT – number of the patients n (%)
	Reader 1	Reader 2	
Rib fracture, right	21 (34.4)	21 (34.4)	23 (37.7)
Rib fracture, left	30 (49.2)	33 (54.1)	32 (52.4)
Sternal fracture	6 (9.8)	6 (9.8)	10 (16.4)
Scapular fracture	7 (11.5)	9 (14.8)	11(18)
Clavicular fracture	7 (11.5)	5 (8.2)	7 (11.5)
Vertebral body fracture	7 (11.5)	8 (13.1)	7 (11.5)
Posterior vertebral process fracture	13 (21.3)	11 (18)	16 (26.2)

CT – computed tomography; MDCT – multidetector computed tomography.

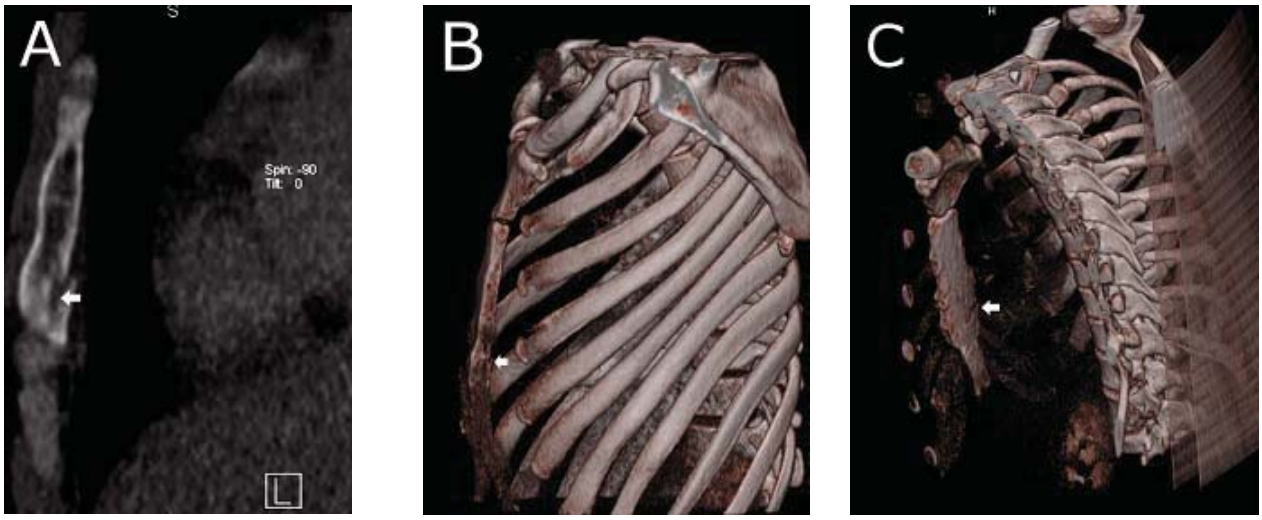

Fig. 1 – A fracture of the posterior aspect of sternal body (A, B, C arrow). Note the more accurate presentation of the fracture using sagittal reformatted computed tomographic image (A, arrow) compared to volume rendering technique (B, arrow). The fracture was more obvious on volume rendering technique only after the removal of the ribs on the left side in post-processing (C, arrow).

No statistically significant difference in displaying fractures of clavicle (n = 7; 11.5%) using 5 mm CT and MDCT was recorded (χ^2MCN, $p > 0.05$).

Comparison of the results of examination of vertebral bodies showed no statistically significant difference regarding the results of mutual comparison of 5 mm CT and MDCT (χ^2MCN, $p > 0.05$). However, MDCT examination showed a higher number of fractures and more accurately presented injuries of vertebral bodies compared to the standard 5 mm axial CT examination. Considering fractures of posterior vertebral processes, similar results were obtained (χ^2MCN, $p > 0.05$).

Discussion

Rib fractures are the most common injuries in blunt chest trauma, as was the case in our study, with 64% of patients with rib fracture on one or both sides of the body. By themselves, rib fractures are not life-threatening unless a flail chest occurs. The main advantage of using CT in evaluation of potential rib fractures is its capability of showing costal chondral fractures which could not be attributed to chest radiography. In addition, chest radiography may miss 50% of rib fractures [6]. Analyzing our results, the value of MDCT compared to 5 mm CT could be seen in depicting greater number of rib fractures, though not statistically significant, and in better displaying of the level of injury. The contribution of volume rendering technique (VRT), as a part of MDCT examination, in diagnosing rib fractures is in time-efficiency which is a crucial factor in an emergency setting [7]. It was estimated that the mortality incidence and probability of respiratory failure was greater in those patients who had higher number of rib fractures. Moreover, the incidence of respiratory failure was doubled in patients with rib fracture in more than one anatomic region [8]. Holcomb et al. [9] concluded that patients above 45 and with more than 4 four fractured ribs were at risk of prolonged intensive care unit stays and overall hospital days. Since the patients in our study had fractures of 5 ribs on the right side on average, it could be estimated that they were at risk of developing respiratory failure regarding their age. The fact that the 4th to 10th ribs are the most frequent fractured is in concordance with our results [6]. It should not be forgotten that each rib fracture may be associated with pneumothorax, hemothorax or extrapleural hematoma [10, 11].

Sternum fracture is usually overlooked on conventional chest radiography unlike CT examination [11]. In our study the percentage of patients with sternal fractures (16.4%) was higher than in previously published results (7%–10%) that relied mostly on evaluation of axial CT scans [2, 10–12]. Empirically, we noticed that MPR is more accurate than VRT in diagnosing sternal fractures (Figure 1), though it was not the aim of our study. Traditionally, patients with an isolated sternal fracture are admitted to the hospital for observation in order not to miss other injuries (heart, great vessels, spinal cord) [13]. However, since there are no evidence-based data that would support this clinical approach, it is recommended that patients with no hemodynamic instability, dysrhythmia or previous history of ischaemic heart disease can be safely discharged home provided pain control is adequate [14].

The association of scapula fractures with rib fractures was recorded in 81.8% of the patients on the right and in 100% of the patients on the left side. Scapula fracture suggests that the high energy influenced on the chest, therefore injury of deeply placed structures or organs must be suspected [15]. MDCT is particularly useful in the diagnosis of fracture of coracoid process, scapular spine and glenoid cavity [16]. Scapulo-thoracic dislocations must be identified on a conventional radiography or CT examination, due to its 100% association with brachial plexus injury. The advantage of CT in this condition is clear depiction of subscapular hematoma which is responsible for lateral displacement of the scapula [12].

Fractures of the thoracic spine make 16%–30% of all spinal fractures [17]. Unfortunately, despite the severity of injury, fractures of the thoracic spine often remain unrecognized during the initial critical period for patient evaluation and treatment [10, 18]. There are technical difficulties in performing conventional radiography, often requiring additional examinations which demand time, radiological units, higher radiation exposure and manipulation of the patient [18]. Historically, monoslice CT was considered as additional diagnostic modality in order to assess the extent and stability of spinal fractures diagnosed at conventional radiography due to its inability to display the entire spine and, to make a reconstruction, which is already sufficient advantage of MDCT [19]. Roos et al. [20] concluded that injuries of thoracolumbar spine can be fully assessed using standard MDCT protocol for trauma of the thorax and abdomen with targeted reconstructions. Based on clinical experience, in our study MDCT was expected to show statistically significant higher number of fractures of spine compared to 5 mm CT examination, however, MDCT accurately and easily determined the level of the fracture. We assume that this might be the result of a relatively small number of patients with fractures of the spine.

Although the benefit of using CT in trauma patients from the aspect of treatment and outcome is indisputable, high doses of detrimental ionizing radiation and high costs associated with its use are growing concern. As of now, there has not yet been established a protocol for referring trauma patients to CT examination in our emergency ward. Most of the injured patients, especially during a motor vehicle collision, underwent a so-called "pan-CT" examination including scanning from the head to the pelvis. Justification of this procedure certainly is debatable, however, reviewing the literature Brink et al. [21] concluded that no predictor can possibly exclude all pertinent traumatic injuries that can be depicted by CT. On the other hand, the same group of authors in their next prospective study concluded that if CT is performed only in patients who suffered high-energy blunt trauma with any of strong independent predictors [age $\geq$ 55 years; abnormal chest physical examination (PE); altered sensorium; abnormal thoracic spine PE; abnormal chest and thoracic spine conventional radiography (CR); abnormal abdominal ultrasonography or pelvic CR; hemoglobin < 6 g/dL and arterial blood gas base excess < -3 mmol/L], the sensitivity for the presence of chest injuries on CT is 95%. Therefore, the number of CT examinations can be considerably reduced with low risk of missing relevant injuries [22].

The weakness of the study were: the unblinded observers to referring diagnosis, though they were uninformed about the results of PE and laboratory findings; no clear difference was distinguished between MPR and other 3D reconstructions such as VRT and surface shaded display. Consensually, the findings of MDCT were ultimately regarded as a real true finding.

Conclusion

MDCT is a powerful diagnostic tool that can depict higher number of bone fractures of the chest in traumatized patients compared to 5 mm CT, especially in the region of the sternum where a statistical significance using MPR was obtained. Moreover, the importance of MDCT is in easier and more accurate characterization of the level of bone injury.

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GENERAL REVIEW

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Pseudoexfoliation syndrome

Pseudoeksfolijativni sindrom

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Introduction

Pseudoexfoliation (PEX) syndrome is a daily challenge for ophthalmologists because of aggressive secondary glaucoma and cataract surgery complications caused by its presence. Although it has been known since the beginning of the 20th century, the interest for its research has been increasing during the last few decades. It has been found that PEX syndrome exists in diseases of other organs and systems. In addition, the origin and nature of PEX material has not been explained yet, which further arouses the interest of scientists.

Discovery of pseudoexfoliation syndrome

Research of Finnish ophthalmologist John G. Lindberg was inspired by the works of his senior colleague, the German ophthalmologist Axenfeld, who described changes in the iris of older people. Obeying his own research needs, Lindberg worked on the thesis, examining the patients at the slit lamp, which he constructed himself. Thus, he observed and described the changes in the form grayish villi and flakes at the pupillary edge and lens in patients with cataract older than 50. In patients with chronic glaucoma, changes were found in 50% of cases. In his doctoral thesis, presented in 1917 at the University of Helsinki, Lindberg described in detail the observed changes in the iris and lens, and he personally illustrated his work.

A few years later, Lindberg gave a copy of his thesis to his Swiss colleague A. Vogt. But Vogt published his first work on PEX and glaucoma, not even once mentioning Lindberg and his work. Lindberg also borrowed his thesis to the Swedish ophthalmologist, Malling, who shortly afterwards published papers on this subject, also without mentioning Lindberg and his discovery.

Lindberg received a full recognition posthumously, a few decades later, thanks to Finnish ophthalmologists [1]. Interest in PEXs was awakened later, in the 80s and in 1998, the International Association for the Study of PEX was established, which was named after Lindberg – the Lindberg's Society.

At first, it was thought that PEXs originated from anterior lens capsule [exfoliation superficialis capsule anterioris (Malling, Vogt)], and then they were only deposited on the front lens capsule (Busacca). Georgiana Dvorak-Theobali first suggested the term PEX (1954) [2], in order to make the difference with the lamellar exfoliation of the lens capsule within glass blowers [1–3]. In 1964, Bertelsen, Drablos and Flood found deposits located in the very lens epithelial cells and suggested the term fibrillopathia epitelio-capsularis. Today, we use the term pseudoexfoliation syndrome (PEX syndrome, PXF syndrome, XFS, PXS, PES) and, less frequently, exfoliation syndrome and senile lens exfoliation.

Clinical diagnosis of pseudoexfoliation syndrome

PEX syndrome is a systemic disorder of basement membrane. Until recently, as a direct cause of disease, the syndrome has been recognized only in the eye, where it causes aggressive secondary glaucoma and cataract surgery complications.

Initially, the syndrome is typically manifested unilaterally. However, electron microscopy indicates that subclinical changes mostly exist in the other eye, as well. In the later course, PEX syndrome becomes clinically manifested in both eyes. A patient usually has no symptoms or complains about sight quality, caused by cataract or a deficit in the visual field due to glaucoma. The diagnosis is made by standard ophthalmic examinations. It is sufficient to use biomicroscope

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with slight light (sensitivity of the examination is 85% and specificity of up to 100%) [4]. PEXs can be clearly seen on the pupillary margin. Overview of the anterior eye segment, with pharmacological mydriasis, recognizes "3-ring" or "eye bull'' sign on the front surface of the lens. It comprises a central disc and peripheral ring covered with PEX material and pure annular zone between them. Pure zone is probably caused by pupillar movement, leading to removal of PEX from the part of lens capsule and its dispersal into the environment. Total, white cataract may make it difficult to see this sign.

Pupilla pure dilates with the use of mydriatics. The entire anterior segment of the eye is ischemic to a certain degree and iris neovascularization is possible [5, 6]. PEXs are found in the front parts of hyaloid membrane of the vitreous body, ciliary processes, lens zonules, cornea and trabeculum. Primarily, they are created by epithelium of ciliary body, iris pigment epithelium and pre-equatorial lens epithelium. It is possible that corneal endothelium, the trabecular cells and vascular endothelium are included in the creation of PEX material. PEX are also found in the orbit, conjunctiva, bulbomotors, vorticous veins, central retinal artery, optic nerve envelopes and skin of eyelids [7, 8].

The movements of the iris and pupillary dynamics do not only lead to the removal of PEX from lens capsule, but also to the pigment dispersion from the pigment layer of iris. In this way, there are some places of iris transparency and pupillary edge atrophy. Pigment granules accumulate in the chamber angle. Chamber angle is intensely and inhomogeneous pigmented and pigmentation of the angle is most pronounced in the Schwalbe's line and in front of it (Sampaolesi line).

Lens capsule is thin and easily and unexpectedly torn during capsulorhexis of cataract surgery. Lens suspensory zonules (Zinni zonules) are weakened, leading to a tendency towards dislocation of the lens during minor trauma, and during the operational lens extraction. Split of the lens capsule, prolapsed vitreous body and lens or intraocular artificial lens dislocation are common complications of cataract surgery in subjects with the syndrome [4, 9]. Changes on the corneal endothelium predispose the development of endothelial decompensation during cataract surgery and corneal edema during the postoperative period.

Aimed at preventing some operational complications, in longer use are several instruments and surgical modifications in cataract extraction, which increase comfort to an ophthalmologist during surgery and, to some extent, affect the incidence of some early and late postoperative complications [10].

Weak suspensory zonules allow movement forward of the entire lens, so in some persons with PEX syndrome the anterior chamber looks shallow and chamber angle narrowed. In advanced stages of the syndrome, phacodonesis is visible (lens shakes while moving eyes).

Cataract is frequently associated with the PEX syndrome [4, 9–11]. It is a mixed, cortico-nuclear type, with large, pigmented nucleus (brunescent). The findings that PEX syndrome is a risk factor for age-related macular degeneration are not consistent [4, 12, 13].

Pseudoexfoliation glaucoma

Today, it is believed that PEX syndrome is the most common cause of open angle glaucoma. It is expected that up to 50% of people with PEX syndrome have elevated intraocular pressure. In people with PEX syndrome, intraocular pressure is significantly higher, even when it was within normal limits [9, 14, 15]. The risk of conversion of intraocular hypertension to glaucoma is twice as high in patients with PEX syndrome. In 5 years, 7%–30% of persons with PEX syndrome will develop glaucoma [16]. Although PEX syndrome itself does not produce optic nerve damage, people with diagnosed PEX syndrome should be inspected once a year [3, 4, 17]. Sometimes, glaucoma may firstly develop in an eye without clinically manifest PEXs [16].

PEX glaucoma is secondary open-angle glaucoma. Earlier, it was known as capsular glaucoma (glaucoma capsulare). Glaucoma can manifest as closed/narrow-angle glaucoma. Lens weakened zonules and instability of lens allow moving forward of iridolental membrane, and thus the emergence of a narrow chamber angle and shallow anterior chamber. In addition, the pupilar bloc development is helped by the presence of iridolental synechies and iris rigidity within the syndrome [3, 4].

Glaucoma is a secondary event. Blockage of the trabecular spaces by PEX material promotes accumulation of pigment and cellular debris, which causes obstruction of the aqueous channels and limits access to the Schlemm canal. Accumulation of PEX material in the juxtacanicular tissue adjacent to the Schlemm canal leads to narrowing of the canal lumen, collapse of its walls, disruption of its endothelium, and partial obliteration. These changes appear to be the causative factors for chronic intraocular pressure elevation and PEX glaucoma.

PEX glaucoma has an aggressive course and an unfavourable prognosis. In its course there are high values and large day-night variations of pressure, rapid deterioration of nerve fibers of the retina and optic neuropathy, rapid progression and development of blindness. Eyes with primary open-angle glaucoma (POAG) were found to have axon loss associated with more connective tissue in the septa and surrounding the central retinal vessels and a decrease in the density of capillaries as compared with eyes with PEX glaucoma where the capillary density did not change with axon loss [18]. Optical coherence tomography (OCT) and Heildeber retina tomography (HRT) have been used to help in the diagnosis and follow-up of patients with glaucoma. Both OCT and HRT have shown a high correlation between the retinal nerve fiber layer thickness and the visual field mean defect during achromatic perimetry.

PEX glaucoma responds poorly to medical management of POAG, glaucoma simplex. Glaucoma is often unilateral, compared to POAG, and if the syndrome is manifested bilaterally, the eye with glaucoma has a more intense pigmentation of the chamber angle [19]. Angle pigmentation is positively correlated with the amount of IOP, but not with severity of glaucoma. Pressure rise in PEX glaucoma happens due to the increased resistance of aque-

ous humor outflow in the trabeculum and reduced uveoscleral flow [20].

Medical therapy does not lead to a long-term compensation of IOP. Initially, combinations of drugs are often used in therapy, because of the weak response to monotherapy [21]. Myotics have worsening potential for the pupillary block development. Argon laser trabeculoplasty shows a great initial success, but the percentage of decompensation of pressure is up to 50%, three years after the intervention [22]. Selective laser trabeculoplasty shows a similar success, but it can be repeated several times, because it does not cause thermal damage [23]. Trabeculectomy and other filtration penetrant and nonpenetrant surgical procedure should be usually performed in earlier stages of the disease than in POAG. Postoperative complications in the form of inflammation, hypotony, and cataract are more common than in POAG.

Differential diagnosis include POAG and pigmentary glaucoma.

Epidemiology of PEX syndrome and PEX glaucoma

PEXs syndrome prevalence increases with age, but shows considerable variations, depending on geography and ethnicity. Judging from early reports, it was considered that PEX syndrome and PEX glaucoma dominate in Scandinavian countries [24]. Later, analyzing the results of population studies, it was realized that these entities existed throughout the world. This syndrome is rarely manifested before 50, and it is most commonly recorded with patients between 69 and 75 years. In Scandinavian countries PEX syndrome was found in 23% of people in their seventh decade [14].

In the same study, after a 20-year follow-up period, Swedish authors [14] reported that the elevated IOP was 6 times more common in people with PEX syndrome. They found identical incidence of glaucoma in both sexes and bilateral manifestation of PEX syndrome that was initially recorded in one eye in 55% of persons observed for 2 decades. During the follow-up, 25% of those patients developed glaucoma before they were 87 years of age, and in almost 60% of cases they had characteristics of PEX glaucoma. In the same study, the prevalence of PEX syndrome in people who were 87 was even 61%.

On Iceland, PEX syndrome and PEX glaucoma has recorded annual growth of 10% in people older than 50 [25]. The syndrome has not been found in the Inuits living in the polar regions of Canada, but has a high prevalence in the Samis, who live on the same latitude in Europe [26]. The prevalence is 4.7% in England, 6.3% in Norway, 4.4% in Germany and 1.1% in Greece. Similar prevalence exists in India, Pakistan (hospital studies) and in some African tribes [3]. In China, there are only 0.4% of respondents aged between 60 and 91, hospitalized for other diseases [27]. In USA, 6–12% cases of open angle glaucoma seem to be PEX glaucoma and the prevalence of PEX syndrome ranges from 0.4% to 2.7% in different regions and racial groups, with white patients predominance [28, 29].

In some areas, PEX syndrome and PEX glaucoma are manifested in several generations of the same family and suggest a genetic predisposition [12, 28, 30]. However, in different populations examined, the findings suggest mitochondrial, X linked and autosomal form of inheritance [31].

Earlier papers have reported significantly more common occurrence of PEX syndrome in women [26]. Howerever, it has not been confirmed by authors [12, 13] from Iceland and Greece.

Studies on PEX syndrome have been conducted on very diverse population and also dealt with variability in the glaucoma definition, especially in early publications. These make it often difficult to compare different series, even when age-specific rates are available [31].

Extraocular localization of pseudoexfoliations and syndrome comorbidity

PEXs have been found in the skin, lungs, liver, heart, kidney, gallbladder and meninges, mainly in areas containing connective tissue [32, 33]. They are also in blood vessels in the body, but the results documenting their frequent association with cardiovascular diseases such as angina, hypertension and stroke [33–36] or abdominal aortic aneurysm [37], are contradicted by the results of researchers who have found similar frequency in the clinical syndrome and control group [38–41]. A greater number of studies with the same design, clinical and control subjects and methods of analysis are required for more consistent conclusion. Cardiac arrhythmia is the most common finding in patients with this syndrome [41]. Systemic vascular endothelial dysfunction has been found in persons with PEX syndrome and PEX glaucoma [42].

PEX syndrome is often associated with Alzheimer's disease [43, 45]. Due to similarities in the pathogenesis, some authors call pseudoexfoliation glaucoma “eye Alzheimer's disease”. However, the syndrome does not result in an increased rate of mortality from cerebrovascular disease [40, 41]. Quality testing of cerebral blood flow in patients with PEX glaucoma shows a reduced speed and increased resistance [46], and magnetic resonance imaging test is periventricular zone of damage, regardless of optic neuropathy in patients who have not only PEX syndrome, but also PEX glaucoma [47, 48].

Hearing disorders have also been detected in patients with this syndrome, especially in hearing the frequency range of human voice [49].

PEX syndrome is rare in patients with diabetic retinopathy [50] yet initiated a series of new tests. However, better selection of groups of respondents, particularly in relation to age, has showed that diabetes is not rare in patients with this syndrome [41, 42] or even that it is more common [50].

Origin and composition of pseudoexfoliation material

Today, the accepted concept suggests that PEX syndrome is a pathological process of the extracellular matrix, which is characterized by the excessive production of abnormal extracellular material aggregating and accumulating, but not decomposing in the organism. Based on known

characteristics of PEX material, PEX syndrome is one of the systemic elastosis that primarily affects elastic microfibriles [51].

PEX material belongs to glycoprotein or proteoglycan. It consists of a protein core surrounded by a mass of conjugated complex sugars. It contains glycosaminoglycans (heparan sulfate, chondroitin sulfate, dermatan sulfate and hyaluron acid), as well as many components of noncolagen ingredients basement membrane and elastic microfibrile (elastin, vitronectin, amyloid P, laminin, nidogen, fibrillin 1, latent TGF binding protein 1 and 2, microfibrille assosciated glycoprotein [21]. It is not known which are integral parts of molecules and which are adhered. Human natural killer 1 carbohydrate component is considered to be responsible for the adhesive nature of PEX. The components are connected among themselves by enzymes and they stabilize the complex. Proteolytic imbalance observed in syndrome may be caused by a disturbed relationship between matrix metalloproteinases and their tissue inhibitors [31]. Because of similarities in the painting composition, PEX material is often described as amyloid [4, 31, 32].

The origin of syndromes is associated with different risk factors. In addition to these age and genetic predisposition, other factors with potential cumulative but mutually dependent effects are also mentioned: ultraviolet radiation, oxidative stress, chronic inflammation in the autoimmune process, infections caused by herpes viruses, hepatitis C virus and *Helicobacter pylori* bacteria, as well as diet habits [4, 25, 30, 52–57].

Hyperhomocysteinemia has been found in persons with PEX syndrome and PEX glaucoma [29, 51, 58]. Genetic polymorphism lysil oxidase like 1 gene and its homozygous presence represent the risk for the disorder of homocysteine metabolism, abnormal hepatic fibrosis and aggregation of elastic components of PEX, especially in a disturbed folate status in the body. Similar disturbances in the plasma levels of homocysteine, folic acid and vitamins B6 and B12 are evident with Alzheimer's disease and certain cardiovascular diseases. However, hyperhomocysteinemia exsists in patients with POAG and normotensive glaucoma.

Elevated serum levels of connective tissue growth factor in a number of diseases accompanied by fibrosis has been detected in patients with PEX syndrome [57]. Again, matrix metalloproteinases are involved in its regulation. Trasforming growth factor β1, as a regulator of most genes that are expressed in PEX syndrome, is increased in aqueous humor of patients with this syndrome and is considered to be one of the key mediators in the fibrotic process syndrome [59].

The system of production and accumulation of PEX makes it complex and interdependent set of elements of such a specific, stress-induced elastosis [31].

Conclusion

Considering population aging worldwide, including our country, we can expect an increasing prevalence of PEX syndrome and PEX glaucoma. Although there is no clear pathogenesis, since the first PEX glaucoma is prevalent among other types of secondary open angle glaucoma, it should be studied during the regular medical studies.

Diseases mentioned in comorbidity of syndrome, which include the risk of mortality and disability with a significant reduction in quality of life, can be suspected earlier if the ophthalmologic overview of the anterior eye segment is done. The possibility of preventing such diseases or reducing their progression is the chance that should be taken. Further studies PEX syndrome origin and nature may help in shedding light on its etiology and pathogenesis, which may open new perspectives in its prevention.

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CURRENT TOPIC

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News in the pathophysiology of asthma

Novine u patofiziologiji astme

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Ključne reči:
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Introduction

Asthma is a complex disorder that displays heterogeneity and variability in its clinical expression both acutely and chronically [1, 2]. This heterogeneity is influenced by multiple factors including age, sex, socioeconomic status, race and/or ethnicity, and gene by environment interactions [3, 4]. Understanding the immunopathology of airways in asthma has been markedly advanced with the use of bronchoscopy and biopsy. Airway samples can then be analyzed by using histologic and immunologic methods, and identified features can be evaluated in relationship to clinical features of asthma to more fully understand the contribution of cellular and molecular events to the resulting physiology and response to treatment [5, 6]. It is helpful to arbitrarily consider asthma in terms of the traditional T-helper2 (Th2) inflammatory processes.

In the acute inflammatory aspects of asthma, allergen-IgE–directed processes are predominant features of airway pathology. Mast cells, Th2 lymphocytes and eosinophils are the predominant histologic features. The cytokine network associated with these processes includes IL-3, IL- 4, IL-5, IL-9 and IL-13 [7, 8].

Mast cells are important contributors to the initiation of asthma with release of acute-phase mediators, including cysteinyl leukotrienes, and also inflammatory cytokines, which serve to perpetuate inflammatory events in the airway [8]. Subpopulations of lymphocytes polarized toward a Th2 profile further sustains the inflammatory process by the release of cytokines, including IL- 4, IL-5 and IL-13. These factors serve to drive inflammation (e.g., recruitment of eosinophils) and also regulate IgE production [4].

Eosinophils are a characteristic feature of allergic inflammation [3]. Eosinophils that are recruited to the airway in asthmatic subjects by the families of cytokines and chemokines [e.g., IL-5, Regulated on Activation, Normal T Cell Expressed and Secreted (RANTES) and eotaxin] undergo cell activation through processes not fully identified and release highly inflammatory mediators.

Recent years have been marked by rapid progress in understanding cellular and chemical mechanisms in the pathogenesis of asthma and other allergic disorders. Studies published in the Journal of Allergy and Clinical Immunology [9, 10] described advances in our knowledge of signaling molecules and pathways, cytokines and activation and tolerance in asthma and murine models of this disease. Additional studies provided novel information about the induction and regulation of allergic inflammation and the genetic determinants of asthma and responsiveness to asthma therapy.

The news in asthma genetics

Recent articles explored novel variants in candidate genes potentially involved in asthma and in the regulation of glucocorticoid responsiveness [9, 10]. Several studies have suggested that chromosome 19q13.1–3 contains asthma susceptibility genes. The microsatellite analyses provided tentative support for an asthma/lung function susceptibility locus, and fine mapping localized modest association to the plasma urokinase plasminogen activator receptor gene (PLAUR, also known as urokinase receptor or CD87). PLAUR SNPs in the 5’ region, intron 3, and the 3’ region were found to be associated with asthma and bronchial hyperresponsiveness. The same 5’ region and 3’ region SNPs were found to be determinants of forced expitatory volume in 1 second (FEV1) decrease in subjects with asthma. This is the first report to identify PLAUR as a potential asthma susceptibility gene. The association of PLAUR with lung function decrease supports hypothesis for PLAUR role in airway remodeling.

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Mediators production by epithelial cells repairing and the epithelial mesenchymal trophic unit activation

The bronchial epithelium is a barrier to the external environment and plays a vital role in protection of the internal milieu of the lung. It functions within the epithelial-mesenchymal trophic unit (EMTU) to control the local microenvironment and help maintain tissue homeostasis. However, in asthma, chronic perturbation of these homeostatic mechanisms leads to alterations in the structure of the airways, termed remodeling. Damage to the epithelium is now recognized to play a key role in driving airway remodeling. Several important mediators of remodeling have been identified, most notably transforming growth factor-β, which is released from damaged/repairing epithelium or in response to inflammatory mediators, such as IL-13. In summary, the cross talk between the epithelium and the underlying mesenchyme appears to be central in driving remodeling responses in asthma. The expression of the asthma susceptibility gene ADAM33 in the EMTU and its involvement with airway remodeling helps place these processes at the center of asthma pathogenesis.

In asthma there is an evidence that epithelial injury and repair are abnormal. Several studies have reported increased susceptibility to injury [11, 12] and abnormal repair responses, including increased expression of the epithelium growth factor receptor (EGFR) in bronchial biopsies from adults and children with asthma, as well as expression of the cyclin-dependent kinase inhibitor p21waf1 [13, 14]. More recent studies using differentiated epithelial cultures have confirmed that damage causes release of TGF- β and have shown that coculture of epithelial cells and fibroblasts results in sustained TGF-β release [15]. In this coculture model, there was also marked synthesis of interstitial collagen, which was deposited in close proximity in basal surface of the epithelium, closely mirroring the thickening of the lamina reticularis seen in asthmatic bronchial biopsies.

Although the epithelium was initially considered to function solely as a physical barrier, it is now evident that it plays a central role in the Th2-cell sensitization process due to its ability to activate mucosal dendritic cells [16]. Cytokines are inevitable factors in driving immune responses. To the list of numerous cytokines already known to be involved in the regulation of allergic reactions, new cytokines were added, such as thymic stromal lymphopoietin (TSLP), IL-25 and IL-33. IgE is also a central player in the allergic response. The activity of IgE is associated with a network of proteins, especially with its high- and low-affinity receptors for immunoglobulins (Fc receptors).

Mucosal dendritic cells (DCs) are extremely efficient sentinels in the defense against antigen challenge. They are strategically positioned within the epithelium in the basolateral space, separated from the inhaled air only by the epithelium tight junction barrier. Despite the fact that most inhaled antigens are transported to the lymph nodes by DCs, the usual outcome following the inhalation of harmless protein antigens is the induction of tolerance. This is because they cannot fully activate DCs to induce an effective T-cell response [17, 18]. It follows that DCs have to be somehow activated to break tolerance. Conventional DCs express numerous pattern-recognition receptors, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain and C-type lectin receptors [19–21].

As most inhaled allergens, such as those derived from cockroaches and house-dust mites, are contaminated with lipopolysaccharides (LPSs) and peptidoglycans, they can activate DCs. In fact, it was shown that the main house dust mite allergen, Der p 2, acted as a functional homologue of myeloid differentiation factor 2 (MyD2) that drove airway inflammation in a TLR4-dependent manner [22].

MyD2 physically associates with the extracellular domain of human TLR4 and binds the lipid A region of LPS without the need for LPS-binding protein. Given that many allergens, including Der p 2, are members of the MyD2-like lipid-binding protein family and that more than 50% of major allergens are lipid-binding proteins, such mimicry could also explain the immunogenicity of these allergens.

In the absence of contaminating TLR ligands, some allergens can activate DCs by triggering protease-activated receptors (PARs). The ligation of TLRs and PARs leads to a cascade of events that culminates in the production of chemokines that attract neutrophils, monocytes, and DCs to the airways and to the production of cytokines that can induce DC maturation and Th2 polarization [23]. Thymic stromal lymphopoietin (TSLP), granulocytemonocyte colony-stimulating factor (GM-CSF), and interleukin (IL)-25 are among the most important mediators.

Mast cells have a key role in asthma. They are concentrated in the mucosal tissues and are recruited to the surface of the airways by stem-cell factor released from epithelial cells. In addition, CXCL8 and CXCL10, (chemokine ligands) produced by airway smooth muscle cells, are important in the recruitment of mast cells by interacting with their receptors, CXCR2 and CXCR3, respectively. Moreover, these chemokines also prime mast cells for enhanced mediator secretion. Reversely, mast cells secrete CCL19 which, through its CCR7, stimulates airway smooth muscle cell migration and contributes to smooth muscle hyperplasia [24, 25].

The cross-linking of IgE-FcεRI complexes on mast cell surfaces by allergens leads, within minutes, to the so-called "early phase" of the allergic reaction, which involves their degranulation and release of histamine, tryptase and other proteases, heparin and some cytokines, which are preformed and stored in granules, as well as newly formed eicosanoids (LTC4, LTD4, LTE4, PG2, and TXA2). These mediators are potent smooth muscle contractile agents and also increase microvascular permeability. Both PGD2 and LTD4 interact with cell-surface receptors on eosinophils, macrophages, basophils, and mast cells, where they serve as chemoattractant as well as priming agents. Cytokines and chemokines liberated in this early phase initiate the "late phase", which peaks some hours later. The inflammation that occurs in asthma is often described as eosinophilic [26].

Eosinophils are a rich source of granule basic proteins, such as major basic protein, eosinophil peroxidase, and eosinophil cationic protein, and also have the capacity to generate eicosanoids such as prostacyclin (PGI2) and leukotrienes. They also release potentially tissue damaging superoxide and a range of cytokines and chemokines. Eosinophil-derived neurotoxin is released by eosinophils as well. It was recently shown that it had the capacity to activate Th2-polarizing DCs by triggering the TLR2-MyD88 signaling pathway and to enhance the Th2-based immune response [27, 28].

Cytokines network

Cytokines play a key role in orchestrating the chronic inflammation of asthma and chronic obstructive pulmonary disease (COPD) by recruiting, activating, and promoting the survival of multiple inflammatory cells in the respiratory tract. Over 50 cytokines have now been identified in asthma and COPD, but their role in the pathophysiology of these complex airway diseases is often unclear. For the purpose of this review, cytokines are classified into lymphokines (cytokines that are secreted by T- cells and regulate immune responses), proinflammatory cytokines (cytokines that amplify and perpetuate the inflammatory process), growth factors (cytokines that promote cell survival and result in structural changes in the airways), chemokines (cytokines that are chemotactic for inflammatory cells) and antiinflammatory cytokines (cytokines that negatively modulate the inflammatory response), although many of these functions may overlap [29].

T-helper 2 (Th2) cytokines. In patients with asthma, there is an increase in the number of CD4+ Th cells in the airways, which are predominantly of the Th2 subtype. Th2 cells are characterized by secretion of IL-4, IL-5, IL-9, and IL-13. The transcription factor GATA-binding protein 3 (GATA3) is crucial for the differentiation of uncommitted naive T-cells into Th2 cells and regulates the secretion of Th2 cytokines [30, 31]. There is an increase in the number of GATA3+ T cells in the airways of stable asthmatic subjects. Following ligation of the TCR and CD28 coreceptor by antigen presenting cells (APCs), GATA3 is phosphorylated and activated by p38 MAPK, resulting in translocation from the cytoplasm to the nucleus, where it activates transcription of genes characteristic of Th2 cells [32]. Nuclear factor of activated T cells (NFAT) is a T cell–specific transcription factor and enhances the transcriptional activation of the Il4 promoter by GATA3. Finally, IL-33, a member of the IL-1 family of cytokines, promotes differentiation of Th2 cells by translocating to the nucleus and regulating transcription through an effect on chromatin structure [33], but it also acts as a selective chemoattractant of Th2 cells [34].

T-helper 1 (Th1) and Tc1 cytokines. The transcription factor T-bet is crucial for Th1 cell differentiation and secretion of the Th1-type cytokine IFN-gama. Consistent with the prominent role of Th2 cells in asthma, T-bet expression is reduced in T cells from the airways of asthmatic patients compared with airway T cells from nonasthmatic patients. After phosphorylation, T-bet associates with and inhibits the function of GATA3 by preventing it from binding to its DNA target sequences [35, 36]. In turn, GATA3 inhibits the production of Th1-type cytokines by inhibiting STAT4, the main transcription factor activated by the T-bet inducing cytokine IL-12 [37]. Th1 cells are the prominent CD4 + T cells, and Tc1 cells the predominant CD8 + T cells expressed in COPD lungs [37], but their role in the pathogenesis of COPD is not yet certain.

Interleukin-12 (IL-12) and related cytokines. They play an important role in differentiating and activating Th1 cells and is produced by activated macrophages, DCs, and airway epithelial cells. IL-12 induces T cells to release IFN-γ, which regulates the expression of IL-12Rβ2 and so maintains the differentiation of Th1 cells, whereas IL-4 suppresses IL-12R β 2 expression and thus antagonizes Th1 cell differentiation.

T-helper 17 (Th17) cytokines. Th17 cells are a subset of CD4 + T cells that play an important role in inflammatory diseases and are regulated by the transcription factor retinoic acid orphan receptor- γ t (ROR γ t). IL-6, IL-1 β, TGF-β, and IL-23 are all involved in the differentiation of human Th17 cells [38, 39]. Little is known about the role of Th17 cells in either asthma or COPD, but levels of IL-17A (the predominant product of Th17 cells) are increased in the sputum of individuals with asthma and Th17 cells are increased in the airways of asthmatic subjects. More work is needed to understand the role and regulation of Th17 cells in asthma and COPD, as they may provide important new targets for future therapy.

The role of proinflammatory cytokines in asthma and chronic obstructive pulmonary disease

Proinflammatory cytokines, such as TNF-α, IL-1β, and IL- 6, are found in increased amounts in the sputum and bronchoalveolar lavage (BAL) fluid in individuals with asthma and COPD and amplify inflammation, in part through the activation of NF- κB, which leads to the increased expression of multiple inflammatory genes.

Many cells have the capacity to secrete TNF-α, including macrophages, mast cells, T- cells, epithelial cells, and airway smooth muscle cells. TNF-α is expressed in various cells in asthmatic airways, particularly mast cells, and may play a key role in amplifying asthmatic inflammation through the activation of NF- κB .

IL-6 often works in concert with other cytokines and provides a link between innate and acquired immunity. IL-6 is found in increased amounts in induced sputum of asthmatic patients after mast cell activation. It may play a role in the expansion of Th2 and Th17 cells and therefore have a proinflammatory effect in asthma.

Thymic stromal lymphopoietin (TSLP) is a cytokine belonging to the IL-7 family that shows a marked increase in expression in airway epithelium and mast cells of asthmatic patients [40, 41].

Growth factors

Several cytokines implicated in airway inflammation either promote the differentiation and survival of inflamma-

tory cells or result in proliferation and/or activation of structural cells, contributing to airway remodeling granulocyte-monocyte colony-stimulating factor (GM-CSF). GM-CSF plays a role in the differentiation and survival of neutrophils, eosinophils, and macrophages and has been implicated in asthma and COPD.

It is the ligand of the c-Kit tyrosine kinase receptor SCF, which is expressed by several structural and inflammatory cells in the airways. SCF is produced by epithelial cells, airway smooth muscle cells, endothelial cells, fibroblasts, mast cells, and eosinophils. It is a critical growth factor for mast cells and promotes their generation from CD34+ progenitors [38, 41].

Neurotrophins. They are cytokines that play an important role in the function, proliferation, and survival of autonomic nerves. In sensory nerves, neurotrophins increase responsiveness and expression of tachykinins. Nerve growth factor (NGF) may be produced by mast cells, lymphocytes, macrophages, and eosinophils as well as structural cells, such as epithelial cells, fibroblasts, and airway smooth muscle cells.

Chemokines play an important role in the recruitment of inflammatory cells from the circulation to the airways in both asthma and COPD [38–41].

Anti-inflammatory cytokines

Although most cytokines increase or orchestrate the inflammation process in asthma and COPD, some cytokines have inhibitory or anti-inflammatory effects. As discussed above, IL-12, through the release of IFN-γ. from Th1 cells, can suppress Th2 cytokine release and allergic inflammation.

Conclusion

Bronchial asthma was once considered a purely allergic disorder dominated by Th2 cells, IgE, mast cells, eosinophils, macrophages and cytokines. However, it is now clear that the disease also involves local epithelial, mesenchymal and vascular events that are involved in directing allergic reactions to the lung which eventually result in remodeling of the bronchial wall. Better understanding of genetics, environmental factors, and immunopathogenesis of asthma can lead to improved therapeutic aproaches.

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Treatment options for childhood medulloblastoma

Izbor lečenja meduloblastoma kod dece

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Key words:
medulloblastoma; radiotherapy; drug therapy; neurosurgical procedures; child, preschool; treatment outcome.

Ključne reči:
meduloblastom; radioterapija; lečenje lekovima; neurohirurške procedure; deca, predškolska; lečenje, ishod.

Introduction

Medulloblastomas, infratentorial or cerebellar, primitive neuroectodermal tumors (PNETs) account for 20% of all childhood brain tumors and 40% of all cerebellar tumors [1, 2]. Peak occurrence is at 4 years of age. Approximately 10% to 15% are diagnosed in infancy and require specific treatment approach [3]. Treatment protocols are based on risk stratification, which takes into account age at presentation, residual disease [residual tumor at the primary site after surgery measured by postoperative gadolinium – enhanced magnetic resonance imaging (MRI)] as well as evidence of disseminated disease at the time of diagnosis. Patients older than 3 years of age with minimal residual disease (if postoperative MRI showed residual disease of 1.5 cm^2 or less) are classified as an average risk group. Patients are defined as high-risk group if they had metastatic disease at the time of diagnosis (confirmed by gadolinium-enhanced MRI of the head and spine and if lumbar cerebrospinal fluid assessed after resection contained tumor cells); if they had residual disease of more than 1.5 cm^2 (measured by postoperative gadolinium – enhanced MRI); if they are younger than 3 years of age at the time of diagnosis.

Over the past decades there has been progressive improvement in the results of treatment of this group of patients with overall survival rates higher than 70% [4].

There are several reasons for this which include advances in neuro-radiological imaging leading to more accurate localization, improvements in neuro-surgical techniques, better perioperative care, improvement in radiotherapy equipment and techniques including greater and more precise dosage delivered to the tumor and refinements in the timing and dosing of chemotherapy [5].

Treatment options

Surgery

Surgical resection remains the mainstay of therapy with the goal of gross total resection (GTR). All patients who present with a posterior fossa tumor will undergo an open craniotomy. Studies have shown that patients with less then 1.5 cm^2 residual disease had improved survival [6–8]. Some patients might require a ventricular shunt or third ventriculostomy prior to resection of the tumor. The majority of patients will have resolution of the hydrocephalus after tumor resection, but approximately 40% will require permanent shunt placement. Prognostic factors for permanent shunting are young age, significant pre-surgical hydrocephalus and large tumors [9].

For most patients, treatment started within 28 days of surgery, the extent of which was defined as: gross total resection if followed by no evidence of residual disease; near-total resection if postoperative MRI showed residual disease of 1.5 cm^2 or less; and subtotal resection if 25% or more of the tumor remained. The extent of resection is defined by using the neurosurgeons operative notes and by postoperative MRI.

Postsurgical complication characteristically developing after posterior fossa tumor resection is the cerebellar mutism syndrome (CMS) also referred to as the posterior fossa syndrome. This entity typically starts within 1 to 2 days after surgery, persists for weeks to months and consists of paucity of speech leading to mutism, hypotonia, ataxia and emotional instability. In addition, brainstem dysfunction can be seen, including dysphagia, facial weakness and abducens paralysis. In a large study of 450 children, CMS developed after surgery in 107 (24%). Only brainsteam involvement was predictive for the development of CMS [10]. Another series analyzed 253 children in which CMS developed in 20 children.

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All of these cases had brainsteam involvement [11]. Evidence of hydrocephalus also appears to exacerbate the development of CMS [12]. Individual case studies report on successful use of dopamine agonists, such as bromocriptine for the treatment of CMS but unfortunately children are often left with dysarthic speech [13, 14]. Therefore, careful resection is recommended, especially in children with brainstem involvement.

Radiation therapy

Radiation therapy was the first adjuvant treatment for brain tumors and was initially applied to the treatment of adult gliomas and pituitary tumors in the early 1900s. It remains very effective therapy for many malignant pediatric brain tumors, contributing substantially to duration of survival and the chance of cure.

Medulloblastomas are very radiosensitive tumors and adjuvant therapy with radiation has been the standard of care in children older than 3 years of age [15]. The reported, long-term side effects of radiation therapy, such as hearing loss, cognitive decline, endocrine abnormalities, vascular complications, as well as secondary malignancies have inspired many investigators over the years to try to reduce the radiation dose as well as the radiation field [16–19]. The Pediatric Oncology Group (POG) and Children's Cancer Group (CCG) now known as the Children's Oncology Group (COG) compared in a prospective trial (POG 631/COG 923) reduced neuroaxis radiation of 23.6 Gy to the standard regimen of 36 Gy with equal posterior fossa radiation (54 Gy) for children with average risk medulloblastoma. The interim analysis indicated an increased risk of early relapse with reduced radiation [20]. Since then, many studies have focused on the introduction of chemotherapy to reduce radiation dose but maintain adequate survival.

The introduction of conformal radiotherapy enabled radiation oncologists to reduce the radiation field. In previous treatment strategies after craniospinal irradiation boost radiotherapy was delivered to the complete posterior fossa [21, 22]. Currently, most investigators used a boost dose to the tumor bad, instead of irradiating the entire posterior fossa using conformal radiation therapy with 5-year overall survival rates of 84% for an average risk-group of patients [23].

Proton beam therapy is another alternative to conventional radiation therapy. The benefit of using proton beams is the higher proportion of tumor versus normal tissue distribution. Proton beam therapy is not currently used in Serbia.

Radiosurgery can successfully be used for local tumor control in patients with recurrent or residual disease [24]. However, stereotactic radiation as primary treatment modality is limited given the propensity of medulloblastomas for dissemination and treatment failure can occur due to subclinical craniospinal metastas.

The current standard for average risk medulloblastoma includes postoperative craniospinal irradiation of 23.4 Gy, plus a boost to the posterior fossa of 54 Gy followed by 12 months of chemotherapy. This regimen has resulted in a 5-year overall survival rates of 80% or better [25]. In high-risk disease, 36 Gy craniospinal irradiation, plus a boost at the posterior fossa of 54 Gy, followed by chemotherapy is standard. Ongoing trials are investigating the benefit of chemotherapy during irradiation.

Chemotherapy

There has been a progressive improvement in the results of treatment of children with medulloblastoma with overall survival rates of 70% or better [4, 26].

Reducing the radiation dose without adding chemotherapy has led to worse outcomes in children with medulloblastoma. Many studies have investigated the role of chemotherapy in addition to radiotherapy with the goal to reduce the amount of radiation. Different chemotherapeutic agents has been used and are now standard in the management of children with medulloblastoma in all risk groups. Alkylators and platinum compounds such as lomustine, cyclophosphamide and cisplatin remain key therapeutic agents. Vincristin is often administrated weekly during radiotherapy and as adjuvant chemotherapy. Children with average risk disease, who were treated with craniospinal radiotherapy of 23.4 Gy and 55.8 Gy to the posterior fossa, and adjuvant chemotherapy (lomustine, vincristine and cisplatin) showed a progression-free survival (PFS) of 86% at 3 year and 79% at 5 years [27]. The European Hirntumor (HIT) 91 trial compared outcome in patients with average risk medulloblastoma receiving either neoadjuvant chemotherapy (prior to radiation therapy) or postradiation chemotherapy. The 5-year PFS in the postiradiation chemotherapy arm was 78% and in the neoadjuvant chemotherapy arm was 65% [28].

These and some other studies confirm the benefit of adjuvant chemotherapy for the treatment of average risk medulloblastoma and regimen reported by Packer [5] and Packer et al. [27] as previously described remains the standard of therapy for average-risk medulloblastoma patients.

For high-risk medulloblastoma patients, the priority remains to improve survival. Average event-free survival (EFS) at 5 years for high-risk medulloblastoma ranges from 34% to 40% across studies [29]. Multiple studies have used different chemotherapy protocols, including neoadjuvant chemotherapy in combination with surgery and radiation to improve survival with moderate success [28]. The use of prolonged neoadjuvant chemotherapy resulted in inferior outcomes compared with those obtained with shorter times between surgery and radiation therapy [28, 29]. The best outcome for high-risk medulloblastoma patients to date was achived by craniospinal irradiation (36 Gy M0-1; 39.6 Gy M2-3) with a boost to the primary tumor site after maximal surgical resection followed by dose-intensive cyclophosphamide, vincristine and cisplatin chemotherapy with autologous peripheral blood stem rescue. The 5-year EFS was 70% [30]. The COG (COG 99701) treated 57 patients with metastatic medulloblastoma with vincristine and carboplatin while receiving radiation therapy (36 Gy for craniospinal irradiation), followed by monthly treatment with cyclophosphamide and vincristine. The 4-year OS and EFS were reported at 81% and 66%, respectively [31].

All these studies indicate that chemotherapy is pivotal for the treatment of high-risk medulloblastoma patients and ongoing studies are investigating the best regimen for these patients.

Treatment for children less than 3 years of age

Small children with medulloblastoma have poorer survival than older children when treated with standard radiotherapy and even more significantly they sustain much greater treatment-related neurotoxicity. Also, it is believed that medulloblastoma in a very young child have a more aggressive behavior and a higher incidence of metastasis at the time of diagnosis, although the data is limited. Evans at al. [32] reported that 34% of children under the age of 4 years presented with disseminated disease compared with only 14% of children aged 4 years or older. Similar results were reported separately with 62% of children less than 5 years of age demonstrating metastatic disease versus 38% in children older than 5 years of age[3].

The impact of age on prognosis is difficult to assess because younger patients normally receive different treatment modalities than older children. In an attempt to delay or obviate radiation therapy, multiple studies have been performed using different chemotherapy regimens.

In the mid 1980s, the POG conducted a trial (referred to as Baby-POG I) enrolling 102 children less than 3 years of age with brain tumors in which prolonged postoperative chemotherapy was given with an attempt to delay radiation therapy. The 5-year PFS of 62 children with medulloblastoma less than 3 years of age was reported at 31.8% and the 5-year OS at 39.7% using a combination of cyclophosphamide, vincristine, cis-platinum and etoposide. Radiation was delayed until 3 years of age. The main predictor for survival was extent of surgical resection. Twenty children undergoing GTR had a 5-year OS of 60% compared with 33 children who had subtotal resection and who had a 5-year OS of 32% [33].

Other studies investigated a similar approach. The CCG used the "8-in-one-day" regimen followed by either radiation after two cycles of chemotherapy versus craniospinal irradiation 1 year after diagnosis and completion of maintenance chemotherapy. Forty-six children with medulloblastoma were less than 18 months old with 3-year PFS of 22%. Thirty percent were alive and disease-free at a mean follow-up of 72 months [34]. The poorer outcome in the "8-in-one-day" regimen is probably best explained by the less intensive chemotherapy regimen in this study compared to the Baby-POG I trial.

Also because of concern for neurotoxicity of radiotherapy in young children, the use of high-dose ablative chemotherapy with autologous bone marrow transplant or stem cell rescue for children with recurrent or newly diagnosed tumor is being explored. In view of the chemo-sensitivity of medulloblastoma there have been preliminary studies in which a small number of newly-diagnosed infants were successfully treated in consolidation with high-dose chemotherapy supported by autologous peripheral stem cell rescue; this approach may have a larger role in treating young children, as it may for patients who relapse after standard therapy [35].

Investigational therapy

Better therapy for medulloblastoma undoubtedly will have its basis in clarification of tumor molecular biology. Improved understanding of the molecular signature of individual tumors will help in determining prognosis and more accurate tumor risk-stratification, permitting children at lower risk for recurrence to safety receive less toxic therapy and reserving more intensive treatment for those at higher-risk. Knowledge of the molecular defects critical in tumorigenesis could also provide the means to use them as targets for novel therapeutic approaches.

A number of studies have identified several possible molecular traits that could serve as prognostic factors, as well as potential targets for therapy of medulloblastoma. Among these are the amplification or overexpression of several oncogenes, including epidermal growth factor receptor B2 (ERBB2), C-Myc, and N-Myc, loss of caspase-8 expression, and mutations in several other signal transduction pathways including the PTCH1/"Sonic Hedgehog" pathway, "Wingless" (WNT/WG)/beta catenin pathway and platelet-derived growth factor-alpha (PDGF-a) and RAS/MAP tyrosine kinase pathway [36–39].

Understanding mechanisms of tumorigenesis for future molecular classification and prognosis is also the first step in the development of molecular-targeted therapies. Specific small molecule tyrosine kinase inhibitors could prove effective against targets in some medulloblastoma (and other brain tumors). These include imatinib mesylate (Gleevec), a PDGFa/RAS/MAP tyrosine kinase inhibitor, Erlotinib (Tarceva), which inhibits the oncogene, ERBB2 tyrosine kinase and Iressa (gefitinib), which inhibits the epidermal growth factor receptors (EGFR) tyrosin kinase [39, 40]. Some of these agents are tested in ongoing clinical trials.

The retinoid, cis-retinoic acid, is another therapeutic agent soon to be evaluated in a randomized fashion in the upcoming COG protocol for high-risk medulloblastoma/PNET tumors. Retinoids mediate apotosis in medulloblastoma cells in vitro, and suppres tumor growth in xenograft models [41].

Conclusion

Childhood medulloblastomas remain a challenging oncologic condition.

The main goal for patients with average-risk disease is to improve morbidity of current treatment regimens and maintain adequate survival. For patients with high-risk and recurrent disease, survival remains poor; therefor, improving outcome is the focus of current investigations. Advances in understanding molecular profile and associated clinical outcome will eventually lead to better risk stratification and enable neuro-oncologist to better determine risk-benefit profiles for each individual patient. Therapy for childhood medulloblastoma requires a delicate balance between the need to intensify therapy for some group of patients and the desire to reduce potentially neurotoxic therapy and risk for other malignancies, so as to have greater number of survivors with cognitive, psychological and endocrinologic abilities allowing them to have a better quality of life.

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CASE REPORTS

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Primary nasal tuberculosis: A case report

Primarna tuberkuloza nosa

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Abstract

Introduction. During the past two decades, tuberculosis (TBC) both pulmonary and extrapulmonary, has emerged to be a major health problem. Nasal tuberculosis is a specific inflammatory process which is, in most cases, joined by the inflammation of neck lymph nodes. **Case report.** Thirty-year-old man presented with shortness of breath through the nose and periodical headaches. Clinical examination showed signs of chronic rhinitis, with slight granular changes of nasal septal mucosa. Laboratory analyses were within the reference ranges. Nasal and throat swabs for bacteria and fungi were normal. Skin allergy testing to standard inhalatory allergens was positive. Computer rhinomanometry showed increased nasal resistance at medium difficulty level, on the right. Radiography of paranasal sinuses indicated chronic polysinusitis on the right. Anti-allergy therapy was prescribed. The patient came for checkup after a month with subjective deterioration and a neck tumefact on the right. Nasal endoscopy revealed the presence of dark red infiltrates with the 3 mm diameter on nasal septal mucosa, dominantly on the right, with small greyish nodules. This findings indicated a potential specific nasal inflammatory process. In the upper jugulodigastric area, on the left, painless tumefact 3 × 5 cm in size was palpated, it was mobile comparing to supra- and infrastructure, with unaltered skin above. The definite diagnosis was established on the basis of the results of nasal mucosa biopsy. After histopathological diagnosis was obtained, we started with antituberculosis therapy at once. **Conclusion.** Due to actual trends of TBC incidences, otolaryngologist should have in mind nasal TBC, when granulomatose lesions are found in nose.

Key words:
tuberculosis; nasal septum; diagnosis, differential; antitubercular agents.

Apstrakt

Uvod. Tokom protekle dve decenije tuberkuloza pluća i vanplućna tuberkuloza javljaju se kao veliki zdravstveni problem. Tuberkuloza nosa predstavlja specifični zapaljenski proces koji u velikoj meri prati zapaljenje limfnih čvorova vrata. **Prikaz bolesnika.** Tridesetogodišnji muškarac javio se otorinolaringologu zbog otežanog disanja na nos i povremenih glavobolja. Kliničkim pregledom našli smo znake hroničnog rinitisa sa malim granuliranim promenama na sluznici septuma. Bris nosa i grla na bakterije i gljivice bio je uredan. Kožne alergijske probe na standardne inhalatorne alergene bile su pozitivne. Rinomanometrijski nalaz ukazao je na povišen nosni otpor srednjeteškog stepena desne nosne šupljine. Radiografija paranazalnih šupljina ukazala je na hronični polisinuzitis, desno. Ordinirana je antialergijska terapija. Bolesnik se javio na kontrolu nakon mesec dana sa subjektivnim pogoršanjem i sa velikim tumefaktom na vratu desno. Klinička ispitivanja pokazala su na sluznici septuma infiltrirate promera 3 mm, tamnocrvene boje, s malim čvorićima sive boje. Posumnjali smo na specifični zapaljenski proces u nosu. U gornjem jugulodigastričnom regionu uočen je tumefakt promera 3 × 5 cm, pokretan u odnosu na supra- i infrastrukturu, nepromenjene kože iznad, palpatorno bezbolan. Definitivna dijagnoza postavljena je na osnovu kliničkog pregleda, radiološkog nalaza i nalaza biopsije. Nakon dobijanja histopatološke dijagnoze odmah smo počeli sa antituberkuloznom terapijom. **Zaključak.** Otolaringolog bi trebalo da ima na umu tuberkulozu nosa uvek kada se u nosu bolesnika nalaze granulomatozne promene. Na ovo ga obavezuju i tendencije učestalosti ove bolesti.

Ključne reči:
tuberkuloza; nos, septum; dijagnoza, diferencijalna; antituberkulotici.

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Introduction

Upper respiratory tract tuberculosis is uncharacteristic and present in 1.8% of all patients suffering from tuberculosis (TBC) [1]. TBC though a rare disease, spread widely at the end of the 20th century, which is explained with the emersion of immunodeficiency diseases and the development of resistant strains of *Mycobacterium tuberculosis* [2]. Since the end of the 20th century there has been a constant and progressive increase of TBC, even in developed countries [3]. Though the frequency of TBC is growing on the global level, primary nasal tuberculosis is still rare [4].

Primary nasal tuberculosis is chronic skin and nasal mucosa inflammation, characterised by granulomas (tuberculoma) which necrotise and turn into scars. The cause is *Mycobacterium tuberculosis*. Typical symptoms are: unilateral secretion, periodical bleeding and nasal congestion. Rhinoscopically, the changes are mostly present on the frontal part of inferior nasal concha or the frontal part of septum, in the form of isolated ulcerous red nodules (tuberculoma). Spreading of disease causes perforation of the frontal part of septum. Granulations in the later phase of disease cause scar stenosis. Some patients who suffer from primary nasal tuberculosis can later develop of TBC lungs or larynx [2].

Diagnosis is not easy to establish since the symptoms and signs of this specific nasal inflammation are quite similar to non-specific nasal inflammation processes [5]. In the sense of differential diagnosis, all other specific nasal diseases as well as malignant processes are possible. A diagnosis is established on the basis of anamnesis, rhinoscopy, nasal endoscopy, biopsy and histopathological verification, as well as additional diagnostic methods (biochemical blood analysis, serology, PPD, isolation of *Mycobacterium tuberculosis* complex, radiological investigation). The definite diagnosis is established by biopsy and histopathological verification of Langerhans cells [5]. The treatment of nasal TBC should follow general guidelines for tuberculosis treatment. The choice of drugs must comply with their availability and local emersion of resistant strains, thus, at least two types of medications are used for the treatment but often three or more types [5].

Case report

A 30-year-old man, presented with shortness of breath through the nose and periodical headaches for about a month. Anamnesis contained no data on nasal bleeding, smell disorder, increased body temperature, coughing, fatigue or weight loss.

Rhinoscopic examination confirmed the picture of chronic rhinitis with hypertrophic mucosa and slight granular changes on nasal septal mucosa. Nasopharynx, oropharynx and larynx did not have visible pathologic changes. Nasal and throat swabs did not isolate pathogenic germs. Allergy testing to standard inhalatory allergens was positive (house mites, house dust and tobacco). Computer rhinomanometry indicated increased nasal resistance of medium difficulty level of the right nasal cavity, whereas the result for the left side was normal. Total nasal resistance was increased in mild level. Rhinomanometric testing after vasoconstriction confirmed mucosal type of nasal obstruction. Radiography of paranasal cavities showed the signs of chronic sinusitis. Due to negative bacteriological result and positive allergy "prick" test, the patient was prescribed intranasal corticosteroids and peroral antihistamines, along with advice to rinse nasal cavities every day using saline solution. The patient came for the checkup after a month, without subjective improvement, with a tumefact in the upper jugulodigastric neck area, on the left.

Endoscopic nasal examination on nasal septal mucosa, dominantly rightwards, in its posterior third, revealed the presence of dark red infiltrates with the diameter of about 3 mm, with tiny white-greyish nodules at the top. In the upper jugulodigastric neck area, a 3 × 5 cm diameter tumefact was noticed; it was mobile comparing it to supra and infrastructures, with unaltered skin above and palpatory painless. Ultrasound neck examination showed that the results for thyroid and both of the submandibular salivary glands were normal; however, on both sides of the neck, more to the left, at the level of submandibular, sublingual and upper cervical area, increased lymph nodes of the changed morphology were identified (some of them had hyperechogenic center and almost all of them had the thickened and hyperechogenic cortex with pronounced hilar vascularisation). Lymph nodes on the right had the diameter of 12–28 mm, and on the left 14–29 mm. Due to our suspicions for a specific inflammatory process, we carried out the biopsy of altered nasal septal mucosa and sent it for histopathologic (HP) examination. The HP result showed that it was tuberculosis (Figures 1 and 2). Basic laboratory analyses were within the reference values, erythrocyte sedimentation was 10/20 mm/h, C reative protein (CRP) – 7 mg/dL. Lung radiography did not show any active pathological changes in lung parenchyma. Computed tomography (CT) of paranasal cavities (coronal and axial intersections) revealed granulomatous lesion in the right nasal cavity at the level of the middle third of the septum (Figure 3).

The definite diagnosis was established by combining clinical picture, clinical examination, nasal endoscopy results, the biopsy of pathologically modified nasal septal mucosa, HP analysis, as well as additional diagnostics (ultrasound of the neck, CT of nasal cavum and paranasal cavities).

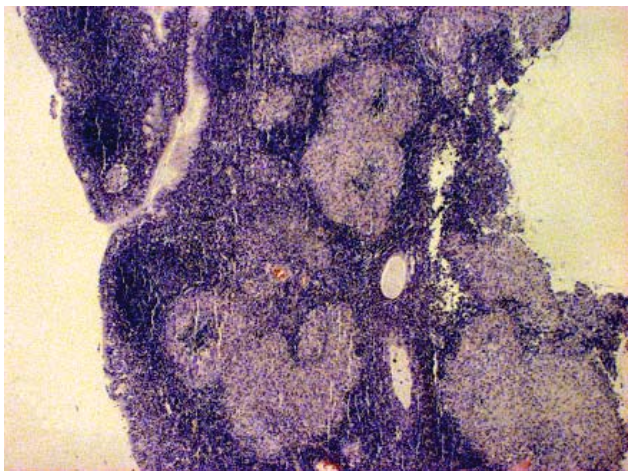

Fig. 1 – Endonasal mucosa with numerous central granulomas which show caseous necrosis (HE, × 100).

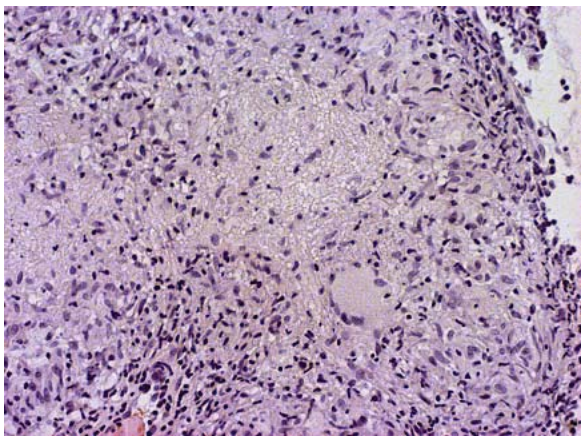

Fig. 2 – Tuberculous granuloma with Langhans' multinucleate giant cells (HE, × 100).

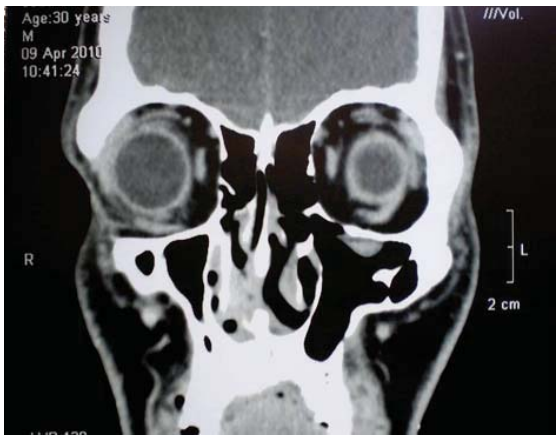

Fig. 3 – Computed tomography of paranasal sinuses (coronal cut): granulomatose lesion of the right nasal cavity at the level of the middle third of septum.

As a therapy, antituberculosis drugs were prescribed. At first checkup, after a month, the patient felt subjectively better, the lump on the neck was in regression, and so were the changes on septal mucosa.

Discussion

Primary nasal TBC is quite a rare disease. Nasal TBC is usually secondary in nature when the process spreads from lungs or larynx. Nasal tuberculosis signs and symptoms can imitate the signs of chronic rhinitis. The most common symptoms are: shortness of breath through the nose, nasal secretion and recidivant epistaxis. Purohit and Gupta [6] state that nasal tuberculosis is a specific inflammatory process usually followed by tuberculous inflammations of neck lymph nodes (scrofula), as seen in the presented patient.

The first suspicion for a specific inflammatory process appeared at the patient's checkup. Until then, the patient was treated for allergic rhinitis. However, when endoscopic examination of his nose revealed infiltrates on nasal septal mucosa and inferior nasal conchae, as well as a tumefact on the left side of his neck, we suspected the specific inflammatory process.

Kim et al. [7] demonstrated eight cases of nasal tuberculosis and six of these patients had primary nasal tuberculosis. The average age of patients was about 30 years, and the localization of tuberculous infiltrates was, as it was in our case, the nasal septum [7]. Varshney et al. [8] demonstrated three cases of nasal TBC and all three patients were male, the average age of 35 years old. Sithinamsuwan et al. [9] demonstrated the case of nasopharynx TBC with the presence of diplopia, and the definite diagnosis was established by biopsy and histopathological verification of the tissue modified by tumor on the roof of epipharynx [9]. Johnson et al. [10] demonstrated the case of nasopharyngeal tuberculosis in a 22-year-old man who presented with recidivant epitaxis, nasal obstruction and a neck lump on both sides. Dixit and Dave [11] reported the case of primary nasal tuberculosis in a ten-year-old girl who came to an otolaryngologist because of recidivant epitaxis and nasal obstruction. The definite diagnosis was established by the nasal endoscopy and multiple biopsies of granulomatose septum lesions.

Nasal TBC treatment should follow general guidelines for tuberculosis treatment. The choice of drugs must comply with the availability and local emersion of resistant strains; thus, at least two types of medications are used for the treatment, often three or more types [5]. According to the protocol, nasal TBC treatment lasts for at least 6 months continually.

Conclusion

Due to actual trends of TBC incidences, otolaryngologist should have in mind nasal TBC, when granulomatose lesions are found in nose.

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CASE REPORT

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Use of mineral trioxide aggregate in the treatement of traumatized teeth in children – Two case reports

Upotreba mineralnog trioksidnog agregata u lečenju povreda zuba kod dece

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Abstract

Introduction. Dental injuries in immature permanent teeth often result in endodontic complications. Apexification technique using calcium hydroxide is associated with certain flaws, such as long treatment time, the possibility of tooth fracture and incomplete calcification. The use of an apical plug employing mineral trioxide aggregate (MTA) is an alternative treatment option. **Case report.** We reported the successful treatment of 4 maxillary incisors (in a 7-year-old boy and a 10-year-old girl) with open apices and periapical lesions. Apical portions of the canals were filled with MTA plugs in both cases. Coronal parts of the root canals were filled with gutta-percha and sealer. Clinical findings were clear 6 months after the definite obturation with no pathological changes on the radiographs in both cases. **Conclusion.** The use of MTA for apical plugging appears to be a valid treatment option in traumatized immature teeth with endodontic complications.

Key words:
tooth fractures; root canal therapy; minerals; child.

Apstrakt

Uvod. Povrede zuba nezavršenog razvoja korena često su praćene endodontskim komplikacijama. Tehnika apeksifikacije u kojoj se koristi kalcijum-hidroksid povezuje se sa brojnim problemima, kao što su dugotrajnost lečenja, mogućnost frakture korena i neadekvatna kalcifikacija apeksne regije. Stvaranje apeksne barijere korišćenjem mineralnog trioksidnog agregata (MTA) predstavlja alternativu konvencionalnoj tehnici apeksifikacije**. Prikaz slučaja**. U radu su prikazana dva slučaja uspešnog lečenja hroničnog apeksnog parodontitisa na maksilarnim sekutićima (ukupno četiri) nezavršenog razvoja korena, prvi kod 7-godišnjeg dečaka, a drugi kod 10-godišnje devojčice. U oba slučaja apikalni deo korena zuba napunjen je MTA cementom. Koronarne partije korena zuba napunjene su gutaperkom i pastom. Šest meseci nakon lečenja nije bilo ni kliničkih ni radiografskih znakova patoloških promena**. Zaključak**. Upotreba MTA kao apeksne barijere pokazuje potencijal validne terapijske opcije kada su u pitanju povređeni zubi nezavršenog razvoja korena sa endodontskim komplikacijama.

Ključne reči:
zub, prelomi; zub, lečenje korenskog kanala; minerali; deca.

Introduction

The goal of endodontic treatment of traumatized immature teeth with periapical lesions is to achieve bone healing and form an adequate seal in the apical area [1]. Apexification technique using calcium hydroxide is still the most widely accepted, but it is associated with certain difficulties, such as long treatment time, the possibility of tooth fracture and incomplete calcification [2–5].

Mineral trioxide aggregate (MTA) has been used in paediatric dentistry as a pulp capping agent in young permanent teeth, as a pulpotomy medicament in primary and permanent teeth, as an apical barrier in immature non-vital teeth, in the coronal fragment of fractured roots, and as a repair material for perforation and resorptive defects [6, 7].

The aim of this study was to present therapeutic application of MTA in the treatment of immature traumatized teeth in children.

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Case report 1

A 7-year-old boy was referred to a control examination after the trauma he had experienced two months before. On that occasion the boy was diagnosed with enamel and dentine fracture without pulp involvement of permanent right central and lateral maxillary incisors, and extrusion and fracture of enamel and dentine without pulp involvement of permanent left central maxillary incisor (Figure 1a). Left central incisor was repositioned and the composite splint placed from left to right upper first primary molars. After removal of the splint, composite restorations were made. During the clinical examination after two months a buccal sinus tract in the apical part of vestibulum between the right central incisor and lateral incisor was noticed. The patient did not have any symptoms (Figure 1b).

The initial treatment included trepanation, removal of the necrotic tissue, biomechanical debridement of the root canal with copious irrigation (1% sodium hypochlorite and saline), placement of the calcium hydroxide dressing into the root canal and temporary filling of the right lateral incisor. The same therapy regiment was used on the right central incisor after a month (Figure 1c). During the next 3 months, calcium hydroxide dressing was replaced monthly with the same canal. After 3 months, the same endodontic treatment was initiated on the left central incisor because of the appearance of dentoalveolar abscess (Figure 1d). During the therapy, the odontometry was performed (Figures 1e and f). Six months after the therapy was initiated, the teeth were obturated with MTA in the apical third, and the coronal part of the root canal was filled with a canal sealer and gutta-percha two days after placement of MTA (Figure 1g). After setting the materials, temporary fillings were removed and the coronal restorations were made with a composite material. The teeth were asymptomatic clinically and radiographies did not show pathological changes at the control examinations after one and six months (Figure 1h).

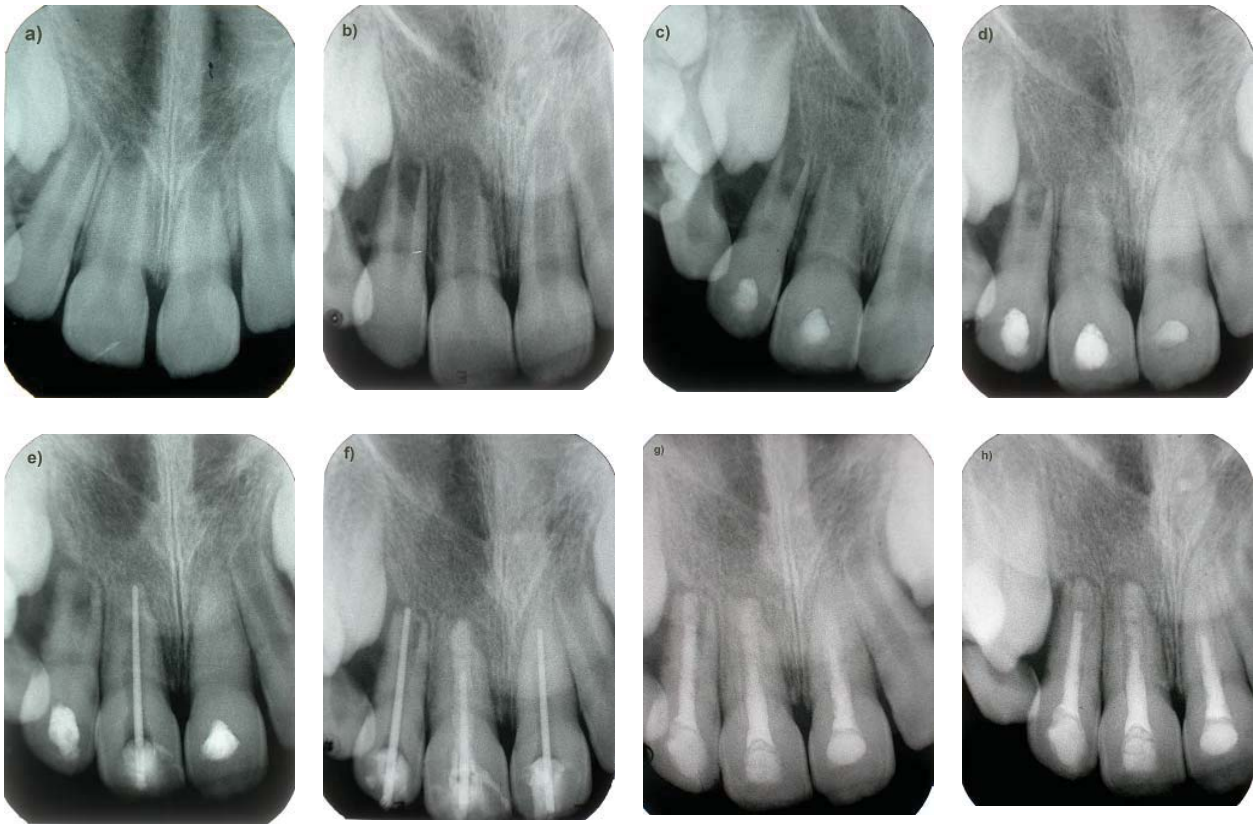

Fig. 1 – a) Radiographic view immediately after trauma; b) Radiographic view after 2 months; c) View after 4 moths, calcium hydroxide dressing; d) Radiographic view after 6 months calcium hydroxide dressing; e) Odontometry of the tooth 11; f) Odontometry of the teeth 12, 21; placement of MTA in the apical third of the tooth 11; g) Definitive obturation with MTA, root canal sealer and gutta-percha; h) Radigraphic follow-up after 6 months.

Case report 2

A 10-year-old girl without any complaint was referred for endodontic treatment of the permanent left central maxillary incisor which had been traumatized 4 years ago. Periapical radiography showed a large periapical lesion (Figure

2a). Clinical examination showed tenderness to percussion, while the vitality test (thermal pulp test) was negative. The therapy included trepanation, removal of the necrotic tissue, biomechanical debridement of the root canal with copious irrigation (1% sodium hypochlorite and saline) and drainage during two following days. After that period, calcium hydroxide dressing was placed into the root canal and the access cavity was closed with a temporary filling. Further therapy included replacement of calcium hydroxide each month (Figure 2b) during a 4 month preiod. The tooth was definitely obturated with the MTA apical plug, and the remaining portion of the root canal was filled with a canal sealer and gutta-percha 2 days after MTA placement (Figure 2c). After setting the materials, coronal restoration was made with a composite material. At the control examination after 3 months, the patient did not report any difficulties, and the tooth was asymptomatic clinically and radiographically (Figure 2d).

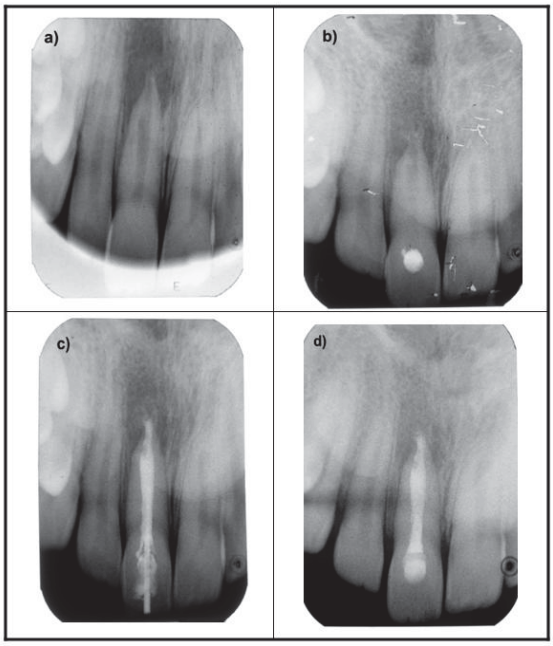

Fig. 2 – a) Radiographic view at the first visit, large periapical lesion; b) Radiographic view after 1 month, calcium hydroxide dressing; c) Radiographic view of completed root canal obturation (MTA, sealer and gutta-percha); d) Radiographic follow-up at 3 months, demonstrating osseous repair of periapical lesion.

Discussion

Mineral trioxide aggregate was developed and used for the first time at the Loma Linda University, California, USA, in the late 1990s, as a root-end filling material in surgical endodontic treatment [7]. MTA is a mixture of Portland cement (3 $CaO{\cdot}SiO_2$ and 2 $CaO{\cdot}SiO_2$) and bismuth oxide and contains trace amounts of CaO, SiO, MgO, K_2SO_4 and Na_2SO_4 [6]. In contrast to Portland cement, MTA is composed of smaller and more uniform particles and contains lower levels of potentially toxic heavy metals (Mg and Sr), oxide Fe, Al and K [6, 7]. MTA displays better marginal adaptation than amalgam and zinc oxide–eugenol-based root canal sealers [8, 9]. It has been shown that a layer of MTA of 4 mm is the most effective in preventing apical leakage when compared with lesser thickness of the same material, which makes a 4 mm MTA layer the most adequate as a root-end filling material [10].

Dental trauma in permanent teeth is a very frequent clinical finding in children and adolescents. Most of these injuries happen before a complete root formation and can result in pulp inflammation and its necrosis, and consequent root resorption which compromises the further process of apexogenesis. Treatment of endodontic complications at that age is a challenge because of very thin walls of the root canal, a large pulp chamber and a widely open apex [2].

A successful apexification depends on the creation of a calcified barrier from the cells that migrate from the surrounding periapical tissue and then differentiate influenced by specific cell signals into the cells that form the cement or the osteodentin matrix. The remains of the Hertwig sheath in this process are of a crucial importance because they can stay vital even after a complete necrosis of the pulp and initiate the growth of the root after endodontic treatment [2]. The traditional apexification technique with calcium hydroxide has been proved to be successful in forming a calcified barrier and confirmed throughout many years of practice [3–5]. However, treatment with calcium hydroxide comprises a great number of visits during a long period of time (3–21 months), the tendency towards cervical fracture of the treated tooth, and increased risk of reinfection of the root canal [4–5].

The use of MTA as a technique of apexification has so far been documented in rare studies [11–13] and demands further research. The first case in the present paper confirms that MTA acts as an apical barrier which enables regeneration of

the periapical tissue and favourizes the apical obturation of the infected immature teeth. Radiographs of the cases presented in this study document the positive outcome of the therapy after the use of MTA and a definite obturation of the endodontic space. Positive reparatory processes are not compromised even in the case of slightly extruded material in the periapical space and, as Mente et al. [11] reported, that is a proof that MTA does not cause a strong inflammatory reaction of the periapical tissue and it is not an obstacle for its reparation after the therapy.

There is no enough reports, that is no consistency in the final results when we talk about antibacterial and antifungal activity of MTA [14], and that is why before the definite obturation of the root canal with MTA, the application of calcium hydroxide is recommended to eliminate the root canal flora. Finally, the high price of MTA cannot be neglected, which presents a limiting factor for its everyday clinical use in the dental practice.

Conclusion

MTA is an effective therapy when it comes to young infected teeth that have suffered trauma, especially in the case of teeth with immature root development. Advantages of this material are a significant decrease in therapy duration and good apical plugging. In addition to that, having stated the advantages, further clinical research with a long observational period is still needed.

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CASE REPORT

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Central pontine myelinolysis in a chronic alcoholic: A clinical and brain magnetic resonance imaging follow-up

Centralna pontina mijelinoliza kod hroničnog alkoholizma: klinički oporavak uz postojanu leziju na magnetnoj rezonanci mozga

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Abstract

Introduction. Central pontine myelinolysis (CPM) is a noninflammatory, demyelinating lesion usually localised in the basis pontis. Chronic alcoholism is frequently associated with this condition which may have a variable clinical outcome. Until now, brain magnetic resonance imaging (MRI) follow-up in alcoholic CPM cases after alcohol withdrawal has been rarely described. **Case report.** We reported a 30-year-old male with a 12-year history of alcohol abuse, who presented with inability to stand and walk, nausea, vomiting and somnolence. Neurological examination revealed: impared fixation on lateral gaze, dysarthria, mild spastic quadriparesis, truncal and extremity ataxia, sock-like hypesthesia and moderate decrease in vibration sense in legs. Brain MRI showed a trident-shaped non-enhancing pontine lesion highly suggestive of CPM. After an eight-month alcohol-free follow-up period, the patient's clinical status significantly improved, while the extent of MRI pontine lesion was merely slightly reduced. **Conclusion.** The presented case demonstrates that CPM in chronic alcoholics may have a benign clinical course after alcohol withdrawal, which is not necessarily associated with the reduction of lesions on brain MRI.

Key words:
demyelinating diseases; pons; diagnosis; magnetic resonance imaging; alcoholism; treatment outcome.

Apstrakt

Uvod. Centralna pontina mijelinoliza (CPM) je neinflamatorno i demijelinizaciono oštećenje ponsa koje se može javiti kod hroničnog alkoholizma. Prognoza CPM je varijabilna, a prikazi nalaza na magnetnoj rezonanci (MR) mozga bolesnika sa CPM kod alkoholizma koji su prospektivno praćeni nakon alkoholne apstinencije retki su u literaturi. **Prikaz bolesnika.** Prikazan je 30-godišnji bolesnik koji je konzumirao prekomerne količine alkohola tokom 12 godina, kod koga su se subakutno ispoljili nemogućnost samostalnog stajanja i hoda, mučnina, povraćanje i pospanost. U neurološkom nalazu postojali su slabost fiksacije pri pogledu ulevo i udesno, dizartrija, znaci blage spastične kvadripareze, blaga do umerena ataksija ekstremiteta, umerena trunkalna ataksija, hipestezija za površni dodir poput čarapa i skraćen vibracijski senzibilitet na nogama. Na MR mozga utvrđena je lezija oblika trozupca u centralnom delu ponsa koja je imala karakteristike CPM. Nakon osam meseci alkoholne apstinencije klinički neurološki status kod bolesnika značajno se popravio, dok je kontrolnim MR pregledom mozga pokazana samo minimalna regresija ranije verifikovane ekstenzivne pontine lezije. **Zaključak.** CPM kod hroničnog alkoholizma može imati povoljan klinički tok i prognozu nakon prestanka konzumiranja alkohola uprkos održavanju lezije na MR mozga.

Ključne reči:
demijelinizacione bolesti; pons; dijagnoza; magnetna rezonanca, snimanje; alkoholizam; lečenje, ishod.

Introduction

Central pontine myelinolysis (CPM) is a noninflammatory, demyelinating lesion usually localised in the basis pontis. CPM has been reported to affect patients with a history of chronic alcoholism, malnutrition, dysionemia or rapid correction of hyponatriemia [1–4]. Before the introduction of magnetic resonance imaging (MRI), the diagnosis of CPM was frequently established postmortem, so the prognosis of this condition was doubtful [4], but nowadays rare asymptomatic [2] and benign [5] CPM cases have been reported. However, longitudinal brain MRI findings in alcoholic CPM patients following alcohol withdrawal are scarce.

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Case report

A 30-year old male with a 12-year history of chronic alcohol abuse, who presented with inability to stand and walk, nausea, vomiting and slight somnolence, consumed up to 2.5 L of home-made brandy per day over the year preceding the onset of neurological manifestations. Physical examination was normal. Neurological examination revealed the following: impared fixation on lateral gaze, dysarthria, mild bilateral upper and lower extremity weakness with slightly exaggerated tendon reflexes on upper extremities and diminished tendon reflexes on lower extremities; mild upper limb and moderate lower limb ataxia, moderate truncal ataxia; sock-like hypesthesia and moderate decrease in vibration in legs. The patient walked with unilateral assistance. Brain MRI performed at that time revealed a trident-shaped pontine abnormality on axial images (or omega sign) that presented as a non-enhancing T_1-weighted hypointensity (Figure 1A) and T_2-weighted hyperintensity (Figure 1B) which spared the outer rim of the pons. No other brain MRI abnormalities were detected. At that time, the patient refused hospitalization, as well as the participation in any additional diagnostic procedure including blood withdrawal.

Two months after the initial presentation, the patient joined the alcoholism rehabilitation program and his neurological status gradually improved without any specific treatment. After an eight-month alcohol-free period and eleven months after the initial presentation, a follow-up examination revealed normal findings on cranial nerves, no extremity weakness and slightly exaggerated tendon reflexes on upper extremities; a minor upper extremity ataxia which patient was not aware of was noticed, as well as a mild lower limb ataxia, positive Romberg test with the eyes closed and normal walking. Sensory impairment was still present unchanged. At that time the patient accepted to have blood withdrawn for hematology and biochemistry panel, including blood sodium and potassium levels, and laboratory findings were normal. A follow-up MRI showed the persisting trident-shaped pontine lesion whose volume was slightly reduced compared to the baseline scan performed eleven months earlier (Figure 1 C, D).

Discussion

We reported a CPM case in a chronic alcoholic with a significant clinical recovery after alcohol withdrawal, not associated with a significant resolution of a pontine MRI lesion.

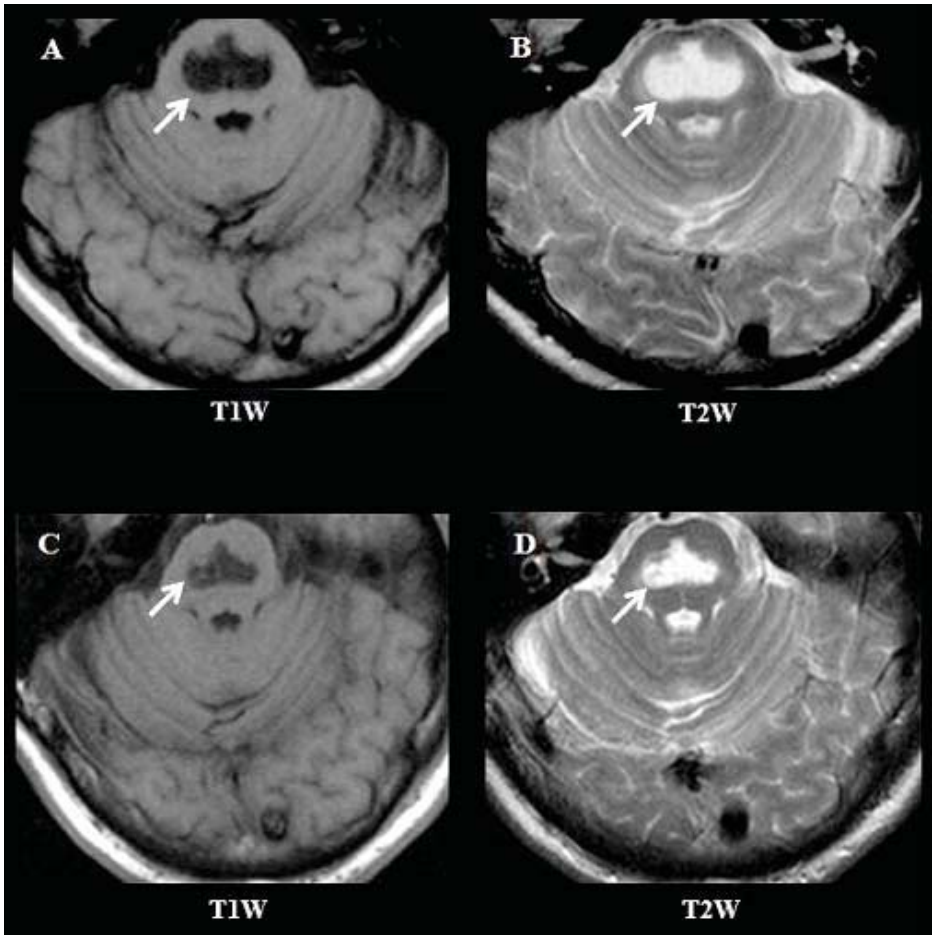

Fig. 1 – Axial brain magnetic resonance imaging scans show a well-defined trident-shaped T1-weighted (T1W) hypointense (A) and T2W hyperintense lesion (B) (arrows) suggestive of central pontine myelinolysis at baseline and 11 months after baseline (C, D).

Brain MRI in our patient showed a trident-shaped symmetrical non-enhancing central pontine abnormality which suggested CPM [6]. Although, a typical CPM lesion usually spares ventrolateral longitudinal fibres and corticospinal tracts [7] our patient had a mild spastic quadriparesis at the initial presentation. The reported patient did not have extrapontine brain MRI lesions. However, until now, symmetrical foci of extrapontine myelinolysis (EPM) have been frequently reported in other brain regions (in descending order of frequency: cerebellum, lateral geniculate body, external capsule, extreme capsule, hippocampus, putamen, cerebral cortex/subcortex, thalamus, caudate nuclei, claustrum, internal capsule, midbrain, internal medullary lamella, mamillary body, medulla oblongata) [4, 8, 9] in subjects with or without CPM, giving a wide spectrum of clinical presentations [4]. In line with the MRI findings in our patient, both CPM and EPM typically present with a non-enhancing hypointensity on T1-weighted brain MRI images, hyperintensity on T2-weighted and fluid attenuated inversion recovery sequences. Additionally, in certain CPM/EPM cases, contrast-enhancement of T1-weighted brain MRI lesions was described in early phases up to 4 weeks after the initial clinical manifestation [10] and diffusion-weighted imaging changes were reported before the development of conventional MRI signal intensity abnormalities [7].

CPM and EPM referred as osmotic demyelination syndromes (ODMS) [11, 12] have been shown to share similar histology [8]. The pathophysiology of ODMS is poorly understood [13], but the proposed mechanisms involved in its development include osmotic injury to the vascular endothelial cells, vasogenic edema and/or brain dehydration, the release of myelinotoxic factors, as well as axonal separation from the myelin sheath which contributes to oligodendrocyte injury and demyelination [13]. It has been shown that edema and/or demyelination in ODMS could be reversible [14], potentially correlating with a resolution of brain MRI lesions, which varies from their complete disappearance to merely slight reduction on conventional MRI [14, 15]. Residual MRI findings have been considered to represent areas of permanent damage [14].

Chronic alcoholism is a common predisposing condition for the development of ODMS [12]. Although alcohol itself could interfere with sodium/water regulation by suppression of antidiuretic hormone, an inadequate nutrition/water intake and/or liver dysfunction in alcoholics might also contribute to ODMS [4]. Additionally, chronic alcoholics might not be able to maintain protective cerebral mechanisms against osmotic stress [13], and could also suffer from direct toxicity of alcohol [16]. Furthermore, oxidative stress in alcoholics may favor apoptosis of brain cells [16, 17] leading to an ireversible damage.

However, it has been suggested that a better outcome of CPM/EPM could occur in chronic alcoholics in which it may be asymptomatic or have relatively few symptoms, than in cases associated with an acute correction of hyponatremia [5] in which a high mortality rate was reported with a significant neurological deficits in the survivors [5, 9, 11]. Clinical recovery of our patient was associated with the alcohol withdrawal without any other specific treatment. Since he did not accept any diagnostic tests in the acute phase of his illness, apart from brain MRI, we can only speculate that CPM in this case developed due to alcohol-related mechanisms and not as a consequence of disturbances in blood sodium level.

Clinical recovery which was not accompanied by a significant resolution of MRI lesions in our patient supports the notion that follow-up MRI in CPM does not have a significant prognostic value for the outcome of this disorder [4, 15].

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CASE REPORT

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Early reconstruction of bone defect created after initial surgery of a large keratocystic odontogenic tumor: A case report

Rana rekonstrukcija defekta vilice nastalog posle prve operacije velikog keratocističnog odontogenog tumora

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Abstract

Introduction. Keratocystic odontogenic tumor (KCOT) is defined as a benign cystic neoplasm of the jaws of odontogenic origin with a high rate of recurrence. The most lesions occur in the posterior part of the mandible. Treatment of KCOT remains controversial, but the goals of treatment should involve eliminating the potential for recurrence while minimizing surgical morbidity. However, another significant therapeutic problem related to the management of KCOT is an adequate and early reconstruction of the existing jaw defect, as well as appropriate aesthetic and functional rehabilitation of a patient, especially in cases of a very large destruction of the jaws bone. **Case report.** We presented a 65-year-old female patient with very large KCOT of the mandible. Orthopantomographic radiography showed a very large elliptical multilocular radiolucency, located on the right side of the mandible body and the ascending ramus of the mandible, with radiographic evidence of cortical perforation at the anterior border of the mandibular ramus and the superior border of the alveolar part of the mandible. The surgical treatment included two phases. In the first phase, the tumor was removed by enucleation and additional use of Carnoy solution, performing peripheral ostectomy and excision of the affected overlying mucosa, while in the second phase, restorative surgery of the existing mandibular defect was performed 6 months later. Postoperatively, we did not register any of postoperative complications, nor recurrence within 2 years of the follow-up. **Conclusion.** Adequate and early reconstruction of the existing jaw defect and appropriate aesthetic and functional rehabilitation of the patient should be the primary goal in the treatment of KCOT, having in mind the need for a long-term post-surgical follow-up.

Key words:
odontogenic cysts; oral surgical procedures; recurrence; reconstructive surgical procedures; mandible; treatment outcome.

Apstrakt

Uvod. Keratocistični odontogeni tumor (KCOT) definisan je kao benigna cistična neoplazma viličnih kostiju odontogenog porekla, sa visokom stopom recidiva. Najveći broj lezija obuhvata bočne delove mandibule. Lečenje KCOT je kontroverzno, ali ciljevi lečenja treba da obuhvate eliminisanje potenecijala za pojavu recidiva, kao i smanjenje pojave hirurških komplikacija. Međutim, drugi značajan terapijski problem u lečenju KCOT jeste adekvatna i što ranija rekonstrukcija postojećeg viličnog defekta, kao i odgovarajuća estetska i funkcionalna rehabilitacija ovih bolesnika, naročito u slučajevima velikih destrukcija viličnih kostiju. **Prikaz bolesnika.** U radu je prikazana 65-godišnja žena sa veoma velikim KCOT mandibule. Na ortopantomografskoj radiografiji uočeno je elipsasto multilokularno rasvetljenje lokalizovano na desnoj strani tela mandibule i susednog dela ramusa donje vilice sa radiografski evidentnim postojanjem kortikalne perforacije prednje ivice ramusa i gornje ivice alveolarnog dela mandibule. Operativni zahvat izveden je u dve faze. U prvoj fazi, tumor je uklonjen enukleacijom uz upotrebu Karnojevog rastvora i perifernom osteotomijom, sa ekscizijom okolno zahvaćene sluzokože. U drugoj fazi, šest meseci kasnije, rekonstruiran je postojeći defekt donje vilice. Postoperativno, nije registrovana nikakva komplikacija niti pojava recidiva dve godine nakon intervencije. **Zaključak.** Adekvatna i rana rekonstrukcija postojećeg viličnog defekta i odgovarajuća estetska i funkcionalna rehabilitacija ovih bolesnika treba da bude primaran cilj lečenja KCOT. Takođe, potrebno je sistematsko i dugotrajno postoperativno praćenje bolesnika.

Ključne reči:
ciste, odontogene; hirurgija, oralna, procedure; recidiv; hirurgija, rekonstruktivna, procedure; mandibula; lečenje, ishod.

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Introduction

Keratocystic odontogenic tumor (KCOT) is defined as a benign cystic neoplasm of the jaws of odontogenic origin [1]. Histological features are characterized by the presence of a thin band-like parakeratinized stratified squamous epithelium [2, 3]. The most lesions occur in the posterior part of the mandible [4].

Clinically, KCOT is one of the most aggressive behavior lesions, with the potential of rapid growth, and tendency for local intraosseal destruction, penetration to adjacent soft tissue and with a high rate of recurrence [5]. Treatment of KCOT remains controversial, but the goals of treatment should involve eliminating the potential for recurrence while also minimizing the surgical morbidity [6].

Certainly, the most important therapeutic problem in dealing with KCOT is the risk of its recurrence, as well as the choice and radicalism of surgical procedure to minimize possibility of recurrence [7–9].

However, treatment of these lesions has another major therapeutic problem, which is the choice of adequate reconstruction of the existing jaw defect after initial surgical treatment, and appropriate aesthetic and functional rehabilitation of the patient, especially in cases of a very large destruction of the jaws, with the aim that patients early regain functioning.

The purpose of this report was to present a patient with a very large KCOT of the mandible, which was removed by enucleation with the additional use of Carnoy solution, as well as peripheral ostectomy and excision of the affected overlying mucosa in the first phase, and restorative surgery of the mandibular defect in the second phase, followed-up postoperatively for 2 years.

Case report

A 65 year-old female patient was admitted to the Department of Oral Surgery, Military Medical Academy (MMA), Belgrade, because of expansion of the buccal cortex of the mandible body on the right side, as well as a large perimandibular soft tissue swelling. The patient had no other clinical symptoms. Orthopantomographic radiography showed a large elliptical multilocular radiolucency, located in the right side of the mandible body and the ascending ramus. Radiographic evidence of cortical perforation at the anterior border of the mandibular ramus and the superior border of the alveolar part of the mandible was also noted (Figure 1).

The patient was advised for surgical treatment after biopsy, because it is the adopted protocol for cases of the suspected KCOT or similar lesion. Due to the existing infection at the time of examination, antibiotic therapy was started (amoxicilin/clavulanic acid and metronidazole, orally). Ten days later, the patient underwent an incisional biopsy of the lesion under local anesthesia (4% Articain chloride™, 3M ESPE).

The histopathologic finding was odontogenic cyst – keratocystic odontogenic tumor, indicating that tumor was completely lined with squamous epithelium of parakeratotic type, without atypia. Secondary acute inflammation in the lumen of the lesion and partially in the epithelium was present (Figures 2–4).

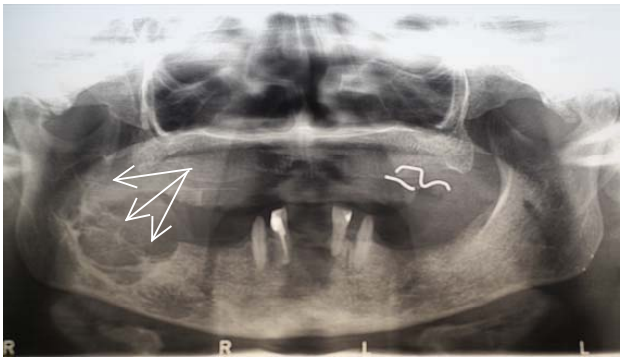

Fig. 1 – Orthopantomographic radiography at the time of examination: a large elliptical multilocular radiolucency, located on the right side of the mandible, with radiographic evidence of cortical perforation (arrows).

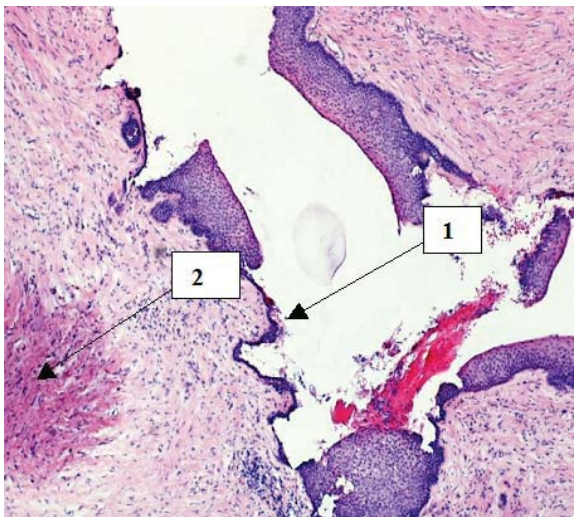

Fig. 2 – Cystic squamous epithelium, showing atrophy and parakeratoticinvaginations into the lumen (arrow 1): focal hemorrhage and fibroblastproliferation in the wall of the lesion (arrow 2) (H&E, 40×).

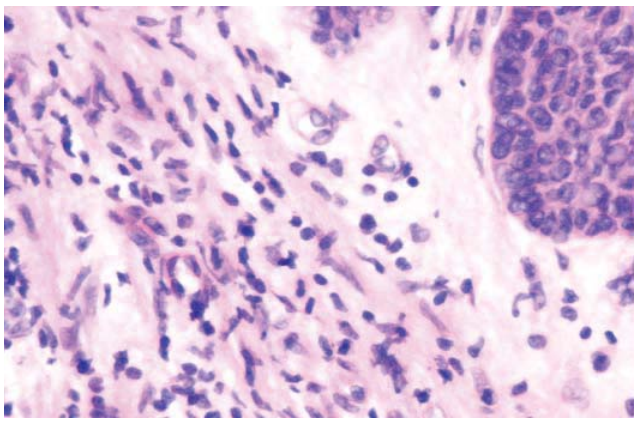

Fig. 3 – Inflammatory components in the wall of KCOT, mainly consisting of plasmocytes, with granulocytes to a lesser degree (H&E, 75×).

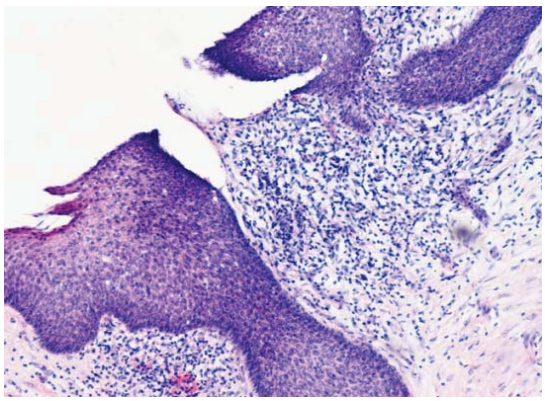

Fig. 4 – Separating granulation tissue between deep epithelial rete pegs at the site of ulceration (H&E, 40×).

The first surgery

Upon receipt of the histopathological findings, the patient was advised to have the operation under general anesthesia on the in-patient basis. At the Clinic for Maxillofacial, Oral Surgery and Implantology of the MMA all the necessary laboratory analysis for surgery under general anesthesia were made, and at the Institute of Pharmacy of the MMA, Carnoy solution was prepared according to the following prescription: absolute alcohol – 6 mL, chloroform – 3 mL, glacial acetic acid – 1 mL and ferric chloride – 1 g.

Surgical procedure started with incision along the anterior border of the right mandibular ramus, then over the alveolar crest to the gingival margin till the right first premolar, including vertical relaxing incision down to the fornix in this region. After mucoperiostal flap uplifting and separating the masseter and medial pterygoid muscle attachments, the defect of the anterior border of the ramus and the superior border of the alveolar part of the mandible, about 60 mm long, was seen. The defect was slightly widened caudally at its lateral aspect in order to approach the lesion and the intralesional septae were eliminated before enucleation of the tumor and application of Carnoy's solution. Then, the lesion was completely enucleated, a defect rinsed with saline, and a sterile gauze swab presoaked with Carnoy solution was placed in the lumen of the defect, for 3 minutes, then rerinsed with saline to be able to see dark brown colored and fixated cystic wall remnants, and easily and completely remove them. After that, a peripheral ostectomy in the caudal and cranial direction was performed and the overlying attached mucosa was excised. After repeated rinsing with saline the wound was sutured, primarily. Postoperatively, the patient was prescribed intravenous antibiotics: ceftriaxon 2 g × 1 daily, aminoglicoside 1 g × 1 daily and metronidazole 0.5 g × 3 daily, for the folowing seven days. The sutures were removed after 7 days. No neurosensory deficit in the inervation area of the inferior alveolar nerve was present, as well as, any other postoperative complications.

The second (restorative) surgery

The restorative surgery was performed about 6 months after the enucleation and peripheral ostectomy procedure, and included intraoral reconstruction of the existing mandibular defect.

The second surgery was also performed on the inpatient basis under general anesthesia. Surgical procedure started with the same incision as in the first surgery. The mandibular defect was debrided of the fibrous tissue, which was taken for histological examination, and *ex tempore* histological report on the fibrous tissue removed from the mandibular defect and the soft tissue removed from the surrounding mucosa before graft placement was negative to KCOT. A heterogenous bone block (Osteovit®, B. Braun Melsungen AG.) was placed into the mandibular defect thereafter, and a spongious bone substitute (Bio-Oss®, Geistlich Pharma AG, Switzerland) was added around the bone block to fill the gaps. The intraoral wound was closed with 4-0 silk sutures, primarily. Postoperatively, the patient was prescribed antibiotics intravenously (ceftriaxon 2 g, once daily and metronidazole 0.5 g, 3 times daily) for the following 7 days. The sutures were removed after 7 days, and the patient was discharged without any postoperative complications.

Functional rehabilitation of the patient was completed with a pair of classic dentures six months after the second surgery, because the patient refused our suggestion for dental implantation and construction of dental implants-supported dentures.

The patient was followed up regularly, radiographically and clinically. After a 2-year period, orthopantomographic radiography confirmed a successfull and complete bone healing of the previuos mandibular defect on the right side of mandible, without recurrence of the lesion (Figures 5 and 6).

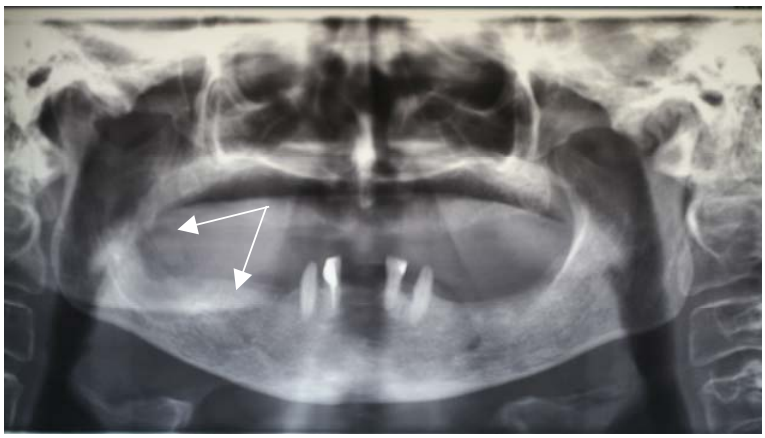

Fig. 5 – The orthopantomographic radiography six months after the second surgery (arrows).

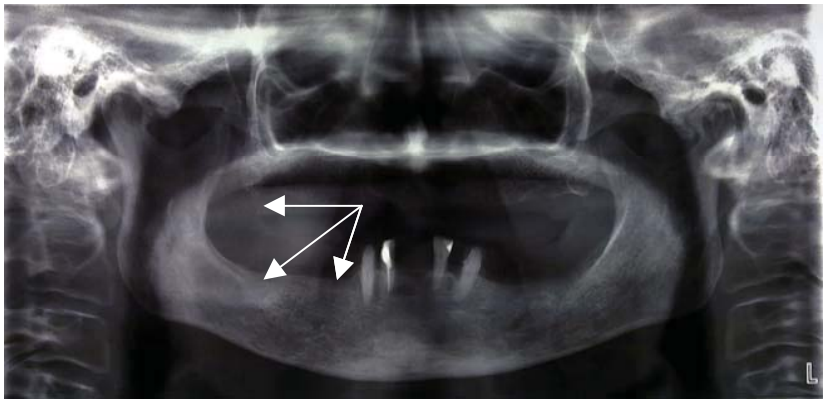

Fig. 6 – The orthopantomographic radiography, 2 years after second surgery, showing a complete and successful bone healing on the right side of the mandible (arrows).

Discussion

KCOT is one of the most commonly encountered odontogenic entities, thus requiring special consideration [5]. Renewed classification of odontogenic keratocysts to the KCOT by the World Health Organization (WHO) was based on the well-known aggressive behavior of this lesion, its histology and new information regarding its genetics [4]. Clinical evidence of its aggressive behavior is supported by reported cases penetrating the cortical bone and involving adjacent soft tissues [5].

Generally, treatments of these lesions are classified as conservative or radical (aggressive), but there are several dilemmas and different opinions concerning the appropriate surgical approach [10]. Kolokythas et al. [5] suggested that the treatment modality should be chosen on the basis of clinical or radiographic evidence of cortical perforation and subsequent soft tissue involvement, and possible history of previous recurrences of the same lesion. The crucial problem is to determine when, and whether aggressive and radical therapy is necessary in treatments of these lesions.

It seems that there are 4 different, but very important and intriguing facts related to treatment of KCOT: firstly, a high rate of recurrence, as many authors reported frequency of recurrences up to 60%, especially within the first 5 to 7 years after initial surgery [11–14]; secondly, there are different surgical options, and the main goal would be to eliminate the potential for recurrence and minimize surgical morbidity; thirdly, there is a need, very often, for early reconstruction of bone defect and an adequate aesthetic and functional rehabilitation of the patient; and fourthly, there is a chance, although rarely, of malignant transformation [15]. Certainly, the first and second therapeutic problems are the most significant because recurrences may have serious consequences [16].

In the literature review, the mayority of studies reported relationship between the incidences of the recurrences and the choice of the surgical approach. A choice of applying completely conservative method in the treatment of KCOT may be justified if there is a risk of injury to the surrounding anatomic structures [17–21], especially in children, where the use of aggressive surgical techniques can cause disturbances in the growth and development of jaws and teeth [18]. However, completely conservative methods (marsupialisation, decompression) have many disadvantages, primarily due to the fact that they imply incomplete removal of the lesion. After decompression and marsupialisation, some authors reported a considerable reduction of the cystic volume and levels of IL1-α and cytokeratin-10, which are related to the cystic expansion and metaplasia of the epithelial lining, meaning conversion to a less aggressive form [11, 20–22]. However, malignant alteration is still possible [23, 24], and a very high rate of recurrence still exists [5, 21, 22, 25]. Finally, after completely conservative approaches, early reconstruction of the jaws defect is not possible.

On the contrary, jaw resection, as the most radical and aggressive therapeutic option, regardless the lowest rate of recurrence, should be used only in cases of frequent recurrences (more than three) and in patients where KCOT is associated with nevoid basal cell carcinoma syndrome [5, 6, 8, 9, 14]. Kolokythas et al. [5] and Tolstunov and Treasure [9] consider jaw resection as the initial surgical treatment in cases of KCOT showing more aggressive behavior (large and/or multilocular tumor). However, jaw resection produces a significant morbidity, the loss of the jaw continuity and facial disfigurement [8]. Although this surgical method offers the possibility of immediate reconstruction of the bone defect with iliac crest bone graft and the early return to function [26], esthetic and functional rehabilitation of the patient might be inappropriate.

Conclusion

We operated on the presented patient successfully for a large, multilocular KCOT by a less aggressive surgical approach (enucleation with the use of Carnoy's solution, peripheral ostectomy and excision of the affected overlying mucosa) without the loss of the jaw continuity. This surgical approach provided a significant possibility for adequate and early reconstruction of the jaw defect, resulting in better quality of life of the presented patient.

Acknowledgements

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Sećanje povodom Dana Sanitetske službe Vojske Srbije: štab-doktor dr Emerih P. Lindenmajer (1806–1883)

Remembrance on the occasion of the Day of Armed Forces Medical Services of Serbia: Staff Doctor Emmerich P. Lindenmayer (1806–1883)

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„Rodio se 1806. godine u Oraovici u Banatu, odrastao u Čakovu, učio u Pešti i Beču, prešao u Srbiju 1835. godine sa željom da po svome znanju i lekarskom iskustvu Srbiji čestito i iskreno služi“ napisao je svojeručno na molbu dr Vladana Đorđevića [1].

Podunavski Švaba rođenjem, lekarski sin, zemljak našeg Dositeja Obradovića, medicinske studije završio je 1832. u Pešti inauguralnom disertacijom „*Hippocrates, homo, philosophus, medicus*“.

„Kada sam ovamo došao, to nisam kao novajlija medicinskog zvanija došao, nego kao doktor, koi sam pre toga tri godine u Pešti prakticirao“, pisao je dr Lindenmajer u pismu knezu Milošu 11. marta 1837. godine [2]. Obnovljena Srbija je u vreme o kome govorimo bila u potpunosti bez sopstvene inteligencije i zavisila je od stranaca ili Srba iz Austrije.

Dr Lindenmajer je u Srbiju došao sredinom 1835. godine, zamenivši u Šapcu dr Karla Paceka na mestu lekara Podrino-savske komande [3]. Odavde je naredbom kneza Miloša u januaru 1837. premešten u Beograd u Soldački špital, koji je ostao bez lekara. Tu je radio kraće vreme jer je već u martu iste godine premešten u Kragujevac za „gvardejskog lekara“, ponovo zamenivši dr Paceka koji je postao knežev lični lekar i intimus. Na njegovo mesto došao je dr Karlo Beloni [3]. Zanimljiva je epizoda tokom njegovog boravka u beogradskom špitalu kada je došlo do kraćeg neslaganja između njega i Tome Vučića-Perišića, Kneževog izaslanika u Beogradu, oko organizacionih problema u bolnici, o čemu ga je Toma tužio knezu, te zamalo nije došlo do njegovog odlaska iz Srbije zbog kneževe ljutnje, iz čega ga je izbavio njegov kolega sa studija dr Pacek [3].

Srbija je tih godina preživljavala ustavnopravne probleme pošto je turska Porta odbila, prema sugestijama Rusije i Austrije, da prihvati slobodoumno pisani, po uzoru na francuski i belgijski, „Sretenjski ustav“ donet na Narodnoj skupštini u Kragujevcu 1835. godine. Kada je najzad u decembru 1838. iz carigradske Porte stigao novi Ustav, nazvan „turski“, pristupilo se obrazovanju državnih organa. Tako je 29. maja (8. juna) 1839. godine doneto „Ustrojenije garnizone vojske“ (koja je potpadala pod Popečiteljstvo vnutrešnjih dela), a na osnovu njega i Glavni vojeni štab, 18. jula, po starom kalendaru, odnosno 30. jula, po novom kalendaru. U okviru toga štaba dr Emerih Lindenmajer imenovan je za glavnog štab-doktora i time je postao prvi načelnik saniteta srpske vojske u nastajanju [4, 5]. U znak sećanja, taj je datum izabran je za Dan sanitetske službe Vojske Srbije [6].

Ubrzano posle toga pristupilo se obrazovanju regularne vojske, pa i saniteta. Imenovan je i prvi štab-hirurg, magistar hirugije Moric Fidler i započeta je regrutacija. Tom priliko javlja se i prvi sanitetski problem u vidu učestalog obolevanja vojnika i povećane smrtnosti. Ispitujući uzroke, dr Lindenmajer je utvrdio, upoređujući podatke sa podacima starijih vojnika, da je uzrok ove pojave velika i nagla promena u načinu života tih seoskih mladića, dovedenih u potpuno novu sredinu i izmenjene uslove života, pa je predložio da se regruti dovode sukcesivno i u manjim grupama radi lakšeg prilgođavanja kasarnskom životu, da se češće kupaju, leti u rekama, a zimi u toplim prostorijama, da se periodično puštaju na otsustva, da se među njima razvija takmičarski duh i nagrađivanje uspešnijih, što je sve dovelo do smanjenja nemilih pojava.

Svakako da je najznačajnije vojnosanitetsko delo dr Lindenmajera nacrt „Zakona o ustrojeniju špitalja centralne vojske“ koji je sačinio još 1839. godine i koji je u skoro neizmenjenom obliku usvojen 19. februara (2. marta) 1844. godine. Njime je predviđeno sve što je neophodno za osnivaje, život i rad vojnih bolnica. Taj dan se danas proslavlja kao Dan Vojnomedicinske akademije [7].

Uredivši vojne bolnice u Kragujevcu, Beogradu i Ćupriji, uvevši red u apotekarskoj službi i izradivši „Tablice ishrane“ koje su se decenijama koristile u srpskoj vojsci, dr Lindenmajer je postavio čvrste temelje na kojima se dalje,

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istina mukotrpno i sporo, zbog kulturne zaostalosti i siromaštva, razvijao srpski vojni sanitet.

Kao rezultat njegovog uspešnog rada dolazi imenovanje za načelnika Sanitetskog odeljenja u Popečiteljstvu vnutrešnjih dela, mesta na kome će ostati punih 14 godina, od 23. juna 1845. do 1. aprila 1859. godine, upravljajući celokupnom sanitetskom službom Srbije, i civilnom i vojnom. Na njegovo mesto u vojnom sanitetu došao je dr Karlo Beloni. Njihovim skladnim odnosom i zajedničkim naporima srpski vojni sanitet se lagano uzdizao, sa povremenim padovima usled društvenopolitičkih teškoća koje su u tom periodu povremeno zaustavljale njegov napredak [2].

Nemoguće je navesti šta je sve dr Lindenmajer za tih 14 godina učinio radeći najčešće potpuno sam, počev od brige za povećanjem broja sanitetskog osoblja i njegove adekvatnije nagrađenosti, preko uređenja sanitetske administracije, jačanja karantinskih pograničnih ustanova radi sprečavanja unosa epidemijskih bolesti, naročito kuge i kolere, stvaranja stručne komisije za borbu protiv endemskog sifilisa („frenga“) i specijalne bolnice u Gurgusovcu (Knjaževac) za lečenje frengavih, osnivanja „Stalne lekarske komisije“ 1852. godine kao najvišeg stručnog tela za rešavanje svih velikih zdravstvenih problema u Srbiji, organizovanje uništavanja legala „golubačke mušice“ koja je u istočnim delovima zemlje nanosila velike štete stočnoj privredi, oživljavanje lekarske čitaonice sa najnovijim knjigama i časopisima iz Evrope, do sakupljanja, zajedno sa Jovanom-Sterijom Popovićem, novčanih priloga za pomoć sirotinji o Božiću i Uskrsu [2].

Od 1846. godine počeo je da uređuje već poznata mesta po Srbiji u kojima se narod okupljao da se leči po barama sa mineralnom vodom: 1846. godine – Bukovički kiseljak kod Aranđelovca, 1850. godine – Brestovačka i Ribarska banja, 1851. godine – Palanački kiseljak i „Smrdan-banja“ kod Loznice. Godine 1852. u Beču je izvršio analizu svih tih voda i napisao, od Sovjeta odobren, „Pravilnik o korišćenju“ kojima se propisuje lečenje u njima i uloga banjskih lekara. O svemu tome je napisao i knjigu koja je objavljena 1856. godine u Beogradu na srpskom i nemačkom jeziku pod nazivom „Opis mineralni i lekoviti voda i njino upotrebljenje voopšte i ponaosob lekoviti voda u Knjaževstvu Srbiji do sad poznati“. Iste godine u Temišvaru štampa knjigu u počast očevom delu o „Mineralnim vodama u Buzjašu“ [2].

Najveće pisano delo dr Lindenmajera na kome mu sve generacije lekara i istoričara u Srbiji moraju biti zahvalne, jeste delo o razvoju zdravstvene službe u Srbiji od 1820. do 1870. godine, izdato u Temišvaru na nemačkom jeziku, kako bi se (po njegovim rečima) i šire o tome moglo saznati, „Srbija i njen razvoj i napredak u sanitetskim poslovima...“ Bez ovoga dela, koje i danas čeka svoje srpsko izdanje, naše znanje o prvim decenijama razvoja srpske zdrvavstvene službe bilo bi minimalno [8].

Nažalost, ovaj čestiti i vredni čovek, koji je Srbiju i srpski narod prigrlio kao svoj („naš narod“, „naša država“, „naša domovina“, kako se na više mesta u svojoj knjizi izražava), služeći samo svojoj struci i srpskom narodu i ne obraćajući pažnju na nekoliko državnih i dinastičkih lomova (smene dve srpske dinastije Obrenovića i Karađorđevića i bune u vezi sa tim događajima), doživeo je 1859. godine, povratkom u Srbiju ostarelog i osvetoljubivog kneza Miloša, tužnu sudbinu da grubo bude maknut sa položaja bez ikakvog usmenog ili pismenog obrazloženja, „prosto zaobiđen kao da ne postoji“ kako je sam rekao i držan „na raspoloženju“ sve do 1865. godine kada se knez Mihailo, ispravljajući greh oca, rešio da ga penzioniše i visoko odlikuje „Takovskim krstom oko vrata“ III reda [9]. Iako do suza uvređen, ćuteći je trpeo nanesenu mu nepravdu, a 1862. godine, kada je u Beogradu izbio poznati oružani sukob sa turskim garnizonom kod Čukur česme i Beograd bio teško bombardovan, pružila mu se ponovo prilika da svojoj Srbiji još jednom priskoči u pomoć – na knežev poziv hitno je, uz pomoć gradskog fizikusa dr Jovana Mašima, organizovao sanitetsku službu u gradu iz koga su mnogi drugi u strahu utekli [2].

Uvažen kao lekar, počasni član Srpskog lekarskog društva, dopisni član bečkog lekarskog društva, ostao je u Srbiji koju je smatrao svojom domovinom sve do smrti. Umro je 12/24 oktobra 1883. godine, a nad njegovim grobom jedan drugi veliki srpski lekar, dr Vladan Đorđević, izgovorio je sledeće reči: „..na grobu dr Lindenmajera Majka Srbija veli: hvala Ti, posinče, što si mi ceo Tvoj vredni život posvetio. A mi, njegovi poslenici, mi Mu velimo: čast Tvojoj uspomeni među nama“ [1].

Nažalost, Srbija je prema njemu i posle smrti ostala nezahvalna, niti mu je sačuvan grob niti ijedna fotografija, koji bi potsećali na ovoga čestitog, vrednog i požrtvovanog lekara. Jedini spomen na njega su natpisi koji se tu-i-tamo pojave, a najveći spomen je svakako dan njegovog imenovanja za prvog srpskog načelnika vojnog saniteta koji se od 2009. godine slavi kao Dan sanitetske službe Vojske Srbije.

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MEETING REPORT/IZVEŠTAJ
SA STRUČNOG SKUPA

Report on the 18th Congress of the Balkan Military Medical Committee held in Instanbul on May 22–26, 2013

Izveštaj sa 18. kongresa Balkanskog komiteta vojne medicine (Istambul, 22–26. maj 2013. godine)

The Balkan Military Medical Committee (BMMC) founded in 1995 included all the founding countries as members, namely Bulgaria, Greece, Romania and Turkey. Serbia was made an equal member of the BMMC in 2006 participating fully in its work ever since then. In 2012, thus, Serbia organized the 17th BMMC Congress in its capital Belgrade.

This year's, that is 18th BMMC Congress was held in Istanbul as Turkey was its organizer and host. A total of 275 delegates from the 5 member countries participated to the Congress presenting 418 papers, out of which 172 orally and 246 as poster presentation. The majority of papers were on military surgery, internal medicine, neuropsychiatry, preventive medicine, air medicine, and navy medicine.

Within the Congress the Heads of Military Medical Corps and Academies of the BMMC member countries had a meeting on "Prophylaxis and preventive medical care before, during and after military forces deployment in military operations".

The experience of Serbian Military Medical Corps was presented at this Congress by Brigadier General Prof. Dr. Zoran Popović, Head of Directorate for Military Health Care, Ministry of Defence, Republic of Serbia, as well as by Brigadier General Prof. Dr. Marijan Novaković, Head of Military Medical Academy, Belgrade, Serbia.

Greek, Romanian, Serbian and Turkish cadets (final year medical students) took part in the Cadets Session III presenting very successfully the results of scientific projects included as young researchers guided by their mentors.

The best both oral and poster presentations from each BMMC member country were especially awarded, particularly the best presented paper of the Congress. All these presentations are to be published in the official journal of the BMMC – Balkan Military Medical Review, now edited and published by the Greek Military Medical Service.

Treatment of war injuries and surgery experience from piecekeeping missions in Afghanistan, Syria, Kenya and other countries called a special attention of all the participants. Introduction of *de novo* cardiological surgical procedures, mobile surgical teams for urgent interventions, as well as introduction of highly sophisticated diagnostic possibilities in everyday application in military hospitals in the regional countries, primarily in the Greek Navy, called the absolute attention of the present.

Balkanski komitet vojne medicine (BKVM) ustanovljen je 1995. godine i činile su ga zemlje osnivači: Bugarska, Grčka, Rumunija i Turska. Srbija je postala punopravni član BKVM 2006. godine i od tada punim kapacitetom učestvuje u njegovom radu. Prošle 2012 godine, organizovala je i 17. kongres BKVM koji je održan u Beogradu.

Osamnaesti kongres BKVM održan je u Istambulu, u organizaciji Turske kao zemlje domaćina. U radu Kongresa učestvovalo je 275 delegata iz pet zemalja članica BKVM. Ukupno, saopšteno je 418 radova, od čega 172 usmeno, a 246 kao poster prezentacije. Najveći broj radova bio je iz vojne hirurgije, interne medicine, neuropsihijatrije, preventivne medicine, te vazduhoplovne i pomorske medicine.

U toku održavanja kongresa načelnici vojnosanitetskih službi i vojnomedicinskih akademija zemalja članica BKVM imali su sastanak na temu „Profilaksa i preventivnomedicinska zaštita pre, tokom i nakon rasporeda snaga u vojnim operacijama". Iskustva našeg vojnog saniteta na ovom sastanku predstavili su brigadni general prof. dr Zoran Popović, načelnik Uprave za vojno zdravstvo Ministarstva odbrane Republike Srbije, i brigadni general prof. dr Marijan Novaković, načelnik Vojnomedicinske akademije u Beogradu.

Kadeti Grčke, Rumunije, Srbije i Turske (studenti medicine završnih godina fakulteta) učestvovali su u radu 3. kadetske sesije u okviru koje su imali izuzetno kvalitetna izlaganja rezultata naučnoistraživačkih projekata u kojima su učestvovali kao mladi istraživači pod vođstvom svojih mentora.

Najbolja usmena i poster saopštenja iz svake zemlje članice BKVM posebno su nagrađena, kao i najbolje usmeno izlaganje na celom kongresu. Ova saopštenja će biti štampana u zvaničnom časopisu BKVM „*Balkan Military Medical Review*" koji trenutno uređuje i izdaje vojnosanitetska služba Grčke.

Lečenje ratnih povreda i iskustva hirurga iz mirovnih misija u Avganistanu, Siriji, Keniji i drugim zemljama izazvali su veliku pažnju među učesnicima skupa. Uvođenje novih hirurških procedura u kardiohirurgiji, mobilne hirurške ekipe za brze intervencije, kao i uvođenje visokosofisticiranih dijagnostičkih sredstava u rutinsku primenu u vojnim bolnicama zemalja u regionu, pre svega u Grčkoj mornarici, izaz-

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Učesnici 18. kongresa Balkanskog komiteta vojne medicine na ceremoniji otvaranja skupa
Partcipants of the 18th Congress of the Balkan Military Medicine Committee at opening ceremony.

The Serbian physicians, specialists in plastic surgery and orthopedics, presented their experience in reconstructive operations and patellar tendons managing, while neurosurgeons showed new bearings and achievements in managing neurosurgical injuries. The general surgeons presented a paper on their experience in the treatment of ileus caused by bile stone. Urology was presented by the results of a surgical technique for designing a new bladder from a part of terminal ileum according to the original Serbian procedure referred to as the "Belgrade pouch". Regarding anesthesiology session, the Serbian specialists explained the approach to patients with serious intubation and injuries of the head, neck and respiratory organs. The other presented papers on internal medicine, preventive medicine, neuropsychiatry, bioterrorism, etc. were also not only very interesting, but presented on the highest level of quality, as well.

So, we could point out that the Congress was not only a chance to share medical experience and the obtained results, but also to share time, make friends, expanding good relations to the colleagues from the region. Our kind hosts patiently introduced us to their tradition, history and cultural heritage.

The next, 19th BMMC Congress will take place in Bulgaria in 2014.

Col. Prof. Dr. Đoko Maksić
President of the Serbian National BMMC Counsel

valo je nepodeljenu pažnju prisutnih. Lekari iz Srbije su u domenu plastične hirurgije i ortopedije izneli su svoja iskustva u rekonstruktivnim zahvatima i zbrinjavanju rupture patelarne tetive. Neurohirurzi su prikazali svoje novine i dostignuća u zbrinjavanju neurohirurških povreda. Opšti hirurzi su izložili rad o svom iskustvu u lečenju ileusa nastalog kod kamena žučne kese. Urologija se predstavila rezultatima hirurške tehnike za izradu neobešike od dela terminalnog ileuma po našoj originalnoj tehnici nazvanoj „*Belgrade pouch*". Na anesteziološkoj sesiji prikazan je pristup bolesnicima sa otežanom intubacijom i povredama glave, vrata i disajnih organa. I ostali izloženi radovi naših kolega iz interne medicine, preventivne medicine, neuropsihijatrije, bioterorizma izazvali su veliko interesovanje, a izlaganje, kvalitet radova kao i način njihovog saopštavanja bili su na najvišem nivou.

Na kraju možemo istaći da je ovo bila ne samo prilika za razmenu stručnih iskustava i prikaz postignutih rezultata, već i za druženje, upoznavanje i produbljivanje dobrih odnosa sa kolegama iz regiona. Ljubazni domaćini su se potrudili da učesnike Kongresa upoznaju sa svojom tradicijom, istorijom i kulturnim nasleđem.

Naredni, 19. kongres BKVM biće održan 2014. godine u Bugarskoj.

pukovnik prof. dr Đoko Maksić,
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Poziv za reklamiranje u 2013. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline*), *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je *on line* dostupan od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2012. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
2.	Oglas u c/b tehnici A4 formata za celu godinu (11-12 brojeva)	200 000,00 dinara
3.	Oglas u boji A4 formata za jedan broj	35 000,00 dinara
4.	Oglas u boji A4 formata za celu godinu (11-12 brojeva)	330 000,00 dinara
5.	Oglas u boji na koricama K3 za jedan broj	50 000,00 dinara
6.	Oglas u boji na koricama K3 za celu godinu (11-12 brojeva)	455 000,00 dinara
7.	Oglas u boji na koricama K2 i K4 za jedan broj	55 000,00 dinara
8.	Oglas u boji na koricama K2 i K4 za celu godinu (11-12 brojeva)	530 000,00 dinara

Za sva obaveštenja, uputstva i ponude obratiti se redakciji časopisa „Vojnosanitetski pregled“. Sredstva se uplaćuju na žiro račun kod Uprave javnih plaćanja u Beogradu broj: 840-941621-02 **VMA (za Vojnosanitetski pregled ili za VSP)**, **PIB 102116082**. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om) na adresu: Vojnosanitetski pregled, Crnotravska 17, 11000 Beograd; tel/faks: 011 2669 689, e-mail: vsp@vma.mod.gov.rs ili vmavsp@hotmail.com

UPUTSTVO AUTORIMA

Vojnosanitetski pregled (VSP) objavljuje radove koji ranije nisu nigde publikovani, niti predati za publikovanje redosledom koji određuje uređivački odbor. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“ neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu potpisanu od strane svih autora da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjava o pojedinačnom doprinosu autora mora biti potpisana od strane svakog autora rada, skenirana i poslata uz rad kao dopunska datoteka. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa. Tim postupkom svi autori postaju odgovorni za ispunjavanje svih postavljenih uslova, čemu sledi odluka o prihvatanju za dalji uređivački postupak. Za objavljene radove VSP zadržava autorsko pravo. **Primaju se radovi napisani samo na engleskom jeziku.**

Od 1. januara 2012. godine Vojnosanitetski pregled prešao je na e-Ur: Elektronsko uređivanje časopisa.

Svi korisnici sistema: autori, recezenti i urednici moraju biti registrovani jednoznačnom e-mail adresom. Registraciju je moguće izvršiti na adresi:

http://aseestant.ceon.rs/index.php

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme** ili **metaanalize**, **kazuistika**, članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga, referati iz naučne i stručne literature i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

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

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina.

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

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje koresponding autoru na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** i **literatura.**

1. ***Naslovna strana***

a) Naslov treba da bude kratak, jasan i informativan i da odgovara sadržaju rada. Podnaslove treba izbegavati.

b) Ispisuju se puna imena i prezimena autora.

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen i mesta u kojima se ustanove nalaze, sa jasnim obeležavanjem odakle je autor, koristeći standardne znake za fus-note.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **uvod i cilj** rada, osnovne procedure - **metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi - **rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt (**250** reči) ima podnaslove: *uvod/cilj, metode, rezultati* i *zaključak*. Za apstrakte na engleskom dozvoljeno je i do **450** reči. Strukturisani apstrakt je obavezan za metaanalize (istog obima kao i za originalne članke) i kazuistiku (do 150 reči, sa podnaslovima *uvod, prikaz slučaja i zaključak*). Ispod apstrakta, pod podnaslovom „Ključne reči“ predložiti 3–10 ključnih reči ili kratkih izraza koji oslikavaju sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost etičkog komiteta.

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

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

Literatura

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

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.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tabele

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

Ilustracije

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

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

Skraćenice i simboli

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

Detaljno uputstvo može se dobiti u redakciji ili na sajtu:

www.vma.mod.gov.rs/vsp/download/uputstvo_za_autore.pdf.

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From January 1, 2012 the Vojnosanitetski pregled has been edited using the service e-Ur: Electronic Journal Editing.

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http://scindeks-eur.ceon.rs/index.php/vsp

VSP publishes: **editorials, original articles**, **short communications**, **reviews**/**meta-analyses, case reports**, from the **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, extensive abstracts of interesting articles from foreign language journals, and other contributions. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided. Observational and experimental articles, reviews and meta-analyses, should not exceed 16 pages (including tables and illustrations); case reports – 6; short communications – 5; letters to the Editor, reports on scientific meetings and book reviews – 2.

All measurements should be reported in the metric system in terms of the International System of Units (SI). Standard, internationally accepted terms should be used.

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs**.** Avoid the use of colors in graphs.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the first author for corrections that should be returned within 3 days. Manuscripts accepted for publication are not being returned.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with key words; Text; References**.

1. ***Title page***

a) The title should be concise but informative. Subheadings should be avoided;

b) Full name of each author;

c) Name and place of department(s) and institution(s) of affiliation, clearly marked by standard footnote signs.

2. ***Abstract and key words***

The second page should carry a structured abstract with the title for original articles, metanalyses and case reports. The abstract should state the purposes of the study or investigation, basic procedures (selection of study subjects or laboratory animals; observational and analytical methods), main findings (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasize new and important aspects of the study or observations. S t r u c t u r e d abstract should contain typical subtitles: *background/aim, methods, results* and *conclusion*. The abstract for metaanalyses and obrginal papers should have up to 450 words, and up to 150 words for case reports (with subtitles *background, case report, conclusion*). Below the abstract authors should provide, and identify as such, 3–10 key words or short phrases that will assist indexers in cross-indexing the article and will be published with the abstract.

3. ***Text***

The text of original articles is divided into sections with the headings: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

In the **Introduction** repeat the title of the article, excluding the names of authors. State the purpose of the article and summarize the rationale for the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

Methods. Describe your selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Identify the methods, apparatus (manufacturer's name and address in parentheses), and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approvement of the Ethnics Committe for the tests in humans and enimals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and important aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

References should be superscripted and numbered consecutively in the order in which they are first mentioned in the text. **The references must be verified by the author(s) against the original document.** List all authors, but if the number exceeds 6, give 6 followed by et al. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be designated as ”in press“. Information from manuscripts not yet accepted should be cited in the text as ”unpublished observations“. References are cited according to the ***International Committee of Medical Journal Editors.*** *Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med 1997; 126: 36–47. Updated October 2001.*

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

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.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tables

Each table should typed double-spaced on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

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Figures are submitted as photos which should be sharp. Letters, numbers, and symbols should be clear and even throughout and of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure. If a figure has been published, acknowledge the original source.

Legends for illustrations are typed on a separate page, with arabic numerals corresponding to the illustrations. Identify and explain each one clearly in the legend symbols, arrows, numbers, or letters used to identify parts of the illustrations. Explain the method of staining in photomicrographs.

Abbreviations and symbols

Use only standard abbreviations. Avoid abbreviations in the title and abstracts. The full term for which an abbreviation stands should precede its first use in the text.

Detailed Instructions are available at the web site:
www.vma.mod.gov.rs/vsp/download/instructions_to_authors.pdf.

VOJNOSANITETSKI PREGLED
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Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2013. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

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3. Virmanom po prijemu profakture.

Datum _____	Potpis _____

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
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Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

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