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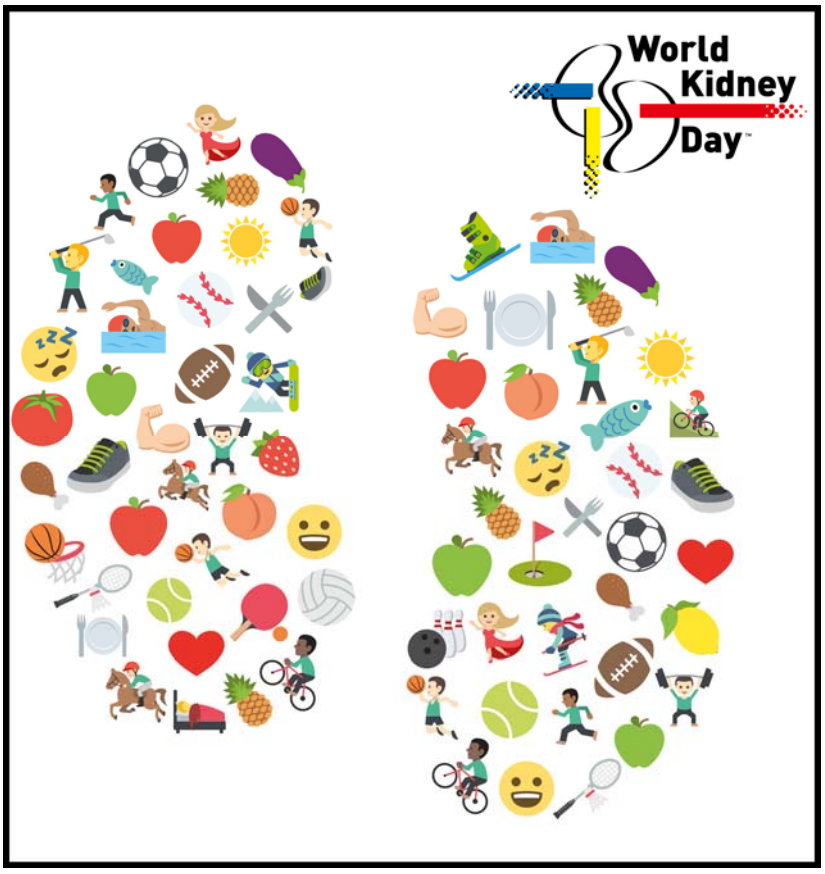

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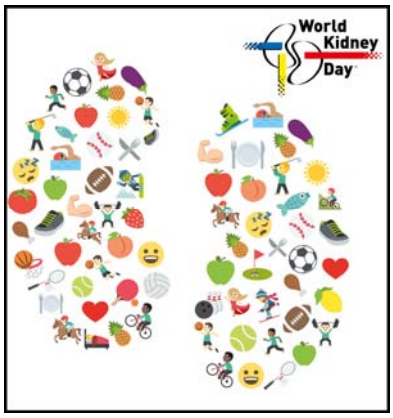

World Kidney Day is established in 2006 as a joint initiative of the International Society of Nephrology and the International Federation of Kidney Foundations. At present, the date is observed in close to 100 countries around the world on March 9 with aim to raise awareness of kidney-related diseases and importance of kidney health to our overall health. It also tries to highlight preventative measures in an attempt to reduce the frequency and impact of kidney diseases and related health problems, on a global scale.

Svetski dan bubrega ustanovljen je 2006. godine od strane Međunarodnog društva za nefrologiju i Međunarodne federacije fondacija za bubreg. Danas se obeležava u gotovo 100 zemalja širom sveta, svake godine 9. marta, sa ciljem podizanja svesti o značaju očuvanja zdravlja bubrega za ukupno zdravlje čoveka. Poseban akcent stavljen je na preventivne mere koje treba da smanje učestalost i značaj bolesti bubrega i pratećih zdravstvenih problema na globalnom nivou.

E D I T O R I A L

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The Author and the Reviewer of the Year 2016 awards by the Vojnosanitetski Pregled

Priznanja Autor i Recenzent godine Vojnosanitetskog pregleda za 2016. godinu

Silva Dobrić

Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia

As it is well-known, every year, since 1996, on March 2 the Day of the Military Medical Academy in Belgrade (the institution in which Editorial Office of the Vojnosanitetski Pregled is situated), the Publisher and the Editorial Board of the Journal assign the Author of the Year award to an author who in the previous year published the largest number of papers in the Journal. This award was established with an intention to emphasize the importance of publishing in medical journals for advancing medical science and profession as well as the impact of the Journal itself in the medical scientific community.

Selecting critera for the award were established in 1996 and include the number and category of articles published in the year for which the award is assigned, and the author's order in the byline. As shown in Table 1, original articles and the first or sole author, provide the highest score.

Besides the Author of the Year award, since 2013 the Reviewer of the Year award of the Vojnosanitetski Pregled has also been assigned with aim to emphasize reviewer's important role in improving the quality of papers published in the Journal. Criteria for this award include the number and quality of reviews, and their submission in a due time.

Having in mind the above-mentioned criteria, the Author of the Year 2016 of the Vojnosanitetski pregled is Branka Roganović, MD, PhD, a gastroenterologist from the Clinic of Gastroenterology and Hepatology, Military Medical Academy (MMA) in Belgrade. She is an Assistant Profesor of Internal Medicine at the Faculty of the MMA, University of Defence in Belgrade. In 2016, she published two original articles as the first author (Table 2) and, among 768 authors, achieved the highest score – 24.

Table 1

Criteria for author and article scoring in the *Vojnosanitertski Pregled*

Article category	Score		
	first author	second author	third author
Original article	12	6	3.6
Preliminary report	5	2.5	1.5
General review	10	5	3
Current topic	8	4	2.4
Case report	4	2	1.2
History of medicine	5	2.5	1.5
Editorial	5	2.5	1.5

Table 2

Articles of Assist. Prof. Branka Roganović published in the *Vojnosanitetski pregled* in 2016

No.	Article category	Authors and title of article
1	Original article	***Roganović B**, Perišić N, Roganović A*. The usefulness of endoscopic ultrasonography in differentiation between benign and malignant gastric ulcer. Vojnosanit Pregl 2016; 73(7): 657-62.
2	Original article	***Roganović B**, Perić S, Janković S.* The impact of in-hospital nutritional status deterioration on treatment outcome of adult gastroenterological patients. Vojnosanit Pregl 2016; 73(8):764-9.

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The Reviewer of the Year 2016 of the Vojnosanitetski Pregled is again Col. Slobodan Obradović, MD, PhD, a cardiologist from the Clinic of Emergency Internal Medicine, MMA, a Full Professor of Internal Medicine at the Faculty of Medicine of the MMA, University of Defence in Belgrade. This is the third time that Prof. Obradović receives this award. In last year he reviewed the largest number of papers, totally 23, submitted to the Vojnosanitetski Pregled.

I have a great pleasure to, on behalf of the Publisher, the Editorial Board and the Editorial Staff of the Vojnosanitetski Pregled, as well as in my own name, congratulate Assist. Prof. Branka Roganović and Prof. Slobodan Obradović on their awards wishing them all success in future, hoping that they will remain loyal to the Journal and continue fruitful cooperation as its authors and reviewers.

I also wish to thank all other authors and reviewers of the Vojnosanitetski Pregled on their effort to improve its quality and impact in the fields which thematically covers.

The short Curriculum Vitae of the Author of the Year 2016 by the Vojnosanitetski Pregled – Assist. Prof. Branka Roganović, MD, PhD

Assist. Prof. Branka Roganović, MD, PhD – the Author of the Year 2016 by the *Vojnosanitetski Pregled.*

Curricula vitae of the Author and the Reviewer of the Year 2016 of the Vojnosanitetski Pregled are given beneath.

Assist. Prof. Branka Roganović was born in Kraljevo, Serbia, in 1960, where she finished elementary and high school. She graduated from the Faculty of Medicine, University of Belgrade in 1984, after which in the period 1985-1990 worked as a general physician in the Medical Center "Dr. Ljubinko Đorđević" in Kraljevo. She finished specialization in internal medicine at the Faculty of Medicine in Belgrade in 1995, and since October that year has worked in the Clinic for Gastroeneterology and Hepatology, Military Medical Academy in Belgrade. Subspecialization in gastroenterology she finished at the at the Military Medical Academy in 2006. In this institution she defended her master's thesis "Endoscopic ultrasonography in the diagnosis of benign and malignant gastric ulcers" in 2001, and PhD thesis "Factors deteriorating nutritional status of gastroenterological patients during hospitalization and their impact on the course and outcome of disease" in 2007. She continued studies in the field of clinical nutrition, which is the subject of her narrow interests, in Graz (2002) and Prague (2006). The results of research in the field of nutrition she presented at several international and domestic congresses (Prague 2007, Florence 2011, Belgrade 2008).

Assist. Prof. Branka Roganović is a member of the National Association for Clinical Nutrition of Serbia since its establishment in 2000, the European Society for Clinical Nutrition and Metabolism (ESPEN) since 2001, the Serbian Medical Society, and the Gastroenterology Association of Serbia.

Dr. Branka Roganović was elected teaching assistant of internal medicine at the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgarde in 2012, and last year (November 2016) Assistent Professor of internal medicine at the same faculty.

She was the author or co-author of 21 professional and scientific papers published in domestic and international peer-reviewed journals.

The short *Curriculum Vitae* of the Reviewer of the Year 2016 by the Vojnosanitetski Pregled – Col. Prof. Slobodan Obradović, MD, PhD

Col. Prof. Slobodan Obradović, MD, PhD - the Reviewer of the Year 2016 by the *Vojnosanitetski Pregled.*

Curriculum vitae of Col. Prof. Obradović was already published in Editorials of the third issues of the Journal in 2013, and 2016, when he received the Reviewer of the Year of the Vojnosanitetski Pregled award. However, because of our new readers, a short presentation of his biography is again given.

Col. Professor Slobodan Obradović, a full professor of Internal Medicine at the Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade, graduated from the Faculty of Medicine, University of Belgrade in 1994 with the average mark of 9.74. In a period 1995–1999 and in 2002 he specialized in internal medicine and cardiology, respectively, in the Military Medical Academy in Belgrade.

Since the end of 1999, Prof. Slobodan Obradović has been working at the Clinic for Emergency Internal Medicine, Military Medical Academy, Belgrade, where has been the Head since October 2013.

Prof. Slobodan Obradović defended both his master's thesis and PhD dissertation in the Military Medical

Academy, related to the field of hemostasis in acute coronary syndromes and percutaneous coronary intervention. He was chosen for Assist. Prof, Assoc. Prof, and Full Prof. of Internal Medicine in 2004, 2011, and 2016, respectively (Faculty of Medicine of the Military Medical Academy, University of Defence in Belgrade).

Prof. Slobodan Obradović was a 3-year (2008–2012) leading investigatior of the project "Stem Cells in Ischemic Heart Disease Treatment" on which he published a few scholarly papers including a chapter in the book "Stem Cells in Clinic and Research" (Gholamrezanezhad A., editor, Rijeka: In Tech; 2011). The results obtained in that project were included in the meta-analysis that was published in „Circulation Research" in April, 2015. This article was proclaimed as the best article in the Journal that year.

Prof. Slobodan Obradović has been doctorial dissertation advisor/mentor of numerous PhD students in cardiology, being very active in advising young researchers. He is one of the authors of the monograph "Pulmonary thromboemboly – case reports" published in 2011 reporting on 32 cases treated in the Clinic for Emergency Internal Medicine, Military Medical Academy, Belgrade, and accounting for the major diagnostic and therapeutic procedures in this condition.

Prof. Slobodan Obradović published numerous scientific articles on hemostasis, percutaneous coronary intervention, acute coronary syndrome and stem cells transplantation in cardiology. A period of 2009–2011 was marked by presidency of Prof. Slobodan Obradović in the Group for Thrombosis and Hemostasis, Serbian Association of Cardiologists. At the moment he is a member of several advisory bodies (councils) for anticoagulant and antiplatelet drug use in Serbia. Also, it is worth to mention, Prof. Slobodan Obradović has been a member of the Editorial Board of the Vojnosanintetski Pregled since 2010 and co-editor for the field of cardiology since 2014.

It should be emphasized that in spite of very demandable professional and scientific research engagement, Prof. Obradović finds time to write, not only scholarly articles and reviews, but also short stories and lyrics usually on everyday medical practice.

ORIGINAL ARTICLES

UDC: 617.7-089
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Anatomical and functional factors influencing the results of scleral buckling procedure for macula-off rhegmatogenous retinal detachments

Anatomski i funkcionalni faktori koji utiču na rezultate klasične hirurške procedure kod bolesnika sa regmatogenom ablacijom retine

Igor Kovačević*†, Aleksandra Radosavljević*†, Ivan Stefanović*†, Bojana Radović*, Jelena Karadžić*, Dragana Kovačević Pavićević*†, Olgica Djurković-Djaković‡

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Abstract

Background/Aim. Rhegmatogenous retinal detachment is a potentially blinding condition of the posterior segment of the eye. Currently, the only treatment modality is surgery and surgical options include scleral buckling, *pars plana* vitrectomy and pneumoretinopexy. Many factors may influence the outcome of the surgery. Well defined indications are essential for achieving the best postoperative results. The aim of this study was to assess anatomical and functional outcome of treatment with scleral buckling for macula-off rhegmatogenous retinal detachments. **Methods.** This prospective, nonrandomized, interventional study included consecutive patients underwent scleral buckling for macula-off retinal detachment in the tertiary centre for vitreoretinal surgery. **Results.** A total of 168 consecutive patients (mean age 58.2 ± 13.9 years) were included in the study. Postoperatively, anatomical success was achieved in 152 (90.5%) of the patients. Parameters that influenced the anatomical success included the number of retinal breaks ($p = 0.040$), lens status ($p = 0.041$), preoperative proliferative vitreoretinopathy ($p < 0.001$), patients' age ($p = 0.049$), and marginally, the presence of typical symptoms ($p = 0.057$). Duration of macular detachment, previous ocular trauma and refraction of the eye did not affect the anatomical result. Functional success was evaluated using the postoperative visual acuity and depended mainly on the duration of macular detachment prior the surgery. Visual acuity 0.4 or better was significantly more often achieved if duration of macular detachment was up to seven days ($p < 0.001$). Refraction and patients' age did not influence the functional result. **Conclusion.** Scleral buckling is an efficient surgical procedure for treatment of patients with retinal detachment. Optimal results are achieved if operation is performed within the first seven days of duration of macular detachment.

Key words:
retinal diseases; retinal detachment; ophthalmologic surgical procedures; treatment outcome.

Apstrakt

Uvod/Cilj. Regmatogena ablacija retine je oboljenje zadnjeg segmenta oka koje, ako se ne leči, može dovesti do slepila. Trenutno, jedini način lečenja je hirurški i opcije su klasična metoda (*scleral buckling*), *pars plana* vitrektomija i pneumoretinopeksija. Mnogi faktori mogu uticati na ishod operacije. Jasno definisane hirurške indikacije su ključne za postizanje dobrih postoperativnih rezultata. Cilj ove studije bio je da se procene faktori koji utiču na anatomski i funkcionalni ishod lečenja klasičnom metodom lečenja bolesnika sa regmatogenom ablacijom retine sa zahvaćenom makulom. **Metode.** U prospektivnu, nerandomizovanu, interventnu studiju bili su uključeni svi bolesnici operisani klasičnom metodom zbog ablacije retine sa zahvaćenom makulom, u tercijarnom centru za vitreoretinalnu hirurgiju. **Rezultati.** Ukupno 168 bolesnika (starosti 58,2 ± 13,9 godina) bilo je uključeno u studiju. Postoperativno, anatomski uspeh postignut je kod 152 (90,5%) bolesnika. Parametri koji su uticali na anatomski uspeh operacije bili: su broj ruptura retine ($p = 0,040$), status sočiva ($p = 0,041$), preoperativno prisustvo proliferativne vitreoretinopatije ($p < 0,001$), starost ($p = 0,049$) i, granično, prisustvo tipičnih simptoma ($p = 0,057$). Dužina odvojenosti makule, ranije povrede oka i refrakcija oka nisu uticali na anatomski rezultat. Funkcionalni uspeh procenjivan je na osnovu postoperativne vidne oštrine i zavisio je uglavnom od preoperativne dužine trajanja odvojenosti makule. Vidna

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oštrina 0,4 ili bolja bila je značajno češće postignuta ako je dužina trajanja odvojenosti makule bila do sedam dana ($p < 0,001$). Refrakcija oka i životno doba bolesnika nisu uticali na funkcionalni ishod operacije. **Zaključak.** Klasična metoda *scleral buckling* je efikasna hirurška procedura za nekomplikovane slučajeve ablacije retine. Kada je makula zahvaćena, najbolji rezultati postižu se operacijom u toku prvih sedam dana od početka simptoma.

Ključne reči:
retina, bolesti; retina, ablacija; hirurgija, oftalmološka, procedure; lečenje, ishod.

Introduction

Rhegmatogenous retinal detachment (RD) is a potentially blinding condition of the posterior segment of the eye that develops as a result of full-thickness retinal defects that allow liquefied vitreal gel to reach into the subretinal space and detach neurosensory retina from the retinal pigment epithelium (RPE) [1]. The only current treatment is surgery, and surgical procedures include scleral buckling (SB), pars plana vitrectomy (PPV) and pneumoretinopexy [2]. Well defined surgical indications are essential for achieving the best postoperative results. Anatomical restitution rates after both SB and PPV are over 90% [3–5]. Clinical features and prognosis of RD mainly depend on the presence of detachment of neurosensory retina in the macular area [6, 7]. In macula-off RD cases, despite the successful surgical repair and excellent anatomical results, functional recovery is limited by the time-dependent damage of the photoreceptors in the macular area [7]. Restitution of visual acuity is possible, but is most commonly incomplete and therefore, time elapsed between macular detachment and surgery, known as duration of macular detachment (DMD), is critical for postoperative results [8].

As mentioned, precisely defined indications and excellent surgical technique are essential for successful postoperative results. There is a long tradition of RD surgery in Serbia, with the first surgical procedure performed in 1926. The most prominent vitreoretinal surgeons that were masters of those methods and teachers of nowadays Serbian ophthalmologists are Milan Blagojević, Zlatimir Kecmanović, Vasilije Misita and Miloš Ignjačev [9–11].

The aim of this study was to evaluate the results of SB for the repair of rhegmatogenous macula-off RD, with the emphasis on the influence of DMD on postoperative anatomical results and visual function.

Methods

A prospective, nonrandomized, interventional study included consecutive patients hospitalized for macula-off RD between January 1, 2013 and December 31, 2013, who had SB performed at the single referral centre for vitreoretinal surgery in Serbia. The patients underwent complete ophthalmological examination including medical history, best corrected visual acuity (BCVA) assessment (measured by Snellen chart at 6 m and converted to decimal notation), applanation tonometry, slit lamp examination and indirect ophthalmoscopy with 90D and 20D lens. DMD was established for each patient, as the period between the onset of decreased vision and surgery.

Only patients with uncomplicated rhegmatogenous RD involving macula were analyzed in the study. The exclusion criteria were complicated cases of RD [multiple retinal breaks in more than two quadrants, giant tears, posterior breaks unreachable for SB or extensive proliferative vitreoretinopathy (PVR), who were operated by PPV], ocular pathology that might affect visual acuity (amblyopia, glaucoma, corneal pathology, macular diseases, previous vitreoretinal surgery), and persons in whom visual function could not be assessed (neurological or psychiatric diseases). The last two groups were operated either by SB or PPV, but were not analyzed in the study (19 and 5 cases, respectively).

SB was performed under general anesthesia, according to a standardized protocol which included localization of the retinal breaks using indirect ophthalmoscopy, transscleral cryopexy of the breaks and suturing of an encircling silicone band and silicon explant, that corresponded in size and localization to the position of the breaks, to the sclera. External drainage of subretinal fluid was not performed due to the risk of potential complications [12].

The postoperative outcome was evaluated three months after surgery and included anatomical and functional results. Anatomical result of the surgery was classified as retina reattached to the RPE or persistent RD. The functional result was assessed using the difference in pre- and postoperative best corrected visual acuity.

The study followed the tenets of the Declaration of Helsinki, and was approved by the Ethics Committee of the Clinical Centre of Serbia.

Data were analyzed using SPSS 15.0. Comparison of categorical variables, such as DMD groups, history of eye trauma, symptoms of RD, number of retinal breaks, the presence of PVR, lens status, refraction of the eye and age groups, was carried out using χ^2 or Fisher test (as appropriate). Comparison of numerical variables, such as change in pre- and postoperative BCVA, was performed using the general linear model for repeated measures (with Bonferroni's adjustment). The level of statistical significance was 0.05.

Results

The study involved a total of 168 patients who underwent SB for macula-off RD and fulfilled other inclusion criteria. Demographic and clinical findings of patients are presented in Table 1.

According to the anatomical outcome, the majority of the patients (90.5%) had retinal reattachment achieved, while 9.5% patients had persistent RD (Table 1). Comparative analysis of their characteristics (Table 2) showed that significantly better anatomical results were achieved in

Table 1

Demographic and clinical characteristics of 168 patients underwent scleral buckling (SB) for macula-off retinal detachment (RD)

Variable	Values
Sex, n (%)	
male	97 (57.7)
female	71 (42.3)
Age in years, $\bar{x}$ ± SD (range)	58.2 ± 13.9 (8–83)
Age in years, n (%)	
< 50	32 (19.0)
50–65	87 (51.8)
> 65	49 (29.2)
Affected eye, n (%)	
right	89 (53.0)
left	79 (47.0)
Refraction of the affected eye, n (%)	
emmetropia	124 (73.8)
hyperopia	1 (0.6)
myopia	43 (25.6)
≥ - 3 D	9 (5.4)
< - 3 and ≥ - 6 D	13 (7.7)
< - 6 D	21 (12.5)
Lens status, n (%)	
transparent	112 (66.7)
cataract	24 (14.3)
pseudophakia	26 (15.5)
aphakia	6 (3.6)
History of ocular trauma, n (%)	
no	157 (93.5)
yes	11 (6.5)
Typical symptoms of RD, n (%)	
none	111 (66.1)
present	57 (33.9)
Number of retinal breaks	
1	103
2	28
3	18
≥ 4	19
PVR, n (%)	
present	13 (7.7)
absent	155 (92.3)
DMD (days), range	
DMD (days), n (%)	2–90
1–10	37 (22.0)
1–4	7 (4.2)
5–7	12 (7.1)
8–10	18 (10.7)
11–30	76 (45.2)
> 30	55 (32.7)
Anatomical outcome of SB, n (%)	
reattached retina	152 (90.5)
residual RD	16 (9.5)
BCVA of the affected eye, $\bar{x}$ ± SD	
at admission	0.15 ± 0.24
90 days post SB	0.27 ± 0.25
p value	< 0.001

n – number of patients; $\bar{x}$ – mean; SD –standard deviation; PVR – proliferative vitreoretinopathy; DMD – duration of macular detachment; BCVA – best corrected visual acuity.

Table 2

Anatomical outcome of scleral buckling (SB) in patients with macula-off retinal detachment (RD) according to baseline clinical characteristics

Variable	Patients, n (%)		*p*
	reattached retina	persistent RD	
DMD (days)			
1–10	33 (89.2)	4 (10.8)	
11–30	70 (92.1)	6 (7.9)	0.807
> 30	49 (89.1)	6 (10.9)	
History of ocular trauma			
yes	10 (90.9)	1 (9.1)	0.960
no	142 (90.4)	15 (9.6)	
Typical symptoms of RD			
present	55 (96.5)	2 (3.5)	0.057
none	97 (87.4)	14 (12.6)	
Number of retinal breaks			
1	97 (94.2)	6 (5.8)	0.040
≥ 2	55 (84.6)	10 (15.4)	
PVR			
present	7 (53.8)	6 (46.2)	< 0.001
absent	145 (93.5)	10 (6.5)	
Lens status			
transparent	105 (93.8)	7 (6.2)	0.041
cataract or post cataract surgery	47 (83.9)	9 (16.1)	
Refraction of the affected eye			
emmetropia and hyperopia	112 (89.6)	13 (10.4)	0.509
myopia	40 (93.0)	3 (7.0)	
Age (years)			
< 50	26 (81.2)	6 (18.8)	0.049
50–65	83 (95.4)	4 (4.6)	
> 65	43 (87.8)	6 (12.2)	

PVR – proliferative vitreoretinopathy; DMD – duration of macular detachment.

patients with a single retinal break ($p = 0.040$), without PVR ($p < 0.001$), with a transparent lens ($p = 0.041$) and in those 50–65 years old ($p = 0.049$). The outcome in the patients presenting typical symptoms (flashes of light, floaters and visual field defects) was better but this difference was only close to statistically significant ($p = 0.057$). On the other hand, parameters including DMD, the history of ocular trauma and refraction of the eye did not affect the anatomical result of SB ($p > 0.05$).

Among 152 of the patients who achieved retinal reattachment, we further investigated functional outcome. The change in the BCVA between admission and 90 days post-SB was used as a parameter of functional recovery. Overall, a significant increase in visual acuity was observed ($p < 0.001$) (Table 1). The patients with shorter DMD (≤ 10 days) had significantly better both preoperative and post-SB BCVA compared to those with DMD 11–30 days ($p = 0.001$) or > 30 days ($p < 0.001$) (Table 3). On the other hand, there was no difference in post-SB BCVA between patients with DMD 11–30 and > 30 days. The DMD group 1 was further divided into subgroups with DMD 1–4, 5–7 and

Table 3

Functional outcome of scleral buckling (SB) in patients with macula-off retinal detachment (RD) according to duration of macular detachment (DMD)

DMD (days)	BCVA, $\bar{x} \pm SD$		$p^{\#}$
	at admission	90 days post SB	
1–10	0.29 ± 0.33	0.42 ± 0.28	< 0.001
11–30	0.12 ± 0.21***	0.26 ± 0.24**	< 0.001
> 30	0.09 ± 0.16	0.18 ± 0.18	0.001
1–4	0.44 ± 0.35	0.51 ± 0.28	0.427
5–7	0.35 ± 0.34	0.53 ± 0.25	0.013
1–7	0.39 ± 0.34	0.52 ± 0.25	0.012
8–10	0.18 ± 0.30*	0.30 ± 0.26**	0.034
1–7	0.39 ± 0.34	0.52 ± 0.25	0.001
8–30	0.13 ± 0.23***	0.26 ± 0.24***	< 0.001
> 30	0.09 ± 0.16	0.18 ± 0.18	0.001

#p* – value between best corrected visual acuity (BCVA) at admission and 90 days post-SB; *,**,, statistical significance at level $p < 0.05, < 0.01, < 0.001$, respectively, *vs* previous entry in column; $\bar{x}$ – mean; SD – standard deviation.**

8–10 days (comprising 7, 12 and 18 patients respectively), among which no difference in post-SB BCVA was observed (Table 3), presumably due to a small number of patients and consequential lack of statistical power. As the BCVA was quite similar in DMD subgroups 1–4 and 5–7 days, they were aggregated and compared to the 8–10 days DMD subgroup, and significantly higher both preoperative and post-SB visual acuities were observed in the 1–7 days subgroup (Table 3). Therefore, patients from DMD subgroup 8–10 days were added to those in DMD 11–30 days group, and BCVA was analyzed in the transformed groups (1–7, 8–30 and > 30 days). The patients with the shortest DMD had a significantly better visual recovery ($p < 0.001$) (Table 3).

Finally, analysis of to the postoperative visual function showed that BCVA 0.4 or better was more likely to be reached in patients with DMD 1–7 days (72.2%), as compared to DMD 8–30 (23.5%) and > 30 days (16.3%) ($p < 0.001$) (Table 4).

when compared to multiple breaks, was associated with better anatomical result, confirming that multiple breaks increase the risk for failure of SB [8, 16, 19]. It is more likely to overlook a retinal break or fail to seal them all, in cases with multiple breaks. Early PVR was a significant predisposing factor for unfavourable anatomical outcome [7, 17]. PVR restricts the retina and SB cannot prevent this process. In cases with extensive proliferative vitreoretinopathy, PPV should be performed.

An association of anatomical success with the presence of typical symptoms may be expected since those patients were operated earlier and had a lower chance of developing retinal changes including PVR. Indeed, significantly better anatomical results in the presence of typical symptoms (82% and 77% respectively, $p = 0.014$), were shown in the MUSTARD study [1]. We achieved anatomical success in 96.5% of the patients with typical symptoms *vs* 87.4% of those who did not present symptoms. However, this difference was only marginally significant.

Table 4

Functional outcome of scleral buckling (SB) 90 days post-SB in 152 patients with anatomic success according to the duration of macular detachment (DMD)

DMD (days)	Patients, n (%)		*p*
	BCVA ≥ 0.4	BCVA < 0.4	
1–7	13 (72.2)	5 (27.8)	
8–30	20 (23.5)	65 (76.5)	< 0.001
> 30	8 (16.3)	41 (83.7)	
Total	41 (27.0)	111 (73.0)	

BCVA – best corrected visual acuity.

The functional results of SB were the same in patients with different refractive errors ($p > 0.05$). Emmetropic (including one hyperopic patient) and myopic patients alike achieved significantly better post-SB BCVA ($p < 0.001$). The patients below 50 years of age had better preoperative BCVA when compared to those older than 65 years ($p = 0.047$). Postoperative BCVA, although better than the initial one in all age groups, did not differ significantly.

Discussion

SB is a conventional surgical procedure for the repair of uncomplicated RD. The rate of anatomical success in the presented series was 90.5%. This is comparable to 94% and 88.5% the data of bicenter studies by Pastor et al. [13], and Falkner-Radler et al. [14] respectively, and even better 80.4% than the large multicenter MUSTARD study [1].

The anatomical success rate did not depend on DMD, ocular trauma and refraction of the eye, but depended on the number of retinal breaks, the presence of PVR, lens status and patients' age and marginally on the presence of typical symptoms. These results, with small differences, confirm previous findings [7, 15–19]. We did not find any difference in anatomical success in the groups of patients with different refractive errors. Interestingly, the MUSTARD study reported better anatomical results in moderate myopes (-2.75 to -8 dioptres, 86.7%) [18]. The presence of a single retinal break

Patients with a transparent lens had a significantly higher rate of retinal reattachment than those with cataract or previous cataract surgery. The presence of cataract in phakic or capsule opacities in pseudophakic eyes can reduce visualisation of peripheral retina and make the detection of retinal breaks more difficult. Therefore, those patients can have poorer surgical outcomes, as shown by Vukosavljević et al. [20]. However, Thelen et al. [1] reported equal success in patients with a natural lens, aphakia and pseudophakia (80.49%, 79.18% and 81.95% respectively), but the results could not be compared since their study did not analyze patients with natural lens according to its transparency.

The best anatomical results were achieved in patients of 50–65 years of age. In contrast, in the study of Hassan et al. [21] no significant difference was observed in the rate of retinal reattachment in different age groups (≤ 60, 61–75 and > 75 years of age). Thelen et al. [1] reported that the best anatomical results were achieved in the younger age group (21–30 years; 87.79%) and poorest in children up to 10 years (77%) and very old patients (71–80 and 81–90 years; 74.07% and 70.48% respectively). The great diversity of reported results warrants further investigations.

In patients with anatomical success, functional results were analysed. Many preoperative factors including visual acuity [6, 7, 19, 22], DMD [6, 8, 19, 21], height of RD [22], vitreomacular traction [12], as well as postoperative factors including epireti-

nal membranes [23] and persistent submacular fluid [22–24] can influence visual recovery. Functional results mainly depend on the time lapse between the separation of neurosensory retina from the RPE in the macular area and surgical repair. In our series, the best functional recovery was achieved if SB was performed within the first 7 days after the symptoms occured. After that period postoperative BCVA decreased significantly. Postoperative visual acuity was significantly higher than preoperative BCVA in all patients irrespective of DMD, except for the patients with 1–4 days of DMD. In this group, improvement in visual acuity did not reach statistical significance due to good initial BCVA. Postoperative visual acuity was equally good within the period of 1–7 days of DMD and the majority of these patients (72%) achieved visual acuity of 0.4 or better (mean BCVA 0.52). Previous studies reported shorter, equal or longer DMDs as critical for the satisfactory postoperative results, including 3 [25], 6 [8], 7 [3, 7, 15] and 10 days [21]. In a series of 96 patients, Liu et al. [15] reported a BCVA ≥ 0.4 achieved in 68% of patients with DMD of 1–7 days (mean BCVA 0.45) and concluded that postoperative BCVA was the same within this period. On the other hand, Hassan et al. [21] analyzed 94 patients with macula-off RD and concluded that DMD of 1–10 days was a significant period for satisfactory functional results (when compared to DMD groups 11 days to 6 weeks and > 6 weeks). In our study, the patients with DMD 1–10 days had overall better visual recovery, but when stratified into subgroups of 1–7 and 8–10 days, a significantly better postoperative BCVA was observed in favor of DMD 1–7 days. Diederen et al. [8] reported similar findings, concluding that the critical DMD was 6 days.

Refraction of the eye did not affect the postoperative BCVA and in all groups significant improvement was achieved. However, Yang et al. [26] reported that low grade myopic patients (up to -6 D) regained significantly better postoperative visual acuity as compared to both high grade myopic (> -6 D) and emmetropic patients.

Functional outcome of SB did not differ among the age groups, although it was slightly better in younger patients due to the best preoperative BCVA. The fact that functional results are better in younger patients has been shown in studies of Pastor et al. [13] and Hassan et al. [21].

Conclusion

SB is an efficient surgical procedure for uncomplicated macula-off rhegmatogenous RD cases with a single retinal break, transparent lens and without PVR. The presented results show that equally good postoperative results are achieved if operation is performed at any time within the first seven days of DMD, whereas after this period functional recovery significantly decreases.

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Declaration of interest

Authors declare no conflict of interest.

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Differences in IL-1β and IL-6 levels in the gingival crevicular fluid during acute phase of orthodontic tooth movement between juveniles and young adults

Različiti nivoi citokina IL-1β i IL-6 u gingivalnoj tečnosti tokom početne faze ortodontskog pomeranja zuba kod dece i mladih

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Abstract

Background/Aim. There is little information, about the difference in cytokine levels in the gingival crevicular fluid (GCF) during orthodontic tooth movement (OTM), between juveniles (children) and young adults (adults). The aim of this study was to examine the levels of interleukins IL-1β and IL-6 in GCF of these two age groups during the acute phase of OTM. **Methods.** The subjects, 10 children and 10 adults, underwent OTM of a single tooth, with an untreated antagonistic tooth used as the control group. GCF was sampled from both the control and treatment sites right before the beginning (the baseline) and 24 h, 72 h and 168 h upon initiation of OTM. Cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA). **Results.** The levels of both GCF IL-1β and IL-6 showed a bimodal peak during early phase of OTM, at 24 h and 168 h, in both age groups. As the statistic has shown, the increase in IL-1β levels was more prominent after 168 h in treated teeth of children, compared to both children's control teeth and treated teeth of adults, whilst the GCF IL-6 levels in the same group increased significantly after 24 h, as well as after 168 h, approximately 70 and 55 fold, respectively. In the same time periods the increase of IL-6 levels in GCF of adults was notably lesser, averaging approximately 5 and 10 fold, respectively, compared to the control teeth. In addition, the amount of tooth movement was statistically larger for children than for adults 168 hours upon the initiation of OTM. **Conclusion.** GCF IL-1β and IL-6 were increasingly expressed during initial phase of OTM in both children and adults. However, excretory response of cytokines in children's GCF, especially the concentration of IL-6, was at a significantly higher level than that of adults', which accords to the finding that the initial OTM is faster in children.

Key words:
tooth movement; child; adolescent; adult; interleukin-6; interleukin-1 beta; gingival cervicular fluid; acute-phase proteins.

Apstrakt

Uvod/Cilj. Postoji malo podataka o razlikama u nivou citokina u gingivalnoj tečnosti (GT) kod dece i odraslih u toku ortodontskog pomeranja zuba (OPZ). Cilj ove studije bio je da se ispitaju koncentracije citokina IL-1β i IL-6 u GT kod dece i odraslih u toku rane faze OPZ. **Metode.** Ispitavanje je obuhvatilo dve grupe ispitanika – 10 dece i 10 odraslih osoba, kod kojih je postavljen ortodontski separator između drugog premolara i prvog molara na jednoj strani, a suprotna strana je služila kao kontrolna. Uzorci GT uzimani su i sa lečenih i sa kontrolnih zuba i to pre, 24 h, 72 h i 168 h nakon postavljanja separatora. Nivo citokina određivan je *enzyme-linked immunosorbent assay* (ELISA) metodom. **Rezultati.** Praćenje koncentracije IL-1β i IL-6 u GT u toku rane faze OPZ pokazalo je dva pika vrednosti za oba citokina u obe grupe ispitanika – 24 h i 168 h od postavljanja separatora. Međutim, porast vrednosti IL-1β bio je statistički značajno veći kod dece nakon 168 h u GT lečenih zuba u odnosu na kontrolne, kao i u GT lečenih zuba dece u odnosu na lečene zube odraslih ispitanika. Što se tiče koncentracije IL-6 u GT, ona je kod dece bila statistički značajno viša u GT lečenih zuba o odnosu na kontrolne vrednosti kako nakon 24 h posmatranja (povećanje od oko 70 puta), tako i nakon 168 h posmatranja (povećanje od oko 55 puta). Kod odraslih se zapažao isti trend u povećanju koncentracije ovog citokina u GT lečenih zuba u odnosu na kontrolne u posmatranim periodima, ali je to povećanje, iako statistički značajno, bilo manje (oko 5 puta posle 24 h i 10 puta posle 168 h posmatranja) u odnosu na

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isto kod dece. Uz to, stepen pomeranja zuba izražen u mm kod dece bio je statistički viši od istog kod odraslih ispitanika nakon 168 h od primenjenog ortodontskog lečenja. **Zaključak.** IL-1β i IL-6 se povećano luče u GT u toku rane faze OPZ kako kod dece, tako i kod odraslih ispitanika. Međutim, sekretorni odgovor, naročito u pogledu sekrecije IL-6, daleko je veći kod dece nego kod odraslih ispitanike, što je u skladu sa nalazom da je stepen početnog pomeranja zuba brži u ovoj populaciji ispitanika.

Ključne reči:
zub, pomeranje; deca; adolescenti; odrasle osobe; interleukin-6; interleukin-1 beta; gingivalna sulkusna tečnost; proteini akutne faze.

Introduction

During orthodontic correction of tooth position, the remodelling of periodontal ligament and alveolar bone takes place, in response to mechanical load. The early phase of this process is characterized by an aseptic inflammation. Various pro-inflammatory cytokines have been suggested to play their role in it. Inflammatory mediators may trigger the biological processes associated with alveolar bone resorption and apposition [1]. In order to study these factors in humans, non-invasive methods have been developed, which rely on results from gingival crevicular fluid (GCF) samples. The GCF content is presumed to reflect the physiological status of the periodontal ligament [2]. Interleukins (IL) IL-1β and IL-6 are some of the first cytokines to increase in levels in GCF, during the application of orthodontic force. IL-1β is a key mediator, involved in a variety of immune and acute-phase inflammatory response activities, having been detected in GCF during orthodontic tooth movement (OTM) [2–4]. IL-6 interacts directly with bone cells, playing an important role in the local regulation of bone remodelling, as well as in the acute inflammation associated with the OTM [5, 6]. IL-1β is an inducer of IL-6 [7], and they both participate in the complex mechanism of mediators that regulate inflammation.

Although numerous studies on concentration of various cytokines in GCF during OTM have been conducted, there is little data available on the effect of age on cytokine production in humans. The purpose of this work was to examine and compare the expression of IL-1β and IL-6 cytokines in GCF in children and adults, during the early phase of OTM.

Methods

Subjects

The subjects undergoing orthodontic treatment were 10 children (ages 9–14, mean age 13) and 10 adults (ages 19–24, mean age 20), without any health issues, selected according to following criteria: 1) good general health; 2) no antibiotic therapy within 3 months prior to the study; 3) no anti-inflammatory drugs, nor analgesics in the month preceding the study; 4) healthy periodontium with generalized probing depth of 2 mm. The study was performed with the informed consent of the adult patients and the children's parents, and was approved by the Ethics Committee of the Faculty of Medicine in Kosovska Mitrovica.

Application of force

Orthodontic elastic separator (Dentalastics separators blue 2.1 mm, Dentaurum, Germany) was inserted between the second premolar and the first permanent molar in the mandible (experimental site). The untreated antagonistic tooth served as the control group. Both control and experimental sites showed good periodontal status. The amount of tooth movement was measured by digital nonius, using new splint for each patient. The precision of nonius measurement was 0.1 mm.

GCF collection

GCF was sampled at the control and treatment sites right before (the baseline), 24 h, 72 h, and 168 h (the checkpoints) after initiation of orthodontic treatment. Paper strips were inserted into the gingival crevice for 60 s, then transferred into the plastic tubes and stored at -70 °C until use. GCF was eluted from each strip into 250 μL phosphate buffered saline (PBS) and extracted by 5 min centrifugation at 15 G.

Cytokine levels determination

Cytokines levels in GCF were determined using enzyme-linked immunosorbent assay (ELISA) kits specific for each cytokine (Quantikine® HS ELISA Assay, R&D systems Inc. USA), and reported as the total mass (in pg) *per* 60 s GCF sample. The lower detection limits were 0.125 pg/mL and 0.156 pg/mL for IL-1α and IL-6, respectively.

Statistics

Statistical analysis was performed using Mann-Whitney tests and SPSS for Windows $p < 0.05$ was considered significant.

Results

In general, analysis of GCF samples showed detectable amounts of both IL-1β and IL-6, in control and treated teeth of all subjects, throughout the observation period. Moreover, the presence of the IL-1β significantly exceeded that of IL-6 cytokines in all the examined fluid samples.

Control GCF concentrations of IL-1β and IL-6 in children and adults

Concentrations of IL-1β and IL-6 in GCF of the control teeth of children and of adults were similar (Table 1). Both IL-1β and IL-6 were present in measurable amounts in all GCF samples of the control teeth, in both examined groups. Generally, GCF IL-1β concentrations significantly exceeded those of IL-6 in both examined groups, in normal conditions. Mean values of GCF IL-1 β concentrations were between 8.5 pg/min and 10.3 pg/min, and 5.3 pg/min and 6.1 pg/min in the children and adult groups respectively, in observation period, while the mean values of GCF IL-6 concentrations were

Table 1

Control GCF concentrations of IL-1β and IL-6 in children and adults

Interleukin		Baseline	24 h	72 h	168 h	*p*
IL-1β (pg/60 sec)*	Children	8.9 ± 2.4	8.7 ± 2.4	10.3 ± 3.7	8.5 ± 2.4	n.s.
	Adult	6.0 ± 1.9	6.1 ± 1.1	6.0 ± 1.2	5.3 ± 2.2	
IL-6 (pg/60 sec)*	Children	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	n.s.
	Adult	0.05 ± 0.02	0.10 ± 0.03	0.10 ± 0.04	0.02 ± 0.00	

***Values were done as mean ± standard error; ns – non significant; GCF – gingival crevicular fluid; IL – interleukin.**

between 0.03 pg/min and 0.04 pg/min, and 0.02 pg/min and 0.1 pg/min in the children and adult groups, respectively, in the same period. The control values of IL-1 β in GCF were notably higher in children than in adults in all time intervals, but these differences were not statistically significant. However, control GCF IL-6 values were somewhat lower in children than in adults in the same time intervals, also without statistical significance.

GCF concentrations of IL-1β in children and adults during the acute phase of OTM

The concentrations of IL-1 β in GCF of children and adults are shown in Figure 1. Our results indicate a bimodal peak of IL-1β levels in GCF during the early phase of OTM, in both children and adult groups: the baseline concentrations increased 24 h and 168 h after the application of the separator. In addition, by comparison, this increase was more prominent in adults group after 24 h, but in children's group after 168 h. However, 24 h into OTM, the peak IL-1β value of the children group exceeded the said value in the adults group, but this difference was not statistically significant. The only statistically significant difference in GCF IL-1β concentrations was at 168 h between the control and treated teeth of the children ($p < 0.05$).

The comparison of GCF concentrations of IL-1β of orthodontically treated teeth of children and adults is offered in Figure 2. The higher concentrations of IL-1β in GCF of treated teeth were first observed in children, then in adults, at

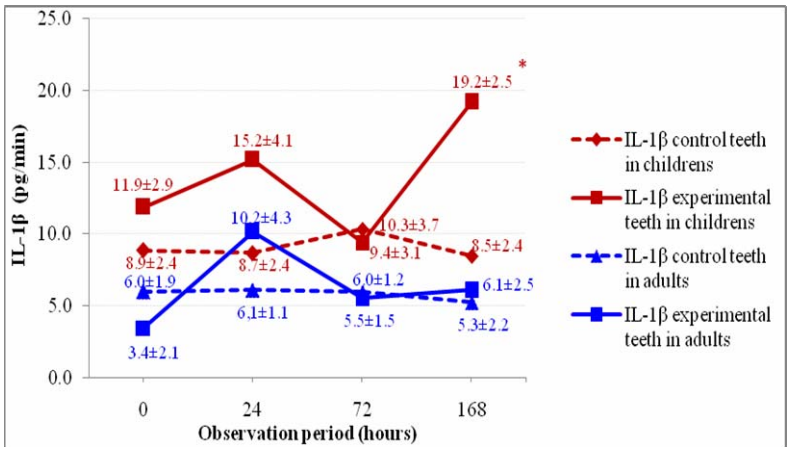

Fig. 1 – Gingival crevicular fluid (GCF) concentrations of interleukin-1β (IL-1β) in children and adults during the acute phase of orthodontic tooth muvement (OTM); *$p < 0.05$ [treated (experimental) teeth in children *vs* control teeth in children].

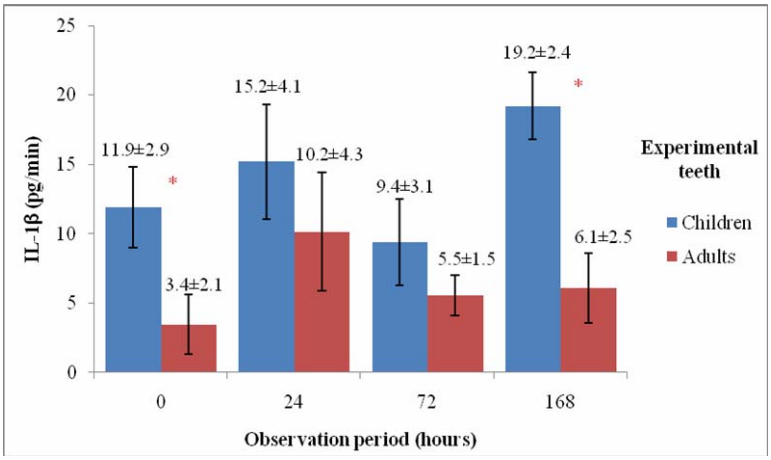

Fig. 2 – Gingival crevicular fluid (GCF) concentrations of interleukin-1β (IL-1β) during the acute phase of orthodontic tooth movements (OTM); *$p < 0.05$ (children *vs* adults).

all checkpoints, but the only statistically significant difference was at the baseline and again at 168 h. Apparently, children had a statistically significantly higher level of IL-1β at the starting point of the experiment ($p < 0.05$), as well as 168 h into OTM ($p < 0.05$).

GCF concentrations of IL-6 in children and adults during acute phase of OTM

The concentrations of IL-6 in GCF in children and adults are shown in Figure 3. Mechanical load applied to tooth triggered a significant release of IL-6 24 h upon initiation ($p < 0.05$), as well as after 168 h ($p < 0.05$), in both examined groups. Peak values at 24 h were higher than at 168 h. Moreover, in addition to statistically significant differences of IL-6 concentrations in GCF between the treated and the control teeth in both examined age groups, in the said checkpoints, higher concentrations were found in the children group. Whilst the GCF levels of IL-6 in the children group increased at 24 h and 168 h approximately by 70 and 55 fold, respectively, in adults they increased approximately by 5 and 10 fold, respectively compared to the control teeth (data not shown). Also, statistically significantly higher IL-6 concentrations in GCF of treated teeth were observed 72 h into OTM only in the children group ($p < 0.05$), approximately a 10 fold increase, compared to the corresponding control teeth (data not shown).

When we compared IL-6 concentration in GCF of treated teeth in adults to those in children, the higher concentrations of IL-6 were observed in children at all checkpoints, but the only statistically significant differences were at 72 h and 168 h ($p < 0.05$) (Figure 4).

The amount of tooth movement after OTM

The amount of tooth movement for children (1.08 ± 0.04 mm) was larger than for adults (0.89 ± 0.04) after 168 h of acute phase of OTM. This difference was statistically significant ($p < 0.003$) (Figure 5).

Correlation between the GCF levels of IL-1b and IL-6 and the velocity of tooth movement after acute phase of OTM

A positive nonsignificant correlation between the levels of both cytokines in GCF and the average velocity of tooth movement was evident in children, while in adults such correlation was registered only for IL-1β (Figure 6).

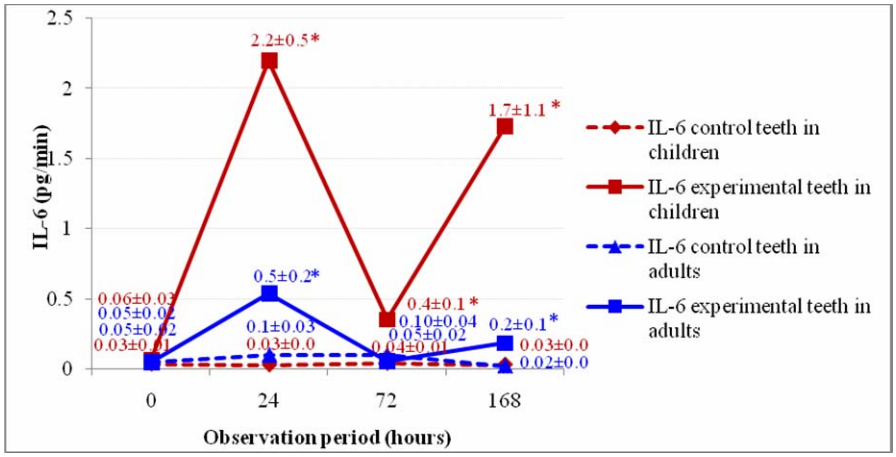

Fig. 3 –Gingival crevicular fluid (GCF) concentracions of interleukin-6 (IL-6) in children and adults during the acute phase of orthodontic tooth movements (OTM); *$p < 0.05$ (children *vs* adults).

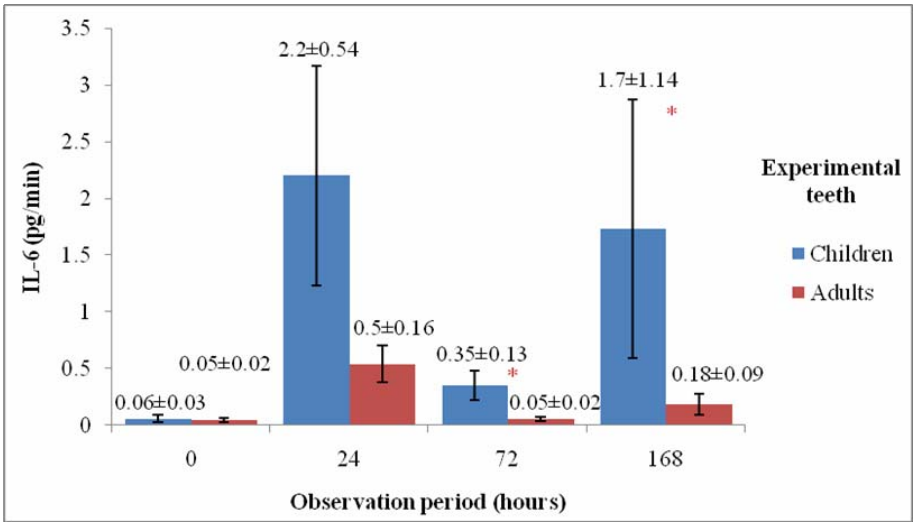

Fig. 4 – Gingival crevicular fluid (GCF) concentrations of interleukin-6 (IL-6β) during the acute phase of orthodontic tooth movements (OTM); *$p < 0.05$ (children *vs* adults).

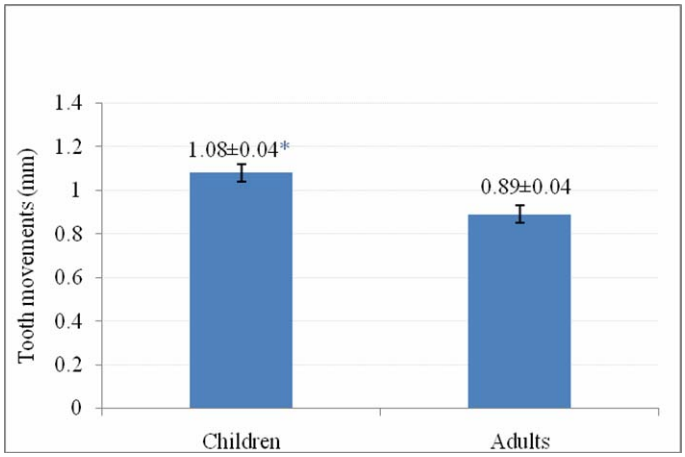

Fig. 5 – Tooth movements (mm) at 168 h after acute phase of orthodontic tooth movements (OTM) in children and adults,*$p < 0.003$ (children *vs* adults).

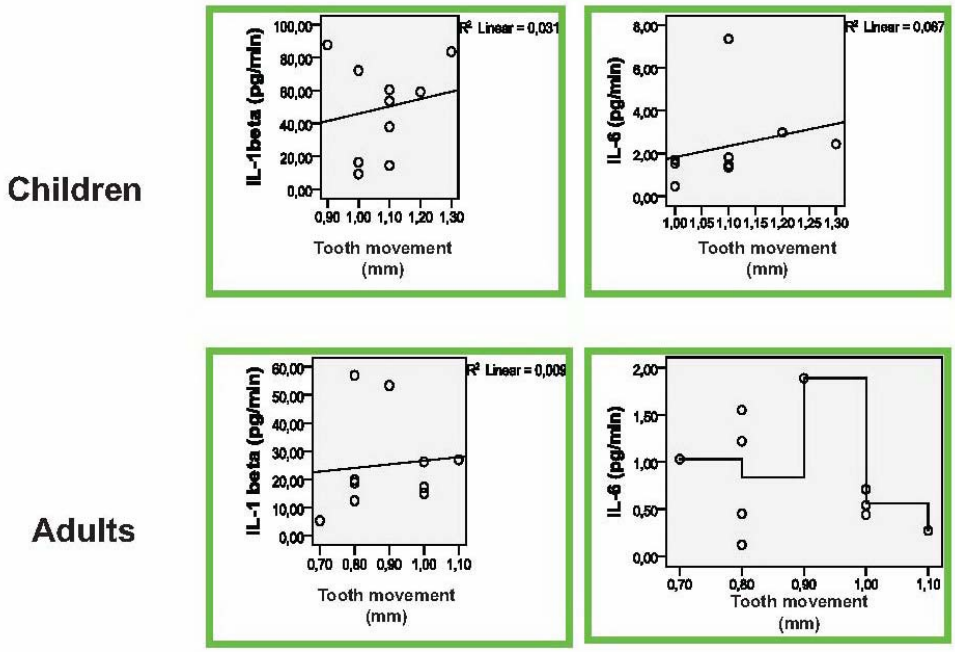

Fig. 6 – Correlation between cytokine levels and the velocity of tooth movement.

Discussion

Age has to be considered as a contributing factor compromising the remodelling potential, *ie* inflammatory response of parodontium during OTM, but the information about cytokine levels and other signal molecules in the GCF regarding patients of different age are sparse. Some of the previous studies demonstrated variations in the levels of different cytokines and signal molecules, including IL-1β, IL-4, IL-6, IL-8, GM-CSF, prostaglandin E2 (PGE2), receptor activator of NF κB ligand (RANKL), osteoprotegerin (OPG) and pentraxin-3, regarding age and orthodontic activation period correlation. Those studies might explain why the speed of orthodontics treatment differs between children and adults [8–13] to such a large extent. The present study was designed to evaluate changes in expression of IL-1β and IL-6 in GCF during the early phase of orthodontic treatment, the difference in this process between children and adults (different age groups), as well as its effects on amount of tooth movement in these two groups.

The contents of IL-1β and IL-6, as detected by ELISA, were measured as total cytokine mass *per* GCF volume secreted in 60 s *per* strip, and expressed in pg/60 s. We believe, as do some other authors, that in the described manner the amount of cytokines in GCF, secreted by periodontal tissues, is presented most realistically [11, 14], and taken most accurately, considering the specific need for the concentration to be expressed in pg/μL GCF or pg/g protein of GCF [15].

The contents of the IL-1β significantly exceeded those of IL-6 cytokines in the fluid of both control and treated teeth, both in children and adults, as had also been reported in other studies [2, 16–18]. Our results indicate that IL-1 β levels in GCFs during the early phase of OTM showed a bimodal

peak in both children and adult groups, but after 24 h this increase was more prominent in the adults group, but after 168 h it was in children. There were statistically significant higher levels of IL-1β in the very beginning of OTM, as well as 168 h from the beginning of treatment, in GCF samples of children's treated teeth, in comparison to those of adults. Our findings are consistent with previously reported data on increased levels of proinflammatory cytokines, including IL-1β and IL-6, in GCF during human OTM [2, 8, 9, 16–18]. In the present study, we found that the content of IL-1β in GCF increased 24 h into OTM in both children and adults, this being the most consistent result reported in the literature [2, 3, 10–15]. However, individual variations of IL-1β in GCF levels were very high, for which reason the only statistically significant difference in concentration of IL-1β in GCF was the one occurring after 168 h between the treated and the control teeth in the children's group. Results of other studies indicate that equivalent force systems during OTM induce individually different cytokine production, which correlates with individual differences in the velocity of canine retraction [15]. There is evidence that IL-1β, IL-1 receptor antagonist (IL-1RA) and IL-1 gene polymorphisms probably play a part in OTM. The speed of tooth movement is related to stress and levels of IL-1β, IL-1RA and IL-1 gene polymorphisms in GCF [19–21]. Three factors that significantly affected the speed and provided the best predictive model for effective teeth movement were: activity index [AI = experimental (IL-1β/IL-1RA)/control (IL-1beta/IL-1RA)], concentration of IL-1RA in GCF and genotype of IL-1B [22].

The source of IL-1β, as well as other cytokines and regulatory molecules, in GCF, during OTM, may more likely come from the compressed periodontal ligament, the resorbing bone adjacent to the root surface or the adjacent gingiva. Hence patients involved in orthodontic treatment belong to different age groups, the age has to be taken into consideration as a contributing factor compromising the remodelling potential of periodontal tissues, as proposed in sparse publications. One recent study proposed that decreased periodontal ligament (PDL) metabolic activity is the reason for lower protein expression of signal molecule including fibroblast growth factor-basic (bFGF), fibroblast growth factor receptor 1 (FGFR1), IL-6, matix metalloproteinase 8 (MMP-8), and matix metaloproteinase 9 (MMP-9) in older patients, and that activity of remodelling process of periodontal tissue decreases with aging and expression of signalling molecule decreases in adults [23]. An explanation of data regarding the statistically significant rise of IL-1β level in children at the start and after 168 h of OTM, compared to the adult group, as well as the explanation to why the peak IL-1β value in 24 h of OTM of the group of children exceeds the equivalent value of adults', lies within the above mentioned finding that there is increased PDL metabolic activity in younger patients [17], and that the advanced level of IL-1β in GCF reflects higher cell activity in the periodontium during OTM [11]. It must be emphasized once again that in our study the second peak of IL-1β after 168 hours of OTM was registered only in the group of children.

IL-6 also showed the highest peak 24 h after placing the separator, in both groups, with a statistical significance, the increase in relation to the initial values being much higher in children than in adults. When we compared IL-6 concentration in GCF of the treated teeth between the adults and the children, the higher concentrations of IL-6 were observed in the later, throughout the observation period, but the only statistically significant differences were after 72 h and 168 h. The explanation for such results could be found in reports of other researchers, stating that inflammatory mediator levels, including IL-6, advance quicker in children than in adults [9].

Although varying in the quantitative level, the observed changes of the two cytokines are matched time-wise. This finding is mostly accordant to the data available in the literature, especially those considering the group (not all authors have examined both cytokines simultaneously), showing that the level of IL-1β and IL-6 both increases rapidly 24 hours after placing the separator [2, 3, 9, 14, 16, 17, 24–27]. Placing separators led to the early inflammatory response of local tissue, which is consistent with the generally accepted view of acute inflammation as a driving force of the process that leads to the remodelling of the periodontal tissues upon mechanical stress. Released at the site of inflammation, whether directly or indirectly (through the substance affected by the synthesis and secretion), they react with bone cells, initiating the process of bone resorption [6, 27–29].

It is known that IL-1β affects the initiation of IL-6 [5], so an increase in the content of IL-6, along the line of increasing IL-1β level, may be due to the described effect of IL-1β. IL-6 is generally considered a proinflammatory cytokine, and it is possible that the increase of its concentration 24 h after the initiation of orthodontic treatment was in function of mediation in the process of acute inflammation. Finding that IL-6 has increased many times over, shows that it is possible this cytokine is indeed part of the feedback mechanism regulating value of IL-1β.

It turned out that teeth movement in relation to the initial position was statistically higher in children (1.08 ± 0.11 mm) than in adults (0.89 ± 0.12 mm). Therefore, a positive correlation, though not statistically significant, was drawn between the tooth movement and the content levels of IL-6 and IL-1β, in children. In the group adults the trend was observed only for IL-1β. There is not much data on the effect of the concentration of cytokines on the degree and rate of tooth movement in the literature. Iwasaki et al. [15] had showed that there is a positive correlation of contents of IL-1β and the rate of tooth movement *per* day, having observed seven patients (mean age 13 years) for 84 days. The trend is somewhat consistent with our finding that in a 7-day period the content levels of IL-1β correlated with the amount of tooth movement. Under identical conditions, large interindividual differences occur regarding the speed of movement of the teeth [30], in both humans and animals, including genetically homogeneous individuals [15–17, 22, 31–38]. For differences in the degree of tooth movement between children and adults, where orthodontically treated teeth of children manifested significantly greater movement than those of adults, we have no clear explanation. Despite the occasional doubts about the effectiveness of orthodontic treatment in adults, clinical experience has shown that the movement of teeth through alveolar bone in adults is workable, but requires more time [38]. The opinion is that adults' biological ability to move the

teeth is reduced by one-third in comparison to that of children's [10, 39, 40]. This assertion is based on thoroughly familiar limitations of adult biological bones, the composition of which changes with aging, in reflection of the cells becoming less reactive, due to the slowing of metabolism.

Conclusion

GCF IL-1β and IL-6 were increasingly expressed during the initial phase of OTM both in children and adults. However, the levels of these cytokines, especially IL-6 concentrations, advance quicker in juveniles than in young adults, which concurs the finding that the initial OTM in juveniles is faster than in adults.

Acknowledgement

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Most common bacterial agents in hospitalized patients with acute exacerbations of chronic obstructive pulmonary disease

Najčešći bakterijski uzročnici kod hospitalizovanih bolesnika sa akutnim pogoršanjem hronične opstruktivne bolesti pluća

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Abstract

Background/Aim. Infection is the major cause of acute exacerbation of chronic obstructive pulmonary disease (AE-COPD). The aim of the study was to establish the most common bacterial agents of AE-COPD in patients hospitalized in a tertiary medical care institution. **Methods**. This retrospective study included the patients hospitalized due to infective AE-COPD in the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica in a 12-month period. We evaluated clinical data, spirometry, pathogen etiology in the patients with positive sputum findings and disease outcome. **Results**. The study included a total of 81 patients, 47 (58.02%) males and 34 (41.97%) females of the mean age of 65.7 years. The mean history of the disease was 14.5 years. The median forced expiratory volume in one second (FEV_1) was 30.12%. The most common isolated pathogens was *Pseudomonas aeruginosa* (n = 36; 38.29%), followed by *Haemophilus influenzae* (n = 25; 26.59%) and *Streptococcus pneumoniae* (n = 16; 17.02%). Coinfections were present in 9 (9.57%) of the patients. The median FEV_1 values of 28.67%, 37.23%, and 42.26% were registered in the patients with *Pseudomonas aeruginosa*, *Haemophilus influenza*, and *Streptococcus pneumonia* induced infection, respectively. The case fatality rate (CFR) was 6.2%. Of the deceased, 100% had *Pseudomonas aeruginosa*-induced infection. A statistically significant difference in FEV_1 values was registered between the patients with and without *Pseudomonas aeruginosa* in their sputum finding ($p = 0.016$). **Conclusion**. The most common pathogens in patients with AE-COPD were *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Streptococcus pneumonia*. The CFR was 6.2%. All (100%) deceased patients had the infection induced by *Pseudomonas aeruginosa*.

Key words:
pulmonary disease, chronic obstructive; acute disease; diagnostic techniques and procedures; sputum; bacteriological techniques; pseudomonas aeruginosa; haemophilus influenzae; streptococcus pneumoniae; mortality.

Apstrakt

Uvod/Cilj. Infekcija je glavni uzročnik akutnog pogoršanja hronične opstruktivne bolesti pluća (AEHOBP). Cilj rada bio je da se odrede najčešći bakterijski uzročnici AEHOBP kod bolesnika koji su hospitalizovani u ustanovi tercijernog ranga. **Metode**. Ova retrospektivna studija obuhvatila je bolesnike hospitalizovane na Institutu za plućne bolesti Vojvodine zbog infektivne AEHOBP u periodu od jedne godine. Analizirali smo kliničke podatke, spirometriju, bakterteriološki nalaz sputuma i ishod bolesti. **Rezultati**. U studiju je bio uključen ukupno 81 bolesnik, 47 muškaraca (58,02%) i 34 žene (41,97%), prosečne starosti 65,7 godina, prosečnog trajanja bolesti 14,5 godina. Prosečni forsirani ekspiratorni volumen u jednoj sekundi (FEV_1) bio je 30,12%. Najčešći izolovani patogen bio je *Pseudomonas aeruginosa* (n = 36, 38,29%), potom *Haemophilus influenzae* (n = 25, 26,59%) i *Streptococcus pneumoniae* (n = 16, 17,02%). Mešana infekcija bila je prisutna kod 9 (9,57%) bolesnika. Prosečni (FEV_1) kod bolesnika sa izolovanim *Pseudomonas aeruginosa* iznosio je 28,67%, sa izolovanim *Haemophilus influenzae* 37,23% i sa izolovanim *Streptococcus pneumoniae* 42,26%. Ukupan letalitet iznosio je 6,2%. Svi (100%) preminuli imali su infekciju uzrokovanu *Pseudomonas aeruginosa*-om. Postojala je statistički značajna razlika u vrednosti FEV_1 između grupa bolesnika sa ili bez izolovanog *Pseudomonas aeruginosa* u sputumu ($p = 0.016$). **Zaključak**. Najčešći izolovani uzročnici AEHOBP bili su *Pseudomonas aeruginosa*, *Haemophilus influenzae* i *Streptococcus pneumoniae*. Ukupan letalitet iznosio je 6,2%. Svi (100%) preminuli imali su infekciju uzrokovanu *Pseudomonas aeruginosa*-om.

Ključne reči:
pluća, opstruktivne bolesti, hronične; akutna bolest; dijagnostičke tehnike i procedure; ispljuvak; bakteriološke tehnike; pseudomonas aeruginosa; haemophilus influenzae; streptococcus pneumoniae; mortalitet.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic progressive pulmonary disease which has a substantial morbidity and mortality [1, 2]. Over three million people died of this disease in 2005. COPD was the sixth most frequent mortality cause in 1990. Furthermore, it was predicted to take the third top position among death causes by 2020 [1]. With the disease progression, exacerbation occurs more frequently, inducing a faster lung function and life quality deterioration [3–6].

The role of microorganisms, particularly bacteria, in COPD exacerbations has been in the focus of research for several decades. Over 70% of COPD exacerbations are due to respiratory infections, and bacterial infections are registered in more than 50% of the cases [2, 4,7–9]. Other exacerbation causes, including air pollution and temperature oscillations, are registered in 30% of the cases, [2, 10] while the causes of exacerbations remain obscure in even one third of the cases [4].

The objective of this study was to identify the infectious agent of COPD exacerbations among the patients hospitalized in a tertiary medical care institution in one-year period. We analyzed if there existed a correlation between the bacterial agent and lung function (forced expiratory volume in one second – FEV_1), or the correlation between the bacterial agent and the treatment outcome, that is a fatal outcome of the disease.

Methods

This retrospective study, included the patients with acute exacerbation of COPD (AE-COPD) hospitalized in the Institute for Pulmonary Diseases of Vojvodina in 2012. The diagnosis was established in compliance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria, with post-bronchodilation FEV1/FVC (FVC – forced vital capacity) of < 70%. Acute exacerbation was diagnosed if the usual daily symptoms changed in terms of deteriorated dyspnea, cough and sputum production, requiring the treatment change.

The following characteristics of all the patients included in the study were reviewed: demographic features, symptoms, smoking habits, disease outcome; they were all submitted to physical examination, spiroplethysmography, staging of the disease in compliance with the GOLD criteria, arterial blood gas analyses, complete blood count, fibrinogen measurement and chest radiography. It was also analyzed which patients required the non-invasive or invasive mechanical ventilation and who had a fatal outcome of their disease. The fatal outcome of the disease was analyzed in correlation to the FEV1 value and the presence of *Pseudomonas aerugionsa* in the sputum.

The patients with radiographycally verified pneumonia were excluded from the study.

Microbiology

Expectorated sputum specimens were collected into sterile containers and processed according to the standard procedures [11]. The microscopic sputum preparations were Gram stained, viewed under low magnification (100 ×), and used to establish leucocyte and epithelial cell counts. The presence of ≥ 25 leucocytes and ≤ 10 epithelial cells in a single field of vision determined the specimen suitable for cultivation [12]. Sputum samples were sputolysine-homogenized, and then, using the calibrated 1 μL loop, cultivated in culture media: blood agar, chocolate agar, and MacConkey agar. Culture plates were then incubated at 37°C, the blood and chocolate agar in the presence of 5% CO_2, and the MacConkey agar in aerobic conditions. The culture plates were examined 24 hours later in order to establish the clinically relevant pathogens. To isolate *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae* and *Staphylococcus aureus*, blood and chocolate agar were used. MacConkey agar was used to isolate *Pseudomonas species*, Acinetobacter species and *Enterobacteriaceae* spp. Bacterial agents were identified by the conventional methods, in compliance with the standard procedures [13]. The bacterial growth was interpreted semiquantitatively, determining the number of Colony Forming Unit (CFU) in one milliliter of the sample. The growth of the isolate with ≥ 10^5 CFU/mL was considered diagnostically relevant [11].

Statistical analysis

Statistical analysis was performed using descriptive statistics, absolute and relative frequencies, mean ± standard deviation (SD) values, $\chi 2$ test, Student *t*-test, and nonparametric test (Mann-Whitney test).

Results

During the examined 12-month period, the positive bacteriological sputum finding was registered in 81 patients treated in the Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, with the total of 94 (27.6%) positive isolates of 340 examined sputum samples in total. Of these patients, there were 47 (58.02 %) males and 34 (41.97%) females. The mean age of the patients was 65.7 ± 8.48. The disease had a 14.5-year long mean history (SD = ± 8.75). Among the examined patients, 56 (69.1%) were active smokers, 17 (21.0%) were ex-smokers, and 8 (9.9%) were non-smokers. The patients' mean FEV_1 amounted to 30.12 ± 12.35%. Demographic features of the examined patients are presented in Table 1.

The patients' clinical features are presented in Table 2. Most patients (69; 85.2 %) had concurrent symptoms of dyspnea and productive cough, while in 12 (14.8%) patients the latter symptoms were accompanied with fever. The mean white blood count was 11.86 ± 5.02 × 10^9/L. Four (4.9%) of the patients required non-invasive mechanical ventilation (NIV). Two (2.5%) of the patients were treated in the Semi-Intensive Care Unit and none in the Intensive Care Unit. The overall CFR amounted to 6.2%.

The most common pathogen was *Pseudomonas aeruginosa* isolated in 36 (38.29%) patients, followed by *Haemophilus influenza* isolated in 25 (26.59%) of the patients, and *Streptococcus pneumoniae* isolated in 16 (17.02%) patients. The mixed bacterial infection type was registered in 9 (9.57%) of the patients. The sputum bacteriology is presented in Table 3.

Table 1

Demographic features of the patients with positive sputum bacteriology hospitalized in the Institute for Pulmonary Diseases of Vojvodina, n = 81

Parameter	Values
Age (years), $\bar{x} \pm SD$	65.7 ± 8.48
Sex, n (%)	
males	47 (58.02)
females	34 (41.97)
Disease history (years), $\bar{x} \pm SD$	14.5 ± 8.75
Smoking habits, n (%)	
active smokers	56 (69.1)
ex-smokers	17 (21.0)
non-smokers	8 (9.9)
Home oxygen concentrator, n (%)	
available	14 (17.3)
unavailable	67 (82.7)
Mean FEV_1 (%), $\bar{x} \pm SD$	30.12 ± 12.35

FEV_1 – forced expiratory volume in one second; n – number of patients.

Table 2

Clinical features of the patients with positive sputum bacteriology hospitalized for AE-COPD, n = 81

Parameter	Values
Hospitalization length in days	14
Dyspnea, cough, sputum production, n (%)	69 (85.2)
Fever, n (%)	12 (14.8)
White blood count, $\bar{x} \pm SD$	$11.86 \pm 5.02 \times 10^9$/L
NIV, n (%)	4 (4.9)
Stay in the semi-ICU, n (%)	2 (2.46)
Overall mortality (%)	6.2

AE–COPD – acute exacerbation of chronic obstructive pulmonary disease; ICU – intensive care unit; NIV – non-invasive mechanical ventilation; n – number of patients.

Table 3

Sputum bacteriology in AE-COPD patients, n = 81

Parameter	n (%)
Total number of isolates	94
Pseudomonas aeruginosa	36 (38.29)
Haemophilus influenzae	25 (26.59)
Streptococcus pneumoniae	16 (17.02)
Acinetobacter spp.	7 (7.45)
Moraxelacatarrhalis	3 (3.19)
Serratia spp.	3 (3.19)
Proteus spp.	2 (2.13)
Stenotrophomonasmaltophilia	2 (2.13)
Mixed infection	9 (9.57)

AE–COPD – acute exacerbation of chronic obstructive pulmonary disease; n – number of patients.

FEV_1 findings were analyzed in correlation to the sputum bacteriology. The patients with isolated *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Streptococcus pneumoniae* had the mean FEV_1 values of 28.67%, 37.23%, and 42.26%, respectively. The correlation of sputum bacteriology and FEV_1 values are reviewed in Table 4.

The CFR was 6.2%. Of the deceased, 100% had the infection induced by *Pseudomonas aeruginosa*. No statistically

Table 4

Sputum bacteriology correlated to FEV_1

Sputum bacteriology	FEV_1 (%)
Pseudomonas aeruginosa	28.67
Haemophylus influenzae	37.23
Streptococcus pneumoniae	42.26
Acinetobacter spp.	24.23
Moraxela catarrhalis	35.20
Serratia spp.	37.73
Proteus spp.	38.25
Stenotrophomonasmaltophilia	18.9

FEV_1 – forced expiratory volume in one second.

significant difference in FEV_1 values was registered between the patients who died and those who recovered (p = 0.307). A statistically significant difference in FEV_1 values was, however, registered between the patients with and without *Pseudomonas aeruginosa* isolated in their sputum samples (p = 0.016).

Disscusion

This retrospective one-year study, carried out in the Institute for Pulmonary Diseases of Vojvodina, analyzed demographic features, bacteriological sputum findings, clinical characteristics and disease outcome in the patients hospitalized for an acute exacerbation of the chronic obstructive pulmonary disease in 2012.

In our study, we found that 81 patients had positive sputum bacteriology, with the total of 94 positive isolates of 340 examined sputum samples in total, or 27.6% positive isolates of all the examined samples. In their report published in the Chest in 2007, Ko et al. [14] reported 32.3% positive sputum isolates of all the examined ones. In their article published in 2015, Ko et al. [15] also reported 37.8% of positive sputum findings, while Boixeda et al. [2] in their study in 2012 reported 24.1% of positive bacteriological sputum findings, correlating well to our results.

Pseudomonas aeruginosa was the most common bacterium isolated in our study, registered in 38.29% of the patients, followed by *Haemophilus influenzae*, isolated from 26.59% of the patients, and *Streptococcus pnaumoniae*, registered in 17.02% of the patients. In the study carried out by Ko et al. [15] in 2005, similar results were obtained for *Haemophyllus influenzae*, which was isolated in 23.1% of their cases, but not for *Pseudomonas aeruginosa* isolated in 6.3%, and *Streprococcus pneumoniae* isolated in only 4.0% of the cases, unlike our study results. Furthermore, Groenewegen and Wouters [16] reported *Haemophilus influenzcae* for the most common bacterium isolated in ther study (in 45% of the cases), followed by *Streptococcus pneumoniae* isolated in 27%, and *Pseudomonas aeruginosa* registered in 15% of their cases, deviating from the results obtained in our study. Our results also differ from those obtained in the study carried out in Taiwan by Lin et al. [5] in 2007, who isolated *Klebsiela pneumoniae* most frequently (19.6%), then *Pseudomonas aeruginosa* (16.8%), and *Haemophilus influenzae* (7.5%).

In our study, *Haemophilus influenzae* was more frequently isolated in the patients with FEV_1 > 30%, while Ko et al. [15] in their study carried out in 2005 isolated this bacterium more frequently in the patients with FEV_1 > 50%, correlating to the results reported by Lin et al. [5] In our study, *Pseudomonas aeruginosa* was more frequently isolated in the patients with FEV_1 < 30%, as it was the case in the study by Ko et al. [14] performed in 2007. In their study carried out in 2009, Larsen et al. [6] reported the patients with FEV_1 < 1.0 L had *Pseudomonas aeruginosa*-induced infections more frequently. Lin et. al. [5] also reported *Pseudomonas aeruginosa* was more frequently isolated in very severe COPD cases, while Groenewegen et al. [16] reported the positive sputum bacteriology was more frequently registered in the patients with lower FEV_1 in their study, but with no significant difference in the isolated bacterial species related to the severity of the disease.

All the deceased patients in our study had *Pseudomonas aeruginosa*-induced infection. Lin et al. [5], applying the multivariate regression analysis in their study, have established the finding of *Pseudomonas aeruginosa* for one of independent case fatality risk factors, quite unlike Larsen et al. [6] who reported the presence of this bacterium in the sputum had no influence on the CFR, which was primarily affected by the patients' age and low FEV_1. In our study, we registered no statistically significant difference in FEV_1 levels between the groups of deceased and recuperated patients (p = 0.307). However, the statistically significant difference in FEV_1 was established between the patients with and without the sputum finding of *Pseudomonas aeruginosa* (p = 0.016).

The major limitation of our investigation is that it included only bacteriological sputum findings, lacking the opportunity to perform virology analyses. Another limitation is that it provided no information on bacterial agents' sensitivity to antibiotics. In addition, our analysis included only hospital-treated exacerbations, lacking the insight into out-patient treated exacerbations. We will try to overcome these limitations in our additional future research work.

Conclusion

The most common bacterial agents isolated in our AECOPD patients were *Pseudomonas aeruginosa*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. All the deceased patients had the infection induced by *Pseudomonas aeruginosa*. The patients with advanced disease stages had *Pseudomonas aeruginosa* sputum finding more frequently.

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Prognostic value of serum parathyroid hormone in ST-elevation myocardial infarction patients

Prognostička vrednost paratireoidnog hormona u serumu kod bolesnika sa infarktom miokarda sa elevacijom ST segmenta

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Abstract

Background/Aim. Parathyroid hormone (PTH) is an important messenger in the regeneration process which might influence the outcome of patients with ST-segment elevation myocardial infarction (STEMI). The aim of this study was to investigate the role of PTH in comparison to other traditionally used markers for the prediction of heart failure in STEMI patients. **Methods.** In 165 consecutive patients with STEMI treated with primary percutaneous coronary intervention (PCI), blood concentrations of PTH, C-reactive protein (CRP), B-type natriuretic peptide (BNP), creatine kinase MB (CK-MB) and admission glycaemia (AG) were measured during the first three days after admission and correlated to the primary outcome – episodes of acute heart failure in the period of six months. **Results.** The area under the ROC curve of the maximal serum concentration of PTH was the largest among the measured biomarkers (0.867 *vs* 0.835 *vs* 0.832 *vs* 0.627 *vs* 0.619, for PTH, CRP, BNP, CK-MB and AG, respectively) for the prediction of primary outcome. The maximal PTH level adjusted to several risk factors had an independent prediction value for primary outcome ($p < 0.001$). In addition, PTH improved the prediction of primary outcome when added to the other markers in the model [c-statistic with BNP, CRP, CK-MB and AG was 0.908 (95% CI 0.849–0.967)], and when PTH was added, it was 0.931 (0.883–0.980), with $p < 0.001$ for the discrimination. **Conclusion.** Serum concentration of PTH early in the course of STEMI can predict acute heart failure episodes in the first six months in patients treated with primary PCI.

Key words:
myocardial infarction; heart failure; biological markers; parathyroid hormone; natriuretic peptides; creatine kinase; c-reactive protein; blood glucose; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Paratireoidni hormon (PTH) značajan je glasnik u regeneracionim procesima i može uticati na ishod kod bolesnika sa infarktom miokarda sa ST elevacijom (STEMI). Cilj ovog rada bio je da se ispita uloga PTH u poređenju sa drugim uobičajenim markerima za predviđanje srčane slabosti kod bolesnika sa STEMI. **Metode.** Kod 165 bolesnika sa STEMI, lečenih primarnom perkutanom koronarnom intervencijom, prva tri dana hospitalizacije merene su vrednosti u serumu: PTH, B-tipa natriuretskog peptida (BNP), kreatin-kinaze miokarda (CK-MB), C-reaktivnog proteina (CRP) i glikemije na prijemu, te je ispitivan uticaj na primarni ishod – epizode akutne srčane slabosti u periodu od šest meseci. **Rezultati.** Površina ispod ROC krive bila je najveća u poređenju sa ostalim markerima (0,867 *vs* 0,835 *vs* 0,832 *vs* 0,627 *vs* 0,619), za PTH, CRP, BNP, CK-MB i glikemiju na prijemu za predviđanje primarnog ishoda. Maksimalna vrednost PTH imala je nezavisnu vrednost predviđanja za primarni ishod ($p < 0,001$). **Zaključak.** Serumske koncentracije PTH u ranoj fazi STEMI mogu predvideti epizode akutne srčane slabosti u prvih šest meseci kod bolesnika lečenih primarnom perkutanom koronarnom intervencijom.

Ključne reči:
infarkt miokarda; srce, insuficijencija; biološki pokazatelji; paratireoidni hormoni; natriuretski peptidi; kreatin kinaza; c-reaktivni protein; glikemija; osetljivost i specifičnost.

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Introduction

The most important role of parathyroid hormone (PTH) is to maintain of calcium homeostasis. However, PTH has several important cardiovascular effects which may be relevant for some pathophysiological states, such as myocardial infarction or acute heart failure [1]. In experimental studies, PTH showed positive inotropic and chronotropic action on myocardium, as well as vasodilatatory effect on arteries [2]. Parathyroid hormone in myocardial infarction may be important messenger in the regenerative process because it takes important role in the mobilization and homing of stem cells [3–5].

On the other hand, chronically higher levels of PTH are associated with arterial hypertension and increased mortality in patients with chronic renal failure, stable coronary disease, chronic heart failure and even healthy elderly men [6–8].

However, not much is known regarding PTH blood concentrations, dynamics and prognostic value in acute myocardial infarction (AMI). More than 20 years ago Ljunghall et al. [9] found elevated levels of PTH in patients with AMI. However, the role and potential prognostic significance for PTH in AMI patients are unknown. Several studies have shown a significant derangement in Ca-VitD-PTH axis in severely ill patients in correlation with higher mortality rate [10, 11].

Therefore, the aim of our study was to determine the serum concentration of PTH in patients with ST elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (PCI) during the first three days of hospitalization and to investigate the association between serum PTH level and episodes of the acute heart failure in a six-month period of time after STEMI.

Methods

The study included 165 consecutive patients with the first STEMI, admitted to the Coronary Care Unit of the Military Medical Academy in Belgrade, between March 2010 and November, 2012. The diagnosis of STEMI was established if a patient had typical chest pain lasting > 20 minutes less than 12 hours before admission, and typical electrocardiographic changes with increase of serum creatine kinase-MB (CK-MB) or troponin concentration elevation above 99% of the reference value. All the patients were treated with the reperfusion therapy, primary percutaneous coronary intervention (pPCI) or thrombolysis with adjunctive urgent PCI according to the guidelines [12, 13]. There was no age limit for study enrollment. The main exclusion criterion was elevated admission serum creatinine level above 115 μmol/L. The other exclusion criteria were the presence of known malignant, infectious or autoimmune disease and death inside the first 24 hours from the symptom onset. All the patients have had scheduled follow-up in the first six months.

The control group presented as the cohort of patients who did not develop acute heart failure symptoms through the 6-months follow-up period.

The study was conducted according to the Declaration of Helsinki and was approved by the hospital Ethical Committee. Written informed consent was obtained from all the participating patients.

Outcomes

The primary outcome was the presence of signs and symptoms of acute heart failure during the 180 days of follow-up after STEMI. The primary outcome was diagnosed during initial hospitalization and through the scheduled visits at 30 and 180 days from the day of admission by the physicians who do not participate directly in the study. Acute heart failure was defined as the presence of typical symptoms and signs required intravenous application of diuretics, re-hospitalization or the need for the increased dosage of oral diuretic therapy.

Laboratory testing

Glycaemia was measured at admission by using commercial Dimension® Clinical Chemistry System. C-reactive protein (CRP) extended range was determined in the serum of patients with turbidimetric immunoassay on commercial Dimension system at the first, second and third day in the morning before meal. Creatine kinase-MB was determined in the serum of patients by the immunoinhibition method on the commercial Dimension® Clinical Chemistry System, at admission and every 6 hours during the next 24 hours, and every 8 hours during the next 48 hours. B-type natriuretic peptide was determined in plasma samples on the commercial ADVIA Centaur analyzer (Siemens Medical Solutions, Fernwald, Germany) using direct chemiluminescence immunoassay.

Parathyroid hormone and total calcium serum concentrations

Intact parathyroid hormon and total calcium serum levels were determined from the venous blood sample withdrawn at the first, second and third morning after admission before meal. Intact PTH was measured in fresh serum inside the 4 hours from the sampling by a commercial two-site sandwich immunoassay using chemiluminometric detection technology. Intact PTH is measured on the ADVIA Centaur analyzer (Siemens Medical Solutions, Fernwald, Germany). The reference range from the test was 1.60–7.00 pmol/L and intra-assay coefficient of variation was 2.7%.

Total serum calcium levels were measured using the calcium o-cresolphtalein method adapted to commercial colorimetric assay on the Siemens Dimension® Clinical Chemistry System with the intra-assay coefficient of variation of 1.9% .

Statistics

Following the test of statistical normality (Kolmogorov-Smirnov test), continuous variables were presented as mean ± standard deviation (SD), or with a skewed distribution as median [interquartile range (IQR)] and quartiles. Biomarker levels were analyzed as continuous variables and as categori-

cal values – quartiles of values for all patients in the study. Categorical variables were reported as counts with percentages. Differences in categorical variables were tested by χ^2 test and between continuous ones with Student's *t*-test or Fisher's oneway ANOVA with Boferroni adjustment. Oneway ANOVA with repeated measurements was applied when the values of PHT and total calcium in three time points were analyzed.

To assess the diagnostic value of each marker, nonparametric receiver operating characteristic (ROC) curves were generated by plotting the sensitivity *vs* 1-specificity. For each marker the optimal cut-off point, sensitivity and specificity were obtained. The areas under the curves (AUC), 95% confidence interval (CI), significance of discriminative power of the marker according to Hanley and McNeil test, and the differences between ROC curves according to de-Long test were calculated.

Unadjusted and adjusted (for all variables which can influence the hazard rate) Cox proportional hazard regression models were used to show the hazard rate for the positive outcome with comparison of the IV quartile of each marker with the other three quartiles.

The Kaplan–Meier method with Log rank test was used to describe and analyze significant differences between survival curves based on quartiles of PTH values.

All analyses were performed using SPSS version 21 (SPSS Inc, Chicago, IL, USA), Stata Version 10.1 (Stata-Corp, College Station, TX), or R-statistical software.

Results

The characteristics of the patients are shown in Table 1. During a 180-day follow-up, 36 (21.8%) of the patients had at least one episode of acute heart failure. The patients with acute heart failure episodes were older, had higher thrombolysis in myocardial infarction (TIMI) risk score at admission and more frequently TIMI2 flow through the infarction related coronary artery after PCI comparing to the control group.

Table 1

Basic demographic and procedural characteristics in patients with and without congestive heart failure symptoms

Patients characteristics	Congestive heart failure symptoms		*p*
	Yes (n = 36)	No (n = 129)	
Age (years), mean ± SD	71 ± 11	60 ± 11	< 0.001
Female, n (%)	12 (33.3)	33 (25.6)	0.399
Risk factors, n (%)			
history of hypertension	31 (86.1)	92 (71.3)	0.085
active smoking	13 (36.1)	73 (56.6)	0.038
diabetes	11 (30.6)	34 (26.4)	0.673
hypercholesterolemia	17 (47.2)	77 (59.7)	0.189
Time from pain onset to reperfusion (hours)			
median	4.0	4.0	0.447
interquartile range	3.0–7.7	2.5–9.0	
TIMI score			
median	7.0	3.0	< 0.001
interquartile range	5.2–9.5	2.5–9.0	
Reperfusion therapy, n (%)			
primary PCI	33 (91.7)	118 (91.5)	1.000
urgent PCI after thrombolysis	3 (8.3)	11 (8.5)	
Multivessel disease, n (%)	28 (77.8)	82 (63.6)	0.161
Infarct related artery, n (%)			
left anterior descending	19 (52.8)	62 (48.1)	0.707
ramus cirkumflexus	6 (16.7)	20 (15.5)	0.592
right coronary artery	11 (30.6)	47 (36.4)	0.559
TIMI flow before PCI, n (%)			
TIMI 0/1	25 (69.4)	99 (76.7)	0.388
TIMI 2	8 (22.2)	12 (9.3)	0.046
TIMI 3	3 (8.3)	18 (14.0)	0.572
TIMI flow after PCI, n (%)			
TIMI 0/1	1 (2.8)	1 (0.8)	0.390
TIMI 2	12 (33.3)	13 (10.1)	0.001
TIMI 3	23 (63.9)	115 (89.1)	0.001
Stent implantation, n (%)	30 (88.9)	117 (90.7)	0.753
Negative ST segment resolution after reperfusion, n (%); (157 patients)	17 (50.0)	40 (32.3)	0.070

TIMI score – thrombolysis in myocardial infarction; PCI – percutaneous coronary intervention.

Parathyroid hormone and total calcium levels in the patients with acute myocardial infarction

Serum concentrations of PTH decreased significantly over three days in the control group, but the decrease did not rich significance in the group of patients who already had symptoms of heart failure, or who had episodes of acute heart failure during the next 6 months (Figure 1a). Among the patients with primary outcome, 31 (86.1%) of them had PTH concentration at the first day above the upper limit of the normal range for the assay (1.6–7.0 pmol/L). However, in the control group 35 (27.1%) of the patients had PTH serum concentration higher than the upper limit of the normal value. Concentrations of serum PTH were significantly higher for all three measurements between the patients with positive primary end-point and the control group [10.84 (7.62–15.65) *vs* 5.31 (3.90–7.50) pmol/L, $p < 0.001$; 9.20 (6.90-13.59) *vs* 4.42 (3.52–5.42) pmol/L, $p < 0.001$; 7.31 (6.03–9.64) vs. 3.95 (3.15–5.51) pmol/L, $p = 0.001$ for measurement at 24, 48 and 72 hours after admission, respectively].

Total serum Ca^{2+} did not significantly change during the first three days after admission in both groups (Figure 1b). There was no significant difference in total serum Ca^{2+} levels during the first three days between the patients with acute heart failure and controls.

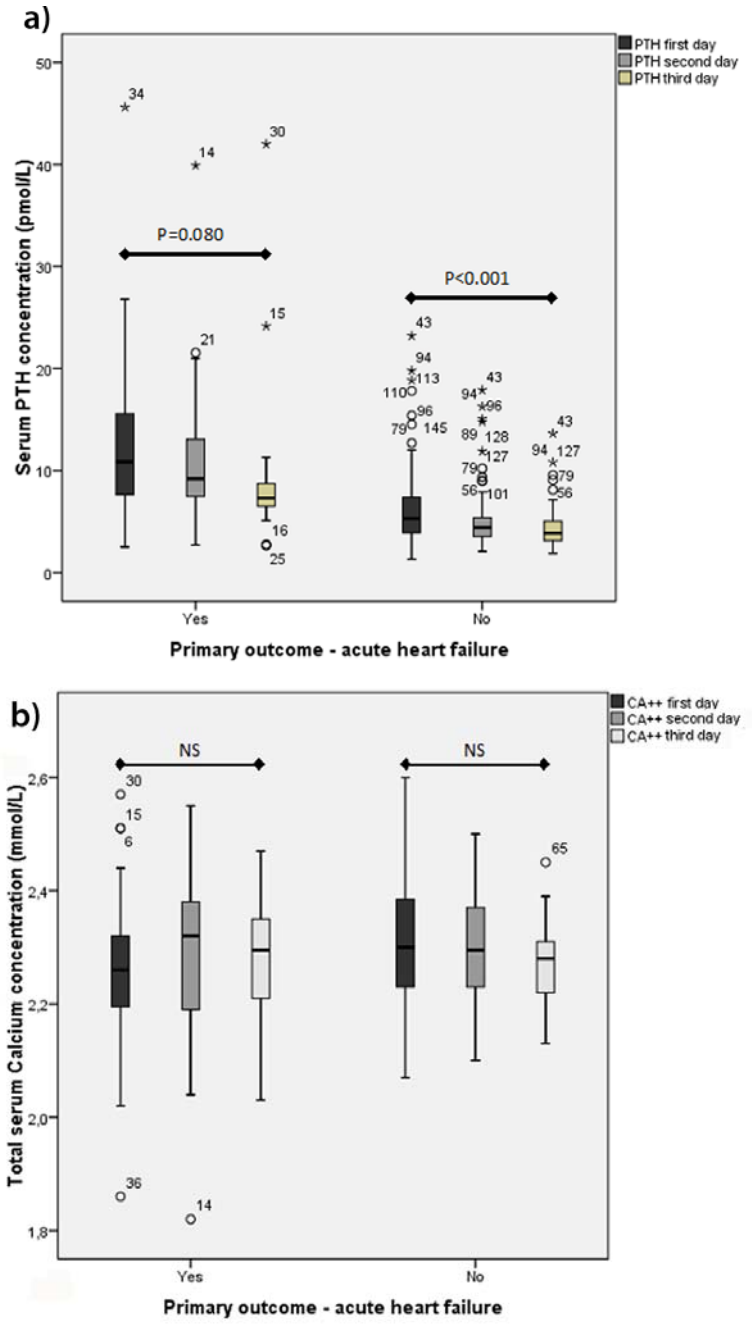

Fig. 1 – a) Serum values of parathyroid hormone (PTH), and b) total serum Ca^{2+} concentration according to the presence of primary outcome – acute heart failure at six months. Boxes in dark gray – the first day, light gray – the second day, and white – the third day from admission.

Biomarkers and clinical outcome

During 180 days follow-up 36 (21.8%) of the patients had at least one episode of non-fatal heart failure. Cox proportional hazards models were created using quartiles of all five biomarkers separately in the univariate and multivariate analysis where important variables (age, gender, the presence of diabetes, hypercholesterolemia, hypertension, TIMI risk score for STEMI, total ischemic time, and TIMI-flow before and after PCI) were included into the model for the adjustment of hazard ratios (Table 2). In the unadjusted models values in the upper quartile compared to other three quartiles of admission glycaemia (HR 1.73; 95% CI 0.87–3.42; $p = 0.113$), maximum CK-MB (HR 1.55; 95% CI 0.77–3.10; $p = 0.215$), maximum C-reactive protein (CRP) (HR 4.23; 95% CI 2.18–8.18; $p < 0.001$), maximum BNP (HR 7.14; 95% CI 3.55–14.35; $p < 0.001$) and maximum PTH (HR 9.78; 95% CI 4.68–20.42; $p < 0.001$) were associated with the occurrence of acute heart failure during the six months follow-up. In multivariable analysis adjusted hazard ratios on age, gender, smoking, the presence of diabetes, hypertension and hypercholesterolemia, time from the pain onset to reperfusion, TIMI risk score and the TIMI flow before and after PCI, were similar for admission glycaemia and CK-MB, slightly attenuated for CRP and BNP and unchanged for PTH: HR 1.51, 95% CI 0.60–3.78, $p = 0.373$ for admission glycaemia; HR 1.42, 95% CI 0.68–2.99, $p = 0.344$ for maximum CK-MB; HR 2.62, 95% CI 1.24–5.54, $p = 0.011$ for maximum CRP; HR 4.19, 95% CI 1.84–9.49, $p = 0.001$ for maximum BNP and HR 8.98, 95% CI 3.58–22.52, $p < 0.001$ for maximum PTH, respectively.

The areas under the ROC curves (Table 3 and Figure 2) for the primary outcome were the greatest for maximum PTH comparing to other four markers. Pairwise comparison

Table 2

Comparison of the levels of biomarkers in the patients with and without congestive heart failure symptoms with Cox proportional hazard regression models

Biomarkers*	Congestive heart failure symptoms		Unadjusted Hazard rate, (95% CI); *p*	Adjusted †Hazard rate, (95% CI); *p*
	with (n = 36)	without (n = 129)		
Admission glycaemia (mmol/L),				
mean ± SD	10.70 ± 5.65	8.83 ± 3.76	1.34 (1.04–1.72); 0.025§	1.68 (1.10–2.55);0.014§
median (IQR)	8.40 (7.55–11.32)	7.70 (6.55–9.45)	1.62 (0.82–3.16); 0.160	1.31 (0.57–2.99); 0.522
Q1 – n, %‡	4, 11.1	36, 27.9	R	R
Q2 – n, %‡	10, 27.8	32, 24.8	e	e
Q3 – n, %‡	9, 25.0	32, 24.8	f.	f.
Q4 – n, %‡	13, 36.1	29, 22.5	1.73 (0.87–3.42); 0.113	1.51 (0.60–3.78); 0.373
Maximum CK-MB (IU/L),				
mean ± SD	248.86 ± 130.70	202.74 ± 151.13	1.25 (0.94–1.66); 0.122§	1.22 (0.89–1.67);0.215§
median (IQR)	237.0 (145.25–334.75)	159 (89.50–278.00)	2.35 (1.16-4.79); 0.018	2.01 (0.93-4.36); 0.076
Q1 – n, %‡	4, 11.1	37, 28.7	R	R
Q2 – n, %‡	7, 19.4	34, 26.4	e	e
Q3 – n, %‡	13, 36.1	29, 22.5	f.	f.
Q4 – n, %‡	12, 33.4	29, 22.5	1.55 (0.77–3.10); 0.215	1.42 (0.68–2.99); 0.344
Maximum CK-MB (IU/L),				
mean ± SD	101.02 ± 80.48	35.25 ± 41.10	1.64 (1.37-1.96); < 0.001§	1.42 (1.10–1.83);0.007§
median (IQR)	79.55 (43.05–133.93)	17.36 (9.25-48.05)	12.32 (3.77–40.23); < 0.001	7.62 (2.23-26.70); 0.001
Q1 – n, %‡	-	41, 31.8	R	R
Q2 – n, %‡	3, 8.3	38, 29.5	e	e
Q3 – n, %‡	13, 36.1	29, 22.5	f.	f.
Q4 – n, %‡	20, 55.6	21, 16.3	4.23 (2.18-8.18); < 0.001	2.62 (1.24–5.54); 0.011
BNP (pg/mL),				
mean ± SD	831.71 ± 582.50	246.23 ± 246.03	1.88 (1.56–2.28); < 0.001§	1.76 (1.33-2.32); < 0.001§
median (IQR)	768.20 (348.14-1225.00)	182.16 (93.71–304.05)	8.86 (3.13–25.09); < 0.001	4.57 (1.46-14.34); 0.009
Q1 – n, %‡	3, 8.3	38, 29.5	R	R
Q2 – n, %‡	1, 2.8	40, 31.0	e	e
Q3 – n, %‡	8, 22.2	34, 26.4	f.	f.
Q4 – n, %‡	24, 66.7	17, 13.2	7.14 (3.55-14.35); < 0.001	4.19 (1.84-9.49); 0.001
Maximum PTH (pmol/L),				
mean ± SD	15.33 ± 9.97	6.38 ± 3.65	1.65 (1.41–1.94); < 0.001§	1.54 (1.20–1.96); < 0.001§
median (IQR)	12.57 (8.88–18.59)	5.40 (4.00–7.73)	12.38 (3.79–40.42); < 0.001	8.15 (2.39–27.79); < 0.001
Q1 – n, %‡	1, 2.8	40, 31.0	R	R
Q2 – n, %‡	2, 5.6	39, 30.2	e	e
Q3 – n, %‡	7, 19.4	35, 27.1	f.	f.
Q4 – n, %‡	26, 72.2	15, 11.6	9.78 (4.68–20.42); < 0.001	8.98 (3.58–22.52); < 0.001

*** Not normally distributed according to Kolmogorov-Smirnov test (Ln transformation was applied and all calculations were done with transformed values).**

† Hazard rates adjusted by age, gender, smoking, the presence of diabetes, hiperholesteronemia and hypertension, time from the pain onset to reperfusion, TIMI score, and the TIMI flow before and after PCI.

‡ Quartiles generated according to distribution of percentiles for all the patients

§ Hazard rates and confidence intervals are expressed *per* standard deviation increase.

Ref – reference value.

CK-MB – creatine kinase-myocardial band; CRP – C-reactive protein; BNP – B-type natriuretic peptide; PTH – parathyroid hormone; TIMI – thrombolysis in myocardial infarction; PCI – percutaneous coronary intervention.

Table 3

Areas under the curves (AUC) of biomarkers for the prediction of congestive heart failure symptoms

Biomarkers	Congestive heart failure symptoms at 180 days *					
	AUC	95% CI	*p*	Cut-off	Sensitivity	Specificity
Admission glycaemia (mmol/L)	0.619	0.518–0.720	0.029	7.1	83.3	39.5
Maximum CK-MB (ng/mL)	0.627	0.532–0.722	0.020	160	75.0	52.7
Maximum CRP (mg/mL)	0.835	0.773–0.898	< 0.001	22.8	100.0	58.1
BNP (pg/mL)	0.832	0.741–0.923	< 0.001	320	80.5	78.1
Maximum PTH (pmol/L)	0.867	0.799–0.936	< 0.001	8.8	77.8	83.7

***Significance levels of pairwise comparison (deLong et al. [14]) of ROC curves: Admission glycaemia *vs* Maximum CK-MB, Maximum CRP, BNP, Maximum PTH (0.941, 0.0002, 0.0006, 0.0001, respectively); Maximum CK-MB *vs* Maximum CRP, BNP, Maximum PTH (0.0001, 0.0008, 0.0001, respectively); Maximum CRP *vs* BNP, Maximum PTH (0.9554, 0.4719, respectively); BNP *vs* Maximum PTH (0.5199). CK-MB – creatine kinase-myocardial band; CRP – C-reactive protein; B-type natriuretic peptide; PTH – parathyroid hormone.**

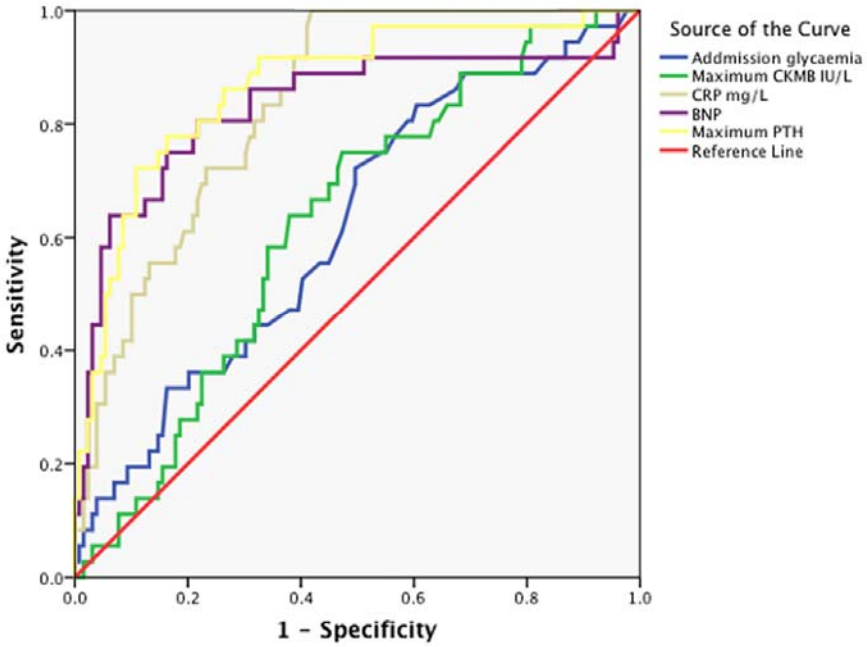

Fig. 2 – Receiver operating characteristic (ROC) curves of biomarkers for primary outcome. CK-MB – creatine kinase-myocardial band; CRP – C-reactive protein; BNP – B-type natriuretic peptide; PTH – parathyroid hormone.

of ROC curves showed that the ROC curve of maximum PTH was better predictor of a six-month primary outcome than admission glycaemia and maximum CK-MB ($p < 0.001$ and $p < 0.001$, respectively). However, there was no significant difference between ROC curves of maximum CRP and PTH as well as BNP and PTH ($p = 0.472$ and $p = 0.520$, respectively) for the prediction of primary outcome. ROC curve analysis was used to establish the best cut-off values of biomarkers for the prediction of primary outcome. For the prediction of primary outcome, maximum PTH > 8.8 pmol/L had sensitivity 77.8 and specificity 83.7, and BNP, for the value greater than 320 pg/mL, similarly had sensitivity 80.5 and specificity 78.1.

Kaplan-Meier plots discovered a higher risk of the six-month primary outcome in the fourth and third quartile of maximum PTH ($p < 0.001$, Breslow test, Figure 3).

Discussion

Our study demonstrates that an increased serum level of PTH is associated with episodes of acute heart failure in the first six months after STEMI, in patients treated with contemporary reperfusion therapy. When we analyzed models of predictive value of PTH for this primary outcome with other four well established biomarkers: admission glycaemia, maximum CK-MB, maximum CRP and BNP, measured during the first three days of infarction, PTH significantly improved predictive value with all individually, but BNP. PTH was not inferior as a predictor of primary outcome compared to conventionally used BNP.

Different biomarkers are used for the prognosis in patients with myocardial infarction [15–21]. We used the most widely and routinely applied biomarkers for the comparison

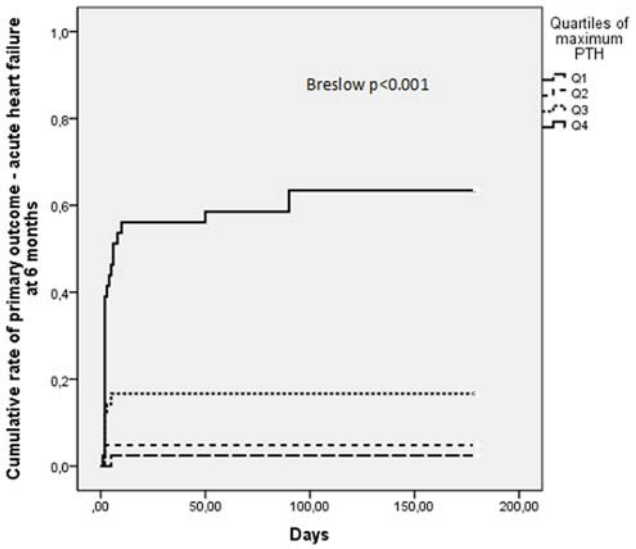

Fig. 3 – Kaplan-Meyer curve for parathyroid hormone (PTH) maximum. Time to acute heart failure symptoms at 180 days according to the quartiles of maximum levels of serum PTH.

with PTH to investigate the predictive models for one of the most important consequences of myocardial infarction and that is the episode of acute heart failure in the first six months after admission. Admission glycaemia in STEMI patients is the consequence of excessive secretion of several hormones and catecholamines into blood and it is associated with heart failure and death irrespective of their diabetic status [15, 16]. Several studies proved the predictive value of serum CRP levels for the outcome in STEMI patients treated with primary PCI [17–19]. BNP is a good prognostic determinant for left ventricular remodeling as well as for other primary outcomes like all-cause mortality in STEMI patients [20, 21]. Creatine kinase and its' isoenzyme creatine kinase-MB was also a good marker of infarction size and can be useful for the prognosis of STEMI [22, 23]. In the large cohort of STEMI patients, Nienhuis et al. [22] have shown that peak CK-MB values were an independent predictor of left ventricle ejection fraction and one-year mortality in STEMI patients treated with primary angioplasty.

Several biochemical markers involved in calcium homeostasis are investigated as prognostic factors in acute coronary syndrome. A low serum calcium concentration at admission in the large cohort of Chinese STEMI patients was an independent predictor of in-hospital mortality [24]. Similarly, low serum levels of 25(OH)D vitamin levels in STEMI predicted well in-hospital and one-year mortality in patients with acute coronary syndrome [25]. In our study, all four traditionally used markers had good predictive value for the primary outcome. Analysis of ROC curves illustrated that maximum PTH level has had the largest area under the curve among all markers, but that the difference between PTH and BNP was not significant and the specificity and sensitivity for their cut-off values, as predictors for primary outcome, were similar.

The increase of serum PTH in STEMI patients are probably due to increased neurohumoral activation with high blood levels of catecholamines and sympathetic activity [26–28]. A significant linear correlation between plasma and platelet epinephrine concentrations and plasma PTH blood levels was found in patients with AMI [29].

What would be potential explanation for the role of PTH in patients with AMI? Direct cardiovascular effects of PTH and its involvement in the regeneration process might be the explanation. Adult cardiomyocytes have PTH-1 receptor which is up-regulated in the state of ischemia [30]. PTH causes influx of Ca^{2+} into cardiomyocytes with positive inotrope and chronotrope action which may represent a compensatory process in patients with myocardial infarction and acute heart failure [1]. On the other hand, PTH effects L-type Ca channels on smooth muscle cells and causes arterial vasodilatation which contribute to the decreased afterload and better myocardium perfusion in the state of acute heart failure [31]. In the mice model of myocardial infarction, PTH reduced the infarction size by the inhibitory effect on apoptosis which is important mechanism of cell death in ischemic myocardium [32].

The role of PTH as important messenger in the regeneration process after myocardial infarction is very intriguing. Through the receptors on osteoblasts, PTH induced secretion of several cytokines, including interleukin (IL)-6, IL-11, vascular endothelial growth factor (VEGF), chemokine stromal cell-derived factor 1 (SDF)-1 and granulocyte colony-stimulating factor (G-CSF) and regulated the stem cell niches environment in the bone marrow and proliferation and mobilization of stem cells [33]. PTH has the pivotal role in mobilization and homing of bone-marrow-derived stem cells into the ischemic myocardium and can provoke neovascularization, decrease the infarction size and improve survival of animals with myocardial infarction [3–5].

Mechanisms which influence the increment of PTH in myocardial infarction depend on the infarction size and the hemodynamic disturbance. Elevated blood levels of catecholamines, increased sympathetic activity, hypocalcemia and hyperphosphatemia, are all associated with severe, large myocardial infarction and signs of acute heart failure and can inc-

rease blood levels of PTH [24–27]. Therefore, cardiovascular and regenerative action of PTH is proportional to the extent of the myocardial damage and that is possible reason for the good predictive value of serum PTH level early in the course of infarction.

Limitation of the study

There are several limitations of this study. Admission PTH level was not obtained. A relatively small number of patients precludes the statistically powered separate analysis for death outcome. Some newer biomarkers which are more expensive and not widely available [34] were not used in this trial.

Conclusion

We concluded that data from our trial show for the first time that one hormone – PTH has a good predictive value for the six-month outcome in STEMI patients, at least as good as traditionally used markers such as admission glycaemia, CRP, CK-MB and BNP. This finding implies the possible important role of PTH in STEMI and further investigation is needed.

Declaration of interests

The authors have no conflict of interest to declare.

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Affective temperament, social support and stressors at work as the predictors of life and job satisfaction among doctors and psychologists

Temperament, socijalna podrška i faktori stresa na poslu u predviđanju zadovoljstva životom i poslom kod lekara i psihologa

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Abstract

Background/Aim. Affective temperament, social support and work-related stresors belong to the group of life and job satisfaction indicators. The aim of this research was to examine predictive roles of the basic affective temperament traits, social support and work-related stressors in the feeling of job and life satisfaction among doctors and psychologists. **Methods.** The sample consisted of 203 individuals out of whom there were 28% male and 72% female doctors (61%) and psychologists (39%), 25–65 years old (39.08 ± 9.29), from the two university towns in Serbia. The set of questionnaires included Serbian version of the Temperament Evaluation of Memphis, Pisa, Paris and San Diego – autoquestionnaire version (TEMPS-A), Satisfaction with Life scale, Job Satisfaction Survey, short Interpersonal Support Evaluation List, and Source of Stress at Work Scale (IRSa) for estimating the frequency of stressors at work. **Results.** According to the existing norms our examinees are satisfied with their life, but considerably less satisfied with their work, specially with pay and benefits, while they are most satisfied with nature of work itself and social relations with co-workers and supervisors. Our results show that depressive and hyperthymic, and to some extent cyclothymic temperament traits of the affective temperament significantly predict 21% of life satisfaction variance. Situational factors, such as stressors at work and social support, are important in predicting job satisfaction (58% of variance) with no significant contribution of temperament traits. The analysis did not point out any significant relation of sex, occupation, and age with life and job satisfaction. **Conclusions.** Affective temperaments can be regarded as predictors of life satisfaction, but in order to better predict satisfaction the aspects of wider social surrounding and sources of stressors at work must be taken in consideration. Future studies should consider other indicators of life satisfaction such as family or health satisfaction, stressors outside of work situations, and negative aspects of caregiving, for example hyperactivation.

Key words:
personality; workplace; occupational exposure; stress, phychological; medical staff; psychology; questionnaires; job satisfaction.

Apstrakt.

Uvod/Cilj. Afektivni temperament, društvena podrška i faktori stresa na poslu spadaju u grupu pokazatelja kojima se može predvideti zadovoljstvo životom i poslom. Osnovni cilj ovog istraživanja bio je da se ispita mogućnost predviđanja zadovoljstva životom i poslom lekara i psihologa, na osnovu afektivnog temperamenta, socijalne podrške i faktora stresa na poslu. **Metode.** Uzorak se sastojao od 203 ispitanika od kojih 28% muškaraca i 72% žena, lekara (61%) i psihologa (39%), 25–65 (39,08 ± 9,29) godina starosti, iz dva univerzitetska grada u Srbiji. Set upitnika uključivao je srpsku verziju *Temperament Evaluation of Memphis, Pisa, Paris and San Diego – autoquestionnaire version* (TEMPS-A) skale, *Subjective Life Satisfaction* (SLS) skalu zadovoljstva životom, *Job Satisfaction Surgery* (JSS) upitnik zadovoljstva poslom, kraću verziju *Interpersonal Social Support* (ISL) skale socijalne podrške, i *Source of stress at work Scale* (IRSa), za procenu učestalosti stresora na radu. **Rezultati.** Prema postojećim normama naši ispitanici su zadovoljni svojim životima, ali značajno manje zadovoljni poslom, naročito platom i beneficijama, dok su najzadovoljniji prirodom posla i odnosima sa kolegama i nadređenima. Rezultati su pokazali da depresivne, hipertimne i donekle ciklotimne crte afektivnog temperamenta mogu značajno predviđati 21% varijanse zadovoljstva životom. Situacioni faktori, kao što su stresori

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na poslu i socijalna podrška, važni su za predviđanje zadovoljstva poslom (58% varijanse), bez značajanog doprinosa crta afektivnog temperamenta. Analizom nisu dobijene značajne razlike u nivou zadovoljstva životom i poslom u odnosu na pol, zanimanje i starost ispitanika. **Zaključak.** Crte afektivnog temperamenta mogu se smatrati prediktorima zadovoljstva životom, ali se moraju uzeti u obzir i aspekti šireg socijalnog okruženja i izvora stresa na radu, kako bi ovo predviđanje bilo potpunije. Buduća istraživanja trebalo bi da uzmu u obzir i druge pokazatelje zadovoljstva životom, kao što su zadovoljstvo porodicom i zdravljem, stresore van konteksta na radu i negativne aspekte brige o ljudima, na primer hiperaktivaciju.

Ključne reči:
ličnost; radno mesto; profesionalna izloženost; stres, psihički; lekari; psihologija; upitnici; posao, zadovoljstvo.

Introduction

Life satisfaction is a concept which represents the cognitive component of the subjective evaluation of personal well-being and happiness, as well as the most frequently used indicator of the quality of life [1]. One of certainly most important components of life satisfaction among adult population is job satisfaction, *ie* attitude people have toward their job, and aspects of the job [2].

There are three perspectives in literature which try to explain the relationship between job and life satisfaction [3]: according to the 'bottom-up' perspective, job satisfaction is an integral part of life satisfaction and together with other life domains it influences total life satisfaction; the 'bottom-down' perspective suggests that life satisfaction influences job satisfaction; regarding the third perspective, much of the relationship between job and life satisfaction is spurious, resulting from common influences, such as environmental variables (*eg*, job income) and personality traits.

In accordance with the last perspective, differences in basic personality traits and 'core self-evaluations' are pointed out as relevant predictors of both life and job satisfaction [4, 5].

Affective temperaments

Contemporary theory of affective temperaments based on Aristotle, Krecmer and Kraepelin's ideas about basic temperamental types [6], refers to temperament as a stable behavior trait with strong affective coloring. These different temperaments (depressive, cyclothymic, hyperthymic, irritable and anxious) are described as sub-syndromic conditions of the affective pathology, and they are likely to represent liability factors in the subsequent development of affective disorders [7].

The majority of earlier studies on temperaments is used for predicting ill-being, or psychological disfunctioning and only a few studies explore where the traits of the affective temperament predict positive characteristics of mental health and concepts of life and job satisfaction. According to Diener et al. [8], the affective component of the subjective well-being construct is comprised of the frequency and intensity of positive and negative affect experience, which is essentially the description of temperament. Fogle et al. [9] established that the traits of extraversion and neuroticism, as major temperament dimensions, are important predictors of life satisfaction among adolescents. Moreover, neuroticism trait has been a significant negative predictor of life satisfaction among medical professionals [10].

It was previously established that some aspects of job satisfaction are also moderately correlated with emotionality [11]. For instance, the correlation of stress at work with affective temperament traits is confirmed [5]. Moreover, the studies showed that choosing occupational orientation can also correlate with temperament traits [12]. These data point out that the affective temperament characteristics should be considered for predicting job satisfaction as well as life satisfaction, with obligatory observation of situational factors in that correlation [9]. Job satisfaction results from the characteristics of the job and experience a person has with certain aspects of job such as: pay, chances of promotion, managers, the system of rewarding, work procedures, co-workers and the nature of work [13].

Helping professions

Helping professions are professions that provide support to people with physical, psychological, and social problems. Doctors and psychologists were the subjects of this research because the area of work in which they are engaged, covers all areas of helping professions such as medicine, nursing, social work, psychotherapy, counseling, education and coaching. These professions are high risk professions for ill-being, low life and job satisfaction [14]. Both medical doctors and psychologists are exposed to numerous factors that can have negative influences on mental health such as high intensity of work, constant exposure to pain, psychic and physical suffering of clients, disbalance between made effort and the reward for that effort, personal and family stresses that are reflected through job, etc.

The impact of organizational factors on subjective well-being and life satisfaction among health workers and other helping professions have been confirmed in numerous studies all over the world [15–17]. This research examined if and to what extent the affective temperament traits were inserted in the correlation between the situational factors (*eg* work-related stress) and life/job satisfaction among psychologists and doctors. According to the model of Argyle and Lu [18] social competence plays the role of the mediator in the correlation between temperament and happiness, due to which perception of social support, as one of the objective measures of the social competence, was introduced in our model.

The aim of this research was to investigate predictive roles of the basic affective temperament traits, social support and work-related stressors in the feeling of job and life satisfaction among doctors and psychologists.

Methods

Sample

The sample consisted of 203 male and female medical doctors and psychologists, 25–65 years old, from the two university towns in Serbia (Table 1). The doctors were employees in governmental health institutions, while psychologists were employees at schools, kindergartens, centres for social work and health institutions. The total number of administered questionnaires was 320 (reply rate of 63%).

The research was conducted from January till March, 2015 after the approval of the Ethics Committee at the Faculty of Medical Sciences in Kragujevac. All the included examinees signed written consent for participating in this research.

Table 1

Sample characteristics

Characteristics	Values
Number of participants	203
Sex, n (%)	
male	56 (27.6)
female	147 (72.4)
Profession, n (%)	
physician	124 (61.1)
psychologist	79 (38.9)
Age (years), $\bar{x}$ ± SD (range)	39.08 ± 9.29 (24–61)
Working experience (years), $\bar{x}$ ± SD (range)	12.19 ± 8.91 (1–33)
Relationship status, n (%)	
in a relationship	164 (81)
single	39 (19)

Instruments

The short form of Temperament Evaluation of Memphis, Pisa, Paris and San Diego – autoquestionnaire version (TEMPS-A) was used in the research, namely the standardized Serbian version of the scale [19]. So far, this self-evaluation questionnaire has been validated in more than twenty languages, representing the most frequently used instrument for assessing basic temperament traits [19]. The scale measures emotional reactivity (*eg*, depressiveness, irritability), cognitive components of the emotional reactions (pessimism or optimism), psychomotor reactions and circadian rhythm (the energy of reactions, sleep schedule, etc.), and social aspects of emotional reactions [20]. In the Serbian version, 41 yes-or-no items are grouped into six instead of five subscales (the anxious temperament is divided into two subdimensions): depressive, cyclothymic, hyperthymic, irritable, anxious-somatic and anxious-cognitive temperament. The scale showed good internal consistency and the construct validity ($\alpha = 0.83$), as well as moderately high alpha coefficient of subscales (0.73–0.80) among adult non-clinical population. The average test-retest coefficient (*rho* = 0.82) suggests stable reliability [19].

The scale Satisfaction with Life (SWLS) measures the cognitive component of life satisfaction, namely it is referred to the personal assessment of well-being and happiness as the most frequent indicators of the quality of life [1]. Participants state the level of agreement with five items (*eg*, "In most ways my life is close to my ideal") on the seven-point Likert scale ranging from 1 – strongly disagree to 7 – strongly agree. The scores range from 5 to 35, while higher scores indicate greater life satisfaction. Test-retest correlation of the scale is 0.82, and Cronbach's α ranges from 0.83 to 0.87 [21].

The Job Satisfaction Survey (JSS) is a nine facet scale, comprised of 36 items describing employees' attitudes about their job and aspects of the job: pay, chances of promotion, supervision, fringe benefits, contingent rewards, operating conditions, co-workers, nature of work, and communication [22]. The summative score, which points out the general degree of job satisfaction, can also be calculated. Although the JSS was originally developed for use in human service organizations, it is applicable to all types of organizations which are the subject of our research, including medical and mental health centres, schools, social services [2]. It is translated into more than 20 languages, while the official Serbian version was used in this research [23]. The realiability of the whole instrument is $\alpha = 0.91$, while the reliability of certain subscales ranges from 0.62 to 0.82 [2].

The Serbian version [24] of the short Interpersonal Support Evaluation List (ISEL) [25, 26] was used in this research. The scale comprises 12 items and measures the perceived availability of *Appraisal* (advice or guidance), *Belonging* (empathy, acceptance, concern), and *Tangible* (material or financial aid) social support [26]. Items are valued on a four-point scale ranging from 1 (definitely false) to 4 (definitely true). Considering general population, the scale reliability ranges from 0.80 to 0.90 [25]. Moreover, there is a positive correlation of this scale with other scales of social support (*eg*, Inventory of Socially Supportive Behaviors), with the number of close friends, as well as with the level of the quality of marital relationships [26].

The IRSa is a multidimensional scale for estimating the frequency of stressors at work [27]. The scale consists of 38 items, out of which 34 have the form of the five-point Likert scale (from "hardly ever" to "almost always") and assesses

the presence of adverse job conditions and events, *eg*, "How often you do not have time to complete all your tasks?" or "Can you decide when to take a break?". The other four Yes/No items assess job insecurity, *eg*, "Are you worried that you'll get fired?". The IRSa comprises seven subscales that assess different sources of stress at work (demands at work, supervision, the support of the manager, the support of colleagues, relations and working environment, position at work, distribution of work assignments). The reliability of subscales ranges from 0.72 to 0.82, with the average reliability of the whole scale $\alpha = 0.74$.

Statistical analyses

The data were analyzed using SPSS Statistics v.20. Pearson's correlation coefficients were used to examine associations between continuous variables, while *t*-test was used to determine the significance of differences among arithmetic means. Kolmogorov-Smirnov test was used for analyzing normality of the distribution of scores on the scales of life and job satisfaction. Furthermore, the hierarchical and logistic regression analyses were used for establishing the degree where predicting variables indicate criterion variables.

Results

All the scales showed relatively high reliability, for the subscales TEMPS-A ranging from 0.70 to 0.80, ISEL 0.78, IRSa 0.91, SWLS 0.84 and JSS 0.92.

Kolmogorov-Smirnov test showed normal distribution of scores on the scales of life and job satisfaction. Considering life satisfaction, the values are to some extent higher than theoretical frameworks, therefore the curve is slightly curved toward higher values, but it is still within the bounds of significance. Concerning job satisfaction, the values are in the range of normal distribution (Table 2).

The analysis of sociodemographic characteristics did not point out any significant difference considering sex and occupation (doctors and psychologists) in regard to life and job satisfaction. The relationship status of the examinees (in a relationship/single) showed that those who are in a relationship are more satisfied with life than those who are single (Table 3).

Life satisfaction is positively correlated with the number of children and the years of education, while job satisfaction is negatively correlated with the years of working experience (Table 4).

According to the already existing norms in the world [28], our examinees are satisfied with their life, but considerably less satisfied with their work [$t(2) = 11.20$; $p < 0.01$)] [29]. Namely, our workers are the least satisfied with pay (9.03 ± 4.61) and benefits (9.37 ± 4.45), while they are most satisfied with nature of work itself (18.72 ± 3.52), social relations with co-workers (15.56 ± 4.15) and supervisors (15.57 ± 5.33).

The correlation among affective temperaments, life and job satisfaction, social support, and stress at work is shown in Table 5. The effects of sex and age variables were controlled in

Table 2

Descriptive indicators of life and job satisfaction

Parameters	Min-Max	AS	SD	Skewness	Kurtosis	K-S
Life satisfaction	9–35	26.42	5.42	-0.72	0.36	0.058
Job satisfaction	38–185	113.36	28.31	0.08	-0.06	0.200

K-S – Kolmogorov-Smirnov test; AS – average score; SD – standard deviation.

Table 3

Differences in life/job satisfaction according to demographic characteristics

Parameters	AS		SD		*t*	*df*	*p*
	male	female	male	female			
Life satisfaction	26.77	26.10	4.83	5.54	0.792	201	0.429
Job satisfaction	109.88	111.59	26.86	27.82	-0.397	201	0.692
	Psych	Doctors	Psych	Doctors			
Life satisfaction	25.73	26.87	5.79	4.99	-1.966	201	0.058
Job satisfaction	110.34	111.61	26.89	27.98	-0.320	201	0.749
	In a relationship	Single	In a relationship	Single			
Life satisfaction	26.70	24.54	4.82	6.97	2.293	201	0.023*
Job satisfaction	111.72	108.59	26.64	31.14	0.638	201	0.524

***$p < 0.05$; AS – average score; SD – standard deviation.**

Table 4

Correlation of life and job satisfaction with demographic characteristics

Parameters	Age	Number of children	Years of education	Working experience
Life satisfaction	0.044	0.246**	0.138*	0.042
Job satisfaction	-0.114	0.047	-0.004	-0.159*

****$p < 0.01$; *$p < 0.05$; Kolmogorov-Smirnov test.**

Table 5

Correlation between examined psychological constructs

Temperament and job and life satisfaction	Cyclo	Hyper	Irrit	AnxC	AnxS	Life Sat.	Job Sat.	Soc.Sup.	Work Stress
Depressive	0.427**	-0.103	0.332**	0.409**	0.304**	-0.340**	-0.212**	-0.216**	0.273**
Cyclothymic (Cyclo)	-	-0.089	0.300**	0.397**	0.402**	-0.324**	-0.213**	-0.215**	0.240**
Hyperthymic (Hyper)		-	0.063	-0.093	-0.056	0.280**	0.080	0.209**	-0.094
Irritable (Irrit)			-	0.176*	0.200**	-0.164*	-0.180**	-0.084	0.220**
Anxious-Cognitive (AnxC)				-	0.481**	-0.216**	-0.210**	-0.241**	0.216**
Anxious-Somatic (AnxS)					-	-0.209**	-0.168*	-0.077	0.168*
Life Satisfaction (Life Sat.)						-	0.384**	0.250**	-0.349**
Job Satisfaction (Job Sat.)							-	0.392**	-0.744**
Social Support (Soc.Sup.)								-	-0.353**

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

this analysis owing to the fact that there are certain differences of dominant temperament traits in regard to these variables [19, 30].

Life and job satisfaction are in low to moderate negative correlation with almost all temperament traits, moderate positive correlation with social support, and moderate to high negative correlation with sources of stress at work (Table 5). Life satisfaction has moderate correlation with all the three types of social support (Appraisal $r = 0.254$**, Belonging $r = 0.259$**, and Tangible $r = 0.239$**), and the situation is similar regarding job satisfaction (Appraisal $r = 0.351$**, Belonging $r = 0.298$**, and Tangible $r = 0.346$**). In regard to the sources of stressors at work, the lack of the manager's support ($r = 0.712$**), distribution of work assignments ($r = 0.650$**) and bad work environment ($r = 0.640$**) are highly correlated with job satisfaction, as well as the support of colleagues ($r = 0.528$**).

In order to examine the relation between explanatory and criterion variables, analyses of hierarchical and linear regression were conducted. According to the theoretical model and the fact that temperament is a basic personality trait, in the first part of these analyses, life satisfaction was the dependent variable, while the independent ones were those temperament traits that moderately correlated with life satisfaction. In the second step, social support and sources of stress at work were introduced in the model (Table 6).

When life satisfaction is predicted by temperament traits, the model is significant and presents 21% of the variance in life satisfaction. Introducing additional variables, work-related stress and social support, the additional 6% of the variance in life satisfaction is explained and this change is also significant.

Linear regression analysis of job satisfaction considered only variables 'social support' and 'stress at work', because the other ones did not show moderate correlation with the dependent variable. Approximately 58% of the life satisfaction variance was explained by this model (Table 7).

Discussion

The aim of this paper was to examine the predictive role of affective temperaments in the feeling for life satisfacti-

Table 6

Hierarchical regression for life satisfaction as criterion variable

Life satisfaction	B	SE b	β	R	R^2	ER^2
Step 1				0.45	0.21	0.19
constant	24.81	1.01				
depressive	-1.21	0.34	-0.25**			
cyclothymic	-0.49	0.21	-0.17*			
hyperthymic	0.63	0.18	0.22**			
Step 2						
constant	25.36	3.37		0.52	0.27	0.25
depressive	-0.87	0.33	-0.19*			
cyclothymic	-0.36	0.21	-0.12			
hyperthymic	0.54	0.18	0.19*			
work stress	-0.06	0.02	-0.21**			
social support	0.11	0.06	0.12*			

Hierarchial and linear regression analysis; n = 210; *$p < 0.05$; ** $p < 0.01$.

Table 7

Linear regression for job satisfaction as criterion variable

Job satisfaction	B	SE b	B	R	R^2	ER^2
Step 1				0.76	0.58	0.57
constant	167.20	13.09				
work stress	-0.95	0.07	-0.69**			
social support	0.76	0.23	0.17*			

Linear regression analysis; n = 210; * $p < 0.05$; **$p < 0.01$.

on among doctors and psychologists, as to well as examine their intermediary roles in predicting job satisfaction.

Regarding the life satisfaction variance, depressive and hyperthymic and to some extent cyclothymic temperament traits, make the greatest contribution. These results are expected since hyperthymic temperament refers to cheerful, optimistic, energetic (active) and self-confident persons [31], and optimism and self-esteem are actually the traits that are positively correlated with life satisfaction [32–34]. Moreover, it is also expected that depressive temperament has negative influence on life satisfaction, because it can be described as the contrast to hyperthymic temperament. Cyclothymic temperament showed the weakest predictive power, particularly when situational variables are included in the model. Nevertheless, it is obvious that persons with rapid mood and energy swings, which are characteristics of the cyclothymic temperament [31], are less satisfied with their life. This is proved by numerous studies that present great power of this type of temperament for predicting early depressivity and suicidal tendencies [35], higher rates of relapse of depression, higher rates of irritability and mixed feelings, as well as addictions [36]. Introducing stressors at work and social support in the model, the significance of predicting is to some extent increased which points out their potential intermediary role in this model. Other authors also came to similar conclusions [37, 38], emphasizing that approximately 50% of individual differences referred to experiencing happiness can be explained by the inborn predisposition for a certain level of positive affect, 10% can be explained by life experience, while the other 40% can be under our own control.

In respect to demographic variables, the relationship status, the number of children and years of education showed a correlation with life satisfaction. It is expected that people in relationships have the possibility to satisfy psychological, reproductive and social needs, but first of all, the need for belonging as one of basic psychological needs. Being married/cohabiting and social support explained a half of the total variance of life satisfaction among physicians [10]. However, until now most papers either did not confirm the correlation, or showed a negative correlation between the number of children and life satisfaction. According to Angeles [39], previous researches did not manage to identify these effects, because the key role of marital status was not taken into consideration. The experience of individuals who are married and share duties and problems, differs from the experience of the ones who are separated, or have never been married and who have to take care of children themselves. Furthermore, financial status has not been considered in the earlier researches. Regarding the fact that our research includes highly educated people who are employed and have stable incomes, the number of children does not imply worrying for family existence.

National studies in Sweden and Spain confirms our results considering the correlation between years of education and life satisfaction [40, 41]. Incomes and level of education have a direct and indirect impact on most aspects of life satisfaction in these studies. More educated people can better organize their free time, take various activities, make numerous social contacts in their life, and all these issues indirectly influence life satisfaction.

The second model confirms that situational factors such as stressors at work and social support are significant predictors of job satisfaction, while it does not confirm our hypothesis that temperament traits also play a role in such a model. Stressors at work as the most significant predictors for job satisfaction together with social support predict 58% of the variance in job satisfaction. As we have already mentioned, researches in different countries show a high prevalence of work stress among helping professions [15–17]. Exposing oneself to numerous kinds of stressors, among healthcare professionals, can cause increased anxiety, depressivity, irritability, sleep disorder, lack of concentration and aggressive behavior, and it can also contribute to the development of burnout syndrome, mental problems, absence from work, earlier retirement [42]. The sources of stressors at work have a tendency to make its effect complex and multiplied because they are combined together with the sources of stressors from other domains of life (family life, relationship with friends), which all together contribute to not only lower job satisfaction but to low life satisfaction in general.

Family and social relationships, as segments of social and emotional support a person perceives, are also important for maintaining the quality of life [43], though this variable made only a small contribution in our models. Researches emphasize the relevance of social relationships for mental health, therefore the individuals with weak social relationships are liable to numerous health and social problems [44]. Social support is correlated with many positive indices of mental health, such as optimism, happiness, and life satisfaction, but also negatively correlated with perceived stress [45].

Regarding sociodemographic variables our study points out that only working experience is (negatively) correlated with job satisfaction. Some researches show that age is positively correlated with job satisfaction, but more and more authors suggest that the relationship between age (or working experience) and job satisfaction is U-shaped, declining until around the mid-30s and gradually increasing until the late 60s [46, 47]. On the other hand, our research considers specific professions, so we have to regard only the studies with similar population. The correlation between working experience and depression, anxiety and chronic diseases among doctors can be seen in Caplan's [48] research, where he found that older doctors are more exposed to the significant amount of stress. Working experience results in chronic diseases, general exhaustion, impossibility to adapt to new technologies, which all cause harder fulfilling work obligations and less job satisfaction.

Finally, the aspects of job satisfaction that we examined in this paper should also be mentioned. Our examinees are least satisfied with those aspects of job referred to extrinsic motivation, namely external rewarding, such as pay and benefits. These results are expected considering the fact that Serbia belongs to developing countries with budget activities of limited financial possibilities, and organizations mostly relied on other motivation factors for achieving employees' efficiency at work [49]. On the other hand, they are most satis-

fied with aspects of intrinsic motivation, characteristics of job, but with social relationships at work as well, which probably contributed to better total job satisfaction, because support from superiors and co-workers are significant components of high job satisfaction [49–51].

The studies show that caring for others is associated with improvement in health indicators of caregivers, which supports the hypothesis that providing support to others can sometimes be more advantageous than receiving it [50]. The effect of caregiving on the person who gives care quite depends on some psychological characteristics, therefore it would be good to examine the influence of negative aspects of caregiving among helping professions in some future researches. This considers particularly caregiving hyperactivation, which represents self-sacrificing approach to caregiving with an intense desire for increased closeness [51]. Hyperactivated caregivers put demands on themselves so they are constantly stressed which results in lower quality of life and job satisfaction.

Conclusion

The results of this study are mainly theoretical and point out that affective temperaments can be regarded as predictors of life satisfaction, but in order to predict job satisfaction we have to take in consideration the aspects of wider social surrounding and sources of stressors at work.

Future researches should also consider other indicators of life satisfaction such as family satisfaction, health satisfaction, free time etc. It is entirely possible that these aspects are differently influenced not only by personality traits, but also by situational factors that we examined. Furthermore, there are evidences that domestic stresses as well as the aspects of work environment lead to psychology disorders in healthcare professionals, therefore the lack of this study is that we did not examine stressors outside of work situations. This fact is important because it is known that job interference with family life and constant interruptions correspond with high levels of job dissatisfaction and negative well-being in healthcare professionals.

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ORIGINAL ARTICLE

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Efficacy and safety of bevacizumab in combination with irinotecan and capecitabine in first-line treatment of metastatic colorectal cancer

Efikasnost i sigurnost bevacizumaba u kombinaciji sa irinotekanom i kapecitabinom u prvoj liniji lečenja metastatskog kolorektalnog karcinoma

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Abstract

Background/Aim. The efficacy and safety of bevacizumab (BEV) in combination with capecitabin and irinotecan in first-line therapy for patients with metastatic colorectal cancer (mCRC) were studied. In order to improve safety and efficacy of chemotherapy, as well as to reduce adverse reactions to a minimum, doses of active agents applied were changed in relation to previously employed schedules. **Methods**. Patients with histologically documented mCRC with no previously received chemotherapy or with received adjuvant or neoadjuvant chemotherapy, which ended 6 months before capecitabin treatment (1000 mg/m^2 *per os* from the 2nd to 8th day of each cycle), irinotecan (175 mg/m^2 *iv* every 2 weeks), plus bevacizumab (5 mg/kg *iv* every 2 weeks) were observed. **Results**. This prospective study included 35 patients of both sexes. The overall response rate (ORR) of 28.6%, partial response (PR) of 28.6%, progressive disease (PD) of 28.6% and stable disease (SD) of 42.8% were found. The progression-free survival (PFS) of the analyzed patients was 11.3 (95% CL: 9.1–12.9) months while overall survival (OS) of the included patients was 25.2 (95% CL: 17.4–28.4) months and 117 adverse effects were recorded in 24 patients. Alopecia, nausea and vomiting, hemorrhage, hand-foot syndrome, diarrhea, abdominal pain, proteinuria, and hypertension (51.4%, 37.1%, 37.1%, 25.7%, 22.8%, 20.0%, 20.0% and 17.1%, respectively) were most frequently observed adverse effects. **Conclusion**. The results of this clinical trial support and recommend the use of bevacizumab plus capecitabin and irinotecan in the doses and schedule applied throughout this study as the first-line treatment of mCRC patients.

Key words:
colorectal neoplasms; neoplasm metastasis; antineoplastic combined chemotherapy protocols; drug toxicity.

Apstrakt

Uvod/Cilj. U radu je ispitivana efikasnost i bezbednost terapije prve linije protokolom XIA (kapecitabin/irinotekan/bevacizumab) kod bolesnika sa metastatskim kolorektalnim karcinomom (mCRC). U cilju povećanja podnošljivosti i efikasnosti hemoterapije, kao i da bi se neželjeni efekti sveli na minimum, doze aktivnih agenasa su unekoliko promenjene u odnosu na ranije primenjivane šeme. **Metode**. Ispitanici sa mCRC koji nisu prethodno primali hemioterapiju, ili su primali adjuvantnu ili neoadjuvantnu hemioterapiju završenu šest meseci pre početka lečenja lečeni su prema sledećoj terapijskoj shemi: kapecitabin (1000 mg/m^2 *per os* od 2. do 8. dana svakog ciklusa), irinotekan (175 mg/m^2 *iv* svake 2 sedmice) u kombinaciji sa bevacizumabom (5 mg/kg *iv* svake 2 sedmice). **Rezultati**. Ovo prospektivno ispitivanje vršeno je na ukupno 35 ispitanika oba pola. Ukupni odgovor [*overall response rate* (ORR)] bio je postignut kod 28,6%, parcijalan odgovor (PR) kod 28,6% bolesnika, do progresije bolesti (PD) došlo je kod 28,6% i stabilna bolest (SD) kod 42,8%. Preživljavanje bez progresije bolesti (PFS) iznosilo je 11,3 meseca (interval pouzdanosti – 95% CL: 9,1 – 12,9 meseci). Ukupno preživljavanje [overall survival (OS)] ispitanika bilo je 25,2 meseca (95% CL: 17,4 – 28,4 meseca). Kod 24 ispitanika zapaženo je 117 neželjenih reakcija. Najčešće neželjene reakcije bile su alopecija, mučnina i povraćanje, hemoragija, sindrom šaka-stopalo, dijareja, abdominalni bol, proteinurija i hipertenzija (51,4%, 37,1%, 37,1%, 25,7%, 22,8%, 20,0%, 20,0% i 17,1%, respektivno). **Zaključak**. Rezultati ispitivanja podržavaju i opravdavaju dodatak bevacizumaba hemioterapijskoj kombinaciji kapecitabin/irinotekan u prvoj liniji lečenja bolesnika sa mCRC, kao i primenjene doze prema korišćenoj shemi.

Ključne reči:
kolorektalne neoplazme; neoplazme, metastaze; lečenje kombinovanjem antineoplastika, protokoli; lekovi, toksičnost.

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Introduction

Colorectal cancer (CRC) represents the third most frequently diagnosed malignancy and the second main cause of fatal outcome of cancer patients in the world [1].

The introduction of novel drugs in the systemic treatment of mCRC during the last two decades leads to increased median survival in clinical trials from 6–9 months to over 2 years [2].

Irinotecan in combination with 5-fluorouracil (5-FU)-based chemotherapy and bevacizumab (BEV) represent an established option in the treatment of mCRC [2]. Continuous infusion of 5-FU in addition to irinotecan (FOLFIRI) has been found to be more effective and tolerable than bolus of 5-FU [3]. However, this regimen requires hospitalization or the placement of central venous line. In contrast, the irinotecan-capecitabine combination (XELIRI) appears to be more convenient [3]. In the Bolus, Infusional, or Copecitabine with Camptosar-Celecoxil (BICC-C) randomized trial, XELIRI in comparison with FOLFIRI, was associated with higher rates of severely expressed undesirable side effects such as nausea, vomiting, diarrhea, dehydration, hand-foot syndrome and as a consequence, treatment discontinuation. Also, progression-free survival (PFS) was shorter in patients treated with XELIRI, when only patients who had completed the treatment, were compared [3].

In the beginning of the 21st century, the European Medicines Agency (EMA) approved bevacizumab (BEV), a recombinant human monoclonal antibody, targeting vascular endothelial growth factor (VEGF), for first-line therapy of patients with advanced CRC, based on the data from the phase III American Venous Forum (AVF)2107g trial [4]. This trial demonstrated an increased response rate (RR), with a prolonged median duration of survival, as well as a longer median PFS.

After its introduction, fluoropyrimidine-based chemotherapy has been the mainstay for CRC treatment. Capecitabine represents an oral fluoropyrimidine of similar efficacy to 5-fluorouracil/leucovorin (5-FU/LV) as first-line treatment of advanced or mCRC [5, 6]. However, it is advantageous in comparison with 5-FU/LV because of its comfortable oral administration and satisfactory safety profile [7].

The combination of BEV and capecitabin was shown to act synergistically, with a prolonged tumor inhibition period than achieved with either agent alone [1]. Similarly, in several phase I and II trials it has been observed that capecitabine and irinotecan (XELIRI) can be equally effective and safely combined in the most convenient alternative XELIRI regimen in individuals with advanced CRC, with no pharmacokinetic interactions [1, 8, 9].

Based on the results of a previous clinical study on mCRC patients, it was clear that a biweekly combination of irinotecan and capecitabine expressed a synergistic effect, with an acceptable response rate (RR) of 32% and a satisfying tolerability as first-line therapy, together with an important time to progression of 9 months and an overall survival (OS) of 19.2 months in this advanced setting [1].

Based on the aforementioned facts, it was to be expected that the combination of BEV with this biweekly XELIRI treatment scheme would be at least as effective as the standard FOLFIRI regimen with a more satisfactory safety profile.

The data on efficiency of BEV administered together with capecitabine and irinotecan in patients with mCRC are relatively sparse in the available literature. This prompted us to evaluate the efficacy and safety of capecitabine, irinotecan, bevacizumab (XIA) regimen, as first-line treatment of mCRC patients.

The primary objective of this work was to determine the PFS, safety and tolerability to the XIA regimen. Secondary objectives included overall response rate (ORR) and OS.

Methods

A total of 35 patients suffering from initially unresectable chemotherapy-naïve mCRC were included in the present study. The examined group was formed according to the following criteria: adults of both sexes, age range 27–69 years; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2; adequate bone marrow function (neutrophil count ≥ 1.5×10^9/L, platelet count ≥ 100×10^9/L, hemoglobin ≥ 9 g/dL); serum creatinine < 1.25 mg/dL; alanine aminotransferase or aspartate aminotransferase or alkaline phosphatase < 3 times the upper limit of normal and keratin ≤ 1.5 times the upper limit of normal. Previous adjuvant or neoadjuvant chemotherapies had been completed at least 6 months before enrolment in the study.

The patients were treated with BEV 5 mg/kg on day 1 as 90/60/30–min intravenous infusion, followed by irinotecan 175 mg/m^2 as a 120-min intravenous infusion on day 1 and capecitabine 1,000 mg/m^2 orally twice daily from the day 2 to the day 8 (XIA schedule). All the patients were receiving serotonin 5-HT_3 (chemoreceptor trigger zone) inhibitors for nausea and vomiting prophylaxis. They were subjected to this treatment schedule (XIA) every 2 weeks in *continuo* until 12 cycles were completed except in the cases of disease progression, patient refusal, unacceptable toxicity or death. Appropriate dose interruptions/reductions were implemented in the case of specific toxicities, depending on their nature and intensity. The next course of treatment began only when the neutrophil count reached > 1.5×10^9/L, the platelet count > 100×10^9/L, and while any other treatment-related toxicity was lower than or equal to that found at grade 1.

A screening assessment including medical history, physical examination and chest radiography was conducted within 2 weeks before the onset of the treatment. Within 7 days before starting the treatment, further assessments included vital signs, ECOG performance status and laboratory tests (hematology, blood chemistry including liver and renal function tests and urine analyses). The assessment of the response was based on investigator-reported measurements according to the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [10].

The study was performed in accordance with the Declaration of Helsinki. In addition, the approval of the responsible Ethics Committee was provided.

Statistical methods

Toxicity and safety were assessed in terms of toxicity and evaluated according to the National Cancer Institute

Common Toxicity Criteria for Adverse Events (NCI-CTCAE), Version 3.0.

Descriptive data were reported as proportion and medians. PFS was defined as the period from the date of the first dose of treatment applied to the first observation of disease progression or death by any cause. The OS was calculated as the period from the date of the first cycle of treatment until death of any cause or until the date of the last follow-up at which data point was censored. Survival analysis (PFS and OS) was estimated by the Kaplan–Meier method [11].

Results

A total of 35 consecutive mCRC patients were treated by XIA regimen. The first patient was included in the study on October 19, 2005 and the latest one on November 30, 2010. From all 35 patients, seven were alive in December, 2012 and three of them had a second-look operation. One of the patients of this group of three was first included in the study in October, 2006 and one in July, 2010.

Baseline characteristics for the evaluable patients are summarized in Table 1. Median age was 50.8 (range 27–69) years. All the 35 patients had an ECOG performance status of < 2 at baseline, half of them had multiple sites of metastases mostly located in the liver. A total of 22 out of 35 patients had initial mCRC.

Toxicity and dose administration

Out of 35 patients 33 received 12 cycles of XIA (94.3%) and a total of 396 XIA cycles were administered. Overall, 11.4% (n = 4) of patients required reduction of the dose by 25%. Treatment interruption because of BEV-related toxicity was required in a single patient. Treatment delays due to toxicity caused by capecitabine, irinotecan and BEV were required in two patients. The treatment was rather well tolerated and most of the reported undesirable side effects were mildly expressed according to the National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE grade 1 or 2).

The main hematology and non-hematology toxicities are summarized in Table 2.

Table 1

Baseline patient characteristics

Characteristics of the patients (n = 35)	Values
Median age (years), mean (range)	50.85 (27–69)
Sex, n (%)	
male	16 (45.7)
female	19 (54.3)
Grade of disease in the initial diagnosis, (II/III/IV), n	25/5/0
State of disease in the initial diagnosis (2/3/4), n	6/7/20
Localization (colon/rectum/colorectal), n	19/10/6 (54.3/28.6/17.1)
Previous therapy, n (%)	
chemotherapy	10 (28.6)
radiotherapy	0 (0)
Chemotherapy (Adjuvant therapy), n (%)	
5FU/FA	7 (20)
FUP	2 (6)
capecitabine	1 (3)
Surgery of primary tumor, n (%)	34 (97)
Number of metastatic sites, n (%)	
1	17 (48)
2	14 (40)
3	2 (6)
4	2 (6)

5FU/FA – 5 fluorouracil/folinic acid; FUP – follow-up protocol.

Table 2

Most frequent treatment-related adverse events *per* patient

Adverse event	Grade 1/2		Grade 3/4		Total	
	n	%	n	%	n	%
Alopecia	18	51.4	—	—	18	51.4
Vomiting and Nausea	13	37.1	—	—	13	37.1
Hemorrhage	13	37.1	—	—	13	37.1
Leukopenia	9	25.7	1	2.8	10	28.6
Hand–foot syndrome	9	25.7	—	—	9	25.7
Diarrhea	8	22.8	—	—	8	22.8
Abdominal pain	7	20.0	—	—	7	20.0
Proteinuria	7	20.0	—	—	7	20.0
Hypertension	6	17.1	—		6	17.1
Fever	4	11.4	—	—	4	11.4
Mucositis	3	8.6	—	—	3	8.6
Local pain	2	5.7	—	—	2	5.7
Thrombocytopenia	2	5.7	—	—	2	5.7
Hyperbilirubinemia	2	5.7	—	—	2	5.7
Numbness of extremities	2	5.7	—		2	5.7
Anorexia	2	5.7	—	—	2	5.7
Enteritis	—	—	1	2.8	1	2.8
Ileus	—	—	1	2.8	1	2.8

The most common grade 1/2 toxicities were: alopecia, vomiting and nausea, hemorrhage, leukopenia, hand-foot syndrome, diarrhea, abdominal pain, proteinuria and hypertension (51.4%, 37.1%, 37.1%, 28.6%, 25.7%, 22.8%, 20.0%, 20.0% and 17.1%, respectively).

The adverse reactions, toxicity grade 3/4 were: leukopenia, enteritis and ileus (2.8% each).

No treatment-related deaths were reported.

During the present study, a total of 117 adverse reactions were observed and in 24 out of 35 patients involved in the trial, the number of adverse events grade 1 was 94 (80%), those of grade 2 was 18 (16 %) and those of grade 3 was 5 (4 %). No adverse reactions of grade 4 were recorded.

A total of 11 (31%) patients did not express the signs of adverse reactions, while 7 (20%) patients suffered one of adverse reactions. Two adverse reactions were observed in 4 (11%) patients and only a single patient suffered from twelve of these reactions.

Efficacy and survival

As shown in Table 1 XIA regimen led to a partial response in 10 out of 35 (28.6%) patients. Fifteen (42.8%) patients had a stable form of the disease and 10 (28.6%) had a progressive disease (Table 3).

Table 3

Response of the patients to the treatment applied

Response to the treatment	Patients, n (%)
Complete response	0 (0)
Partial response	10 (28.6)
Stable disease	15 (42.8)
Progressive disease	10 (28.6)
R0 resection	3 (8.6)

R0 – nula resection (microscopically margin-negative resection).

PFS was 11.3 months (95% confidence interval CI: 9.1–12.9) (Figure 1). OS was 25.2 months (95% CI: 17.4–28.4 months) (Figure 2), and ORR was 28.6%.

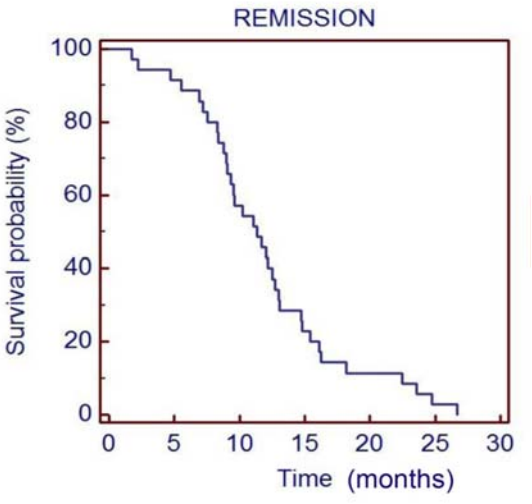

Fig. 1 – Kaplan-Meier survival estimates of progression-free survival.

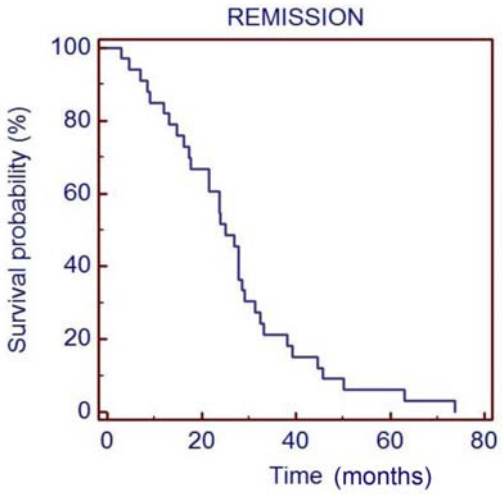

Fig. 2 – Kaplan-Meier survival estimates of overall survival.

Discussion

Based on the results obtained in 2 phase III randomized controlled trials (RCTs) several authors have demonstrated the improved survival of advanced CRC patients upon BEV addition to standard 5-FU-based chemotherapy regimens in combination with irinotecan (IFL) and oxaliplatin (FOLFOX4) [4, 12, 13]. It was also shown that PFS was significantly improved in the bevacizumab-containing arms of all three studies [12, 14]. Both survival and response rates were similarly improved in randomized phase II trials comparing 5-FU/FA combined with BEV with 5-FU/FA alone in advanced CRC patients [12, 15, 16]. Survival benefit was observed after BEV had been added to 5-FU regimens given by bolus injection (IFL) and by continuous infusion (FOLFOX). BEV in combination with 5-FU-based chemotherapy has been shown to be effective in both first- and second-line treatments of advanced CRC [12].

BEV in combination with any fluoropyrimidine-based chemotherapy was more effective than any fluoropyrimidine-based chemotherapy alone. This conclusion was confirmed by two extensive registry trials in first-line mCRC — the Bevacizumab Regimens Investigation of Treatment Effects and Safety (BriTE) trial in the United States [12] and the First BEV Expanded Access Trial (BEAT) performed in Europe and Canada [12, 17]. The above-mentioned trials were designed to evaluate safety events of BEV applied in combination with a variety of chemotherapy regimens in a broad community-based population of mCRC patients. These observational data strongly suggest that BEV in combination with a variety of fluoropyrimidine-based chemotherapy regimens was safe, with efficacy similar to that seen in prospective randomized clinical trials. However, achievement of OS and PFS benefits in BEV-supplemented chemotherapy led to a significant toxicity increase. Commonly observed undesirable side effects in clinical trials with BEV included bleeding, thrombosis, hypertension and proteinuria. Luckily, the hypertension could be managed using oral antihypertensive drugs, but it required frequent blood pressure monitoring [12].

Reported and ongoing phase III trials have excluded patients with cerebral metastases, advanced atherosclerotic disease, or proteinuria. Therefore, these conditions should be considered contraindicative to BEV application. Throughout the above clinical trials, rare cases of BEV-associated gastrointestinal perforation and poor wound healing were seen [11, 17–19]. At present, there is no evidence to support the use of BEV as monotherapy in advanced CRC [12, 13]. Thus, BEV should not be taken as an alternative in the third-line setting of systemic treatment of advanced CRC.

Regarding colon cancer, the results of Wagner et al. [20] were supported by other meta-analyses evaluating the addition of BEV to chemotherapy in the metastatic setting [12, 20–22]. These should be interpreted in the light of the disease specific survival of different malignancies. Therefore, 3 months of survival benefit in metastatic colon cancer when the expected OS is over 20 months differs from a 3 month-benefit in a patient with metastatic lung or pancreatic cancer in which the median survival is under 12 months [19, 23]. The role of PFS as a surrogate for overall survival has been extensively debated in metastatic cancer [19].

To date, there has been limited data on the XELIRI plus BEV regimen [1]. Available data presenting preliminary results from a study using BEV with irinotecan plus capecitabine showed that this combination had a promising clinical activity. Garcia-Alfonso et al. [1] reported an ORR of 40%, with an overall disease control rate of 86% and a year progression-free rate of 49%. At The Annual Meeting of the American Society of Clinical Oncology (ASCO) held in 2009, these authors presented preliminary results of the phase II, non-comparative, randomized FNCLCC ACCORD 13/0503 trial, in which a total of 145 patients, age range 18–72 years, were randomized to receive either BEV plus XELIRI (irinotecan 200 mg/m^2 on day 1, capecitabine 1000 mg/m^2 twice daily on days 1 – 14 plus BEV 7.5 mg/kg on day 1, every 3 weeks) or BEV plus FOLFIRI (irinotecan 180 mg/m^2 on day 1 plus 5-FU 400 mg/m^2 plus leucovorin 400 mg/m2 on day 1 followed by 5-FU 2400 mg/m^2 as a 46-hour-infusion plus BEV 5 mg/kg on day 1, every 2 weeks). Preliminary results from the first 6-month follow-up showed an ORR of 58% (95% CI: 47–70%) in the BEV plus XELIRI arm similar to 58% (95% CI: 53–65%) in the BEV plus FOLFIRI arm. The most common grade 3/4 advese reactions reported in the XELIRI and FOLFIRI groups were neutropenia (17% *vs* 26%), diarrhea (12% *vs* 5%) and cardiovascular events (13% *vs* 11%). The authors concluded that XELIRI and FOLFIRI plus BEV expressed similar efficiency in the treatment of mCRC patients with manageable toxicity.

Garcia-Alonso et al. [1] in a single-institutional study applied the combination of biweekly XELIRI plus BEV for previously untreated mCRC patients and observed beneficial effects of the treatment with an ORR of 67.4%, a median PFS of 12.3 months and a median OS of 23.7 months. The overall disease control rate was 93.5%.

The results of our single-institutional study with the XIA regimen applied to previously untreated mCRC patients also revealed a meaningful clinical activity, with an ORR of 28.6%, a median PFS of 11.3 months, and a median OS of 25.2 months. Analysis of efficacy results demonstrated a higher percentage of stable disease (SD) in our test protocol, in relation to the partial response (PR), as compared to a comparative study. This difference could be ascribed to a lower grade of main adverse events in our study. Also, it should be noted that during the study protocol, there was no complete response (CR), but 7 patients (20.0%) were alive and 3 of them operated on. In general, this drug combination was relatively well tolerated, with most of adverse events grades being 1/2. Interestingly, the overall safety profile of this combination differs from those achieved with the XELIRI regimen [1, 24]. In the BICC-C trial, the XELIRI arm was associated with a significantly higher incidence of grade 3/4 diarrhea (48%), neutropenia (32%) and dehydration (19%) [3]. In the 40015 clinical trial conducted by the EORTC group, XELIRI was associated with increased mortality, as well as an almost 40% incidence of grade 3/4 diarrhea [1, 24]. In these two clinical trials, the increased toxicity clearly impacted the clinical activity of the XELIRI regimen in a negative manner. However, it is worth mentioning that the doses of XELIRI applied in these studies were higher comparing to those used in our XELIRI plus BEV combination described here.

Compared to the recent findings of the Ducreux's trial employed by García-Alfonso et al. [1] who investigated the combination of XELIRI and FOLFIRI along with BEV, the clinical activity of the data reported in the present study in terms of ORR was similar to that reported for the FNCLCC ACCORD 13/0503 trial. However, better toxicity profile achieved in our study could be interpreted to be the result of the lower dose of chemotherapeutic agents in XELIRI plus BEV regimen described here.

Comparative analyses of the efficacy results of the XIA protocol and those obtained in the corresponding studies of others demonstrated a higher percent of SD in relation to a partial regression. Besides, the percent of low grade adverse reactions (grades 1 and 2) found throughout the present study could be connected to a high percent of SD. Doses of capecitabine and irinotecan in XIA protocol are the same in comparison with other regimens with capecitabine, irinotecan and bevacizumab, but in our XIA schedule doses of irinotecan and bevacizumab are differently deployed, and because of that we got better results of the tolerance of therapy.

The patients had the same response as patients in similar protocols, so efficiency is comparable to existing protocols (equal to them). But toxicity is lower, that is, the safety is improved, because the dose of irinotecan is better tolerated if distributed in the manner that the irinotecan is administered for 14 days, but not on the day 21 (as is customary).

The XIA protocol examined here expressed a better tolerance but equal PFS and OS suggesting a beneficial effect achieved after dividing daily dose of the drugs applied into several lower doses. In this way toxicity decrease of the therapy employed can be achieved while PFS and OS remain unchanged. At the same time lower percent of total and partial regression of the disease was observed strongly suggesting the application of this newly created protocol for therapeutic maintenance during continual and intermittent treatments. This could also result from lower grade of adverse re-

actions comparing to those reported in comparative studies of other authors. In addition, it should be noticed that CR was absent when XIA protocol was applied, but six patients were operated on and three of them were still alive.

The distribution and percent of adverse reactions were oscillating both in the present study and in the reports of the others. The reasons for these variations could be ascribed to the differences in evaluation procedures, but also to the differences in time schedules of hormonal therapy (HT) + BEV application (every two or three weeks). Also, the differences in bioavailability and equivalency of novel and generic drugs should be taken into account.

Conclusion

Based on the results obtained throughout the present study it can be concluded that the combination of BEV with the XELIRI regimen is feasible with manageable toxicity. Besides, it is associated with a promising efficacy in terms of PFS, ORR and OS in previously untreated mCRC patients.

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SHORT COMMUNICATIONS

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Interleukin 1-beta analysis in chronically inflamed and healthy human dental pulp

Analiza interleukina 1-beta u hronično zapaljenoj i zdravoj zubnoj pulpi

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Abstract

Background/Aim. Proinflammatory cytokines can act like endogenous pyrogen interleukin 1 (IL-1), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF α) which regulate the synthesis of secondary mediators and other proinflammatory cytokines through macrophages and mesenchymal cells. They stimulate acute-phase proteins and attract inflammatory cells. The aim of this study was to determine interleukin 1-β (IL-1 β) concentrations in chronically inflamed and healthy dental pulps. **Methods.** A total of 41 pulps (19 from patients with *pulpitis chronic causa* and 22 from patients with *pulpatis chronic aperta*), divided into two groups, were obtained from teeth with chronic pulp inflammation. The control group consisted of 12 teeth with healthy pulp. After extirpation, pulp samples were immediately placed in sterile Eppendorf tubes and frozen. After that, homogenisation was performed by a Teflon® pestle in ice-cold phosphate buffer solution at pH 7.4 whose volume was adjusted according to the weight of tissue. The supernatant was then frozen at -70°C until the performance of appropriate biochemical analyses. Cytokine IL-1 β value was determined by a commercial enzyme-linked immunosorbent assay (ELISA test). We applied the high sensitivity system technique, which may register low levels of cytokines, ranging from 0.125 to 8.0 pg/mL for IL-1 β. **Results**. By comparing the mean value of IL-1β, in the pulps we can see a statistically significant difference ($p < 0.01$) among them. The highest value of IL-1 β was in the subjects with *pulpitis chronica clausa* and it was 6.21 ± 2.70 pg/mL. **Conclusion**. Proinflammatory cytokine IL-1 β is present in detectable quantities in the pulp tissue of all vital pulps. Its highest concentrations were found in the sample group with *pulpitis chronica clausa.*

Key words:
dental pulp diseases; chronic disease; inflammation; interleukin-1 beta.

Apstrakt

Uvod/Cilj. Proinflamatorni citokin, interleukin 1 (IL-1), interleukin 6 (IL-6) i tumor nekrozni faktor alfa (TNF α) mogu delovati poput endogenih pirogena koji regulišu sintezu sekundarnih medijatora i ostalih proinflamatornih citokina preko makrofaga i mezanhimskih ćelija. Oni stimulišu proteine akutne faze ili privlače ćelije inflamacije. Cilj ove studije bio je da se utvrdi koncentracija interleukina 1-beta (IL-1 β) u hronično zapaljenoj i zdravoj zubnoj pulpi. **Metode.** Ukupno 41 pulpa, podeljena u dve grupe (19 od pacijenata sa *pulpitis chronica clausa* i 22 od pacijenata sa *pulpitis chronica aperta*), dobijene su od zuba sa hroničnim oboljenjem pulpe. Kontrolnu grupu činilo je 12 zdravih pulpi. Uzorci pulpi odmah po ekstirpaciji stavljani su u sterilne ependorf epruvete i zamrzavani. Zatim je vršena homogenizacija telefonskim tučkom u ledenom fosfatnom puferu pH 7,4 čija je zapremina bila prilagođena težini tkiva. Supernatant je nakon toga smrznut na -70°C do izvođenja odgovarajućih biohemijskih analiza. Citokin IL-1β određivan je komercijalnom metodom sendvič enzim-imunoesej tehnikom (ELISA test). Primenjena je tehnika sistema visoke oseljivosti, kojim se mogu registrovati niske koncentracije citokina, u opsegu 0,125–8,0 pg/mL za IL-1β. **Rezultati.** Poređenjem srednjih vrednosti IL-1β (pg/mL), u pulpama uočena je statistički značajna razlika ($p < 0,01$) među njima. Najviše vrednosti Il-1β bile su kod ispitanika sa *Pulpitis chronic clausa* i iznosila je 6,21 ± 2,70 pg/mL. **Zaključak.** Proinflamatorni citokin IL-1β prisutan je u primetnim količinama u pulpnom tkivu kod svih vitalnih pulpi. Njegove najviše koncentracije nađene su u uzorcima zubne pulpe ispitanika sa *pulpitisa chronic clausa.*

Ključne reči:
zub, bolesti pulpe; hronična bolest; zapaljenje; interleukin-1 beta.

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Introduction

Inflammatory and immune system reactions within dental pulp occur as a response to microorganisms and their products which penetrate dentinal tubules [1, 2]. *Pulpitis* is an inflammatory disease of the pulp which is characterised by the local accumulation of inflammatory mediators, including cytokines and chemokines [3]. Depending on the nature of pulp changes and whether the process takes place in the open or closed pulp cavity, we can make a difference between ulcerative and hyperplastic *pulpitis*. Chronic open *pulpitis* is characterised by local vasodilatation and infiltration of mononuclear leukocytes, exudation and cellular infiltration by neutrophil leukocytes. At the pathohistological level we can find granulation tissue present in hyperplastic *pulpitis* which is rich in capillaries and cellular infiltration [4]. Initial infiltration is composed of lymphocytes, macrophages and plasma cells [1]. We can make a difference between the two forms of chronic closed *pulpitis – pulpitis clausa alternativa seu parenchymatosa* and *granulomatosa interna*.

Closed *pulpitis* is characterised by cellular infiltration of small round cells and we can also find degenerative changes of different intensity and nature. Pathohistological examination of internal granuloma indicates the presence of granulation tissue which is rich in vascularised infiltrated round cells which are covered by odontoclasts [4].

The inflamed area is swarmed by inflammatory cells, neutrophils, neutrophil leukocytes, monocytes, macrophages, lymphocytes and plasma cells [5]. Apart from the local accumulation of inflammatory mediators, *pulpitis* is characterised by the presence of cytokines and chemokines [3]. Inflammation degree assessment is a diagnostic problem influencing the decision on what therapy should be applied. However there are no objective, quantitative and clinically practical methods to assess the inflammation degree.

Synchronisation of immune and inflammation reaction depends on the communication between cells through soluble molecules which are generically called cytokines. They include: chemokines, interleukins (IL), growth factors and interferons (IFN) [6]. Interleukins are signal substances which transmit information between different types of leukocytes [7]. Interleunkin-1 beta (IL-1β) is responsible for numerous activities and mediations in host inflammatory response. It is dominantly produced by monocytes/macrophages, fibroblasts, bone cells, endothelial cells, keratinocyte, astrocytes, lymphocytes B, activated lymphocytes T, smooth muscle cells, microglial cells and dendritic cells [8, 9].

Its induction may be caused by microorganisms, microbiological products, inflammatory agents, and antibodies. It is present in pulp tissue and cell culture, which makes it one of the most important interleukins in the inflammatory process occurring in the pulp of teeth. Its main role at low concentrations is to mediate in the local inflammatory process. At high concentrations it has endocrine effects [10]. IL-1 local effects imply the increase in leukocyte adhesion to the endothelial wall, lymphocyte stimulation, neutrophil potentiation, activation of prostaglandin and proteolytic enzymes [11].

Numerous studies suggest that cytokines are markers of pulp inflammation, but the assessment of cytokine level is possible only after pulp extirpation. IL-1β is an important mediator of inflammatory response and local response and, apart from that, it participates in numerous cellular activities such as: proliferation, differentiation and apoptosis. This led us to investigate the level of IL-1β in chronically inflamed and healthy dental pulp, in order to investigate a possible connection between IL-1β level and chronic *pulpitis*. An important and potentially useful outcome of this study would be to find out if the proof of the presence and assessment of IL-1 β level can help in making the diagnosis.

Methods

The research was conducted on 41 human dental pulps with chronic *pulpitis*. The control group consisted of 12 healthy human teeth, which had their pulps extirpated because of prosthetics reasons. After the diagnosis was established on the basis of anamnestic data and basic and auxiliary diagnostic methods, tested teeth were divided into three groups, including the control group. The group 1 (n = 19) consisted of dental pulps with the diagnosed *pulpitis chronica clausa* (*granulomatosa internum s. granuloma internum, alterativa s. parenchymatosa*); The group 2 (n = 22) consisted of dental pulps with the diagnosed *pulpitis chronica aperta* (*ulcerosa huperplastica s. polyposa s. granulomatous*); The group 3 (n = 12), the control group, consisted of healthy teeth and encompassed subjects who had their pulps extirpated because of the prosthetics reasons (Table 1).

Table 1

Classification of investigated pulps into groups according to the diagnosis

Diagnosis	n (%)
Pulpitis chronica clausa	19 (35.83)
Pulpitis chronica aperta	22 (41.5)
Control group	12 (22.64)
Total	53 (100)

After making the diagnosis, the teeth of all the groups were processed in the same manner. Vital pulpectomy was performed. Before the start of the procedure, teeth, gingiva and mucous membrane were cleaned in order to get aseptic working conditions. Anaesthesia with 2% lidocaine was administered in the projection of the root tip for the upper jaw teeth, or mandibular for the lower jaw teeth. Aseptic working conditions were provided by a dental dam and aspirator. Pulp chamber trepanation was performed by a round dental burr. After that, the access cavity was prepared and pulp extirpation was done with a device of an appropriate size. After extirpation, pulp samples were immediately placed in marked sterile Eppendorf tubes and frozen. Then, homogenisation was performed by a Teflon® pestle in ice-cold phosphate buffer solution at pH 7.4 whose volume was adjusted according to the weight of tissue, so that the final homogenate concentration was 10%. The supernatant was then frozen at -70°C until the performance of appropriate biochemical analyses.

Determination of IL-1β concentration – enzyme-linked immunosorbent assay (ELISA) test

In order to measure the IL-1β content in dental pulp, we applied the procedure called ELISA test. For ELISA test we used specific commercial systems of kits particular to IL-1β. The kits are produced by R&D Systems Inc. Minneapolis, USA. We applied the high sensitivity system technique, which may register low levels of cytokines, ranging from 0.125 to 8.0 pg/mL for IL-1 β.

The procedure applied in order to quantify tested cytokines is based on the so-called enzyme-linked immunosorbent assay (Quantikine HS ELISA Assay Principle). Sample density value reading during ELISA test was done by the Ascent plate reader (Thermo Labsystems).

Monoclonal antibodies specific for IL-1β are bound to the surface of a microtitre plate. Standards and samples were added to the sample wells and, during incubation, were bound to antibodies. Microtitre plate wells were then washed out by buffer solution thus removing the excess of unbound cytokines. Enzyme-linked polyclonal antibodies specific for particular cytokines were added to the wells. After incubation the excess of antibody-enzyme reagent was removed by rinsing. Addition of substrate causes colour development which is proportionate to the quantity of cytokines bound in the initial reaction. In order to stop colour development we added 2 N sulphuric acid and colour intensity was read on the wavelength of 450 nm and the obtained values were expressed in pg/mL.

For primary data analysis we used descriptive statistics and statistical hypothesis test. Within descriptive statistics we used measures of central tendency (arithmetic mean), measures of the variability of distribution (standard deviation) and relative numbers. In order to test the hypothesis of the significance of the difference of mean values for independent samples we used Student's *t*-test and analysis of variance (ANOVA) with Tukey *posthoc* test. The statistical significance criterion was $p < 0.05$ or $p < 0.01$. Statistical processing of the results was done by a software package used for statistical analysis, SPSS (version 21).

Results

Cytokine concentration in a homogenate of healthy and chronically inflamed dental pulps was determined by the application of ELISA test. Tests encompassed 53 pulp samples (including the control group) and the cytokine concentration was analysed by the following groups: chronically inflamed, open and closed *pulpitis*.

Examination of the IL-1β concentration in pulp tissue showed a significant cytokine concentration in all samples. Figure 1 shows IL-1β concentrations in the patients with chronic *pulpitis* (n = 41) and the control group (n = 12). The arithmetic mean of IL-1β in the patients with chronic *pulpitis* was 4.24 ± 2.64 pg/mL (range, 1.54–8.82 pg/mL), and in the patients from the control group 3.94 ± 1.26 pg/mL (range, 2.5–6.89 pg/mL). There was no statistical difference between the patients with chronic *pulpitis* and the control group related to IL-1β values ($t = 0.543$, $p = 0.590$) (Figure 1).

The arithmetic mean of IL-1β in the patients with chronic closed *pulpitis* was 6.21 ± 2.70 pg/mL (range, 1.71–8.82 pg/mL), in the patients with open *pulpitis* 2.54 ± 0.66 pg/mL (range, 1.54–3.67), and in the patients from the control group 3.94 ± 1.26 pg/mL (range, 2,5–6,89 pg/mL). Among the patients with chronic closed *pulpitis*, chronic open *pulpitis* and the control group there was a statistically significant difference related to IL-1β values ($F = 21.883$, $p < 0.01$).

IL-1β values were statistically significant higher in the group with chronic close *pulpitis* when compared to the group of the patients with chronic open *pulpitis* ($p < 0.01$), and in the group with chronic closed *pulpitis* when compared to the patients from the control group ($p < 0.01$), but the difference was not statistically significant between the patients with chronic open *pulpitis* and the patients from the control group ($p = 0.081$) (Figure 2).

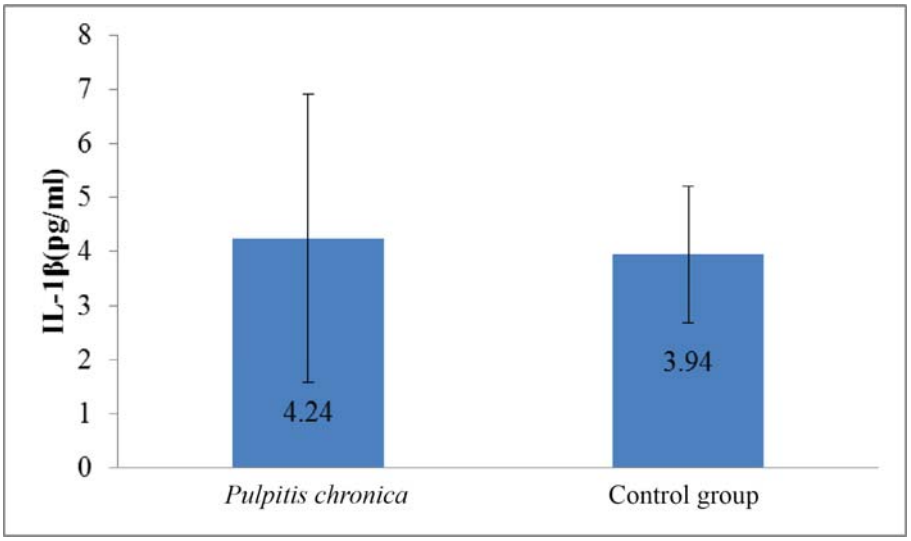

Fig. 1 – Interelukin-1 beta (IL-1β) values in subjects with chronic pulp inflammation and the control group

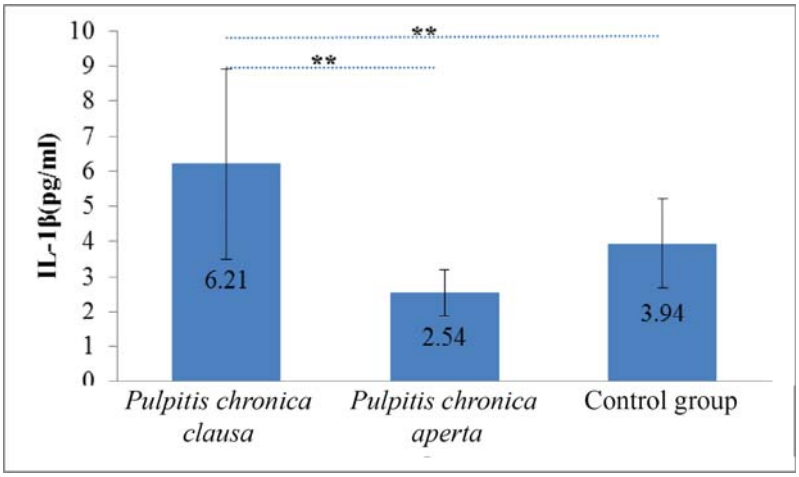

Fig. 2 – Values of Interelukin-1 beta (IL-1β) in the subjects with chronic pulpitis clausa, pulpitis chronica aperta and the control group.
****statistically significant difference ($p < 0.01$).**

Discussion

Biological response of pulp to stimuli and events which control these processes are still not clear [12]. Contemporary methods of molecular biology and genetic engineering make it possible for all laboratories to quantitatively and qualitatively determine cytokines in body fluids or tissue cell cultures. Due to a more frequent use of cytokines as possible modulators of inflammation, immunity and haematopoiesis, they are often called biological response modulators. Several biological molecules [tumor necrosis factor α (TNF α), IL-8, IL-2)] are identified and found in inflamed pulp in higher concentrations than those registered in healthy pulp. These substances attract immune cells such as polymorphonuclear cells and macrophages [12]. Diagnostic assessment of biologic markers may improve validity of predicting or treating inflammatory pulp disease [13]. In our research, we analysed the concentration of proinflammatory cytokine in pulp tissue of chronically inflamed and healthy dental pulp. IL-1 was the first described as a lymphocyte activating factor and it was then discovered that IL-1 has a number of other biological activities and that there are at least two major types of IL-1 (alpha and beta) which bind to the same receptor [14].

Present researches are related to the examination of pulp tissue and cytokine expression in healthy and inflamed pulp. Production of IL-1, IL-2, IL-6, IL-8, IL-18 and TNFα and cyclooxygenase-2 (COX-2) in pulp tissue with symptomatic inflammation was published by numerous authors in their studies [15–21].

After damaging dental pulp, some cells produce cytokines important for initiating and controlling the inflammation process. In this case, the most important cytokines are: IL-1β and IL-8. Silva et al. [10] in their study analysed the location, distribution and concentration of IL-1β and IL-8 in healthy and inflamed dental pulps. Immunohistochemical results confirm that the samples of inflamed pulps were stronger positive for both cytokines than those from healthy pulps. The results of our study do not completely match those from the abovementioned studies [10, 14, 22–24]. The difference in the concentration of IL-1β in chronically inflamed and healthy dental pulp has no statistical significance ($t = 0.543$, $p = 0.590$). However, the difference is statistically significant in the group with chronic close *pulpitis* when compared to the group of the patients with chronic open *pulpitis* ($p < 0.01$), and in the group with chronic closed *pulpitis* when compared to the patients from the control group ($p < 0.01$). Higher level of IL-1β in closed *pulpitis*, in comparison to the control group is expected, which is also in accordance with other studies [10, 15, 25–27], since it is known that IL-1β stimulates the inflammation process. The difference is not statistically significant between the patients with chronic open *pulpitis* and the control group ($p = 0.081$). This level of IL-1β concentration in chronic open *pulpitis* remains somewhat a mystery and poses a question for a new, expanded study. Maybe the reason of the decrease in IL-1β concentration in chronic open *pulpitis* can be linked to the fact that pulp is in an advanced inflammation stage leading to pulp necrosis. In 1985 Hes [28] wrote that chronic open *pulpitis* is always total, meaning that infection and inflammation engulfs the pulp up to the apex. Systematic assessment of the results of the earlier studies, ours included, suggests that gene expressions of cytokines increases in pulp tissue during inflammation. Findings about the important roles of IL-1β, IL-6, IL-17 and TNF-α in pathogenesis of chronic inflammation may be used in finding the most efficient therapy for the treatment of chronic diseases. Herman et al. [29] think that in the future we can expect the development of new medicine which will act as anti-cytokine, thus preventing cytokines to bind with their receptors. In this manner we would be able to selectively and with less side effects block a particular cytokine [29].

Conclusion

On the basis of the obtained data we conclude that IL-1β could be found in all vital pulp tissues. The highest concentrations of this protein were found in the sample group with *pulpitis chronica clausa*.

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Ipsilateral humeral neck and shaft fractures

Ipsilateralni prelom vrata i dijafize humerusa

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Abstract

Background/Aim. Fractures of the proximal humerus or shaft are common, however, ipsilateral neck and shaft humerus fracture is a rare phenomenon. This combination injury is challenging for orthopaedic surgeons because of its complex treatment options at present. The purpose of this study was to review a series of ipsilateral humeral neck and shaft fractures to study the fracture pattern, complications and treatment outcomes of each treatment options used. **Methods.** A total of six patients (four female and two male) with the average age of 42.8 years (range: 36–49 years) was collected and reviewed retrospectively. Two of them were treated with double plates and four with antegrade intramedullary nail. According to the Neer's classification, all proximal fractures were two-part surgical neck fractures. All humeral shaft fractures were located at the middle of one third. Five fractures were simple transverse (A3), one fragmented wedge fracture (B3). One patient had associated radial nerve palsy. **Results.** All surgical neck fractures except one united uneventfully in the average time span of 8.7 weeks. Four humeral shaft fractures healed in near anatomic alignment. The remaining two patients had the nonunion with no radiological signs of fracture healing. The average University of California, Los Angeles End-Results (UCLA) score was 23.1. On the contrary, the average American Shoulder and Elbow Surgeon's (ASES) score was 73.3. The patients treated with antegrade intramedullary nails presented 70.5 points. The ASES scores were 79 in the double plates group. **Conclusions.** Ipsilateral humeral shaft and neck fracture is extremely rare. Both antegrade intramedullar nailing and double plates result in healing of fractures. However the risk of complication is lower in the double plating group.

Key words:
humeral fractures; fractures, multiple; fracture fixation, intramedullary; bone plates; treatment outcome.

Apstrakt

Uvod/Cilj. Prelomi proksimalnog humerusa ili dijafize humerusa su vrlo česti prelomi, međutim, ipsilateralni prelom proksimalnog humerusa i dijafize je vrlo redak. Ova kombinacija preloma je izazov za ortopedske hirurge zbog složenih opcija lečenja. Cilj ove studije bio je da se prikaže serija ipsilateralnih preloma proksimalnog humerusa i dijafize, razmotre komplikacije i ishod dve različite metode hirurškog lečenja. **Metode.** Šest bolesnika (četiri žene i dva muškarca) prosečne starosti 42,8 godina (raspon 36–49 godina); analizirani su retrospektivno. Dva bolesnika lečena su duplim pločama, a četiri anterogradnim plasiranjem intramedularnog klina. Prema Nerovoj klasifikaciji, svi prelomi proksimalnog okrajka humerusa bili su dvodelni. Svi pelomi dijafize bili su locirani u srednjoj trećini, pet preloma bila su tipa A3, a jedan tipa B3, prema AO klasifikaciji. Jedan bolesnik imao je leziju radijalnog nerva. **Rezultati.** Svi prelomi proksimalnog okrajka (vrata) humerusa, sem jednog, zarasli su bez komplikacija za prosečno vreme od 8,7 nedelja. Četri preloma dijafize humerusa zaraslo je u dobroj poziciji, dok kod dva preloma nije došlo do zarastanja (bez radioloških zarastanja). Prosečni *University of California, Los Angeles* (UCLA) skor bio je 23,1. Naprotiv, prosečan *American Shoulder and Elbow Surgeon's* (ASES) skor iznosio je 73,3. Bolesnici lečeni anterogradnim intramedularnim uklinjavanjem imali su prosečni ASES skor 70,5, dok je skor kod bolesnika lečenih duplom pločom iznosio 79. **Zaključak.** Ipsislateralni prelomi proksimalnog humerusa i dijafize vrlo su retki. Primenom anterogradne intramedularne fiksacije ili osteosinteze duplom pločom postiže se zarastanje preloma, međutim, rizik od komplikacija niži je kod ipsilateralnih preloma rešenih osteosintezom duplom pločom.

Ključne reči:
humerus, prelomi; prelomi, multipli; prelomi, intramedularna fiksacija; pločice za kost; lečenje, ishod.

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Introduction

Fractures of the proximal humerus or shaft are common; however, simultaneous fracture of the ipsilateral humeral neck with shaft is an uncommon occurrence. This phenomenon has been seldom reported in the literature [1]. Ipsilateral femoral neck and shaft fracture is well-reported, and this unique fracture is encountered in younger patients after high-energy trauma [2, 3].

There has been no report on the fracture pattern, complications and surgical outcomes regarding ipsilateral humeral neck and shaft fracture in the literature, nor suggested fracture mechanism. This combination injury is challenging for orthopaedic surgeons because of its uncommon occurrence and there is a lack of consensus about surgical management of this complex trauma pattern. The goal of the treatment is fracture healing and avoiding potential complications such as avascular necrosis, nonunion or delayed union.

The aim of this study was to review a series of this rare ipsilateral humeral neck and shaft fractures, to study this fracture pattern in detail, to understand the treatment options used and highlight the potential complications.

Methods

Between January 1999 and December 2006, a total of six patients with ipsilateral humeral neck and shaft fractures were collected and reviewed. All medical notes and radiographs were reviewed retrospectively.

There were four female and two male patients, the average age of 42.8 years (range, 36–49 years). The right side was affected in three patients and this was the dominant arm in all the patients. Four of the patients were injured in road traffic accidents, and two by fall from a significant height. Four patients had other associated injuries: rib fractures of the same side in two patients, tibia and femur fractures in one, and anterior cruciate ligament injury in one.

Facture classification

Proximal humerus fracture was classified based on both Neer's [4] and Müller [5] AO Classification of Fractures. According to the Neer's classification, all fractures were included in two-part surgical neck fractures, except one which was one-part undisplaced fracture. There was no two-part tuberosity or three-, four-part fractures. Based on the Müller AO Classification of Fractures, there were two A32 (simple neck fracture with translation), one A31 (simple neck fracture with angulation), and the other three included in A2 (impacted metaphyseal fracture) without displacement (Figure 1).

Humeral shaft fractures were also classified based on the Müller AO Classification of Fractures. All humeral shaft fractures were in the middle of one third. Five fractures were included in simple transverse (A3), one in fragmented wedge fracture (B3). Of the five A3 fractures, three were spiral and two were oblique. One patient had associated radial nerve palsy (Table 1A).

Surgical technique

The average interval from the injury to surgery was 2.8 days (range: 1–5 days), and surgery was conducted as soon as the patients' overall medical condition was suitable.

Double plates technique

The deltopectoral approach was used for surgical neck fracture and the anterolateral Henry approach, splitting brachialis was used for shaft fracture fixation. One patient was treated with a T-shape dynamic compression plate for surgical neck fracture and the other one with a proximal humeral locking compression plate. The conventional 4.5 mm AO/ASIF limited contact dynamic compression plate (Synthes, Paloli, PA) was used for shaft fractures (Figure 2).

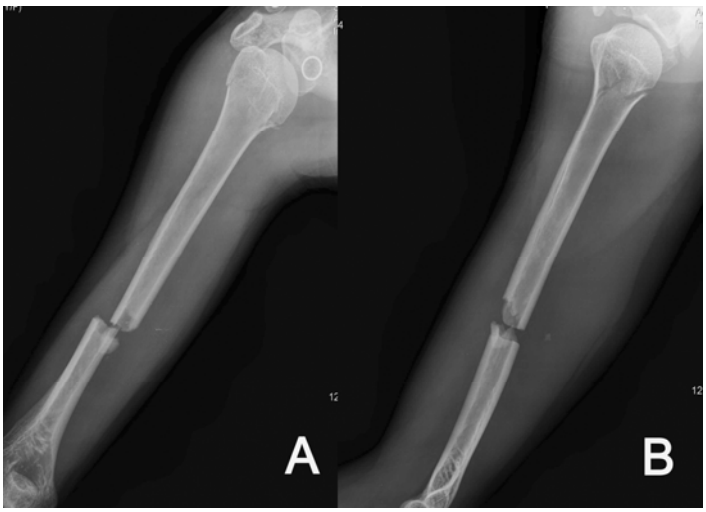

Fig. 1 – 47 year-old lady (case No 6) sustained multiple rib fractures and fractures of the right upper extremity. Proximal humeral neck fracture, initially not recognized, was identified. Non-displaced two-part surgical neck fracture, Müller AO Classification A2 (impacted metaphyseal fracture) was diagnosed for humeral neck fracture intraoperatively. Shaft fracture was classified as simple transverse fracture (AO Classification A31).

Table 1A

Details of the patients with ipsilateral humeral neck and shaft fracture

Case No	Age	Sex	Cause of injury	Associate injury	Diagnosis of the neck fracture	Side	Neck fracture (Neer)	Neck fracture (AO)	Shaft fracture (AO)	Op for neck	Op for shaft
1	49	F	RTA	None	Preop	L	2 part SN	A32	A32	CR/IF c AIN	OR/IF c AIN+cable
2	45	F	RTA	Radial n palsy	Intraop	L	2 part SN	A2	A31	CR/IF c AIN	OR/IF c AIN+cable
3	36	F	RTA	femur, tibia SI fx, MRF	Preop	R	2 part SN	A31	B	CR/IF c AIN +percu screw	CR/IF c AIN
4	40	M	Fall	None	Preop	R	2 part SN	A32	A31	OR/IF c Plate	OR/IF c Plate
5	40	M	RTA	ACL	Preop	L	2 part SN	A2	A32	CR/IF c AIN	CR/IF c AIN
6	47	F	Fall	MRF	Intraop	R	2 part SN	A2	A31	OR/IF c Plate	OR/IF c Plate

F – female; M – male; RTA – road traffic accident; Radial n palsy – radial nerve palsy; SI fx – sacroiliac fracture; MRF – multiple rib fractures; ACL – anterior cruciate ligament injury; Preop – preoperative; Intraop – intraoperative; L – left; R – right; SN – surgical neck; CR/IF c AIN – closed reduction and internal fixation with antegrade intramedullary nail; OR/IF c plate – open reduction and internal fixation with plate; percu screw – percutaneous screw fixation.

Fig. 2 – The previously described patient was treated with open reduction and internal fixation with double plates. Nine months after the operation, there was persistent fracture gap which required autogenous bone graft. However, there were no radiographic signs of avascular necrosis in the humeral head.

Antegrade intramedullary nail

Four of the patients were treated with antegrade type intramedullary nail. The anterolateral deltoid splitting approach between anterior and middle fibers of deltoid was used. After vertical incision in the *supraspinatus*, an entry portal was made at 2 cm posterior to the biceps groove. At least three proximal interlocking screws and two distal interlocking screws were used. Three of the patients were treated with Polaris intramedullary nail (Acumed, Beaverton, OR) (Figure 3). Two of them required additional circulate wiring for fixation of the spiral fracture of the shaft.

Functional outcome

Functional outcome of the shoulder was evaluated using the American Shoulder and Elbow Surgeons' (ASES) Score, and University of California, Los Angeles (UCLA) Score system [6, 7].

Postoperative regimen

A functional brace with arm sling was applied after the operation in all patients. Active elbow and wrist range of motion exercise was allowed immediately after the operation. Pendulum and passive shoulder exercise started two weeks after the surgery, depending on the patients' tolerance. The active shoulder range of motion was only allowed after identification of fracture healing at radiographs.

Results

There were no open fractures identified. All the patients were followed-up for the mean of 35.6 (range, 12–60) months postoperatively.

Surgical neck fracture was not initially appreciated in two of the patients (cases 2 and 6), because of inadequate preoperative radiographs; thus the diagnosis was humeral shaft fracture preoperatively. During the operation for hume-

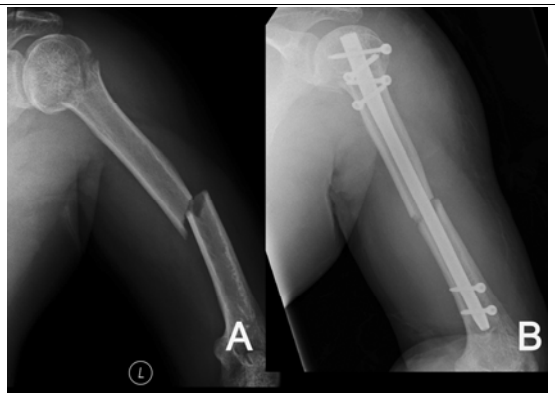

Fig. 3 –A 40-year-old man (case No. 5) sustained fracture after road traffic accident: A) Plain radiographs showed ipsilateral humeral neck and shaft fractures; B) The patient was treated with antegrade intramedullary nail Polaris (Acumed, Beaverton, OR) and postoperative radiographs presented 3 mm gap between the fracture fragments.The patient developed shaft nonunion which required autogenous bone graft.

ral shaft fixation, they were identified and diagnosed with AO type A2 (impacted metaphyseal fracture).

Surgical neck fracture outcome of treatment

All surgical neck fractures except one were united uneventfully on the average of 8.7 (range, 8–12) weeks**.** There was one nonunion in displaced surgical neck fracture (Müller AO Classification A32), which was treated initially with antegrade intramedullary nail. Nonunion was identified three months after the operation with re-displacement of a fracture fragment and screw loosening. Revision surgery was performed with a proximal humeral locking compression plate and autogenous bone graft, which resulted in union three months postoperatively (Figure 4).

Plain radiographs review until the final follow-up showed no patients with signs of avascular necrosis of the humeral head; none of the patients showed localized osteopenia, sclerosis, subchondral fracture, depression or flattening of head and fragmentation.

Humeral shaft fracture outcome of treatment

Four fractures healed in near anatomic alignment without significant shortening (1 cm or less) or angular deformity (over 10 degrees). The remaining two patients resulted in nonunion with no signs of fracture healing and persistent fracture gap in plain radiographs, both presented simple transverse fracture at initial examination (Müller AO Classification A3). These two patients required autogenous bone graft for bone union only. The average time to union was 12 weeks (range, 10–14 weeks) for shaft fractures. When the fracture healed the time for union was found to be similar in the double plate and nailing groups.

Functional outcome of the shoulder

The average UCLA score was 23.1 (range, 18–29). There were two good results, while four were poor (Table 1B).

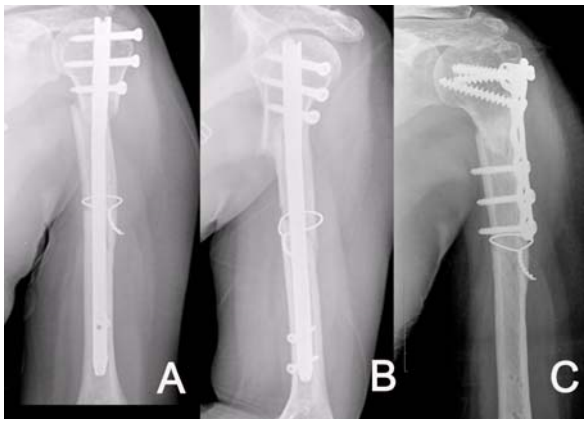

Fig. 4 – A 49-year-old lady (case No. 1) sustained fracture after road traffic accident: A) Plain radiographs showed ipsilateral humeral neck (two part surgical neck fracture, Müller AO Classification A32) and shaft fracture (Müller AO Classification A32). This was initially treated with antegrade intramedullary nail; B) persistent fracture gap seen was developed at 3 months after the operation with re-displacement of fracture fragment and screw loosening; C) Revision surgery was performed with proximal humeral locking compression plate and autogenous bone graft, which resulted in union three months after the revision surgery.

Table 1B

Details of the patients with ipsilateral humeral neck and shaft fracture

Case No	Age	Sex	Union time for neck (weeks)	Union time for shaft (months)	Complications → subsequent revision surgery	UCLA score	ASES score	Flexion of Shoulder	Follow up (months)
1	49	F	Nonunion	3	Nonunion of the neck → Plate/BG	21	77	140°	28
2	45	F	12	3		18	60	140°	12
3	36	F	8	3	Flexible rod protrusion → implant removal	29	85	160°	28
4	40	M	12	3		22	73	160°	60
5	40	M	13	Nonunion	Nonunion of the shaft → BG	20	60	150°	38
6	47	F	8	10	delayed union of the shaft	29	85	160°	48

F – female; M – male; BG – bone graft; UCLA score – University of California, Los Angeles End-Result Score system; ASES – the American Shoulder and Elbow Surgeons'.

One patient with revision surgery for nonunion of neck fracture and another one with radial nerve palsy resulted in a poor functional result. One patient complained shoulder pain and motion limitation after antegrade nailing, because of protrusion of flexible intramedullary nail over the greater tuberosity. The symptoms were resolved with removal of the nail after fracture healing. The overall poor results were present in the patients who had revision surgery or delayed union or associated neurologic impairment in the same extremity.

Complications

There were no complications related to surgery, such as deep infection or neurovascular injuries. Two patients with nonunion (one in the humeral neck and the other one in the humeral shaft) from the antegrade intramedullary nail group and one nonunion from the double plates group underwent the additional autogenous bone graft procedure.

Discussion

Ipsilateral humeral shaft and neck fracture is rare, and has been paid less attention in the literature compared to ipsilateral femoral neck and shaft fracture. The reported incidence of ipsilateral femoral neck and shaft fracture ranged from 2.5% to 6% of all femoral shaft fractures [2]. It is about 0.5% in our series for the last seven years.

Ipsilateral humeral neck and shaft fracture occur in the relatively young patients (the average age, 42) and they were the victims of high-energy injuries (four sustained their fracture after road traffic accident and two from a fall). However, none of these patients presented ipsilateral elbow or wrist injuries, although knee injuries are comparatively common in ipsilateral femoral neck and shaft fracture [8].

In this series, three out of six neck fractures presented linear or minimally displaced surgical neck fractures classified as type A2, two-part surgical neck fracture. There were no two-part tuberosity fractures or three/four-part fractures of the humeral neck in this series. Shaft fractures were mostly simple. Spiral fractures were more common than oblique or transverse pattern.

The femoral neck fracture is often missed and some authors recommended preoperative CT scan in high risk patients [9]. In this series, two neck fractures were identified intraoperatively, which were missed due to inadequate preoperative film. None of the patients presented associated glenoid or scapular fractures.

Numerous surgical treatment options are available for the treatment of humeral neck and shaft fracture, such as non-operative treatment, internal and external fixation, shoulder arthroplasty, intramedullary nail, and minimally invasive percutaneous osteosynthesis [10–12]. However, only two techniques of intramedullary nail and plate fixation were used for ipsilateral humeral neck and shaft fracture [2, 3, 8]. In this series, four patients were treated with reconstruction type antegrade intramedullary nailing using proximal interlocking screws for surgical neck fracture fixation. The other two had double plate fixation. In this series, we found a less chance of nonunion in the plate fixation group. In addition, the risk of shoulder stiffness related to surgical violation of rotator cuff violation was another concern in the patients treated with antegrade intramedullary nailing.

We had two patients with nonunion in humeral shaft fracture after antegrade intramedullary nailing, and both required additional bone graft for achievement of union. Previous studies showed that intramedullary nailing is associated with an increased risk of shoulder impingement, with the related increase in restriction of shoulder movement and the need to remove it, and is associated with the increased risk of the delayed healing rate [13, 14]. However, in our study we found that the reconstruction type antegrade nailing for ipsilateral humeral neck and shaft fracture has a potential risk of nonunion in shaft fracture (two patients out of four had nonunion).

Complication such as avascular necrosis of the head was not observed in this series. There was no fracture dislocation in the shoulder and none of the neck fractures were identified late after the operation.

The limitation of this study is a small number of patients, retrospective review and no controlled study, which makes direct comparison of the two groups difficult. It also includes the inherent disadvantage of retrospective study, because there were no distinct indications of each different modes of fixation. However, considering the rarity of this injury, this series can provide valuable information to predict the prognosis to treating doctors who might encounter this complicated injury.

Conclusion

This was the first report of ipsilateral neck and shaft fracture, which delineated that concomitant neck fractures are usually undisplaced or minimally displaced and shaft fractures are usually unstable. Considering the fact that there is no evidence-based medicine regarding optimal management of these combinations, it is useful to know that both antegrade intramedullary nailing and double plates produced reliable outcome for the treatment of this combination injury, but the risk of complication was lower in the double plating group.

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Artificial intelligence in clinical medicine and dentistry

Veštačka inteligencija u kliničkoj medicini i stomatologiji

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Introduction

The involvement of computer systems in clinical medicine and dentistry has of late become a necessity. These computer systems monitor the admission and treatment of patient, supported by health care professionals, from the moment of setting the time and date of examination, *via* opening a digital patient file (record), patient history taking, support in clinical diagnosis of a disease, analysis of radiographs, help in laboratory analyses, and all the way to the assistance in determination and administration of appropriate treatment. Although the field of use of computer systems in modern medicine and dentistry is very wide and diverse, the most sophisticated area is unquestionably artificial intelligence (AI) [1].

As the expression, it itself suggests that AI enables machines to think and present the results of such thinking. These are most commonly the suggestions regarding the choice among the existing options or solutions in insufficiently clear or doubtful situations, such as the selection of the most appropriate site in the jaw bone where dental implants could be placed without injury to the adjacent tissues and with maximum static product [2], but also in identifying new, unknown solutions. This paper aims at informing the readers about contemporary achievements in the field of AI use in clinical medicine and dentistry.

The concept of AI

Roughly speaking, we may say that AI studies and projects intelligent systems are able to "experience" their own environment and take measures to maximize their own chances for success in it [1, 3]. AI is being used in various disciplines such as medical diagnosis, exchange market, control of robots, law, science, or entertainment. Although the origin of AI can be traced back to more distant past (e.g. to the machines and sketches of Leonardo da Vinci, to ancient Greek machines such as the *Antikythera* mechanism), it is usually associated with the invention of first usable computers after the Second World War, or in the year 1956 when John McCarthy used the term in its full meaning for the first time [3]. In general, AI consists of the knowledge base, research methods, problem-solving systems, reasoning systems, planning systems, learning systems (from previous examples/instances and from the knowledge base), genetic programming, and decision-making or conclusion-drawing systems [3, 4]. In fact, since ancient times, philosophers and mathematicians have presented their own interpretations of the course of human thinking and decision-making, striving to delineate general patterns and rules underlying the process. Although there have been attempts throughout history to create „intelligent" machines based on mechanical scientific achievements, the first real successes have occurred only with the discovery of digital electronic computers and scientific papers of Alan Turing [5], a British mathematician, who laid the groundwork of artificial intelligence and created the Turing test – the test measuring a machine's ability to imitate human intelligence.

Since the 1950s, supported by numerous discoveries in neurology, information technology and cybernetics, statistics and mathematics, and by reviewing and summarizing the avai-

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lable knowledge in the fields of logic and philosophy, AI has made a huge progress. Perhaps the best example in that regard are the modern systems for the game of chess, where even the world chess champions, such as Garry Kasparov, have lost the matches playing against computers. Further on, there are modern Internet browsers able to predict the users wishes, and various other systems such as logic games, software packages assisting planning and construction in mechanical engineering, electronic engineering, simulations in aircraft industry, and so on.

The difference between human and AI is still a huge one. AI still bases mainly on the algorithms that rely on step-by-step reasoning, similar to the human brain solving a puzzle or making logical deductions [3, 6]. That is the reason why more difficult tasks require huge computer resources. In order to cope with the situation, researchers have created the methods and algorithms able to process uncertain and incomplete information, involving the concepts of the probability theory.

In contrast to machines, humans employ intuitive decision-making to resolve a large number of problems and do not always employ their thinking skills and probability consideration and reasoning (consideration of all possible situations; in computer science commonly called the brute force method). AI tries to get as close as possible to this "subsymbolic" way of thinking [7–9]. Perhaps the biggest step in that direction has been made in programming of logic games, when instead of considering all the possible combinations from the ongoing moment to the end, consideration of only the few steps in advance is used according to predetermined value functions (the Minimax procedure employed for searching the tree of moves) or the move is chosen based on the table prepared in advance [10].

The ability to learn and acquire new knowledge is an essential component of AI. AI has to be able to decide whether and in what degree the obtained pieces of information are true (correct), but also to learn to cope with false information, without endangering the aspect of the whole of applied resources.

AI primarily utilizes several basic forms of logic: mathematical logic, the statements of which can be true or false [11]; first-order logic or predicate calculus of the first order, representing a formal deduction system allowing the use of quantifiers and predicates, able to express the facts about objects, their characteristics, and relations with one another [12, 13]; fuzzy logic, allowing the truth of a statement to be represented as a value between 0 and 1, instead of simply true (1) and false (0). Fuzzy logic can be used for uncertain reasoning in the systems in which there are no certain or precise statements [14]; other forms.

AI is required to cope successfully with incomplete and not always true information. That is the reason why it involves different methods and mechanisms that can be of use in that regard: Bayesian networks is a graphical model encoding the complete probability of association/relationships of the variables of interest [15]; probability algorithms (Markov chain, Kalman filter, Coin-tossing, Monte Carlo and others) used in filtering, prediction, and understanding of a chain of information [16, 17]; others.

AI requires excellent classifier behavior and utilizes: Naïve Bayes classifier, a simple probability classifier based on the Bayes' theorem, introducing simplification in the form of supposed independence of each other of the values of attributes in an ordered set of finite n-elements [18]; k-nearest neighbors algorithm, a very intuitive method classifying unlabeled samples based on primer similarity [19, 20]; Gaussian Mixture Model (GMM), representing a parametric function of probability distribution [21]; Decision Tree Classifiers, breaking down complex decision-making processes into a series of simple decisions, enabling solutions that can be easily understood and used [22, 23], others.

In the processes of artificial reasoning, an essential part of AI are artificial neural networks, as an alternative to traditional analytics. Artificial neural networks are modelled based on the discovery of biological mechanisms of operation of the human brain [24]. The concept itself is similar to the transmission of neural signals and operation of the human brain [24]. Programming languages for AI are the principal tool in the assessment and creation of computer programms and understanding of symbolic information in a context. Although for a lower level of symbolic programming the use of standard programming languages is appropriate (such as C/C++, C#, Fortran, Pascal, and similar), for Rapid Application Development of AI applications, the following specialized programming languages are commonly used: Prolog; Lisp; others [25].

Overview of AI application in clinical and dental medicine

The use of AI in medicine is a more recent phenomenon, and it has been developing very quickly; nowadays, it is the focus of interest of many scientists in the field of computer use and applications of robotics in medicine. The following references present the latest advancements and available achievements in the field.

Azarkhish et al. [26], investigating alternative options of determination of iron in serum, have shown that by way of analysis of other standard laboratory data, such as mean carpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular Hb concentration (MCHC), Hb/red blood cell (RBC), using the system of artificial neural network (ANN) and adaptive neuro-fuzzy inference system (ANFIS), the level of iron in serum can be predicted with an acceptable degree of precision. This analysis illustrated how an AI system, based on peripheral laboratory results, drew conclusions and guided physicians regarding the possible presence of anemia as the consequence of iron deficiency. Qidwai et al. [27] have proposed a new strategy to be of use to neurologists, neurosurgeons, and orthopedic surgeons, aiming to predict the recovery and health status of a patient after surgery of the spinal column, based on the analysis of preoperative data by the Standard Fuzzy Inference System. The results suggested a high precision rate of predictions (of about 88%) in the population of 501 patients. The system was thus able to contribute to quality decision-making regarding whether to administer or not surgical treatment,

especially for benign lesions, where surgery could be avoided. Tsuchihashi [28] has found the accuracy of 95% in automatized diagnosis of prostate cancer by an AI system and automatized marking of cancerous regions. Syed et al. [29] have proposed and evaluated a system of fully automatic non-parametric learning and drawing conclusions using the AI method, supported by support vector machines (SVMs) on large datasets, in order to calculate the probability of risk for perioperative patient complications, morbidity, and mortality. The system translated individual Current Procedural Terminology (CPT) codes into procedural risk values, and analyzed the robust risk model producing more reliable assumptions compared to the National Surgical Quality Improvement Program (NSQIP) – traditional, individual programme created by the American College of Surgeons (ACS).

Uzuner et al. [30] have observed the relationships of medical complaints of patients in the context of semantic relation (SR) and cross-interference of diagnoses, tests, and therapies. These were classified according to the Unified Medical Language System (UMLS) and organized according to their meaning [31]. Each medical complaint in a patient was represented as a disease or a symptom, creating a unique SR relation for the classification and processing of medical records. In this way, a significant help in drawing inferences was provided to AI systems, and the consequences were new scientific insights and discoveries. Roberts et al. [32] have demonstrated that it is possible to extract meaning from medical texts in patient records and set meaningful relations in order to use the algorithms of machine learning (ML). Large datasets could thus be analyzed in order to identify patterns of interest. This study was performed on the Clinical E-Science Framework (CLEF) system for storing, integration, and presentation of clinical information for research purposes. The study utilized clinical data from 20,000 patients of the Royal Marsden Hospital (Great Britain). Zolnoori et al. [33] have created an extensive classification of AI applications in asthma-like diseases. They looked into the use of AI in the techniques of diagnosis and work-up of patients with asthma, but also in the automated production of new knowledge and prediction of major events and exacerbations in such patients. Kim et al. [34], using an AI system based on the neural network model, have been able to predict toothache in 80% of the examined cases. Nieri et al. [35] using the Bayesian network analysis, have been able to successfuly identify several substrates with direct effect on the final outcome of treatment of impacted maxillary canines. Xie et al. [36] assisted by the ANN, have been able to use a decision-making expert system in order to identify the factors of prevailing influence in decision-making, evaluating the need for tooth extraction before orthodontic treatment of patients with malocclusion. They were able to reach a decision with the success rate of 80% whether a treatment with tooth extraction should be preferred to a treatment without it in a given situation.

Kitporntheranunt and Wiriyasuttiwong [37] created a software medical expert system of assistance in diagnosing ectopic pregnancy. Using an interactive backward chaining inference algorithm, the system analyzed patient medical records and the available information, successfully detecting ectopic pregnancy in 31 out of 32 cases, while recognizing all cases of normal pregnancy. Tamaki et al. [38] formed a data mining tool to identify associations, anomalies, and statistically significant patterns in large datasets, using a system of AI. The system successfully identified a high level of *Streptococcus mutans and Lactobacillus* as the factors of high risk for caries development in school children. Sakai et al. [39] studied the possibility of prediction of acute inflammation of the appendix using a Bayesian model. Based on the data about conditions preceding the disease in patient medical records, the AI system created a model and evaluated probability of the disease in new cases. Käkilehto et al. [40], using the data mining AI technique, analyzed a large set of patient electronic dental records in order to obtain scientifically acceptable conclusions about the duration of dental restorations. The results showed that the life span of dental amalgams and composite fillings was over 15 years, while 60% of silicate dental cement restorations were replaced as early as 5 years after the initial placement, and more than 50% of glass ionomer cement restorations were replaced after 7 years. Korhonen et al. [41], using the data mining technique, have established that dentists more successfully detect caries in their new patients, compared to their old patients. The study was done using an AI system with the data obtained at general physical examinations. Kawaguchi et al. [42] used the data mining technique in order to find complex interactions between the risk factors and clinical evidence of non-hepatitis B virus/non-hepatitis C dependent hepatocellular carcinoma (NBNC-HCC). Since the disease is usually diagnosed in more advanced stages, in order to identify the risk factors, in a cohort of 663 patients with the diagnosis of NBNC-HCC, the Milan criterion was used and artificial analysis was done using the decision tree algorithm. Six factors were identified: date of diagnosis of hepatocellular carcinoma, the diagnosis of cirrhosis of the liver, values of serum aspartate transaminase, alanin aminotransferase, α-fetoprotein, and the level of des-γ-carboxy prothrombin. Miladinović et al. [4] demonstrated that the use of AI enabled successful collection, triage, sorting, numbering, and analysis of raw electronic data related to tooth extraction samples, and that the applied AI algorithms were able to execute non-parametric evaluation of impact of possible non-dental attributes, drawing scientifically acceptable conclusions. In this way, an AI system was able to "understand" and communicate the reasons for tooth extraction, i.e. to indicate the principal substrates that may have influence on such an outcome. Miladinović et al. [43] have also assessed possible use of medical data usage for research purposes and analysis using the AI methods. They suggested a design model of a huge medical data base of collected medical information which would make possible subsequent precise analysis using AI algorithms. In his PhD Thesis, Miladinović [44] presented an AI model of automatization of numerous scientific processes using an AI system, suggesting that AI systems can be of significant diagnostic help in oral surgery, identification of potentially successful measures of disease prevention, and giving new insights into the diseases and health in oral surgery.

Stanley et al. [45] presented their model of automated search and ordering of clinically obtained diagnostic images (histograms, x-rays, etc.) with an image data base for known diseases. In this way, independent computer search is performed, someting similar to fingerprint identification, facilitating the job of a clinician in diagnosis and confirmation of a disease. Korhonen et al. [46] have created and tested a data mining system able to determine independently the life-long mean index of carious teeth, extracted teeth, and/or filled permanent teeth. They found the method feasible, but not suitable for persons below 20 years of age. De Bruijn et al. [47] investigated the applicability of ANN in their analysis of speech nasalization in patients treated for oral or oropharyngeal cancer in order to evaluate hypernasalization in their speech. After the speech sound samples were recorded from 51 patients, using 18 control sounds, they evaluated hypernasalization, articulation, intelligibility, etc. The analysis of nasalization properties was done in the whole speech range, and based on their final results the study was considered feasible, while parameter prediction was graded as medium to poor. Bas et al. [48] have used ANN to predict two subgroups of internal disturbances of the temporomandibular joint and normal joint using characteristical clinical signs and symptoms of disease. Clinical symptoms and diagnoses of 161 patients with temporomandibular joint disorders were set as a gold standard and used for learning by the neural network. After the learning process, AI was presented for analysis with the results from 58 new patients for proper diagnosis to be made. The experiment was successfully finished in about 90% of cases, with the conclusion that ANN application in the diagnosis of subtypes of the disease can be a valuable diagnostic tool especially to dentists. Cilia et al. [49] obtained some promising results in the prediction of viral mutations. They used machine learning methods on the experience of existing viral resistencies developed against the present drugs. Wright et al. [50], using the Constrained Sequential Pattern Discovery using Equivalent Class (CSPADE) algorithm with 90% success rate, have made predictions as to which would be the next drug in the treatment of diabetic patients, based on the patterns observed for present drugs. Wu et al. [51], evaluating computer-aided diagnosis of early knee osteoarthritis from magnetic resonance imaging (MRI) findings, have concluded that AI methods alone were able to make the accurate diagnosis in 75% of cases.

We have to bear in mind that the use of AI in clinical medical and dental practice is still at an early stage of development and still in the investigation phase. The above studies could serve to foretell the bright future for AI in clinical medicine and dentistry. However, despite sky-high expectations, we should always remember to be patient but firm in our purpose in these developmental stages. If we do, we will be rewarded with the full capacity AI used in the resolution of many clinical problems. We should look at some concrete examples of practical use of AI. The AI within the Secretary-Mimicking Artificial Intelligence (SMILE) system designed to aid pathologists is able to listen to voice commands and perform numerous supplementary tasks in the analysis of pathological sections, creating semiautomatically pathological reports [52]. AI softwares are able to make the diagnosis of dementia easily and reliably from structural MRI images [53, 54]. AI softwares process electronic patient records and identify those with systemic sclerosis with the risk of scleroderma renal crisis [55]. In computerized drug prescription systems, AI improves system efficiency and reduces the risk of wrong drug choice by a physician [56].

Suggestions for possible further research directions

We believe that the scope of further research in the field should be increased. The research should be integrated with clinical practice as much as possible, thus obtaining benefits of this technology of the future as early as possible. All patient medical records should be stored in digital form, adequately processed and prepared, ready to be subjected to AI algorithm analysis. AI systems should constantly, repeatedly process and develop such data sets. Numerous essential correlations between the data items could thus emerge and new knowledge breakthroughs are certain without even starting individual targeted studies. This could help us save our resources and capacities, creating at the same time the basis for continual monitoring of patient health and long-term surveillance of the effects of drugs and therapeutic procedures. This type of research also constitutes the basis for identification of health risks which could be monitored using patient electronic medical records.

Conclusion

Results of the studies undertaken so far to investigate the use of AI in clinical medicine and dentistry have shown that this, most sophisticated area of computer use in health care, has some excellent prospects, but that further research and advancements in the field are required, even more intense than at present. The research has to be extensive and strong, so that high quality automation processes could be devised in the discovery of new drugs and new therapeutic methods.

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PRACTICAL ADVICES
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Oral appliances in the treatment of obstructive sleep apnea syndrome

Oralni aparati za lečenje sindroma prekida disanja tokom spavanja

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Key words:
sleep apnea, obstructive; orthodontic appliances; treatment outcome.

Ključne reči:
apneja u snu, opstruktivna; ortodontski aparati; lečenje, ishod.

Introduction

Obstructive sleep apnea (OSA) is the most common respiratory sleep disorder in clinical practice. It occurs as a result of decreased muscle tone in the musculature that dilates the pharynges, and is especially manifested during sleep. Sleep apnea/hypopnea syndrome is described as the occurrence of a minimum of five interruptions in breathing and/or the decrease in ventilation during one hour of sleeping apnea/hypopnea index (AHI) with the existence of daytime and night-time difficulties [1, 2]. Daytime symptoms present with excessive daytime sleepiness, difficulty in concentrating, loss of movements coordination, the feeling of fatigue and exhaustion, impotence, mood disorders like depression, which altogether not only interferes with normal daily functioning and working abilities, but also increases risk of car accident and occupational injury [3]. Night-time symptoms usually are observed by family members of the one suffering from OSA, first of all apneas during sleep and snoring, which can exceed 90 dB in intensity (traffic noise) [4].

A main phenomenon as a consequence of repeated episodes of respiratory disruptions is the presence of so-called intermittent hypoxia, that stimulates the mechanisms of inflammatory response and accelerates atherosclerosis and carcinogenesis [5–7]. It is important to stress that because of disrupted airflow with a constant inspiratory effort the intrathoracic pressure increases, baroreflex function decreases, causing bradycardia with the risk of cardiac arest [8]. Followed by hypoxia-induced sympathetic activity, which leads to tachycardia with arousal and arterial hypertension peak, these events repeat themselves during sleeping for over a hundred times [8]. Hence, sleep fragmentation, intermittent hypoxia, systemic inflammation and chronic sympathetic stimulation caused by OSA induce cardiovascular disorders, including the most common systemic hypertension, ischemic heart disease, congestive heart failure and heart arrhythmias [9, 10]. The occurrence or aggravation of metabolic dysbalance is also possible, specially the deterioration of carbohydrates and lipids metabolism, with the development of a metabolic syndrome Z [1, 2, 11–14]. OSA exacerbates clinical course of respiratory disorders, chronic obstructive pulmonary disease (COPD) asthma and deepens respiratory insufficiency [15]. Gastrointestinal disorders, gastroesophageal reflux disease and hepatic dysfunction are also present [16]. Neurological deficits, cerebrovascular diseases and nerve damage, caused by hypoxia and accelerated atherosclerosis are a repercussion of OSA, as well [17]. Mental health conditions, among which depression mostly, are the complication of OSA [1, 2, 18].

OSA is caused by abnormal anatomy and physiology of the upper airway [19, 20]. That includes obesity, hypothyroidism, tongue and pharyngeal muscle hypotonia, enlarged tonsils, nasal obstruction, prolonged soft palate, enlarged uvula, mandibular retrognathia and micrognathia, macroglossia, narrow maxilla, gothic palate and other [1, 2, 11–13, 21, 22].

OSA therapy

The diagnosis of sleep-disordered breathing is made by polysomnography testing in specialized centers. Depending on the type and severity of the disorder (mild - AHI 5-15, moderate - AHI 15-30, severe - AHI >30), different therapeutic modalities are available, which can roughly be divided

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into conservative and surgical therapy [23]. More practically four groups of treatment are distinguished [19]: hygienic-dietary regimen and lifestyle; surgical approach to upper airways; use of oral appliances (OA) and noninvasive ventilation with continuous positive airway pressure (CPAP). CPAP is a gold standard in sleep apnea syndrome treatment, especially in severe forms of OSA. Depending on the severity of OSA different types of CPAP machines are used [1, 2]. Its efficacy is practically provided in the elimination of apneas and daytime sleepiness, which Sullivan et al. [24] proved back in 1981. Nevertheless, not infrequently patients barely tolerate and accept this device [23]. Only one year after Sullivan et al. [24], Cartwright and Samelson [25] introduced, as a therapy option, tongue-retaining device, the first oral appliance in use.

Oral appliances in the OSA – the Dentist's Role

The name "oral appliance" varies. There are synonyms for both terms: dental, intraoral or mandibular can be used instead of oral, and instead of appliance there are devices, splint or prosthesis [26]. In general terms, this treatment approach relies on repositioning of the mandible and/or tongue and related soft tissues in such a way that the upper airway calib [27]. The potential advantages of such an approach, particularly relative to the current gold standard CPAP, include its simplicity, portability, lack of noise and independence from a power source, and potentially lower cost [19]. Aside from therapeutic effect, one of the advantages is sleep and rest quality improvement of the bed partner, thus promoting partner's health and relationship [26]. All of this has a positive impact on patient acceptance.

Types of oral appliances

Oral appliances used for OSA generally fall into one of two classes: mandibular advancement splints (MAS) (Figure 1), and tongue retaining devices (TRD).

MAS induce protrusion of the mandible by anchoring a removable device to part of or the entire upper and lower dental arches, while TRD uses a negative pressure of a suction cavity to protrude the tongue out of the mouth. MAS are far more widely used in clinical practice and there is the extensive literature on their use, compared to TRD. There are many designs available, but they generally fall into either one-piece (monobloc) or two-piece (duobloc) configurations. Beyond this, they can differ substantially in size, type of material, coupling mechanism, amount of occlusal coverage, degree of customization to the patient's dentition, titratability of mandibular advancement, degree of mandibular mobility permitted, and allowance for oral respiration. Although prefabricated appliances are commercially available, it has been proven that the best clinical outcome is achieved with custom-made oral appliances [23, 26]. Using intraoral devices increases the volume of upper airways, primarily on the account of lateral and partly anteroposterior dimensions, along with decreased pharyngeal wall collapsibility [28], which has been proven clinically by computerized tomography (CT) [29, 30], magnetic resonance imaging (MRI) [31, 32] and video endoscopy [33]. Most of the radiological studies were performed on patients while awake, therefore it is unknown if the same alterations occur during sleep [26].

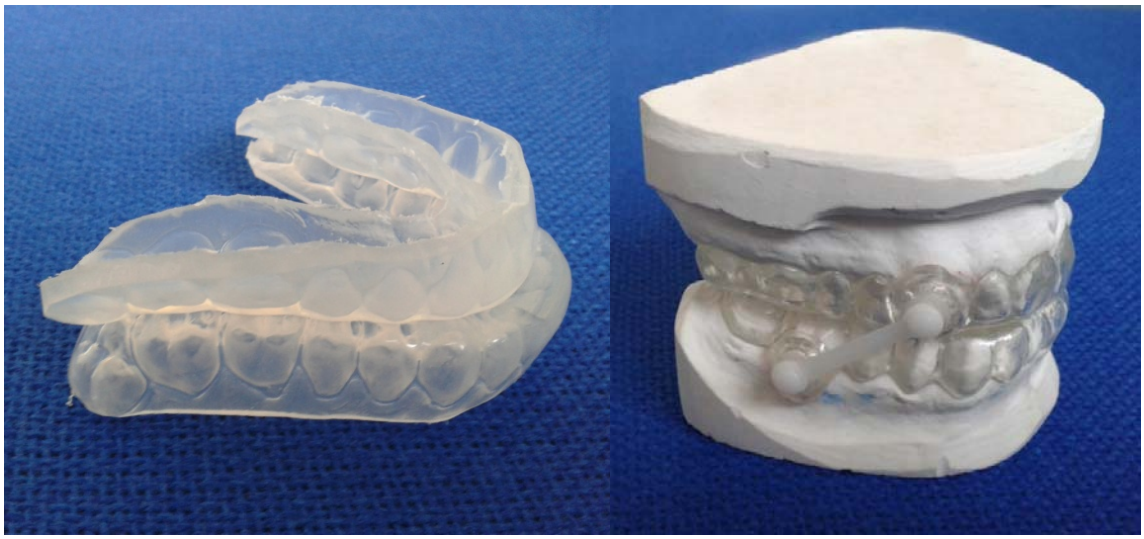

Fig. 1 – Mandibular advancement splints.

Indications

Major indications for this appliance are mild-to-moderate OSA-hypopnea syndrome, in case a patient does not tolerate, is unwilling to accept or unable to comply with CPAP therapy; as well as severe OSA-hypopnea syndrome, in case of CPAP therapy failure [23, 26].

Clinical trials and findings

The effect of OAs on polysomnographic outcomes has been extensively evaluated, and there is strong evidence of clinical benefit in controlling or significantly reducing the

number of obstructive breathing events and arousals, and improving arterial oxygen saturation, particularly in the mild-to-moderate OSA range [19, 23, 34]. It has been confirmed that intraoral devices decrease daytime sleepiness, as well as neurocognitive functioning [35, 36]. The overall success rate is dependent on the definition used, with almost 70% of patients achieving a greater than 50% reduction in AHI [36], and up to 50% achieving an AHI < 5/hour [30, 31, 37, 38]. Previous studies have demonstrated that oral appliances use results in the reduction of blood pressure [36–38]. Beneficial impact was found also in other cardiovascular diseases, in cardiac and endothelial function, as well as in oxidative stress markers, which needs to be investigated in further studies [26, 36–43]. Concerning snoring, intraoral appliances decrease snoring frequency in 40–60%, and intensity to 3 dB [44, 45]. Similarly, MAS has a good therapeutic effect on bruxism coupled with OSA [26].

Clinical predictors of efficacy and tolerance of OA are not well designed so far [26, 34]. From one side, persistent snoring while using the MAS could be a sign of inefficiency in preventing apneas [46]. From the other, milder sleep apnea syndrome, supine position dependent apnea, female sex and lower obesity grade have positive influence on the treatment outcome [24].

Period of adjustment

It is important to note that the patient requires a period of adjustment between two weeks and a month before oral therapy appliances demonstrate their effectiveness. The biggest factor is to adjust the patient's response to the new position of the mandible, which the OA causes during sleep [44, 47, 48]. A period of up to six months of everyday use of MAS with progressive adjustment of mandibular protrusion degree is necessary for the patient's adjustment and satisfactory response [26].

Side-effects

All oral appliances, regardless of design, have potential short- and long-term side effects. Most patients experience acute adverse effects during the initial phase of treatment. Excessive salivation (38–50%) and transient dental discomfort (33%), particularly of the upper and lower front teeth, for a brief time after awakening, are commonly reported with initial use and may preclude early acceptance of an oral appliance [36]. Temporomandibular joint discomfort (12.5–33%), dryness of the mouth (28–46%), gum irritation (20%), headaches and bruxism (12.5%) are other side effects that have been reported [44, 46, 49]. Although these acute side effects are common, for most patients these are minor and transient, subsiding with continued use of the OA. Potential long-term adverse effects can be broken and/or loosened teeth, dislodgement of existing dental restorations, tooth mobility, periodontal complications, muscle spasms, and otalgia [50–54]. These complications can often be avoided by simple recognition and appropriate early response to initial complaints. To monitor for these potential problems, it is suggested that patients with OAs should make periodic visits to the treating dental clinician.

Contraindications

According to numerous clinical trials, OAs are not recommended in case of severe periodontopathy [26]. The Food and Drug Administration of USA does not recommend OAs in case of coexisting central sleep apnea, severe respiratory disease, loose teeth and periodontopathy, as well as for the population younger than 18 years because of insufficient evidence [55]. Despite lateral mouth openings, they are contraindicated in severe nasal obstruction [33, 56]. In case of limited mandibular protrusion, enlarged tongue, edentulousness and edentulism the tongue-retaining device has advantage, while mandibular protrusion splint requires eight stable teeth in each jaw [26]. Pain in temporomandibular joint is not a contraindication. Use of OAs in bruxism (crunching of teeth) during sleep is not contraindicated, but recommended as a mode of therapy.

It could be said that, there has been a significant improvement in the quantity and quality of research and the use of intraoral appliances [45, 57] in the previous decade. Despite great progress, it is still a challenge to make a final and definitive recommendation on the use of intraoral appliances for various types that are used, as well as different types of patients and their anatomical configuration [23].

Conclusion

The cooperation between sleep medicine laboratories and dentists is essential for appropriate selection of patients that can benefit from MAS treatment for OSA.

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CASE REPORTS

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Successful usage of intravenous lipid emulsion in treatment of acute verapamil poisoning – A case report

Uspešna primena intravenskih emulzija masti u terapiji akutnog trovanja verapamilom

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Abstract

Introduction. During the last few years, intravenous lipid emulsions have been effectively used in treatment of acute poisonings with lipophilic substances, including verapamil. **Case report.** A 37-year-old women presented 1 hour after ingestion of 2.8 g verapamil with hypotension and complete heart block. Because of the applied standard therapy failure and further patients impairment, Intralipid® 20% was used. Sinus rhythm was restored, arterial blood pressure increased and verapamile concentrations, both total and free decreased. **Conclusion.** Intravenous lipid emulsion can be important in treatment of severe acute intoxication and cardiotoxicity caused by verapamil.

Key words:
poisoning; suicide; verapamil; calcium channel blockers; heart block; fat emulsions, intravenous; treatment outcome.

Apstrakt

Uvod. Poslednjih godina intravenske emulzije masti uspešno se koriste u terapiji akutnih trovanja lipofilnim supstancama, uključujući i verapamil. **Prikaz bolesnika.** Prikazana je 37-godišnja pacijentkinja primljena jedan sat nakon ingestije 2,8 g verapamila. Na prijemu je bila hipotenzivna, sa kompletnim srčanim blokom. Zbog izostanka efekta primenjene standardne terapije i daljeg pogoršanja stanja pacijentkinje primenjen je Intralipid® 20%, nakon čega je uspostavljen sinusni ritam. Zabeležen je porast arterijskog pritiska, uz smanjenje koncentracije verapamila, kako ukupnog, tako i slobodne frakcije leka. **Zaključak.** Intravenske emulzije masti mogu zauzeti važno mesto u terapiji teške akutne intoksikacije i kardiotoksičnosti uzrokovane verapamilom.

Ključne reči:
trovanje; samoubistvo; verapamil; kalcijum, blokatori; srce, blok; masne emulzije, intravenske; lečenje, ishod.

Introduction

Verapamil is a phenylalkylamine calcium channel blocker used in treatment of angina pectoris, arrhythmia and arterial hypertension. Acute poisonings with this agent are not frequent, but can be severe with high mortality which can be predicted by serum verapamil concentration 1. Clinical manifestations include rapid development of hypotension, bradycardia or other types of dysrhythmias and cardiac conduction abnormalities. Severity of clinical manifestations depends on ingested doses, drug formulation and patient's comorbidity. Toxicity development may be delayed when sustained release tablets are ingested.

Standard treatment protocols include gastrointestinal decontamination, administration of fluids, atropine, calcium, glucagon, inotropic agents, hyperinsulinemia/euglycaemia protocol, temporary pacemaker insertion and supportive measures 2. Very often, cardiocirculatory shock remains refractory to applied antidote, inotropic and vasopressive therapy.

According to the above mentioned, definition and adoption of new and more successful treatment protocols are required.

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During the last years, intravenous lipid emulsions (ILE) have been effectively used in treatment of acute poisonings with lipophilic substances, including verapamil [3]. We presented a case of severe verapamil intoxication successfully treated with ILE. Early administration of therapy led to fast and complete recovery and decrease of serum verapamil level.

Case report

A 37-year-old woman, with a medical history of psychosis and palpitations, presented after 1 hour of ingestion of 2.8 g verapamil in a suicide attempt. On admission the patient was alert, oriented, and eupnoic. The patient was hypotensive with blood pressure of 75/45 mmHg, heart rate was 65/min and oxygen saturation 97%. An electrocardiography (ECG) revealed complete heart block with widened QRS complex and frequent multifocal ventricular extrasystoles (Figure 1).

The initial therapy included isotonic fluids, glucagon (total dose of 10 mg) and calcium chloride (total dose of 1 g), and dopamine. One hour after starting the treatment the patients' condition deteriorated. The patient was somnolent, pale and more hypotensive with blood pressure of 70/30 mmHg. Intralipid® 20% (20% *iv* fat emulsion), 100 mL in *iv* bolus followed by continuous infusion of 400 mL over 30 min and additional 500 mL over the next 1 h was given. Approximately 45 min after starting with Inralipid®, sinus rhythm was restored and arterial blood pressure increased to 105/60 mmHg. The patient remained hemodynamically stable and dopamine infusion discontinued after six hours. ECG showed sinus rhythm with first degree AV block (Figure 2). On the day 3 ECG was normal.

Laboratory tests on arrival showed glucose level of 8.4 mmol/L (normal range 4.1–5.9 mmol/L) and blood urea nitrogen 1.7 mmol/L (normal range 2.5–7.5 mmol/L). Other initial laboratory investigations, comprising complete blood count, electrolytes, renal and liver function tests were in reference ranges and remained so during hospitalization.

The treatment completed without any complications. The patient was discharged on the day 6 and transferred to psychiatric hospital.

Determination of verapamil in serum samples was performed by high performance liquid chromatography with photo diode array detection (HPLC-PDA). After applying Intralipid® the lipid phase was removed by ultracentrifugation of sample on 0ºC and 13,500 rpm for 10 min. Lipid volume was determined by the difference in total serum volume with and after removing lipid phase. Verapamil in lipid phase was calculated as a difference between its quantity in the lipid serum and the serum after removing the lipid phase.

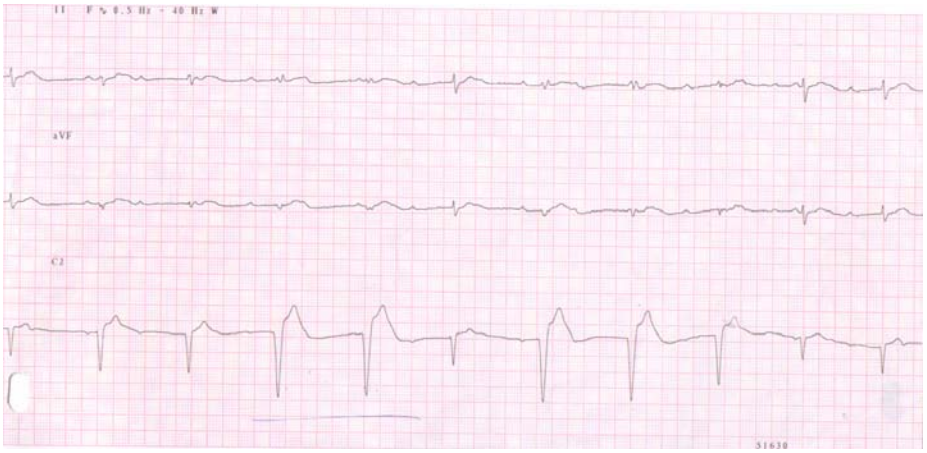

Fig. 1 – Electrocardiographic changes in the patient acutely poisoned by verapamil, noted before Intralipid® 20% administration, showed complete heart block with multifocal ventricular extrasystoles.

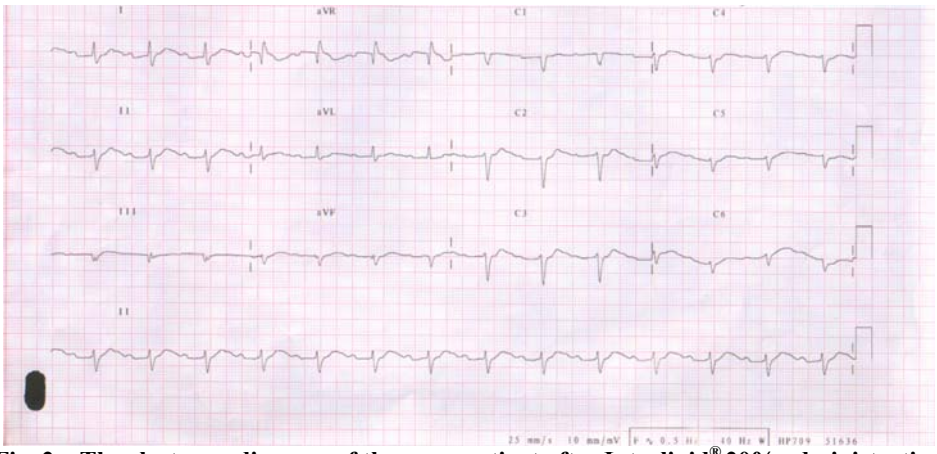

Fig. 2 – The electrocardiogram of the same patient after Intralipid® 20% administration showed sinus rhythm with first degree AV block.

The serum level of verapamil on admission was 2.2 mg/L and metabolites were positive (verapamil therapeutic values range 0.02–0.25 mg/L) [4]. Six hours after Intralipid® 20% administration, the total verapamil level was 1.04 mg/L, the level after removing lipid phase was 0.95 mg/L and the calculated level of verapamile in lipid phase was 11.34 mg/L.

Discussion

ILE have been used in parenteral nutrition for the last 50 years [5]. Over the last decade the use of ILE as antidote has opened new era in clinical toxicology. At the beginning, it has been recommended for the treatment of circulatory collapse and cardiac arrest due to local anaesthetics toxicity [6]. For these cases current dosage regimen is recommended by the Association of Anaesthetists of Great Britain and Ireland. The starting dose is *iv* bolus of Intralipid® 20% 1.5 mL/kg over 1 min with the subsequent 15 mL/kg/h. Bolus doses can be repeated and rate of infusion can be increased up to max 12 mL/kg [7]. There are many dilemmas about indications and dosage in the case of other liposoluble agents toxicity. The number of published case reports and animal studies that show effectiveness of ILE to reverse ECG changes, hemodynamic and neurological parameters in poisoning by lipophilic agents including verapamil, is increasing [3]. The mechanism of action and effectiveness of ILE in acute poisoning by lipophilic agents is basically explained by the 'lipid sink' theory. Lipid intravascular compartment, that is created, pulls lipophilic substances from the tissue and site of action (myocytes, brain) due to high lipid solubility and the established concentration gradient. This shift results in decreasing the free fraction substance concentration and clinical improvement [6, 8]. Increasing intracellular fatty acid and providing energy substrate for myocytes, cardioprotective effect, direct cardiotonic effect and activating calcium channels are some of the other mechanisms that contribute to efficacy of ILE [8].

In an animal model of severe verapamil toxicity it is demonstrated that ILE significantly prolong survival time, prevent the development of bradycardia, increase median lethal dose of the drug and increase mean arterial pressure (MAP) [9, 10].

Effectiveness of Intralipid® in acute verapamil poisoning has been confirmed in case reports [11–13]. The first case of a successful lipid rescue of verapamil poisoning was published by Young at al. [11] in 2009. A patient ingested 13 g of sustained released tablets of verapamil. He had refractory hypotension and junctional bradycardia. Intralipid® 20% was used in a dose of 100 mL over 20 min followed by infusion at 0.5 mL/kg/h for the next 23 h. During the first hour, systolic blood pressure increased and the patient was stabilized. In the report there were no data about ECG changes reversal rapidity. Total serum concentrations of verapamil and norverapamil, measured at 20 h and 36 h after ingestion were 0.99/1.11 µmol/L and 0.62/0.91 µmol/L, respectively.

French at al. [12] demonstrated reducing serum verapamil concentration after removing lipid phase and increasing in MAP associated with ILE administration. The ingested dose was 6.3 g, and the patient was hypotensive with complete heart block. Two 100 mL boluses of Intralipid® 20% followed by 500 mL *iv* infusion over 30 min were administered nineteen hours after ingestion. That resulted in improvement of patient's MAP and lowering of verapamil concentration in the serum. Deterioration of patient's condition with blood pressure decrease was reported 29 h after ingestion. Verapamil concentration increased and 100 mL bolus of Intralipid® was given. Again, hemodynamic improvement was followed by reducing verapamil concentration, but it remained elevated for the next few days. This can be explained by large amount of the sustained release formulation ingested and a lower Intralipid® 20% dose than necessary.

Clinical presentation and high serum verapamil concentration in the presented patient on admission, clearly indicated a serious cardiotoxicity. Failure of the applied standard therapy and further impairment of the patients' condition was the reason for ILE use. During administration, sinus rhythm was restored, arterial blood pressure increased and verapamil concentration, both, total and free serum fraction, decreased.

Efficiency of ILE is shown in acute poisoning with other Ca^{2+} - channel blockers, including diltiazem, nifedipine and amlodipine [8]. Knowing the similar characteristics of those drugs (liposolubility - partition coefficient and volume of distribution), such results are expected [14]. Yet, in assessment of ILE efficacy, we also should bear in mind the possibility of bias, because cases of successful treatment are mainly reported. There were rare case reports in which ILE was not effective. For example, in polydrug poisoning with prevailing effects of amlodipine or nifedipine, fatal outcomes due to respiratory and cardiocirculatory shock occurred despite the use of ILE [15]. However, other drugs probably contributed to the severity of poisoning in described cases and to the failure of ILE therapy.

Conclusion

Successful early usage of Intralipid® 20% in the presented patient, along with other cases in the literature supports lipid rescue treatment in severe verapamil-induced cardiotoxicity. In cases of severe acute intoxication and cardiotoxicity caused by verapamil we believe that there is no reason to delay the use of ILE.

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CASE REPORT

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Modified technique of the treatment for proximal tibiofibular joint dislocation

Modifikovana tehnika lečenja iščašenja gornjeg golenjačnolišnjačkog zgloba

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Abstract

Introduction. Dislocation of the proximal tibiofibular joint (PTFJ) is a rare injury. The diagnosis requires an accurate history of the mechanism and symptoms of the injury, and adequate clinical and radiographic evaluation of both knees. In the literature there is no larger series, only several cases of PTFJ dislocation treated by different methods have been published so far. The aim of the study was to present a modified technique for the treatment of the unstable PTFJ that results in faster recovery of the patient. **Case report**. A 24-year-old football player was injured at the beginning of training; when tackling the ball he felt a sharp pain in his right knee. He was immediately brought to the Emergency Center of Vojvodina and diagnosed with anterolateral dislocation of the PTFJ. Close reduction in general anesthesia was tried but we failed and then open reduction and internal fixation (ORIF) were performed with a single three cortical screw. We preferred not to immobilise the knee after the procedure and immediately employed passive and active exercises in the knee, without bearing weight to the injured leg. After 6 weeks we removed the screw and gave full weight support to the leg and continued physical treatment. **Conclusion**. In case of acute PTFJ dislocation, the first method of choice is closed reduction in sedation or general anesthesia. If closed reduction fails, ORIF must be performed. ORIF without immobilization and early start of physical therapy lead to the rapid return to sports activities.

Key words:
knee dislocation; joint instability; diagnosis; orthopedic procedures; treatment outcome.

Apstrakt

Uvod. Dislokacija gornjeg golenjačnolišnjačkog zgloba je retka povreda. Postavljanje dijagnoze se zasniva na anamnezi, kliničkom pregledu i RTG dijagnostici oba kolena. U literaturi nisu zabeležene velike serije ovih povreda, te su opisani kao pojedinačni slučajevi koji su lečeni različitim tehnikama. Cilj rada bio je da se predstavi modifikovana tehnika lečenja nestabilnosti gornjeg golenjačnolišnjačkog zgloba koja rezultuje brzim oporavkom bolesnika. **Prikaz bolesnika**. Fudbaler, star 24 godine, povredio se na početku treninga prilikom uklizavanja kada je osetio jak bol u desnom kolenu. Odmah je dovezen na pregled u Urgentni centar Vojvodine gde je dijagnostikovano prednje spoljašnje iščašenje gornjeg golenjačnolišnjačnog zgloba. Pokušana je ortopedska repozija u kratkotrajnoj intravenskoj anesteziji, ali nije bila uspešna te je pacijent bio pripreman za hirurški zahvat – otvorene repozije golenjačnolišnjačnog zgloba i njegovu fiksaciju sa jednim zavrtnjem kroz tri korteksa. Postoperativno nije postavljena imobilizacija i odmah su započete pasivne i aktivne vežbe u kolenu, ali bez oslonca na operisanu nogu. Nakon isteka šest postoperativnih nedelja odstranjen je zavrtanj i dat je pun oslonac na nogu i nastavljeno je fizikalno lečenje. **Zaključak**. U slučaju iščašenja gornjeg golenjačnolišnjačkog zgloba prvi metod izbora lečenja je ortopedska repozicija, a ukoliko ona ne da rezultate, pristupa se otvorenoj repoziciji i unutrašnjoj fiksaciji. Otvorena repozicija i unutrašnja fiksacija bez postoperativno postavljene imobilizacije i odmah započeta rehabilitacija dovode do bržeg oporavka i brzog vraćanja sportskim aktivnostima.

Ključne reči:
koleno, iščašenje; zglob, nestabilnost; dijagnoza; ortopedske procedure; lečenje, ishod.

Introduction

Dislocation of the proximal tibiofibular joint (PTFJ) is a rare injury. The first case was described by Nelaton [1] in 1874. Injuries of the PTFJ account for less than 1% of all injuries of the knee joint. Some authors believe that this condition is more common than previously thought and, as such, is rarely diagnosed immediately [2, 3]. These injuries usually occur in athletes and in sportsmen with twisting often in the flexed knee [4]. In 1974 Ogden [2] collected 43 cases from the literature; his classification describes four types of traumatic PTFJ dislocations (subluxation – type I, anterolateral – type

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II, posteromedial – type III, upper – type IV) [3]. The role of the PTFJ is to reduce torsional forces in the ankle and transfer the vertical force in standing position [5]. The diagnosis is based on the medical history, clinical examination and X-ray diagnostics. In the literature there is no larger series; only several cases of PTFJ dislocation treated by different methods have been published so far. There are reports on individual cases treated differently: nonoperatively, with closed reduction and immobilization [6–8] or open reposition and transfixation with Kirschner-wires (K-wires) or screws [9, 10].

In case of the late diagnosis, when reduction is not possible, the fibular head resection is made [7, 11] arthrodesis of the upper tibiofibular joint [3, 12], or reconstruction of the tibiofibular joint with femoral biceps muscle tendon [13, 14] or hamstring graft [15].

There is no agreement about the type of transfixation (three cortical or four cortical) and regarding immobilization after open reduction and transfixation of the joint. Recommendation by some authors is to use soft dressing of the leg with modified weight bearing [16, 17], while others advocate leg casting for 1 to 6 weeks [9, 18]. We presented a case of traumatic dislocation of the PTFJ and the modified technique of the treatment.

Case report

A 24-year-old football player was injured at the beginning of training session when he was tackling the ball and then felt a sharp pain and said that something snapped in his right knee. He was immediately brought to the Emergency Center.

Clinical examination revealed palpable pain and swelling in the area of the outer edge of the tibia and diagnosed anterolateral dislocation of the PTFJ which was based on the history, clinical examination and X-ray (Figure 1).

Closed reduction was attempted in general anesthesia, but with no result, then open reduction internal fixation (ORIF) was performed with a single screw. The operations were performed under spinal anesthesia in pale ischemia. A slightly curved skin incision was made on the lateral side of the knee at fibular head, and the fibular nerve was identified. After the fibular head repositioning, the PTFJ was transfixed with one cortical screw with canvas (Figure 2).

Position of the PTFJ was checked by X-ray and the wound was closed in layers.

After these procedures we did not put any immobilization and immediately started with passive and active exercises in the knee (full range of motion) without weight bearing to the injured leg. Six weeks after operation the screw was removed under local anesthesia and full weight support to the leg was allowed and rehabilitation was continued.(Figure 3.)

Twelve weeks after the injury, the patient returned to his sports activities. Follow-up one year later showed the full knee range of motion, the patient did not complain and continued with his active soccer playing. On X-ray examination there were no signs of PTFJ arthrosis.

Fig. 1 – Anteroposterior (AP) and profile radiographs of both knees indicates the right anterolateral proximal tibiofibular joint (PTFJ) dislocation – Ogden type II.

Fig. 2 – Profile and anteroposterior (AP) postoperative radiographs of the right knee after repositioning of the proximal tibiofibular joint (PTFJ) and transfixation with one cancellous screw with canvas.

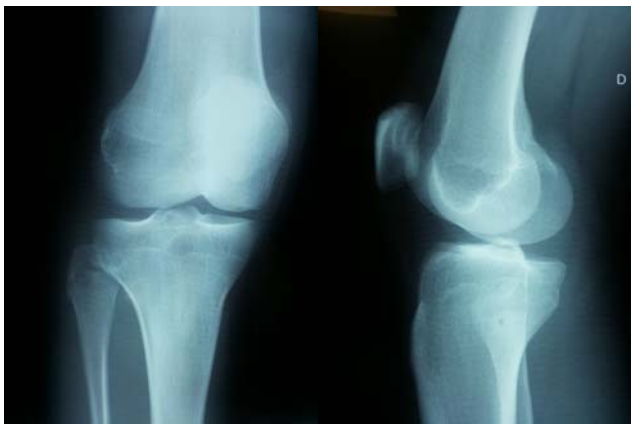

Fig. 3 – Postoperative anteroposterior (AP) and profile X-ray findings of the right knee after removing the screws.

Disscusion

The PTFJ is a synovial joint of lateral tibial condyle and fibular head [19]. In 10–60% of the whole population there is a communication between the knee joint and PTFJ and because of that they are often called PTFJ as the fourth knee department [20]. Generally, the PTFJ is a stable joint. His stability is provided by his joint capsule, ligaments (anterior tibiofibular ligament, a group of ligaments at the postero-laterlal angle, lateral collateral ligament) muscles (biceps femoris tendon) [21, 22]. Traumatic PTFJ dislocation is mostly seen in males of 17–30 years of age, as in the presented case [15]. The elementary function of the PTFJ is the dissipation of torsional loads applied at the ankle, the dissipation of lateral tibial bending moments, and the transmission of axial loads in weight-bearing [5]. Injuries usually occur by force of twisting on the flex knee [23] during the sports activities: soccer [22] (as in the presented case), volleyball [15], skiing [22], basketball [24], trampoline jumping [25], or it results from high-energy trauma as usually seen in polytraumatized patients [26]. Morrison et al. [27] described the case of atraumatic instability of the PTFJ [27]. Ogden [2] divided PTFJ dislocations into four types: subluxation – type I (excessive anterior-posterior motion without dislocation, as usually seen in adolescents with lax joints); anterolateral dislocation – type II (most common and accounts for about 85% of all dislocations of the PTFJ) [7], posteromedial dislocation – type III (occurs in 10% of PTFJ dislocations, and is often seen as a result of direct hit to fibular head) [28], type IV, upper dislocation (represents 2% of all PTFJ dislocations, and is usually seen in high-energy injuries, often associated with fractures of the tibia, fibular head, upper dislocation of lateral malleolus and tear of interosseus membrane) [29]. The presented patient had type II by Ogden classification. A patient with the PTFJ dislocation presents with pain, swelling and asymmetry of lateral side of the knee, while the knee joint is without swelling and the range of motion is not limited [2]. Because this injury can be associated with injury of the fibular nerve, it is very important to examine the fibular nerve function [11].

The differential diagnosis can be: partial rupture of the *ligamentum collaterale laterale* (LCL), meniscal cyst or lateral meniscal tear and distal iliotibial band syndrome [30]. No, or the late diagnosis of the PTFJ dislocation leads to chronic pain in the knee and development of arthrosis PTFJ and because of this it is recommended to compare clinical examination and radiographs with the healthy leg [31].

In case of acute PTFJ dislocation, the first choice method is closed reduction in sedation or general anesthesia, which success depends on the good knowledge of the mechanism of dislocation. The reduction is performed while the knee is flexed, the foot is in eversion and dorsiflexion, and the fibula is in external rotation, with the direct front-to-back pressure on the fibular head, which clicks back into the place. After the successful repositioning, the above-the-knee plaster cast is applied for 3–6 weeks, and then follows functional rehabilitation [20]. Some authors recommend soft dressing (no cast immobilization) of the leg with modified weight bearing [32].

If closed reduction fails, as it was in the presented case, ORIF will be performed with K-wires or screws [9, 10]. In our case the PTFJ was transfixed with one cortical screw with canvas, through three bone cortices. A three bone cortices screw is a stable type of fixation for PTFJ dislocation, and there is no need to transfix the screw through all four bone cortices [10]. If it is not possible to perform reduction of the PTFJ in open surgery, the next step is resection of the origin of the extensor digitorum longus muscle off the fibular head, which makes the reduction easier. After the reduction is done, a joint capsule has to be reconstructed, and then the fibular head is to be transfixed temporarily.

Van den Bekerom et al. [33] recommended that postoperative immobilization is not necessarily required, and they allowed to bear weight immediately after the operation although the knee may not be flexed more than 90° for the first two weeks. We preferred not to immobilize the knee after the procedure also but in the other side we gave no bear weight but preferred immediately a full range of motion in the knee joint. After six weeks we took out the screw although it is recommended after 3–6 months [33], because we wanted to avoid cracking the screws as it was in two cases. Some authors also removed the screw after six weeks [10]. In the presented case full weight bearing was given immediately after removing

the screw, but without the previously placed postoperatively immobilization which resulted in faster patient recovery and his earlier return to sports activities. Full sports recovery after non-surgical treatment of luxation PTFJ with or without immobilization and without bear weight was six months [34, 35]. Robinson et al. [18] said that their patient had full sports recovery after nine months, but when treated operatively with immobilization and without bear weight after eight weeks.

At our Clinic for Orthopaedic Surgery and Traumatology we described a similar case of dislocation of the PTFJ in a football player three years ago (published in 2013), who was injured during football game, when he suddenly changed the direction while his foot was fixed to the ground, unlike this case, where injury was caused by a direct blow on bended knee when he was tackling the ball [10]. The same type of dislocation of the PTFJ was in both cases and it was type II by Ogden's classification. We used a previously described stabilization principles for PTFJ dislocations but in the first case, stabilization was conducted with cancellous screw and in this case we used cortical screw, also we had changes in the postoperative period. The main difference of this modified treatment which proved to be better (in this two extremely rare and similar cases) was based on greater joint stability using cortical screw and immediately starting with physical therapy (full range of motion in the knee joint and strengthening the muscles of the whole leg), without use of postoperative immobilization which was used in the earlier described case by Milankov et al. [10]. This consequent delay of rehabilitation for about two months resulted in longer recovery period. The case reported by Milankov et al. [10] was back to his sports activities four months after the injury, they treated the patient operatively with above-the-knee plaster cast and after six weeks full weight bearing was allowed and physical therapy started. The presented patient was treated without immobilization and weight bear, and we immediately started with physical therapy (immediately full range of motion in the knee joint) and as a result of that the patient returned to football playing without any limitations 12 weeks after the injury.

Conclusion

Prolonged pain in the knee can be induced by not diagnosed dislocation of the PTFJ and it must be taken into consideration in the differential diagnosis of chronic pain in the knee.

Adequate clinical examination and analysis of X-ray of the knee can be relatively easy to establish a diagnosis and further treatment. In case of acute PTFJ dislocation, the first choice method is closed reduction in sedation or general anesthesia, if closed reduction fails, ORIF must be performed. Three cortical fixation of PTFJ with a cortical screw, without immobilization and early start of physical therapy lead to rapid return to sports activities.

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CASE REPORT

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Tolosa-Hunt syndrome: is it really necessary to show granuloma? – The report on eight cases

Tolosa-Hunt sindrom: da li je neophodno prikazati granulom?

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Abstract

Introduction. Tolosa–Hunt syndrome (THS) is a rare entity, characterized by unilateral orbital pain associated with paresis of one or more of the oculomotor cranial nerves and caused by a granulomatous inflammation in the cavernous sinus, superior orbital fissure or orbit. The low prevalence of THS with a broad spectrum of other disorders that could cause painful ophtalmoplegia resulted in a stricter diagnostic criteria of THS in the latest edition of the International Classification of Headache Disorders. Current criteria require demonstration of granuloma by magnetic resonance imaging or biopsy. The diagnosis could be difficult and the initiation of treatment delayed due to a high variablity of clinical presentation of TSH. Reducing the number of patients that, based on clinical presentation, could be classified as having THS, but do not fullfil all diagnostic criteria further complicates establishing of correct diagnosis. **Case report**. Hereby we presented eight patients diagnosed with and treated for THS. Inspite the exclusion of other causes of painful ophtalmoplegia, granuloma could not be demonstrated in a half of patients. Clinical presentation of THS in patients with and without shown granuloma, did not significantly differ concerning headache characteristics (localization, intensity, quality, duration preceding cranial nerve palsy, response to steroids), the affected cranial nerve, disease course and response to the treatment, as well as types of diagnostic procedures that were performed in ruling out other diseases from the extensive differential diagnosis of painful ophthalmoplegia. **Conclusion.** There is no significant difference between the THS patients with and without demonstrated granuloma.

Key words:
tolosa-hunt syndrome; brain; diagnosis; diagnosis, differential; magnetic resonance imaging.

Apstrakt

Uvod. Sindrom Tolosa–Hunt (THS) je redak entitet koji se odlikuje jednostranim orbitalnim bolom udruženim sa parezom jednog ili više okulomotornih nerava, uzrokovanim granulomatoznim zapaljenjem kavernoznog sinusa, gornje orbitalne fisure ili orbite. Niska prevalencija ovog sindroma, kao i široki spektar drugih oboljenja koja bi mogla uzrokovati bolnu oftalmoplegiju, doprineli su pooštravanju dijagnostičkih kriterijuma za THS u poslednjem izdanju Međunarodne klasifikacije glavobolja. Važećim kriterijumima zahteva se dokazivanje prisustva granuloma magnetnom rezonancom ili biopsijom. Visoka varijabilnost kliničke prezentacije THS može biti uzrok otežanog postavljanja dijagnoze, uz odlaganje početka lečenja. Smanjenje broja bolesnika koji bi na osnovu kliničke slike mogli imati THS, ali ne ispunjavaju sve dijagnostičke kriterijume, dalje komplikuje i odlaže pravovremeno postavljanje tačne dijagnoze. **Prikaz bolesnika**. Prikazali smo osam bolesnika lečenih pod dijagnozom THS. I pored isključenja drugih uzroka bolne oftalmoplegije, granulom nije bilo moguće prikazati kod četiri bolesnika. Klinička prezentacija THS kod bolesnika sa i bez dokazanog prisustva granuloma, nije se razlikovala značajno kada su u pitanju osobine glavobolje (lokalizacija, intenzitet, kvalitet, trajanje pre razvoja slabosti okulomotora, odgovor na kortikosteroidnu terapiju), zahvaćenost kranijalnih nerava, trajanje bolesti i odgovor na terapiju, kao ni dijagnostički postupak sproveden sa ciljem isključivanja drugih bolesti koje bi mogle biti uzrok bolne oftalmoplegije. **Zaključak**. Ne postoji značajna razlika između bolesnika sa THS bez obzira na to da li je granulom dokazan ili ne.

Ključne reči:
tolosa-huntov sindrom; mozak; dijagnoza; dijagnoza, diferencijalna; magnetna rezonanca, snimanje.

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Introduction

Tolosa–Hunt syndrome (THS) [1, 2] is a rare entity, described in the latest, 3rd, beta version of The International Classification of Headache Disorders (ICHD), as unilateral orbital pain associated with paresis of one or more of the III, IV and/or VI cranial nerves caused by a granulomatous inflammation in the cavernous sinus, superior orbital fissure or orbit [3].

The low prevalence of THS with broad spectrum of other disorders that could cause painful ophtalmoplegia resulted in stricter diagnostic criteria of THS in ICHD-III.

Current criteria require demonstration of granuloma by magnetic resonance imaging (MRI) or biopsy, while the prompt positive response on steroid treatment, as not specific treatment only of THS, had been excluded from diagnostic criteria.

In clinical practice, THS is highly variable in its presentation [4], thus, reducing the number of patients that, based on clinical presentation could be with THS, but do not fullfil all diagnostic criteria, complicates establishing of the correct diagnosis on time to start treatment.

Hereby we present eight patients diagnosed with and treated as THS in our Headache Center during the period of 13 years, picked up from over 5,800 examined patients. In further reading it will be shown that granuloma could not have been demonstrated by MRI examination in a half of the patients meeting clinical criteria for THS.

Case reports

Case 1

During the period of six years, from her 48 to 54 years of life, this woman suffered from five episodes of right-sided headache separated by headache free periods lasted from 1 to 4 years. All episodes had similar presentation consisting of constant pain localized on the right side of the forehead and in the right orbital region, that was sharp and of moderate intensity, irreseponsive to analgesics. The headache used to last for ten days and then inability to make complete abduction of the right eye with double vision occurred. Additionally, during the second episode, besides the six cranial nerve palsy, the ptosis of the right palpebra, inability to adduct the right eye, or move it in vertical direction, was noticed, pointing to the affection of the 3rd cranial nerve, too. On neurological examination, there was no other abnormalities. In the personal history, mild hypertension and chronic gastritis, without permanent therapy, were recorded.

Extensive diagnostic procedures had been performed and repeated during multiple hospitalizations. Blood examination, considering biochemical parameters, blood cells, coagulation, immunology and thyroid hormones levels, were completely normal. The biochemical examination and cytological examination of cerebrospinal fluid (CSF) were completely normal, as well. Oligoclonal bands were not detected. Echosonography of orbits and MRI of the brain, particularly cavernous sinus regions (1.5T, 3 mm thickness) were normal. Control MRI of the brain, performed during every single relaps showed normal findings.

The patient was treated with steroids, in some episodes orally with 60–80 mg of prednisolone *per os* daily, while in others intravenously with methylprednisolone, 120 mg to 1,000 mg daily for 7–14 days followed by oral steroid therapy for several months. The response to steroid treatment, concerning headache, was prompt, resolving pain in 72 hours. Still, the recovery was incomplete concerning bulbomotor paresis, with diplopia lasting for several months.

Case 2

During the period of seven years, from her 60 to 67 years of life, this woman suffered from six episodes of left-sided constant intensive orbital pain, being after ten days associated with ptosis of the left palpebra and paralysis of the left bulb. Hypoesthesia in the distribution of V1 and V2 branches of the trigeminal nerve were present, as well. In personal history, mild hypertension and dyslipidemia were recorded. Bilateral mild hearing impairment was present too, for several years.

Blood examination, considering biochemical parameters, blood cells, coagulation, immunology and thyroid hormones levels, were completely normal. Biochemical and cytology examinations of CSF were completely normal, as well. Repeated MRI of the brain, with the special sections through the *ala minor* (1.5T, 3 mm thickness), showed multiple ischemic lacunar lesions, localized in paraventricular regions, bilaterally, without any other abnormality, particularly in cavernous sinus regions. The result of digital subtraction angiography (DSA) was normal. MRI of orbits, MRI angiography and phlebography were normal, as well.

In each episode, the patient was treated with oral steroid terapy, prednisolone 60 mg *per os* daily, which set her pain free with remaining hypoesthesia in the distribution of V1 branch on the left. The steroid dose was tapered to 10 mg daily, which became permanent because of symptoms relapses on every attempt of steroid withdrawal. After several years, the patient developed diabetes mellitus.

Case 3

A 56-years-old women was examined because of onset of intense, throbbing-like headache located at the base of the nose on the left side. The pain was irradiating to the left supraorbital and frontotemporal region. During the next month the patient experienced everyday, mostly bilateral facial pain treated unsuccessfully with antibiotics and analgesics. More than 5 weeks after the first symptoms, the patient noticed weakness of her left upper eyelid palpebra. Over the next 2 weeks this was accompanied by decreased mobility of the left eye bulb. Neurologic examination revealed ptosis of the left palpebra, plegia of the left bulb with the possibility of only mild abduction and hypoesthesia in the area of V2 nerve distribution. The rest of examination was unremarkable.

MRI examination (1.5T, 3 mm thickness) showed a mass in the left cavernous sinus without propagation to the near structures and without contrast enhancement (Figure 1).

Radiologic appearance was mostly consistent with inflammatory/granulomatous infiltration. Subocclusion, due to external compression, of the intracavernous segment of the left internal carotid artery was visualized on cerebral panangiography (Figures 2 a and b). Biochemistry panel tests, full blood count, coagulation screening tests and urine analysis were within reference ranges. Additional testing included immunoserology (circulating immunocomplexes, antinuclear, antinucleocytoplasmatic and anticardiolipine antibodies), thyroid function tests, 24 h calciuria, serum angiotensin-converting enzyme (ACE) level and chest computed tomography – all normal. Lumbar puncture was performed, with protein levels 0.51 g/L, 3.5 mmol/L of glucose and 1 lymphocyte. CSF was analyzed for syphilis *Treponema pallidum* haemagglutination (TPHA), *Borrelia burgdorferi* (ELISA), tuberculosis (acidoresistent staining, culture), *Cryptococcus* (direct staining) and common parasitic pathogens (*Cysticercus*, *Toxoplasma*, *Echinococcus*), with negative results. CSF sediment was without pathologic elements.

High-dose pulse corticosteroid therapy (methylprednisolone 1 g, *iv* during 7 days) was initiated. Orbital pain responded dramatically after 24 h and resolved completely after < 36 h of steroid introduction. Very discrete, but progressive ocular motility was observed during the next 7 days and after that the patient was followed as outpatient. The therapy switched to oral prednisolone (80 mg daily) and tapered down in one month intervals to the maintenance dose of 20 mg, every second day. Full ocular motility was established 3 weeks after the initial steroid dose, but mild ptosis of the left palpebra and hypoesthesia in V1 distribution were still present three months after. Control MRI showed infiltration of the left cavernous sinus, without progression or regression in comparison to the former imaging (Figure 3).

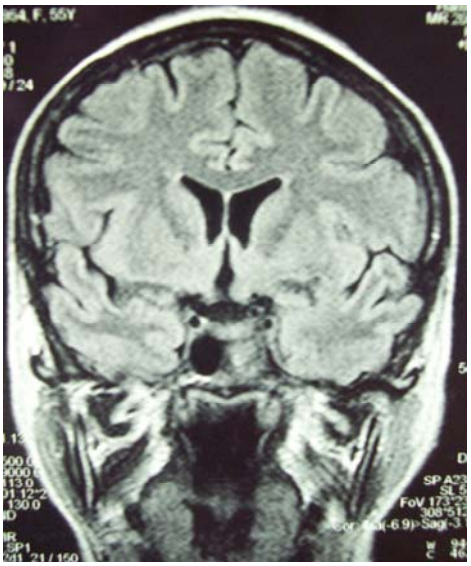

Fig. 1 – Magnetic resonance imaging (MRI) focussed on sellar and parasellar region – infiltration of the left cavernous sinus.

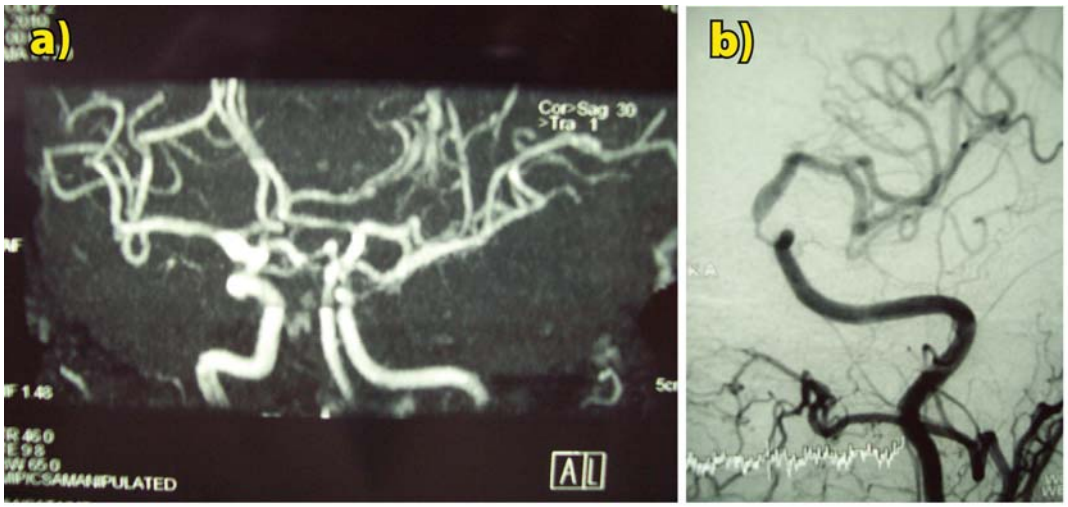

Fig. 2 – a) Magnetic resonance angiography (MRA) shows subocclusion of the cavernous portion of the left internal carotid artery; b) Digital subtraction angiography (DSA) finding of subocclusion of the left internal carotid artery.

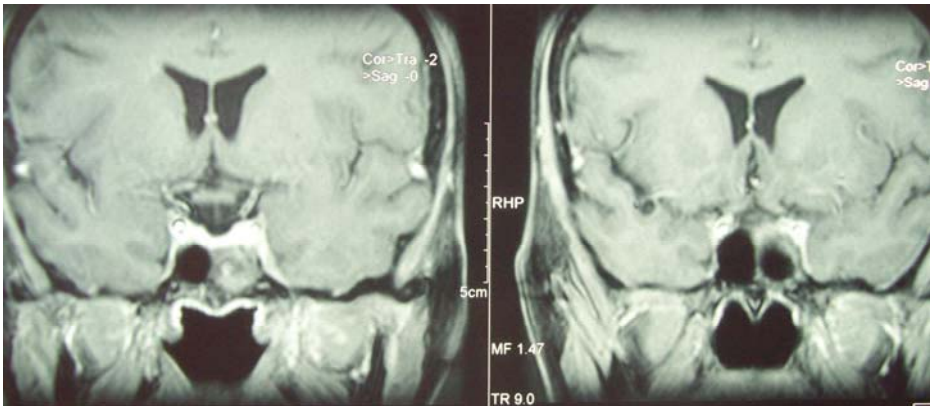

Fig. 3 – Control magnetic resonance imaging (MRI) focussed on sellar and parasellar region – no progression, no regression [in comparison with the previous finding (Fig. 1)]

Case 4

A 53-years-old man was admitted two days after experiencing left gaze diplopia and dull, constant left-sided retroorbital headache. During the first week of hospitalization his status progressed with the development of left-sided ptosis, mydriasis and complete left external ophtalmoplegia. Neurological examination revealed complete paralysis of the oculomotor, trochlear and abducens nerves with the involvement of the first two trigeminal nerve branches. There were no other pathological signs in neurological examination.

MRI of the brain showed infiltration of the left cavernous sinus, most consistent with granulomatous infiltration. Computed tomography (CT) angiography of the brain was normal. Laboratory analyses included general biochemistry panel, complete blood count, urinalysis, immunoserology panel (antinuclear, antinucleocytoplasmatic and anticardiolipin autoantibody titers), ACE, chitotriosidase levels and thyroid hormone levels, all showing normal results. Cerebrospinal fluid biochemistry, cytology, polymerase chain reaction (PCR) for *Mycobacterium tuberculosis*, bacteriological cultures, analysis for *Cryptococcus neoformans*, TPHA, ELISA for *Borrelia burgdorferi* and ACE were all also normal or within the reference range.

The patient was treated with high-pulse corticosteroid therapy (methylprednisolone, 1gr *iv* for 7 days) with resolution of pain (within 72 h) and progressive recovery of affected cranial nerve function. Three weeks after the therapy was started, the patient was pain free with discrete mydriasis of the left pupil and minimal diplopia in extreme lateral gaze. Therapy was continued with oral prednisolone (60 mg), the patient was discharged and followed as an outpatient. In the next two years, the patient was asymptomatic.

Case 5

A 38-year old women experienced abrupt onset of left gaze diplopiae and intense, permanent left hemicrania, with a gradual localization of pain in the left orbital region. Neurologic examination revealed impaired function of the left lateral rectus muscle. In the past medical history, the patient had partial thyroidectomy one month prior to the actual symptom onset which revealed a solitary colloid cyst.

MRI of the brain was notable for asymmetry in the cavernous sinus regions, with diffuse enlargement of the left cavernous sinus without post-contrast enhancement. CT angiography disclosed patent intracranial vessels. Laboratory analyses included general biochemistry panel, complete blood count, urinalysis, basic immunologic screening which all yielded normal results. Cytology, biochemistry, mycobacterial cultures, staining for *Cryptococcus neoformans*, TPHA and ELISA detecting *Borreliosis*, as well as ACE retrieved the results within the reference range in CSF. We, however, noted slightly increased levels of chitotriosidase in CSF, but complementary tests aimed at detecting sarcoidosis were normal.

After introduction of pulse corticosteroid therapy (methylprednisolone, 1g for 7 days) a substantial, but not complete, improvement of left eye bulb motility was noted. Pain intensity was reduced but did not extinct. Two months later and after corticosteroid dose had been tapered to 10 mg of prednisolone on the second day, the patient condition got worse with the same symptoms and signs as at disease onset, which required additional methylprednisolone pulse to control the symptoms. Follow-up examination, performed two months after the relapse, revealed a complete recovery maintained for the next 18 months, till the last check-up. The latest, control MRI of the brain was normal.

Case 6

A 68-years old woman, examined because of headache that occurred suddenly, presented with pain in the left eye bulb and forehead, lasting for days, followed by left palpebra ptosis and diplopia. Neurological examination revealed paralysis of the left oculomotor, ophthalmic and abducens nerves.

Blood examination, considering biochemical parameters, blood cells, coagulation, immunology and thyroid hormones levels, tumor markers, was completely normal. The biochemical and cytology examination of CSF was completely normal, as well. MRI of the brain showed minimal infiltration of the apical region of the left orbit and the left cavernous sinus with post-contrast enhancement in signal intensity, dominantly on T1W FS MR tomograms. MRI angiography did not show abnormalities in blood vessels of the brain.

The high pulse corticosteroid therapy (methylprednisolone 1 g, given intravenously for 7 days) led to incomplete recovery with still present impaired function of the left abducens, in one week. The therapy was continued with oral prednisolone (80 mg daily), gradually tapered and switched of in one month.

Six months later, paresis of the left abducens was still present, the patient went through symptoms of impairment of the left ophthalmic nerve again.

Control MRI of the brain did not show infiltration of the apical region of the left orbit and the left cavernous sinus that had been previously shown. The patient was advised to continue with oral prednisolone (20 mg daily) for the next four weeks, recovered completely, and on the last check-up, six months later, was without pathological symptoms and signs.

Case 7

A 69-years old woman, was examined because of intensive, throbbing headache localized in the right eye bulb, constant and lasting for 20 days, followed by right palpebra ptosis and diplopia. Neurological examination revealed paralysis of right oculomotor and trochlear nerves.

Blood examination, considering biochemical parameters, blood cells, coagulation, immunology and thyroid hormones levels, tumor markers, was completely normal. Biochemical and cytology examination of CSF was completely normal, as well. MRI of the brain, particularly cavernous sinus regions (1.5T, 3 mm thickness) was normal. MRI angiography did not show abnormalities in blood vessels of the brain.

The high pulse corticosteroid therapy (methylprednisolone 1g, given intravenously, for 7 days) in 48 hours brought pain relief and improvement of motility of the right bulb, with still present semiptosis and incomplete adduction. The therapy was continued with oral prednisolone (60 mg daily), gradually tapered and switched of in six months.

Six months later, after steroids withdrawal, the patient went through pain of the same characteristics as she did before, still without symptoms of impairment of cranial nerves.

Control MRI of the brain did not show any pathological finding. The patient was advised to continue with oral prednisolone (20 mg daily). In ten days, the patient was pain-free, continuing with this therapy for the next six weeks. On the last check-up, two months later, the patient was without pathological symptoms and signs.

Case 8

A 53-year-old man was examined due to moderate pain in the right orbit, abrupt in its onset, followed by diplopia and ptosis of the right palpebra. Neurological examination revealed dysfunction of the 3rd and 4th cranial nerves. Extensive blood and CSF examination showed all results normal. MRI of the brain showed granulomatous infiltration of the right cavernous sinus. Introduction of pulse corticosteroid therapy (methylprednisolone, 1 g for 7 days) set him pain-free in 3 days, with residual semiptosis and incomplete adduction. Corticosteroid therapy was continued orally with 40 mg of prednisolone daily until check-up scheduled in 6 weeks. Control MRI of the brain was scheduled in 3 months.

Summary of clinical presentations of THS, meaning characteristics of headache and cranial nerves paresis, in our patients, are given in Tables 1 and 2. MRI findings are given in Table 3.

Disscusion

According to the ICHD-III criteria for THS, to establish THS diagnosis patients should have unilateral headache localized around the brow and the eye that precedes in the period up to two weeks ipsilateral paresis of oculomotor nerve(s) and confirmed granulomatous inflammation of the cavernous sinus, superior orbital fissure or orbit, by MRI or biopsy [3].

The presented patients completely satisfied clinical criteria for THS diagnosis. In all the patients, the same panel of diagnostic procedures was performed in order to rule out other pathological disorders involving cavernous sinus and orbit. Nowadays, biopsy is rarely performed in order to confirm the diagnosis of THS, so that the diagnosis relies on MRI finding. The four of eight presented patients lack MRI confirmation; otherwise, their clinical presentation was similar to the presentation of patients with the confirmed cavernous sinus granuloma.

ICHD-3 do not give specified features for headache in THS, besides the unilateral pain localized around the brow and

Table 1

Headache features in the presented Tolosa-Hunt syndrome patients

Patient	Localization	Intensity of pain	Quality of pain	Time gap between the headache onset and cranial nerve(s) paresis (days)	Disappearance of pain after initiation of steroids (days)
1	R forehead and orbit	moderate	sharp	10	3
2	L orbit	intensive	sharp	10	3
3	L forehead, orbit and temple	intensive	throbbing	35	2
4	L retro-orbital	moderate	dull	immediately	3
5	L orbit	intensive	sharp	immediately	7
6	L forehead and orbit	intensive	sharp	10	7
7	R orbit	intensive	throbbing	20	2
8	R orbit	moderate	dull	5	3

R – right; L – left.

Table 2

Cranial nerves palsy in the presented Tolosa-Hunt syndrome patients

Patient	Affected cranial nerve(s)	Disappearance of paresis after initiation of steroids period	Residual deficit	Number of relapses	Monitoring time duration
1	III, VI	> 3 months	VI	4	6 years
2	III, V_1, V_2, VI	> 3 months	V_1	5	7 years
3	III, IV, V_1,V_2, VI	3 weeks	III, V_1	0	2 months
4	III, IV, V_1,V_2, VI	3 weeks	no	0	2 years
5	VI	> 3 months	no	1	18 months
6	III, V_1, VI	> 3 months	no	1	6 months
7	III, IV	> 3 months	no	1	6 months
8	III, IV	3 weeks	III	0	3 months

Table 3

Magnetic resonance imaging (MRI*) findings in the presented Tolosa-Hunt syndrome patients

Patient	The first MRI	Control MRI
1	Normal	Normal
2	Normal	Normal
3	Granulomatous infiltration in the left cavernous sinus	Unchanged
4	Granulomatous infiltration of the left cavernous sinus	Not performed
5	Diffuse enlargement of the left cavernous sinus	Normal
6	Infiltration of the apical region of the left orbit and the left cavernous sinus	Normal
7	Normal	Normal
8	Granulomatous infiltration of the right cavernous sinus	Not performed

***Examinations performed with 3 mm thickness coronal and axial T1-SE and T2-SE sequences and with T1 FS weighted images after gadolinium administration at the level of the orbit and cavernous sinus.**

eye. Analyses of headache features, summarized in Table 1, revealed that pain was always localized in the orbit, and in more than half of the patients, in the ipsilateral part of the forehead. Pain was mostly intensive, rarely moderate, mainly sharp and throbbing, exceptionally dull. According to the literature [4, 5], in THS pain is typically periorbital, or in some cases retroorbital, frontal or temporal. It is described as intense, sometimes as "boring" or "stabbing." In our group of THS patients, headache was immediately, or within 10 days, followed by paresis of the cranial nerves, with the exception of one patient who had headache lasting for 5 weeks before cranial nerves paresis occurred. The time gap exceeding two weeks was reported in 11% of patients in large THS cohort, reported by Zhang et al. [6].

Concerning pain, all the patients had good response to corticosteroids, becoming pain-free within 2 to 7 days after the initiation of the therapy. Colnaghi et al. [7] reported pain resolution within 72 hours after initiation of corticosteroids in 78% of 38 cases of THS, picked-up from 536 articles published between 1999 and 2007.

The most commonly affected cranial nerve was the 3rd cranial nerve being affected in all but one patients (Table 2). The 6th cranial nerve was affected in six cases, the first or the second division of the trigeminal nerve in four cases and the 4th cranial nerve in four patients. Zhang et al. [6] reported that the 3rd cranial nerve was affected in three quarters of THS patients, being the most commonly affected of all, as well. Four out of eight patients reported sensory disturbances in distribution of ophthalmic branch of trigeminal nerve and additionally, of maxillary branch in three of them. In our patients, the involvement of the V2 occurred only in patients with the simultaneous involvement of other nerves of cavernous sinus. Looking the cavernous sinus anatomy, the position of the V2 is in the lowest portion of the sinus, exiting outside the orbital apex [8]. Clinical presentation with V2 involvement could point to the more extensive pathological process visible by MRI. Still, MRI lesion was found in the Case No 2.

Concerning paresis of the cranial nerves, in all the patients, response to corticosteroids was delayed comparing to headache resolution, which is in accordance with findings of other studies [9]. Recovery was incomplete in a half of our patients (Table 2).

The most prominent observation in these patients is that the lack of granuloma verified by MRI was noticed in two patients with multiple relapses (Table 3).

So, in those patients the diagnosis of recurrent painful ophthalmoplegic neuropathy, coded with 13.9 in ICHD-3, could not be excluded. Namely, for establishing this diagnosis, at least two attacks of unilateral headache, accompanied by ipsilateral paresis of ocular motor nerves, are required, with the exclusion of intracranial lesions. The time gap between headache and nerve palsy should not exceed two weeks, just like in THS. Moreover, recent data demonstrate gadolinium enhancement or nerve thickening by MRI in these patients [10, 11], still that type of MRI changes is not shown in any of our patients.

Corticosteroid treatment is beneficial in some patients, just like in THS. So, it seems that the term "recurrent painful ophthalmoplegic neuropathy", coined in order to reject an old and inappropriate term of ophtalmoplegic migraine, now overlaps with THS, at least in some patients.

Three out of eight patients had negative findings on MRI, and two of them had clinical course with multiple relapses. Although it has been suggested that a normal MRI

study should exclude the diagnosis of THS [3, 12], in the lack of more suitable diagnosis, the authors considered those patients as patients with Tolosa-Hunt syndrome. In accordance with the opinion of some authors [13, 14], our opinion is that the diagnosis of THS is clinical and finding of granuloma using MRI or biopsy should not be obligatory.

Conclusion

There is no significant difference in clinical presentation of THS in patients with and without demonstrated granuloma.

The diagnosis of THS is clinical and finding of granuloma using MRI or biopsy should not be obligatory.

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CASE REPORT

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Burkitt lymphoma as a cause of intussusceptions – The significance of positron emission tomography scan in the follow-up

Barkitov limfom kao uzrok intususcepcije – Važnost pozitronske emisione tomografije za praćenje bolesti

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Abstract

Introduction. Burkitt lymphoma (BL) is a high-grade, high-risk type of non-Hodgkin lymphoma characterized by a very rapid cell progression. Although BL is a rare cause of intussusceptions, it must arouse suspicion if the intussusception occurs outside the characteristic peak. **Case report.** A previously healthy 7-year-old boy was hospitalized for recurrent abdominal pain, loss of appetite and body weight, a fever of up to 38°C, persistent vomiting and with soft tumefaction ileocecally. Nuclear magnetic resonance (NMR) revealed intussusceptions due to a tumor mass, which was followed by a surgical procedure (right hemicolectomy and termino-terminal anastomosis). Histopathological examination confirmed the diagnosis of primary abdominal Burkitt lymphoma. In spite of the subsequent oncologic treatment lasting for four months, an undifferentiated mass was detected in the retroperitoneal space below the lower pole of the right kidney. Positron emission tomography (PET) scan was performed in order to detect the metabolic activity of the deposit in the retroperitoneal space. A high metabolic activity of Burkitt lymphoma confirmed its presence, as well as the need for additional surgical intervention for tumor extirpation. PET scan undoubtedly contributed to distinguishing the visibility of tumor cells and to the detection of the subcentimeter tumor mass. **Conclusion.** The timely diagnosis, high tumor sensitivity to chemotherapy as well as the great importance of PET scan in the early detection of tumor recurrence, significantly increase the survival rate in patients with BL.

Key words:
burkitt lymphoma; diagnosis, differential; intussusceptions; child; positron-emission tomography; digestive system surgical procedures; antineoplastic combined chemotherapy protocols.

Apstrakt

Uvod. Barkitov limfom (BL) je visokomaligni tip ne-Hočkinovog limfoma koji se odlikuje jako brzom ćelijskom progresijom. Iako je BL retko uzročnik intususcepcije uvek mora da se posumnja na njega ukoliko se ista pojavi van karakterističnog uzrasnog pika. **Prikaz bolesnika.** Prethodno zdrav 7-godišnji dečak primljen je na lečenje zbog abdominalnog bola, gubitka apetita i telesne mase, febrilnosti do 38°C, upornog povraćanja i tumefakcije u projekciji ileocekalne regije. Nuklearna magnetna rezonanca (NMR) ukazala je na postojanje invaginacije posledično na terenu tumorske mase, zbog čega je usledila hirurška intervencija (desna hemikolektmja sa termino-terminalnom anastomozom). Patohistološki pregled potvrdio je dijagnozu primarnog abdominalnog BL. Uprkos sledstvenoj onkološkoj terapiji, četiri meseca nakon operacije detektovana je nejasno lokalizovana masa u retroperitonealnom prostoru, ispod donjeg pola desnog bubrega. Pozitron emisiona tomografija (PET) učinjena je u cilju otkrivanja metaboličke aktivnosti depozita u retroperitonealnom prostoru. Visoka metabolička aktivnost BL potvrdila je njegovo postojanje i potrebu za ponovnom hirurškom intervencijom u cilju tumorske ekstirpacije. PET sken nesumnjivo doprinosi vizualizaciji tumorskih ćelija u detekciji subcentimetarskih tumorskih masa. **Zaključak.** Pravovremena dijagnoza, veliki senzibilitet tumorskih ćelija na hemoterapiju i veliki značaj i uloga PET u ranom otkrivanju tumorskog recidiva, značajno doprinose povećanju preživljavanja bolesnika sa BL.

Ključne reči:
limfom, burkitt; dijagnoza, diferencijalna; intususcepcija; deca; tomografija kompjuterizovana, emisiona; hirurgija digestivnog sistema, procedure; lečenje kombinovanjem antineoplastika, protokoli.

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Introduction

Burkitt lymphoma (BL) is a high-grade, high-risk type of non-Hodgkin lymphoma, first described as a distinct clinical entity in 1958 [1, 2]. It represents an aggressive, rapidly growing B-cell neoplasm [3], characterized by a very rapid progression of tumor cell pools and the cell multiplication taking place every 24 hours. A high reproduction of tumor cells and a high tumor growth often lead to a late diagnosis when the tumor is already disseminated, resulting in a higher incidence of recurrence and a lower survival percentage.

Although BL is a rare cause of intussusceptions, it must arouse suspicion if the intussusception occurs outside the characteristic peak (after two years).

Case report

A previously healthy boy, aged 7, was referred to the Pediatric Surgery Clinic because of a recurrent abdominal pain and the loss of appetite and body weight. Symptoms had begun a few weeks earlier when he was subfebrile and vomited several times. For the next few days he had constipation, persistent vomiting after every meal and was persistently febrile (38°C). On admission, the child was pale and sweaty, with a soft, distended abdomen, sensitive to palpation in the ileocecal region, and had stools without blood.

The patient was anemic and the results of blood count were as follows: leucocytes (Le) 7.7 × 10^9/L, erythrocytes (Er) 4.19 × 10^{12}/L, hemoglobin (Hb) 7.4 g/dL, hematocrit 26.7%, platelets (PLT) 648 × 10^9/L. He was also hypoproteinemic (total protein 54 g/L) due to hypoalbuminemia (albumin 26.3 g/L). Lactate dehydrogenase (LDH) was 825 U/L, with the C-reactive protein values 326 mg/L.

Plain abdominal radiography in the standing position indicated the presence of multiple aero-liquid levels predominantly in the upper and the right parts of the abdomen (Figure 1). Echosonography revealed free fluid in the subhepatic and the right paracolic space accompanied by some very dilated aperistaltic intestinal convolutions with a "target sign" in the ileocecal region.

Nuclear magnetic resonance (NMR) examination of the upper abdomen detected colonic stenosis in the distal part of the ascending colon immediately before the hepatic flexure with the maximum luminal diameter of 4 mm, spreading 4.5 cm in length. Stenosis was caused by the characteristic tubular "target sign" (Figure 2a) formation which corresponds to invagination; however, the presence of lymphatic tumor could not be excluded.

Following the stabilization and the corrections of the metabolic status, and the transfusion of washed red blood cells, surgical intervention was performed. The surgical procedure revealed the presence of a solid bloody friable surface tumor in the terminal ileum, located 10 cm from the ileocecal valve, measuring approximately 5 cm in diameter with a firm consistency that occupied almost all of the lumen and invaded the antimesenteric ileal wall. Right hemicolectomy was performed with the termino-terminal ileocolic anastomosis. The boy spent seven days in the intensive care unit receiving dual antibiotic therapy. The histopathology examination of the excised tumor mass proved to be Burkitt lymphoma.

After the recovery, the patient underwent 2 courses of oncologic treatment. Four months after the operation, follow-up ultrasound examination revealed changes measuring 10 × 7 mm in the retroperitoneal space near the lower pole of the right kidney. In the forthcoming period, the mass had the tendency to increase progressively reaching the dimensions of 36 × 33 mm as reported during one of the following examinations conducted 2 months later. Position emission tomography (PET) scan detected a change in standardized uptake values (SUV) of 16.06, which corresponded to metabolically active deposit recurrence of the underlying disease (Figure 2b). After the standard preoperative preparation, the boy was operated on, and the tumor mass was completely enucleated. Histopathology examination of the excised tumor mass confirmed to be relapsed BL. To date, all the performed check-up results were within normal ranges.

Fig. 1 – Plain radiography of the abdomen with multiple aero-liquid levels in the upper parts and right parts of the abdomen.

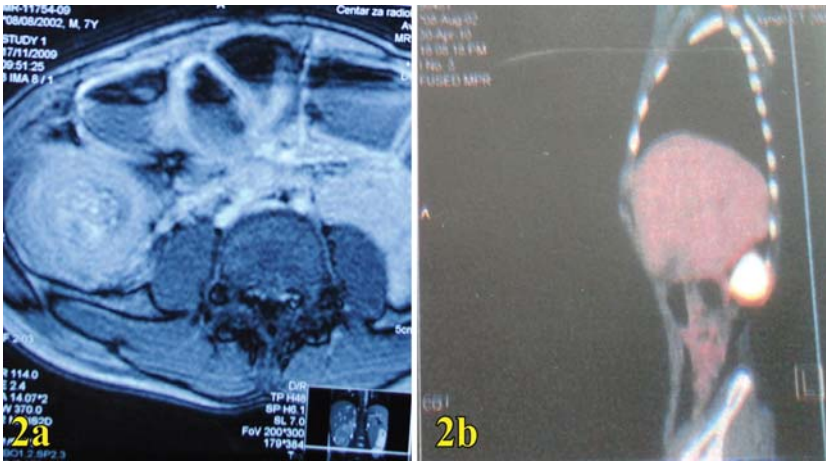

Fig. 2 – a) Nuclar magnetic resonance examination of the upper abdomen detected colonic stenosis in the distal part of the ascending colon immediately before the hepatic flexure with maximum luminal diameter of 4 mm, spreading to the length of 4.5 cm; b) Positron emission tomography scan corresponds to the metabolically active deposit in the retroperitoneal space near the lower pole of the right kidney.

Discussion

Abdominal pain is a very common surgical problem in pediatric population, caused by a variety of mechanical factors, although metabolic, hormonal, neuromuscular disorders and even malignancy cannot be excluded.

BL is a highly malignant, aggressive, rapidly growing B-cell neoplasm [2].

During the last 30–40 years, sporadic forms of BL have increasingly spread worldwide manifesting themselves in direct or indirect symptoms all over the digestive tract (causing invagination, constipation, bleeding or mechanical intestinal pressure) [3].

Ultrasound is an essential diagnostic procedure with a high sensitivity rate (over 98%), that may indicate the existence of a "target sign" caused by invagination due to occult tumors most commonly appearing in the ileocecal junction [4].

The prevalence of gastrointestinal BL in childhood is not known with much certainty, as the data on only a few series of patients have been published in the literature. Bethel et al. [5] claimed that BL accounts for less than 1% of all pediatric gastrointestinal malignancies. It is usually localized in the ileocoecal region, which is to be expected due to the increased concentration of lymphoid tissue. The colon, stomach and rectum are rarely affected.

The onset of BL in children is often abrupt, with rapid development and progression of neoplasm. BL appears to be the fastest growing human malignancy, with doubling time of 24–48 hours, regardless of the type, with the peak incidence between the ages of 11–14 years and with boys predomination of 3 : 1 [6, 7]. In BL relapses, the bone marrow is most often involved. Intra-abdominal disease occurs in 20–40% of childhood cases with male predominance, involving predominantly ileum or ileocecal valve [8].

The most frequent symptom is intestinal obstruction, caused by intussusceptions, which can be acute (complete) or chronic (partial). Our patient had subacute onset of symptoms with the loss of weight, constipation and two out of three typical signs of invagination: vomiting and pain in the ileocecal region.

It is still a subject of debate which diagnostic procedures provide credible information in the diagnosis of intussusceptions caused by BL. Some experts believe [9] that ultrasound as a noninvasive diagnostic tool in the pediatric age has priority, while others believe that computer tomography (CT) and NMR offer a much more visual picture of the abdomen, giving a precise overview of the intestinal wall condition, clearly delineating the tumor. CT provides better information about cancer dissemination in the abdomen.

During PET scan, exposure to radiation is extremely short without affecting the normal processes in the body. Sensitivity of the method is very high, forming three-dimensional pictures used to calculate the body volume and the size of focal lesions. PET is the most effective method in detecting the recurrence of cancer (especially lymphoma and melanoma), detecting a higher tumor-cell metabolic activity *versus* normal rates. PET scanning is also a dominant method for assessing the effectiveness of chemotherapy.

Fortunately, BL is very sensitive to chemotherapy as published by Enlgand et al. [10]. They also stated that patients with invagination as the initial sign of BL (like in our patient) require a less extensive and shorter course of chemotherapy compared to patients where the symptoms appear progressively later.

In the presented patient, we performed right hemicolectomy (with complete tumor resection) followed by the subsequent termino-terminal ileocolic anastomosis. PET scan was crucial in the diagnosis of retroperitoneal tumor mass confirming recurrence of the underlying disease.

Conclusion

Although BL is a very rare leading point for the development of intussusceptions, it should always arouse suspicion, especially if the onset of symptoms occurs in older children and preadolescents. In BL patients, the role of surgery is based on complete enucleation of the tumor mass with a "free edge" and the resolution of acute abdomen signs. A complex multidisciplinary approach should be adopted for each patient. The timely diagnosis, high rate tumor sensitivity to chemotherapy and also the great importance of PET scan in early detection of tumor recurrence significantly increase the survival rate in patients with BL.

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E R R A T U M

In the Editorial [1] published in the first (January) issue, 2017, of the Vojnosanitetski pregled, there was an error in the surname of one of the reviewers. In the Table 2 in which names of reviewers of the Journal in 2016 were given, on the page 7, 4th column right, the name of a reviewer Vanović Mirjana should have read as Životić-Vanović Mirjana.

The correction has been made to the online version of that issue of the Journal which is available at: http://www.vma.mod.gov.rs/vsp_1_2017.pdf

The author would like to apologise for any inconvenience caused.

1. Dobrić S. Towards 2017. Vojnosanit Pregl 2017; 74(1):5–7.

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

3. ***Tekst članka***

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

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 nadležnog 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

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

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Tabele

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

Ilustracije

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

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

Skraćenice i simboli

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

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