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Dr Vladan Đorđević, prvi srpski vojni hirurg-operator i prvi sanitetski pukovnik Srpske vojske, načelnik njenog saniteta u tri rata koje je vodila u drugoj polovini 19.veka (1876, 1877 /1878. i 1885), vojni pisac i istoričar srpskog vojnog saniteta - vojnički deo njegovog života
Dr. Vladan Djordjevic, the first Serbian military surgeon-operator and the first medical colonel of the Serbian Army, chief of its medical care in the three wars it fought in the second half of the 19th century (1876, 1877/1878 and 1885), military writer and historian of the Serbian military medicine - A military part of his life 1351

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Vladan Đorđević (21. novembar 1844 – 31. august 1930) bio je čuveni srpski lekar, osnivač Srpskog lekarskog društva i Srpskog Crvenog krsta, diplomata, gradonačelnik Beograda, premijer Srbije, pisac,... O njegovom bogatom životu napisano je mnogo dela. U ovom članku, koji je pisan povodom 90-godišnjice njegove smrti, detaljnije je obrađen njegov rad kao vojnog lekara i pisca istorije srpskog vojnog saniteta (vidi str. 1351 – 1358).

Vladan Djordjevic (November 21, 1844 – August 31, 1930) was a famous Serbian physician, the founder of the Serbian Medical Association and the Serbian Red Cross, a diplomat, the mayor of Belgrade, the Prime Minister of Serbia, a writer, etc. Many papers have been written about his prosperous life. This article, which was written on the occasion of his 90th death anniversary, deals in more detail with his work as a military physician and writer of the Serbian military medicine history (see pp. 1351 – 1358).

Dear Authors, Editors, Peer Reviewers and Readers of *the Vojnosanitetski pregled,*

On behalf of members of the Editorial Office, I thank you for cooperation and support in 2020 and wish you all the best in the upcoming 2021!

Marry Christmas and a Happy and Healthy New Year!

Cordially,

Prof. Silva Dobrić, PhD
Editor-in-Chief

Dragi autori, urednici, recenzenti i čitaoci *Vojnosanitetskog pregleda,*

Uz zahvalnost na saradnji i podršci u 2020, u ime članova Redakcije časopisa i u svoje lično ime želim vam sve najbolje u nastupajućoj 2021. godini!

Srećna i zdrava Nova godina i Božićni praznici!

Srdačano,

prof. dr Silva Dobrić
glavni i odgovorni urednik

ORIGINAL ARTICLES

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Clinical characteristics of posterior segment penetrating eye injuries treated in tertiary referral hospital in Serbia

Kliničke karakteristike penetrantnih povreda zadnjeg segmenta oka lečenih u tercijarnoj zdravstvenoj ustanovi u Srbiji

Igor Kovačević*†, Aleksandra Radosavljević*†, Jelena Karadžić*†, Ivan Stefanović*†, Jovana Vukadinović*

Clinical Center of Serbia, Hospital for Eye Diseases, *Department for Ocular Trauma and Vitreoretinal Surgery, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, †Ophthalmology Department, Belgrade, Serbia

Abstract

Background/Aim. Ocular trauma is a significant cause of visual impairment worldwide. The aim of the study was to analyze clinical characteristics of patients with penetrating eye injuries with retained intraocular foreign body (IOFB) in posterior segment of the eye. **Methods.** The retrospective study enrolled medical records of consecutive patients hospitalized in a five year period (2012–2016) in a tertiary referral center in Serbia. Surgical repair included *pars plana* vitrectomy and IOFB removal. Postoperative results were evaluated using the best corrected visual acuity (BCVA). **Results.** Out of 130 patients who suffered penetrating eye injury with retained IOFB, 72 had IOFB in posterior segment of the eye (43 in retina, 25 in vitreous, 3 in ciliary body, 1 on the optic nerve). Patients were predominantly young adults (aged 44.3 ± 14.9 years, 97.2% men). Average BCVA at admission was poor (0.27 ± 0.38, measured by Snellen chart), and 52.8% of patients had BCVA of counting fingers at 1 meter or less. The majority of patients had corneal wound (70.8%), medium sized IOFB (48.6%), iris injury (61.1%) and traumatic cataract (69.4%). Complications included retinal detachment (16.7%) and endophthalmitis (15.3%). Two patients had the eye enucleated due to severe endophthalmitis, which could not be otherwise controlled. Significant risk factors for postoperative outcome were: initial BCVA ($p < 0.001$), ocular hypotony ($p = 0.013$), medium size of IOFB ($p = 0.037$), presence of traumatic cataract ($p = 0.036$), retinal detachment ($p = 0.032$) and endophthalmitis ($p = 0.045$). **Conclusion.** Treatment of posterior segment penetrating eye injuries remains a challenge due to high frequency of low initial visual acuity, retinal detachment and endophthalmitis, all of which are risk factors for poor visual outcome. Patients with better initial BCVA, normal intraocular pressure and small IOFB have better postoperative results.

Key words:
endophthalmitis; eye foreign bodies; eye injuries; retinal detachment; risk factors; treatment outcome.

Apstrakt

Uvod/Cilj. Trauma oka je značajan uzrok vidne nesposobnosti širom sveta. Cilj studije bio je da se ispitaju kliničke karakteristike blesnika sa penetrantnim povredama oka i zadržanim stranim telom u zadnjem segmentu oka. **Metode.** U retrospektivnoj studiji analizirana je medicinska dokumentacija bolesnika uzastopno hospitalizovanih tokom petogodišnjeg perioda (2012–2016) u tercijernom zdravstvenom centru u Srbiji. Hirurške procedure su obuhvatale *pars plana* vitrektomiju i uklanjanje stranog tela. Postoperativni rezultati su procenjivani pomoću najbolje korigovane vidne oštrine. **Rezultati.** Od ukupno 130 bolesnika koji su pretrpeli penetrantnu povredu oka sa zadržanim intraokularnim stranim telom, njih 72 su imala strano telo zadržano u zadnjm segmentu oka (43 na retini, 25 u staklenom telu, 3 u cilijarnom telu, 1 na vidnom živcu). Bolesnici su najčešće bili mlađe odrasle osobe muškog pola (prosečna starost 44,3 ± 14,9 godina, 97,2% muškarci). Prosečna vidna oštrina na prijemu bila je niska (0,27 ± 0,38, mereno na Snellen optotipu), a čak 52,8% bolesnika imalo je vidnu oštrinu brojanje prstiju na 1 metar ili manju. Većina bolesnika je imala ranu lociranu na rožnjači (70,8%), intraokularno strano telo srednje veličine (48,6%), povredu dužice (61,1%) i traumatsku kataraktu (69,4%). Najznačajnije komplikacije bile su ablacija retine (16,7%) i endoftalmitis (15,3%). Dva bolesnika su morala da budu podvrgnuta enukleaciji oka zbog teškog endoftalmitisa koji nije mogao biti kontrolisan drugim metodama. Značajni prognostički faktori rizika od postoperativnog ishoda bili su vidna oštrina na prijemu

Correspondence to: Aleksandra Radosavljević, Clinical Centre of Serbia, Hospital for Eye Diseases, Pasterova 2, 11 000 Belgrade, Serbia. E-mail: alexandra.radosavljevic@gmail.com.

($p < 0,001$), prisustvo hipotonije oka ($p = 0,013$), strana tela srednje veličine ($p = 0,037$), prisustvo traumatske katarakte ($p = 0,036$), ablacija retine ($p = 0,032$) i endoftalmitis ($p = 0,045$). **Zaključak.** Lečenje penetrantnih povreda zadnjeg segmenta oka još uvek predstavlja izazov, zbog visoke učestalosti teških povreda sa niskom početnom vidnom oštrinom i komplikacijama poput ablacije retine i endoftalmitisa, koji svi predstavljaju faktore rizika od lošeg ishoda po vid. Bolesnici sa boljom vidnom oštrinom na prijemu, normalnim vrednostima intraokularnog pritiska i malim intraokularnim stranim telima imaju najbolje postoperativne rezultate.

Ključne reči:
endoftalmitis; oko, strana tela; oko, povrede; retina, ablacija; faktori rizika; lečenje, ishod.

Introduction

Ocular trauma still remains significant cause of visual impairment, particularly in developing countries [1]. Mechanical injuries of the eye are classified into open and closed depending on the integrity of the wall of an eye. Penetrating injuries are subcategory of open globe injuries, defined as full thickness defects of an eye wall, usually caused by a sharp object. Sometimes, the object that caused the injury is retained within the eye as intraocular foreign body (IOFB) [2]. The initial type and extent of injury are decisive for the final outcome. Severity of the injury can vary greatly, and is generally accepted that visual acuity at presentation, the zone of eye injury, presence of ocular hypotony, retinal detachment, posttraumatic endophthalmitis and afferent pupillary defect are the most significant factors influencing the postoperative visual acuity [3, 4]. The aim of primary surgical repair is to restore the integrity of the globe and to prevent vision threatening complications. Timing of surgery is essential, since early repair can impede complications such as endophthalmitis, retinal detachment, proliferative vitreoretinopathy, IOFB associated metallosis and sympathetic ophthalmia [5, 6].

Patients with penetrating eye injuries and retained IOFB in the posterior segment of the eye remain the most difficult cases for surgical repair. The postoperative visual recovery is often not complete. The aim of this study was to analyze clinical characteristics and visual outcome in a group of patients with this type of injury, as well as clinical parameters that are important for prognosis and postoperative outcome.

Methods

The retrospective study included consecutive patients, hospitalized for penetrating eye injury between January 1, 2012, and December 31, 2016. All patients underwent primary surgical repair according to the standardized protocol, at the Department for Traumatology and Vitreoretinal Surgery of the Hospital for Eye Diseases in Belgrade, which is the tertiary referral center in Serbia. At admission, patients were fully assessed including medical history, best corrected visual acuity (BCVA), measured by Snellen chart at 6 m and converted to decimal notation, applanation tonometry (when appropriate/possible), slit lamp examination, indirect ophthalmoscopy with 90D and 20D lens and ultrasound examination. Furthermore, patients with suspect metal foreign bodies had undergone orbital radiography. All patients had surgical repair, that included pars plana vitrectomy (PPV) and IOFB removal, performed according to the standardized protocol, within the first 12 hours from the admission [7]. Cataract extraction, endolaser or cryopexy were performed as needed. Postoperative results were evaluated using BCVA at the discharge from the hospital. Furthermore, in patients who had postoperative complications that required additional hospitalizations, all were analyzed and shown in the results.

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

Data were analyzed using SPSS 15.0. Methods used included Student's *t*-test or Wilcoxon test for comparison of numerical variables, χ^2 or Mann-Whitney U test for categorical variables (as appropriate). Correlation analysis included Pearson's correlation for numerical variables and Spearman's correlation for categorical variables (as appropriate). The level of statistical significance was 0.05.

Results

During the study period, a total of 130 patients with penetrating eye trauma with retained IOFB were hospitalized. Out of them, 13 had an old injury that lasted more than 10 days and 45 had IOFB retained in the anterior segment of the eye and were excluded from the study. Totally, 72 patients had IOFB in the posterior segment of the eye and were further analyzed. The vast majority of patients were men (70 patients, 97.2%) and 84.7% of the patients of both sexes belonged to working population (average age 44.3 ± 14.9, range 12–82 years). Detailed clinical characteristics of patients are shown in Table 1.

Postoperative results of IOFB removal and wound repair are shown in Table 2. Majority of patients had pars plana vitrectomy with instillation of silicon oil and phacoemulsification (63.9%). IOFB was completely removed in 87.5% of the cases. The remaining 12.5% had an IOFB positioned in such place (for example, within the globe wall) that it was not possible to remove it without significant damage to the eyeball. Postoperative BCVA was significantly better ($p = 0.046$, data not show in Table 2) and the majority of patients had normal IOP (76.4%). Secondary glaucoma was present in 13.9% of the patients, and in all cases IOP control could be achieved with conservative treatment. Average number of hospitalizations was 1.5 ± 1.1,

Table 1

Clinical characteristics of patients with posterior segment penetrating trauma and retained intraocular foreign body (IOFB)

Variable	Values
Sex	
male	70 (97.2)
female	2 (2.8)
Age (years)	44.3 ± 14.9; 12–82
11–20	2 (2.8)
21–30	12 (16.7)
31–40	13 (18.0)
41–50	21 (29.2)
51–60	15 (20.8)
61–70	6 (8.3)
71–80	2 (2.8)
81–90	1 (1.4)
Affected eye (side)	
right	30 (41.7)
left	40 (55.5)
both	2 (2.8)
Time lapse between injury and hospital admission (days)	1.7 ± 2.6; 0–10
0–1	49 (68.1)
2–4	13 (18.0)
5–10	10 (13.9)
BCVA at admission	0.27 ± 0.38; amaurosis - 1.0
0.7–1.0	17 (23.7)
0.4–0.6	5 (6.9)
0.2–0.3	5 (6.9)
0.05–0.1	5 (6.9)
0.02–0.04	2 (2.8)
< 0.02 or light perception	36 (50.0)
no light perception	2 (2.8)
IOP at admission (mm Hg)	13.3 ± 6.4; 5–44
Localization of the wound	
cornea	51 (70.8)
sclera	20 (27.8)
both	1 (1.4)
Localization of the IOFB	
in vitreous cavity	25 (34.7)
in the ciliary body	3 (4.2)
on the retina	43 (59.7)
on the optic nerve	1 (1.4)
Size of the IOFB (mm)	3.6 ± 2.2; 1–11
small (< 2)	19 (26.4)
medium (3–5)	35 (48.6)
big (> 5)	10 (13.9)
not known	8 (11.1)
Injury of the iris	
absent	28 (38.9)
present	44 (61.1)
Lens status	
transparent	22 (30.6)
traumatic cataract	50 (69.4)
Retinal detachment	
absent	60 (83.3)
present	12 (16.7)
Endophthalmitis	
absent	61 (84.7)
present	11 (15.3)

***Note*: The values are given as mean ± standard deviation; range (minimum-maximum) or number (percentage) of patients.**

BCVA – best corrected visual acuity; IOP – intraocular pressure.

Table 2
Postoperative results of intraocular foreign body (IOFB) removal and surgical repair of the wound

Variable	Values
IOFB removal	
PPV with SO	12 (16.7)
PPV with SO and CE	46 (63.9)
removal with electromagnet	14 (19.4)
IOFB surgery outcome	
IOFB removed	63 (87.5)
IOFB not completely removed	9 (12.5)
BCVA at discharge	0.34 ± 0.38; amaurosis – 1.0
0.7–1.0	17 (23.6)
0.4–0.6	10 (13.9)
0.2–0.3	9 (12.5)
0.05–0.1	10(13.9)
0.02–0.04	1 (1.4)
< 0.02 or light perception	20 (27.8)
no light perception	5 (6.9)
IOP at discharge	15.0 ± 5.5; 3–34
normotensive	55 (76.4)
hypotensive	7 (9.7)
secondary glaucoma	10 (13.9)
Number of hospitalizations	1.5 ± 1.1
1	50 (69.4)
2	14 (19.4)
3	3 (4.2)
4	3 (4.2)
5 or more	2 (2.8)

***Note*: The values are given as mean ± standard deviation; range (minimum-maximum) or number (percentage) of patients.**
PPV – pars plana vitrectomy; SO – silicon oil; CE – cataract extraction (phacoemulsification or lensectomy); IOP – intraocular pressure; BCVA – best corrected visual acuity; NLP – no light perception.

and majority of patients (88.8%) had one or two hospitalizations (Table 2).

The most significant factors that influenced final BCVA are shown in Table 3, and included: initial BCVA (Pearson's corelation $p < 0.001$, data not shown in Table 3), ocular hypotony ($p = 0.013$), medium sized IOFB ($p = 0.037$), traumatic cataract ($p = 0.036$), retinal detachment ($p = 0.032$) and endophthalmitis ($p = 0.045$). In our study group, final BCVA was not affected by the time lapse between the injury and admission to the hospital, localization of globe wound, presence of iris injury, or localization of IOFB ($p > 0.05$, data partially shown in Table 3).

The occurrence of retinal detachment was not affected by duration of period between the injury and admission to hospital, localization of the wound, presence of iris injury, size or localization of IOFB, or presence of endophthalmitis ($p > 0.05$, data not shown in Table 3). Interestingly, patients with retinal detachment more often had postoperatively retained IOFB in the globe ($p = 0.029$).

Occurrence of posttraumatic endophthalmitis was not associated with any of the investigated parameters including period between the injury and admission to

hospital, localization of the wound, and size or localization of IOFB ($p > 0.05$, data not shown in Table 3). In our study group, two patients (2.8%) had severe endophthalmitis on a

Table 3

Clinical factors that influence the postoperative best-corrected visual acuity (BCVA)

Variable	BCVA at discharge (mean ± SD)	*p*-value
IOP		
normal	0.41 ± 0.40	0.013
ocular hypotony	0.02 ± 0.04	
secondary glaucoma	0.30 ± 0.31	0.034
ocular hypotony	0.02 ± 0.04	
normal	0.41 ± 0.40	> 0.05
secondary glaucoma	0.30 ± 0.31	
Wound localization		
corneal wound	0.31 ± 0.37	> 0.05
scleral wound	0.43 ± 0.41	
iris injury present	0.29 ± 0.38	> 0.05
iris injury absent	0.41 ± 0.37	
IOFB size		
small	0.50 ± 0.36	0.037
medium	0.28 ± 0.36	
small	0.50 ± 0.36	> 0.05
large	0.24 ± 0.42	
medium	0.28 ± 0.36	> 0.05
large	0.24 ± 0.42	
Lens status		
transparent lens	0.48 ± 0.40	0.036
traumatic cataract	0.28 ± 0.36	
Posterior segment complications		
retina attached	0.38 ± 0.40	0.032
retinal detachment	0.13 ± 0.19	
no endophthalmitis	0.38 ± 0.39	0.045
present endophthalmitis	0.13 ± 0.24	

IOP – intraocular pressure; IOFB – intraocular foreign body; SD – standard deviation.

blind eye, that did not respond to any kind of treatment (both medicamentous and surgical). Due to a high risk of further propagation of infection, eventually, eyeballs had to be enucleated.

None of the patients developed sympathetic ophthalmia during the study period.

Discussion

Posterior segment penetrating eye injury is often associated with poor visual acuity at admission [1] and in our study group more than 50% of the patients initially had BCVA of counting fingers at 1 meter or less. Furthermore, those patients have worse prognosis regarding the postoperative visual outcome [1, 8] and this was confirmed in our study group as well.

The majority of our patients were working adults (84.7%) and males (97.2%), and similar findings are found world-wide in studies of ocular trauma [1, 9–12]. The time lapse between injury and admission to the hospital varied from 1 to 10 days, however this parameter alone did not affect the final visual acuity. Similar results were found in a study of Mansouri et al. [12].

Postoperative BCVA was significantly better than at admission. However, initial visual acuity was the most important factor that correlated with the visual outcome ($p < 0.001$). Initial visual acuity indirectly can reflect the severity of eye injury and is important prognostic factor for postsurgical visual outcome, which has been shown in studies of other authors as well [1, 8, 9, 13].

Secondary glaucoma was present in 13.9% of the patients, but responded well on conservative treatment, and therefore did not significantly affect postoperative outcome. Secondary glaucoma can be caused by inflammation, trabecular meshwork disruption, or occlusion with erythrocytes, lens particles or silicon oil after PPV [14]. On the other hand, ocular hypotony was present in 9.7% of the patients and was associated with significantly lower postoperative BCVA (0.02 ± 0.04), when compared to both patients with normal IOP or secondary glaucoma ($p < 0.05$). Postsurgical ocular hypotony can reflect loss of intraocular structures (vitreous, uvea etc.), residual retinal detachment or insufficiency of ciliary body, all of which are associated with poor visual outcome. Similar findings are reported in the study of Knyazer et al. [8].

In our study, postoperative BCVA did not differ between groups with different time lapse from the injury and admission to the hospital, or between groups with corneal or scleral localization of the wound, or between groups with or without iris injury, or between groups with different

localizations of IOFB ($p > 0.05$). However, patients with small IOFB, up to 2 mm in size, had better postoperative BCVA when compared to those with medium sized (significantly better) or large IOFB. This could be explained by the fact that due to their smaller size, IOFB can cause less damage to the intraocular structures. Furthermore, surgical extraction of small IOFB is technically easier and has better postoperative prognosis.

Presence of traumatic cataract did influence the postoperative BCVA, since in those cases, cataract surgery is associated with more complications due to often present partial disruption of zonular fibers or lens capsule.

Patients with retinal detachment at presentation, had a significantly lower postoperative BCVA. This was confirmed in the study of Lin et al. [13] and Nicoarã et al. [10], although other authors found no correlation [1, 8]. The occurrence of retinal detachment was not affected by the time lapse between the injury and admission, localization of the wound, size or localization of IOFB, presence of iris injury or endophthalmitis. Interestingly, patients with retinal detachment more often had postoperatively retained IOFB in the globe ($p = 0.029$). This could be explained by the fact that IOFB passed through retina (causing retinal tear and detachment) and than was buried deeply within the globe wall. Therefore, it was not possible to surgically remove it without further damage to the eye.

Endophthalmitis is one of the most severe complications of penetrating eye injuries, with potentially devastating consequences. The known risk factors for development of endophthalmitis are delay in the wound closure, posterior segment eye injuries with disruption of crystalline lens, rural settings of injury and retained IOFB [15]. Since our study included only penetrating eye injuries with IOFB retained in the posterior segment of the eye, a slightly higher rate of posttraumatic endophthalmitis was observed (15.3%), when compared to other studies that included both anterior and posterior segment injuries [16]. However, in a study of Nicoarã et al. [10], considerably higher incidence of posttraumatic endophthalmitis (28.6%) was noted.

In our study group, two patients had the eye enucleated due to severe endophthalmitis and high risk of propagation of infection in the surrounding structures. Clinically, the main reasons for enucleation of the eye after penetrating injury are: development of sympathetic ophthalmia in the fellow eye, painful blind eye and, more rarely, severe endophthalmitis [17, 18]. However, this rate is significantly lower than in a study of Rahman et al. [19] (12%), which included all types of open globe injuries.

In our group of patients, there were no signs of development of sympathetic ophthalmia during the study period due to timely and adequate surgical treatment and similar results were reported by Nashed et al. [20] as well.

The limitations of our study include a relatively small number of patients with retinal detachment and endophthalmitis (12 and 11, respectively), making it less possible to statistically assess risk factors for those complications of posterior segment penetrating trauma. Further studies should include multicenter collaboration in assessing the severe vision threatening complications of eye injuries, in order to obtain more statistically powerful results.

Conclusion

The study was carried out in a tertiary setting and included patients with posterior segment penetrating eye injury with retained IOFB. Those patients had high frequency of low initial visual acuity, retinal detachment and endophthalmitis, all of which were shown to be risk factors for poor postoperative visual outcome. Furthermore, presence of traumatic cataract and postoperative hypotony were also associated with lower final visual acuity. Patients with smaller IOFB had better postoperative results.

Conflict of interest

The authors declare no conflict of interest.

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Teucrium polium induces apoptosis in peripheral blood lymphocytes isolated from human chronic lymphocytic leukemia

Teucrium polium indukuje apoptozu limfocita iz periferne krvi kod obolelih od hronične limfocitne leukemije

Milan Zarić*, Radica Živković Zarić†, Marina Mitrović*, Ivana Nikolić†, Petar Čanović*, Zoran Milosavljević‡, Danijela Jovanović§, Ivanka Zelen*

University of Kragujevac, Faculty of Medical Sciences, *Department of Biochemistry, †Department of Pharmacology and Toxicology, ‡Department of Histology and Embriology, §Department of Internal Medicine, Kragujevac, Serbia

Abstract

Background/Aim. Chronic lymhocytic leukemia (CLL) is considered more as a disease of cells accumulation due to the defect in apoptosis rather than deregulated cell's proliferation. The activation of apoptosis is one of the main molecular mechanisms responsible for anti-cancer activities of most of the currently studied potential anti-cancer agents, including natural compounds. *Teucrium polium* (TP) extracts exhibited strong cytotoxic effects in murine leukemia cell line, RAW 264.7 and human melanoma cell line, C32, but their cytotoxic effects against human leukemia cells were unknown. **Methods.** The viability of human leukemia cell lines (MOLT 4 and JVM 13), lymphocytes isolated from 28 patients with CLL (CLL cells), and peripheral blood mononuclear cells (PBMCs) isolated from 16 healthy subjects treated with TP leaves methanolic extract, was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Apoptosis of TP treated CLL cells was measured by flow cytometry applying Annexin V/7AAD staining. The expressions of active proapoptotic protein Bax, antiapoptotic protein Bcl-2, cytochrome c and the percentage of cells containing cleaved caspase-3 in treated CLL cells was determined by flow cytometry and immunocytochemistry. **Results.** The TP methanolic extract decreased the viability of all tested human leukemia cells but it had no effect on the viability of PBMCs isolated from healthy subjects. The cytotoxic effect of TP was caused by its induction of CLL cells' apoptosis. TP disarranged the ratio of the expressions of proapoptotic Bax and antiapoptotic Bcl-2 protein in favor of Bax, consequently inducing apoptosis by cytochrome c mitochondrial release and activation of caspase-3 in treated CLL cells. **Conclusion.** The TP leaves methanolic induced selective apoptosis in CLL cells and it affected the expressions of key proteins involved in the regulation of programmed cell death.

Key words:
teucrium extract; apoptosis; leukemia, lymphocytic, chronic, b cell; cell line; mice; humans.

Apstrakt

Uvod/Cilj. Hronična limfocitna leukemija (HLL) se pre smatra bolešću akumulacije ćelija usled defekta u njihovoj apoptozi, nego bolešću ćelijske proliferacije. Aktivacija apoptoze je jedan od glavnih molekulskih mehanizama odgovornih za antitumorsku aktivnost većine agenasa koji se sada ispituju, uključujući i agense prirodnog porekla. Ekstrakti biljke *Teucrium polium* (TP) su ispoljili snažne citotoksične efekte na ćelije mišje leukemije, RAW 264.7, i ćelije humanog melanoma, C32, ali su njihovi citotoksični efekti na ćelije humane leukemije nepoznati. **Metode.** Vijabilnost ćelija humane leukemije (MOLT 4 i JVM 13), limfocita izolovanih iz krvi 28 bolesnika sa HLL (HLL ćelije) i mononukleara periferne krvi (PBMCs) izolovanih iz krvi 16 zdravih ispitanika je određena 3-(4,5-dimetiltazol-2-il)-2,5-difeniltetrazolium bromid (MTT) testom nakon tretmana ispitivanih ćelija metanolskim ekstraktom lista TP. Apoptoza HLL ćelija tretiranih ovim ekstraktom merena je protočnom citometrijom korišćenjem bojenja Annexin V/7AAD kita. Ekspresija aktivnog proapoptotičnog proteina Bax, antiapoptotičnog proteina Bcl-2, citohroma c i procenat ćelija koje sadrže aktivnu kaspazu-3 u tretiranim HLL ćelijama, određivana je pomoću protočne citometrije i imunocitohemijskim metodama. **Rezultati.** Metanolni ekstrakt lišća TP je smanjivao vijabilnost svih leukemijskih ćelija, ali nije uticao na vijabilnost PBMCs. Pokazano je da ovaj ekstrakt deluje citotoksično indukujući apoptozu HLL ćelija, kao i da utiče na odnos ekspresije Bax i Bcl 2 proteina u korist Bax, posledično indukujući apoptozu preko citohroma-c i aktivacije kaspaze-3 u HLL ćelijama.

Correspondence to: Radica Živković Zarić, University of Kragujevac, Faculty of Medical Sciences, Svetozara Markovica 69, 34 000 Kragujevac, Serbia. E-mail: radica_zivkovic@yahoo.com.

Zaključak. Metanolni ekstrakt lista TP selektivno indukuje apoptozu HLL ćelija menjajući ekspresiju ključnih proteina uključenih u proces programirane ćelijske smrti.

Ključne reči:
teucrium ekstrakt; ćelija, smrt; leukemija, b ćelije, hronična; ćelijska linija; miševi, ljudi.

Introduction

Chronic lymhocytic leukemia (CLL) originates from the antigen-stimulated mature B lymphocytes that either avoid death through the intercession of external signals or die by apoptosis, only to be replenished by proliferating precursor cells [1]. For that reason, CLL is considered more as a disease of cells accumulation due to the defect in apoptosis rather than deregulated cells proliferation [2]. The activation of apoptosis is one of the main molecular mechanisms responsible for the anti-cancer activities of most of the currently studied potential anti-cancer agents, including natural compounds [3, 4]. Several, novel drugs designed to interfere with the proteins regulating the cell cycle, the apoptotic machinery or leukemic microenvironment, are currently being tested in *in vitro* or *in vivo* studies, as well as in clinical trials [5–9].

There are two main cellular death pathways leading to caspase activation and apoptosis: the extrinsic pathway, initiated by "death" receptors and the intrinsic pathway, initiated after cytosolic discharge of mitochondrial derived cytochrome c and the other apoptotic proteins, caused by the mitochondrial outer membrane permeabilization induced by the formation of the proapoptotic proteins oligomerisation pores, such as Bax. Both pathways merge into the activation of caspase-3, the executioner caspase, that ultimately finalizes the apoptosis. The mitochondrial outer membrane permeabilization by pro-apoptotic protein Bax that by following the death signal, translocates from the cytosol to the mitochondrial outer membrane, is suppressed through the actions of cytosolic antiapoptotic proteins such as Bcl-2. Therefore, the changes in cytosolic expressions of Bcl-2 and Bax, play a significant role in the execution of apoptosis [10, 11].

In the present study, for the first time, we investigated the antitumor activity of *Teucrium polium* (TP) leaves methanolic extract against two human leukemic cell lines, MOLT 4 and JVM 13, and peripheral blood lymphocytes isolated from human chronic lymphocytic leukemia patients (CLL lymphocytes). We also determined the cellular pathway responsible for the activation of apoptosis induced by the TP methanolic extract in CLL lymphocytes.

Methods

Chemicals

Unless stated otherwise, all reagents were from Sigma-Aldrich (St. Louis, MO, USA), and all dishes for culturing cells were from Sarstedt (Numbrecht, Germany).

Preparation of drug solution

Leaves of TP were collected in the late summer period of 2015, from the region of Šumarice, Kragujevac, Serbia. The voucher specimen of TP was confirmed and deposited in Herbarium at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac. The sampled leaves were air-dried in darkness at room temperature (20ºC).

Air-dried leaves (10 g) were transferred to the dark-coloured flasks. They were mixed with 200 mL of methanol and stored at room temperature. After 24 h, the extracts were filtered through Whatman No.1 filter paper and residues were again mixed with equal volumes of the solvent. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40ºC using rotary evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4ºC.

Stock solution of the TP extract was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Germany) at a concentration of 20 mM, filtered through a 0.22 mm Millipore filter before use, and diluted by the nutrient medium Roswell Park Memorial Institute (RPMI 1640) to various working concentrations, so the final concentration of DMSO in the culture medium never exceeded 0.5% (v/v).

Patients

The local Ethics Committee accepted the study and prior to the initiation of the study, the written informed consent was obtained from all individual participants included in the study according to the Declaration of Helsinki. CLL was diagnosed by establishing the clinical criteria and it was confirmed by immunophenotypic analysis for the expression of CD5, CD19 and monoclonal immunoglobulin in accordance with updated the National Cancer Institute (NCI) Working Group Guidelines [12]. The control group was composed of healthy volunteers without known acute and chronic diseases. Peripheral blood samples from 28 CLL patients and 16 healthy control subjects were included in study.

Cell preparation

All blood samples were obtained in the morning and collected in potassium-ethylendiaminetetracetic acid (EDTA) coated blood collection tubes (Terumo). Peripheral blood samples (9 mL) were centrifuged at 400 x g for 10 minutes to separate plasma and cells. Peripheral blood mononuclear and polymorphonuclear cells were

separated by single step continuous density-gradient centrifugation with Histopaque 1077. The separated mononuclear cells were washed three times with culture medium RPMI 1640 and resuspended in RPMI 1640 supplemented with 10% autologous serum. The monocytes were removed by adhesion on plastic Petri dishes [13].

Cell lines

Human leukemia cell lines, MOLT- 4 (ATCC® CRL-1582™) and JVM 13 (ATCC® CRL-3003™), were acquired as a gift from professor Sonja Denčić, Department of Biochemistry, Belgrade University School of Medicine, Serbia. Both cell lines were maintained in culture medium consisting of RPMI-1640, supplemented with 10% heat-inactivated fetal bovine serum (FBS).

MTT assay

The viability of cultured cells was determined by assaying the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan [14]. In brief, cells were treated with different concentrations of the TP methanolic extract (10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL and 500 µg/mL) or cultivated in the cell culture medium containing the appropriate amount of DMSO (control). After 24 and 48 hours of cells incubation at 37°C in the atmosphere containing 5% CO_2, the 96 well plates were centrifuged for five minutes at 400 x g, the culture medium was removed, and MTT solution (5 mg/mL) was added to the cells. After additional 4h of incubation, the microtiter plates were centrifuged again for five minutes at 400 x g, the culture medium with MTT solution was removed and DMSO (150 µL/well) was added to dissolve the formazan crystals. Absorbance was measured at 595 nm with a multiplate reader (Zenyth 3100, Anthos Labtec Instruments, Austria). The results were presented as relative to the control value (untreated cells).

Detection of apoptosis

Apoptosis of CLL lymphocytes was measured using annexin V–fluorescein isothiocyanate (FITC)/7-amino-actinomycin D (7-AAD) Apoptosis Kit (BD Biosciences) according to manufacturer's instructions. CLL cells were treated with the TP methanolic extract at earlier specified concentrations and the percentage of apoptotic cells were determined by flow-cytometer FC 500 (Beckman Coulter). Data were presented as density plots of Annexin V-FITC and 7AAD stainings.

Assessment of apoptosis mechanism

In order to understand the mechanism of apoptosis induced by the TP methanolic extract, we analyzed the expressions of the active proapoptotic protein Bax, antiapoptotic protein Bcl-2, cytochrome c and the percentage of cells containing cleaved caspase-3. Lymphocytes from 28 CLL patients were incubated for 24 hours with 250 µg/mL and 500 µg/mL of the TP extract investigated or with the culture medium alone (control), washed three times with ice cold (PBS), and then resuspended, fixed and permeabilized (Fixation and Permeabilization Kit, eBioscience). Four types of stainings were separately performed afterward. Intended for Bcl-2 staining, the permeabilized cells were then incubated with Bcl-2 fluorescein isothiocyate primary antibody (mhbcl01, Life technologies) for 15 minutes at room temperature. Other three types of staining included incubation of permeabilzed lymfocytes for 30 minutes with primary antibodies against Bax (N20, sc-493; Santa Cruz Biotech. Inc), cytochrome-c (G7421, Promega) and caspase-3 (#9661, Cell signaling Technology). These cells were washed and then incubated with the appropriate secondary antibodies for 30 minutes. We used Alexa488 goat anti-mouse IgG (H+L) antibody (A-11001, Life Technologies) for cytochrome c, and goat anti-rabbit IgG FITC (Ab6717-1, Abcam) for Bax and caspase-3 staining. All cells were then washed with phosphate buffer saline (PBS) and analysed by flow cytometry and/or immunocytochemistry.

Immunocytochemistry

Observation of cells by fluorescent microscope was performed to localize the presence of Bax, Bcl-2, cytochrome c and cleaved caspase-3 in CLL lymphocytes. The images were aquired with a Olympus BX51 at 1000x magnification.

Flowcytometric evaluation

Fluorescence of at least 10,000 events per sample was measured using flow-cytometer FC500 (Beckman Coulter). Fluorescence intensity was standardized using isotype-matched negative control antibodies. The mean fluorescence intensities (MFIs) of Bax and Bcl-2 were calculated as the ratio of raw mean channel fluorescences to isotype control levels, respectively. Cytochrome c and cleaved caspase-3 levels were evaluated as percentage of cells displaying the fluorescence.

Statistical analysis

All values were expressed as mean ± standard deviation (SD). Each experiment was performed in triplicate and conducted on every sample. Commercial SPSS version 20.0 for Windows was used for statistical analysis. The distributions of data were evaluated for normality using the Shapiro-Wilk test. Statistical evaluation was performed by Student's *t*-test for paired observations, or one-way ANOVA depending on data distribution. *P* values less than 0.05 were considered significant.

Results

Teucrium polium methanol extract decreased viability of human leukemic cells

After 24 hours of incubation of MOLT-4 leukemia cells with 10 μg/mL, 25 μg/mL and 50 μg/mL of the TP methanolic extract, there was no statistically significant decrease in the viability of the examined cells compared to the viability of untreated MOLT-4 leukemia cells ($p > 0.05$). However, at the TP extract concentrations of 100 μg/mL, 250 μg/mL and 500 μg/mL, there was a statistically significant reduction in the viability of MOLT-4 leukemic cells compared to the viability of untreated cells ($p < 0.05$). Specifically, after 24 h of incubation of MOLT-44 cells with 100 μg/mL, 250 μg/mL and 500 μg/mL of the TP extract, the viability of MOLT-4 cells was reduced to 63.56 ± 1.27%, 30.32 ± 2.39% and 41.83 ± 1.42%, respectively.

After 48 hours of incubation with 100 μg/mL of the TP extract, the viability of the MOLT-4 cells was 71.41 ± 2.39% ($p < 0.05$). Also, at concentrations of 250 μg/mL and 500 μg/mL of the extract, after 48 h of the treatment, the extract significantly decreased viability of MOLT-4 cells to 14.41 ± 1.14% and 13.92 ± 0.94%, respectively ($p < 0.05$) (Figure 1).

After 24 hours incubation of JVM-13 cells with 10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL and 250 μg/mL of the TP extract, there was no statistically significant decrease in JVM-13 cells viability compared to the viability of untreated JVM-13 cells ($p > 0.05$). However, 24 h of incubation of JVM-13 cells with 500 μg/mL of the TP extract, reduced viability of these cells to 36.24 ± 1.52% compared to the untreated JVM-13 cells, was observed.

Nevertheless, a statistically significant decrease ($p < 0.05$) in JVM-13 cells viability to 84.96 ± 3.53%, 59.85 ± 8.98% and 11.31 ± 1.35% compared to untreated cells was noticed after 48 h of cells incubation with 100 μg/mL, 250 μg/mL and 500 μg/mL of the TP extract, respectively (Figure 2).

Afterwards, we analyzed effects of the TP extract on viability of CLL cells and peripheral blood mononuclear cells (PBMCs) isolated from healthy subjects. After 24 hours, 250 μg/mL and 500 μg/mL of the TP extract statistically significantly decreased viability of treated CLL cells to 76.85 ± 7.25% and 50.27 ± 9.25%, respectively compared to the control group of untreated CLL lymphocytes ($p < 0.05$).

Also, after 48 hours, 250 μg/mL and 500 μg/mL of the TP extract statistically significantly decreased viability of treated CLL cells to 65.63 ± 8.13% and 40.39 ± 6.67%,

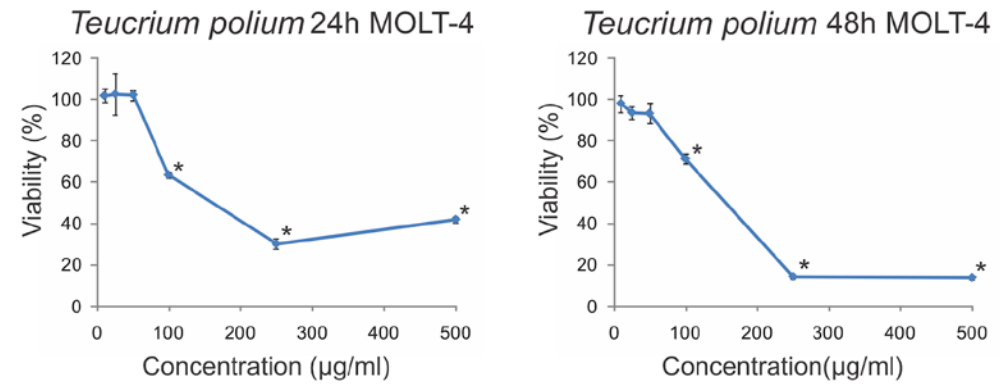

Fig. 1 – Effects of the *Teucrium polium* leaves methanolic extract on viability of MOLT-4 cells (*$p < 0.05$).

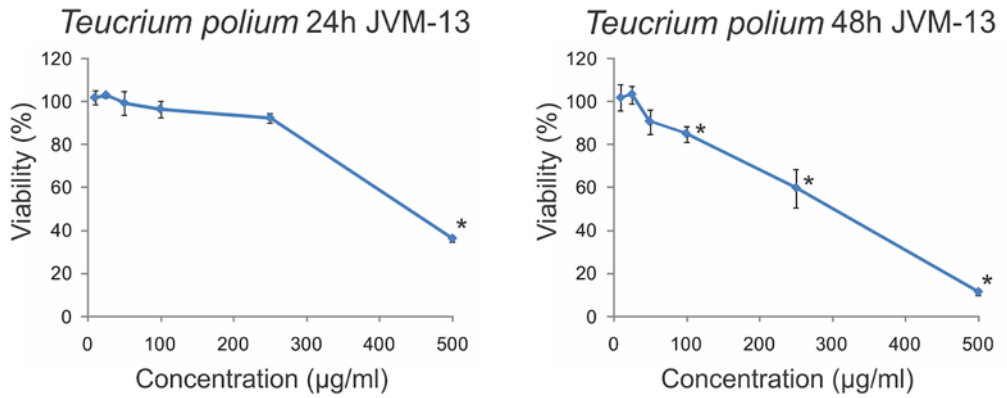

Fig. 2 – Effects of the *Teucrium polium* leaves methanolic extract on viability of JVM-13 cells (*$p < 0.05$).

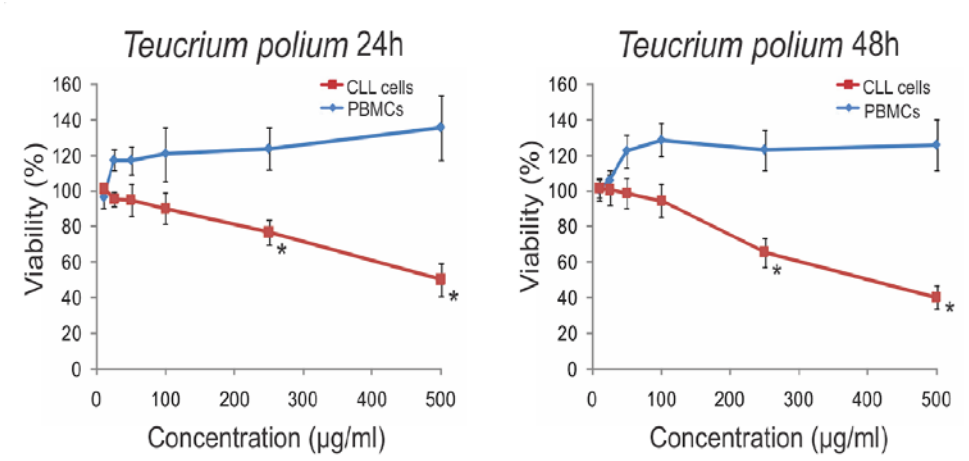

Fig. 3 – Effects of the *Teucrium polium* leaves methanolic extract on viability of chronic lymphocytic leukemia (CLL) cells and peripheral blood mononuclear cells (PBMCs) (*$p < 0.05$).

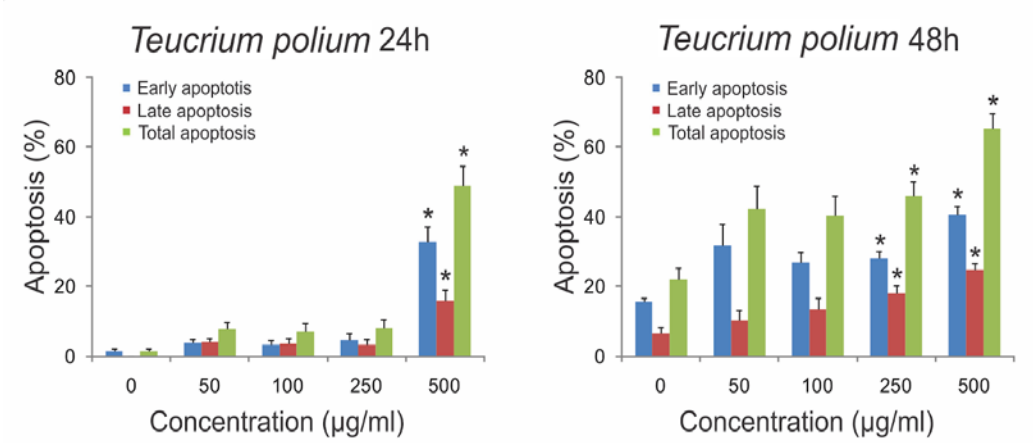

Fig. 4 – The *Teucrium polium* leaves methanolic extract induces apoptosis of chronic lymphocytic leukemia (CLL) cells. The extract induces apoptosis in peripheral blood lymphocytes isolated from human CLL patients via mitochondrial apoptotic pathway (*$p < 0.05$).

respectively compared to the control group of untreated CLL lymphocytes ($p < 0.05$).

After cultivation of PBMCs for 24 and 48 hours with the TP extract at concentrations ranging from 10 µg/mL to 500 µg/mL, there were no statistically significant changes in the viability of PBMCs relative to the control group of PBMCs that were not exposed to the TP extract ($p > 0.05$) (Figure 3).

Teucrium polium methanol extract induced apoptosis of CLL cells

Considering that our previous results showed that the TP extract produced the cytotoxic effect on human leukemia cells, especially on CLL cells, our next goal was to examine the type of cell death induced by the TP extract in CLL cells. The type of cell death was determined by Annexin V/7AAD staining.

Results obtained by Annexin V/7AAD staining after 24 hours of incubation of CLL cells with 500 µg/mL of the TP extract displayed significant increase of percentage of total apoptotic cells of about 50% compared to the untreated cells ($p < 0.05$, Figure 4). Nevertheless, results obtained after 48 hours of incubation of CLL cells with 250 µg/mL and 500 µg/mL of *TP*, also showed significant increase of percentage of total apoptotic cells of 50% and 70%, respectively compared to the untreated cells ($p < 0.05$, Figure 4).

To investigate whether the TP extract activated the mitochondrial apoptotic pathway, CLL cells were either treated with 500 µg/mL of the TP extract or cultivated in complete medium (control) for 24 hours and the localisation of cytochrome c was analyzed by fluorescent microscopy.

In parallel, we examined the localisation of active Bax proapoptotic protein and the fluorescence intensity representing the total expression of Bcl-2 antiapoptotic protein, along with presence of cleaved (activated) caspase-3 in treated and control cells. Our results showed that the TP extract induced the activation and translocation of Bax from cytosol to mitochondria, decreased the expression of cellular Bcl-2 protein, and induced the release of cytochrome c from mitochondria to cytosol and caspase-3 cleavage (Figure 5).

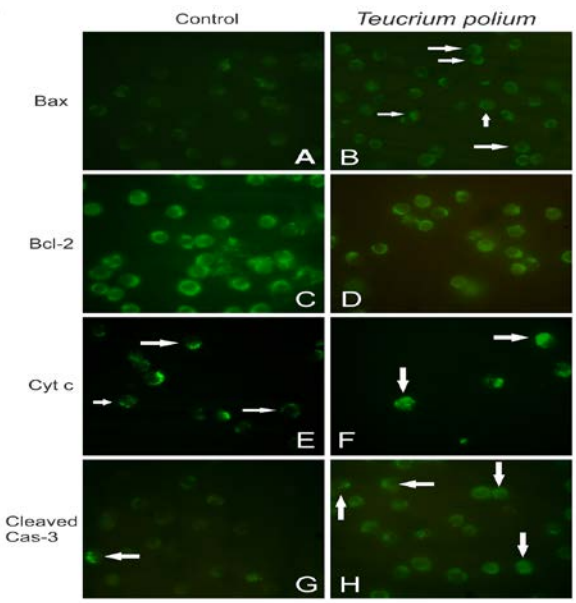

Fig. 5 – *Teucrium polium* (TP) leaves methanolic extract induces Bax translocation, decreases of cellular Bcl-2 protein level, release of cytochrome-c to cytosol and caspase-3 activation.

Chronic lymphocytic leukemia (CLL) lymphocytes were incubated for 24 h with Roswell Park Memorial Institute (RPMI) (control) or 500 μg/mL the TP methanolic extract. In a group of control cells, (A) Bax was localized in the cytosol, while in treated lymphocytes (B), Bax becomes organelle membrane-associated, and especially, mitochondrial membrane associated. Treated lymphocytes (D) displays a reduced amount of fluorescence intensity compared to the control group (C) suggesting decreased amount of antiapoptotic protein Bcl-2. The TP methanolic extract also stimulates cytochrome-c release to cytosol (F) compared to the untreated cells (E). Besides, the number of cleaved caspase-3 positive cells shows a trend of increase in a group of CLL lymphocytes treated with 500 μg/L of the TP methanolic extract (H) compared to the control group of lymphocytes (G).

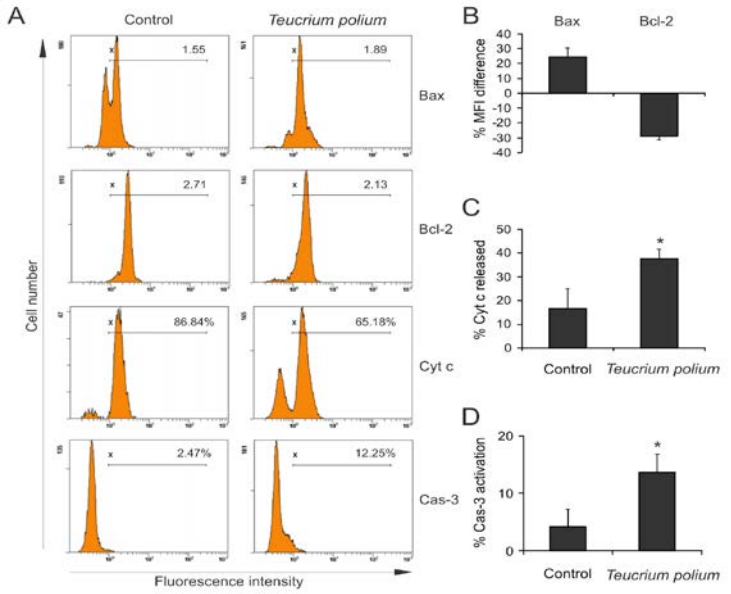

Fig. 6 – *Teucrium polium* (TP) leaves methanolic extract increases active Bax concentration and decreases cytosolic Bcl-2 concentration in treated CLL lymphocytes, consequently inducing apoptosis by cytochrome-c (Cyt c) mitochondrial release and activation of caspase-3 (Cas-3).

Lymphocytes of CLL patients were incubated in RPMI (Control) or 500 μg/mL of the TP methanolic extract for 24 h and stained with antibodies specific to Bax, Bcl-2, Cyt cand cleaved Cas-3. Cells were analyzed using single-colour flow cytometry. (A) Representative histograms that show Bax and Bcl-2 mean fluorescence intensities (MFIs) and percentage of cells displaying fluorescence for Cyt c and cleaved Cas-3 are presented. (B) Percentage of MFIs suppression or increase compared to untreated cells was calculated by formula (TP-C)*100/C where TP and C are MFIs of cells treated with TP methanolic extract or control cells, respectively. (C) Cyt c translocation was determined by selective permeabilisation of plasma membrane followed by flow cytometry. The percentage of cells with low fluorescence (100% of cells displaying fluorescence), where Cyt was translocated during apoptosis, is displayed. (D) The percentage of cells displaying fluorescence for cleaved Cas-3 (*$p < 0.05$ compared to the untreated cells).

Additionally, in order to quantify these apoptotic changes induced by the TP extract in CLL cells, we analysed the expression levels of Bax, Bcl-2 and cytochrome c by flowcytometry. Furthermore, we identified the amount of cells displaying cleaved caspase-3, to verify that the apoptotic pathway induced by the TP extract in CLL cells was caspase-dependent. The expression of active-Bax and cytosolic concentrations of cytochrome c were significantly increased, while the cytosolic expression of Bcl-2 was significantly decreased in treated CLL lymphocytes compared to the untreated cells. Furthermore, the percentage of cells containing cleaved caspase-3 was significantly increased (Figure 6). These finding showed that the TP extract disarranged the ratio of the expressions of proapoptotic Bax and antiapoptotic Bcl-2 protein in favor of Bax, consequently inducing apoptosis by cytochrome c mitochondrial release and activation of caspase-3.

Discussion

The results of our study showed for the first time that the methanolic extract of *Teucrium polium* reduced the viability of MOLT-4 and JVM-13 cells, and of CLL cells isolated from CLL patients after 24 hours and 48 hours of cells incubation. A very important result of our study was the absence of changes in cells viability of treated PBMCs compared to the untreated PBMCs, after 24 and 48 hours of cultivation of these cells with the TP methanolic extract. Although diverse mechanisms of action might contribute to the anti-cancer effects of TP, we showed that inhibition of Bcl-2 protein expression and activation of Bax were directly involved in TP-induced CLL cells apoptosis.

The results of our research demonstrating antitumor effects of the methanolic extract of *Teucrium polium* in human leukemic cells correlated with the results of previous studies showing cytotoxic effects of TP extracts on cell lines of prostate, colon, lung, and skin tumors [15–20]. In these tumor cell lines, as well as in leukemic cells used in our study, TP decreased the viability of examined cells. Stanković et al. [20] have demonstrated that the half maximal inhibitory concentration (IC_{50}) values of TP extracts after 72 hours of cells incubation were between 100 μg/mL and 200 μg/mL that were in correlation with the results of our research. Particularly, the concentration of the TP extract required to reduce the viability of HeLa cervical adenocarcinoma cells to 50% after 72 hours was 148.02 ± 4.99 μg/mL, for Fem-x human melanoma cells was 199.79 ± 0.30 μg/mL, and for K562 cells chronic myelogenous leukemia's cells, the concentration was 116.75 ± 24.40 μg/mL [21].

Species of plants of the genus *Teucrium*, are very rich in phenols and flavonoids, which are the carriers of the strong biological activity of various extracts of this plant. Extracts of TP have recently been subjected to the increasing number of *in vitro* studies in which their anticancer potential was tested. The results obtained using the HCT-116 cell line clearly indicated that the methanol extract of *Teucrium polium* reduced the viability of these cells by the induction of apoptosis. In a study of Stanković et al. [15], it was shown that after 24 hours of HCT-116 cells incubation with 250 μg/mL of the TP methanolic extract, apoptosis occurred in 85% of the total cell population. Our findings were also consistent with the results of a study of Stanković et al [15], since we showed that the TP extract induced apoptosis in CLL cells. Hence, in our research the percentage of apoptotic CLL cells after 48 hours of incubation in the presence of 250 μg/mL of the TP extract was about 46%. Therefore, reasults from our study suggest that active compunds from the TP methanolic extract reduce the viability of CLL cells by inducing apoptosis.

Permeabilization of outer mitochondrial membrane allows apoptotic molecules such as cytochrome c to be released into the cytoplasm that consequently induce the activation of caspases and subsequently execution of apoptosis. Permeabilization of outer mitochondrial membrane is mainly regulated by Bcl-2 family of proteins, such as proapoptotic protein Bax and antiapoptotic protein Bcl-2 [22]. The results of our study for the first time demonstrated that the TP methanolic extract induced selective mitochondrial apoptosis of CLL cells, by activating proapoptotic protein Bax and reducing the cytosolic expression of antiapoptotic protein Bcl-2, that led to the mitochondrial release of cytochrome c into the cytosol and the activation of caspase-3. The results of various studies that have been published to date were only confirming that the methanol extract of *Teucrium polium* could induce apoptosis in some cell lines, but to date there have not yet been accurately investigated the potential cellular mechanisms involved in this process [12]. The results of some previous studies emphasized the antioxidant capacity of the TP methanolic extract, but these studies have not yet been performed on tumor cell lines [15, 22]. It was shown that methanol extracts of other plants of the genus *Teucrium*, such as *Teucrium chamaedris*, *Teucrium montanum*, *Teucrium arduini* and *Teucrium scordium*, could have an antioxidant activity [15].

Conclusion

The methanolic extract of *Teucrium polium* affected key proteins involved in the regulation of programmed cell death of CLL cells. It selectively induced mitochondrial apoptosis in peripheral blood lymphocytes isolated from human CLL and it had no cytotoxic and apoptotic effects on PBMCs of healthy subjects. In order to precisely define the molecular component, one or more of them, carrying the biological activity of methanolic extract of TP leaves, it is necessary to perform additional research. The results of our experiments, therefore, represent a promising start for the future studies in the investigation of cytotoxic and apoptotic effects of specific components of TP in human leukemia cells.

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Conflict of interest

The authors declare no conflict of interest.

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Evaluation of the anticoagulant effect of vitamin K antagonists in patients with non-valvular atrial fibrilation

Ispitivanje antikoagulantnog efekta antagonista vitamina K kod bolesnika sa nevalvularnom atrijalnom fibrilacijom

Zoran Stanojković*†, Ana Antić*, Bela Balint‡§‖¶, Milena Todorović‖, Miodrag Vučić† ††, Nebojša Vacić††, Milan Lazarević‡‡**

***Blood Transfusion Institute of Niš, Niš, Serbia; University of Niš, †Faculty of Medicine, Niš, Serbia; Serbian Academy of Sciences and Arts, ‡Department of Medical Sciences, Belgrade, Serbia; Institute of Cardiovascular Diseases "Dedinje", §Department of Transfusion Medicine, Belgrade, Serbia; University of Belgrade, ¶Institute for Medical Research, ‖Faculty of Medicine, Belgrade, Serbia; Clinical Center of Serbia, **Clinic for Hematology, Belgrade, Serbia; Clinical Center of Niš, ††Clinic for Hematology, ‡‡Clinic for Cardiovascular and Transplant Surgery, Niš, Serbia**

Abstract

Background/Aim. Despite the introduction of new oral anticoagulants (dabigatran, rivoroxaban, apixaban), vitamin K antagonists (VKA), such as warfarin and acenocoumarol are still the most widely used oral anticoagulants for the treatment of nonvalvular atrial fibrillation (NVAF). The time in therapeutic range (TTR) represents a measure of the quality of the anticoagulant effect of these drugs, and it is considered that the lower value of TTR is associated with the adverse effects of therapy. The aim of this study was to evaluate of the effectiveness of VKA therapy in patients with NVAF and to identify factors affecting the anticoagulation efficacy. M**ethods.** A retrospective study was conducted on a population of 725 outpatients with NVAF, treated with VKA and followed in the Blood Transfusion Institute of Niš, Serbia, from January to December 2017. Laboratory control of the INR was done from capillary blood of patients on Thrombotrack Solo (Axis Shield, Norway) and Thrombostat (Behnk Elektronik, Germany). Targeted therapeutic INR was between 2.0 and 3.0. For each patient all available INR values were evaluated to calculate the individual TTR according to the Rosendaal method. **Results**. The study included a total of 725 patients with NVAF which had 6,105 INR measurements, what was 8.13 ± 2.47 INR measurements per patient. The mean value of TTR and was 60.15 ± 17.52%, but 49.72% of patients had TTR less than 60%. Patients were at high risk of thrombosis in 6.15% of time (INR < 1.5) and high risk of bleeding in 2.2% of time (INR > 4.5). The most significant independent factors affecting the quality of VKA therapy were gender, arterial hypertension, diabetes mellitus and the use of amiodarone and antiplatelet drugs (aspirin, clopidogrel). **Conclusion.** The TTR is undoubtedly useful indicator of the VKA treatment effectiveness. The most important predictors of poorer efficacy of VKA therapy are: arterial hypertension, diabetes mellitus, patients' gender and the use of amiodarone and antiplatelet drugs (aspirin, clopidogrel). To improve the quality of VKA therapy, education of patients and better collaboration with them, as well as a successful teamwork of clinicians are also imperative.

Key words:
anticoagulants; atrial fibrillation; blood coagulation tests; dose-response relationship, drug; vitamin k.

Apstrakt

Uvod/Cilj. I pored uvođenja novih oralnih antikoagulantnih lekova (dabigatran, rivoroksaban, apiksaban), antagonisti vitamina K (AVK), kao što su varfarin i acenokumarol, još uvek su najčešće primenjivani oralni antikoagulantni lekovi u terapiji nevalvularne atrijalne fibrilacije (NVAF). Vreme u terapijskom opsegu (*Time in Therapeutic Range* – TTR) predstavlja meru kvaliteta antikoagulantnog efekta tih lekova, te se smatra da su niže vrednosti TTR udružene sa neželjenim efektima terapije. Cilj rada bio je da se utvrde efikasnost terapije AVK kod bolesnika sa NVAF i faktori koji utiču na kvalitet antikoagulantnog efekta tih lekova. **Metode**. Retrospektivnom analizom obuhvaćeno je 725 bolesnika sa NVAF koji su ambulantno praćeni u Zavodu za transfuziju krvi u Nišu, u periodu januar-decembar 2017. godine. Laboratorijsko određevanje međunarodnog normalizovanog

Correspondence to: Ana Antić, Blood Transfusion Institute of Niš, Blvd Dr Zoran Đinđić 48, 18 107 Niš, Serbia.
E-mail: anaantic@sbb.rs.

odnosa (*International Normalized Ratio* – INR) vršeno je iz kapilarne krvi bolesnika na aparatima Trombotrack Solo (*Axis Shield, Norveška*) i Thrombostat (*Behnk Elektronik, Nemačka*). Ciljni terapijski INR bio je između 2,0 i 3,0. Na osnovu svih dostupnih vrednosti INR za svakog bolesnika pojedinačno, određen je individulani TTR metodom po Rosendaal-u. **Rezultati.** Ispitivanjem su bila obuhvaćena ukupno 725 bolesnika sa NVAF kojima je u toku 2017. godine urađeno 6,105 kontrola INR (8,13 ± 2,47 INR kontrola po bolesniku). Srednja vrednost TTR bila je 60,15 ± 17,52%, ali je 49,72% bolesnika imalo TTR < 60%. Bolesnici su imali visok rizik od tromboze u 6,15% vremena (INR < 1,5), a u 2,2% vremena visok rizik od krvarenja (INR > 4,5). Najznačajniji nezavisni faktori koji su uticali na kvalitet AVK terapije bili su: pol i arterijska hipertenzija, dijabetes melitus, upotreba amiodarona i antitrombocitnih lekova (aspirin, klopidogrel). **Zaključak.** Parametar TTR je nedvosmisleno koristan pokazatelj efikasnosti antikoagulantnog efekta AVK. Najznačajniji prediktori lošije efikasnosti AVK su: pol, arterijska hipertenzija, dijabetes melitus, upotreba amiodarona i antitrombocitnih lekova (aspirin, klopidogrel). U cilju unapređenja kvaliteta primene i monitoringa antikoagulantnog efekta AVK neophodna je pravilna edukacija i bolja saradnja sa bolesnicima, ali i bolji timski rad kliničara.

Ključne reči:
antikoagulansi; fibrilacija pretkomora; krv, testovi koagulacije; lekovi, odnos doza-reakcija; vitamin k.

Introduction

Despite the implementation of new oral anticoagulants for the treatment of patients with atrial fibrillation or venous thromboembolism, vitamin K antagonists (VKA) such as warfarin, acenocoumarol and phenprocoumon are still the most widely used oral anticoagulants. The most common indications for their use are atrial fibrillation, mitral or aortic stenosis, mitral or aortic prosthetic valve, venous thromboembolism and intracavitary thrombosis [1, 2]. This therapy is long lasting, for months and years, and in some cases till the end of life. The mechanism of action of these drugs is based on their competition with the vitamin K and reduction the level of vitamin K dependent coagulation factors (FII, FVII, FIX, FX), an anticoagulant protein C and its cofactor protein S [3].

The use of VKA must be regularly and often laboratory controlled in order to ensure the adequacy of therapy and to avoid subdosing or drug overdose. The most commonly used test for the control of oral anticoagulant therapy is the prothrombin time (PT), expressed in international normalised ratio (INR) system, which provides an internationally standardized monitoring of the treatment. Therapeutic range for INR is from 2.0 to 3.5, depending on the indication for which the drug is used [4]. Therapeutic ranges are generally set up on the basis of clinical trials and are determined in order to achieve the required minimum coagulating effect for the prevention of recurrent thrombosis or lasting of existing thrombotic episodes. The treatment carries, on the one hand, the risk of bleeding, and on the other hand, the risk of thrombosis because warfarin and other VKA have a narrow therapeutic index and should be dosed within strictly defined ranges [3, 5].

The time in therapeutic range (TTR) is commonly used to evaluate the quality of VKA therapy and is an important tool for assessing the risks of this therapy. TTR estimates a percentage of time a patient's INR is within the desired therapeutic range and is widely used as an indicator of anticoagulation control [6–8]. Numerous studies have shown that higher TTR correlates with good clinical outcomes, and that there is a strong correlation between TTR and adverse events (bleeding, thrombosis). But although TTR is routinely assessed, there is no consensus on acceptable target for TTR in practice. Active-W study suggested a minimum TTR of 58% in order to show a benefit of oral anticoagulant therapy over antiplatelet therapy in terms of preventing vascular events [9], RE-LY study on Portuguese patients showed mean TTR of 61% [10, 11], Thrombosis Canada states that good INR control is when TTR is more than 60% [12], but there are studies that report elevated level of TTR of 74% as a measure of effective anticoagulation [8, 13]. It is known that many factors correlate with TTR, and the most important are age, sex, smoking, concomitant drugs, alcohol, comorbid medical and psychiatric conditions [14].

The aim of this study was to evaluate the effectiveness of VKA therapy and to identify factors affecting anticoagulation efficacy in patients with NVAF.

Methods

A retrospective study was conducted on a population of 725 outpatients with atrial fibrillation, treated with VKA [warfarin (Farin®), acenocoumarol (Sintrom®, Sinkum®, Acenokumarol®)] and followed in the Department for Hemostatic Disorders Testing in the Blood Transfusion Institute of Niš, Serbia from January to December 2017. The study included patients of both sexes who had strictly determined diagnosis of nonvalvular atrial fibrillation (NVAF) and indication for the use of VKA (the target INR 2.0–3.0), patients who were expected to take VKA throughout the whole period of the study and that control testing of INR would be done only at the mentioned Institute. We excluded patients who had discontinued treatment for any reason at any time of investigation, patients who had interruption in taking VKA for any reason, patients who made any of the control of INR in another facility, patients who had changed target INR during the investigation, as well as patients with INR > 6.0. We recorded demographic and clinical characteristics of the patients, as well as the use of other drugs (beta-blockers, antiplatelet drugs, statins, amiodarone, ACE inhibitors).

Laboratory control of the INR was done from capillary blood of the patients on Thrombotrack Solo (Axis Shield, Norway) and Thrombostat (Behnk Elektronik, Germany). For each patient we evaluated all available INR values to calculate the individual TTR according to the Rosendaal method [15]. This method uses linear interpolation to assign an INR value to each day between successive observed INR values [INR-DAY software program (Dr FR Rosendaal, Leiden, Netherlands)]. The primary outcome was to determine TTR, and the secondary outcomes were to determine time under (INR < 2.0) and over therapeutic range (INR > 3.0), time with increased thrombotic risk (INR < 1.5) and time with increased hemorrhagic risk (INR > 4.5), as well as to determine independent factors for increased risk of worse anticoagulation therapy.

Statistical analysis was performed using Statistical Package for Social Science (SPSS Software GmbH, Germany), version 15.0. The results are presented in tables and graphs, using the mean values and standard deviations. Qualitative characteristics of the investigated variables are given as frequency (n) and the percentage (%). The continuous data were analyzed using χ^2 test. Multivariate logistic regression analysis was performed to identify independent risk factors for TTR < 60%. The results were considered to be statistically significant at a $p < 0.05$. Since it was "*post-hoc*" analysis from the prospective observational registry, we could not exclude the presence of unmeasured selection bias, and statistical analyses were not specified before the data were seen, which could be some kind of study limitation.

Results

Out of the total of 725 patients in this study, there were 430 (59.40%) men and 295 (40.60%) women. The average age of patients was 71.05 ± 10.42 years, range from 22 to 88 years. There was no statistically significant difference in the age structure of patients by gender ($t = 1.125$; $p = 0.043$). Table 1 shows the main characteristics of the patients.

Table 1

Characteristics of patients with nonvlavular atrial fibrillation (n = 725)

Characteristics	Values
Age (years)	71.05 ± 10.42
Gender (male/female)	430 (59.40) / 295 (40.60)
Previous stroke/TIA	111 (15.35)
Hypertension	524 (72.30)
Previous AMI	232 (32.00)
Vascular disease history	138 (19.10)
Diabetes mellitus	162 (22.40)
Concomitant drugs	
β-blockrers	624 (86)
statins	565 (78)
aspirin	275 (38)
clopidogrel	152 (21)
amiodaron	138 (19)
ACE-inhibitors	522 (72)

***Note*: Values are given as number (percentage) of the patients or mean ± standard deviation.**
AMI – acute myocardial infarction; TIA – transient ischemic attack; ACE – angiotensin-converting enzyme.

During the one year follow-up of patients on VKA therapy, a total of 6,105 INR measurements were done, which was 8.13 ± 2.47 INR measurements per patient. Average number of days between INR measurements was 34.89 ± 17.26. Characteristics of anticoagulant therapy during the investigated period are shown in Table 2.

Table 2

Characteristics of anticoagulant therapy in patients with nonvalular atrial fibrillation (n = 725)

Characteristics	Values
Total number of INR mesurements	6,105
Number of INR mesurements per patient	8.13 ± 2.47
Number of days between INR measurements	34.89 ± 17.26
Drug	
warfarin	436 (60.10)
acenokoumarol	259 (39.90)
Daily dose of drug (mg)	
warfarin	4.7 ± 1.26
acenokoumarol	3.58 ± 1.47

***Note*: Values are given as number (percentage) of the patients or mean ± standard deviation.**
INR – international normalized ratio.

The mean TTR was 60.15 ± 17.52%. More than a fifth of time, the patients had INR under therapeutic range (INR < 2.0 in 21.05% of time), while in 18.10% of time, patients had INR > 3.0. A high risk of thrombosis (INR < 1.5), patients had in 6.15% of time, and in 2.20% of time, they were at high risk of bleeding (INR > 4.5).

During the period of examination there were no major bleedings, while 65 (8.96%) of the patients had minor bleedings, mainly in the form of bruises, haematoma and epistaxis, whereas 4 (0.55%) of the patients had haematuria and 3 (0.41%) of the patients had bleeding from the gastrointestinal tract. After adjusting the dose of VKA, bleedings were stopped.

Distribution of TTR values is shown in Figure 1. It can be seen that 49.72% of the patients had TTR less than 60% which means that almost half of the patients was at increased risk for serious complications of treatment.

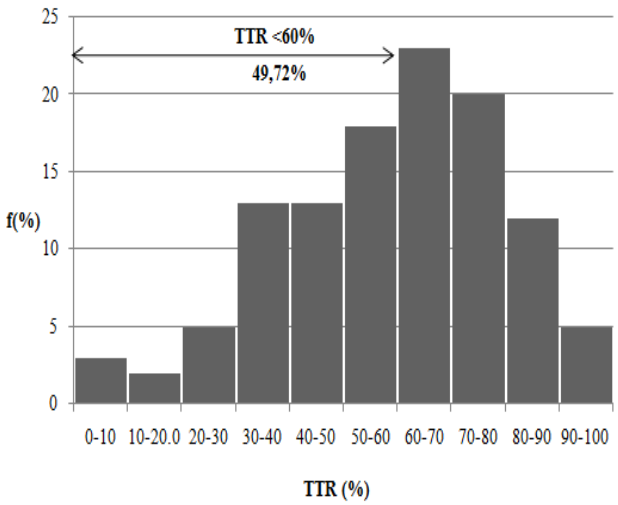

Fig. 1 – Histogram with relative frequencies of time in therapeutic range (TTR).

Table 3 shows logistic regression model of independent factors for the assessment of increased risk of poor effect of anticoagulation therapy. The whole model was highly significant [χ^2 (df = 9, n = 725) = 20.637; $p < 0.001$] and explained 57.81% of the variance of efficiency of VKA. Factors that gave statistically significant contribution to the model were: gender, arterial hypertension, diabetes mellitus and the use of amiodarone, aspirin and clopidogrel.

Table 3

Logistic regression model of independent factors for assessing the efficiency of vitamin K antagonists

Factors	OR	95% CI	*p*
Age	1.223	0.065–8.480	0.092
Gender	3.870	1.065–12.060	0.040
Previous stroke/TIA	1.590	0.951–2.682	0.076
Hypertension	2.082	1.049–4.133	0.036
Previous AMI	0.502	0.050–2.880	0.061
Diabetes mellitus	3.100	2.330–4.150	0.240
Amiodarone	11.360	4.870–26.520	< 0.001
Aspirin	4.820	1.150–20.190	0.031
Clopidogrel	5.200	1.520–12.760	0.008

OR – odds ratio; CI – confidence interval; TIA – transient ischemic attack; AMI – acute myocardial infarction.

Discussion

Anticoagulant drugs are used in the treatment or prevention of thromboses and thromboembolic complications. Traditional VKA, which have been in use for over 50 years are the gold standard in therapy for all that time. They provide the necessary protection from thromboembolic events and have proven to be sufficiently effective over many years of use. One of the most common indications for VKA therapy is atrial fibrillation and guidelines recommend that patients who are at low risk may be treated only with aspirin, while in patients at high risk, it is recommended to use VKA [2, 16, 17]. Anticoagulant therapy reduces stroke rate by 64% and mortality rate by 26% in this group of patients [18]. But, VKA therapy has disadvantages and the most important are: unpredictable response, narrow therapeutic window, routine monitoring, slow start/stop action, often dose adjustment, numerous interactions with food and drugs, resistance to warfarin, procoagulant effect of warfarin at the beginning of the therapy. However, the most severe complication of VKA therapy is intracranial hemorrhage, whose rate is about 1% in clinical studies [19].

The efficiency and safety of VKA depend strongly on the TTR value, which is a measure of the period in which a patient is in an optimal INR range. However, although TTR is generally accepted as a measure for monitoring of the anticoagulant effect of drugs and the successful conduction of this therapy, there are no strengthened data what is accepted value of TTR. Recent trials related to the introduction of new oral anticoagulants have provided data of actual TTR values in different countries of the world. In the ROCKET-AF study, the mean TTR was 55.2%, but the values in Western Europe and North America were significantly higher, 63% and, the mean TTR was 66% [21], in the RE-LY study 67.2%, with the highest values of 77% in Sweden and 74% in Finland and Australia [10, 11]. On the other hand, Gateman et al. [8] calculated the mean TTR in the St. Paul Family Health Network in Ontario of 58.05% [8], while the mean TTR in the study of Ciurus et al. [1] is 76% that is considered to represent excellent anticoagulation control [1]. According to our study, the mean value of TTR is 60.15% during a follow-up of one year, and it is lower than that reported from big clinical trials, but still correlates with the number of the existing data in the literature. Also, the value is greater than the minimum TTR of 58% at which there is a benefit of anticoagulant therapy over antiplatelet therapy in terms of preventing vascular events [9]. Especially important result of our study was the fact that 49% of patients had TTR less than 60%, indicating that almost half of the patients were at increased risk of serious adverse events, both of bleeding and thrombosis.

This fact imposes a deeper analysis of management of the anticoagulant therapy in our institution, which involves the study of the relationship between patient and transfusion physician, identifying and understanding the factors which may have the influence on the quality of the therapy, the behavior of the patients in accordance with established criteria, as well as the modification of VKA therapy in accordance with comorbidities and other drugs that must be introduced into therapy afterward. The INR values that are out of therapeutic range require high-speed control (in a short period of time), which enhances the number of patients on a daily and monthly basis, increasing the cost of treatment, and, additionally, they are the risk factor for complications of VKA treatment which may be potentially very serious for patients.

Great variations in the values of TTR show that the anticoagulant effect of VKA is affected with a great number of factors. Our investigation showed that gender, arterial hypertension, diabetes mellitus and the use of amiodarone, aspirin and clopidogrel were associated with lower probability of staying within the target INR. The strongest independent factor for bad anticoagulation control was use of amiodarone, which is the most widely used antiarrhythmic in atrial fibrillation. It is known that amiodarone has a negative impact on the anticoagulant effect of VKA, because it inhibits the hepatic metabolism of warfarin, potentiating its anticoagulant effect and resulting in high INR values and increased risk of bleeding [22, 23]. The same effect has the concomitant use of antiplatelet therapy (aspirin and/or clopidogrel), which also potentiates the anticoagulant effect of VKA and increases the risk for bleeding. A large number of studies have shown that although this combination of drugs can potentially prevent both thromboembolism and atherothrombotic events, it is also associated with an increased risk of severe bleeding and requires careful consideration of all the risks and benefits [24, 25]. A large, nationwide investigation in Denmark showed that a risk for severe bleeding in patients taking VKA and aspirin was 1.8-fold increased, 3.5-fold increased in patients taking VKA and clopidogrel, and 4-fold increased in patients taking triple therapy [26]. Looking at the same problem from the other hand,

our recent investigation of different preparations of aspirin (acetylsalicylic acid) in patients with stable coronary disease has also shown that there is an increased effect of aspirin in patients receiving anticoagulant therapy, so there is an increased risk for bleeding [27].

Gender also stands out as a significant predictor of bad anticoagulation implying that women respond poorer to VKA treatment, so there is far more difficult to achieve good control than in men. The reason for this effect is unclear, but previous studies have confirmed this fact and have shown that women are at greater risk of atrial fibrillation-related stroke during VKA treatment, as a result of poor anticoagulant effect of warfarin [14, 28, 29].

The impact of arterial hypertension on anticoagulant therapy has not been precisely defined, although it has been studied in numerous investigations. Therefore, Apostolakis et al. [14] have shown that hypertension is associated with lower TTR, while on the other side, the Veterans AffaiRs Study to Improve Anticoagulation (VARIA) [30] did not confirm this relationship. Our investigation showed that arterial hypertension is a predictor of poor anticoagulation, and possible explanation of this influence may be associated with interaction of drugs [31]. Finally, diabetes mellitus, as a predictor of the poorer effect of VKA is associated with increased levels of the procoagulant clotting factors (FII, FVII) and a decrease of anticoagulants, such as thrombomodulin, with abnormal fibrinolytic pathway and decreases fibrinolysis [32, 33]. In these patients, most often there is a disorder of renal function, which leads to the abnormal elimination of these drugs and the poorer anticoagulant effect.

Since of the various effects of VKA and the impact of a number of factors to this therapy it is developed a new era of anticoagulation which is a crucial for all patients who do not have sufficient anticoagulant protection or where the TTR is less than 60%. These are direct oral anticoagulants or new oral anticoagulants (also called a target-specific anticoagulants): on one side, dabigatran, which is a direct inhibitor of thrombin, and on the other side inhibitors of FXa: rivaroxaban, apixaban, edoxaban. A number of meta-analyses have shown that these drugs have a better safety profile than VKA, lower incidence of bleeding, especially intracranial or gastrointestinal, have fewer interactions with food than VKA, achieve faster antithrombotic effect, and during their use, there is no need for regular monitoring because of predictable pharmacokinetics [34–36]. Compared with warfarin, dabigatran is associated with a reduced risk of ischaemic stroke, intracranial haemorrhage and mortality, but with an increased risk of major gastrointestinal bleeding. It is the only anticoagulant with a specific antidote idarucizumab. Inhibitors of FXa are recommended for patients with mild renal impairment (only 1/3 of the drug is renal eliminated), and those with high risk of bleeding, and/or potential drug-drug interactions.

Conclusion

The TTR is undoubtedly proved and useful indicator of the effectiveness of VKA anticoagulant treatment. The most important predictors of poorer VKA therapy efficacy are: arterial hypertension, diabetes mellitus, patients' gender and the use of amiodarone and antiplatelet drugs (aspirin, clopidogrel). To improve the quality of VKA therapy, an education of patient and better collaboration with them, as well as a successful team-work of clinicians are also imperative.

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Surgical fear questionnaire (SFQ) – Serbian cultural adaptation

Upitnik za merenje straha od operacije – kulturološka adaptacija na srpski jezik

Slobodan M. Janković, Gordana V. Antonijević, Snježana N. Mirković, Katarina M. Raspopović, Ljiljana R. Radoičić, Srdjan S. Putnik, Marija N. Živković-Radojević, Ivana R. Vasić, Boško V. Nikolić, Dragan R. Stanojević, Sladjana D. Teofilov, Katarina V. Tomašević, Valentina D. Opančina

University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia

Abstract

Background/Aim. After having established an indication for surgery, some patients experience sense of fear, unpleasantness and embarrassment due to the expectance of adverse consequences of surgical intervention. Recently an instrument for measuring fear of surgery – the Surgical Fear Questionnaire (SFQ) – was developed and validated on a sample of Dutch patients awaiting surgery. The objective of this study was to translate the SFQ to Serbian language, make cultural adaptation of the translation and test its reliability and validity in a sample of outpatients in Serbia. **Methods.** The SFQ was translated and adapted according to the accepted international standards (double forward translation, harmonization, backward translation, and piloting). The study was multicentric, involving patients from 7 cities in 3 countries: Serbia, Montenegro, Bosnia and Hercegovina. It was conducted at state-owned health facilities. The sample was of consecutive nature and consisted of 330 outpatients who visited specialists of either internal medicine or general surgery. **Results.** Translated SFQ showed excellent reliability, both when rated by the investigators (Cronbach's alpha 0.915), and by the patients themselves (Cronbach's alpha 0.917). It is temporally stable, and both divergent and convergent validity tests had good results. Factorial analysis revealed one domain on the whole study sample and two domains like in original on the subsample of patients without experience with surgery in general anesthesia. **Conclusion.** Identification of patients with high level of fear of surgery by this questionnaire should help clinicians to administer measures which may decrease fear and prevent avoidance of absolutely necessary surgery by such patients.

Key words:
fear; surgical procedures, operative; surveys and questionnaires; serbia; language.

Apstrakt

Uvod/Cilj. Nakon uspostavljene indikacije za operaciju, neki bolesnici imaju osećaj straha, neprijatnosti i stida zbog očekivanja nepovoljnih posledica hirurške intervencije. Upitnik za merenje straha od operacije (engl. *the Surgical Fear Questionnaire* – SFK) je instrument koji se razvijen i validiran za primenu kod bolesnika iz Holandije podvrgnutih operaciji. Cilj ovog rada je bio da se nakon prevoda SFK na srpski jezik, izvrši kulturološka adaptacija prevoda i testira njegova pouzdanost i validnost na uzorku bolesnika iz Srbije, lečenih ambulantno. **Metode.** SFK je preveden i prilagođen srpskom jeziku u skladu sa prihvaćenim međunarodnim standardima (dvostruko prevođenje unapred, usklađivanje, prevođenje unazad i pilot studija). Studija je bila multicentrična i sprovedena je na 330 bolesnika iz 7 gradova Srbije, Crne Gore i Bosne i Hercegovine, lečenih ambulantno u državnim zdravstvenim ustanovama od strane specijalista interne medicine ili opšte hirurgije. **Rezultati.** Preveden SFK je pokazao odličnu pouzdanost, i prilikom ocene istraživača (Cronbach-ov alpha 0,915), kao i samih bolesnika (Cronbach-ov alpha 0.917). Vremenski je bio stabilan, a i divergentni i konvergentni testovi validnosti su imali dobre rezultate. Faktorska analiza je otkrila jedan domen na celom uzorku i dva domena kao u originalom upitniku na poduzorku bolesnika bez iskustva sa opštom anestezijom u hirurgiji. **Zaključak.** Identifikacija bolesnika sa visokim stepenom straha od operacije pomoću ovog upitnika trebalo bi da pomogne lekarima da primene mere koje mogu da smanje strah i spreče izbegavanje apsolutno neophodne operacije od strane takvih bolesnika.

Ključne reči:
strah; hirurgija, operativna, procedure; ankete i upitnici; srbija, jezici.

Correspondence to: Slobodan Janković, University of Kragujevac, Faculty of Medical Sciences, Svetozara Markovića Street, 69, 34 000 Kragujevac, Serbia. E-mail: sjankovic@medf.kg.ac.rs.

Introduction

Fear of medical treatment relates to the fear of diagnostic and/or therapeutic procedures involving medical staff in healthcare settings [1]. Preoperative or surgical fear is frequently encountered in patients who are waiting for surgical intervention and is associated with prolonged psychophysical recovery [2, 3]. Within the fear of surgery, researchers have found the following components: fear of needles, blood and injuries, fear of pain or fear of infections which may happen during the invasive diagnostic and surgical procedures [4–7]. The observational study [8] devoted to the fear of anesthesia revealed the following main sources of anxiety in patients waiting for surgery: concerns about postoperative pain (84%), prolonged unconsciousness after the surgery (64.8%) and injury by catheters or needles (59.5%). It was also shown in the same study that women are more anxious preoperatively than men (85.3% vs. 75.6%, respectively; $p = 0.014$).

Although several rating instruments were developed for measuring fear from blood, injury or dental treatments [9–11], only recently, reliable and valid instrument was developed for measuring fear of surgery. The Surgical Fear Questionnaire (SFQ) was developed in Dutch and its aim was to assess the level of fear of surgical intervention in patients who are waiting for elective operation [12]. It has 8 questions and two-factor structure: one factor is related to fear of the short-term consequences of surgery (items 1–4) and the other to fear of the long-term consequences of surgery (items 5–8). Up to now, this instrument was translated only to Portuguese and validated in that cultural setting, showing similar psychometric properties as the original.

The aim of this study was to translate the SFQ to Serbian language, make cultural adaptation of the translation and test its reliability and validity in a sample of outpatients in Serbia.

Methods

Translation and cultural adaptation

Translation and cultural adaptation of the SFQ was made according to International Society for Pharmacoeconomics and Outcomes Research (ISPOR) guidelines [13]. Permission for translation of the SFQ (version with 8 items) from English to Serbian was granted by the first author of the original scale Mr. Maurice Theunissen, MSc, epidemiologist from the Maastricht University Medical Center, Netherlands. The original scale was first translated to Serbian by two independent translators who were Serbian native language speakers. They translated the scale independently of each other, and then the translations were harmonized to one Serbian version at the meeting of the study investigators and the translators. The harmonized Serbian version was then translated back to English by native English speaker, citizen of Australia. When translated back to English, the translator was not aware of the original English version of the SFQ. The back-translation to English was then compared with original English version by the study investigators at the new meeting of the investigators, where final Serbian version of the SFQ was agreed on. The final translation of the SFQ to Serbian was then tested on 5 PhD students (at the Faculty of Medical Sciences, University of Kragujevac, Serbia) for clarity and comprehension. After the pilot, a few minor changes were made, and then the final Serbian version of the SFQ was copied and prepared for reliability and validity testing.

The study was approved by the Ethics Committee of Clinical Center Kragujevac, Serbia. The patients were treated, with due respect and care, according to the principles stated in Declaration of Helsinki.

Population and the sample

The final Serbian version of the translated (SFQ) questionnaire was tested for reliability on outpatients who visited specialists of either internal medicine or general surgery at state-owned health facilities in seven cities, in three countries [Serbia (SER), Montenegro (ME) and Bosnia and Herzegovina (B&H)]: Belgrade (SER), Podgorica (ME), Kragujevac (SER), Bijeljina (B&H), Vršac (SER), Kraljevo (SER) and Soko Banja (SER). The visits took place in April and the first two weeks of May, 2016. The inclusion criteria were literacy, and age over 18. The exclusion criteria were pregnancy, lactation, cognitive disorders, mood disorders, mental retardation and incomplete patient's files. The sample of the patients was of consecutive nature, i.e. all patients who visited their general practitioner on the survey day (and satisfied inclusion and exclusion criteria) were offered the questionnaire. During the first encounter the questionnaire was completed in two ways: at first, by the investigators who were questioning the patients, and second, by the patients themselves.

Reliability testing

Reliability of the questionnaire was tested using three methods. First, internal consistency was determined through calculation of Cronbach's alpha for the questionnaire as a whole. Second, the questionnaire was divided by split-half method to two parts with the same number of questions, and Cronbach's alpha for each of the parts was calculated. Using the alphas for both parts, number of questions in each part and average correlation between questions in both parts of the original questionnaire, the Spearman-Brown coefficient for the questionnaire as a whole was calculated by the Spearman-Brown "prediction" formula [14]. Third, for each question mean score and their variances were calculated, in order to check their suitability for measurement of whole extent of fear.

Factorial analysis

Exploratory factorial analysis of the questionnaire was made in order to discover principal factors [15]. First, suitability of the questionnaire and sample for factorial

analysis was tested by the Kaiser-Meyer-Olkin measure of sampling adequacy and by the Bartlett's test of sphericity. Then, the factors were extracted at first without rotation, with conditions that Eigenvalues had to be greater than 1.0, and using Scree-plot (the extracted factors were above the "elbow" of the graph). Second, referent axes were rotated orthogonally, by the varimax method, and another extraction of the factors was made, using the same criteria as for the unrotated solution. Extracted factors were than named accordingly.

Validity

Construct validity of the questionnaire was evaluated by an independent panel of three experienced clinicians at the Clinical Center Kragujevac, Serbia: a psychiatrist and two general surgeons.

The criterion validity was tested by two methods: (1) convergent validity testing by comparison of the SFQ score with the Visual Analogue Scale (VAS) value measuring fear of hospitalization, and (2) divergent validity testing by comparison of the SFQ score with the score of the Short Subjective Well-being Scale (SSWS). The permission to use the SSWS in Serbian language (which measures feeling of well-being, and was previously validated in Serbian population [16, 17]) was granted by a psychologist. The correlations between scores on the questionnaires and/or VAS values were calculated. All calculations were performed by the SPSS statistical software, version 18.0.

The results are presented in the multi-method, multi-trait matrix.

Temporal stability

Temporal stability of the SFQ results was tested by second completion of the questionnaires by the investigators who repeatedly interviewed the patients one month after the first encounter. The patients were then invited to the second encounter by phone.

Results

The study sample consisted of 330 outpatients: mean age 45.9 ± 16.1 years, male/female ratio 141/189 (42.7/57.3%), years of formal education 14.0 ± 3.6, place of residence, urban/rural = 246/84 (74.5/25.5%), living alone/in a family = 37/293 (11.2/88.8%), previous experience with surgery in general anesthesia (194 [58.8%] yes /136 [41.2%] no). The distributions of diagnoses within the study sample was as following: hypertension (17%), chronic heart failure (0.6%), coronary disease (2.4%), chronic obstructive pulmonary disease (COPD) (3.9%), asthma (6.1%), diabetes mellitus (0.9%), cancer (3.6%), surgical disease (24.2%), other (18.2%) and no diagnosis of a chronic disease (23%).

Reliability testing

Results of testing original 10 items from the questionnaire, and examining results of correlation matrix, mean values, variance, skewness and kurtosis of distributions of responses for each of the items, are shown in Table 1. Cronbach's alpha of the version with 10 items was 0.915, when the scale was rated by the investigators. After division of the questionnaire by the split-half method, the Spearman-Brown coefficient for the questionnaire as a whole was calculated by the Spearman-Brown "prediction" formula, and its value was 0.822. When the scale was rated by the patients themselves (a week after the rating by the investigators), Cronbach's alpha was 0.917.

Factorial analysis

Factorial analysis was made by the principal components method. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.884 and the Bartlett's test of sphericity was significant (p = 0.000). Only one factor was extracted, explaining in total 62.85% of the variance. This factor bore 5.028 eigenvalues, and included all 8 items.

Validity

Construct validity of the questionnaire was confirmed by the panel of experts, who also helped with slight rephrasing of the questions.

Divergent criterion validity was tested through non-parametric correlation between scores of the SFQ (when it

Table 1

Mean values, standard deviation, skewness and kurtosis of responses to items of the Surgical Fear Questionnaire (SFQ)

Item	Mean response*	Standard deviation	Skewness	Kurtosis
I am afraid of the operation	5.14	3.26	-.051	-1.168
I am afraid of the anaesthesia	4.48	3.49	.174	-1.347
I am afraid of the pain after the operation	4.94	3.10	.056	-1.045
I am afraid of the unpleasant side effects (like nausea) after the operation	4.35	3.13	.286	-1.031
I am afraid my health will deteriorate because of the operation	3.41	3.07	.691	-.576
I am afraid the operation will fail	3.79	3.12	.462	-.895
I am afraid that I won't recover completely from the operation	3.81	3.05	.455	-.777
I am afraid of the long duration of the rehabilitation after the operation	4.16	3.15	.312	-.972

* **the responses are rated from 0 (not at all afraid) to 10 (very afraid).**

Table 2

Multi-method, multi-trait correlation matrix (non-parametric Spearman's coefficients)

	SSWS score, rated by an investigator	SSWS score, rated by a patient	SFQ score, rated by an investigator	SFQ score, rated by a patient	VAS score
SSWS score, rated by an investigator	1	0.919**	-0.113	-0.109	0.004
SSWS score, rated by a patient	0.919**	1	-0.108	-0.107	0.032
SFQ score, rated by an investigator	-0.113	-0.108	1	0.950**	0.645**
SFQ score, rated by a patient	-0.109	-0.107	0.950**	1	0.652**
VAS score	0.004	0.032	0.645**	0.652**	1

SFQ – Surgical Fear Questionnaire; SSWS – Short Subjective Well-being Scale; VAS – Visual Analogue Scale.
****significant correlation at $p < 0.001$.**

was rated by investigator and by patients themselves) and scores of the SSWS scale (when it was rated by investigator and by patients themselves). Convergent criterion validity was tested through non-parametric correlation between scores of the SFQ (when it was rated by investigator and by patients themselves) and VAS scores. Non-parametric correlation was chosen due to non-normal distribution of some of the scores. Spearman's correlation coefficients are shown in the Multi-trait, multi-method matrix (Table 2).

Temporal stability

The SFQ showed satisfactory temporal stability: when rating (by the investigator) was repeated on the same patients one month later, the correlation between the scores (Spearman's coefficient) was 0.930 ($p < 0.001$). Cronbach's alpha after the repeated rating was 0.892.

Discussion

Version of the SFQ scale with 8 questions showed excellent reliability, both when rated by the investigators, and by the patients themselves. It was temporally stable, and both divergent and convergent validity tests had good results. Factorial analysis revealed only one domain, unlike the analysis of original scale, where two domains were established: the fear of the short-term consequences of surgery and the fear of the long-term consequences of surgery.

Although short- and long-term consequences of surgery are well defined clinical entities [18], there is no research about characteristics of fear of these entities. In a Portuguese study, on 203 women undergoing hysterectomy, it was shown that preoperative anxiety was strong predictor of chronic or persistent postsurgical pain (PPSP), which is one of the long-term adverse consequences of surgery [19]. Division of the SFQ scale into two parts (the first 4 questions relate to fear of short-term and next four questions to fear of long-term consequences of surgery) seems intuitively logical, and worked well in the studies of Theunissen et al. [12] on patients awaiting surgery. In our study sample, which was composed of outpatients currently not scheduled for any surgical intervention in close future, the SFQ behaved as a whole, i.e. the patients had the same attitude towards the possible short- and long-term consequences of hypothetical surgery. Only one factor emerged when analysis was made on the questionnaires rated by investigators, by the patients themselves and when the rating was repeated by the investigators a month later (results not shown, available on request).

However, when we tried factor analysis on subsamples of patients who had and who had not previous experience with surgery in general anesthesia, those who had the experience behaved as the whole sample, i.e. only one factor was extracted. On the other hand, the factor analysis of the SFQ on the subsample of patients without previous experience with surgery in general anesthesia revealed two factors after rotation: the first composed of the questions 1–4 (explaining 34.3% of variance), and the second composed of questions 5–8 (explaining 38.5% of variance). Inexperienced patients also scored higher on the first 4 questions than the patients with previous experience and then on the questions 5–8 (results not shown, available on request), showing that they were more afraid of short-term consequences of surgery. Experience with surgery and general anesthesia obviously has alleviating effect on fear of next surgery, at least when short-term adverse consequences are in question. It would be very interesting to see whether the two-factor structure of original instrument would remain as such if factor analysis was made on a subsample of patients having previous experience with surgery in general anesthesia.

Main limitation of this study was the fact that the patients from the study sample were not scheduled for a surgery in close future, since this was the main characteristic of the study sample on which original instrument was developed and validated. This is probably the reason why the translated instrument did not show the same factorial structure as the original. Future studies with the same translated questionnaire should be conducted on a group of patients who are scheduled for surgery in near future, in order to get complete insight into its functionality.

Conclusion

The translated SFQ to Serbian language is reliable and valid instrument for the surgical fear measurement. Identification of patients with high level of fear of surgery by

this questionnaire should enable administration of measures which may decrease that fear and prevent avoidance of absolutely necessary surgery by such patients.

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Conflict of Interest

The authors declared that they had no competing interests.

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Deep inspiration breath-hold radiotherapy for left-sided breast cancer after conserving surgery: A dose reduction for organs at risk

Radioterapija karcinoma leve dojke pri zadržanom dahu u dubokom udisaju nakon konzervacione hirurgije: smanjenje doze na organe u riziku

Dražan Jaroš*†, Goran Kolarević*†, Milovan Savanović‡, Slavica Marić*§

Affidea, International Medical Centers Banja Luka, *Center for Radiotherapy, Banja Luka, Bosnia and Herzegovina; University of Banja Luka, †Faculty of Medicine, Banja Luka, Bosnia and Herzegovina; Hospital Tenon, ‡Department of Radiotherapy, Paris, France; University of Paris-Saclay, §Faculty of Medicine, Le Kremlin-Bicetre, France

Abstract

Background/Aim. For patients with left-sided breast cancer, a major concern is the dose of radiation delivered to the heart, because of increased risk of exposure and consequently increased risk of major coronary events and side effects. In order to reduce the dose to the heart during breast irradiation, deep inspiration breath-hold (DIBH) technique was implemented in our institution. The aim of this retrospective study was to compare dosimetric parameters of DIBH on the heart, left anterior descending artery (LAD) and ipsilateral lung (IL), compared with free breathing (FB) technique. **Methods.** Twenty patients who underwent radiotherapy with DIBH at our institution were retrospectively analyzed. Two computed tomography (CT) scans were acquired for each patient, FB-CT and DIBH-CT. Plans consisted of two opposed tangential segmented beams and one direct beam with small dose contribution. Doses to the heart, LAD, and IL were assessed. **Results.** Dosimetric comparison between FB and DIBH for mean dose to the heart was 5.17 Gy vs. 3.68 Gy, respectively ($p < 0.0001$), and the mean percentage of the volume receiving 25 Gy was 4.63% vs. 0.85%, respectively ($p < 0.0001$). Mean dose for LAD was 26.09 Gy vs. 11.89 Gy, respectively ($p = 0.00014$). Mean percentage of the volume receiving 20 Gy for the IL was 15.16% vs. 13.26% ($p = 0.0007$) for FB and DIBH, respectively. **Conclusion.** Implementation of DIBH technique in radiotherapy treatment of patients with left-sided breast cancer statistically significantly reduces the dose delivered to the surrounding organs at risk, particularly to the heart and LAD, with optimal target coverage.

Key words:
breast neoplasms; postoperative period; radiotherapy; respiration; heart; lung.

Apstrakt

Uvod/Cilj. Za bolesnice sa dijagnozom karcinoma lijeve dojke, značajan problem predstavlja doza koju će primiti srce, te povišen rizik od koronarne bolesti srca i drugih neželjenih efekata. Kako bi smanjili dozu na srce tokom zračenja tangencijalnim poljima, implementirana je *deep inspiration breath-hold* (DIBH) tehnika u našem radioterapijskom centru. Cilj ove retrospektivne studije bio je poređenje dozimetrijskih parametara DIBH tehnike na srce, levu prednju descendentnu arteriju (LAD) i ipsilateralno plućno krilo (IL), u odnosu na radioterapijski tretman tokom slobodnog disanja. **Metode.** Retrospektivno je analizirano dvadeset bolesnica koje su ozračene DIBH tehnikom u našem radioterapijskom centru. Za svaku bolesnicu napravljene su dvije serije kompjuterizovane tomografije, jedna tokom slobodnog disanja i druga za DIBH tehniku. Planovi su se sastojali od dva tangencijalna segmentna polja i jednog direktnog polja sa malim doprinosom doze. Urađena je komparacija doze na organe od rizika: srce, LAD i IL. **Rezultati.** Izmerena vrednost srednje doze na srce između slobodnog disanja i DIBH tehnike bila je 5,17 Gy i 3,68 Gy, redom ($p < 0,0001$), dok je srednja procentna vrednost volumena koji prima 25 Gy bila 4,63% i 0,85%, redom ($p < 0,0001$). Srednja doza na LAD je iznosila 26,09 Gy i 11,89 Gy, redom ($p = 0,00014$). Srednja procentna vrednost volumena IL koji prima 20 Gy bila je 15,16% i 13,26%, redom ($p = 0,0007$). **Zaključak.** Uvođenje DIBH tehnike u radioterapijski tretman kod bolesnica sa karcinomom lijeve dojke statistički značajno smanjuje dozu koju će primiti okolni organi od rizika, naročito srce i LAD, uz optimalnu pokrivenost ciljnog volumena.

Ključne reči:
dojka, neoplazme; postoperativni period; radioterapija; disanje; srce; pluća.

Correspondence to: Dražan Jaroš, Affidea, International Medical Centers Banja Luka, Center for Radiotherapy, Dvanaest beba bb, 78 000 Banja Luka, Bosnia and Herzegovina. E-mail: jarosdrazan@yahoo.com.

Introduction

Breast cancer is the most common malignant disease in the female population. The estimated number of new breast cancer cases worldwide during 2012 was 1,676,600 and the estimated number of breast cancer deaths was 521,900. Breast cancer is the most frequent cause of death from malignant diseases in female population in less developed countries, and the second one in more developed countries [1].

Radiotherapy significantly improves local control rates and an overall survival for patients with early breast cancer, who previously underwent the breast conserving surgery [2]. Effects on the heart constitute a potentially significant and serious clinical problem in radiation therapy of early breast cancer. Increased cardiac mortality among irradiated patients may compromise potential benefit in terms of a reduced risk of recurrence or death from breast cancer [3]. Radiation exposure of the heart can lead to coronary artery disease, congestive heart failure, valvular heart disease, pericardial disease, conduction abnormalities and sudden cardiac death [4]. In combination with systemic therapy, anthracyclines and trastuzumab, it may increase the risk of cardiac toxicity [5]. The heart and left anterior descending artery (LAD) exposure during breast cancer radiotherapy, increases the subsequent rate of the ischemic heart disease. The increase is proportional to the mean dose to the heart. It begins within a few years after the exposure and continues for at least 20 years [6].

In a conventional radiotherapy (RT) technique, scan is taken with the patient in the treatment position, but without taking breathing motion into account. Different techniques were developed in order to decrease radiation dose to the heart during breast cancer treatment. Deep inspiration breath-hold (DIBH) radiotherapy is a promising one. In deep inspiration there is separation between target and organs at risk: the heart and LAD. That is basic concept for introduction of DIBH in left breast cancer treatment. The goal is to reduce dose to the heart and LAD by delivering radiation only in the breathing phase in which the anatomical position of organs of risk and target is optimal. In the breath-hold technique, the respiratory phase, during which irradiation is delivered, is selected as the most favorable in terms of distance between the target volume and the organs at risk. Respiratory gating allows the radiation beam to be turned on and off whenever patient moves in and out of a planned position (which is usually close to the maximal inspiration) [7, 8].

In the studies of Lin et al. [9] and Hong et al. [10] one can find dose reduction for the heart and LAD during breathing adopted radiotherapy of left breast cancer but in this study, we also evaluated doses for the ipsilateral lung (IL) and contralateral breast.

Other studies [11–13], using modern techniques such as intensity modulated radiation therapy (IMRT), volumetric modulated arc therapy (VMAT) or tomotherapy for breast cancer treatment, showed that there is an advantage of field in field (FIF) DIBH technique over IMRT, VMAT and tomotherapy, which reduces the maximum dose and increases lower dose regions and the mean dose to organs at risk.

The primary aim of this study was to determine and compare dose distributions to the heart, LAD and ipsilateral lung (IL) during adjuvant left-sided breast cancer radiotherapy with DIBH and free breathing (FB) technique. Doses to clinical target volume (CTV) and planning target volume (PTV) and contralateral breast were also assessed and compared.

Methods

Patient selection

In the period from January 2015 to October 2017, 20 patients referred for adjuvant RT after left breast cancer preserving surgery were treated in our institution with DIBH technique using Real-Time Positioning Management (RPM) system (Varian Medical Systems, Palo Alto, CA, USA) [14]. Patients with left-sided breast cancer with negative regional nodes after breast conserving surgery were candidates for this study. Generally, only patients who could reproduce a breath-hold, were included.

Methodology

All patients went through educational sessions with a radiation therapist, who introduced them with DIBH technique. They were coached to hold breath at the same inhale respiratory phase for at least 15 seconds.

Data acquisition

FB and DIBH computed tomography (CT) series (2.5 mm slice thickness) were made for all patients in supine position with arms above their head (immobilization – the All-In-One breast and lung board solution – Orfit Industries Wijnegem, Belgium), on a GE Lightspeed CT (General Electric Medical Systems, Waukesha, WI) equipped with the RPM system. The RPM Respiratory Gating technology enables correlation of the target position with the patient's respiratory cycle [14]. An infrared tracking (Charged-Couple Device – CCD) camera mounted in the room for CT simulation and in the tretment room was used with a reflective marker usually placed over the xiphoid process. Position was marked on patient's skin so it could easily be reproduced during the whole treatment process. The system measures the patient's respiratory pattern and range of motion and displayes them as a waveform [14]. After precise determination of the target volume movement in relation to the waveform, gating thresholds were set along the waveform, in order to mark the target volume in the desired portion of the respiratory cycle [14]. These thresholds determined when the automatic gating system should turn the treatment beam on and off on the LINAC. We used audio and visual guidance during the treatment.

Delineation

For breast-conserving operations, CTV was delineated according to the Radiation Therapy Oncology Group (RTOG) consensus guidelines [15]. Contouring of organs at risk was performed in accordance with the guidelines published by the RTOG [16].

PTV was made separately in FB-CT and DIBH-CT, for plan evaluation. PTV was generated using a 8 mm and 7 mm margin from CTV for FB and DIBH, respectively, limited to the midline, and shrunk 4 mm from the skin. The ipsilateral lung was contoured using an automatic contouring tool.

The LAD coronary artery was delineated in the anterior interventricular groove from its initiation down to the apex of the heart. Delineation was done according to the CT-based cardiac atlas by Feng et al. [17]. In order to reduce the interobserver variability, the same physician delineated LAD for each patient. Target volumes were checked by an experienced radiation oncologist. Only non-contrast CT scans were used, because intravenously contrast enhanced CT scans did not improve the delineation accuracy [18].

Treatment planning and dose constraints

Field-in-field RT technique were performed on DIBH and FB CT series with two opposed tangential medial-lateral segmented beams and one direct beam (with dose weight < 10%). The prescribed dose was 50 Gy in 25 fractions. The energy of the beams was 6 MV, and the fields were shaped with Varian Millennium 120 multileaf collimator. Treatment planning was performed with the Varian Eclipse 10.0 treatment planning system (TPS) and calculated with Anisotropic Analytical Algorithm (AAA). Dose variation between +7% and 5% was accepted in PTV following ICRU 50 and 62. Dose–volume histograms were extracted and compared for each of DIBH and FB plans. For PTV we determined coverage of 95% of PTV and mean dose. For the heart, the volume covered with 25 Gy (V25) as well as the mean heart dose were measured. For the IL, the volume covered with 20 Gy (V20) was analysed. For the LAD, the mean dose was analyzed. For the contralateral breast we compared volume receiving 5Gy and 10Gy (V5 and V10, respectively).

Before the first fraction, it was mandatory to perform the treatment plan dosimetry verification by MapCHECK2 (SunNuclear, Melbourne, FL) device. The criteria was that 95% of the pixels pass with a 3% dose tolerance of reference values and a distance to agreement (DTA) 3 mm (gamma: 3%, 3 mm).

Statisticaly analysis

Paired-samples Student's *t*-test (2-tailed hypothesis test) was used for statistical analysis of the comparison of PTV and CTV dose coverage but for the organs at risk we used Wilcoxon 2-tailed test because distribution of data for organs at risk was not normal and standard deviation was big. We have tested normality of distribution with SPSS statistical software version 23.0. Data were considered statistically significant at $p < 0.05$.

Results

The mean age ± standard deviation (SD) of the observed twenty patients was 47 ± 7.2 years (range 34–60 years. In Table 1 we presented comparision of mean doses in Gy for the heart and LAD during FB and DIBH.

Result of the volume dose constraint for organ at risk – the heart (V25) for FB was 4.63%, and for DIBH 0.85% ($p = 0.000089$), with a decrease of 81.6% (Figure 1). The mean heart dose for FB was 5.17 Gy vs. 3.68 Gy for DIBH ($p < 0.0001$), with a decrease of 28.8%.

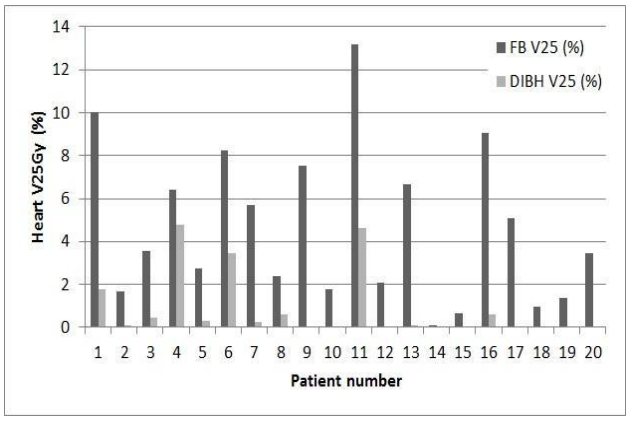

Fig. 1 – Heart V25 in % values for free breathing (FB) vs. deep inspiration breath hold (DIBH) for all 20 patients. V25 – the volume covered with 25 Gy.

In comparison with FB, the mean dose delivered to LAD was decreased for 54% if DIBH technique was used (26.09 Gy vs. 11.88 Gy, respectively; $p = 0.00014$) (Figure 2).

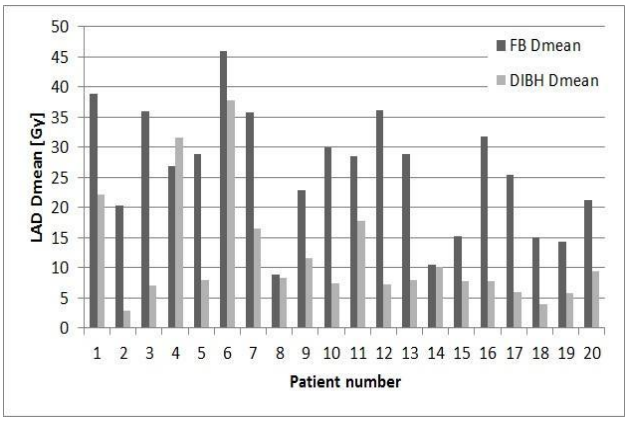

Fig. 2 – Left anterior descending artery (LAD) mean dose in Gy for free breathing (FB) and deep inspiration breath hold (DIBH) for all 20 patients.

Result of the volume dose constraint for organ at risk – the IL (V20) was 15.16% vs. 13.26% for FB and DIBH, respectively ($p = 0.04$) (Figure 3). The volume was decreased by 144.73 cm^3 (42.8%) with FB compared to DIBH technique.

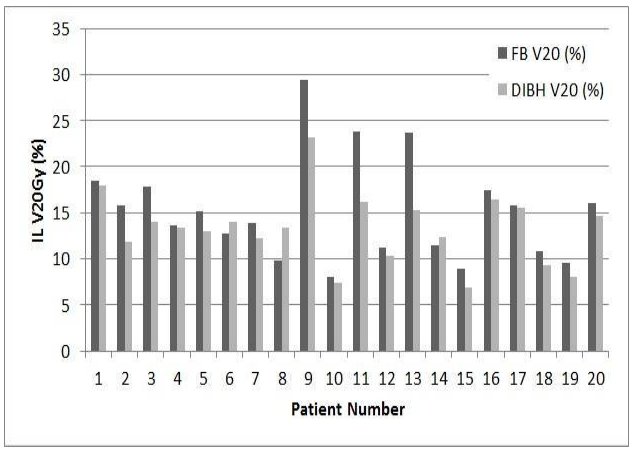

Fig. 3 – Comparison between free breathing (FB) and deep inspiration breath hold (DIBH) for ipsilateral lung (IL) V20 in %, for all 20 patients. V25 – the volume covered with 20 Gy.

Usually a small volume of the right breast was exposed and the comparison between FB and DIBH for V5 was 1.20% vs. 0.81%, (p = 0.157) and for V10, it was 0.87% vs. 0.49% (p = 0.133), respectively (Figure 4).

The mean 95% coverage of PTV for FB and DIBH plans was 96.58% vs. 96.33%, respectively (p = 0.186). Mean coverage value for 95% of CTV was 98.13% for FB vs. 97.85% for DIBH (p = 0.297).

A survey of mean irradiation doses for the heart and LAD during FB and DIBH used in this and earlier published studies is given in Table 1.

Discussion

The heart dose parameters were significantly reduced in the present study. It is directly related to the position-distance from the chest wall and the relative curvature of the anterior chest wall, size and position of the breast, etc. [19]. The published results of the left-sided breast cancer radiotherapy indicate reduction of the irradiated heart and LAD volumes, although there were no literature data available to correlate a given risk of cardiac complication [20, 21].

Based on QUANTEC, the heart V25 value should be less than 10%, with probability of cardiac mortality less than 1% [22]. The heart V25 values for FB and DIBH in this study

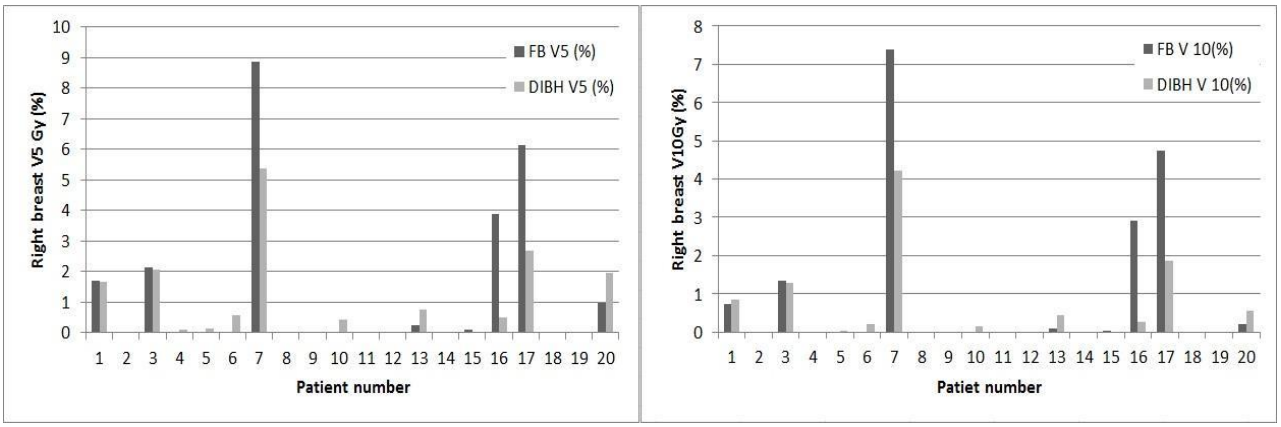

Fig. 4 – The comparison of free breathing (FB) and deep inspiration breath hold (DIBH) for the right breast, V5 and V10 in %. V5 and V10 – the volume covered with 5 Gy and 10 Gy, respectively.

Table 1

Survey of mean irradiation doses (Dmean) for the heart and left anterior descending artery (LAD) during free breathing (FB) and deep inspiration breath-hold (DIBH) used in different studies

Study	Heart (Dmean), Gy		LAD (Dmean), Gy	
	FB	DIBH	FB	DIBH
Bruzzaniti et al.[20]	1.68	1.24	9.01	2.74
Vikström J et al.[24]	3.7	1.7	18.1	6.4
Hjelstuen et al.[25]	6.2	3.1	25	10.9
Wang W et al.[27]	3.17	1.32	20.47	5.94
Lee HY et al.[28]	4.53	2.52	26.26	16.01
Lin A et al.[9]	1.41	0.82	12.24	4.25
Joo et al.[29]	7.24	2.79	40.79	23.69
Rochet N et al.[30]	2.5	0.9	14.9	4.0
Hayden AJ et al.[31]	6.9	3.9	31.7	21.9
Bolukbasi Y et al.[32]	1.74	0.66	1.71	0.78
This study	5.17	3.68	26.09	11.88

and the studies of Yeung et al. [23] (0.8% vs 0.1%, respectively), Vikstrom et al. [24] (2.0% vs 0.0%, respectively) and Hjelstuen et al. [25] (6.7% vs 1.2%, respectively), had a similar volume reduction. DIBH technique also showed better result for the heart V25 compared to that of a study of Fan et al. [26] (7.89%) for patients treated in FB prone position.

Clinical evaluation and comparison of mean heart doses and LAD doses are very important, because cardiac toxicity due to radiation therapy has a prolonged latency period. Patients with breast cancer and good prognostic factors have a long life expectancy, and it is very important to reduce potential acute and late side effects due to radiation therapy of breast cancer. DIBH is promising technique in terms of reduced cardiac morbidity and mortality. In several studies, DIBH has been associated with significant improvement in both mean heart doses and mean LAD doses, when comparing the same patients planned with FB and DIBH [9, 20, 24, 25, 27–32].

Retrospective data from a large analysis of both community and academic centers demonstrates that patients treated with DIBH had an average lower heart doses than those treated with FB [10].

In this study, all patients had dosimetric benefit from DIBH technique, in terms of cardiopulmonary dose reduction. However, the mean dose to LAD exceeded 20 Gy in case of three patients. This result was related to the position-distance of the LAD from the chest wall and the curvature of the anterior chest wall. Position of the breast and understanding of coaching during CT simulation were also reasons for higher doses to the LAD.

Using DIBH technique the same target dose coverage was achieved compared to FB technique.

The relatively small sample size was a limitation to this study because the achievement of DIBH criteria was not possible for all patients. Patient coaching before CT simulation was necessary but also time consuming [33].

The strength of this study was using exactly the same patient cohort in both DIBH and FB plans.

Negative side of the use of DIBH technique is increased radiotherapy treatment room workflow due to a complex set-up procedure. Treatment time is also increased when several breath-holds are needed to complete the irradiation of a beam, therefore we are using maximal dose rate to decrease the beam-on time.

Secondary malignancies related to radiation are a problem in breast cancer patients. In this study, there was no significant difference in contralateral breast doses between DIBH and FB plans. This was also demonstrated by Johansen et al. [34]. Relative risk estimates for secondary contralateral breast cancer are almost the same either using DIBH or FB technique. If modern radiotherapy techniques are in use for breast cancer irradiation, one should carefully evaluate the region of lower doses in contralateral breast.

Conclusion

DIBH radiotherapy of left-sided breast cancer, utilizing audio and video guidance, statistically significantly reduces radiation doses to the heart and LAD without compromising target coverage. More effort should be given to patient coaching before CT simulation because it is the only factor that is not anatomy related and can result in lower doses to the heart and LAD.

Future research should be done in the field of precise dose constraints determination for cardiac specific tissues (pericardial tissue, valves, etc.) and to find out prediction factors that point out the patients with highest benefit from DIBH technique.

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The accuracy of ultrasonography for detection of enlarged parathyroid glands in patients with different forms of hyperparathyroidism

Tačnost ultrasonografije u detekciji uvećanih paratireoidnih žlezda kod bolesnika sa različitim oblicima hiperparatireoidizma

Dara Stefanović*†, Milan Petrović‡, Sanja Dugonjić†§

Military Medical Academy, *Institute for Radiology, ‡Clinic for Surgery, §Institute for Nuclear Medicine, Belgrade, Serbia; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Ultrasonography is a cheap, easily available and convenient method for diagnosis. The aims of this study were: to determine the utility of ultrasonography for preoperative identification and localization of enlarged parathyroid glands (PTG) in patients with different forms of hyperparathyroidism (HPT); to examine the frequency of PTG detection in patients previously non-suspected for HPT but having symptoms relevant to the disease; to determine sensitivity and positive predictive value (PPV) of ultrasonography for identification of PTG in HPT and to compare obtained results with those obtained by scintigraphy. **Methods.** This investigation was designed as a retrospective–prospective study. The total number of patients undergoing ultrasonography prior to surgery was 179 and the number of those subjected to scintigraphy, mostly by the ^{201}Tl/^{99m}Tc method, was 112. The patients (52 male, 128 female) were divided into the following four groups: group A – patients with primary (p)HPT (n = 78); group B – patients with secondary (s)HPT (n = 47); group C – patients with tertiary (t)HPT (n = 13); group D – patients with unrecognized (u)HPT, but with anamnestic data implying the disease (n = 42). High resolution ultrasonography was performed by a single experienced observer. Diagnosis of HPT was based on characteristic clinical and biochemical parameters. Final proof of HPT diagnosis was surgery followed by histopathological examination. **Results.** Ultrasonography detected enlarged PTG in 93.85% of total patients, whereas scintigraphy uncovered 75.89% of positive cases ($p < 0.05$). The total number of positive PTG detected by ultrasonography was 211 *vs* 225 detected by surgery (sensitivity – 95.9%; PPV – 99.4%). Histopathology confirmed the predominance of adenoma in the A and D groups in comparison with the B group of patients having PTG hyperplasia. The group C was characterized by the presence of adenomas in hyperplastic PTG. The mean size of PTG measured by ultrasonography was 17.59 ± 8.0 mm (n = 164) *vs* 18.36 ± 8.54 mm (n = 179) measured after surgery. Ultrasonography proved itself as an accurate technique in all HPT groups, regarding its high sensitivity (range 93.6–100%) and PPV (95.6–100%). In contrast, scintigraphy was shown to be less reliable, especially in the sPTH group (sensitivity: 51.7%; PPV: 78.4%). **Conclusion.** Ultrasonography is more sensitive and accurate method for pre-operative localization of PTG in comparison with ^{201}Tl/^{99m}Tc scintigraphy. It can be also efficiently used for detection of PTG and diagnosis of HPT in patients previously not suspected for this disease.

Key words:
parathyroid glands; hyperparathyroidism; ultrasonography; diagnosis, differential; radionuclide imaging; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Ultrasonografija je jeftina, lako dostupna i pogodna dijagnostička metoda. Ciljevi ove studije su bili: odrediti korisnost ultrasonografije kod preoperativne detekcije i lokalizacije uvećanih paratireoidnih žlezda (PTŽ) kod bolesnika sa različitim oblicima hiperparatireoidizma (HPT); odrediti učestalost detekcije uvećanih PTŽ kod bolesnika kod kojih se prethodno nije sumnjalo na ovo oboljenje, ali koji su imali simptome HPT; odrediti senzitivnost i pozitivnu prediktivnu vrednost (PPV) ultrasonografije u identifikaciji PTŽ kod HPT i uporediti ih sa rezultatima dobijenim scintigrafijom. **Metode.** Istraživanje je dizajnirano kao retrospektivno-prospektivna

Correspondence to: Sanja Dugonjić, Military Medical Academy, Institute for Nuclear Medicine, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: sanja.dugonjic14@gmail.com.

studija. Ukupan broj bolesnika kod kojih je urađena ultrasonografija pre hirurškog zahvata je iznosio 179, a broj bolesnika kod kojih je urađena scintigrafija, pretežno $^{201}Tl/^{99m}Tc$ metodom, iznosio je 112. Bolesnici (52 muškarca i 128 žena) bili su podeljeni u sledeće četiri grupe: grupa A – bolesnici sa primarnim (p)HPT (n = 78); grupa B – bolesnici sa sekundarnim (s)HPT (n = 47); grupa C – bolesnici sa tercijarnim (t)HPT (n = 13); grupa D – bolesnici sa neprepoznatim HPT, ali čija anamneza ukazuje na ovo oboljenje (n = 42). Visoko-rezoluciona ultrasonografija korišćena je za dijagnostiku od strane samo jednog iskusnog radiologa. Dijagnoza HPT postavljena je na osnovu karakterističnih kliničkih i biohemijskih parametara. Konačna potvrda bila je na hirurškom i patohistološkom nalazu. Uvećane PTŽ bile su detektovane kod 93,85% bolesnika pomoću ultrasonografije, a kod 85,89% bolesnika pomoću scintigrafije ($p < 0,05$). Ukupan broj pozitivnih PTŽ detektovanih ultrasonografijom iznosio je 211 u odnosu na 225 PTŽ detektovanih na osnovu hirurškog nalaza (senzitivnost – 95,9%; PPV – 99,4%). Histopatološkom analizom potvrđena je najveća zastupljenost adenoma u grupama A i D, dok je u grupi B bila dokazana hiperplazija. Grupu C je karakterisalo prisustvo adenoma u hiperplastičnim PTŽ. Prosečna veličina PTŽ izmerena ultrasonografijom je iznosila 17,59 ± 8,0 mm (n = 164), a veličina žlezda izmerenih nakon hirurškog zahvata je bila 18,36 ± 8,54 mm (n = 179). Ultrasonografija se pokazala kao tačna metoda kod svih formi HPT u pogledu senzitivnosti (93,6–100%) i PPV (95,6–100%). Nasuprot ovoj metodi, scintigrafija se pokazala manje pouzdanom i tačnom metodom kod preoperativne lokalizacije PTŽ, posebno kod sHPT (senzitivnost: 51,7%; PPV: 78,4%). **Zaključak.** Ultrasonografija je senzitivnija i tačnija metoda za preoperativnu detekciju PTŽ u poređenju sa $^{201}Tl/^{99m}Tc$ scintigrafijom. Ova metoda se, takođe, može uspešno koristiti za detekciju uvećanih PTŽ, a time i dijagnoze HPT kod bolesnika kod kojih se na ovo oboljenje prethodno nije sumnjalo.

Ključne reči:
paratireoidne žlezde; hiperparatireoidizam; ultrasonografija; dijagnoza, diferencijalna; scintigrafija; osetljivost i specifičnost.

Introduction

Normal sized parathyroid glands (PTGs) are very small [approximately 6 mm (craniocaudal) and 3–4 mm (transverse) dimensions], and could not be usually identified by most imaging methods. Therefore, a parathyroid gland that is imaging-visible is very suspicious for the presence of a pathological lesion which is a cause of primary hyperparathyroidism (pHPT) [1, 2]. The pathologic entities of PTGs include solitary adenoma (80%–85%), multiglandular disease (15%–20%), and rarely carcinoma (< 1%). Multiglandular PTG diseases include hyperplasia of all of the parathyroid glands or, occasionally, double/triple adenomas [2, 3].

Primary HPT is the third most frequently diagnosed endocrine disorder [4] with serious complications on the skeletal system due to bone demineralization, recurrent peptic ulcers, renal stones and many neurological, psychiatric and vascular disturbances. Secondary (s) HPT is caused by hyperplasia of PTGs due to renal insufficiency. A decrease of calcium (Ca) levels in plasma, as a result of renal failure, results in an increase of parathyroid hormone (PTH) secretion. In addition, sHPT can be caused by malnutrition, vitamin D deficiency, increased Ca^{2+} excretion and by an influence of certain drugs [4, 5]. Tertiary (t) HPT is developed as an autonomous PTG hyperfunction in patients on renal dialysis and the pathological lesions include diffuse or nodular PTG hyperplasia [4, 6, 7].

The treatment of HPT involves primarily surgical approach which is the most successful therapy of HPT. The traditional technique has been a bilateral neck exploration under general anaesthesia, involving the evaluation of all four glands. Subsequent removal of pathological PTGs by skilled surgeons provides a high rate of cure exceeding 95% [8]. Parathyroid surgery is also indicated for patients with hipercalcemia, high PTH levels and/or renal osteodystrophy in sHPT which cannot be successfully medicated. However, there is still no consensus whether any asymptomatic HPT patient needs [5, 9]. Nowadays, due to the availability of preoperative PTG imaging techniques, less invasive surgical alternatives are used such as minimally invasive parathyroidectomy and endoscopic parathyroidectomy [9, 10]. These techniques demand an accurate preoperative localization of enlarged PTGs; this is especially important for patients with solitary PTG adenomas. The main reason for insufficiently successful surgical intervention is failure to localize the ectopic PTG and undiagnosed multiple PTGs in pHPT [9–11].

Localization of an abnormal PTG preoperatively can reduce operative time, postoperative morbidity, costs and the requirement for repeated surgery. The other reasons for preoperative localization include ectopic PTG adenoma and familiar HPT with multiglandular disease [10, 12].

Different methods for localization of PTGs have been used in the last three decades, such as high-resolution ultrasonography, scintigraphy imaging, computerized tomography (CT) (conventional and new 4D), and magnetic resonance imaging (MRI) [11]. All these methods have varying rates of success, so it is difficult to suggest any single imaging modality to be routinely used before surgical neck exploration [13–15].

High-resolution ultrasonography was first described as a method for detection of PTG tumours in 1979 by Edis and Evans [16]. Subsequently, many studies have confirmed its efficacy for preoperative localization of abnormal PTGs. However, the results of these studies have been quite varying with sensitivities of PTG detection ranging from 34% to 82% and an unacceptably high false-positive rates of 4–25% [17]. Among PTG imaging techniques, ultrasonography has the advantage of convenience, easy availability and low cost,

and is preferred by some authors [18–20]. Ultrasonography shows an abnormal PTG as an oval, bean-shaped, or infrequently, multilobulated hypoechoic mass with a well-defined margin, located posteriorly or inferiorly to the thyroid gland [2, 19–21]. PTGs are usually very vascular, typically showing a peripheral vascular arc and a prominent polar feeding artery that arises from the branches of the inferior thyroidal artery. Its identification can distinguish PTGs from lymph nodes, which usually have a hilar blood supply. Other features include asymmetrically increased vascularity in the thyroid gland on the side of identified PTGs and in the hyperechoic capsule [21].

In 1989, a new approach using the radiopharmaceutical 99m Tc-MIBI (sesta methoxyisobutylisonitrile) was reported for identification and localization of PTGs and this imaging method gradually replaced the previous subtraction method based on $^{201}Tl/^{99}Tc$ [22]. Several investigators confirmed the use of this technique for the identification of abnormal PTGs using either MIBI alone or with subtraction imaging. The sensitivity was in range of 71–93% [23–25]. Numerous studies comparing scintigraphy and ultrasonography suggest that both methods have similar sensitivities and specificities in the detection of solitary adenomas with a range of 68%–95% for scintigraphy and a range of 72%–89% for ultrasonography [25–27]. Both methods have significantly lower sensitivities in the detection of the multiglandular disease [27]. However, very often, these methods are not comparable as suggested by meta-analyses based on a large number of publications [26, 27]. It is generally suggested that a preoperative approach that combines both ultrasonography and scintigraphy is more accurate than technique alone [11, 28, 29].

There are many factors influencing the accuracy of ultrasonography for detection of pathological PTGs, but it seems that the careful examination by a very experienced observer is of crucial importance [19]. This was the reason why we wanted to present our own results, which show very high sensitivity of this imaging method in identification and localization of pathological PTG. The concrete aims of the study were: to determine the utility of ultrasonography in preoperative identification and localization of enlarged PTGs in patients with different forms of HPT; to examine the frequency of PTGs detection in patients previously non-suspected for HPT, but having symptoms relevant to the disease; to determine sensitivity and positive predictive value (PPV) of ultrasonography for identification of PTGs in HPT and to compare them with results obtained by scintigraphy.

Methods

This was a retrospective–prospective study on patients with HPT, conducted at the Military Medical Academy (MMA) in Belgrade, Serbia during the period between 1989 and 2014. The study was approved by the Ethics Committee of the MMA. The number of patients was 180 and all of them were subjected to surgery in order to remove pathological PTGs. There were 52 males and 128 females. Their main age was 51.78 years (range: 18–79 years). Only one patient was false positive on surgery and thus excluded from the study. The patients were divided into four groups. The group A (n = 78) was consisted of patients with primary HPT (pHPT); the group B (n = 47) and group C (n = 13) included patients with secondary HPT (sHPT) and tertiary (tHPT), respectively. The group D consisted of patients with previously unrecognized HPT (uHPT) both by clinical and biochemical means. They were directed for the ultrasonographic examination of abdomen and pelvis. After a carefully conducted anamnesis related to kidney stones, peptic ulcers, skeletal and joint problems, neuromuscular and psychiatric disturbances, the patients gave their consent for ultrasonographic examination of PTGs. This group consisted of 523 patients of which 124 had enlarged PTGs. Of them, only 42 were fully processed and included in the study. The main demographic characteristics of these patients were given in Table 1.

Table 1

Demographic characteristics of patients with hyperparathyroidism (HPT)

Group	Total number	Male	Female	Age (years), mean ± SD
A (pHPT)	78	22	56	50.95 ± 12.39
B (sHPT)	47	17	30	51.09 ± 8.40
C (tHPT)	13	8	5	48.92 ± 9.78
D (uHPT)	42	5	37	55.00 ± 10.52

***p* – primary; s – secondary; t – tertiary; u – unrecognized; SD – standard deviation.**

Ultrasonography

The ultrasonography of the neck was performed at the Institute for Radiology, MMA, by a single experienced radiologist (D.S.), by using a high resolution transducer (Diasonics type CV 400 aparatus equipped with 10 MHz array transducer or SPECTRA, 7.5 MHz transducer). In some patients, Doppler and Color Doppler examination was performed by using SPECTRA probe 7.5 MHz and Acuson128 xp multifrequent probe of 7.5 MHz. The ultrasonography examiner was aware about clinical and laboratory parameters characteristic for the HPT groups A, B and C, while being unaware of any prior scintigraphy imaging results. The ultrasonographic examination was performed with the patient supine and the neck extended. The central neck was examined from the subclavian vein to the submandibulary glands using the thyroid gland as a reference point. PTGs were recognized as hypoechoic, oval/round encapsulated structures laying posterior and adjacent to the upper one third of the thyroid lobes, adjacent to the lower pole of the thyroid lobes, or variably inferior to the thyroid lobe in the case of ectopic localization. Both cross-sectional and longitudinal images were obtained. In some cases, the examined area was extended to the superior part of mediastinum. The Color Doppler was used to detect

the feeding artery entering one pole of PTGs. The size of abnormal PTGs was measured by taking the largest dimension.

Scintigraphy

In most patients, subtraction scintigraphy by using ^{201}Tl/^{99m}Tc was performed as described [30]. In brief, scintigraphy of the neck region was done in dinamic mode during 25 min, after iv. injection of 2 mCi (74 MBq) 201Tl. After Tl scintigraphy, dynamic scintigraphy during 25 min in the same position was done after *iv* injection 5 mCi (185 MBq) 99mTc. A direct subtraction view was obtained by subtracting the ^{99}Tc image from the ^{201}Tl image. Only some images were obtained with a new scintigraphy method by using 740MBq of ^{99m}Tc-MIBI followed by ^{99m}Tc-pertechnetate, exactly as was described [31]. Scintigraphy was carried out at the Institute for Nuclear Medicine, MMA.

Biochemical parameters, surgery and statistical analysis

Biochemical parameters, such as plasma concentration of Ca and phosphorus (P), serum activity of alkaline phosphatase (ALP) and serum concentrations of PTH were taken from medical history of patients.

All patients were operated on by using classical bilateral neck exploration in the Clinic for Surgery, MMA. After removal, the size of PTGs was measured and then the glands were processed for histopathology and examined by light microscopy (Institute for Pathology, MMA). The histopathological reports were taken from medical history of patients and used for definitive diagnosis. Histopathological diagnosis was classified as: adenoma, atypical adenoma, hyperplasia, combination of adenoma and hyperplasia, and carcinoma.

Data were expressed as mean ± standard deviation (SD) or mean ± standard error (SE). Comparisons between groups were analyzed by the Student *t*-test, Mann-Whitney *U* test or Kruskal-Wallis test (multiple groups). Categorical data were compared by the Chi-square (χ^2) test. Correlations were analyzed by the Spearman rank test. Sensitivity was defined as the ratio of true positive (TP) tests to the sum of TP and false negative (FN) tests. PPV was defined as the ratio of TP tests to the sum of TP and false positive (FP) tests. Statistical significance was accepted at $p < 0.05$. For statistical analysis, SPSS computer program was used.

Results

Characteristics of PTGs detected by ultrasonography

Ultrasonography was performed in 179 patients in which the diagnosis of HPT was confirmed by surgery and subsequent histopathological evaluation. The enlarged PTGs were detected in 169 (93.9%) patients with HPT.

The total number of ultrasonographically detected PTGs was 211, out of 225 detected during surgery. Of them, 199 were TP, 6 were TN, and 20 were FN. Ultrasonography did not detect 12.9% of PTGs. There was a statistically significant correlation between ultrasonography and surgery in the number of PTGs (Spearman range correlation, r = 0.79; $p < 0.001$). Histopathology confirmed 67.6% of adenomas, 24.0% of hyperplasia, 7.3% of adenomas combined with hyperlasia and 1.1% of carcinoma. Adenomas had typical ultrasonographic characteristics: ovoid/round shape with homogenic echogenicity lower in comparison to that of the thyroid gland. Their size was higher than 5 mm × 3 mm × 1 mm. Those PTGs were located in the close proximity to the posterior capsule of the thyroid gland. The fibro-fatty capsule of PT adenomas was usually presented as hyperechoic line separating them from the thyroid gland (Figure 1). When combined with Power Doppler or Color Doppler, extrathyroidal feeding artery entering one pole of PT adenomas (Figures 2a and 2b, Figure 3) or diffuse blood flow within them were visible.

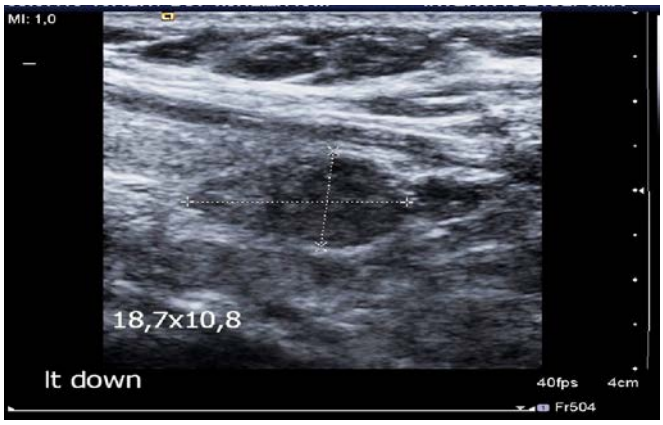
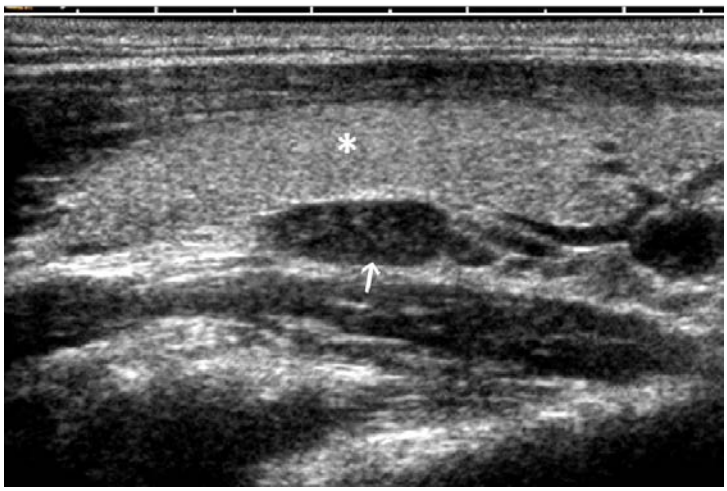
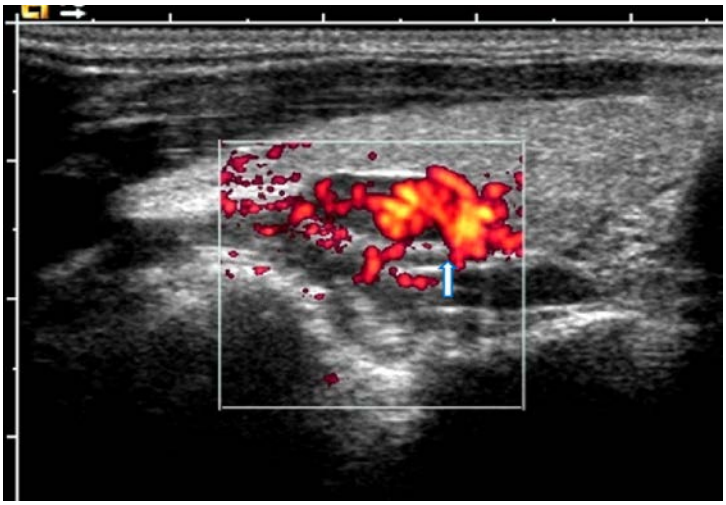


Fig. 1 – Adenoma (primary hyperparathyroidism – pHPT): ultrasonographic appearance of a parathyroid adenoma (gray scale) appearing as a solid, encapsulated, hypoechogenic lesion with a well-defined margin, located adjacent to the lower pole of the left thyroid lobe.



a)



b)

Fig. 2 – Adenoma (primary hyperparathyroidism – pHPT): a) ultrasonographic appearance of a parathyroid adenoma (gray scale) appearing as a solid, oval, hypoechogenic lesion with a well-defined margin, located under the upper pole of the right thyroid lobe (marked by an asterix); b) the same adenoma visualized by Power Doppler. Note extrathyroidal feeding blood vessels entering at the parathyroid gland (white arrow).

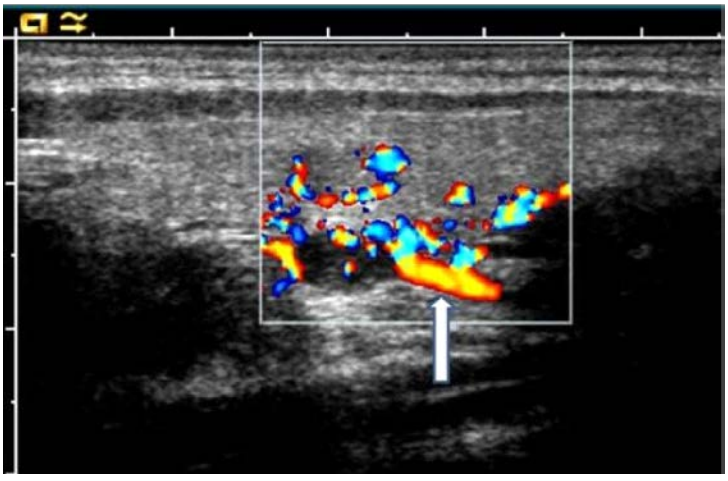


Fig. 3 – Adenoma (primary hyperparathyroidism – pHPT): Color Doppler of the right inferior parathyroid gland. Note an artery entering at the one pole of the gland. A thyroid parenchymal node with a typical intrathyroid vascularization is visible above the parathyroid adenoma.

The PTG had various side and site localizations. Most of them had lower side position. (left lower: 38.2%; right lower: 22.7%; left upper: 6.6%; right upper: 5.7%). Of the total PTG number, 18.2% were multiple; 6.2% had atypical topic localization, and 2.4% were localized ectopically in the upper mediastinum (Table 2). The collision between ultrasonography and surgery regarding localization was observed in 27 (13.5%) of PTGs.

Table 2

Distribution of parathyroid glands (PTGs) detected by ultrasonography

Localization	PTGs (%)
Right upper	5.7
Left upper	6.6
Right lower	22.7
Left lower	38.2
Multiple	18.2
Topic atypic	6.2
Ectopic	2.4

The mean size of PTGs measured by ultrasonography was 17.59 ± 8.0 mm (n = 164) *vs* 18.36 ± 8.54 mm (n = 179) after the surgery.

Biochemical, anamnestic and clinical parameters in patients with different forms of HPT

Biochemical, anamnestic and clinical parameters in HPT patients divided into different groups were studied. Biochemical parameters included serums levels of PTH, Ca, P and ALP activity.

As shown in Figure 4, mean values of serum ALP activity were above normal values (120–180 IU/L) and those in the group C were statistically significantly higher ($p < 0.05$) compared to other groups.

The concentrations of PTH were in the range between 25.2–2,300 pMol/L (normal range, 60–120 pMol/L). The levels of this hormone in patients with secondary and tertiary HPT (the groups B and C, respectively) were higher than in the groups with primary HPT (the groups A and D), ($p < 0.05$).

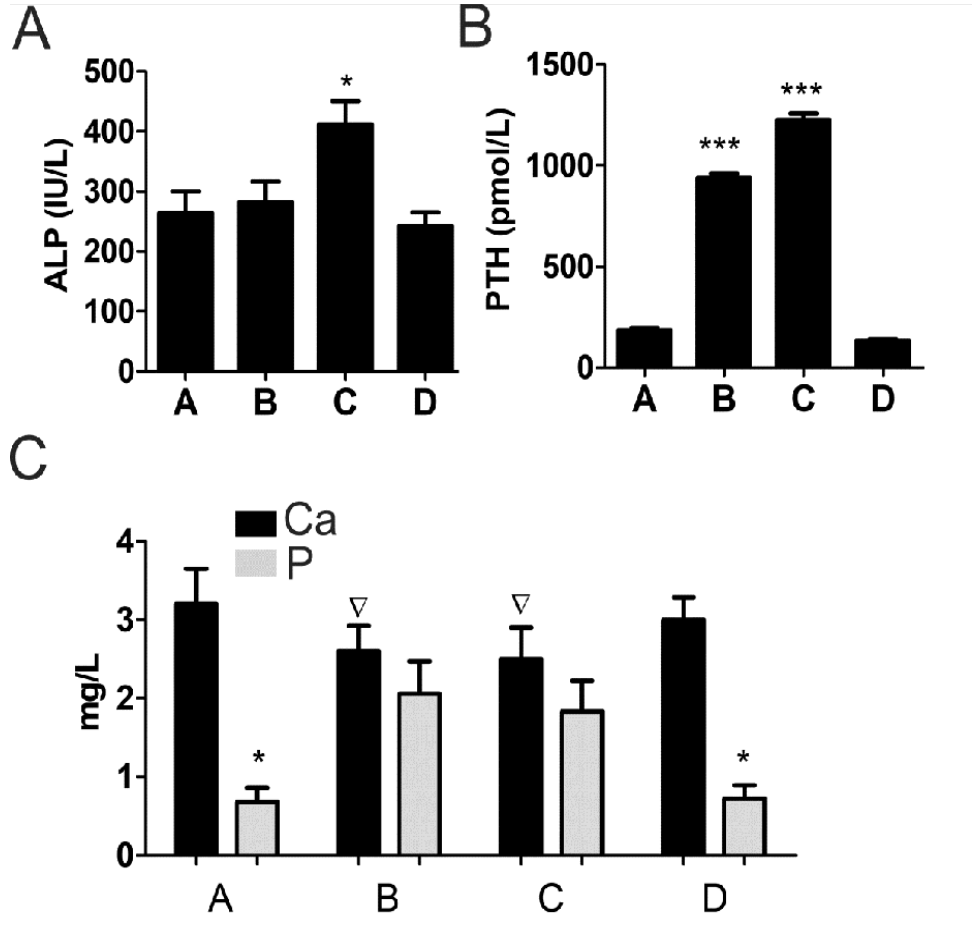


Fig. 4 – Biochemical parameters in patients with different forms of hyperparathyroidism (HPT): ALP – alkaline phosphatase; Ca – calcium; P – phosphorus. Values are given as mean ± SE for n = 78 (group A), n = 47 (group B), n = 13 (group C) and n = 42 (group D). (A) * $p < 0.05$ compared to A, B and D groups; (B) *** $p < 0.001$ compared to group A and D; (C) *$p < 0.05$ compared to B and C groups, ▽$p < 0.05$ compared to A and D groups.

Table 3

Dominant anamnestic and clinical parameters in patients with different forms of hyperparathyreoidism (HPT)

Parameters	Group A (n = 78)	Group B (n = 47)	Group C (n = 13)	Group D (n = 42)
Symptoms				
renal colica	$72^{B,C}$	11	8	$76^{B,C}$
kidney stone	$71^{B,C}$	14	8	$69^{B,C}$
peptic ulcus	30	51^{D}	46	21
bladder stone	12	15	8	8
bone pain	63	$96^{A,D}$	$100^{A,D}$	45
joint stiffness	34	$60^{A,D}$	$92^{A,D}$	22
bone fracture	11	23	31	3
psych. symptoms	49	64^{D}	$92^{A,D}$	34
neurol. symptoms	32	$94^{A,D}$	$100^{A,D}$	24
Clinical signs				
osteoporosis	75	100	100	76
positive EMNG	58	$14^{A,C}$	61	ND
positive findings of urinary system	73	100	100	76
positive findings of GD system.	45	40	38	16

Group A – primary HPT; Group B – secondary HPT; Group C – tertiary HPT; Group D – unrecognized HTP. EMNG – electromyoneurography; GD – gastroduodenal; ND – not done.
Superscript letters point out statistically significant differences (χ^2 test; $p < 0.05$): ^{A} in relation to the group A; ^{B} in relation to the group B; ^{C} in relation to the group C, and ^{D} in relation to the group D

Table 4

Comparison of positive parathyroid glands (PTGs) findings between ultrasonography and scintigraphy in patients with different forms of hyperparathyroidism (HPT)

Group*	Imaging method	Total number of patients**	Number of patients with positive PTGs	χ^2 value	*p*
Total	Ultrasonoraphy	179	169	21.29	< 0.0001
	Scintigraphy	112	85		
A (pPTH)	Ultrasonography	78	71	4.28	< 0.039
	Scintigraphy	50	39		
B (sPTH)	Ultrasonography	47	44	13.82	< 0.0002
	Scintigraphy	32	19		
C (tPTH)	Ultrasonography	13	13	1.36	0.244
	Scintigraphy	10	9		
D (uPTH)	Ultrasonography	42	41	1.71	0.191
	Scintigraphy	20	18		

***For explanation see under Table 1; **Total number of patients with positive PTGs detected by surgery/pathology.**

The concentrations of Ca in plasma in the groups A and B were higher than physiological ones, and the differences in relation to values in the groups C and D, were statistically significant ($p < 0.05$). In contrast, the concentrations of P in the groups B and C were statistically significantly higher ($p < 0.05$) than those in the groups A and D. Certain patients in the group D were normocalcemic. In most patients, total Ca levels correlated with the concentrations of ionized Ca and their levels were normalized after one year following surgery (data not show).

Dominant anamnestic and clinical data relevant to HPT are presented in Table 3. Patients from the groups A and D had dominant anamnestic and clinical signs of urinary system pathology, which were more frequent than in the groups B and C ($p < 0.05$). In contrast, the symptoms/signs associated with the skeletal system, psychiatric disturbances and neurological disorders were higher in the groups B and C in comparison to the other two ($p < 0.05$). It is interesting that the percentage of patients with positive electromyoneurography (EMNG) results in the groups A and C was higher in comparison to the group B ($p < 0.05$). The percentage of patients with the amanestic data of peptic ulcer was higher in the group B than in the group D ($p < 0.05$). However, these differences were not confirmed by gastroscopy (Table 3).

Comparison of positive PTG findings between ultrasonography and scintigraphy

One of the aims of the study was to compare the accuracy of ultrasonography and scintigraphy for abnormal PTGs detection, in patients with different clinical forms of HPT. Results are given in Table 4. The number of patients with positive PTGs detected by ultrasonography *vs* surgery was as follows: total number of patients – 169 *vs* 179, respectively; the group A – 71 *vs* 78, respectively; the group B – 44 *vs* 47, respectively; the group C – 13 *vs* 13, respectively; the group D – 41 *vs* 42, respectively.

The number of patients with positive PTG findings detected by scintigraphy *vs* surgery was as follows: total number of patients – 85 *vs* 112, respectively; the group A – 39 *vs* 50, respectively; the group B – 19 *vs* 32, respectively;

Table 5

Comparison of detected parathyroid glands (PTGs) numbers between ultrasonography and scintigraphy in patients with different forms of hyperparathyroidism (HPT)

Group*	Imaging method	Total number of PTGs**	Number of positive PTGs	χ^2 value	*p*
Total	Ultrasonography	225	211	12.31	0.0004
	Scintigraphy	133	109		
A (pHPT)	Ultrasonography	80	77	29.28	< 0.0001
	Scintigraphy	80	49		
B (sHPT)	Ultrasonography	141	79	30.92	< 0.0001
	Scintigraphy	96	19		
C (tHPT)	Ultrasonography	33	20	2.97	0.0849
	Scintigraphy	24	9		
D (uHPT)	Ultrasonography	44	41	1.083	0.298
	Scintigraphy	20	17		

***For explanation see under Table 1;**Total number of PTGs detected by surgery/pathology.**

the group C – 9 *vs* 10, respectively; the group D – 18 *vs* 20, respectively.

When the success was analyzed according to the number of patients with positive PTGs, it can be seen that in total group, the groups A and B, ultrasonography was significantly superior to scintigraphy. In the groups C and D, there were no statistically significant differences between these two imaging methods.

The total number of PTGs detected by ultrasonography *vs* surgery was: total group – 211 *vs* 255, respectively; the group A – 77 *vs* 80, respectively; the group B – 79 *vs* 141, respectively; the group C – 20 *vs* 33, respectively; the group D – 41 *vs* 44, respectively. The total number of PTGs detected by scintigraphy *vs* surgery was: total group – 109 *vs* 133, respectively; the group A – 49 *vs* 80, respectively; the group B – 19 *vs* 96, respectively; the group C – 9 *vs* 24, respectively; the group D – 17 *vs* 20, respectively. The results are presented in Table 5. When the results were calculated in this way, they were very similar as those presented in Table 4.

Comparison of sensitivity and positive predictive values between ultrasonography and scintigraphy in detection of PTGs

The final aim of this study was to check sensitivity and PPV of ultrasonography and scintigraphy for detection of pathological PTGs in different groups of HPT patients. The results are summarized in Table 6. When sensitivity and PPV were analyzed by assessing the number (percentage) of the patients with detected PTGs by ultrasonography, it can be seen that both parameters were very high in all groups of HPT patients (sensitivity: range, 91.0–100%; PPV: range, 95.6–100%). In the total group, sensitivity was 96.4% and PPV was 99.3%.

Scintigraphy results showed lesser sensitivity. In the total group, sensitivity was 74.6% and PPV 94.6%. The lowest sensitivity (59.3%) and PPV (72.2%) was detected in the group B. In other groups (A, C, and D) PPV did not significantly differ compared to ultrasonogrpahy (Table 6, A imaging).

The sensitivity and PPV, calculated on the basis of total PTGs number identified by ultrasonography, were similar to those analyzed by assessing the number of PTGs positive patients. Similar to the previous results, sensitivity and PPV, determined by scintigraphy, were lower compared to ultrasonographic findings, while being lowest in the group B (Table 6, B imaging).

Discussion

This clinical study was designed with the aim to analyze the accuracy of ultrasonography for pre-operative detection

Table 6

Comparison of sensitivity and positive predictive value (PPV) in detection of positive parathyroid glands (PTGs) (A imaging) or total number of PTGs (B imaging) between ultrasonography and scintigraphy

Imaging method	Parameters	Total groups	Group A	Group B	Group C	Group D
Detection of positive PTGs						
ultrasononography	Sensit. (%)	96.4	91.0	93.6	100	100
	PPV (%)	99.3	98.6	95.6	100	100
scintigraphy	Sensit. (%)	74.6*	78.0*	59.3***	90	90
	PPV (%)	94.6	93.9	79.2*	100	100
Detection of total number of PTGs						
ultrasonography	Sensit. (%)	95.9	94.5	93.6	100	100
	PPV (%)	99.4	97.4	98.7	100	100
scintigraphy	Sensit. (%)	80.2*	77.7*	51.7***	88.8	88.8
	PPV (%)	89.6	87.3	78.4*	93.4	80.9*

***Note*: For explanation about groups see under Table 3.**
***$p < 0.05$; ***$p < 0.001$ compared to corresponding parameters of ultrasonography.**

of pathological PTGs in HPT patients. Although several hundred papers cover this topic, we wanted to present our experience in the MMA, Belgrade and to show some specificities. The study included ultrasound imaging of a relative large cohort of patients (n = 179) performed by only one radiologist, simultaneous comparison of all tree forms of HPT and inclusion of one group of patients with no prior recognized HPT. However, as many other studies, its limitation is related to the relatively small number of patients in the tHPT group, unequal number of patients imaged with ultrasonography in comparison with scintigraphy and the fact that scintigraphy was performed in most patients with an old $^{201}Tl/^{99m}Tc$ method. In addition, the investigation was a retrospective-prospective study.

Our cohort consisted of 71.0% female and 29.0% male, which is in agreement with the literature data reporting a female-to-male ratio in pHPT of approximately 3–4:1 [32]. The prevalence of pHPT in female is most probably associated with estrogens, but their role in pathogenesis of HPT is still unclear. The average age of our patients was 51.8 years, suggesting that the disease evolution is slow and thus its detection is late.

The analysis of diagnostic parameters in our study was aimed just to illustrate their differences between groups, but not comparison with imaging data, since they are explored too much in literature. The increased serum level of PTH is a hallmark of HPT. It is known that the secretion and synthesis of PTH is controlled by the ambient of circulating ionized Ca concentration. Under normal conditions, an increase in serum Ca concentration, which might not be detected by biochemical methods, will instantly suppress PTH secretion. Similarly, a reduction in serum Ca concentration will immediately simulate PTH secretion. This inverse sigmoidal association between these two parameters is regulated by the calcium-sensing receptor. The other principal regulator of PTH secretion is 1,25-dihydroxyvitamin D concentration, which also inversely correlates with PTH concentration [4].

Primary HPT in our groups of patients was characterized by both increased levels of serum concentrations of PTH and Ca, simultaneously with reduction of serum P levels. Abnormal secretion of PTH raises the serum Ca level by promoting the renal tubular reabsorption of Ca, decreasing tubular reabsorption of phosphate, and stimulating osteoclasts. In addition, PTH stimulates vitamin D production, which, in turn, raises serum Ca by promoting its absorption in the gastrointestinal tract [4, 32]. We found elevated concentrations of PTH in almost all groups of HPT patients and less than 5% of them have normal values. The concentrations were higher in the groups B and C compared to other two groups, suggesting higher activity of PTGs and more severe form of the disease. Although we found that both PTH and Ca levels were increased in most patients, some inconsistency was observed, especially in the group D (unrecognized HPT), such that PTH is increased and Ca was normal or *vice versa*. The literature data also suggest similar findings [4, 6, 32]. We did not find any differences between normocalcemic and hypercalcemic patients regarding ultrasonographic findings of abnormal PTGs (data not shown).

In our study we observed much higher number of patients having symptoms and clinical signs of HPT than others did. For example, Reid et al. [8] showed that a history of nephrolithiasis was present in 10.0% of their patients with pHPT in contrast to 90% in our study. It is interesting that the anamnestic data about bone fracture were very similar (15–16%). However, symptoms and clinical parameters of skeletal system in our patients were very often and the results correlated with increased ALP activity. These and other clinical findings clearly indicate that diagnosis of HPT in our patients was established too late.

Histopathology of PTGs in our patients from different HPT groups did not significantly differ from published results [1, 2, 7, 9], showing the predominance of adenomas in the groups A and D (pPTH), hyperplasia in the group with sPTH and combination of adenomas with hyperplasia in the group with tPTH. In addition, we also found the predominant localization of pathological changes in the inferior PTGs, predominantly in the left lower quadrant. Some authors reported predominant right lower localization [8, 33], or equal right-left lower localization [34]. The exact localization of abnormal PTGs is of particular importance for planning the adequate surgical procedure [35].

In the 1980s, sensitivity of ultrasonography for PTGs localization in patients with pHPT without previous surgery ranged between 34% and 82% [17]. However, since 1996, sensitivity of ultrasonography has been improved, especially in patients with solitary PT adenoma, reaching sensitivity of 77–91% [13]. A meta-analysis [36] based on 43 studies showed that ultrasound had pooled sensitivity and PPV of 76.1% and 93.2%, respectively, for preoperative localization of abnormal PTGs in pHPT. Ruda et al. [37] reviewed the literature from 1995 to 2003 and reported a sensitivity of 79% for ultrasound in PTGs detection. A limitation of this study was the inclusion of reoperative patients with persistent disease. In another meta-analysis, sensitivity of ultrasound ranged from 48.3% to 96.2%. In a retrospective cohort study on 477 patients, Stern et al. [34] demonstrated that ultrasonography correctly localized the adenoma in 76% of patients with pHPT, with a sensitivity of 76.2% and PPV of 86.8%. Measurements were least accurate for adenomas measuring less than 1 cm in diameter. In a recent study of Reid et al. [8], performed in 374 patients, neck ultrasound was able to detect adenomas only in 66.0% of patients with pHPT. The failure in adenoma detection was associated with older age, lower peak Ca, lower PTH and higher creatinine levels. However, when an adenoma was identified on ultrasound, the laterality was confirmed to be correct at surgery in 94.5% of cases, which is very similar to our results.

Our results are closest to those published by Bradley and Knodle [18] who showed that the sensitivity of neck ultrasonography in detecting pathological PTGs was 97.5% (number of adenomas) and 85% (localization). In addition, similarly with our results, image size of PTGs correlated

well with the measured size of the adenoma on final pathological examination.

It is obvious that our results regarding sensitivity (96.4%) and PPV (99.3%) are better than most results published to date. We think that the main reason for such a success is careful ultrasound examination by only one radiologist with long-term experience in the neck ultrasound diagnostics. This assumption is supported by many publications. For example, Stern et al. [34] showed that ultrasound scans made by a single senior operator specializing in neck had a higher sensitivity than scans made by multiple examiners. The operator dependence of ultrasound is also recognized through meta-analysis of Cheung et al. [36]. It is interesting that experienced surgeon-performed ultrasound may be comparable or superior to radiologist-performed ultrasound.

One aim of our study was to compare the sensitivity and PPV of ultrasonography and scintigraphy in detecting abnormal PTGs. We showed that ultrasonography is more reliable method than scintigraphy and this is especially important for sHPT. There are many papers which compared the accuracy of ultrasonography and scintigraphy in detecting pathological PTGs [13–15, 24, 28, 38, 39]. However, only few of them are relevant to our study, because the dominant ^{211}Tl/^{99m}Tc scintigraphy method that we performed is no longer in use. In this context, Gooding et al. [30] reported that parathyroid scintigraphy using a double-tracer (^{211}Tl/^{99m}Tc) subtraction technique discovered 74% of parathyroid adenomas in patients with and without previous neck operations. High-resolution (10-MHz) ultrasound depicted 78% of these adenomas. Alone, neither modality was particularly sensitive in the detection of primary hyperplasia of PTGs, but combined techniques were more effective than the use of a single modality. Roses et al. [38] analyzed 36 patients with pHPT in whom either high-resolution real time ultrasonography, ^{211}Tl/^{99m}Tc subtraction scintigraphy or CT scaning were performed. Overall sensitivity of correctly localizing the abnormal PTGs with these techniques was relatively low: 34% for ultrasonography, 49% for the ^{211}Tl/^{99m}Tc scintigraphy, and 41% for CT scanning. The authors concluded that these three imaging techniques did not provide reliable information for initial bilateral exploration of the neck.

Most comparisons in literature refer to scintigraphic methods that are now in use. As one can see from several selected publications, mainly related to pHPT, results are very variable indicating that scintigraphy is superior, inferior or equivalent to ultrasonography [13–15, 24, 28, 38–40]. The results depends on many factors such as type of scintigraphy, localization and size of pathological PTGs, form of HPT, histopathological characteristics of PTGs and many others.

Haber et al. [28] studied 120 patients with pHPT. Ultrasonography detected enlarged PTGs in 77% of unselected patients and correctly predicted surgical findings in 74% of patients undergoing surgery. Sestamibi scintigraphy was positive in 88% of unselected patients and the difference, compared to ultrasonography, was statistically significant. Sestamibi scintigraphy was clearly more sensitive for ectopic parathyroid adenomas, providing correct localization in all 8 cases. When one test was negative, testing with the second method was usually positive, improving the likelihood of a positive result to 98% when both tests were employed. Equal sensitivity and PPV between these methods were demonstrated in a meta- analysis of Cheung et al. [36]. They showed that ultrasound had pooled sensitivity and PPV of 76.1% (70.4–81.4%) and 93.2% (90.7–95.3%), respectively. Sestamibi-SPECT had pooled sensitivity and PPV of 78.9% (64–90.6%) and 90.7% (83.5–96.0%), respectively. Lumachi et al. [41] analyzed 22 papers published between 1996 and 2000, and showed that sensitivity detected by various scintigrafic methods varied between 56.9% and 100% for solitary adenomas but sensitivity was significantly lower when multiglandular PTGs were analyzed (35.5–80%). Gotthardt et al. [26] found median sensitivity of 72% (range 39–92.5%) of sestamibi-SPECT in a meta-analysis that was not limited to pHPT patients undergoing initial parathyroidectomy since studies included patients with secondary HPT, as well as those with persistent and recurrent disease. Our results are comparable to those and pointed out that the reliability of scintigraphy is lowest in the group of sHPT, manifested by PTG hyperplasia. However, recent results from our hospital show how new scintigraphic methods can improve the detection of hyperplastic PTG. Namely, Dugonjic et al. [42] demonstrated that subtraction parathyroid scintigraphy (^{99m}Tc-MIBI followed by ^{99m}Tc-pertechnetate) is a reliable and very sensitive diagnostic tool in detecting abnormal PTGs in parathyroid hyperplasia, reaching 100% sensitivity in detecting a “dominant gland” and sensitivity *per* localized gland of 70%.

In patients with sHPT, the four glands are not uniformly enlarged and therefore preoperative localization is difficult in comparison with pHPT. In one study, sensitivity and PPV, were 47.3% and 97.8%, respectively for MIBIscintigraphy, and 69.5% and 96.9%, respectively for ultrasonography. The sensitivity of combined techniques was 84.2% [43]. In a recent meta-analysis, the pooled sensitivity of PTG scintigraphy in patients with sHPT was 53% and the pooled specificity was 93% [27]. Based on a recent study, McHenry and Shi [44] concluded that, compared to patients with a single adenoma, patients with hyperplasia were more likely to have negative sestamibi, ultrasound or both exams and lower gland weights. Therefore, parathyroid hyperplasia should be suspected in patients with lower gland weights and negative imaging. Our findings showed that ultrasonography in patients with sHPT, although slightly less sensitive in detection of hyperplastic PTGs than PTG adenomas, is very accurate diagnostic procedure.

In our opinion, the inclusion of the group D in this study is of great importance in order to show that ultrasonography could be the first diagnostic option for

pHPT. This group represented patients without prior suspicion to HPT. The patients were examined ultrasonographically mainly due to the renal stone. After careful analysis of their symptoms, the patients gave consent for neck ultrasound. We detected abnormal PTGs in 23.7% of the patients. Therefore, this group, although very similar by ultrasonography parameters to the group A (pHPT), deserves more careful analysis.

Conclusion

Ultrasonography is an accurate imaging method for detection of pathological PTGs. Its high sensitivity and PPV, independently of the HPT forms, which were higher than those achieved by $^{201}Tl/^{99m}Tc$ scintigraphy, make it as reliable tool for preoperative surgical procedure. Ultrasonography can be also efficiently used for detection of PTGs and diagnosis of HPT in patients previously not suspected for this disease.

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Correlation between somatic complaints, personality traits and positivity

Odnos između somatskih tegoba, crta ličnosti i pozitivnosti

Vesna Petrović, Dragan Žuljević, Zorica Knežević

Faculty for Law and Business Studies "Dr Lazar Vrkatić", Department for Psychotherapy, Novi Sad, Serbia

Abstract

Background/Aim. Many recent studies have focused on investigation the differences in personality traits and its role in promoting health and in moderating vulnerability to adversities and illness. The aim of our study was to investigate the role of somatic complaints in moderating the relationship between personality traits operationalized in Cybernetic Battery of Conative Tests (KON-6) model and positivity. **Methods.** The sample consisted of 512 students, 23.83 years old in average, 56.3% were female, 23% reported the presence of somatic complaints. In investigation, the Positivity Scale measuring positivity and the KON-6 measuring the activity of 6 dynamic personality traits – activity regulation system (ε), organic function regulation system (χ), defense reactions regulation system (α), attack regulation system (σ), homeostatic system coordination(δ) and regulation system integration (η) were used. **Results.** All of the conative personality traits were significantly correlated with positivity, except for σ. The subsample with somatic complaints reported higher scores in α and η traits, suggesting higher levels of anxiety and social reality impairment. The personality traits together with presence of somatic complaints significantly explained 26% of positivity variance, positively predicting positivity with higher activity of ε, and negatively with higher activity of α and δ. The moderating role of somatic complaints was found in two specific relations. Positivity can be predicted in a reverse manner by homeostatic system coordination (δ) but only in students with low somatic complaints, which was also the case for organic function regulation system (χ) but only in the presence of high somatic complaints. **Conclusion.** The positivity represents a good organizational and regulation disposition for regulating the cognitive, dynamic and motoric functions. It is also partially moderated by presence of somatic complaints. Practical benefit of these findings represents a concrete support for developing positivity in people, in order to empower people's health.

Key words:
personality; somatoform disorders; optimism; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Nedavna istraživanja su se bavila utvrđivanjem razlika u crtama ličnosti i njihove uloge u promociji zdravlja i moderiranju osetljivosti na nesreće i bolest. Cilj ovog rada bio je da se istraži moderatorska uloga somatskih tegoba i relacija crta ličnosti operacionalizovanih putem modela Kibernetske baterije konativnih testova (KON-6) i pozitivnosti. **Metode.** Istraživanje je sprovedeno na uzorku od 512 studenata, prosečne starosti 23,83 godine, 56,3% ženskog pola, 23% sa somatskim tegobama. U istraživanju je korišćena Skala pozitivne orijentacije koja meri pozitivnost i KON-6 koja meri šest dinamskih crta ličnosti – Sistem regulacije aktiviteta (ε), Sistem regulacije organskih funkcija (χ), Sistem regulacije odbrambenih reakcija (α), Sistem regulacije napada (σ), Homeostatsku koordinaciju sistema (δ) i Integrativnu regulaciju sistema (η). **Rezultati.** Sve konativne dimenzije ličnosti značajno su i adekvatno korelirale sa pozitivnošću, izuzev σ, dok je poduzorak sa prisustvom somatskih tegoba demonstrirao više skorove crtama α i η, upućujući na viši nivo anksioznosti i socijalne usklađenosti. Na kontrole efekata pola i starosti, crte ličnosti zajedno sa prisustvom somatskih tegoba objasnile su ukupno 26% varijanse pozitivnosti, koju pozitivno predviđa viša aktivnost ε, te negativno viša aktivnost α i δ. Moderacioni uticaji prisustva somatskih tegoba bili su detektovani u dve specifične relacije. Pozitivnost se mogla negativno predvideti aktivnošću homeostatske koordinacije sistema (δ), ali samo kod studenata sa malim brojem somatskih tegoba, što je bio i slučaj sa sistemom regulacije organskih funkcija (χ), ali samo kod snažnog prisustva somatskih tegoba. **Zaključak.** Pozitivnost predstavlja dobru organizacionu i regulacionu dispoziciju za regulaciju kognitivnih, konativnih i motoričkih funkcija. Takođe, delom je moderira

Correspondence to: Vesna Petrović, Faculty for Law and Business Studies "Dr Lazar Vrkatić", Department for Psychotherapy, 21 000 Novi Sad, Serbia. E-mail: v.petrovic581@gmail.com.

na prisustvom somatskih tegoba. Praktične implikacije ovih nalaza predstavljaju konkretnu podršku za razvoj pozitivnosti kod ljudi kako bi se osnažilo zdravlje ljudi.

Ključne reči:
ličnost; psihofiziološki poremećaji; optimizam; ankete i upitnici.

Introduction

An interesting question that attracted a researcher's attention for a long period of time was about the differences in personality traits and their role in promoting health and in moderating vulnerability to adversities and illness. A personality can be viewed as the self-regulating system that is responsible for many different ways that people manage themselves and interact with the outside world. Also, individual differences in personality traits, self-beliefs, attitudes and habits are in different levels associated with biological variations affecting health and well-being.

In a psychological literature, it is well-known that self-esteem [1–3], life satisfaction [4] and dispositional optimism [3] are repeatedly associated to well-being and successful adaptation. Life satisfaction refers to a person's general evaluation of various activities and relationships that make someone's life worth living [5]. Self-esteem denotes an individual's general self-regard and the level of self-acceptance [6]. Optimism refers to one's perspective on future personal and social events, in which there will be an abundance of good things and a scarcity of bad things [7].

Also, in a few studies were shown a relatively high level of inter-correlation among the judgments people hold about themselves, their life and their future [8, 9]. Caprara et al. [10] further explored mentioned inter-correlation and discovered that this judgment leads to a common latent component affecting the ways people understand their experiences and prepare them to action. This component was originally named positive thinking and then positive orientation or positivity [10–12].

A question that further attracted attention of the researchers was a relation of positivity with other basic dispositions like the ones that are commonly investigated under the Five-factor model [13, 14].

The results of research obtained on a large sample of Italian participants (n = 3,589; 58% women) aged 17 to 75 years (mean = 39.01 years) suggested that all dimensions of the Five-factor model correlated with positive orientation[15]. The psychological instruments used in the study were: the Positivity Scale, a short measure of positive orientation [15] and a short version of the Big Five Questionnaire [16]. Results revealed positive correlations between positive orientation and: energy (equivalent of extraversion; 0.38), emotional stability (reverse of neuroticism; 0.30), agreeableness (0.29), conscientiousness (0.25) and openness (0.19) (all $p < 0.01$) [15]. Summarizing the results, we can see that positive orientation is correlated with all of the basic personality traits in the Five factor model. Similar research was done by Miciuk et al. [17], although with somewhat different results. In this research the strongest correlations were also obtained between positive orientation, neuroticism and extraversion. However, it is important to note, that Caprara et al. [18] emphasize that Positive orientation, differs from other basic dispositions like the ones that are commonly investigated under the Five-factor model [13, 14]. Whereas the so-called Big Five is related to behavioral dispositions that enable people to deal with the fundamental tasks of agency and communion, positive orientation represents a basic attitude that is present and is important in facing major challenges of human life like illness, aging and death.

In our research we did not want to replicate previous research which used the so called Big Five Model, but deliberately, we used another model of personality. The idea behind this choice was to test the relation between positivity and personality in the context of another model and learn something more and further understand a status or a role of positivity. The model of personality traits used in this research is a reformulated basic cybernetic personality model [19], originally formulated by Powell and Royce [20]. We chose this model as it is designed inside the conceptual frame of neurological systems of regulatory functions and has a good instrument which has been successfully and frequently used in Serbia and ex-Yugoslavia. The instrument assesses six personality dimensions which are conceptualized as manifestations of six neurological systems of regulatory functions. As we were interested also in the role that somatic complaints play in and its relation to personality dispositions and positivity, it looked like an interesting and good opportunity to use this model and understand better the relation between the positivity, personality and somatic complaints.

An important question for individual existence and functioning is on what structure, attitude, belief, view, individuals can rely on to sustain a good health. Having that in mind, an important question is, what is an impact of Positivity to human's health?

Research done in the context of biological base of positivity further supported the importance of Positivity for human health. Studies addressing biological base of Positivity attested biological underpinning of Positivity and its protective function under stress through immune response [21]. Also, an EEG study connected positive orientation to the activity of the brain structures that previous studies cite as mostly engaged in self-evaluative process [22].

Having in mind all of the findings mentioned, the aim of our research was twofold: 1) investigation of the relations between personality traits and positive orientation in a Serbian sample, and 2) evaluation of potential

moderating effect of present somatic complaints in this relation.

Methods

The total of 512 students were included in our investigation. All participants were Serbian speaking individuals, in average 23.83 years old [mod = 21, standard deviation (SD) = 3.92, ranging from 18 to 40 years], 56.3% were female. The research was organized by following the principles of voluntaristic participation, without any kind of compensation. The total sample was collected by students of the Faculty for Law and Business Studies "Dr Lazar Vrkatić" in Novi Sad, Serbia, engeged in the snowball sampling principle [23]. The study was approved by the local Ethic Committee.

In our investigation we used the Positivity Scale [15] and the Cybernetic Battery of Conative Tests (KON-6) [24].

The Positivity Scale [15] is an 8-items self report questionaire aimed to assess positive orientation, or in short the positivity. The respondents provide their responses by using a 5-point scale ranging from 1 (strongly disagree) to 5 (strongly agree) for each of the items. All of the items are positively worded, except one (At times, the future seems unclear to me) which needed recoding into reverse values. The total score (ranging from 8 to 40) indicates the level of general positive orientation towards self (eg. On the whole, I am satisfied with myself), other people (eg. Others are generally here for me when I need them) and the future (eg. I have great faith in the future). The instrument was translated to Serbian by back-translation procedure with the consent of the author and under his supervision. In this research, the scale demonstrated an acceptable internal consistency (Cronbach $\alpha = 0.77$).

The KON-6 [24] was used to evaluate the conative personality dimensions. The instrument is designed to assess the 6 personality dimensions which are conceptualized as manifestations of 6 neurological systems of regulatory functions. The model was presented by Horga et al. [19], as a result of reformulation of basic cybernetic personality model by Powell and Royce [20]. The activity of every system is operationalized with 30 items worded in the same direction, followed by a 5-point scale ranging from 1 (Not true at all) to 5 (Absolutely true), forming a score in a theoretical range from 30 to 150.

Activity regulation system (epsilon, ε) is conceptualized as the basic regulation system regulating the activation role of the reticular formation. The activity of ε directly determines the activation levels of the other remaining subsystems, including the cognitive and motor processors. Its basic function is keeping the balance between excitation and inhibition of neural activity and is related to the speed of informational processing within the central nervous system. Dysregulation of ε could lead to depressive or hypomanic reactions and could also affect the cognitive and motoric functioning. Behaviorally, this system is manifested throughout the extraversion–introversion dimension. Higher scores on this dimension indicate extraverted personality operationalized by items like "I like to make people laugh" or "I like to make contacts with various unknown people".

Organic function regulation system (chi, χ) is conceptualized as a functional interaction of cortical systems for organic control and regulation with subcortical centers for the regulation of organ functions, predominantly located in the hypothalamic region. Dysregulation of χ, indicated by higher scores on this dimension, could lead to functional disorders of the basic organic systems, disorders of the sensory and motor system, and also could lead to increase of ideas hypochondria and subsequent hypochondriac behavior. This system is operationalized with items like "Sometimes my hearts pounds so intensively that I have the impression it will explode" or "Something is wrong with my senses".

Defence reactions regulation system (alpha, α) is conceptualized as a defense reaction center located in the limbic system. It modules the tonic excitation, probably on the basis of reactions formed under the influence of various forms of conditioning. This system is responsible for all behavioral patterns caused or followed by anxiety, and regulates the reactions to situations which include explicit or implicit threat to the physical or psychological integrity. Most of the neurotic disorders could be associated to dysregulation of α, especially various states characterized by high levels of fear, anxiety, as well as emotional and sensory hypersensitivity. Higher scores indicate potential dysregulation of the system, which is operationalized with items like "My feelings can be easily hurt" or "I am always frightened of doing something stupid".

Attack reaction regulation system (sigma, σ) is conceptualized as an attack control center, also potentially located in the limbic system. It modulates and controls various behavioral patterns based on aggressive impulses, triggered by various stimuli and situations which a person interprets as a signal of frustrations. Dysregulation of this regulation system indicated by higher scores is manifested in various forms of aggressive reactions, externalizing behavior patterns, and also in poor control of impulses. The system is operationalized with items like "I like to participate in a fight" or "I often have trouble because I cannot keep my mouth shut" or "Even when they help others, people do so for their own advantage".

System for homeostatic coordination of regulation systems (delta, δ) is conceptualized as a system of higher order which coordinates the functions of the other cognitive and conative subsystems that differ, functionally of hierarchically, including the cognitive processors functions. This system is functionally superior to the organic functions regulators such as α and σ. Its basic function is the homeostatic regulation of all behavior patterns, as well as synchronizing uncoordinated behavior. Dysregulation of this system indicated by higer scores on this dimension could cause disorganization and dissociation of both cognitive and conative functions, as well as disorders of motoric functions. The system is operationalized with items like "I absolutely

cannot do anything right" or "Someone is trying to influence my thoughts".

System for integration of regulation systems (eta, η) is conceptualized as the highest in hierarchy of conative regulatory functions, potentially located in the frontal cortex. Its basic function is to integrate conative processes into a coherent psychological field, as conceptualized in gestalt psychology. It is mainly determined by socialization and other social factors such asconditioning, reinforcement, internalization within the educational process. Dysregulation of this system indicated by higher scores on this dimension could cause various forms of social incompatibility. The system is operationalized with items like "I have entered the wrong public transport vehicle much more than once" or "I find it difficult to formulate what I want even in casual conversations".

The presence of somatic complaints was assessed by an open ended question regarding the presence of an acute somatic complaints or chronic illness. The total of 118 participants (23%) reported the presence of somatic complaints. The results were summed into a binary variable indicating the presence of somatic complaints.

Statistical analysis

In statistical analysis, descriptive data analysis was included calculating the mean value and SD.

In order to test whether the presence of somatic complaints moderates the relation between conative personality dimensions and positivity, a hierarchical linear regression analysis [25] was performed. Significant interaction effects were presented on two separate graphs. In order to reduce the possibility of data multicollinearity, the moderation analysis was performed on mean centered data [26].

Statistical analysis was carried out using IBM SPSS (Statistical Package for the Social Sciences) software version 20.0.

Results

As we can see from Table 1, positivity was significantly associated in an adequate manner with all of the personality traits, except for σ.

Table 1

Spearman product-moment correlation between positivity and personality traits

Parameters	ε	χ	α	σ	δ	η
Personality traits						
ε	1	-0.14**	-0.28**	0.20**	-0.25**	-0.13**
χ		1	0.66**	0.36**	0.63**	0.66**
α			1	0.32**	0.55**	0.70**
σ				1	0.41**	0.39**
δ					1	0.69**
Positivity	0.41**	-0.31**	-0.37**	-0.02	-0.36**	-0.26**

ε – activity regulation system; χ – organic function regulation system;
α – defense reactions regulation system; σ – attack regulation system;
δ – homeostatic system coordination; η – regulation system integration.
**** *p* < 0.01.**

The mean differences in positivity and personality traits between participants with somatic complaints (n = 118) and without them (n = 394) are presented in Table 2. The Levene's test of variance equality demonstrated the inequality only for χ personality trait, but not affecting the mean difference. As we can see, the subsample suffering from somatic complaints demonstrated significantly higher scores in α and η traits, suggesting higher levels of anxiety and social reality impairment for this subsample.

Table 2

Mean differences in personality traits and positivity between subsamples

Parameters	Somatic complaints mean (SD)	No somatic complaints mean (SD)	F	*p*	*t*	*p*
Personality traits*						
ε	111.69 (18.12)	111.76 (17.61)	0.62	0.42	-0.03	0.87
χ	55.88 (14.53)	54.21 (18.58)	5.17	0.02	0.89	0.30
α	76.41 (19.30)	70.92 (20.82)	0.36	0.55	2.53	0.01
σ	90.55 (18.72)	87.16 (18.86)	0.01	0.91	1.70	0.09
δ	50.13 (17.17)	48.47 (16.31)	0.37	0.54	0.95	0.34
η	62.13 (15.37)	58.48 (16.76)	2.65	0.10	2.11	0.03
Positivity	32.01 (4.14)	31.93 (4.25)	0.55	0.46	0.19	0.84

***For explanation see under Table 1.**
SD – standard deviation.

The analyses tested tree subsequent hierarchical models are presented in Table1. The purpose of the first and also the lowest model was to control the effects of gender and age to subsequent relations. The second model was built up to the first and aimed to evaluate the main effects of conative personality dimensions and presence of somatic complaints. The third model was built by adding the interactions between personality predictors and the presence of somatic complaints to the second model. The ANOVA suggested the significance of only two subsequent models (Table 3).

Table 3
The significance of the models predicting positivity

Model		Sum of squares	df	Mean square	*F*	*p*
1	Regression	30.68	2	15.34	0.90	0.41
	Residual	8426.27	492	17.13		
	Total	8456.95	494			
2	Regression	2242.83	9	249.20	19.45	0.00
	Residual	6214.12	485	12.81		
	Total	8456.95	494			
3	Regression	2477.08	15	165.14	13.23	0.00
	Residual	5979.87	479	12.49		
	Total	8456.95	494			

Model 1: age, gender; Model 2: age, gender, personality dimensions, presence somatic complaints; Model 3: age, gender, personality dimensions, presence somatic complaints, interaction of personality dimensions and presence of somatic complaints.

As we can see on Table 4, the personality dimension and somatic complaints explained 26% of positivity variance. By adding the interactions between the predictors, the total of the variance explained rose for 3% of additionally explained criterion variance. By reaching the statistical significance, this change in explained variance suggested the possible moderation role of somatic complaints.

In more details, the models are presented in Table 5. The second model suggested that the higher levels of ε would predict the higher level of positivity, while the levels of α and δ predict positivity in the reverse manner. The activity of other systems as well as the presence of somatic complaints did not reach the statistical significance of prediction. On the other hand, there were some interesting points. First of all, the standardized regression coefficients of ε and α remained relatively unchanged, determining that their main effects on positivity were direct and not by any mean moderated by somatic complaints. Secondly, there was absence of main effects of other systems. This also included δ, whose main effect was overtaken by the interaction with somatic complaints. Finally, there were two mild but significant interaction effects of χ and δ systems with the presence of somatic complaints. These moderation effects are illustrated in Figures 1 and 2.

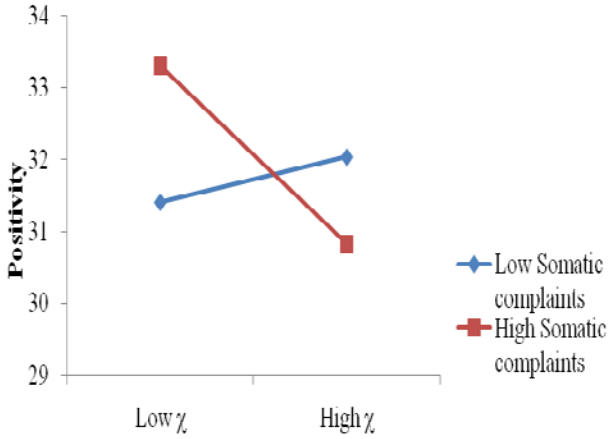

Fig. 1 – The moderation role of somatic complaints in the relation between the organic function regulation system (χ) and positivity.

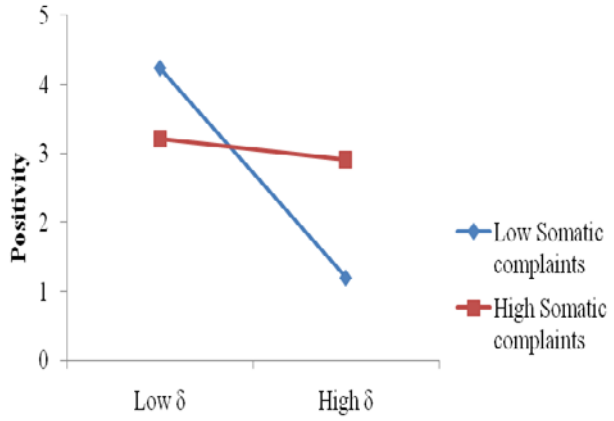

Fig. 2 – The moderation role of somatic complaints in the relation between the homeostatic coordination of regulation systems (δ) and positivity.

As presented in Figure 1, the higher activity of the organic function regulation system (χ) predicted lower positivity, but only in the case of presence of somatic complaints, while in the case of low somatic complaints, the relation between this system and positivity could not be detected.

Table 4
The parameters of models predicting positivity

Model*	R	R^2	AR^2	SE	Change Statistics				
					ΔR^2	F	df1	df2	*p*
1	0.06	0.00	0.00	4.14	0.00	0.90	2	492	0.41
2	0.52	0.26	0.25	3.58	0.26	24.66	7	485	0.00
3	0.54	0.29	0.27	3.53	0.03	3.13	6	479	0.00

***For explanation see under Table 3.**

Table 5

Standardized regression coefficients in models predicting positivity

Model*	β	*t*	*p*
1			
Intercept		21.91	0.00
Gender	0.06	1.34	0.18
Age	0.00	0.07	0.95
2			
Intercept		25.56	0.00
Gender	0.08	2.03	0.06
Age	-0.04	-1.04	0.30
Personality traits			
ε	0.26	6.08	0.00
χ	-0.06	-1.06	0.29
α	-0.23	-3.62	0.00
σ	0.07	1.41	0.16
δ	-0.18	-2.85	0.00
η	0.05	0.81	0.42
Somatic complaints	0.03	0.86	0.39
3			
Intercept		26.16	0.00
Gender	0.08	1.96	0.05
Age	-0.06	-1.43	0.15
Personality traits**			
ε	0.24	4.35	0.00
χ	-0.23	-2.68	0.01
α	-0.23	-3.11	0.00
σ	0.05	0.88	0.38
δ	-0.09	-1.39	0.16
η	0.03	0.39	0.69
Somatic complaints	0.04	1.03	0.30
somatic complaints x ε	-0.05	-1.00	0.32
somatic complaints x χ	-0.22	-2.56	0.01
somatic complaints x α	0.00	0.06	0.95
somatic complaints x σ	-0.00	-0.11	0.91
somatic complaints x δ	0.19	2.85	0.01
somatic complaints x η	-0.11	-1.55	0.12

***For explanation see under Table 3;**
****For explanation see under Table 1.**

On the other hand, the higher activity of the homeostatic coordination of regulation systems (δ) predicts lower positivity, but only in the case of absence of somatic complaints, while in the case of high somatic complaints, the relation between this system and positivity cannot be detected.

Discussion

In this research, the correlation between personality traits and positive orientation in a sample of Serbian students was investigated and explored moderating effects of somatic complaints in the relation between personality traits and positivity.

Here is also worth to note that excellent psychometric properties and structural validity of the positivity scale were confirmed across various cultural contexts including Brazil, China, Columbia, Germany, Izrael, Italia, Japan, Mexico, Poland, Pakistan, Spain, Turkey, the Nederlands, UK and the USA. In most of these contexts, the scale demonstrated an unidimensional structure as well as excellent psychometric properties [27–31].

As it can be seen in the results, epsilon, which is conceptualized as the basic regulatory system which regulate the activating role of the reticular formation, has a direct relation to positivity, as the higher level of epsilon predicts the higher level of positivity.

Behaviorally, the epsilon system is manifested throughout the extraversion–introversion dimension. Having in mind that the activity of epsilon is defined in the model of personality dimensions that was used in this research, like the one that directly determines the activation

levels of the other remaining subsystems, including the cognitive and motor processors, the role of the positivity in relation to personality traits could be understood better like the one of the self-regulative system. Some kind of indirect support could be the findings obtained in a recent study that used the so called Big Five Model [32]. In that research, a latent variable representing the positivity construct fully mediated the relation of extraversion with happiness. Anyway, positivity and extraversion are directly related.

Alpha, defined like the regulation system of defense reactions is directly related with Positivity, but in different direction. It is expected results, as alpha is defined also as responsible for all behavioral patterns caused or followed by anxiety. According to Caprara et al. [18], positive orientation represents a basic attitude that is present and is important in facing major challenges of human life like illness, aging and death. Positive attitudes and reactions in facing challenges are not related to fear and anxiety although they could represent defense reactions, but, of the opposite directions. And, according to authors of the Cybernetic battery of conative tests, most of the neurotic disorders could be associated to dysregulation of alpha. It seems that non-neurotic or emotionally stabile individuals are related to higher scores on Positivity.

Both findings, epsilon and alpha and their relation to Positivity are fully supporting the results obtained in previous research that explore relation between personality traits and Positivity [15, 17].

Delta, in the model of the personality used in the research, is conceptualized as a system of higher order which coordinates the functions of the other cognitive and conative subsystems. Dysregulation of this system indicated by higher scores on this dimension could cause disorganization and dissociation of both cognitive and conative functions, as well as disorders of motoric functions. Results obtained in the research showed that delta predicts Positivity in the opposite manner. Following this finding, positivity could be understood as a good organizational and regulational concept which regulates cognitive, conative and motoric function.

The results revealed that activity of other systems presented in the model of personality used in this research, as well as the presence of somatic complaints did not reach statistical significance of prediction for Positivity. This means that chi, sigma and eta did not predict the Positivity. Chi mainly regulates organ functions and could lead to increase of ideas of hypochondria and subsequent hypochondriac behaviour. Sigma is mainly regulating attack reactions and is manifested in various forms of aggressive reactions and poor control of impulses. Eta, or system for integration of regulation systems, is mainly determined by process of socialization. Dysregulation of eta manifests itself in various forms of social incompatibility. To some extent these results were expected. It seems that Positivity was not in a direct relation with ideas of hypochondria, aggressive reactions and social incompatibility. On the other hand, it could be seen that the presence of somatic complaints did not relate directly to positivity in any manner.

But, the picture is changed in the light of the results of standardized regression coefficients. It became obvious that only epsilon and alpha were directly related to Positivity and not moderated by somatic complaints.

Commenting the relation of delta with Positivity, it could be seen that its main effect was overtaken by the interaction with somatic complaints. What is the meaning of this finding?

One hypothesis is that somatic complaints represent some kind of or are attempt to balance the disorganization and dissociation of both cognitive and conative functions, as well as disorders of motoric functions. In this case, where there is a presence of some somatic complaints, there is no relation between the Positivity and delta. Somatic complaints are supporting delta, or are a support for disorganization and dissociation in personality. It could be one more reason to conclude that Positivity represents a good organizational and regulation concept which regulates cognitive, conative and motoric function. Also, further hypothesis is that somatic complaints are a sign that there are some reasons to believe that there is no good organization in personality functions or there is some dissociation.

Further, commenting the relation of chi with Positivity, it is clear that the higher activity of the organic function regulation system (χ) predicts lower positivity, but only in the case of presence of somatic complaints, while in the case of low somatic complaints, the relation between this system and Positivity cannot be detected. Again, one could hypothesized that Positivity is protecting health, as there is no relation between chi and Positivity when there is a low level of somatic complaints. And, vice versa, when there is a higher chi, and higher somatic complaints, there is lower level of Positivity.

A crucial point of the discussion can be a hypothetical status of positive orientation and its relation to health. As Caprara et al. [10, 15] argue, positive orientation fulfills important biological functions, for example it underlies an individual's need to grow, to flourish, to successfully cope with life in spite of occurring adversities, failures, and losses, as well as to keep on caring about living in the face of aging and closeness of death. Authors of previous research already cite that Positive orientation is not a trait but it probably represents the same level of personality – basic dispositions or processes [33–36]. Results in this research give the base for the hypothesis that Positive orientation represents a good organizational and regulation dispositions or a process which regulate cognitive, conative and motoric function. Also, somatic complaints could be a sign that there are no good regulations of personality functions.

Limitations of the study

All of the presented findings should be viewed with having in mind a few shortcomings of this research. First of all, this study was performed predominantly on general population of students, with only 23% of them suffering from some kind of somatic complaints. Although they did not differ significantly from the rest of the sample in

personality scores or positivity, we must have in mind that to some extent different results could be gained if this subsample would be equally represented in the total sample. Secondly, the student population is generally healthy, so the somatic complaints within our sample are to great extent represented with minor health problems. This shortcoming should direct some future research to include more complex samples representing general population, especially elderly people, as we have in mind that the increase of somatic complaints will be pronounced both in frequency and in intensity as people get older. Thirdly, we are aware that these findings should be retested on various clinical samples suffering from some kind of acute or chronic somatic complaint, in order to investigate is there any specific connection between personality and positivity under these specific circumstances. The last but not the least, we find that the transversal research design is the biggest shortcoming of our research. As all of the variables were assessed at the same point in time, we could not make any conclusion of potential causality or time sequence of their relation. For instance, it would be interesting to see does the appearance of somatic complaints
alter positivity itself, as well as its relations to personality, so the biggest suggestion for future research is to investigate these relations by using a longitudinal research design.

Conclusion

A better understanding of the relationship between positive orientation and the personality traits requires further explorations. The data about this relationship might help us to better understand even Positivity itself. Also, further exploration of the function and place of positive orientation for human's health is precious. This kind of research could strongly benefit psychological practices and psychotherapy aiming at inspiring people to be healthy, self-regulated, living good and full lives.

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Benefits of self-etch adhesives active application with rotary brush to enamel

Korist od aktivne primene samovezujućeg adheziva na gleđ korišćenjem rotirajuće četkice

Muhammet Kerim Ayar

Usak University, Faculty of Dentistry, Department of Restorative Dentistry, Usak, Turkey

Abstract

Background/Aim. Theoretically, agitation of self-etch adhesive and solutions can be significantly accelerated by the use of the rotary brush. The aim of this study was to evaluate application of a universal adhesive in a self-etch mode and a self-etch adhesive using a rotary brush active application technique by microshear bond strength (μSBS) test. **Methods.** The crown parts of 20 bovine teeth were separated from their roots, embedded in acrylic blocks and enamel surfaces were prepared. The prepared crowns were divided into four groups according to the adhesive system tested (Nova Compo-B Plus, and Optibond All-in-one), and application technique (manual active application and rotary brush active application). Bonded samples were immersed in distilled water for 24 h before bond strength testing by μSBS test at 1.0 mm/min. Data were analyzed using non-parametric tests ($p = 0.05$). **Results.** Both adhesives, applied by rotary brush active application technique, showed significantly higher enamel bonding strength compared to the manual active application technique. **Conclusion.** Application of self-etch adhesives to enamel with a rotary brush active application technique can significantly increase initial resin-enamel bond strength compared to the manual active application.

Key words:
adhesives; dental enamel; dental materials; materials testing.

Apstrakt

Uvod/Cilj. Teorijski, mućkanje samovezujućeg adheziva i rastvora može biti značajno ubrzano korišćenjem rotirajuće četkice. Cilj studije bio je procena primene univerzalnog adheziva u samovezujućem modu i samovezujućeg adheziva primenom tehnike aktivne aplikacije rotirajućom četkicom, korišćenjem testa mikro-smicanja (μSBS). M**etode.** Delovi krunica 20 goveđih zuba odvojeni su od korenova, ugrađeni su u akrilatne blokove i pripremljene su površine gleđi. Pripremljene krunice su podeljene u četiri grupe prema testiranom adhezivnom sistemu (*Nova Compo-B Plus* i *Optibond All-in-one*) i tehnici primene (ručna aktivna aplikacija i aktivna aplikacija rotirajućom četkicom). Uzorci su, potom, bili potopljeni u destilovanu vodu 24 sata pre ispitivanja jačine veze μSBS testom na 1,0 mm/min. Podaci su analizirani pomoću neparametrijskih testova ($p = 0,05$). **Rezultati.** Oba adheziva, primenjena tehnikom aktivne aplikacije rotirajućom četkicom, pokazali su značajno veću čvrstoću vezivanja za gleđ u poređenju sa tehnikom ručne aktivne aplikacije. **Zaključak.** Aplikacija samovezujućih adheziva na gleđ tehnikom aktivnog nanošenja rotirajućom četkicom može značajno više povećati inicijalnu snagu vezivanja smole za gleđ u poređenju sa tehnikom aktivne ručne aplikacije.

Ključne reči:
adhezivi; zub, gleđ; stomatološki materijali; materijali, testiranje.

Introduction

Resin adhesive systems available in the dental market are divided into two main categories depending on bonding strategies: etch-and-rinse (ER) and self-etch (SE) adhesive systems. ER adhesive systems have two or three application step versions, and SE adhesive systems have one or two application steps versions [1, 2]. Newly, manufacturers have introduced more versatile adhesive systems, able to be used in both bonding strategies. The manufacturers suggest that clinicians may choose to use bonding strategies according to their preference and type of tooth tissue [3, 4]. Recently introduced to the dentistry market, this novel group of dental adhesives are called “multi-mode” or “universal” adhesives and constitute the newest generation of adhesives [5, 6].

Correspondence to: Muhammet Kerim Ayar, Usak University, Faculty of Dentistry, Department of Restorative Dentistry, 64 200 Usak, Turkey. E-mail: Muhammet.ayar@usak.edu.tr and mkayar82@gmail.com.

However, studies have shown that these new adhesives provide lower bond strength when applied to enamel in self-etch mode [5, 7, 8]. This is similar to the low bond strength of the previous single-stage self-etch adhesives with enamel [9, 10]. Compared to phosphoric acid, self-etch adhesives and universal adhesives have lower acidity. Due to these characteristics, the demineralization capacities are not sufficient to produce satisfactory micro-retentive porosity. As a result, the force they provide for enamel bonding is not at the desired level [2, 11].

This may not be considered as a new problem; the successful methods for solving such problems have already been tested to increase the enamel bonding, such as application of phosphoric acid etching prior to adhesive application [3, 12]. On the other hand, in clinical studies, it was not detected that the universal adhesives used by selective enamel-etching had a positive effect on the survival time of cervical restorations [13–15]. However, it has been found to reduce the marginal staining [15, 16]. In addition, phosphoric acid pretreatment increases the number of clinical application steps of the adhesive system.

In recent studies, some researchers have shown that when universal adhesives are used in self-etch mode, active application may be an alternative to selective enamel-etching technique in terms of enamel bonding strength [12]. It has already been shown that the active application increases the bond strength of one-step self-etch adhesives with low enamel bonding strength [17, 18]. In a clinical trial, it has been reported that active application of one-step self-etch adhesive provides a higher 2-year retention rate and lower marginal coloration. These clinical findings support the findings of above laboratory investigations [19]. As another clinical method, self-etch adhesive agitation has been attempted with sonic waves by special device, and it has been reported to increase enamel bonding [20].

Theoretically, agitation of self-etch adhesive and solutions can be significantly accelerated by the use of a rotary brush. The bristles of the brush may disrupt the integrity of the smear layer on the enamel surface during rotation. At this time, a circulation in the adhesive solution can be made between the fresh acidic monomers on the surface and the buffered passive monomers after initial superficial demineralization on the enamel surface. As a result, a deeper and more continuous interaction can take place between the enamel and the acidic monomers. All of these facts can improve the enamel bonding of self-etch adhesives and solutions, which do not have good enamel bond strength. For this reason, the aim of this study was to analyze how the active application of self-etch adhesives and universal adhesives, applied in self-etch mode with rotating brushes, affects the bond strength of these adhesives.

Methods

Study design

The adhesive system (brand of adhesives) and the adhesive application mode (application according to manufacturer instructions and rotary micro-brush application) are independent variables. The microshear bond strength (μSBS) mean was dependent variable. The contents of the adhesive systems and user instructions are shown in Table 1. In total, there were four groups in the study. Five teeth were used for each group. In the microshear test, more than one composite button could be placed on a single tooth. According to the width of the enamel surface, 6–9 bonding test specimens *per* tooth were prepared.

Table 1

Description of the tested adhesive systems

Adhesive	Chemical composition	pH	Instructions for use
Nova Compo-B Plus (Imicryl, Konya, Turkey)	Bis-GMA, HEMA, ethanol, 10-MDP, 4-META, silanated nano silica, initiators, water.	2.5–2.7	Apply with agitation for 20 s. Gently air-dry for 5 s. Light cure for 10 s.
Optibond All-in-one (Kerr, Orange, CA, USA)	GPDM, HEMA, GDMA, Bis-GMA, water, acetone, ethanol, CQ, silica filler	2.5–3.0	Apply with agitation for 20s. Start with gentle air blowing, followed by stronger air blow.

10-MDP – 10-methacryloyloxydecyl dihydrogen phosphate; Bis-GMA – bisphenol glycidyl methacrylate; HEMA – 2-hydroxyethyl methacrylate; 4-META – 4-methacryloxyethyl trimellitate anhydride.

Sample preparation

In total, 20 extracted bovine incisors were used. The teeth were kept in a solution of 0.1% sodium azide for a maximum of six months after tooth extraction prior to be used. The root parts of the teeth were cut from the crown parts by cutting with a low-speed diamond disc under water cooling. The crowns were individually embedded in the plexi molds with autopolymerized acrylic, with the vestibular surface of the tooth upward. The exposed enamel surfaces were first smoothed with 180-grit silicon carbide abrasive paper, then polished with 600-grit silicon carbide abrasive paper for one minute under water-cooling.

Resin-enamel microshear bond strength (μSBS)

Procedures of resin-enamel μSBS test and bonding procedures were shown in Figure 1. Universal adhesives were applied in self-etch mode according to their manufacturer's instructions, except for rotary brush groups (Table 1). A single operator applied all bonding procedures as described below.

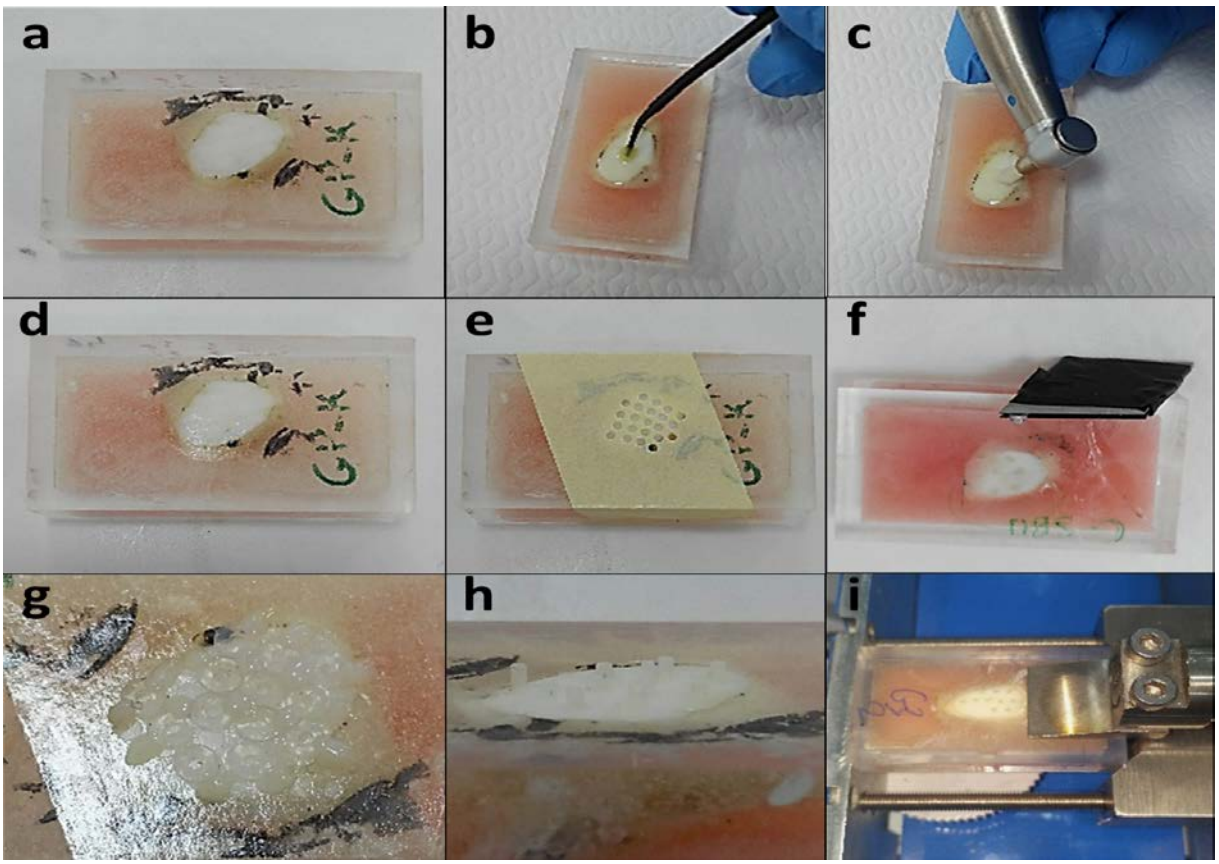

Fig. 1 – Procedures of specimen preparation for microshear bond strength test and rotary-brush active application technique: (a) the crowns of the bovine incisors were embedded in acrylic as seen in the Figure, and the enamel surface was finished with 600-gram SiC abrasive paper under water cooling; (b) in manual active application groups, adhesive was applied to enamel surfaces with an applicator; (c) in rotary brush active application groups, adhesive was applied to enamel surface with micro-brush with 3-mm diameter. The brush was applied to the entire enamel surface with light pressure and sweeping movements until the solvent of the adhesive was visibly evaporated. This time was 30–40 seconds at 1,200 revolutions per minute (rpm); (d) after the adhesive is applied to the enamel surface by a rotary brush the adhesive was seen before it is polymerized; (e) in order to keep the Tygon tubes fixed on bonding surface during packing resin composite, a double-sided tape with holes with diameter of 1.5 mm was placed on the surface. The area where the holes were placed was limited to the enamel surface; (f) 0.5 mm high Tygon tubes are prepared with a simple utility knife assembly; (g) Tygon tubes were placed onto holes on the adhesive tape by using a 2.5× magnification loop. Flowable composite filled spaces between tubes to ensure better fixation of the Tygon tubes; (h) the finished, composite buttons are seen in the figure. Adhesive and composite placement took an estimated 40–60 min for a micro-shear sample of a single tooth; (i) the prepared micro-shear test sample was fixed to the test device as shown in the figure. Shear force was applied to each sample by knife at a speed of 1 mm / min from the closest possible location of the resin-mine until the sample failed.

Manual active application: adhesives were actively applied to the enamel according to the manufacturer's instructions.

Rotary brush active application: the 3 mm-diameter prophylaxis brush was attached to the micromotor-mounted contra-angle handpiece operating at 1,200 revolutions per minute (rpm). An adhesive was dispensed to the enamel surface with a regular applicator and then agitated with rotary brush in sweeping motion. The application took 20–30 s on average. When there was no visible solvent on the surface, the application was finished. Furthermore, the adhesive was not dried by air-water spray to evaporate the solvent. After the adhesive application procedures were finished, the adhesives were polymerized with the LED light source for 20 s.

With the rubber dam puncher, 6–8 holes having inside diameter of 1 mm were punched on the double-sided adhesive tape. This adhesive tape was adhered to the prepared enamel surface after adhesive polymerization. The number of perforations was adjusted according to the width of the enamel surface. After adhesive polymerization, Tygon tubes with an inner diameter of 0.8 mm and a height of 0.5 mm were placed corresponding to the holes on the double-sided tape. Because this procedure is sensitive, the operator used a dental loupe with 2.5× magnification during the application.

For the Tygon tubes to remain stable, a flowable composite was placed in the spaces between the Tygon tubes. In this way, the possibility of moving the Tygon tubes when placing the composite into them was minimized. Each adhesive was used with the resin composite of its own manufac-

turer (Table 1). Resin composites were carefully placed into the Tygon tubes by the operator using the dental loupe. The composites were polymerized with 20 s LED light source. After polymerization of the composites, the Tygon tubes were cut with scalpel. Bonded samples were immersed in distilled water for 24 h before bond strength testing.

The bonded samples were fixed to the shear-test jig and tested on the universal test device. The blade was placed as close as possible to the resin-enamel surface interface. The speed of the crosshead was set to 1 mm/min. The blade was moved downwards until it broke the composite. The μSBS values were calculated as the MPa unit by dividing the load causing the failure by the surface area. In this way, shear bond strength was determined. Failure modes, only enamel or composite covering cohesive (C), resin-enamel interface that occurs in the adhesive (A) or partially enamel or composite were classified as a mix failure (M). Failure modes were determined by digital microscope with the aid of 20–30× magnification.

Statistical analysis

The normal distribution of μSBS means and the homogeneity of the variance between the averages were analyzed by the Kolmogorov-Smirnov test and Levene's test, respectively. According to the Levene's test, since the variance was heterogeneous among the means, parametric tests could not be applied to the μSBS data. Therefore, the Kruskal-Wallis and Mann-Whitney *U*-tests were applied to the data ($p < 0.05$).

Results

Findings of μSBS test are summarized in Table 2. When all adhesives were applied by rotary brush active application technique, they showed significantly higher enamel bonding strength compared to the manual active application technique ($p < 0.05$). The Nova Combo B-Plus adhesive gave significantly higher bonding strength than the Optibond All-in-one adhesive in both application techniques ($p < 0.05$). Most of the samples showed adhesive or mixed rupture (Table 2).

Discussion

In this study, the technique of applying the adhesives with a rotary brush was tested in order to increase enamel bonding strengths of a universal adhesive applied in self-etch mode and self-etch adhesive as a different method. Null hypothesis of the study was that active application with rotary brush will not have effect on the bond strength of the adhesives tested. Our results suggest that null hypothesis was rejected because the active application technique with rotary brush showed that the two adhesives tested significantly increased their enamel bond strengths.

Since pH values of the self-etch adhesives are usually mild or ultra-mild, these adhesives cannot demineralize the enamel as well as phosphoric acid. Therefore, the enamel bonding strengths of self-etch adhesives are generally lower than those of the etch-and-rinse adhesives [9, 10]. However, this problem also applies to universal adhesives applied in the self-etch mode [6]. In order to solve this problem, methods such as pretreatment of enamel surfaces with phosphoric acid, and active application of adhesive with sonic devices have been proposed [12, 20].

The problem of low enamel bonding strength, which is an issue of self-etch solutions, can be solved by active application of the rotary brush. In previous studies, it has been shown that the self-etch protocol does not produce retentive patterns on the enamel surface [8, 21]. Probably, the superficial interaction of the self-etch solutions with enamel prevents the acidic monomers from demineralizing to give the enamel sufficiently strong bond strength.

When applying the self-etch solution to the enamel surface with a rotary brush active application, it is undoubted that the brush's bristles will impair the integrity of the smear layer as a result of the rotation of the brush. As a result, the acidic monomers can pass through the smear layer to the underlying intact enamel. In addition, the rotating effect of the brush can act in the self-etch solution itself, allowing the movement of the fresh monomers towards the enamel and demineralization waste being transported outwardly. This may have led to a deeper chemical interaction between fresh acidic monomers and enamel. Finally, self-etch solution applied by rotary brush may have increased capacity to produce retentive area in the enamel for further mechanical

Table 2

Means, standard deviations of the microshear bond strength (μSBS; MPa) and failure modes

Application technique	Adhesive	μSBS (MPa) mean ± SD	n	Failure modes (%)		
				A	C	M
Manual active application	Nova Combo B-Plus	14.88 ± 6.7 [a]	32	58.2	12.7	21.8
	Optibond All-in-one	10.82 ± 5.2 [b]	40	72.7	7.3	16.4
Rotary brush active application	Nova Combo B-Plus	19.82 ± 7.8 [c]	28	67.3	9.6	19.3
	Optibond All-in-one	14.62 ± 6.0	31	61.6	10	26.7

A – adhesive failure mode; C – cohesive failure mode; M – mix failure mode; SD – standard deviation.
[a] – significant difference ($p < 0.05$) vs. the means of Optibond All-in-one (Manual active application) and Nova Combo B-Plus (Rotary brush active application); [b] – significant difference ($p < 0.05$) vs. the means of all other groups;
[c] – significant difference ($p < 0.05$) vs. the means of all other groups.

interlocking. In a previous study [20], it has been reported that active application with sonic device enables self-etch solutions to make better demineralization in enamel. The similar mechanism of action may also apply to the rotary brush active application.

The problem of low enamel bonding strength of self-etch solutions can be solved by phosphoric acid pretreatment. But this solution makes the adhesive application less user-friendly. Furthermore, poor taste of phosphoric acid gel is not comfortable for the patients. Application of a self-etch solution with a rotary brush has no such disadvantages. Moreover, this new technique evaporates the solvent in the adhesive solution during accelerated active application. It does not require the solvent to be dried by air spray after adhesive application. When the adhesive is applied for a sufficient time with a rotating brush, the enamel surface covered by uncured adhesive solution has a clinical appearance as if it were air-dried. In this respect, it can be argued that this technique makes the adhesive application procedures more user-friendly. However, the issue that the rotary brush active application may eliminate the solvent evaporation step would be an important improvement that will significantly simplify the application of self-etch solutions. For this suggestion, further studies are needed.

When the literature is reviewed, it is seen that sonic application of self-etch solutions also improves the enamel bonding performances of these adhesives [20]. In this method, sonic waves are transmitted to the adhesive solution by a special device. It has been suggested that the sound waves make a circulation in the adhesive solution, facilitating solvent evaporation. An advantage of the rotary brush active application from sonic application is that this technique does not require any special equipment. An active application of self-etch solutions can be made with a small diameter brush attached to any micromotor with adjustable rotation speed. In this technique, the smear layer is physically deteriorated, the circulation of the monomers within the adhesive solution is maintained, and the solvents in the adhesive are evaporated.

One of limitations of the rotary-brush active application technique is that it is yet a novel technique. The rotational speed of the brush, the diameter of the brush, the application pressure of the brush, the geometric structure of the cavity, the adhesive materials that being used, etc., would affect the effect of the rotating brush active application on the resin-mine bond strength. Therefore, it is seen that further studies are needed on this respect in the future.

Conclusion

Application of self-etch adhesives to enamel with a rotary brush active application technique can increase the resin-enamel bond strength compared to the manual active application. Therefore, the active application technique with rotary brush can be an alternative method to increase the enamel bonding strengths of the self-etch adhesives or universal adhesives applied in self-etch mode.

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Disclosure statement

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Surgical outcome of the transobturator tape procedure for management of female urinary incontinence – A single center experience

Ishod hirurškog lečenja urinarne inkontinencije kod žena primenom transobturatornih traka – iskustvo jednog centra

Branko Košević*, Ivica Nikolić*, Vladimir Bančević*†, Predrag Marić*, Mirko Jovanović*, Dušica Stamenković†‡, Aleksandar Spasić*, Predrag Aleksić*†

Military Medical Academy, *Department of Urology, ‡Department of Anesthesiology and Intensive Care, Belgrade; University of Defence, †Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. The transobturator tape (TOT) procedure is considered as a gold standard of surgical treatment option for stress urinary incontinence (SUI). The aim of this study was to determine the efficacy of this procedure in the surgical management of SUI by analyzing a single centre short-term results. **Methods.** From April 2011 until January 2018, 40 patients with predominantly SUI were operated by the standard TOT procedure. A polypropylene tape was placed in the mid-urethra by a percutaneous transobturator approach. The postoperative assessment considered cough tests and post-void residual urine volume at a week following the operation with additional clinical examination and urine culture at one, three and six months. **Results.** The mean age of the patients was 58 (42–78) years. Predominantly SUI was present in 32 patients (80%) and mixed urine incontinence in 8 patients (20%). At the initial (one week) assessment, the cough test was positive in 3 patients (7.5%), and 4 patients (10%) needed an indwelling urethral catheter because of voiding difficulties. At the second follow-up, 2 (5%) patients still had a positive cough test, 2 patients (5%) had still the need for an indwelling catheter because of significant postvoid residual (PVR) urine volumes and 2 patients (5%) had a positive urine culture. At the three and six months, postoperative assessment. 3 patients (7.5%) still had a positive cough test. After six months 36 patients (90%) were considered as cured, 1 patient (2.5%) improved and 3 patients (7.5%) were classified as a failure. **Conclusion.** These results concur with the results of the other published short-term studies that analyzed the surgical outcome of the TOT procedure for female urinary incontinence. This allows us to confirm that the transobturator tape technique is a safe, effective and straightforward procedure after adequate training.

Key words:
urinary incontinence, stress; female; surgical procedures, operative; suburethral slings.

Apstrakt

Uvod/Cilj. Procedura plasiranja trake suburetralno, transobturatornim putem (engl. *transobturator tape* – TOT) predstavlja jedan od zlatnih standarda hirurškog lečenja stres urinarne inkontinencije (SUI) kod žena. Cilj ove studije bio je da se odredi efikasnost ove procedure u hirurškom lečenju SUI analizom kratkoročnih rezultata dobijenih u jednom centru. **Metode**. U periodu od aprila 2011. do januara 2018. godine, standardnom TOT tehnikom operisano je 40 bolesnika sa preovlađujućom SUI. Polipropilenska traka pozicionirana je u visini srednje uretre prekutanim transobturatornim pristupom. Postoperativna procena sprovedena je nedelju dana, jedan, tri i šest meseci nakon operacije. Inicijalna procena (nedelju dana po operaciji) podrazumevala je test kašljanja i odredjivanje rezidualnog volumena urina nakon mokrenja sa dodatim kliničkim pregledom i određivanjem urinokulture posle jednog, tri i šest meseci. **Rezultati.** Prosečna starost bolesnica iznosila je 58 (42–78) godina. Sa SUI je bilo 32 (80%), a sa mešovitom inkontinencijom 8 (20%) bolesnica. Na inicijalnoj (prva nedelja) proceni, test kašljanja bio je pozitivan kod 3 (7,5%) bolesnice, a kod 4 (10%) je, zbog problema sa mokrenjem, plasiran urinarni kateter. Na drugom kontrolnom pregledu, 2 (5%) bolesnice su imale pozitivan test kašljanja, kod 2 (5%) bolesnice je ostavljen urinarni kateter zbog značajnog rezidualnog volumena urina, a 2 (5%) bolesnice su imale pozitivnu urinokulturu.

Correspondence to: Branko Košević, Military Medical Academy, Department of Urology, Belgrade, Crnotravska 17, 11 040, Belgrade, Serbia. E-mail: bkosevic@gmail.com.

Na tromesečnoj i šestomesečnoj postoperativnoj proceni, 3 (7,5%) bolesnice su i dalje imale pozitivan test kašljanja. Nakon 6 meseci od operacije, kod 36 (90%) bolesnica je konstatovano izlečenje, kod jedne (2,5%) poboljšanje, a kod 3 (7,5%) bolesnice neuspeh operativnog lečenja. **Zaključak.** Rezultati naše studije slažu se sa rezultatima drugih objavljenih kratkoročnih studija gde je analiziran hirurški ishod TOT procedure u lečenju ženske urinarne inkontinencije. Ovo nam dozvoljava tvrdnju da je TOT procedura sigurna, efikasna i jednostavna nakon adekvatne obuke.

Ključne reči:
inkontinencija, urinarna, stres; žene; hirurgija, operativne procedure; trake, suburetralne.

Introduction

Stress urinary incontinence (SUI) is defined by the International Continence Society (ICS) as "the complaint of any involuntary loss of urine on effort or physical exertion (e.g sporting activities) or on sneezing or coughing" [1]. Petros and Ulmsten [2] with their 'Integral theory' and DeLancey [3] with his 'Hammock hypothesis' determined the anatomical and structural factors for female continence and its impact on the pathophysiology of female incontinence. As a result of their research, a great variety of surgical procedures for the treatment of SUI have been developed. Recently proposed new aspect that takes into consideration that the active reflex urethral closing mechanism is the most important factor in the pathophysiology of incontinence is still too be verified [4]. The appearance and development of mid-urethral slings (MUS) had an impact on the change of approach for surgical management of SUI. Based on the 'Integral theory' by Ulmsten and Petros [5], the retropubic tapes (TVT) were released and introduced in 1996. Their role was to imitate the pubourethral ligament and became widely adopted [6]. Taking into consideration the complications associated with the retropubic approach, Delorme [7] promoted the TOT outside–in and de Leval [8] the TOT inside–out approach based on the 'Hammock hypothesis' [3, 7, 8]. No superiority between these two variations of the TOT procedure has been determined [9].

Clinical practice and worldwide publications of results for this procedure have led to that TOT is considered as one of the gold standard surgical treatment options for SUI [10].

Methods

This is a case series study that presents a single centre experience of 40 patients who underwent surgical treatment for female urinary incontinence performing the TOT procedure from April 2011 until January 2018. Patients with SUI and mixed incontinence were included. Following initial evaluation proposed by the guidelines, all patients were conservatively treated before the definitive decision for surgical treatment of urinary incontinence. This included appropriate lifestyle changes, weight reduction in obese patients and enforcement of pelvic muscle floor training (PFMT). Duration of conservative treatment in accordance with the guidelines must not be less than 8 to12 weeks [11, 12]. Our experience showed that the duration of this treatment option was exceeded in all of the patients due to the fact that the majority of them were referred from other medical institutions. In all of the patients diagnosed with mixed urinary incontinence (MUI) by initial evaluation, it was defined that they had stress-predominant MUI. In accordance with the guidelines, all were conservatively treated that also included application of antimuscarinics. The treatment not lasted less than 12 weeks. In these patients, the specialized assessment included urodynamic studies – filling cystometry. No detrusor overactivity (DO) was registered by filling cystometry in these patients. The absence of DO does not exclude MUI and in 40% of female patients with MUI, it is possible not to identify DO [13, 14]. According to the guidelines for specialized management after initial management failure, one of the possible treatment options is MUS surgery [11, 12].

Preoperative evaluation of all the patients consisted of previous medical history, clinical uro-gynecological examination, urine dipstick and urine culture, measurement of post-void residual (PVR) urine by ultrasound and cough test. In our study one patient was with concomitant vaginal prolapse (cystocele). Eight patients had previously undergone a hysterectomy. All patients with previous hysterectomies underwent cystoscopy in preoperative assessment.

All of the patients were operated on by the standard operative technique described by Delorme [7] (TOT outside–in) using a monofilament polypropylene tape and tunnellers for the tape to be exteriorized. The patients were operated under general or spinal anesthesia. A Foley catheter 16 Fr was left in place overnight and all of the patients were discharged the first postoperative day. The patients were operated on by two surgeons who finished a hands-on training course. The learning curve patients were also included in our study.

Postoperative assessment of the patients was performed in an outpatient setting a week, month, three and six months subsequently. Initial assessment performed one week after surgery included cough test and ultrasonic post voiding urine residual measurement followed by additional clinical examination and urine culture (one, three and six months after operation). The provocation cough tests were performed in the supine position with a full bladder (ultrasound confirmed).

The outcome of the operation, on the registered follow-ups, was then classified as cured, improved or failure. Cured was defined if the patient declined that there was leakage in everyday activities and during the provocation cough tests, improved if the leakage was less than prior to the operation and used less protection, and failure was considered if there was no postoperative improvement.

Prior to the operation, a written consent form was obtained from all of the patients included in this case series.

Results

In the monitored group, 40 patients underwent surgical treatment for urinary incontinence by the standard TOT outside–in operative technique. The baseline patients' characteristics are shown in Table 1.

Table 1
Baseline patients' characteristics

Parameter	Values
Age (years), mean (range)	58 (42–78)
Type of incontinence, n (%)	
SUI	32 (80)
MUI	8 (20)
Type of anesthesia, n (%)	
general	33 (82.5)
spinal	7 (17.5)
Previous hysterectomy, n (%)	8 (20)

SUI – stress urinary incontinence;
MUI – mixed urinary incontinence.

All of the patients in preoperative assessment had a positive cough test, negative urine culture and without significant postvoid urine volume (< 100 mL).

The results of postoperative assessment performed one week, a month, three and six months following the operation are shown in Table 2. Analyzing these results, we could confirm that there was no significant difference in the positive cough test in 3 (7.5%) of the patients throughout the postoperative assessment periods, and decrease in patients with significant PVR in the first two assessment periods with no abnormalities diagnosed in the additional clinical examination.

Table 2
Postoperative assessment

Parameter	Week 1 n (%)	Month 1 n (%)	Month 3 n (%)	Month 6 n (%)
Positive cough test	3 (7.5)	2 (5)	3 (7.5)	3 (7.5)
Significant PVR	4 (10)	2 (5)	0	0
Positive urine culture	/	2 (5)	0	0
Clinical examination	/	NAD	NAD	NAD
Total patients	7 (17.5)	6 (15)	3 (7.5)	3 (7.5)

NAD – no abnormality detected;
PVR – postvoid residual urine.

Throughout the postoperative assessment, no vaginal extrusion or urethral erosion by the tape was reported. Transitory pain in the route of the TOT was reported in 6 patients which spontaneously resolved in all of the patients during the first month following the operation.

The objective cure rate results determined at the time of postoperative assessment are shown in Table 3. Analyzing the cure rates, we could confirm that 36 (90%) of the patients were considered cured from the second, and unchanged throughout the following postoperative assessment periods.

Table 3
Objective cure rate

Cure rate	Week 1 n (%)	Month 1 n (%)	Month 3 n (%)	Month 6 n (%)
Cured	33 (82.5)	36 (90)	36 (90)	36 (90)
Improved	/	/	1 (2.5)	1 (2.5)
Failure	7 (17.5)	4 (10)	3 (7.5)	3 (7.5)

Discussion

Following the relevant guidelines for surgical management of female urinary incontinence, the TOT procedure has its clear indications (SUI and MUI), complications (perioperative and postoperative), cure rates (subjective and objective) and unfortunately failure rates [15, 16]. Studies have proven that the outcome of MUS procedures is independent of the specific type of anesthesia used. Previously, there was a conviction that the use of spinal anesthesia was important to achieve the adequate tensioning of the sling and control of continence performing the cough test during the procedure [17]. As in other studies, in our study, the use of general or spinal anesthesia was in accordance with the surgeons or anesthesiologists preference and the anesthesiological requirements with no proven impact on the surgical outcome. The decision whether to use general or spinal anesthesia was in general brought by the anesthesiologist taking into consideration the patients age and comorbidities and, in most events, their preference.

Besides the fact that the TOT procedure was designed to avoid and decrease the TVT intraoperative complication rates (bladder perforation and vascular injury), it still has its registered complications. The most frequent intraoperative complications are bladder and vaginal perforations and hemorrhage [18]. The reported overall complication rates for TOT are in the range from 10 % to 31.3% [19]. Laurikainen et al. [20] in their randomized controlled trial (RCT), in the short term follow-up in the TOT group (131 patients), showed that there were no significant intraoperative complications. Vaginal perforation occurred in 2.3% of the patients. In our study, no intraoperative bladder perforation or excessive bleeding (> 200 mL) were reported. This can be explained by adequate surgical training and experience of the conducting surgeon. Intraoperatively, one case of perforation of the lateral vaginal fornix by the tunneller was recognized and immediately resolved by repositioning the tunneller and with an additional suture of the perforated vaginal fornix wall. Stav et al. [21] in their study analyzed the influence of different prolapse repairs taking into consideration compartments (anterior, posterior, vault or uterine prolapse) and concluded that they had no significant influence on the success rate. But, when they analyzed them as one group (any repair), they proposed that concomitant prolapse and the TOT surgery could have an influence on decreasing the incidence of recurrent SUI. In our study, in one patient with concomitant anterior vaginal wall prolapse (cystocele),

beside the TOT procedure, an anterior colporrhaphy was performed simultaneously. She was the only patient in our study that had a significant concomitant anterior vaginal wall prolapse and the decision was made to perform both procedures at the same time.

Postoperative complications of the TOT procedure can be immediate or late: voiding difficulties, groin pain, *de novo* urgency, urinary tract infections, urethral erosions and vaginal extrusions. Voiding difficulties can be presented as a weak stream with an intermittent flow pattern, straining, with a feeling of inadequate emptying that results with a significant PVR or even complete urinary retention [18]. Observed risk factors can be preoperative voiding difficulties or exceeding the tension on the tape. Ahn et al. [22], in their study, reported that 10.5% of the patients had postoperative voiding difficulties, 2,2% needed prolonged catheterization due to retention, and 0.4% underwent sling incision as a definite solution of voiding difficulties. Kim et al. [23] reported that 9.5% of the patients had transient retention (TR) and suggested that preoperative PVR can be noted as a risk factor for developing TR. In our study, one week after the operation, 4 (10%) of the patients needed an indwelling urethral catheter because of voiding difficulties, transient incomplete or complete urinary retention. In 2 of these patients, the catheter was taken out between the two initial follow-ups. On the first month follow-up, 2 patients (5%) had still the need for an indwelling catheter because of significant PVR. Between the second and third follow-up after several tries without catheters and because of the patient incompliance for intermittent clean self-catheterization (CISC), 2 patients underwent tape incision. Following that procedure, one of the patients had recurrent incontinence and the second had minimal signs of incontinence, less than initial incontinence. In all of our patients, no preoperative PVR was determined in the preoperative assessment.

The previous hysterectomy can have, as a result, a change of local anatomy, scaring of the vaginal wall, or even neurophysiological damage. This has shown to have a possible influence on the success of the TOT procedure due to inadequate tape positioning or even change in the dynamics of the sphincter mechanism [26]. Reviewing the adverse events in our series, 8 (20%) of the patients had a history of previous hysterectomies in which all the adverse events occurred. This might be a part of the explanation of the cause of complications but must not be viewed isolated from the fact that the majority of the complications occurred in the learning curve cases. A very important factor is the experience of the conducting surgeon that must be taken into consideration with referment of the patients and analyzing their results [15, 16].

Paick et al. [27] presented a success rate for TOT procedures in patients diagnosed with MUI up to 94%. The only risk factor for failure is the simultaneous presence of DO. Gamble et al. [28] have reported a postoperative reduction of DO in 31.5% of the patients with MUI who underwent surgical treatment by TOT. In the conclusion of Committee 14 who considered TOT in special populations (MUI), it was specified that these patients have benefited from this procedure. They also have the largest improvement of problems related to urgency, decrease in DO as well as the lowest rate of *de novo* urgency [29].

Defining the surgical outcome of the TOT procedure as cured, when the patient declines that there was leakage in everyday activities and during the provocation cough tests, has been analyzed in various studies [30]. In the Cochrane review of randomized controlled trials, the short-term results have shown an objective cure rate for TOT to be 85.7%, compared to 78% reported in the TOMUS study and 88% in the E-TOT study [31–33]. In our study, from the first month follow-up till the last postoperative assessment, the objective cure rate was 90% with just a slight decrease in the failure rate between the first and third month follow-up from 10% to 7.5%. This was a result of one patient (2.5%), after incision of the tape that was performed because of voiding difficulties and a significant PVR, and it was defined as improved (less than initial incontinence).

Conclusion

Taking into consideration and analyzing our data (intraoperative/postoperative complications, postoperative assessment and cure rates) of the patients that underwent the TOT procedure and comparing them to data from referent studies, no major differences were established and confirmed. There might be some limitations of this study due to the short time of analyzed postoperative assessment. This can be resolved by future studies with a longer postoperative follow-up. We can conclude that the TOT procedure is a safe, effective and straightforward procedure after adequate training for surgical treatment of female urinary incontinence.

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Zyxin expression levels in non-small cell lung cancer patients

Ekspresija ziksina kod obolelih od nesitnoćelijskog karcinoma pluća

Dejan Ilić*, Milan Rančić†, Tatjana Stoimenov Jevtović‡, Veljko Petrović§, Marina Petrović‖

***Special Hospital for Lung Diseases "Ozren", Sokobanja, Serbia; University of Niš, Faculty of Medicine, †Department of Internal medicine, ‡Institute of Biochemistry, Niš, Serbia; University of Novi Sad, §Faculty of Technical Sciences, Serbia; University of Kragujevac, Faculty of Medical Sciences, ‖Department of Internal Medicine, Kragujevac, Serbia**

Abstract

Background/Aim. Non-small cell lung cancer (NSCLC) is the most common cause of cancer-related mortality worldwide. Early detection represents one of the most promising approaches to reduce lung cancer mortality. Zyxin (ZYX) is a member of the focal adhesion protein family, recently identified as a potential early diagnostic marker for NSCLC. The aim of this study was to evaluate ZYX expression levels in NSCLC patients and compare its serum expression profiles between early and advanced clinical stages, different histological subtypes and histological grades. **Methods.** Blood samples were obtained from 90 patients diagnosed with NSCLC in all clinical stages and 30 patients without the clinical and radiological findings and previous history of malignancy. For the quantitative determination of human ZYX concentrations in the serum we used enzyme-linked immunoadsorbent assay (ELISA). **Results**. ZYX exhibited higher serum levels in NSCLC patients as compared to the control samples with exceptionally significant difference (p = 0.00). The ROC curve demonstrated a high specificity with AUC = 0.912. There were no statistically significant differences in the ZYX values between two most common NSCLC types, adenocarcinoma and squamous cell carcinoma (p = 0.758). There were no statistically significant differences in the ZYX values among different clinical stages (p = 0.518). Only 3 patients had well-differentiated tumor, and no useful data may be extracted from their samples. There were no statistically significant differences in the ZYX values between patients with moderately differentiated tumor and poorly differentiated tumor (p = 0.48). **Conclusion.** We found that ZYX was overexpressed in NSCLC, but its expression level was not closely correlated with the tumor size and advanced tumor, node, metastasis (TNM) stage. Our results suggest that ZYX has potential to be an early diagnostic plasma-based tumor marker for NSCLC with the same importance for both adenocarcinoma and squamous cell carcinoma.

Key words:
zyxin; carcinoma, non-small-cell lung; biomarkers, tumor; diagnosis, differential.

Apstrakt

Uvod/Cilj. Nesitnoćelijski karcinom pluća (engl. *non-small cell lung cancer* – NSCLC) je najčešći uzrok smrti od malignih tumora širom sveta. Rano otkrivanje bolesti najviše obećava u smislu smanjenja smrtnosti od ovog tipa karcinoma. Ziksin (ZYX) je član porodice proteina fokalnih adhezija, nedavno identifikovan kao potencijalni marker za rano otkrivanje NSCLC. Cilj studije bio je procena nivoa ekspresije ZYX kod obolelih od NSCLC i poređenje profila njegove ekspresije u serumu između ranih i odmaklih kliničkih stadijuma bolesti, različitih patohistoloških suptipova i različitih histoloških gradusa tumora. **Metode**. Uzorci krvi dobijeni su od 90 bolesnika sa verifikovanim NSCLC u svim kliničkim stadijumima bolesti i od 30 bolesnika bez kliničkih i radioloških znakova malignoma i bez prethodno verifikovane maligne bolesti. Za kvantitativno određivanje koncentracije humanog ZYX u krvi koristili smo ELISA (eng. *enzyme-linked immunoadsorbent assay*) test. **Rezultati**. Utvrđen je viši nivo ZYX u serumu bolesnika obolelih od NSCLC u poređenju sa kontrolnom grupom, sa izuzetno značajnom razlikom (p = 0,00). ROC kriva pokazala je visoku specifičnost testa sa AUC = 0,912. Nije bilo statistički značajne razlike u vrednostima ZYX kod dva najčešća tipa NSCLC, adenokarcinoma i skvamocelularnog karcinoma (p = 0,758). Nije utvrđena statistički značajna razlika u nivoima ZYX kod različitih kliničkih stadijuma bolesti (p = 0,518). Kod samo tri bolesnika verifikovan je dobro diferentovani tumor, pa nije bilo moguće izvući korisne podatke iz ovako malog uzorka.

Correspondence to: Dejan Ilić, Special Hospital for Lung Diseases "Ozren", Naselje Ozren bb, 18 230 Sokobanja, Serbia.
E-mail: dejan@soko-banja.org.

Nije utvrđena statistički značajna razlika u dobijenim vrednostima ZYX kod bolesnika sa srednje diferentovanim i loše diferentovanim tumorom (p = 0,48). **Zaključak**. Utvrdili smo da je ZYX prekomerno eksprimiran kod NSCLC, ali nivo ekspresije nije značajnije korelisao sa veličinom tumora, niti uznapredovalim *tumor, node, metastasis* (TNM) stadijumom bolesti. Naši rezultati sugerišu da serumski ZYX ima potencijal kao dijagnostički tumor marker za rano otkrivanje NSCLC, bez obzira da li se radi o adenokarcinomu ili skvamocelularnom karcinomu pluća.

Ključne reči:
ziksin; pluća, nesitnoćelijski karcinom; tumorski markeri, biološki; dijagnoza, diferencijalna.

Introduction

Lung cancer is the leading cause of cancer deaths worldwide. The incidence is approximately 14% in both genders (second after prostate cancer in men and breast cancer in women) [1]. Every year, lung cancer causes more than 1.7 million deaths, more than breast, colon and prostate cancers combined. Lung cancer is classified into two major types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is divided into adenocarcinoma (approximately 63%), squamous cell carcinoma (approximately 30%), and large-cell carcinoma (approximately 7%) subtypes, accounting for approximately 85% of all new lung cancer cases [2]. Five-year survival rate of NSCLC still remains < 20% [1]. Most patients are diagnosed in older age (≅ 65 years) and in late-stage (IIIB–IV) where surgical resection is not a standard procedure anymore, according to the guidelines of the American Joint Committee on Cancer (AJCC) showing low overall survival rates at 5 years (5% for IIIB and 1% for IV stages) [3]. However, detection at an earlier stage and treatment by immediate resection are the cornerstones of reducing NSCLC death rates.

Despite the tremendous efforts made to discover blood-based tests for the early diagnosis during the past decades, no tumor markers are available with selectivity to effectively diagnose lung cancer. The most widely used blood-based tumor marker screening includes carcinoembrionic antigen (CEA), cytokeratin 19 fragment (CYFRA 21–1), squamous cell carcinoma antigen (SCCA), and neuron-specific enolase (NSE), without the evidence for their significance in the early diagnosis of lung cancer [4, 5].

Over the past decade, proteomic analysis has become the main tool for investigation of tumor biology. The goal of proteomics is to characterize proteins by evaluation of their expression, functions and interactions, and also may provide information about posttranslational modifications of proteins and evaluate their value as specific disease biomarkers [6]. Any biomarker is defined as a specific that is objectively measured and evaluated as an indicator of normal physiological processes, pathogenic processes and diseases or pharmacological responses to a specified therapeutic intervention [7]. Many studies reported elevation of serum haptoglobin (HP) in NSCLC patients [8, 9], elevation of serum amyloid alpha (SAA) [10, 11] and tissue metalloproteinase inhibitor 2 (TIMP2) [12], and reduction of pigment epithelium-derived factor (PEDF) in the pleural effusion and the serum samples [13], in comparison to controls. Higher levels of leucine-rich alpha-2-glycoprotein (LRG1) were found in urine samples of cancer patients in comparison to healthy subjects [14], and high level of gelsolin expression was significantly associated with death risk of NSCLC patients [15]. Zyxin (ZYX; Uniprot ID, Q15942) showed potential to be used for early diagnosis of NSCLC. Analysis of ZYX values at the different clinical stages demonstrated that the levels of this peptide were alredy elevated at early stages of NSCLC [16].

Zyxin is a zinc-binding phosphoprotein known as a member of the focal adhesion protein family. In normal cells ZYX is involved in cell adhesion, cytoskeleton remodeling [17], stress fibers self-monitoring and repair in response to mechanical stress [18]. But, during mitosis, ZYX also acts as a participant in mitotic control by forming a complex with h-warts/LATS1 on the mitotic apparatus [19]. Zyxin has been already reported as being associated with tumorigenesis. The role of ZYX as a key player in the epithelial-mesenchymal transition (EMT) mechanism [20] and its association to lung cancer as a down regulator of TGF-β inducing cell motility [21] has been recently discussed. Zyxin expression correlates with cancer cell lines with higher malignancy, its activation may play a critical role in regulating yes-associated protein (Yap) activation during tumorigenesis [22]. Upregulation of ZYX in hepatocellular carcinoma had been previously reported [23], and a peptide fragment apparently derived from truncated ZYX has been identified in serum samples from colorectal cancer patients [24]. The expression level of ZYX corresponding to tumorigenesis or tumor mass in the human body has been quite controversial [20].

The purpose of this study was to evaluate ZYX expression levels in NSCLC patients and compare serum ZYX expression profiles between among different histological subtypes and histological grades.

Methods

A total of 120 patients were recruited from the Clinic for Lung Diseases, Clinical Center Niš, Serbia, between October 2015 and August 2017. Blood samples were obtained from 90 patients diagnosed with NSCLC in all stages and without prior history of other cancers, including adenocarcinoma and squamous cell carcinoma, and 30 patients without the clinical and radiological findings and previous history of malignancy. None of the

patients received chemotherapy, radiotherapy, hormone therapy, or other related antitumor therapies prior recruiting.

The NSCLC patients were classified into clinical disease stages I (n = 9), II (n = 12), III (n = 30) and IV (n = 39) according to the 7th edition of the American Joint Committee on Cancer Tumotr, Node, Metastasis (TNM) staging system [3]. Blood samples were taken prior to surgery for stages I–IIIA and prior to treatment for advanced stage NSCLC patients using serum separator tube. Sera were allowed to clot for two hours at room temperature and then centrifuged at 1000 × g for 15 minutes. Immediately following centrifugation, all specimens were stored at −80°C until being analyzed.

For the quantitative determination of human ZYX concentrations in the serum we used Cusabio Human Zyxin (ZYX) ELISA Kit, Catalog Number CSB-EL027165HU (Cusabio Technology LLC, 8400 Baltimore Avenue, Room 332 College Park, MD 20740, USA). This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for ZYX has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ZYX present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for ZYX is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of zyxin bound in the initial step. The color development is stopped and the intensity of the color is measured. We created a standard curve by reducing the data using computer software "Curve Expert" capable of generating a four parameter logistic (4-PL) curve-fit. The minimum detectable dose of human ZYX is less than 5.8 pg/mL. Detection range between standards is 23.5–1,500 pg/mL.

This study was approved by the Research Ethics Committee of the Clinical Center Niš. The informed written consents were collected from all eligible patients and the entire study was performed based on the Declaration of Helsinki.

Introduction to the data and statistical analysis

The data tracked numerous factors, but the ones relevant for this analysis were merely a dichotomous categorical variable and a continuous variable, specifically whether a patient was diagnosed with lung carcinoma, and what patient's serum level of ZYX was in pg/mL. These two were suffice to establish a link between carcinoma diagnosis and ZYX level. A sample of the data is presented in Table 1.

Once the descriptive statistics was performed, the data were analyzed using simple group-mean comparison. In point of fact, the analysis was conducted using between-groups statistical comparison, with the groups being determined using the dichotomous categorical variable Dg_Ca representing carcinoma diagnosis. The analysis was conducted using the R programming language, version 3.3.1 built for the x 64 processor architecture, using the following packages: WRS2 [25], effsize [26], and pastecs [27].

Table 1

A sample of the data under observation

Number of sample	Diagnosis of cancer (Dg_Ca)	Zyxin concentration (pg/mL)
1	1	773.6667
2	1	531.3333
3	1	427.4333
4	1	484.7667
5	1	858.6667
6	1	818.0000

Results

The 90 blood samples were collected from patients who were diagnosed as NSCLC; the average age was 63.56 ± 6.344 years, and the majority of patients were males and smokers. The blood samples from 30 control subjects with other pulmonary diseases were collected as controls by matching their age (62.43 ± 9.380 years), sex, smoking history and their duration and intensity. Patient demographics and clinical profiles are presented in Table 2.

Descriptive statistics and assumptions of general linear models

Taken as a whole, the data had an number of 120, and the mean of the variable measuring ZYX concentration was 426.6800277 pg/mL, with a median of 426.2166666 pg/mL and a σ of 249.0966559 pg/mL. The large relative value of the standard deviation compared to the mean casted serious doubts on the assumption of globally normal data, and the histogram in Figure 1 shows that the distribution was likely heavy-tailed.

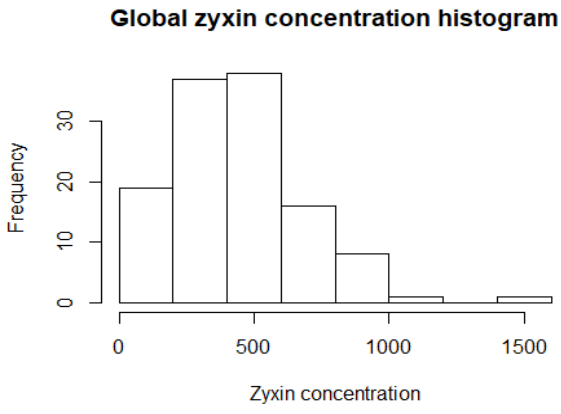

Fig. 1 – Distribution of zyxin concentrations (in pg/mL) in both group of patients (n = 120).

However, more crucial information for our purposes was the analysis of the data as split into groups based on

Table 2

Patient demographics and clinical profiles

Demographics	NSCLC (n = 90)	Control (n = 30)
Age (years), mean ± SD (range)	63.56 ± 6.344 (49–79)	62.43 ± 9.380 (40–82)
Sex, n (%)		
male	68 (75.6)	18 (60)
female	22 (24.4)	12 (40)
Smoke history, n (%)		
yes	76 (84.4)	23 (76.7)
no	14 (15.6)	7 (23.3)
pack-years, mean ± SD	51.167 ± 30.921	44.74 ± 15.235
Clinical histological type*, n (%)		
adenocarcinoma	48 (53.3)	
squamous cell carcinoma	42 (46.6)	
Stage**, n (%)		
IA	5 (5.6)	
IB	4 (4.4)	
IIA	4 (4.4)	
IIB	8 (8.9)	
IIIA	17 (18.9)	
IIIB	13 (14.4)	
IV	39 (43.3)	
Histological grade***, n (%)		
I	3 (3.3)	
II	37 (41.1)	
III	12 (13.3)	
unknown	38 (42.2)	

***Histological type of non-small cell lung cancer (NSCLC) according to the 2015 World Health Organization (WHO) histological classification of lung tumors [2]; **Disease stage according to the 7th Edition of the tumor, node, metastasis (TNM) classification of malignant tumors [3]; *** Histological grade according to the 2015 WHO Classification of Lung Tumors [2]; SD – standard deviation.**

carcinoma diagnosis. To simplify terminology, hence forth the group without the carcinoma diagnosis was referred to as 'control' and the group with the carcinoma diagnosis was referred to as 'effect.' With that said, the descriptive statistics of the control group showed that the number was 30 and that the mean was 168.0501111 pg/mL, while the median was 127.3333334 pg/mL and the σ_D 155.5513055 pg/mL. The distribution of the value in the control group can be seen on the histogram in Figure 2.

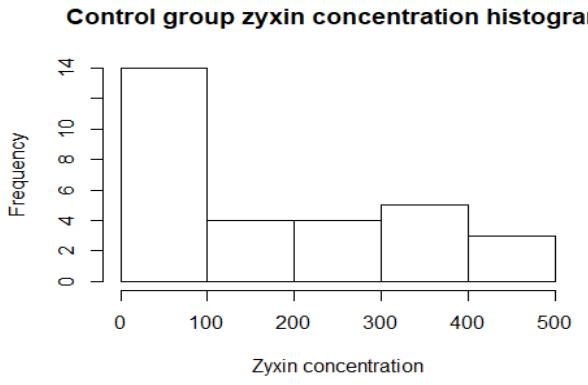

Fig. 2 – Distribution of zyxin concentrations (in pg/mL) in the control group (n = 30).

Despite promising mean/median results, this distribution was not normal which the QQ plot in Figure 3 shows. The s-shape to the curve indicates heavy skew which is also evident in the histogram. Similar results were found by applying standard normality tests with the Shapiro-Wilk test allowing us to reject the H_0 of normality with a p value of 0.0015193.

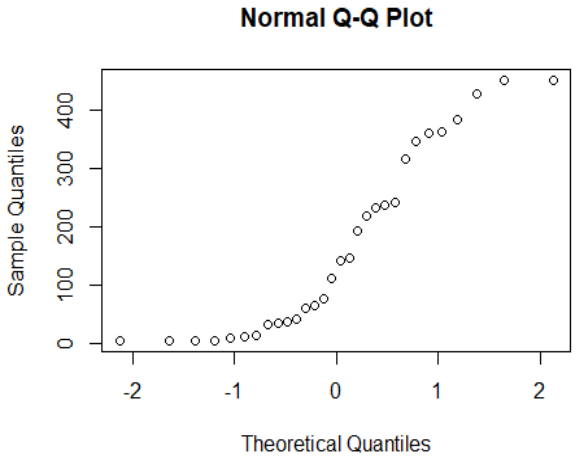

Fig. 3 – The Q-Q plot diagram of mean/median values of zyxin concentrations (in pg/mL) in the control group (n = 30) shows no normality of the data.

The descriptive statistics of the effect group showed that the number was 90 and that the mean was 512.8899999

pg/mL, while the median was 471.7666667 pg/mL and the σ_D was 212.1738935 pg/mL. The distribution of the value with the effect group can be seen on the histogram in Figure 4.

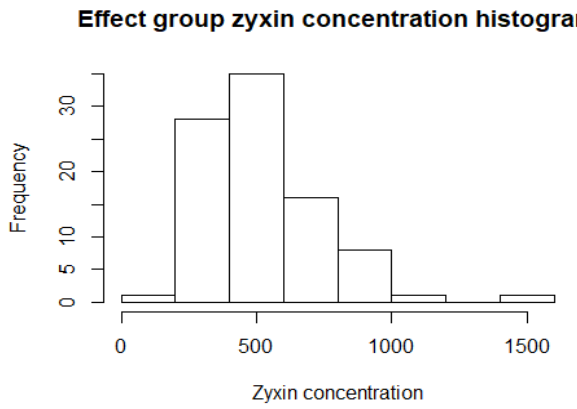

Fig. 4 – Distribution of zyxin concentrations (in pg/mL) in the group of non-small cell lung carcinoma (NSCLC) patients (n = 90).

Despite promising mean/median results, this distribution was not normal which the QQ plot in Figure 5 shows. The deviation from the $\frac{\pi}{4}$ angle to the curve indicated an issue with the kurtosis. This is possible to see by examinging the shape of the histogram suggesting a platykurtic distribution. Similar results were found by applying standard normality tests with the Shapiro-Wilk test allowing us to reject the H_0 of normality with a p value of $2.3073684*10^{-5}$.

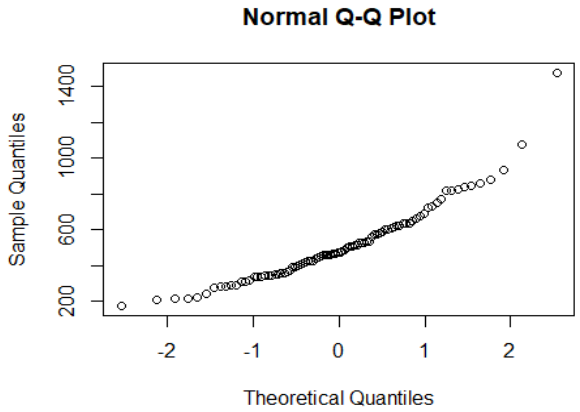

Fig. 5 – Q-Q plot diagram of mean/median values of zyxin concentrations (in pg/mL) in the non-small cell lung cancer (NSCLC) patients (n = 90) shows no normality of the data.

The groups had unequal sizes, unequal variances, and heavily violated the assumption of normality. With the effect size being what it was, we could confirm that the requirements of the General Linear Model (GLM – of which the *t*-test, normally used in situations like this, is an example) were not met, and, indeed, the requirements of parametric models in general were not met. However, the difference between the means of these two groups was large: more than double the size, in fact. This means that the effect we were looking for was very large, and thus, we should be able to identify it with great specificity if we apply a statistical technique with sufficient power and sufficiently lax parametric requirements.

Hypothesis testing for difference in Zyxine level between NSCLC patients and control subjects

We were dealing with two groups of which one was platykurtic and the other was heavily skewed. Assuming their distributions is equal might influence our results. The alternative was to apply a hybrid approach in which we dealt with each of our issues with GLM assumptions in turn by employing a different solution.

Unequal variances are most simply resolved using the Welch modification of the *t*-test [28]. This test is a modification of the familiar Student's *t*-test (and is commonly employed in its stead in various libraries of statistical software) and pools variances in both populations thus producing an altered test statistic

$$t' = \frac{\overline{Y_1} - \overline{Y_2}}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

and a modified measure of degrees of freedom

$$df' = \frac{(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2})^2}{\frac{s_1^4}{n_1^2(n_1 - 1)} + \frac{s_2^4}{n_2^2(n_2 - 1)}}$$

The remainder of the test is the same. To successfully combine a test that deals with heterogeneity of variances and with a violation of the assumption of normality, a combination of the Welch test and the Yuen modification [29] is necessary. Yuen's approach recreates the Welch test using, instead of means, trimmed means, i.e., means with outlier values on the edges of the distribution clipped to some pre-determined value, generally expressed as a proportion in percent. This level is expressed as *y*.

However, Wilcox [30] suggests that if the group sample sizes are unequal, which was true in our case, a more robust bootstrapped version of the Yuen-Welch is employed. This version of the test uses resampling techniques to estimate the confidence interval for the critical value of the test statistic. This helps reduce the probability of Type I error to the nominal level. The procedure, roughly, proceeds by first computing the trimmed means of the sample and Yuen's estimate of the Squared Standard Errors, like so:

$$d_j = \frac{(n_j - 1)s_{wj}^2}{h_j(h_j - 1)}$$

Then, for the *j*-th group analyzed randomly re-sample with replacement from the available data set n_j observations.

Using the samples generated by this Monte Carlo approach computes the same values as the initial Yuen approach and label them $\overline{X^*_{tj}}$ and d^*_j. Then calculate the following:

$$T^*_y = \frac{(\overline{X^*_{t1}} - \overline{X^*_{t2}}) - (\overline{X_{t1}} - \overline{X_{t2}})}{\sqrt{d^*_1 + d^*_2}}$$

This value represents an estimation of the distribution of

$$\frac{(\overline{X_{t1}} - \overline{X_{t2}}) - (\mu_{t1} - \mu_{t2})}{\sqrt{d_1 + d_2}}$$

Repeat the preceding steps generating a sequence of T^*_y values (for our test we selected that this number be $B = 2000$). Then sort these values in ascending order. Let $T^*_{y(i)}$ represent the T-value occupying the i-th place in the sorted array with $i \in [1, B]$. Compute

$$l = \frac{\alpha \cdot B}{2}$$

and round it to the nearest integer and let $u = B - l$.

The confidence interval of μ_ε (being the true mean of the difference in groups) is

$$(\overline{X_{t1}} - \overline{X_{t2}} - T^*_{y(u)}\sqrt{d_1 + d_2}, \overline{X_{t1}} - \overline{X_{t2}} - T^*_{y(l+1)}\sqrt{d_1 + d_2})$$

We used the reference implementation in the WRS2 package with 2,000 resampling steps and a trimming percentage of y = 20. The result of this analysis showed a p-value of 0, indicating a level to small for the computer to measure, with a test statistic of 7.3819, and a 95% confidence interval for the difference in means of 247.1077 - 437.9487.

This was an exceptionally significant result, showing that we could confidently reject the H_0 that the means of the two groups are equal. There was certainly a difference in ZYX concentrations having a significantly larger values in the group diagnosed with NSCLC as compared to the control group of patients without carcinoma diagnosis. Higher plasma levels in NSCLC patients as compared to the control samples are illustrated in Figure 6.

The remaining question was how large the effect was. The traditional approach is to employ Cohen's d effect measure for this purpose, and doing so yields a value of 1.7263458 which corresponds according to Sawilowsky [31] to a very large effect size. However, Cohen's d depends on the same parametric assumptions our data violate. Therefore in accordance to Wilcox and Tian [32], we employ the explanatory measure of effect size, $\hat{\xi}$ which equals 0.889821 and with a 95% confidence interval of 0.8118965 to 0.9635671. The authors of the approach categorize a $\hat{\xi}$ value of 0.5 as a large effect, therefore, we were led to assume that our value represents a very large effect in line with Cohen's d results.

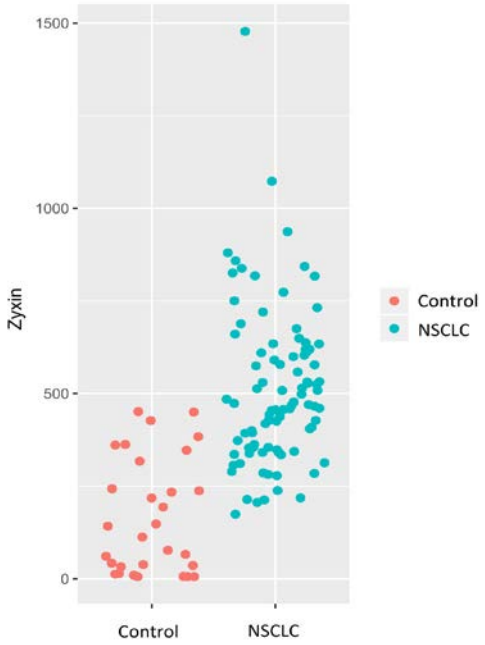

Fig. 6 – Scatter plots of ELISA results for zyxin in samples of the control group (n = 30) and non-small cell lung cancer (NSCLC) group (n = 90).

The receiver operating characteristic (ROC) curve generated using ZYX values demonstrated a high specificity toward NSCLC with AUC = 0.912 as shown in Figure 7.

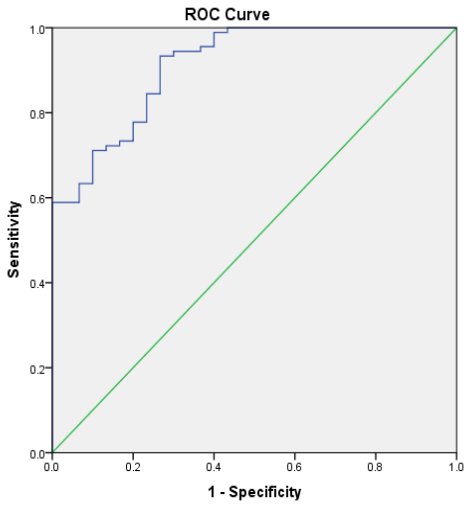

Fig. 7 – Receiver operating characteristic (ROC) curve for zyxin shows its high specificity toward non-smqall cell lung cancer [area under curve (AUC) = 0.912].

Hypothesis testing for difference in Zyxin level among different histological types of NSCLC

Using the exact method outlined above, it was possible to test for a difference in ZYX levels between groups with one of two most common NSCLC types, adenocarcinoma and squamous cell carcinoma. The analysis showed a test statistic of 0.3114692 with an associated p-value of 0.758, showing a difference that was not statistically significant. In practical terms, the actual measured means were 522.1514

pg/mL and 502.3056 pg/mL, respectively, showing the sort of effect only a very large sample size might be able to prove statistically significant difference.

Hypothesis testing for difference in Zyxin level among clinical disease stages

Clinical disease stages of NSCLC delineate four separate groups. Demanding a slight change in approach, specifically, instead of using a modified T-test to determine a difference in means between groups, a robust ANOVA-equivalent was used instead, one which tested the hypothesis of equal trimmed means with a $y = 20$, and a $B = 2{,}000$ without demanding assumptions of normality or heteroscedasticity. Performing this test produced a test statistic 0.8133 and a corresponding *p*-value of 0.518 with an effect size estimate of 0.29. Actual measured means were 443.10 pg/mL for stage I, 562.38 pg/mL for stage II, 439.93 pg/mL for stage III, and 470.10 pg/mL for stage IV. The difference was not large in absolute terms, first, indicating that a larger sample size was required, and second it was not statistically significant, as indicated by the *p*-value. Figure 8 shows a scatter plot of ZYX values for the different clinical stages of NSCLC.

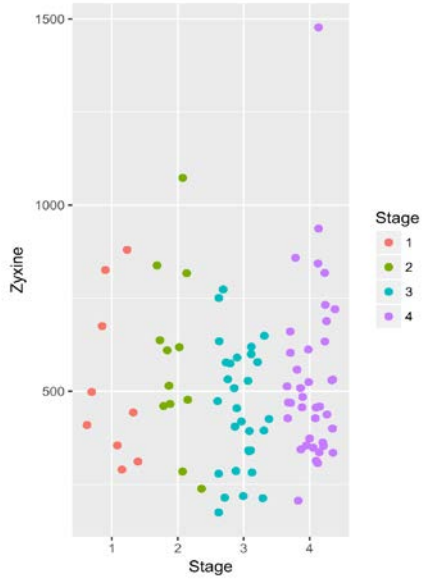

Fig. 8 – Scatter plots of zyxin values for the different clinical stages of non-small cell lung cancer (NSCLC). Number of samples used in each group are: n = 9 for stage I, n = 12 for stage II, n = 30 for stage III, and n = 39 for stage IV.

Hypothesis testing for difference in Zyxin level among histological grades

Given the nature of histological grades, the decision was made to test only grades II and III, since with applying to the grade of 1, no useful data could be extracted from it ($n = 3$). Using the two-group comparison solution described above, it was possible to test for a difference in zyxin levels between groups with grades II and III. The analysis showed a test statistic of -0.646 with an associated *p*-value of 0.4845, showing a difference that was not statistically significant. In practical terms, the actual measured means were 522.3604 pg/mL and 546.1500 pg/mL, showing the sort of effect only a very large sample size might be able to prove statistically significant.

Discussion

Low-dose computed tomography (CT) screening reduces lung cancer-related mortality, at least for subjects fulfilling the National Lung Screening Trial (NLST) inclusion criteria [33] or under the US Preventive Services Task Force (USPSTF) recommendations [34]. The use of lung cancer predictor models could help defining the subjects with higher risks [35]. In the near future, ongoing research on lung cancer biomarkers could increase accuracy of lung cancer low-dose CT screening. Currently, there are no validated biomarkers for early lung cancer detection.

Kim et al. [16] previously demonstrated that ZYX levels were already elevated at early stages of NSCLC. Their study applied highly multiplexed liquid chromatography-selected reaction monitoring (LC-SRM) assay to verify biomarker candidates in plasma samples for lung cancer, and ZYX was identified as a potential early diagnostic marker for NSCLC.

In this study, we have that zyxin was overexpressed in NSCLC, but its expression level was not closely correlated with tumor size and advanced TNM stage. Zyxin protein exhibited higher serum levels in samples of the NSCLC patients as compared to the control samples with exceptionally significant difference.

The NSCLC group was comprised of serum samples of patients at two different histological types of the disease. Analysis of ZYX values at the different histological types of NSCLC demonstrated equal levels for adenocarcinoma and squamous cell carcinoma. There were no statistically significant differences in ZYX values between two most common NSCLC types. To the best of our knowledge, no studies have investigated the possibility of such differences.

Analysis of the ELISA zyxin values at the different clinical stages of the disaese showed significant elevation in the serum of NSCLC patients already at early stages. This result was consistent with the results from the previous report [16]. There were no statistically significant differences in ZYX values among different clinical stages.

A recent study by Ma et al. [22] has shown that ZYX expression correlates with cancer cell lines with higher malignancy. Zyxin is upregulated in human breast cancer and positively correlates with histological stages and metastasis. We did not establish correlation between degree of differentiation of the tumor and ZYX level due to lack of data for histological grade for 38 (42.2%) of the patients. Only 3 patients had well-differentiated tumor, but no useful data may be extracted from their samples.

There were no statistically significant differences in ZYX values between patients with moderately and poorly differentiated tumor.

Conclusion

Our results suggest that zyxin fulfilled the criteria for a potential early diagnostic serum-based tumor marker for non-small cell lung cancer, with the same importance for adenocarcinoma and squamous cell carcinoma. Early detection represents a very promising approach to reduce lung cancer mortality. The results of these analyses give us reason to be hopeful, considering high ELISA zyxin values at clinical stages I and II. As one can anticipate, over the coming decade, effective biomarkers in combination with low-dose computed tomography may provide effective tools for the non-small cell lung cancer early detection and improving survival rates in these patients.

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Depression, anxiety and quality of life in patients with melanoma

Depresija, anksioznost i kvalitet života kod bolesnika sa melanomom

Aleksandra R. Vojvodić*, Gordana Dedić†‡

Military Medical Academy, *Department of Dermatology and Venerology, †Clinic for Psychiatry, Belgrade, Serbia; University of Defence, ‡Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Recent investigations have established a significant correlation between melanoma and quality of life, as well as anxiety and depression in these patients. In prognosis of melanoma, the most important is the stage in which it is diagnosed. The objective of the study was to analyze the quality of life, anxiety and depression in patients with a diagnosis of melanoma at different stages of the disease. **Methods.** In our cross-sectional study, 40 consecutive patients with melanoma, diagnosed and treated at the Department of Dermatology and Venerology, Military Medical Academy in Belgrade during the period from October to November 2015, were included. Twenty respondents were in stages I and II (localized disease) and 20 respondents in the stage IV (distant metastases). We used European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire (EORTC QLQ 30), Beck anxiety inventory (BAI) and Beck depression inventory (BDI). The statistical analysis included parametric and non-parametric descriptive statistics. **Results.** In patients with stages I and II of the disease, anxiety scores were higher in comparison to those in patients with the stage IV (37.5 vs. 14.5, respectively; $p < 0.05$), but depression was more pronounced (6 vs. 2.5, respectively; $p < 0.05$) in patients with the IV stage of the disease. There were statistically significant differences in all segments of quality of life between patients with stages I and II and those with the stage IV of the disease. The global quality of life was significantly worse in patients with the IV stage (33.5 vs. 83), the symptomatology was more pronounced (78.5 vs. 0) and the functioning was significantly worse (31 vs. 85) in relation to patients with stages I and II ($p < 0.01$) for all segments of quality of life. **Conclusion.** Anxiety and quality of life decrease, while depression increases with melanoma stages. The need for adequate social and family support as well as psychological assistance in order to achieve better coping with the illness are necessary in patients with melanoma. Further studies are needed for monitoring of anxiety, depression and quality of life from the moment of diagnosis of the disease over time, as well as the impact of new treatment modalities on these parameters.

Key words:
melanoma; quality of life; depression; anxiety; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Nedavna istraživanja pokazala su značajnu korelaciju između melanoma i kvaliteta života, kao i anksioznosti i depresije kod ovih bolesnika. U prognozi melanoma, najvažnija je faza u kojoj je dijagnostikovan. Cilj ove studije bila je analiza kvaliteta života, anksioznosti i depresije kod bolesnika sa dijagnozom melanoma u različitim stadijumima bolesti. **Metode.** U našoj studiji preseka su bili uključeni bolesnici dijagnostikovani i lečeni od melanoma (n = 40) na Klinici za kožne i polne bolesti Vojnomedicinske akademije u Beogradu od oktobra do decembra 2015. godine. Dvadeset bolesnika bilo je u I i II stadijumu (lokalizovana bolest), a preostalih 20 u IV fazi (udaljene metastaze). U istraživanju smo koristili Upitnik za procenu kvaliteta života obolelih od melanoma Evropskog udruženja za istraživanje i terapiju kancera (EORTC QLQ 30), Bekov upitnik o anksioznosti (BAI) i Bekov upitnik o depresiji (BDI). Statistička analiza uključila je parametarsku i neparametarsku opisnu statistiku. **Rezultati.** Kod bolesnika u I i II stadijumu bolesti anksioznost je bila veća u poređenju sa bolesnicima u IV stadijumu bolesti (37,5 naspram 14,5; $p < 0,05$), ali je depresija bila izraženija (6 naspram 2,5; $p < 0,05$) kod bolesnika u IV stadijumu. Nađene su statistički značajne razlike u svim segmentima kvaliteta života između bolesnika koji su bili u I i II stadijumu i bolesnika u IV stadijumu bolesti. Ukupan kvalitet života značajno je bio lošiji kod bolesnika u IV stadijumu (33,5 naspram 83), simptomatologija izraženija (78,5 naspram 0), a funkcionisanje značajno lošije (31 naspram 85) u odnosu na bolesnike u I i II stadijumu ($p < 0.01$) za sve segmente kvaliteta života. **Zaključak.**

Correspondence to: Aleksandra R. Vojvodić, Military Medical Academy, Department of Dermatology and Venerology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: aleksandravojvodic@live.com.

Anksioznost i kvalitet života opadaju, dok simptomi depresije rastu sa stadijumom napredovanja melanoma. Potreba za adekvatnom socijalnom i porodičnom podrškom, kao i psihološku pomoć neophodna je kod bolesnika sa melanomom, kako bi se što bolje podnela bolest. Dodatna istraživanja su potrebna za praćenje anksioznosti, depresije i kvaliteta života od trenutka dijagnoze bolesti tokom vremena, kao i uticaja novih modaliteta lečenja na sve ove parametre.

Ključne reči:
Melanoma; kvalitet života; depresija; anksioznost; ankete i upitnici.

Introduction

The incidence of melanoma is increasing worldwide, especially in fair-skinned over-exposed white population. Incidence rate of melanoma in Europe is currently 10–25 *per* 100,000 inhabitants. The incidence is continually increasing at all ages and it is predicted that this trend will further continue. The most significant increase in incidence was detected in men over the age of 60 [1, 2].

In prognosis of melanoma, the most important is the stage in which it is diagnosed. If diagnosed occurred at an early stage, at the stage I, the five-year survival rate is 97% for the Ia and 92% for the Ib stage. In the IIa stage, the five-year survival rate is 81% and in the IIc stage 53%, while in the IV stage, it is low – 15% [3].

In recent years, due to important aspect of cancer research, new forms of biological therapy were implemented which have increased survival of patients with melanoma. However, it is not just about the length of life, but also about the quality of life (QoL) of patients with melanoma. There are a few studies dealing with the evaluation of the long-term effect of melanoma on the quality of life and the psychic status of patients with melanoma. In the study carried out in the Netherlands, it was shown that the quality of life of patients with melanoma was not significantly different from the quality of life of the general population [4–6].

According to some studies, about 30% of patients with melanoma suffered from significant distress, especially women and youth. Depression is growing with stages of melanoma and it is higher (approximately about 18–44%) in later stages the disease. The highest level of anxiety is registered in the period of diagnosis and later decreases [7–10].

The aim of this study was to analyze the quality of life, anxiety and depression of patients with melanoma at different stages of the disease.

Methods

A cross-sectional study was conducted in 40 consecutive patients diagnosed with melanoma and treated at the Department of Dermatology and Venerology, Military Medical Academy in Belgrade, during the two-month period, from October to November 2015. Although about 140 patients with melanoma on the average have been hospitalized at our Department of Dermatology and Venerology every year, only patients who were diagnosed and treated during the period when the study was conducted were included. Only patients who volunteered to participate in our study were included and all of them signed an informed consent. The research was approved by the Ethics Committee of the Military Medical Academy in Belgrade and it was carried out according to all the regulations of the Helsinki Declaration. Patients were divided into two groups: the first group consisted of 20 patients in stages I and II (localized disease) melanoma and the second group consisted of 20 patients in the stage IV melanoma (with distant metastases).

Psychological instruments

Assessing of QoL has been done using the validated cancer-specific questionnaire – the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionairre C30. The EORTC QLQ-C30 is a patient self-rating questionnaire consisting of three symptom scales (fatigue, nausea/vomiting, pain), five function scales (physical, role, social, emotional, cognitive functions), and five single items assessing symptoms such as dyspnoea, insomnia, appetite loss, constipation and diarrhea. Basing on those scores we could calculate a global health status/QoL score. All scores of the QLQ-C30 were transformed linearly so that all scales vary from 0 to 100 in a row with the EORTC scoring manual. Patients gave their answers ranked on a 4-point scale (from 1 in general to "4" very much), barring the GH/QoL scale of the EORTC QLQ-C30, which has a 7-point scale (from 1 “very poor” to 7 “excellent”). A linear transformation was used to standardize the raw score, so that overall scores ranged from 0 to 100. For the EORTC QLQ-C30, a higher score in GH/QoL or a functioning scale represents a better level of quality of life and functioning; a higher score in a symptom scale represents a worse level of symptoms [11]. Previously, the licenses for the use of the questionnaire by the EORTC were set out.

The Beck Anxiety Inventory (BAI) is an inventory for self-assessment of the severity of various symptoms related to anxiety in terms of how he felt last week. The BAI contains 21 multiple-choice items [12]. The scale is intended for people over the age of 17 years. For each symptom, four options are offered, and the respondent should choose the one that best describes his condition. The response options are from not at all over mild and moderate to severe. The minimum score is 0, and the maximum score is 63. It is considered that the score above 10 at the BAI

indicates a mild anxiety, the score above 19 shows moderate anxiety and the score above 30 indicates severe anxiety.

The Beck Depression Inventory (BDI), is one of the most appropriate psychometric tests for measuring the severity of depression. The BDI consists of 21 questions for self-reported disability. It shows high levels of internal consistency (alpha coefficient) ranging from 0.73 to 0.95 in psychiatric populations, as has been confirmed so far in many studies [12–14]. The BDI measures the general depression syndrome consisting of three correlated subcars, while for each question and statement, a response of 0 (neutral) to 3 (the most difficult) can be given. Summing the items yields a total score ranging from 0 to 63 [12]. It is considered that the score above 10 indicates a mild depression, the score above 19 shows moderate depression, and the score above 30 indicates a serious depression [13, 14].

Statistical analysis

Statistical analysis included parametric and non-parametric descriptive statistics, depending on the nature of data. Data analysis was carried out using IBM SPSS (Statistical Package for the Social Sciences) software version 20.0. For the normal distribution of all numerical parameters and scores, Kolmogorov-Smirnov test was used. We got the results showing that in all monitored and calculated parameters and scores there was normal distribution (z was less than 1.96, and $p < 0.05$), so that it was possible to apply parametric methods in further analysis.

Results

The average age of the patients in the first group was 54.8 ± 13.76 years and 62.85 ± 11.47 in the second group ($p > 0.05$). Men were also dominated by both groups (60% in the first and 70% in the second group). The presence of some chronic somatic diseases such as hypertension, diabetes, hyperlipidemia and the like, reported 35% patients in the first group and 50% patients in the second group. There were no statistically significant differences between groups in age, gender and presence of chronic somatic diseases (Table 1). In patients with stages I and II melanoma, anxiety scores were higher in comparison to those in the stage IV disease patients (37.5 vs. 14.5, respectively; $p < 0.05$), but depressive symptoms were more pronounced in the IV stage patients (2.5 vs. 6, respectively; $p < 0.05$) (Table 2).

Table 1

Demographic data of the patients with melanoma

Variables	I and II stages (localized disease)	IV stage (distant metastases)	*p*
Age (years), mean ± SD	54.65 ± 13.76	62.85 ± 11.48	ns
Gender (male), %	60	70	ns
Chronic somatic diseases (presence), %	35	50	ns

SD – standard deviation; ns – non significant.

There were statistically significant differences in all segments of QoL between patients that were in stages I and II melanoma and patients in the IV stage melanoma. The global QoL was significantly worse in patients in the stage IV (33.5 vs. 83), the symptomatology was more pronounced (78.5 vs. 0) and the functioning was significantly worse (31 vs. 85) in relation to patients with stages I and II melanoma ($p < 0.01$ for all segments of QoL) (Table 2).

Discussion

Recent investigations have established a significant correlation between melanoma stages and QoL, as well as anxiety and depression symptoms in these patients. Although some researchers were studying the stages of melanoma, anxiety and depression, the QoL of melanoma patients in our country were not analyzed.

In our investigation, we found that patients with stages I and II melanoma (localized disease) had a severe level of anxiety unlike patients with the IV stage of the disease (distant metastases) who had mild anxiety. Our results are in accordance with other investigations. When a patient faces the diagnosis of melanoma, his/her knowledge of malignant disease and its unpredictable prognosis, even when the disease is detected at an early stage, has the consequence of the appearance of fear, anxiety and insecurity. Intensive regular follow-up procedures with radiological and laboratory exams during the first three years can contribute to increase in anxiety. Many studies have shown that anxiety is higher in earlier melanoma stages and that it later slowly decreases. Adaptation to the diagnosis of melanoma and coping with the diagnosis, dealing with various treatment modalities, with or without support of his/her family, friends and social environment, greatly influences the anxiety in later stages of the disease, too [15–19].

Table 2

EORTC QLQ C30, BDI and BAI in the melanoma patients

Questionnaire	Stages I and II (localized disease)	Stage IV (distant metastases)	*p*
BAI score, mean	37.5	14.5	0.05
BDI score, mean	2.5	6	0.05
EORTC-QLQ C30 (Global QoL) score, mean	83	33.5	0.01
symptomatology	0	78.5	0.01
functioning	85	31	0.01

EORTC-QLQ C30 – European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Questionary C30; BAI – Beck Anxiety Inventory; BDI – Beck Depression Inventory; QoL – Quality of Life.

Depressive symptoms occur very rarely in earlier stages of melanoma, but, as the time passes. the patients become aware of the inevitability of the outcome of the disease, regardless to the exhaustion of various modalities of treatment, exhaustion of the disease itself, progression of the disease and depressive symptoms slowly appear. Depression symptoms are in a certain extent present in most melanoma patients at stage IV of the disease, but in minimal level, and the level of depression symptoms is significantly higher than in respondents in stages I and II melanoma. Our results are in accordance with other studies, which also found that depression symptoms occur in later stages of the disease [19–21].

The global QoL, measured by the EORTC C30 questionnaire, showed a very low level in the IV stage melanoma patients. In the IV stage melanoma (distant metastases), the global QoL depends on the type of applied therapy, its side effects and the prevalence and localization of metastases. Our results are in accordance with other studies where the global QoL is low in the IV stagemelanoma patients, too [22–24]. The low level of global QoL in the IV stage melanoma patients was more than twice lower than in stages I and II of the disease. We could explain our results with the fact that the overall global QoL is decreasing during the time, because of the progression of the malignant disease that affects the complication of everyday functioning, including some financial difficulties and symptomatology, considered primarily fatigue, nausea and vomiting, pain, dyspnoea, insomnia, appetite loss, constipation, diarrhea, etc. [24–29]. The data obtained in our study are in accordance with the data obtained in other surveys that support the declining functioning of melanoma patients as well as their global QoL as the symptomology grows [30–32].

In our investigation, we found that in stages I and II of the disease, severe level of anxiety was result of uncertainty and fear of the disease expansion which patients were faced for the first time. In these stages of melanoma, there was not influence on the QoL which stayed uncompromised and high, including low level of depressive symptoms. With progression of melanoma, situation was drastically changed. When metastasis already occurred, anxiety fell down because patients have been yet accepted the disease itself and the current condition. Depressive symptoms slowly increased from the stage I and II to stage IV because patients slowly became aware of the progression and inevitability of the outcome of the disease. In addition, in the stage IV melanoma, functioning in all segments including physical, emotional, cognitive and social functioning was compromised, which also affected the increase of depressive symptoms.

Our findings are very important in clinical practice, because they could help in planning the psychological and psychiatric support in every melanoma stage.

Limitation of the study

The group of 40 patients included in our cross-sectional study was small, and requires further investigations. Further investigation should be focused on determining gender differences in the quality of life, depression and anxiety in patients with different melanoma stages.

Conclusion

The results of our research show that anxiety is highest in the period of melanoma diagnosing and subsequently decreases, while depressive symptoms are more pronounced in later stages. Also, the quality of life of patients with melanoma is significantly worse in the stage IV stage than in the first two stages. Because of that, the need for adequate social and family support as well as psychological help in order to achieve better coping with illness are necessary. Learning techniques to overcome fear and stress would help in better functioning of all affected, regardless of the stage of the disease. The most severe cases of anxiety and depression, in addition to psychotherapeutic interventions, should also be considered for pharmacotherapy. The need for a multidisciplinary team that would be involved in monitoring patients from the moment of the establishing the diagnosis of melanoma is of exceptional importance and includes a dermatologist, surgeon, radiotherapist, neurologist, as well as psychotherapist.

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Teledentistry in dental care of children

Primena telestomatologije kod dece

Lidija Popović*, Dragica Odalović†, Dušan Živković†, Milan Miladinović†, Zoran Lazić‡§, Miloš Duka‡§, Milan Živković†

***Center for Psychophysiological Disorders and Speech Pathology „Prof. Dr Cvetko Brajović“, Belgrade; University of Priština/Kosovska Mitrovica, †Faculty of Medicine, Kosovska Mitrovica, Serbia; Military Medical Academy, ‡Dental Clinic, Belgrade, Serbia; University of Defence, §Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

Key words:
children; dentistry; diagnosis; mobile applications; oral health; telemedicine.

Ključne reči:
deca; stomatologija; dijagnoza; mobilne aplikacije; usta, zdravlje; telemedicina.

Introduction

The use of telemedicine in dental care (teledentistry) has markedly expanded in recent years. The benefits it could afford in pediatric oral and dental health maintenance and improvement are numerous, presenting an attractive challenge to both clinicians and researchers. The essence of its popularity lies in a successful distant transfer of patient information and in a correct interpretation of the transferred patient data [1, 2]. In children, teledentistry is convenient in many ways, but its suitability lies primarily in timely and correct distant clinical diagnosis and in distant interspecialist consultations [3]. Nowadays, teledentistry is used in academic medical institutions, health care centres, managed-care companies, hospitals and health facilities in rural areas, and in international support provided to underdeveloped and developing countries [4]. Due to its close functional and technological interconnection with popular social networks and due to widespread use of mobile devices, teledentistry will certainly prove to be easily acceptable by the adolescent population [5].

Regarding the provision of dental health care advancements made in teledentistry applications, there are some new facts in the area of paediatric dental care.

Teledentistry in screening and diagnosis of dental diseases in children

Kohara et al. [6] have compared the performance of two different smartphone models and a conventional camera with direct clinical inspection in detection of caries and staging of the disease (degree of disease progression) on primary molar teeth in children, They concluded that it was possible to establish teledentistry diagnosis of larger carious lesions and to distinguish them successfully from the healthy teeth by using smartphone photographs. However, the method was not shown to be adequate in the detection of incipient and moderate carious lesions. Al Shaya et al. [7] investigated the reliability of mobile phone based teledentistry in the diagnosis and treatment planning for caries in children during mixed dentition period. The photographs obtained were kept on the Google Drive online platform, and sharing links were sent to investigators using a social network application WhatsApp Messenger (Facebook Corp, Mountain View, CA). The study demonstrated better reliability of teledentistry in primary than in permanent teeth. Moreover, it demonstrated that if used without radiography, teledentistry was not as precise as a clinical examination, but that mobile phone-based teledentistry afforded quite acceptable reliability in the initial diagnosis of caries in children.

Correspondence to: Milan Miladinović, University of Priština/Kosovska Mitrovica, Faculty of Medicine, Anri Dinana b.b, 38220 Kosovska Mitrovica, Serbia. E-mail: milanbetter@gmail.com.

Estai et al. [8] investigated whether traditional clinical dental examination of caries detection in school children could be reliably replaced by a new method – teledentistry examination. The study showed a suboptimal distribution of resources dedicated to dental health care. Specifically, for the children with higher socioeconomic status (predominantly urban dwellers), half of the resources dedicated to dental health care was spent on direct dental examinations. The obtained results suggested that the use of teledentistry methods to examine children with low caries risk had the potential to save around 40 million US dollars a year, so that these resources could be redirected for dental care improvement of children affected by caries.

Teoh et al. [9] investigated the use of teledentistry in specialist dental care at the Royal Children's Hospital for rural and patients dwelling in the region, performing a cost-effectiveness analysis of a teledentistry consultation in comparison to standard consultations in this paediatric health care institution. They found that teledentistry represented an economically viable alternative to the standard practice of face-to-face consultations in this hospital.

Subbalekshmi et al. [10] assessed the reliability and feasibility of teledentistry in screening and diagnosis of dental caries in children aged three to six years. The study enrolled school children in whom the diagnosis of caries was made visually and with digital photographs taken by two investigators using an intraoral camera. It was concluded that in early childhood it was possible to screen for dental caries using digital photography at school-based dental examinations. The road to the use of teledentistry as an effective tool in the diagnosis of dental caries was thus open.

AlKlayb et al. [11] compared effectiveness of mobile phone applications in the education of mothers with children under six years of age within preventive dental care programs. The results showed significant improvement of their knowledge after using the mobile phone application, but even higher effectiveness in mothers with more children compared to those with one child. The study also suggested that the appropriate mobile phone application was able to improve the knowledge of mothers regarding paediatric oral and dental health preservation.

Estai et al. [12] compared the costs of teledentistry approach with those of traditional dental examination among the Australian school children. The cost analysis was accomplished from the perspective of an oral health system, developing a model which simulated the costs incurred by both of these methods for school children aged 5–14 years in Australia. Both fixed and variable expenses were taken into account, expenses for the wages, traveling and accommodation costs, and expenses for the supplies.

It was estimated that the method of teledentistry was able to reduce the overall expenses, and that efforts should be made by dental care service providers to adopt this approach based on new technology.

Pentapati et al. [13] examined reliability of intraoral teledentistry camera in screening oral conditions. They found that intraoral camera was a reliable tool to diagnose common oral diseases, and suggested further research to confirm reliability of teledentistry to diagnose following conditions: sealant retention, pre-malignant lesions, recurrent aphthae, gingival recession and dental malocclusion.

de Almeida et al. [14] used intraoral mobile phone-based photographs as a tool in distant diagnosis of traumatic dental injuries. These diagnoses were compared to the gold standard – in-person dental examination. The degree of agreement between these two methods showed that the accuracy of distant diagnosis was comparable to that made at in-person examination.

Purohit et al. [15] evaluated reliability of a video-graphic method, as a dental caries inspection tool, among 12-year old school children in a rural region in India. The results were compared to the direct visual-tactile examination of children, and the obtained results showed that teledentistry was comparable to clinical dental examination in screening caries among school children. Furthermore, the study provided evidence that teledentistry could be used as an alternative in distant assessment of dental caries and in treatment planning.

Estai et al. [16] estimated effectiveness of teledentistry approach with smartphone camera in distant examination of dental caries. An Android application was designed to collect photographs and upload them to an online server. The photographs were carefully inspected and a diagnosis was established to be compared to the findings obtained by direct clinical dental examination. It was found that, in spite of certain inherent limitations, mobile teledentistry had a possibility of detecting occlusal caries in photographs taken with mobile smartphones, and a combination of telemedicine technologies was recommended to produce a cheap and reliable tool for caries screening.

McLaren et al. [17] performed a study to evaluate accuracy of prediction of dental treatments modalities in children examined by video-based teledentistry. Retrospective reviews of the available dental records were performed in rural paediatric patients. The results indicated that video-based consultations in real time can be an effective way to help us determine the best treatment approach in children with dental diseases. Moreover, the study suggested video-conferencing for consultations in formulation of complex treatment plans for children.

Daniel and Kumar [18] performed a study to compare dental caries identification by dental hygienists using teledentistry compared by dentists using conventional method of clinical examination. The obtained results showed that dental hygienists were able to identify dental caries in photographs of children aged 4–7 years at the same level of accuracy as dentists in clinical settings.

Estai et al. [19] estimated if dental caries could be diagnosed in a valid and reliable way using intraoral digital photographs. In that study, smartphone cameras for taking the photographs and a cloud-server for uploading were used. The obtained results showed that such a teledentistry model had a potential for distant diagnosis of dental caries.

Estai et al. [20] performed a study to assess validity of online cloud-based teledentistry in oral diseases diagnosis. They used a store-and-forward telemedicine system. Their results suggested that such a telemedicine system could be a

valid and reliable alternative to traditional oral examination. They also pointed out a need for further improvement, refinement and robustness testing of such systems.

Torres-Pereira et al. [21] evaluated applicability of telediagnosis in oral medicine using e-mail transfer of digital clinical photographs made by electronic graph and digital camera, which were e-mailed. A distant diagnosis was established and compared to the gold standard. They found that such an approach was able to improve the accuracy of consultations in oral medicine.

Miladinović et al. [22] evaluated the use of telemedicine methods in pathology of odontogenic infections. Their results showed a satisfactory agreement between the diagnoses established using telemedicine and those made by in-person method. Furthermore, in their study, acceptable clinical diagnoses of odontogenic infections could be made using the method of telemedicine, providing a deeper insight into their nature, so that adequate patient management could be planned.

Živković et al. [23] investigated practical use of teledentistry in the diagnosis and routine management planning of endodontic-oral surgery patients and evaluated reliability of telemedicine diagnosis made from a distance. Further, they evaluated a possibility of adequate therapy planning within endodontic-oral surgery treatments. Their results showed that teledentistry can be successfully used in the diagnosis and planning of therapy for periapical lesions affecting frontal teeth, reducing the incurred costs and enabling patient dental management at distant locations. Živković et al**.** [24] studied validity of endodontic-oral surgery consultations using the store-and-forward method of telemedicine. They found that the accomplished consultations between endodontists and oral surgeons were absolutely acceptable and certainly comparable to visual-tactile dental examination of patients.

Comment on previous research in paediatric teledentristy and future research directions

The duty of all doctors is to provide to their patients, especially children, the best they can at the moment. Adverse treatment consequences are primarily the result of incorrect diagnosis, which can be avoided and prevented by using interspecialist consultations [25]. Here, teledentistry emerges as the most convenient method of choice, since it enables rapid, cheap and high quality distant consultation [26].

From a review of the available literature, the focus of teledentistry investigation in children can be seen in early caries diagnosis. The authors agree that a satisfactory distant diagnosis of caries can be obtained and treatment procedures can be decided upon this way as well [6, 8, 10, 12, 15, 16, 18, 19]. It is something to be expected, naturally, since the absence of caries constitutes a basis of oral health in children [27]. Screening for caries using teledentistry approach is possible even at locations without dental health services, while at the same time this approach reduces the costs related to diagnosis and treatment planning.

When other oral conditions are concerned, the authors agree that most of them can be successfully diagnosed from a distance [9, 11, 13, 17, 20, 21]. The situation is similar with the presence and assessment of odontogenic infections treatment [22]. Concerning periapical lesions, it has been reported that distant diagnosis based solely on intraoral examination is not sufficient; it has to be supplemented with radiography [23, 24].

Our opinion and suggestions for further research relate primarily to possibilities of teledentistry in the area of jaw orthopaedics. Further, future research should focus on practical use of distant interdisciplinary consultations in children (especially those with special needs). Then, there is the issue of practical use of teledentistry for interdisciplinary consultation in high risk paediatric dental patients, from the point of view of dentistry and other medical specialties.

Conclusion

Teledentistry has been increasingly used in its different forms in paediatric dental care. Studies designed to assess validity of such supplemental methods report high performance rates and high degrees of practical applicability. All the findings obtained so far encourage further development and use of teledentistry in children.

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CASE REPORTS

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Heart transplantation in a patient with left ventricular assist device after pump thrombosis –The first case report in Serbia

Transplantacija srca kod bolesnika sa ugrađenim uređajem za potporu rada leve komore nakon tromboze uređaja - prvi prikaz takvog slučaja u Srbiji

Duško Terzić*, Svetozar Putnik*, Emilija Nestorović*, Ilija Bilbija*, Ljiljana Gojković Bukarica†, Vladimir Jovičić*, Jovana Rajković†, Miljko Ristić*

Clinical Center of Serbia, *Clinic for Cardiac Surgery, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, †Institute of Pharmacology, Clinical Pharmacology and Toxicology, Belgrade, Serbia

Abstract

Introduction. The device thrombosis is one of the most serious complications of the left ventricle assist device implantation with a high mortality and morbidity rate. **Case report.** A 59-year-old male was implanted by left ventricular assist device Heart Mate II as a bridge to transplantation seventeen months before the onset of a potentially fatal complication – the thrombosis with the complete obstruction of the device. Despite the aggressive pharmacological treatment following the initial suspicion of the pump thrombosis, the patient condition got worse with the final "pump off" alarm that marked the discontinuance of the pump work as a result of the complete obstruction by the thrombus. An appropriate occurrence of an adequate donor resulted in a successful surgical treatment – the heart transplantation. **Conclusion.** The urgent heart transplantation by the first priority rank, or the device replacement, although technically extremely demanding procedures, are successful treatment options for these patients.

Key words:
device removal; heart-assist devices; heart transplantation; thrombosis; treatment outcome.

Apstrakt

Uvod. Tromboza implantiranog uređaja za mehaničku potporu leve komore jedna je od najozbilnijih komplikacija sa visokom stopom morbiditeta i mortaliteta. **Prikaz bolesnika.** Bolesniku starom 59 godina implantiran je uređaj za mehaničku potporu rada leve komore kao most do transplantacije srca, sedamnaest meseci pre nastanka potencijalno fatalne komplikacije - tromboze sa kompletnom opstrukcijom pumpe. Uprkos intenzivnom farmakološkom lečenju posle inicijalne sumnje na trombozu pumpe, stanje bolesnika se pogoršavalo do konačnog oglašavanja finalnog "pump off" alarma, koji je signalizirao zaustavljanje pumpe usled potpune opstrukcije trombom. Pravovremena pojava adekvatnog donora rezultirala je uspešnim hirurškim lečenjem – transplantacijom srca. **Zaključak.** Prioritetna transplantacija srca, po prvom redu hitnosti, ili zamena uređaja, iako tehnički ekstremno zahtevne procedure, predstavljaju uspešne terapijske opcije za tu grupu bolesnika.

Ključne reči:
premeštanje implantiranog aparata; srce, implantabilni mehanički aparati; transplantacija srca; tromboza; lečenje, ishod.

Introduction

The left ventricular assist device (LVAD) implantation has emerged as a relevant option for improving quality of life and survival in patients with the end-stage heart failure. The goals of this procedure include bridge to recovery, bridge to transplant, bridge to candidacy and destination therapy. As the current generation of the continuous flow LVADs activate the coagulation system, anticoagulant therapy is recommended in order to minimize the risk of device thrombosis [1]. The device thrombosis may still occur occasionally, and systemic and local thrombolytic agents are usually applied. Surgical interventions to replace the LVAD device or urgent heart transplantation are in some cases required [2].

Correspondence to: Duško Terzić, Clinical Center of Serbia, Clinic for Cardiac Surgery, Dr. Koste Todorovića 8, 11 000 Belgrade, Serbia. E-mail: terzic.dusko@gmail.com.

Case report

A 59-year-old male with the sudden recurrence of the shortness of breath was admitted to our hospital. Seventeen months before, the patient had been implanted by LVAD Heart Mate II as a bridge to the herat transplantation due to the end-stage heart failure (Figure 1). After the initial uneventful recovery after LVAD implantation, the patient suffered two episodes of gastrointestinal hemorrhage in the fourth and the seventh month postoperatively, which were conservatively, successfully treated. His outpatient anticoagulation regimen was warfarin with a goal international normalized ratio (INR) of 2–3 as well as acetylsalicylic acid, 100 mg daily. His other medications (from last check-up four weeks ago) included ramipril 5 mg daily, amlodipine besylate 5 mg daily, spironolactone 25 mg daily, furosemide 20 mg twice daily, pantoprazole 40 mg twice daily, amiodarone 200 mg daily, levothyroxine 50 mcg daily, atorvastatin 20 mg daily.

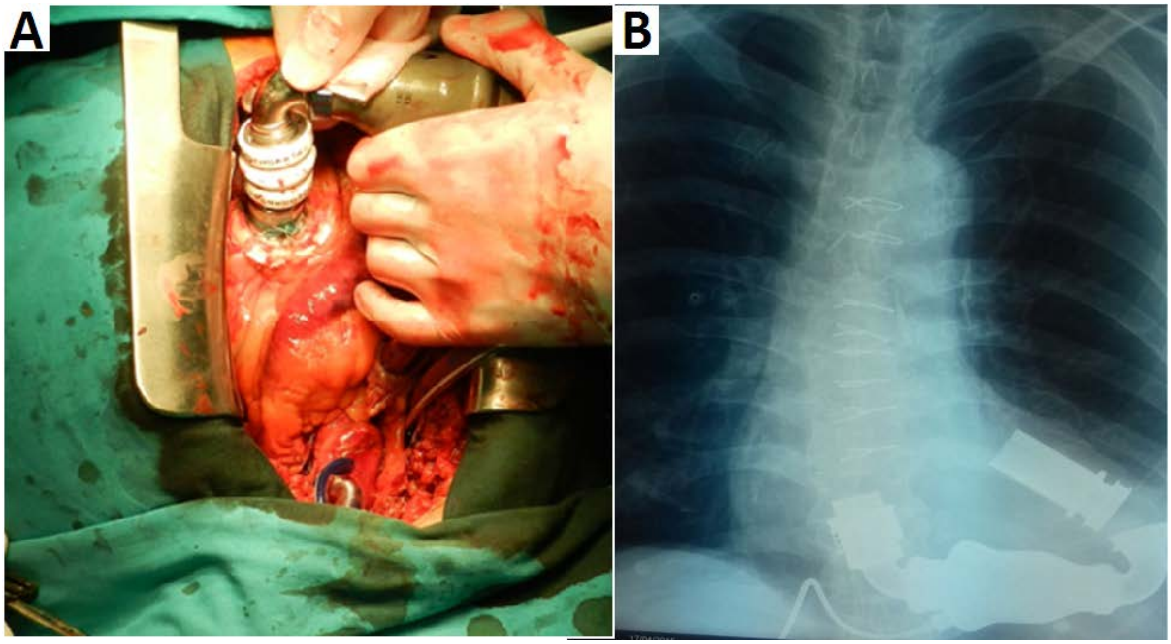

Fig. 1 – A) Left ventricular assist device (LVAD) Heart Mate II implantation as bridge to the heart transplantation; B) Chest X-ray after LVAD Heart Mate II implantation.

At the admission the patient was dyspneic and anxious. The physical examination showed blood pressure of 85/60 mmHg and the heart rate of 80 beats/min. Laboratory tests were consistent with hemolysis. Lactate dehydrogenase (LDH) was elevated at 2,300 IU/L [normal range (nr) 140–280 U/L] (a rise from 400 U/L at the previous check-up four weeks ago), plasma free hemoglobin (pfHGB) 18 mg/dL (does not apear to be above 50 mg/dL) and reticulocytes 2.2% (nr 0.5–2.5%). The INR value was 2.1 (nr 1–2).The LVAD parameters were as following: the pump speed was initially 8,600 rpm but spontaneously dropped down to 8,000 rpm, pump flow (PF) +++ (---), pump power (PP) 15.1–21 W and pulsatily index 1.3. Before the onset of symptoms the LVAD parameters were: pump speed (PS) 8,600 rpm; PF 5.2 L/min; PP 4.6 W; pulsality index (PI) 7.1. The controller device showed a number of "low flow" alarms. The first echocardiogram (ECG) revealed the increase in the dimensions of the left ventricle [both the left ventricular end-diastolic diameter (LVEDD) and left ventricular end systolic diameter (LVESD)] from 6.0 /5.0 cm to 7.3/6.7 cm, respectively. The left ventricle ejection fraction (LVEF) was 15% (Simpson's method). The aortic valve was opening with every beat compared with every third or fourth beat on his last clinic visit. On the color Doppler examination there was no flow through both inflow and outflow cannulas.

The patient was treated by heparin infusion, followed by the infusion of alteplase through the central venous line (started with bolus of 10 mg alteplase during 10 min period, followed by the infusion of 10 mg *per* hour). During the application of the second dose of alteplase on the day 2 after the admission, the patient complained of the acute severe pain on the left side of his chest. At that moment the "pump off" alarm occurred on the monitor display, marking the spontaneous discontinuance of the pump. The patient's LVAD controller was turned off and double drug support with norepinephrine and milrinone was started.

ECG performed after the pump stopped and the inotrope support started showed the mild decrease in the left ventricle dimensions (LVEDD 5.9, LVESD 4.7 cm) with the LVEF in the basal part of 22%. There was a trace of mitral regurgitation. The aortic valve was normaly opening with every beat. There was no flow through both inflow and outflow cannulas as well as through the outflow graft. The chest computed tomography (CT) scan showed the competent outflow graft anastomosis on the ascending aorta as well as the free inflow cannula in the left ventricle. The outflow graft was of substantially narrow lumen with no

signs of kinking. The inflow cannula was in the correct position placed in the direction of the posterior leaflet of the mitral valve. There were no signs of infection around the pump and the drive line (Figure 2).

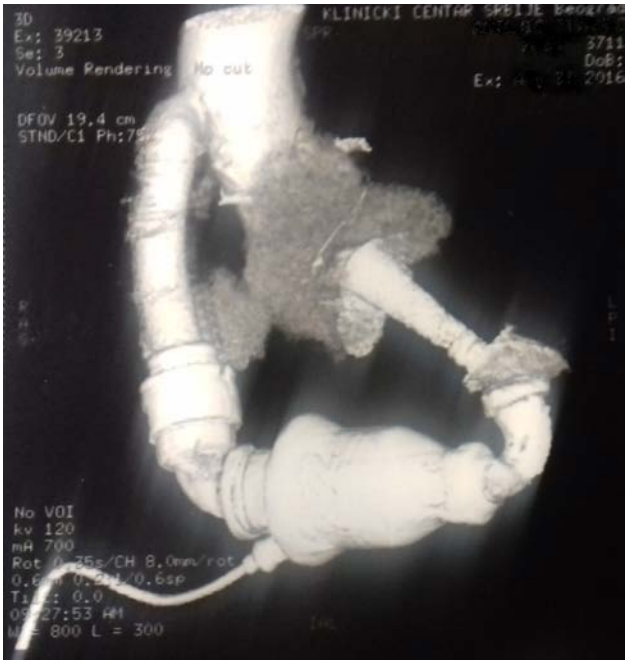

Fig. 2 – Multislice computed tomography (MDCT) of the chest – the outflow graft connection to the ascending aorta and the inflow cannula to the left ventricle are competent.

The haemodynamic inotrope support was continued for the next nine days. The vital sings in that time were: temperature 36.5°C, heart rate of 90 beats/min, mean respiratory rate 24 breaths/ min (nr 12–16 breaths/min) mean arterial pressure of 70 mmHg (nr 70–100 mmHg) and oxygen saturation of 95%. (nr 97–100%). Despite the risks presented, the patient refused suggested pump replacement surgery. He was then en listed on the priority waiting list for the heart transplantation.

At the day 11 upon admission, the favorable occurrence of the adequate donor resulted in the succesfull surgical treatment – the heart transplantation. The operation was carried out as usual under the extracorporeal circulation with the arterial cannulation of the left femoral artery and separate venous cannulation of both caval veins performed after chest reentry. Following resternotomy, it was proceded with the extensive adhesiolysis of the very tough pericardial adhesions. The heart was explanted “en block” with the device**.** After the preparation of the donor heart, the transplantation was performed by biatrial technique (Figure 3). Later inspection of the explanted device confirmed the thrombotic masses in both the inflow and the outflow cannulas and the completely clogged pump rotor.

The operation was completed without complications and the uneventful recovery followed. During the postoperative period the patient was hemodynamically stable, anicteric, eupneic, with good tolerance of physical stress. The regular myocardial biopsy was performed two weeks and one month after the operation. There were no signs of cell or humoral rejection of the transplanted heart.

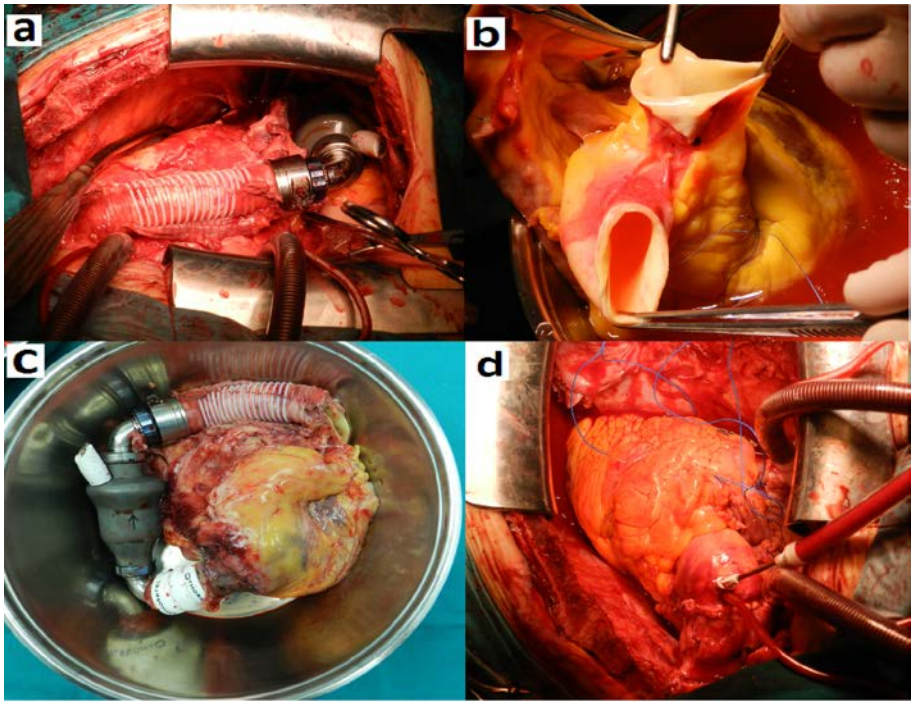

Fig. 3 – Heart transplantation.
a) dissected recipient`s heart with previously implanted left ventricular assist device; b) preparing donor heart; c) explanted heart with the device; d) final result – transplanted heart.

Echocardiography showed normal width of the aorta of 3.1 cm, the normal dimensions of the left ventricle (LVEDD 4.7 cm, LVESD 2.9 cm), excellent systolic function, LVEF 75% by Teicholz, 80% by Simpson. The left atrium size was 3.2 cm. The right ventricle was 2.5 cm with normal tricuspid valve function and morphology.

On the hospitalization day 36 (25 days after the transplantation), the patient was transfered from the isolated care unit to the general care unit and on the day 44, he was discharged home in good general condition. In the regular monthly check-ups, the patient did well. All clinical, histological and laboratory parameters were in the therapeutic range 14 months after the transplantation.

Discussion

Thromboembolic events in patients who recived LVAD are common despite the anticoagulant therapy. The most frequent presentations are: cerebrovascular accident, transient ischemic attack, arterial noncentral nervous system embolism, or pump thrombosis. The device thrombosis of a certain degree was reported in 4% of patients with LVAD implanted as a destination therapy and in 1.5% of patients with LVAD implanted as a bridge to transplantation [3]. There are two main categories of risk factors that predispose to the LVAD thrombosis: device-related and non-device-related [4]. In the screening for and the diagnosis of LVAD thrombosis, laboratory tests are crucial. LVAD thrombosis cause hemolysis and in the early stages it can be identified by elevated LDH, plasma-free hemoglobin and indirect bilirubin levels. Noninvasive diagnostic tests alone are of limited accuracy. Under the certain circumstances, echocardiography can give some indirect evidence like the reduction in diastolic flow velocity through the cannula and/or increased systolic to diastolic flow ratio. Rarely, the direct presentation of LVAD thrombus can be seen. But, the ultrasound “ramp study” is highly sensitive and specific in the detection of axial pump thromosis when used in conjunction with LDH levels [5]. The exact role of routine CT angiography is unclear. Multislice computed tomography (MSCT) is helpful in patients with an unexplained elevated LDH, in order to rule out other causes that may increase this marker and in order to assess patients with a suspected malposition of one of the pump parts. Furthermore, the routine use of MSCT may require a skillful cardiovascular imager with LVAD experience for interpretation [6]. LVAD pump parameters will demonstrate increased pump power or intermittent power spikes. The PF rate will be overestimated with a concomitant decrease in the PI resulting from the reduction of the flow through the LVAD [7]. Ideal strategy for the treatment of LVAD thrombosis is yet to be defined. Medical therapy usually includes the unfractionated heparin infusion, thrombolysis, glycoprotein IIb/IIIa inhibitors, and thrombin inhibitors. These medications are associated with many side effects, mostly bleeding. Most centres weigh the risks associated with the pump replacement vs. thrombolytic therapy in an individualised manner, because it is unclear which patients will have good reponse to medical therapy. If a patient is hemodynamically unstable, the recommended treatment is the pump replacement. On the other hand, if the patient can be hemodynamically stabilized, the other option is the priority heart transplantation [8].

Goldstain et al. [9] formed the algoritam for the diagnosis and therapy of thrombosed LVAD. Patients with LDH elevations or *de novo* power elevations that appear late in the clinical course should be promptly evaluated for frank hemolysis. In the case of the appropriate left ventricle unloading detected by the echocardiography and rump study, the other causes for hemolysis and heart failure symptoms should be searched for. If adequate left ventricle unloading is not confirmed, a MSCT angiogram to evaluate the position of the inflow cannula and outflow graft is indicated. Furthermore, the evidence of unimpeded flow of the contrast from the left ventricle cavity through the outflow graft and into the aorta should be obtained. In the presence of inflow cannula malposition or kinking of the outflow graft, surgical correction should follow. In the absence of pump inflow or outflow abnormalities, the inability to unload the left ventricle on the rump study points to the pump thrombosis. In that case, a patient should be transferred to the intensive care unit for close monitoring and initiation of intravenous heparin and inotropic/diuretic therapy as needed depending on heart failure symptoms. Persistent hemolysis, heart failure symptoms, and/or power spikes may be addressed with more aggressive antithrombotic therapy with direct thrombin inhibitors. If the hemolysis persists despite aggressive antithrombotic therapy, then LVAD replacement should be considered. A patient could be put on the urgent listing for the heart transplantation if the estimated waiting time is no more than a few days and heart failure symptoms can be readily controlled. If the low output state persists and the heart failure progresses, the urgent LVAD replacement is mandatory. If a patient is not potential surgical candidate, in the presence of end-organ dysfunction or hemodynamic compromise, the systemic thrombolytic therapy may be attempted but the prognosis is poor. Some authors advise that patients with pump thrombosis with red alarms-pump stoppage, and in shock unresponsive to battery and controller exchanges, require urgent LVAD replacement [9].

In our clinic, the preffered first line treatment in hemodynamically stable patients with pump thrombosis is thrombolytic therapy with Actylisis®. In the patient reported above, the initial aggressive medical treatment was not successful. On the day 2 following admission, there was a total thrombotic occlusion of the LVAD. After the pump stopped, it was disconnected from the controller and the inotropic support initiated. As the patient was hemodynamically stable and not motivated for the pump replacement, despite the risks presented, he was put on the priority list for the heart transplantation. Nine days later, the adequate donor appeared and the patient underwent the heart transplantation. The operation was successfully completed.

Conclusion

The LVAD thrombosis with total occlusion is one of the most serious complications with a high morbidity and mortality and the treatment must be started immediately. The urgent heart transplantation by the first priority rank or the replacement of the device, although technically extremely demanding procedures, are for the present the successful treatment options for these patients. While our patient had an excellent outcome considering his grave presentation, it again brings up the issue of the optimal management of the LVAD thrombosis.

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CASE REPORT

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Psychogenic diabetes insipidus – A case report of behavioral psychotherapy

Psihogeni dijabetes insipidus – prikaz bihejvioralne psihoterapije

Miodrag M. Stanković*†, Jelena Stevanović‡, Aleksandra Stojanović*, Sandra Stanković§

University of Niš, *Faculty of Medicine, Niš, Serbia; Clinical Centre Niš, †Center for Mental Health Protection, §Clinic for Children's Internal Diseases, Niš, Serbia; ‡General Hospital, Leskovac, Serbia

Abstract

Introduction. Psychogenic diabetes insipidus is potentially a life-threating condition manifesting as a psychogenic thirst disorder with excessive fluid intake (more than 3 L per day) and a preserved function of neurohypophysis and kidneys. **Case report.** We presented a boy aged 4 years and 8 months with symptoms of polydipsia, polyuria, nocturia and malnutrition. Pediatric examination and laboratory analysis were performed, but clear discrimination between psychogenic and nonpsychogenic diabetes insipidus could not be made. A psychiatric consultation was performed to examine the possibility of compulsive fluid taking. The differentiation was performed in two stages. In the first stage, the child was separated from the mother in short intervals. In the second stage, the behavioral psychotherapy interventions were performed: distraction of attention, and positive and negative reinforcement for delaying compulsive fluid taking. The mother was trained to use methods of operant conditioning, privilege and deprivation, as well as methods of exposure and response prevention and relaxation of the child. It was suggested to continue with multidisciplinary treatment (pediatric, liaison psychiatric and behaviour psychotherapeutic). Evaluation of behaviour therapy was performed after 4 and 12 weeks. During 4 weeks of follow-up, the boy reduced the daily fluid intake by 3.5 L, and added 1 kg of body weight. Also, intervals between fluid intake were significantly extended. This therapeutic effect could not be explained by the pediatric treatment introduced prior to the application of behaviour therapy and psychoeducation of the mother. **Conclusion.** Consultations and multidisciplinary approach by different health specialists in resolving of a number of predominantly somatic disorders in children with psychogenic diabetes insipidus should be highlighted as a way of treatment.

Key words:
behavior therapy; child; diabetes insipidus; diagnosis; psychotherapy; treatment outcome.

Apstrakt

Uvod. Psihogeni dijabetes inspidus je potencijalno životnougrožavajuće stanje koje se manifestuje kao poremećaj žeđi sa ekscesivnim unosom tečnosti (više od 3 litre dnevno) i očuvanom funkcijom neurohipofize i bubrega. **Prikaz bolesnika.** Prikazan je dečak uzrasta 4 godine i 8 meseci sa simptomima polidipsije, poliurije, nokturije i malnutricije. Pedijatrijskim ispitivanjem i učinjenim laboratorijskim analizama nije postavljena jasna diferencijalna dijagnoza između psihogenog i nepsihogenog dijabetesa insipidusa. Psihijatrijska konsultacija imala je za cilj sagledavanje mogućnosti postojanja kompulzivnog uzimanja tečnosti i primenu terapije sprečavanja. Diferencijacija se odvijala u dve etape. U prvoj etapi dete je odvojano od majke u kraćim intervalima, a u drugoj fazi uključene su bihejvioralne intervencije: distrakcije pažnje i pozitivno i negativno potkrepljivanje odlaganja kompulzivnog uzimanja tečnosti. Majka je obučavana metodama operantnog uslovljavanja, nagrađivanja i uskraćivanja privilegija, kao i metodama izlaganja, sprečavanja i relaksacije deteta. Predložen je nastavak multidisciplinarnog lečenja (pedijatrijskog, konsultativno psihijatrijskog i bihejvioralno psihoterapijskog). Procena efekata bihejvioralne terapije, sagledana je nakon četiri i dvanaest nedelja. Dečak je smanjio dnevni unos tečnosti za 3,5 L, a dobio je 1 kilogram u telesnoj masi, uz značajno produženje intervala u uzimanju tečnosti. Postignuti terapijski efekat nije mogao biti objašnjen pedijatrijskim tretmanom uvedenim pre primene bihejvioralne terapije i psihoedukacije majke. **Zaključak.** Konsultacije i multidisciplinarni pristup različitih profila zdravstvenih radnika u rešavanju predominantno somatskih poremećaja psihogenog dijabetesa insipidudsa kod dece treba da budu istaknuti kao način lečenja.

Ključne reči:
bihevioralna terapija; deca; dijabetes insipiuds; dijagnoza; psihoterapija; lečenje, ishod.

Correspondence to: Miodrag M. Stanković, Clinical Centre Niš, Center for Mental Health Protection, Radnih bigada 26, 18000 Niš, Serbia. E-mail: doc.stankovic@gmail.com

Introduction

Psychogenic diabetes insipidus (psychogenic – primary polydipsia) is a psychogenic thirst disorder with excessive fluid intake (more than 3 L *per* day) and a preserved function of neurohypophysis (the posterior pituitary) and kidneys [1]. In a prolonged period of time, daily intake of large amounts of fluid results in the development of a functional form of diabetes insipidus, with dilution of extracellular fluid, inhibition of antidiuretic hormone secretion and aqueous diuresis [2]. The clinical presentation is dominated by poliuria with nocturia, followed by extreme thirst and fluid intake (polydipsia). The daily amount of urine output varies, ranging from 16 L to 20 L in severe, to 2.5 L to 6 L in milder cases [3]. Urinating is frequent, at intervals of 30–60 min throughout the day and night. The urine is clear and colorless, with low specific gravity and reduced osmolality. The patients are tired and sleepy, often suffering from constipation. This is potentially a life-threating condition with a constant risk of dehydration and hypovolemia [4]. Diagnosis and treatment of psychogenic insipid diabetes in children often requires liaison approach, engaging several professionals of various specialties: pediatric endocrinologists and nephrologists**,** child psychiatrist, psychologist [5]. There is a small number of case reports of psychogenic polydipsia in preschool children, and even fewer reports of non-pharmacological therapeutic approaches [6], which makes this article more significant.

We presented a case of diabetes insipidus treated by behavioral psychotherapy highlighting the importance of a paediatric psychiatrist in collaborative work, diagnosis and treatment of psychologically conditioned states with predominantly somatic clinical manifestations and potentially serious somatic complications.

Case report

A boy aged 4 years and 8 months with symptoms of polydipsia, poliuria, nocturia and malnutrition (failure to thrive, body mass under -5.3 SD) was hospitalized at the Clinic for Children's Internal Diseases, Clinical Center Niš, Niš, Serbia. Data indicated that mentioned symptoms, with gradual decrease of appetite, had been present during the several months. Child's fluid intake was up to 5.5 liters a day, followed by frequent urination. During the hospitalization, diuresis and fluid intake were closely monitored. Extensive laboratory tests were performed (blood count, biochemical analysis, acid-base and hormone status, general and biochemical urine examination, the chloride concentration in sweat). Craniography, and kidney and abdomen ultrasonographic examination did not indicate any significant pathological changes. Nuclear magnetic resonance (NMR) imaging revealed a change in the pituitary gland which diagnostically correlated with microadenoma. The tubular function of the kidneys was normal. In consultation with the endocrinologist, a fluid deprivation test was performed. The test results were inconsistent with severe symptoms and malnutrition revealing partial deficit of the antidiuretic hormone. The diagnosis of a partial neurohormonal diabetes insipidus was made, and desmopresine nasal spray was administrated (10 mcg, twice daily). Although repeated analysis were performed, a psychogenic diabetes insipidus could not be excluded. During the first month of follow-up, the boy continued to take more fluid than expected, which indicated a presence of compulsive behavior. Finally, a psychologist and a psychiatrist were consulted. The examination showed psychomotor development of the boy within expected limits. The patient lives with his mother in an incomplete nuclear family, as the first and only child. Some chronic somatic disorders in the family history were found**,** mostly cardiovascular ones and epilepsy. There were no data on psychiatric heredity in the context of a close family. During the examination, the boy showed average intellectual scores. He did not exhibit symptoms of neurodevelopmental disorders. Preoccupation with water intake was excessive, persisting and potentially a life-threatening. Water intake was repetitive, volitional and in response to preoccupation. The child was not able to articulate the aim of his behavior. Repetitive behavior was taking more than 1 h per day and cause significant impairment in social functioning. Behavior could not be attribute to the effects of substance, and was not better explained by another mental disorder. A diagnostic interview with the mother pointed to the absence of a structured educational approach, without structured mechanism of privilege and deprivation. Relationship of parent-child was significantly perturbed [Parent-Infant Relationship Global Assessment Scale (PIR-GAS) score 60], mother-child relationship was less than optimal and mother was distressed at home which put development progress of the dyad at risk. Bearing in mind that the use of antidiuretic hormone in the therapy did not lead to improvement of the condition and that behavioral analysis indicated the possibility of compulsive taking of the fluid, we assumed that the disorder was psychogenic.

Applied behavioral therapy

Behavioral therapy was carried out in two stages during 3 sessions. In the first stage, the boy was separated from his mother in an half hour time, and during that time he did not exhibit compulsive behavior nor the anxiety over staying alone with a stranger. In the second phase (another half hour), behavioral interventions were applied: distraction of attention, positive reinforcement for delaying compulsive fluid intake during occupational activities, and a negative reinforcement with the exclusion of mothers' aversive comments when rejoining the child. During three consecutive days, the procedure was repeated with prolonging the second phase for 30 more minutes with the possibility of reunion with the mother after 60 min for a period of 5 minutes. The boy was

allowed to take fluid after 90 min, which meant once during the second phase.

Advising the mother about modification of the child's behavior included psychoeducation (cognitive therapy) and a video footage of working with the child (model learning). It was suggested for fluid intake to be limited to 6 times a day, taking a maximum of 350 mL per taking. Using a substitution such as chewing gum, a small piece of ice, a bottle with a dozer and small sips was suggested. The principle of behavior change by methods of positive and negative reinforcement (operant conditioning) was explained in details, with token economy, tables and stickers.

Follow-up

Evaluation of psychotherapeutic effects was performed after 4 and after 12 weeks. The mother kept a diary of daily fluid intake, body weight and diuresis of the child. By the first visit, the boy reduced daily fluid intake by 3.5 L, and added 1 kg of body weight with extension of the intervals between fluid taking to a total of 6 times a day. The effects sustained on the second visit. The achieved therapeutic effect could not be explained by pharmacotherapeutic treatment (desmopresine) that did not have an effect on compulsive behavior prior to the application of behavioral therapy and psychoeducation of the mother.

Discussion

We described a preschool child with a clinical presentation of diabetes insipidus. Extensive nephrological, endocrinological, laboratory and neuroimaging examinations indicated a partial deficit of antidiuretic hormone and a clinically non-significant pituitary gland microadenoma. Partial neurohormonal diabetes insipidus was diagnosed. Deficit of antidiuretic hormone was supplemented without an effect on excessive fluid intake.

The psychiatric examination was performed to exam the possibility of compulsive fluid taking on the field of insufficiently explained somatic diabetes insipidus. Medical causes of polydipsia, polyuria, and/or hyponatremia were ruled out trough pediatric examinations.

Psychogenic diabetes insipidus, as a psychological component, presents compulsive fluid intake. It differs from developmentally normative preoccupations and rituals by being excessive or persisting beyond the developmentally appropriate age, and cannot be better explained as a direct consequence of another medical condition [7, 8]. Compulsive fluid intake is not classified in psychiatric classifications, but could be viewed as repetitive behavior and respond to the preoccupations, or could be some form of recurrent body focused repetitive behavior. Assessing the relationship of the parent-child dyad, which was significantly perturbed, made possibilities that such a relationship with a little relaxed enjoyment caused anxiety and compulsions that reduced the child anxiety. Differential-diagnostic considerations of the occurrence of clinical manifestations in the time frame indicate that dyad relationship was primarily impaired, and the association of compulsive fluid and coexistence of diabetes insipidus was secondary. This is supported by the fact that behavioral therapy was effective, and that the previously applied therapy was ineffective. The effectiveness of behavioral therapy has been previously reported in a follow-up case [6]. The ability to delay compulsive fluid intake in controlled conditions followed with attention distraction and positive and negative reinforcement, pointed to the importance of the psychogenic component in the appearance and maintenance of the disorder.

The focus of behavioral therapy was controlling the stimuli, and restriction of fluid intake, which included dominantly operant conditioning. Psychoeducation and cognitive therapy of the mother were an integral part of the therapy, aimed at reducing anxiety in the dyad relationship. The effectiveness of the proposed methods of behavioral modification in further psychotherapeutic work confirmed assumption that initially had been made. The achieved therapeutic effect could not be explained by the pediatric pharmacotherapy introduced prior to the application of behavioral therapy and psychoeducation of the mother.

Conclusion

The importance of this case report is to emphasize the importance of liaison and multidisciplinary approach (pediatrician, child psychiatrist, psychologist, psychotherapist) in diagnosis and therapy of a number of predominantly somatic disorders in children with psychogenic diabetes insipidus.

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Should anti-vitamin K be started on the first day in non-high risk pulmonary embolism?

Da li bi trebalo terapiju antagonistima vitamina K otpočeti prvog dana kod bolesnika sa plućnom embolijom koji nemaju visok rizik?

Goran Koraćević*, Dragana Ilić†

University of Niš, Clinical Center Niš, *Clinic of Cardiology, †Department of Radiology, Niš, Serbia

Abstract

Introduction. Protocols and guidelines have been improving results of our clinical practice. Sometimes there have been differences between guidelines on the same topic, but they have not been so important usually. As far as the start of vitamin K antagonists (VKA) in a non-high risk pulmonary thromboembolism (PTE) patients is concerned, there is global consensus (reflected in all comprehensive guidelines) that it should be on the admission day or a day later. However, there are situations in which this VKA administering from the first (or second) day of hospitalization may actually complicate the treatment. **Case report.** As an illustration, our female, 71 years old patient with second unprovoked, intermediate-high risk PTE was given low-molecular-weight heparin (LMWH) + VKA from the second day. Due to lack of improvement in symptoms, oxygen saturation and D dimer after 9 days, computed tomography pulmonary angiography (CTPA) was repeated and it confirmed minimal advancement. The patient already had achieved target international normalized ratio (INR) and it complicated proceeding to fibrinolytic therapy. **Conclusion.** Correction of the therapeutic approach in the PTE treatment may be needed even if the treatment is completely conducted according to the latest guidelines. We recommend postponing VKA from the first (or second) day of hospitalization (as suggested in all available guidelines for non-high risk PTE patients) until satisfying clinical improvement is reached.

Key words:
pulmonary embolism; anticoagulants; computed tomography angiography; lung; fibrin fragment d; treatment outcome.

Apstrakt

Uvod. Protokoli i smernice poboljšavaju rezultate naše kliničke prakse. Ponekad postoje razlike između preporuka o istoj temi, ali obično te razlike nisu toliko važne. U vezi sa početkom primene antagonista vitamina K (VKA) kod bolesnika sa plućnom tromboembolijom (PTE), postoji globalni konsenzus (prisutan u svim savremenim smernicama) da bi to trebalo da bude na dan prijema ili dan kasnije. Međutim, postoje situacije u kojima davanje VKA od prvog (ili drugog) dana hospitalizacije može, zapravo, komplikovati tretman. **Prikaz bolesnika.** Kao ilustracija, naša 71-godišnja bolesnica, sa drugom neprovociranom PTE srednjeg rizika, je dobila heparin male molekulske težine (LMVH) + VKA od drugog dana hospitalizacije. Zbog izostanka poboljšanja simptoma, saturacije kiseonikom i D dimera nakon 9 dana, kompjuterizovana tomografski pulmonarna angiografija (CTPA) je ponovljena i nalaz je potvrdio minimalan napredak. Bolesnica je već postigla ciljni internacionalni normalizovani odnos (INR) i to je komplikovalo prelazak na fibrinolitičku terapiju. **Zaključak.** Korekcija terapijskog pristupa u lečenju PTE može biti potrebna čak i kad se lečenje sprovodi u skladu sa savremenim preporukama. Predlaže se odlaganje primene VKA od prvog (ili drugog) dana hospitalizacije (kao što se preporučuje u svim raspoloživim vodičima za bolesnike sa PTE koji nisu na visokom riziku), dok se ne postigne kliničko poboljšanje.

Ključne reči:
pluća, embolija; antikoagulansi; angiografija, tomografska, kompjuterizovana; pluća; d dimer; lečenje, ishod.

Correspondence to: Goran Koraćević, University of Niš, Clinical Center Niš, Clinic of Cardiology, 18 000 Niš, Serbia.
E-mail: gkoracevic@yahoo.com

Introduction

Pulmonary thromboembolism (PTE) is the third most important cardiovascular disease (following acute myocardial infarction and stroke), as judged by incidence and mortality. The medical importance of PTE increases due to high chance of misdiagnosis, because PTE often presents with insufficiently specific symptoms and signs.

Excluding cardiac arrest, shock state in PTE carries the highest individual mortality risk and fibrinolysis is (clearly) indicated in this subgroup of patients (encompassing 5%–10% of all PTE patients). Situation about fibrinolysis is far less clear in the intriguing and heterogeneous subgroup with intermediate risk. Although individual mortality risk is not so high in these patients, this subgroup is numerous and therefore it results in large number of fatalities. As an illustration, 22% of patients in the subgroup with high risk died during 30 days of PTE, less in the intermediate–high risk subgroup (7.7%) and the intermediate–low risk subgroup (6.0%) and far less (0.5%) in patients at low risk [1]. Moreover, many of the intermediate–risk PTE patients who survive hospitalization are not completely cured – they can suffer "post-PTE syndrome". The "post-PTE syndrome" means that following PTE, a patient has shortness of breath, fatigue, impaired quality of life and abnormalities in heart and lung findings without any other explanation [2]. The "post-PTE syndrome" is not rare at all – it can be expected in around half of all PTE patients [3].

Therefore, PTE patients with intermediate risk can not be considered safe at all. As they can be very heterogeneous subgroup, further refinement in risk stratification is needed. For this purpose, Bova et al. [4] have suggested the score that combines somewhat lower blood pressure (BP), increased heart rate (HR), right ventricle (RV) dysfunction as well as markers of myocardial injury. A non-high risk PTE patient with all above-mentioned has a seven-fold increase in the risk of an adverse 30-day PTE- related outcome predicted. The following criteria are used: systolic BP 90 to 100 mmHg – 2 points; increased cardiac troponin – 2 points; RV dysfunction on echocardiogram or multi slice computed tomography (MSCT) – 2 points and HR $\geq$ 110 beats per minute (b.p.m.) – 1 point. Patients with 0 to 2 points have the first stage, 3 to 4 points the second stage, and with more than 4 points the third stage, with corresponding mortality (30 days following the admission) related to PTE of 1.7%, 5.0% and 15.5%, respectively [4, 5]. Such a score is a certain prerequisite to come closer to the answer to the probably central question in the drug therapy of PTE: whom to thrombolyse among intermediate -risk patients? This remains unresolved issue for decades [6, 7].

To the contrary, the optimal time to start VKA seems to be well-defined, because all available contemporary PTE guidelines suggest it should be on the first or second day, i.e., when the diagnosis is made. For example, the National Institute for Health and Care Exellence (NICE) pathways suggest VKA should be initiated within one day of diagnosis [8]. Similarly, the Anticoagulation Forum recommends that we should start VKA as soon as parenteral anticoagulant's therapeutic concentration is obtained [9]. There are good reasons for such recommendation. One of them is to shorten expensive hospital stay: the sooner we obtain therapeutic INR, the sooner the patient can become an outpatient, with consequent savings. Moreover, the patient may avoid potential in-hospital infection. The reason more is to diminish likelihood of heparin- induced thrombocytopenia (HIT), serious complication of heparin use [10].

However, in PTE patients with an intermediate risk, sudden worsening may occur with hemodynamic compromise, which requires the escalation of therapy. In such situations, already achieved therapeutic level of VKA may increase the bleeding risk and therefore it may complicate an already difficult scenario. As the knowledge of medical community accumulates and new anticoagulants become more widely used, the need for critical evaluation of PTE protocols appears in practice.

Case report

A female patient (71 years old, 70 kg) without any actual medications was admitted because of dyspnea with suspected new PTE. No obvious provoking factor was observed. In the past medical history seven years ago, the patient was hospitalized due to a first recognized unprovoked PTE episode, but she has no medical documentation. Her duplex ultrasound (B-mode imaging and Doppler waveform analysis), and color Doppler of leg veins were then without signs of thrombosis. The patient was treated only by subcutaneous injections at that time. She also had light obstructive lung disease, but no ischemic heart disease diagnosed. Her actual BP was 110/70 mmHg, with diminished respiration sounds at basal part of the right hemithorax on lung auscultation. Her electrocardiogram (ECG) demonstrated sinus rhythm, HR 77 b.p.m., QS in lead III and aVF with ST elevation 0.4 mm in D3 and 0.2 mm in aVF, as well as with negative T in lead III and aVF and Rs in V_2, suggesting recent myocardial infarction; $S_1Q_3T_3$ and negative / biphasic T in V_1–V_4. The absence of negative T in lead I and aVL together with maximal negative T in V_1 (as compared to V_2–V_4) suggested PTE (in differential diagnosis with acute coronary syndrome without ST elevation) [11]. Pathological Q in lead III and aVF resembled description in the American Heart Association statement: "Q in III and aVF (pseudo – infarction)" [12]. Echocardiogram showed dilated RV 34 mm with tricuspid regurgitation 2-3+ (out of 4) with RV systolic pressure 58 mmHg. Vena cava inferior (VCI) was dilated (24 mm), without inspiratory collapse. Left ventricle (LV) diastolic dimension was normal (46 mm), LV ejection fraction was normal (63%), too and regional LV contractility was preserved.

Wells score was 4.5 (previous PTE 1.5 point + alternative diagnosis less likely than PTE 3 points) and Revised Geneva score was 7 (previous PTE 3 points + HR 75–94 b.p.m. 3 points + age > 65 years 1 point). Both scores suggested intermediate clinical probability for PTE. Multislice computed tomography pulmonary angiogram (CTPA) showed the presence of thrombotic

masses in the lobar branches of the pulmonary arteries bilaterally (Figure 1).

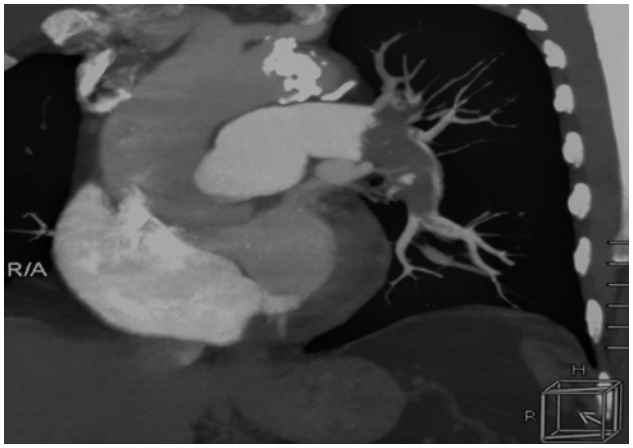

Fig. 1 – Computed tomography pulmonary angiogram (CTPA) shows the central embolic material in the left pulmonary artery (coronal plane). Thrombotic masses were seen in the segmental arteries of the lower lung lobes. There was only a marginal flow of blood in these arteries. There was a small pleural effusion (2cm) on the right side. There was no consolidation of lung parenchyma. Right ventricle end-diastolic diameter/left ventricle end-diastolic diameter (RVEDD/LVEDD) ratio was 1.6 (cut-off value 0.9), as measured 1 cm above and parallel to the annular line in the four chamber view.

D-dimer was high, 3,383 μg/L (age-adjusted cut-off was for her 710 μg/L, using latex method), high sensitive troponin I was 40 ng/L (borderline, normal values < 40 ng/L), brain nariuretic peptide (BNP) was 807 ng/L (normal values < 30 ng/L in chronic and < 100 ng/L in acute setting). Her oxygen saturation was 88% while breathing room air, C-reactive protein was 16.2 mg/L (3.2 times upper normal limit of 5 mg/L), procalcitonin 0.03 ng/ml (in the normal range). A hematologist excluded antiphospholipid syndrome and systemic lupus erythematodes. Neither gynecologist nor gastroenterologist have found carcinoma. Her duplex ultrasound and color Doppler of veins of lower extremities showed no signs of thrombosis, just with small localized dilatation. Pulmonary embolism seventy index (PESI) score was 91 (age in years 71 + arterial oxyhaemoglobin saturation < 90%, 20 points), i.e. Class III, moderate mortality risk (3.2%–7.1%).

Repeated unprovoked PTE of the patient was classified as intermediate-high risk (no hypotension, PESI III–V, present RV dysfunction and cardiac biomarker). Our patient had no hypotension at admission. Therefore, she was treated without fibrinolytic. She received enoxaparin, 1 mg per kg of body weight two times a day (b.i.d.) subcutaneously (s.c.) and warfarin from the second day, according to the guidelines, including the latest 2014 European Society of Cardiology (ESC) Guidelines and 2016 Anticoagulation Forum Pulmonary Embolism Guidelines [13, 14].

Anti-Xa was 0.76 IU/mL on the 5th day and enoxaparin dose was raised to 80 mg b.i.d. International normalized ratio (INR) reached a therapeutic level (≥ 2) on the ninth day – it was 2.2. On the 10th day of hospitalization, the patient was still dyspnoic, her oxygen saturation was 93%, D-dimer was 2,346 μg/L, i.e. three markers of no clinical improvement with the usual protocol. High D-dimer in PTE patients on anticoagulant therapy usually means residual thrombosis [15]. Therefore, we repeated CTPA which showed the continued presence of a thrombotic mass in the pulmonary arteries, predominantly of the lower lobe on both sides, but with a discrete mass reduction. No pleural effusion was seen (Figure 2).

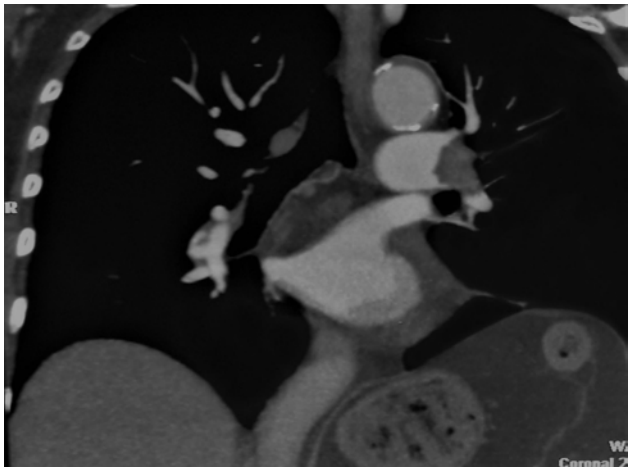

Fig. 2 – Computed tomography pulmonary angiogram (CTPA) in coronal plane shows shows a small reduction of the thrombotic mass in the left pulmonary artery.

We recognized that the effect of previous treatment was minimal and decided to proceed with more effective therapy, according to guidelines [12]. In order to prepare our patient for thrombolysis, we stopped VKA and introduced fondaparinux 2.5 mg next day. When INR dropped below two, we started fondaparinux 7.5 mg once-daily. Unfractionated heparin was not given because frequent activated partial thromboplastin time (aPTT) measurements, e.g. every two hours (when fibrin-selective thrombolytic is applied) was not possible at our institution. The day after beginning treatment with fondaparinux 7.5 mg once-daily, fibrinogen was 4.8 g/L (upper limit of normal 4.6 g/L) and we gave 50 mg of tissue plasminogen activator (TPA) and continued fondaparinux. The rapid clinical improvement was observed: dyspnea disappeared, oxygen saturation increased to 96%, D-dimer decreased to 813 μg/L, RV dimensions and RV systolic pressure got normalized, VCI decreased toward normal (24 mm) and inspiratory collapse appeared. Magnetic resonance pulmonary angiography (MRPA) was done to evaluate eventual residual thrombosis in pulmonary arteries (Figure 3). It showed significant reduction of thrombotic masses in pulmonary arteries. Thrombus was seen in the main artery of the left lung, the diameter was 12 x 10 mm. The reduction of flow through this artery was 30%. Also, the diameters of

both pulmonary arteries were reduced to 20 mm. Color Doppler ultrasound showed no thrombus in her deep leg veins. Her *Clostridium difficile*-induced enterocolitis was well-controlled. Next ECGs showed the presence of r in lead III and aVF. On the day of discharge, D-dimer was 505 μg/L, fondaparinux was ceased and rivaroxaban 15 mg b.i.d. was introduced. The rivaroxaban dose was decreased to 20 mg once daily on the 21st day from the hospital admission. The ergometer bicycle graduated exercise test was negative a month later. In the 5th month following hospitalization, the patient requested the switching from rivaroxaban to VKA (due to financial reasons). Two years from the hospitalization, the patient is without complains, including dyspnea, chest pain, and bleeding. Echocardiography demonstrated normal RV dimensions and pulmonary artery (PA) systolic pressure.

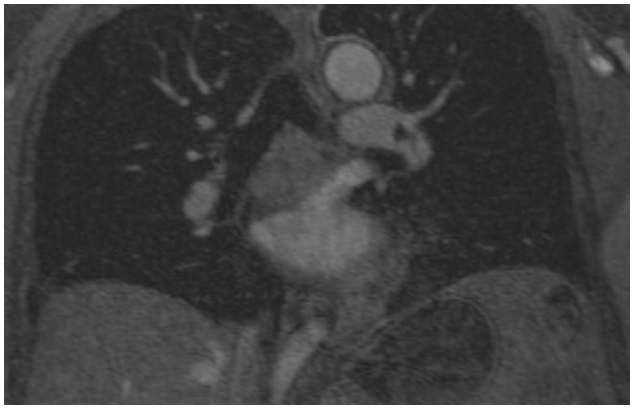

Fig. 3 – Magnetic resonance angiography of pulmonary arteries (MRPA) shows reduced thrombotic mass in the left pulmonary artery.

Diagnostic tests for the detection of the antiphospholipid syndrome were performed with negative result, e.g., Anticoagulant Dilute Russell Viper Venom Test (DRVVT) and silica clotting time (SCT), as well as anticardiolipin antibodies and antibodies to β2-glycoprotein I. Furthermore, in the DNA analysis, Factor V Leiden, FII 20210A, methylenetetrahydrofolate reductase (MTHFR) variant C677T, antithrombin (AT) III, but also Factor XIII were all negative. On the other hand, the patient was found to be heterozygot for plasminogen activator inhibitor-1 (PAI-1) [high risk "4G" for polymorphysm 4G/5G in the position – 675 was present in one gene copy (heterozygot) for PAI-1 (SERPINE1)].

Discussion

As many as 1/4 of all venous thromboembolism (VTE) episodes may occur in patients with malignant neoplasm [16]. An occult cancer was found in as many as 7.6% of 5,863 VTE patients of the large *Registro Informatizado Enfermedad TromboEmbólica* (RIETE) registry. Independent predictors were chronic pulmonary disease, male gender, age over 70 years, anemia, previous episode of VTE, recent operation and increased platelet count [17]. In PTE patients detailed medical history, physical examination as well as usual laboratory analyses are important [14]. If we do sputum cytology plus pelvic and MSCT of abdomen and pelvis, as well as mammography, it is probable that we might not improve survival of the whole group tested [14], but we can double the cancers diagnosed (as judged by meta-analysis of 2287 VTE patients) [16] and early-stage cancers [18]. On the other hand, extensive screening has significant psychological and financial consequences [18]. As a kind of balance, one may follow the NICE guidelines, i.e. add sputum cytology plus pelvic and MSCT of abdomen and pelvis, as well as mammography in patients who are > 40 years old [18]. Other way, we can proceed with tumor marker screening which is adjusted to sex and age (colon, prostate, breast and cervix) [18].

It is also important to decide how long we should recommend oral anticoagulant therapy (OAC) following VTE event. Mostly, it depends on whether the first VTE episode is provoked. In patients with unprovoked VTE longer OAC administration is generally needed. To refine the risk stratification following interruption of OAC, adequate scores were developed, e.g., DASH, Vienna prediction model and HERDOO2 score. For example, the DASH score incorporates high D-dimer concentration (2 points), being ≤ 50 years old (1 point), male gender (1 point) and the use of hormone (-2 points). If the score is >1 it is recommended to proceed with OAC due to high risk of rethrombosis (over 5% a year) [19, 20]. In parallel, it is also important to evaluate patient's risk of bleeding, as the decision to continue OAC or not has to be a balance of both risks (for rethrombosis and for hemorrhage). For bleeding prediction we have several options: RIETE, VTE-BLEED, the Kuijer, mOBRI, Shireman, ATRIA, HEMORR2HAGES, HAS-BLED, modified HAS-BLED, ACCP scores, EINSTEIN model, Hokusai model, ACCP scheme, Outpatient Bleeding Risk Index, etc. [21–24].

The most important reason for guideline authors to recommend VKA from the beginning of PTE treatment has been presumably an intention to avoid both prolonged hospitalization and HIT. This suggestion has not been changed for years, meaning that it has functioned correctly. PTE patients with intermediate risk at admission usually react favorably to anticoagulant therapy and LMWH or fondaparinux are the drugs of choice for most of them [25]. The drawback with starting VKA early arises when such patients during hospitalization experience hemodynamic compromise with the need for therapy escalation, including often "secondary" thrombolysis [25]. Reasons for this hemodynamic worsening are numerous: progression of RV dysfunction, new thromboembolism from concomitant deep venous thrombosis, additional damage to cardiopulmonary function from comorbidities (e.g., infection, anemia, ischemia, arrhythmias), etc. To our opinion, another reason for "secondary" thrombolysis are persistent

symptoms (e.g., severe dyspnea) despite anticoagulant treatment [13].

Half-dose (50 mg) of recombinant tissue-type plasminogen activator (rtPA) is safe in patients with 'moderate' PTE [14] and efficient comparably to 100 mg rtPA [26], which led to the name "safe-dose thrombolysis", as originally suggested by Sharifi et al. [27]. Administration of fibrinolytic agent, while the patient is on VKA and has therapeutic INR, may lead to excessive bleeding. Therefore, it is wise to avoid VKA until it becomes obvious that fibrinolytic treatment will not be needed.

We believe that the right time to start OAC in intermediate-risk PTE patients is when symptoms, ECG, echocardiographic findings, O_2 saturation, etc. get under control [28]. MRPA was useful in our patient for targeted evaluation of particular pulmonary artery to analyze eventual thrombus burden reduction following anticoagulant / thrombolytic therapy. In contrast to CTPA, MRPA can help us to individualize therapy without the risk of excessive radiation.

Conclusion

This case report suggests that even if the treatment of PTE has completely been conducted in accordance with the latest guidelines, the outcome of the treatment may be suboptimal

As our case report illustrates, with VKA from the first day of admission, it is somewhat complicated to administer thrombolytic later during the clinical course (if there is no improvement in dyspnea, ECG, echo, oxygen saturation, etc). We suggest postponing VKA from the first (or second) day of hospitalization (as suggested in all available guidelines for non-high risk PTE patients) until satisfying clinical improvement is reached.

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Minimally invasive approach for the treatment of pancreatic pseudcyst. Transgastric drainage – where we are now?

Minimalno invazivni pristup u lečenju pseudociste pankreasa. Transgastrična drenaža – gde smo sada?

Nenad Perišić*, Mihailo Bezmarević†, Radoje Doder*‡, Darko Mirković†‡, Irina Brčerević*, Stanko Petrović*

Military Medical Academy, *Clinic for Gastroenterology, †Clinic for General Surgery, Belgrade, Serbia; University of Defense, ‡Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Introduction. Minimally invasive approach for the treatment of acute pancreatitis (AP) and its complications has proven to reduce morbidity and mortality rate, length of hospitalization and costs of treatment, and improve quality of life of the patients. This approach for the AP has been implemented in developed countries, but in our region lags behind. In this case report we presented the successful endoscopic transgastric drainage of the large pancreatic pseudocyst (PPC) developed as a complication of AP. **Case report.** A 63-years old male patient was presented with nausea and vomiting as a consequence of the compressive effects of the PPC in the body and tail of the pancreas after episode of AP. On computed tomography (CT) scan, it was shown a cystic formation in the region of the pancreatic body and tail compressing stomach which was verified on upper endoscopy. Under fluoroscopy, using lateral duodenoscope, the biliary plastic prosthesis of 12 French and 8 cm of length was placed throughout posterior stomach wall into the PPC. The intervention was finished uneventfully, without complications. On CT scan performed 7 days after procedure, the reduction of the PPC size was significant and control CT scan one month after the procedure and removal of the prosthesis showed almost complete resolution of the PPC. **Conclusion.** Endoscopic transgastric drainage is safe and effective procedure for PPCs especially when the PPC has propulsion effects on stomach wall.

Key words:
pancreatitis; acute disease; pancreatic pseudocyst; minimally invasive surgical procedures; drainage; treatment outcome.

Apstrakt

Uvod. Minimalno invazivni pristup u lečenju akutnog pankreatitisa (AP) i njegovih komplikacija dovodi do smanjenja stope morbiditeta i mortaliteta, dužine hospitalizacije i troškova lečenja i poboljšava kvalitet života bolesnika. Ovaj pristup u lečenju AP je implementiran u razvijenim zemljama, ali naš region zaostaje u njegovoj primeni. Cilj rada je bio prikaz bolesnika kod koga je učinjena uspešna endoskopska transgastrična drenaža pseudociste pankreasa (PPC) nastale usled komplikacija AP. **Prikaz bolesnika.** Muškarac, starosti 63 godine, javio se zbog mučnine i povraćanja usled kompresivnih efekata PPC u telu i repu pankreasa, a nakon ataka AP. Na kompjuterizovanoj tomografiji (CT) cistična formacija nalazila se u regiji tela i repa pankreasa sa kompresijom na želudac, što je potvrđeno gornjom endoskopijom. Pod fluoroskopijom, uz pomoć lateralnog duodenoskopa plasirana je plastična bilijarna proteza od 12 frenča, dužine 8 cm kroz zadnji zid želuca u PPC. Procedura je protekla bez komplikacija. Na CT pregledu, 7 dana nakon intervencije, potvrđeno je značajno smanjenje veličine PPC, a na kontrolnom CT pregledu, mesec dana od intervencije i odstranjenja proteze, potvrđena je skoro potpuna rezolucija PPC. **Zaključak.** Endoskopska transgastrična drenaža je sigurna i efikasna procedura u lečenju PPC, naročito kada PPC ima propulsivni efekat na zid želuca.

Ključne reči:
pankreatitis; akutna bolest; pankreas, pseudocista; hirurgija, minimalno invazivne procedure; drenaža; lečenje, ishod.

Correspondence to: Mihailo Bezmarević, Military Medical Academy, Clinic for General Surgery, Crnotravska 17, 11 000, Belgrade, Serbia. E-mail: mihailobezmarevic@gmail.com.

Introduction

Acute pancreatitis (AP) accounts over the 50% of all hospital admissions due to pancreatic diseases and still represent one of the most unpredictable disease of the digestive system. The incidence of AP in UK is 30–50/100,000/year which makes around 20,000 hospitalizations per year [1]. However, the highest incidence of AP has registered in USA and Finland [2]. In 2016, in Serbia, 2,768 patients were admitted to the hospital for AP treatment (male/female – 1,630/1,138), whereas 170 patients (male/female – 105/65) due to cystic lesions of the pancreas, which included 6.15% in overall morbidity [3]. Pancreatic pseudocysts (PPCs) account for 75% of the cyst lesions of the gland and they are the most common complication of AP and chronic pancreatitis [4, 5]. The incidence of PPCs is 10% to 20% of patients with AP and may be present in 20% to 40% of patients with chronic pancreatitis [5, 6]. According to the Atlanta 2012 revised classification, the PPC is an encapsulated collection of fluid with a well defined inflammatory wall, minimal or no necrosis, which often requires for maturation more than four weeks after the onset of an acute pancreatic episode [7]. This definition well distinguished PPCs from other entities in AP [acute peripancreatic fluid collections, acute necrotic collections, walled-off pancreatic necrosis (WOPN), and cystic neoplasms]. Necrosis is a region of necrotic pancreatic parenchyma and/or peripancreatic fat. Acute necrotic collections occur within 4 weeks, whereas WOPN persists for more than 4 weeks. WOPN develops only after acute necrotizing pancreatitis and can be intrapancreatic or extrapancreatic. WOPN contains nonliquid material with varying amounts of fluid and has an encapsulating wall. Most PPCs with a diameter < 4 cm will resolve spontaneously, or will remain clinically stable without further complications. PPCs with a diameter between 4–6 cm can be managed by watchful waiting to see if they are asymptomatic or stable on follow-up radiological procedures. Sometimes, these PPCs can resolve spontaneously, but serious complications may occur in 10% of the cases. PPCs > 6 cm that are persistent more than 6 weeks should be treated by invasive approaches [8, 9]. In the last two decades, with continued improvements in medical technology and knowledge regarding treatment options in AP [10], treatment of PPCs dramatically changed. From the traditionally open surgical internal drainage in the past, nowadays, less invasive options including percutaneous, endoscopic and laparoscopic drainage were increasingly reported [11]. The morbidity and mortality rate have been reported as significantly lower for those minimally invasive approaches compared to open drainage surgical procedures [10, 11]. Unfortunately, in Serbia, minimally invasive approaches for the treatment of AP and its complications have implemented just a few years ago. In this case report, we presented the successful endoscopic transgastric drainage of the large PPC developed as a complication of AP.

Case report

A 63-years old male patient was admitted in our hospital due to nausea and vomiting as a consequence of the compressive effects of the PPC in the body and tail of the pancreas. Ultrasound on admission showed the large PPC with more than 15 cm in diameter. Two years ago, the patient was conservatively treated in another hospital due to alcoholic AP and he was discharged with a small acute fluid collection and small unilateral pleural effusion. On admission in our hospital, he was weak, malnourished, dehydrated with palpable painful tumefaction in the stomach region. Laboratory findings showed moderate inflammation with C-reactive protein of 54 mg/L, erythrocyte sedimentation rate of 60 mm/hour, leucocytes of 12.5×10^9/L, hemoglobin of 10.3 g/dL, platelets of 237×10^9/L, serum albumin level of 27 g/L and serum iron of 30 μmol/L with normal serum levels of amylases, lipases, CA 19-9 and liver enzymes including aminotransferases and gamma-glutamyltransferase. On computed tomography (CT) scan (Toshiba Aquilion 64®), it was shown a cystic formation in the region of the pancreatic body and tail compressing stomach which was verified on upper endoscopy (Figure 1).

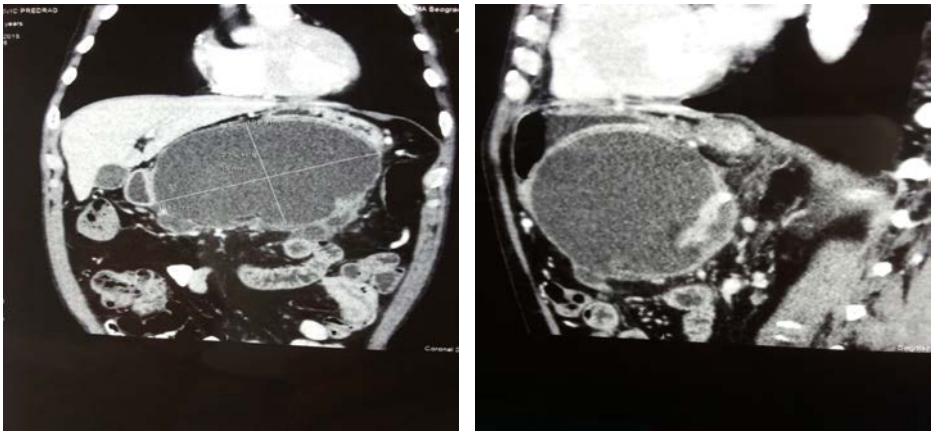

Fig. 1 – Computed tomography (CT) scan with the pancreatic pseudocyst (PPC) in the region of pancreatic body and tail.

Due to clinical condition of the patient (persisting vomiting, delayed gastric emptying and inability for normal food intake), it was decided that minimally invasive PPC drainage should be performed after initial resuscitation. Under fluoroscopy, using lateral duodenoscope (Pentax ED® 3490TK working channel 4.2), a incision on the posterior wall of the stomach was made, afterwards dilatation of the incision whole with biliary balloon diameter and length of 6 mm and 4 cm, respectively. After dilatation, the plastic biliary prothesis of 12 French and 8 cm of length was placed throughout posterior stomach wall into the PPC (Figures 2 and 3).

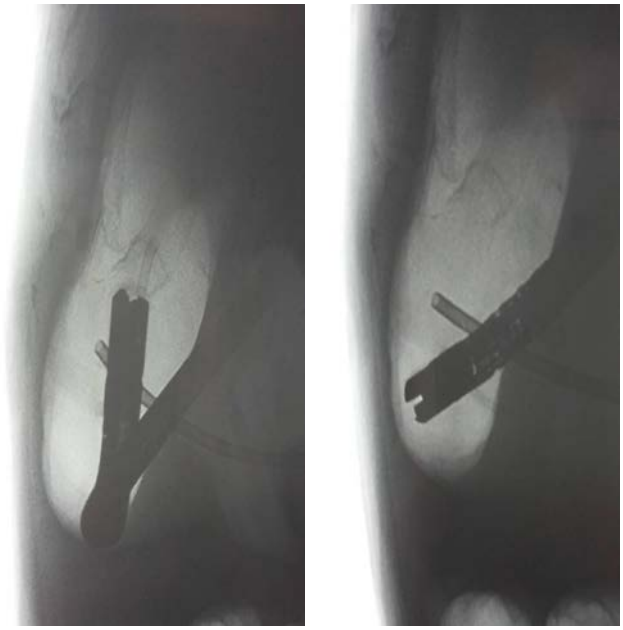

Fig. 2 – Placement of biliary stent under fluoroscopy.

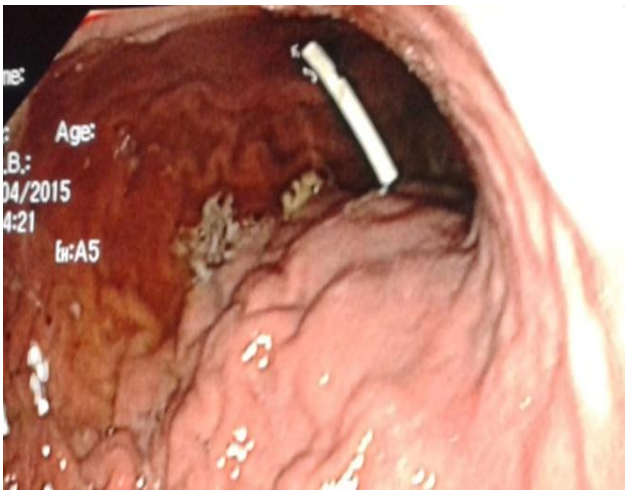

Fig. 3 – Upper endoscopy after placement of the prosthesis.

The intervention was finished uneventfully, without complications. The control abdominal ultrasound showed the reduction of the PPC size, the day after the procedure (Figure 4).

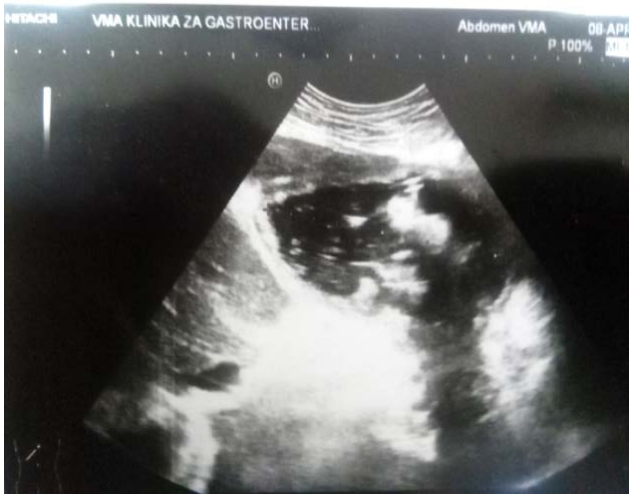

Fig. 4 – Abdominal ultrasound, the day after the procedure.

On the same day after the procedure, the patients started to take liquids and, on the next day, normal food and oral nutritional supplements for the improvements of nutritional status. On CT scan performed 7 days after the procedure, a size of the PPC was decreased for 7–8 cm in diameter (Figure 5).

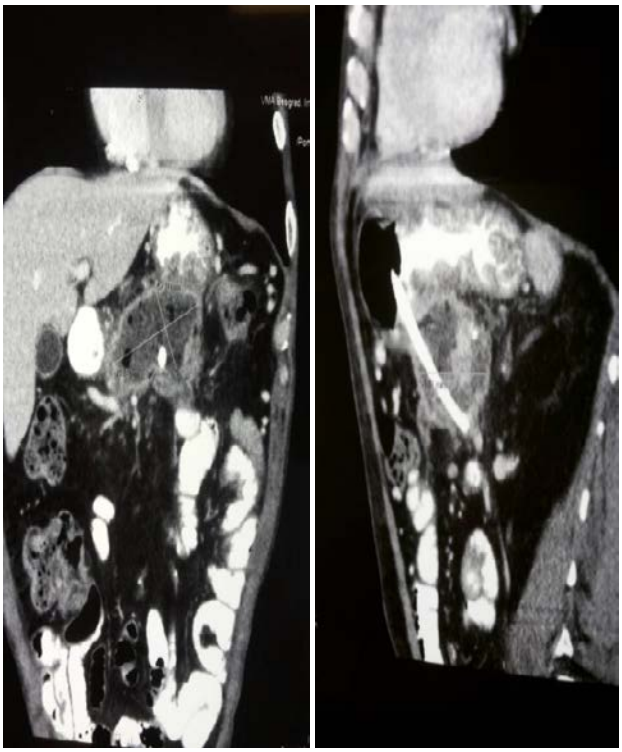

Fig. 5 – Computed tomography (CT) scan, one week after the procedure.

All laboratory findings including parameters of inflammation, blood cells count and serum amylase and lipase levels were in normal ranges one month after the procedure. Control CT scan, performed one month after the procedure and after removal of the prosthesis, showed almost

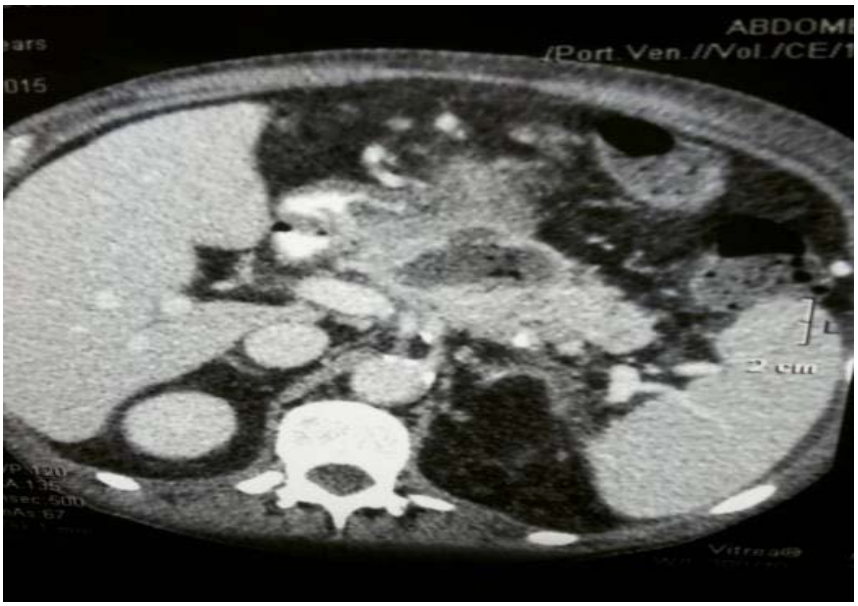

Fig. 6 – Computed tomography (CT) scan, one moths after the procedure and prosthesis removal.

complete resolution of the PPC (Figure 6). The patient had not further complaints on follow-up conducted 3 months after the procedure.

Discussion

Based on the Atlanta revision[7], the acute pancreatic collections need to be managed by drainage when there is abdominal pain, gastrointestinal and/or biliary obstruction, infection and if the size of the collection is greater than 5 cm in diameter. In a recently published review it was suggested that only symptomatic pancreatic collections should be managed, regardless their size [12]. In addition to other minimally invasive procedures for pancreatic collections management, in the last decade endoscopic approach has increasingly being used. Currently, endoscopic drainage is recommended as the first-line treatment for accessible PPCs because it can provide excellent results in terms of costs, duration of hospital stay, and quality of life, as demonstrated in a recent prospective randomized study [13].

A single case and the first reported case of endoscopic transgastric aspiration of a PPC was reported in 1975 by Rogers et al. Coworkers [14]. In the next decade only two reports described this procedure [15, 16]. Kozarek et al. [16] attempted cutting the bulging gastric wall with a needle-knife in 4 patients and reported the first nasocystic tube insertion in 1985. Over the next decade, the procedure was standardized, and retrospective studies proved the safety and efficacy of endoscopic PPC drainage with plastic stents. After introduction of endoscopic retrograde cholangiopancreatography (ERCP) in hepatobiliary and pancreatic pathology management, this procedure was used for PPC drainage [17]. However, there are only few indications for the transpapillary (ERCP) drainage of PPC [18] It is important to mention that endoscopic drainage of PPC which does not compress the stomach is relatively difficult to perform due to uncertain region of the posterior wall of the stomach for initial incision. Indeed, in 42-48% of PPCs, there is no evidence of propulsion or compressive effects of PPC on the posterior stomach wall [19]. This problem has been overridden by introduction of endoscopic ultrasound (EUS) which may measures a distance between PPC and posterior wall of the stomach with visible adjacent vessels and solid and/or necrotic pancreatic masses. The first endoscopic drainage of PPC was reported by Grimm et al. in 1992 [20]. After this report, the subsequent studies were conducted to evaluate a difference between EUS guided and conventional endoscopic PPC drainage. In a study of Kahaleh et al. [21], it was concluded that both techniques have similar efficiency and complications rate in PPC drainage if conventional endoscopic procedure was performed in patients with evident compressive effects on stomach by the PPC and EUS guided drainage if there were no propulsion on stomach wall. In two randomized control trials, it was shown better successful rate and lower complications in EUS guided PPC drainage versus conventional technique, but without significant difference between techniques [22, 23]. The first meta analysis regarding management of PPC was shown that surgical treatment had successful rate of 100% and the lowest recurrence rate (6-8.5%). However, the mortality rate was 1-8%. In contrast, endoscopic drainage had successful rate of 90-94%, recurrence rate of 12%, but mortality rate of 0% [24]. Subsequent study showed that EUS guided PPC drainage should be the first line treatment of this pathology because it has had lower hospital costs and lower hospitalization time as compared to the open surgical approach [25]. In recent review, it was concluded that EUS guided drainage is advantageous in drainage of PPC located adjacent to the stomach or duodenum. In patients with unfavorable anatomy, surgical approach or percutaneous drainage need to be

considered [11]. One of the most challenge conditions for management represents PPC and pancreatic duct disruption. In suspected pancreatic duct disruption, ERCP and/or magnetic resonance cholangiopancreatography should be performed to evaluate the potential lesion of main pancreatic duct and eventual communication with PPC. Nealon et al.[9, 26] reported that altered anatomy of the main pancreatic duct has been associated with lower rate of PPCs resolution. In the follow up of 563 patients, they noticed that spontaneous resolution of PPCs was observed in 87% of patients with normal pancreatic duct versus no resolution in 5% of patients who had pancreatic duct disruption [26]. In addition to this, it is important to evaluate the communication between PPCs and main pancreatic duct due to decrease rate of success after transgastric drainage in cases if this communication is present. Trevino et al. [27] found a reduce rate of successful endoscopic transgastric drainage versus simultaneous endoscopic transgastric and transpapillary drainage (80% versus 97.5%). This combined approach has not had increased mortality rate, length of hospitalization and necessity for additional necrosectomy regarding ERCP.

Overall clinical success of endoscopic transgastric PPC drainage with or without EUS ranges from 33−100%. It is suggested that ultrasound and/or CT scan should be performed after prosthesis placement every one or two months until PPC resolution, or earlier in case of symptoms and complications of the procedure. Following procedure, the complications occurs in around 15−64% of patients, and mortality rate ranges 0−19.6%. The most frequent complications are perforation and bleeding found to be more frequent in endoscopic transgastric drainage without EUS (13.3%) than in other approaches including surgery [11, 28]. Although it is generally advisable to use plastic biliary "pig-tail" prostheses of 7.5 French, in our case we used prosthesis of 12 French with a length of 8 cm due to better drainage of PPC. In the current literature there is no reports regarding usage of classic biliary plastic prosthesis for the PPCs drainage. In several studies with 698 patients observed, a significant difference in clinical success, mortality and recurrence rate after endoscopic PPCs drainage using various and multiple plastic or metal prosthesis were not found [29, 30]. However, one study showed that drainage of PPCs with plastic prosthesis had 2.5 higher complication rate versus drainage using metal stents. Also, complete resolution of PPCs after drainage was higher after metal stents versus plastic prosthesis (98% versus 89%) [31]. Our case is the first presented one of transgastric endoscopic PPC drainage in Serbia and maybe the first one in whom drainage of PPC was performed with plastic prosthesis with 12 French and 8 cm length.

In addition to the established implementation of minimally invasive / "step-up" approach for the treatment of patients with AP in Western countries, our country and maybe the region are lagging behind. Possible reason for this is lack of technical support and relative insufficient trained staff for this kind of treatment. Although there is lacking of data in trials comparing different minimally invasive techniques for management of patients with AP, this kind of treatment has shown overall better results as comparing to the traditionally open surgical approach [7, 8, 10, 11, 18]. In order to have better treatment quality and better care of patients with AP, including lower morbidity, length of hospitalization, treatment costs and quality of patients' life, we need to implement "step-up" approach in a routine medical practice. This will include percutaneous drainage (transperitoneal and retroperitoneal), endoscopic transgastric drainage with or without EUS, videoscopic assisted retroperitoneal debridement and laparoscopic approach.

Conclusion

Endoscopic drainage is safe and effective procedure for PPCs especially when the PPC has propulsion effects on the stomach wall. Transgastric drainage of PPCs with endoscopic ultrasound increases reliability and safety. For adequate treatment, a careful evaluation of patients in multidisciplinary team, including imaging specialists, dedicated interventional gastroenterologists and radiologists, and pancreatic surgeons is essential.

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SARS-COV-2 infection in a patient with Evans syndrome: a silent enemy or an ally?

SARS-COV-2 infekcija kod bolesnika sa Evansovim sindromom: nevidljivi neprijatelj ili saveznik?

Nikola Pantić*, Mirjana Mitrović*†, Marijana Virijević*†, Nikica Sabljić*, Zlatko Pravdić*, Nada Suvajdžić*†

Clinical Center of Serbia, *Clinic for Hematology, Belgrade, Serbia; University of Belgrade, †Faculty of Medicine, Belgrade, Serbia

Abstract

Introduction. During the current outbreak of Coronavirus disease 2019 (COVID-19), the way to manage patients with autoimmune diseases remains elusive due to limited data available. **Case report.** We presented a case of a COVID-19 positive 20-year-old female with prior history of Evans syndrome. The patients remained asymptomatic even though she had been treated with immunosuppressants (prednisolone and azathioprine) together with romiplostim. Moreover, her course of infection was accompanied by thrombocytosis, although her platelet count was mostly below the reference range before the infection. The patient was monitored vigilantly, with special regard to platelet count and signs of thrombotic events. **Conclusion.** Platelet count monitoring and romiplostim administration should be performed more cautiously in chronic immune thrombocytopenic patients infected by SARS-CoV-2.

Key words:
covid-19; evans syndrome; purpura, thrombocytopenic, idiopathic; romiplostim; thrombocytosis.

Apstrakt

Uvod. Tokom pandemije COVID-19 lečenje bolesnika sa autoimunskim bolestima veoma je izazovno, pre svega zbog nedostatka pouzdanih podataka. **Prikaz bolesnika.** Prikazana je dvadesetogodišnja COVID-19 pozitivna bolesnica koja se prethodno lečila od Evansovog sindroma. I pored činjenice da je bila lečena imunosupresivima (prednizon, azatioprin), zajedno sa romiplostimom, tokom celog toka infekcije kod bolesnice se nisu ispoljili simptomi. U krvnoj slici bolesnice uočena je trombocitoza tokom SARS-CoV-2 pozitivnosti, dok je broj trombocita pre infekcije bio ispod referentnog opsega. Bolesnica je praćena vrlo pažljivo, sa posebnim osvrtom na broj trombocita i eventualnu pojavu znakova tromboznih događaja. **Zaključak.** Neophodno je opreznije praćenje broja trombocita i doziranje romiplostima tokom SARS-CoV-2 infekcije kod bolesnika sa autoimunom trombocitopenijom.

Ključne reči:
covid-19; evansov sindrom; purpura, trombocitopenijska, idiopatska; romiplostim; trombocitoza.

Introduction

Patients with certain underlying medical conditions are (or might be) at increased risk for severe forms of Coronavirus disease 2019 (COVID-19). Addressing this issue, experts in different medical fields have provided guidelines on how to treat such patients during the pandemic. Thus, some recommendations regarding chronic primary immune thrombocytopenia (ITP) advise no changes in therapy because of the pandemic, even if it includes steroids and immunosuppressants [1]. Additionally, if a patient shows platelet count (PC) decrease and already uses a thrombopoietin receptor agonist (TPO-RA), a dose could be increased or a second one started. Short-term steroids (1–5 days) could be considered to increase PC, or intravenous immunoglobulins could be administered. Since low molecular weight heparin (LMWH) or heparin are widely employed for thromboprophylaxis in all hospitalized COVID-19 patients, its use is recommended even in patients with ITP. However, the potential benefits vs. risk of LMWH/heparin and the most beneficial dosage and schedule must be evaluated carefully for each ITP patient individually [2]. Herein, we presented a case of

Correspondence to: Nikola Pantić, Clinical Center of Serbia, Clinic for Hematology, Koste Todorovica 2, 11 000 Belgrade, Serbia.
E-mail: drnikolapantic@gmail.com.

a SARS-CoV-2 positive patient with Evans syndrome (ES) treated with romiplostim, prednisolone and azathioprine who experienced transient thrombocytosis during the infection.

Case report

A 16-year-old female was diagnosed with primary ITP in October 2016. Corticosteroid therapy resulted in partial remission and corticosteroid-dependence. Afterwards, the patient was treated with azathioprine, vinblastine and eltrombopag without response. Moreover, at week 8 of eltrombopag usage, the patient developed autoimmune hemolytic anemia (AIHA) and a diagnosis of ES was made. At that point, azathioprine (2.5 mg/kg) and prednisolone (1 mg/kg) were initiated and the hemolysis parameters were normalized after 2 weeks of the therapy. Romiplostim was introduced in December 2019 and given once weekly at the average dose of 6.5 μg/week. Before the SARS-CoV-2 infection, PC had fluctuated (PC_{min} 4×10^9/L; PC_{max} 143×10^9/L; normal range is from 150×10^9/L to 400×10^9/L, Figure 1) but the patient showed no signs of hemorrhage. Azathioprine was continued due to AIHA. Namely, hemolysis occurred each time a reduction in immunosuppressant dose was attempted. In March 2020, a new episode of AIHA was registered. Therefore, prednisolone was reintroduced, which led an increase in hemoglobin level from 56 g/L to 99 g/L (normal range for women is 120 g/L to 150 g/L).

After contact with a SARS-CoV-2 positive person, a nasopharyngeal swab was taken on April 11, and proved negative. The test was repeated 10 days later with a positive result (Figure 1). The patient had an asymptomatic course of COVID-19, with normal serum levels of C-reactive protein (CRP) and interleukin (IL)-6. D-dimer was slightly elevated initially (1.23 mg/L; normal value: < 0.5 mg/L) and then manifested a declining trend. The chest X-ray was normal. At the next check-up (April 14) her hemoglobin and PC were 99 g/L and 160×10^9/L, respectively. Romiplostim was applied in a dose of 6 μg/week. One week later, PC was 768×10^9/L, so romiplostim was omitted and the need for anticoagulant prophylaxis was considered. While white blood cells, neutrophil to lymphocyte ratio and D-dimer levels were low, anticoagulant therapy was not administered. In the forthcoming period, for the first time since the patient was diagnosed with ITP, her PC remained above 100×10^9/L for more than a month. Romiplostim was given once when PC was 144×10^9/L. The first negative polymerase chain reaction (PCR) test was taken on May 21, and at the check-up on May 27, her PC was 7×10^9/L. Afterwards, the patient's PC continued to fluctuate as prior to the infection (Figure 1).

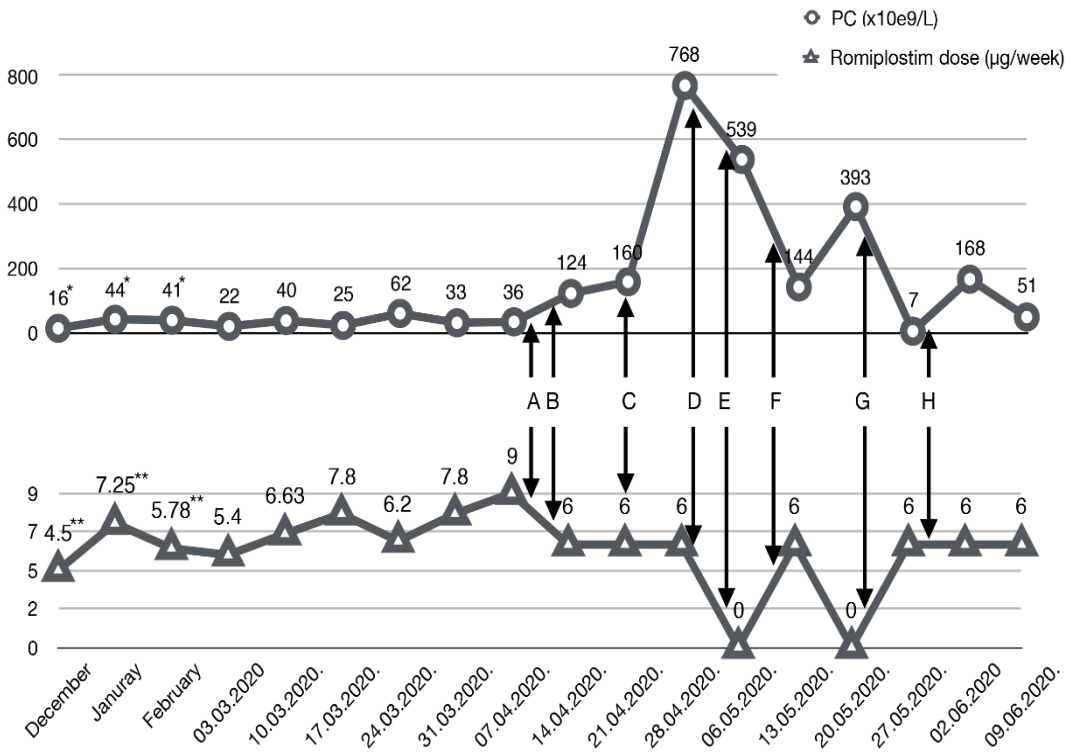

Fig. 1 – Platelet count (PC) and romiplostim dose dynamics before and during the SARS-CoV-2 infection. A – probable exposure date (April 4); B – the first swab test: negative (April 11); C – the first positive test (April 21); D – the second positive test (April 23); E – the third positive test (May 5); F – the fourth positive test (May 12); G – the first negative test (May 21); H – the second negative test (May 28).
***Average PC (× 10^9/L) for the month.**
****Average romiplostim dose applied (μg/week) for the month.**

Discussion

The majority of COVID-19 patients, especially those with nonsevere disease, have normal PC. However, thrombocytopenia has been registered among 20.7% of SARS-CoV-2 positive patients and it has been associated with more severe disease [3]. Moreover, several cases of COVID-19 associated acute ITP or even ES have been recorded [4, 5]. However, to our knowledge, this is the first case of thrombocytosis in a COVID-19 positive patient with chronic ITP.

Although the WHO [6] advised avoidance of corticosteroids in SARS-CoV-2 patients, the systematic reviews by Gao et al. [7] and Minotti et al. [8] showed that immunosuppression and immunodeficiency were not significantly associated with an increased risk of severe COVID-19. Having all this in mind, we decided to continue corticosteroids. Moreover, COVID-19 leads to systemic coagulation activation resulting in thromboembolic (TE) events in up to 40% of critically ill patients. On the other hand, the incidence of TE in ward patients varied between 3–5% [9]. Consequently, a prophylactic dose of LMWH should be introduced in all patients who require hospital admission [10]. However, there are no data regarding TE incidence and thromboprophylaxis in outpatients. Previous studies showed that PC $> 450 \times 10^9$/L, elevated D-dimer, CRP and erythrocyte sedimentation rate at initial presentation, stay in intensive care units, high white blood cells and high neutrophil-to-lymphocyte ratio were predictive of TE events [9, 11]. Additionally, ITP itself is considered a thrombophilic condition, with prevalence of thrombotic events up to 3–4 times greater than for the average control subject [12]. Moreover, the risk of thrombosis could be enhanced by TPO-RA [1, 13]. In addition, our patient had active hemolysis during the infection, which was a supplementary risk factor for venous thromboembolism [14]. Despite several thrombophilic factors, but considering that our patient was asymptomatic, not hospitalized, mobile, with low levels of inflammation parameters and D-dimer, we abandoned the idea of anticoagulant prophylaxis. However, her physical and biochemical status were regularly monitored.

Conclusion

The presented case indicated that PC monitoring and romiplostim administration should be performed more cautiously in ITP patients infected by SARS-CoV-2. Further studies are needed to provide us with information about TE risk factors and anticoagulant prophylaxis in ITP patients with SARS-CoV-2 infection.

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I S T O R I J A M E D I C I N E

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Dr Vladan Đorđević, prvi srpski vojni hirurg-operator i prvi sanitetski pukovnik Srpske vojske, načelnik njenog saniteta u tri rata koje je vodila u drugoj polovini 19.veka (1876, 1877 /1878. i 1885), vojni pisac i istoričar srpskog vojnog saniteta - vojnički deo njegovog života

Dr. Vladan Djordjevic, the first Serbian military surgeon-operator and the first medical colonel of the Serbian Army, chief of its medical care in the three wars it fought in the second half of the 19th century (1876, 1877/1878 and 1885), military writer and historian of the Serbian military medicine - A military part of his life

Aleksandar S. Nedok

Akademija medicinskih nauka Srpskog lekarskog društva, Beograd, Srbija
Academy of Medical Sciences of the Serbian Medical Society, Belgrade, Serbia

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Key words:
history of medicine; history, 19th century; history, 20th century; medical corps; military medicine; surgery.

Uvod

O dr Vladanu Đorđeviću može se pisati iz više uglova, njegov bogati život ga je vodio od vojnog hirurga i rukovođenja vojnim sanitetom u vremenima tri rata XIX veka, pisca znamenitih istorija vojnog saniteta, preko predsednika beogradske opštine i zdravstvenog reformatora, poslanika srpske države u Grčkoj i Turskoj i ministra u vladama, do predsednika srpske Vlade na prelomu vekova i, najzad, do osuđenika na zatvor zbog odavanja državne tajne.

Manje su poznati detalji njegove vojne delatnosti, od vremena kada je bio vojni lekar-hirurg, šef Hirurškog odeljenja beogradske vojne bolnice, potom ratni divizijski referent saniteta i, konačno, načelnik celokupnog srpskog vojnog saniteta u tri rata koje je Srbija vodila, dva sa Turskom i jedan sa Bugarskom. Razlog tome je, manje, u nepovratno pretrpljenim gubicima vojnih arhiva u ratovima, već, pre svega, u njegovom ranom izlasku iz vojske posle ratova sa Turskom 1876/78. i opredeljenju za druge, građanske dužnosti i položaje, sve do kratkotrajnog povratka na rukovodeću dužnost u vojnom sanitetu za vreme rata sa Bugarskom 1885. godine. Zbog toga, danas postoji samo jedna njegova vojna konduit-lista iz 1874. godine i nekoliko Kneževih Ukaza, bilo u dokumentu, dnevnim novinama ili u Službenom vojnom listu.

Ovaj rad, pisan povodom 90. godišnjice njegove smrti, govori o tom delu njegovog života, kao vojnog lekara i rukovodioca i neprevaziđenog pisca vojnosanitetske istorije.

Detinjstvo i mladost, studije i specijalizacija hirurške operatike

Rođen je 21. novembra 1844. godine u Beogradu u grko-cincarskoj porodici od oca „hećima Đorđa“, rodom iz Furke u Epiru, priučenog vojnog apotekarskog i medicinskog pomoćnika, i majke Marije, „lepe Mace“, iz poznate cincarske beogradske porodice Leko. Na krštenju je dobio ime Hipokrat, koje je u kasnijim školskim danima zamenio srpskim imenom Vladan na predlog profesora Đure Daničića, posle nekoliko njegovih uspešnih rodoljubivih sastava. Osnovno obrazovanje je stekao u školi pored Saborne crkve, a sedmorazredno, gimnazijsko, u Prvoj beogradskoj gimnaziji u generaciji 1861/1862, u kojoj je bilo nekoliko kasnije poznatih imena. Posle jedne letnje studentske ekskurzije po srpskim planinama, gde ga je zapazio poznati krajinski lekar i botaničar, dr Stevan Mačaj, na njegov nagovor odlučio je da studira medicinu. Pošto je dr Mačaj o njemu razgovarao sa dr Josifom Pančićem, drugim slavnim lekarom, botaničarem, profesorom Velike Škole, članom Srpskog učenog društva i kasnijim predsednikom Srpske Kraljevske Akademije nauka, ovaj ga je preporučio za državnu stipendiju i tako, dr Vladan u jesen 1863. godine odlazi u Beč na studije zajedno sa budućim istaknutim vojnim lekarima Lazom Dokićem, Mihailom-Radmiom Lazarevićem i Mihailom-Mikom Markovićem. Ne ulazeći u njegovu bogatu vanmedicinsku aktivnost na društvenom i političkom polju u toku studija, treba istaći da je Vladan diplomirao polažući tri

Correspondence to: Aleksandar S. Nedok, Akademija medicinskih nauka Srpskog lekarskog društva, Džordža Vašingtona 17, Beograd, Srbija. E-mail: anedok@sbb.rs.

završna ispita tokom 1869. godine, zahvaljujući kojima je postao doktor medicine, doktor hirurgije i magistar opstetricije. Posle prve od dve doktorske diplome, koje je stekao u Beču, proveo je jedan semestar u Pragu, u velikom porodilištu profesora Sajferta da bi stekao i treću diplomu, a potom se vratio u Beč da učestvuje na konkursu za pitomce-operatore na Prvoj hirurškoj univerzitetskoj klinici profesora Teodora Bilrota [1–3].

Položivši sa uspehom prijemni ispit, postao je lekar-asistent kod profesora Bilrota, ali je uskoro, kao i profesor Bilrot, napravio prekid da bi učestvovao u ratu Francuske i Pruske (1870/71), zatim se vratio i dve pune školske godine, u grupi od osam mladih lekara koji će kasnije postati istaknuti profesori hirurgije germanofonske Evrope (Austrija-Nemačka-Švajcarska), nastavio specijalizaciju. Za vreme specijalizacije se isticao svojim radom, objavivši u bečkom medicinskom časopisu dva rada, jedan o zaustavljanju venskog krvavljenja, a drugi o limforeji, koji su dosta navođeni u udžbenicima hirurgije onog vremena [2].

Kraljevski pruski ordinirajući lekar 1870/71. godine

Upravo u vreme započinjanja njegove specijalizacije, u leto 1870. godine izbio je prusko-francuski rat u kome su se Pruskoj pridružile i ostale, tada nezavisne nemačke državice koje su se posle pobede ujedinile u Prvi nemački rajh.

Zainteresovan kao mladi hirurg da prouči poljsku vojnosanitetsku službu i da se izvežba u ratnoj hirurškoj tehnici, Vladan je zamolio srpsku Vladu za dozvolu da učetvuje u ratu u sastavu udružene nemačke vojske. Vlada mu je dozvolila i poslala još dva sanitetska oficira, dr Filipa Taisića i dr Josifa Holeca i tri oficira (potpukovnik Bojović, kao šef ekipe, major Lešjanin i kapetan Bogićević) da zajedno otputuju na front, kao srpski posmatrači. Međutim, zbog strogih nemačkih vojnih propisa o zabrani prisustva stranaca u zoni operacija, njihov put se završio u Glavnom štabu nemačke vojske u Majncu, gde im je rečeno da se kao lekari moraju prvo isprobati, četiri nedelje u pozadinskoj bolnici i da tek, potom, mogu, uz odobrenje, raditi u nekoj frontovskoj vojnoj bolnici. Kako se dr Holecu i dr Taisiću to nije dopalo, oni su se vratili, dok je Vladan pristao i bio poslat na bolnički brod - rezervnu bolnicu "Jozef Miler", ukotvljen na Majni kod Frankfurta, gde je uspešno proveo jednomesečnu probu i potom se vratio u Beč kod prof. Bilrota koji se i sam spremao da krene u službu svome narodu. On je, u decembru 1870, pre odlaska, pošto je zamoljen od pruskog saniteta da im pošalje u pomoć dva vešta operatora, prekinuo operatorski kurs i poslao Vladana i dr Emila Pernicu u Frankfurt. Tamo je Vladan postavljen za „kraljevskog pruskog ordinirajućeg lekara" u Rezervnoj bolnici br. 1 pri XI pruskom korpusu, u kojoj je vodio dva odeljenja od po 20 kreveta puna težih hirurških bolesnika. Doživeo je čast da u inspekciju dođe glavni vojni inspektor pruske vojske, general-lekar profesor Bernhard fon Langenbek, učitelj Bilrotov, koji je pohvalio njegov rad, posebno u pogledu primene konzervativne hirurgije kojom se izbegavaju amputacije kod komplikovanih povreda zglobova i udova i ranjenici pošteđuju od invaliditeta. Za svoj rad odlikovan je sa dve pruske ratne medalje. Kao nauk iz svojih iskustava, Vladan je usvojio devizu koju je predavao i drugima da „hirurg treba da ima milostivo srce, iako mu ono ne sme oduzeti energiju", t.j. da ima odlučnost da izvrši ono što je neminovno, ali da sačuva sve što je moguće [4–7].

Za vreme svoga rada u nemačkom sanitetu Vladan se nije zadovoljio samo svojim usavršavanjem u ratnoj hirurgiji, već je od svoga šefa dobijenu celokupnu prusku vojnosanitetsku administrativnu i organizacionu dokumentaciju preveo na srpski jezik i predao Ministarstvu vojnom. Kasnije, u okviru priprema za rat sa Turskom, na osnovu te dokumentacije, komisija u kojoj je bio i on sa dr Filipom Taisićem i potpukovnikom Evgenijem Kalinićem, izradila je ustrojstvo i uređenje vojnog saniteta. I ne samo ta dokumenta, već je 1, 8. i 16. jula 1871. poslao iz Frankfurta svoja poznata „Vojno-lekarska pisma" sa svojim zapažanjima i izvedenim zaključcima stečenim tokom frontovskog i pozadinskog rada i, uopšte, zapažanja o organizaciji nemačke vojske i njenog saniteta koja su potom odštampana u posebnoj knjižici u Državnoj štampariji na 206 stranica [4, 8].

Srpski vojni lekar hirurg-operator, 1871-1876. godine

Po okončanju rada u nemačkom vojnom sanitetu Vladan se vraća u Beč da nastavi prekinutu dvogodišnju specijalizaciju i, posle dobijene diplome operatora, u julu 1871. godine vraća se u Beograd i odmah stavlja na raspoloženje Ministarstvu unutrašnjih dela da odsluži svoju stipendiju. Kako se čekanje na posao odužilo, otvorio je privatnu ordinaciju kod današnjeg „Londona" u kojoj je sa uspehom obavljao lekarsku delatnost.

Tadašnji ministar vojni, potpukovnik Jovan Belimarković, kada je saznao da se Vladan nalazi u Beogradu, pozvao ga je na razgovor i, potom, zatražio od Vlade da se on ustupi vojsci, imajući u vidu njegovo ratno iskustvo i diplomu hirurga-operatora. Tada se, po prvi put u svojoj kratkoj istoriji, beogradska vojna bolnica podelila na dva odeljenja, nastavljajući u kasnijim periodima ovaj savremeni pravac u razvoju medicinske nauke i struke koji je podrazumevao njeno rašlanjivanje na pojedine grane. Za ovo je bio posebno značajan razvoj bakteriologija koja je omogućila novi pristup (najpre antiseptični, a kasnije aseptični) u hirurškom zbrinjavanju rana i, uopšte, radu hirurga. Najbolja ilustracija ovog novog pravca u hirurgiji, bile su prve Bilrotove trbušne operacije.

Kneževim Ukazom, Vladan Đorđević postaje „vojeni lekar vtore klase" na mestu šefa Hirurškog („Spoljnog") odeljenja beogradske bolnice, dok je, istovremeno, šef Unutrašnjeg odeljenja postao „vojeni lekar 2. klase" dr Stefan Nedok, a upravnik glavni „vojeni lekar 2. klase" dr Jovan Mašin. Već posle 2,5 godine, 16. februara 1874, Vladan postaje „vojeni lekar 1. klase", a 29. novembra 1874, glavni „vojeni lekar 2. klase", odnosno, izmenama u dotadašnjem zakonu (donesenom 20.03.1864), od 29. januara 1875, sanitetski major [6, 9]. Time je presečena rasprava između lekara o njihovom statusu u vojsci, o lekaru-oficiru, itd, mada su iz toga i oko pitanja ko je dužan da bude dežurni lekar u beogradskom garnizonu, proistekli neki zaostali međusobni animoziteti koji se danas naslućuju iz Vladanovih pisanih dela u njegovim subjektivnim ocenama pojedinih ličnosti ondašnjeg vojnog saniteta. Iz tog prelomnog vremena sačuvana je jedina njegova konduit-lista u kojoj je on popunio svoje opšte podatke, a glavni „vojeni lekar 2. klase", dr Filip Taisić, kao njegov pretpostavljeni, upisao je službenu ocenu koja glasi: „Vrlo je darovit, bistar i s(h)vatljiv. Posve sposoban u lekarstvu, a naročito operativnoj hirurgiji. U svojoj struci za sve je sposoban i vrlo upotrebljiv. U vršenju službe vrlo revnostan, tačan, uredan i iz sopstvenih pobuda zauzimljiv. U službi i van nje je vladanja primernog i ponašanja dostojanstvenog i u svemu prema stanju i položaju. U privatnim svojim poslovima dobar je ekonom i vidi se da je vešt u poslovima. Zaslužuje da se unapredi, no skoro je avanzovao" [1].

Na mestu šefa Hirurškog odeljenja beogradske vojne bolnice ostao je sve do mobilizacije za rat sa Turskom 1876. godine. Za to vreme stekao je ugled odličnog hirurga i, istovremeno, dobrog poznavaoca austrijske i nemačke vojnosanitetske organizacije, kao dobro uređene službe u poređenju sa stanjem u malom i siromašnom srpskom vojnom sanitetu za koje su bili karakteristčni loši uslovi rada, siromašna oprema, loše uređene postojeće vojne bolnice, loši higijenski uslovi u kasarnama i loš odnos prema vojnim lekarima [10].

Odmah po stupanju u vojnu službu, uspeo je da 22. aprila 1872. godine organizuje sastanak sa grupom od 15 beogradskih lekara, na kome je odlučeno da se osnuje Srpsko lekarsko duštvo, a nešto kasnije, 16. septembra iste godine, i njegov časopis Srpski arhiv za celokupno lekarstvo [2].

Te 1872. godine, Vladan izdaje tri knjižice, dve u Beogradu i jednu u Novom Sadu: „Sanitetski poslovi u Srbiji“ (69 stranica u kojoj opisuje, potpomognuto skicama, beogradske bolnice [10], „O kauterisanju rana“ (32 stranice i 13 tablica) i „Narodna medicina u Srba“ (77 strana) [11]. Zapazivši njegov talenat i vrednoću, ministar vojni ga u zimu 1872/73. određuje da u novoobrazovanoj Oficirskoj školi u Beogradu održi za oficire Stajaće i Narodne vojske, t.j. aktivne i rezervne, seriju od 14 predavanja iz vojne higijene, u kojima je izložio savremena evropska shvatanja o tom veoma važnom pitanju čuvanja vojničkog zdravlja, što je kasnije, 1874. godine, štampano u posebnoj knjižici „Načela vojne higijene“ na 392 stranice [12]. Istovremeno, izdata je i knjižica „Bolničarska služba“ na 214 stranica koja će služiti obrazovanju vojnih bolničara, tom neophodnom delu vojnog saniteta [13]. Godine 1875. preveo je Bilrotovu „Opštu hirurška patologiju i terapiju, prvi deo“, koja je štampana u Državnoj štampariji. Kada je početkom 1875. godine u sanitetu kragujevačkog garnizona, u kome je bila tek završena zgrada nove vojne bolnice, došlo do organizacionih i personalnih problema, ministar vojni poslao je dr Vladana da utvrdi stanje i uzroke nastalih problema i da predloži rešenja. Posle izvršene inspekcije, on piše opširan izveštaj i predlaže da se u Kragujevac premesti jedan viši sanitetski oficir koji će da sredi stanje [14]. Na osnovu ovoga izveštaja ministar premešta u Kragujevac glavnog vojnog lekara 2. klase dr Stefana Nedoka i postavlja ga „za upravnika vojne bolnice i da ujedno vrši nadzor nad trupnim lekarima, da u bolnici zavede odeljenja za unutrašnje i spoljne bolesti i da premesti ambulatoriju iz bolnice u kasarnu“ [15].

Iz ovoga perioda potiče njegova Istorija srpskog vojnog saniteta, knjiga I, u kojoj na 816+XXXIV stranica daje ceo njegov razvoj od 1835. do 1875. godine. Knjiga je izdata 1879. godine, pored knjige dr Lindenmajera o Srbiji i razvoju njenog saniteta, što je druga vojnosanitetska inkunabula od neprocenjive vrednosti [6]. Za pisanje ove knjige, načelnik saniteta, dr Beloni, dao mu je svoje uspomene, iz vremena njegovog upravljanja sanitetom, koje su bile napisane na 60 tabaka hartije (danas se one, na žalost, smatraju izgubljenim).

Pripreme za rat sa Turskom

Kada je u leto 1875. godine hercegovački ustanak počeo sve više da uzima maha, u Srbiji je zavladalo uverenje da nije daleko čas kada će ona morati da „zagazi“ u rat radi svoje braće koja su u Hercegovini krvavila. U tom cilju ministar vojni određuje, između ostalih mera, komisiju „da pregleda sadašnje stanje našeg vojnosanitetskog depoa i da pobroji šta bi još valjalo nabaviti za potpunu opremu vojnog saniteta“ i u nju određuje dva saniteska oficira, majore dr Savu Petrovića i dr Vladana Đorđevića. Komisija je podnela opširan izveštaj koji je pokazao katastrofalno stanje: „To je bilo sve što je srpska vojska za 40 godina svoga opstanka nabavila od vojnosanitetskih potreba na slučaj rata“. Stoga je komisija morala mnogo toga da traži, podnevši spisak potreba u materijalu, opremi i ljudstvu. Sve je to koštalo „ravno 110 000 zlatnih dukata. Zbog opšteg državnog siromaštva od svega ovoga ne bi ništa, ministru vojnom se ipak učinilo da možda ipak neće doći do rata“. Te iste godine šef vojnog saniteta, potpukovnik dr Karlo Beloni, u dva maha, 2. i 29. septembra podnosi zahtev ministru „da se zbog sadašnjih, otlaganju netrpećih okolnosti, što pre reši pitanje o ustrojstvu vojnog saniteta, jer vojni sanitet ni posle 40 godina svoga postojanja još nemađaše ustrojstva za ratnu službu, a kamoli uputstva detaljnog za njeno vršenje“ [4]. Tek decembra 1875, ministar je odredio komisiju u sastavu đeneralštabni potpukovnik Jevđenije Kalinić i sanitetski majori dr Filip Taisić i Vladan Đorđević. Taj ogroman posao je, na Vladanovo insistiranje, urađen na bazi najnovijeg ustrojstva pruskog saniteta i pretočen u dva raspisa ministra vojnog 1. marta 1876. godine: „Uređenje i raspored vojnog saniteta“ i „Uputstvo za službu vojnog saniteta srpske narodne i stajaće vojske“. Da bi se sve to uradilo Vladan je bio premešten iz vojne bolnice u ministarstvo vojno za „delovođu vojnog sanitetstva“ u kome su dotle bili dr Beloni, kao referent, i dr Taisić, kao njegov zamenik [16]. Obavivši ogroman rad u dve komisije i, istovremeno, odlučno utičući da se osnuje Društvo za privatnu pomoć ratnim ranjenicima i bolesnicima, buduće Srpsko društvo Crvenog Krsta, o čemu je Vladan održao i dva javna predavanja 2. i 8. januara 1876. (iz prvog je potekla knjižica „Crveni krst na beloj zastavi“ na IV+156 stranica, a u drugom „Sećanje na Solferino“ je evocirao sećanje na prvu bitku u kojoj je organizovano pomagano ranjenicima), on je uspeo, u to vreme, da sa nemačkog jezika prevede i izda knjigu poznatog profesora iz Tibingena, dr Landsbergera, „Ratna hirurška tehnika“.

Dr Vladan je 19. marta 1876, u okviru priprema za rat sa Turskom, Knjaževim Ukazom imenovan za načelnika saniteta Južnomoravske divizije [17].

U to vreme srpska vojska je imala samo 19 lekara: potpukovnika dr Karla Belonija, majore Filipa Taisića, Savu Petrovića, Vladana Đorđevića, Josifa Holeca i Stefana Nedoka, kapetane 1. klase Maksima Nikolić-Miškovičeva, Leonarda Lontkijevića, Lazu Do kića, Aleksandra Verminskog i kapetane 2. klase Jovana Kovača, Juliusa Lenka, Petra Ostojića, Iliju Milijića, Dimitrija Kufasa, Mihaila Lazarevića, Milutina Popovića, Jovana Porubovića i Jaroslava Kuželja, 5 lekarskih pomoćnika (Đorđe Đorđević, Venceslav Švarc, Dimitrije Lomigorić, Pavle Karadžić i Franja Nađ), jednog apotekara (kapetan 1. klase Alojz Helih) i 4 apotekarska pomoćnika (M. Mihailović, M. Birg, M. Herman i N. Cenić), a u Srbiji još 41 građanskog lekara, 5 lekarskih pomoćnika i 25 apotekara i njihovih pomoćnika.

Vladanovo poslednje delo u ministarstvu, pre odlaska na ratnu dužnost, bilo je „Uputstvo za divizijske načelnike saniteta o tome šta imaju pre svega da urade u svojoj, za sve njih tako sasvim novoj službi“ [18]. Uz njega je išlo „Uputstvo odnosno formacije sanitetskih trupa i zavoda“ koje je za sve načelnike divizijskih saniteta izradio Đeneralštab ministarstva vojnog [19].

Mobilizacija vojske i rat sa Turskom 1876. godine

Otišavši u Ćupriju, na svoje mesto opredeljenja, gde se nalazio štab njegove divizije, Vladan se dao na posao sa njemu uobičajenom energijom, trebujući sanitetski materijal, hirurške instrumente, lekove, štampane obrasce i (ranjeničke) cedulje za dijagnozu.

Pri polasku iz ministarstva uspeo je još da isposluje da se svim komandatima brigada i okružnim načelnicima sa teritorije

nadležne divizije naredi sprovođenje 15-dnevnog vežbanja bolničara narodne vojske i „gospode civilnih lekara pomenutih okruga“. On je, pak, lično odlazio u brigadna mesta za vreme izvođenja tih obuka. Osim toga, lično je obišao sva mesta i zgrade gde će biti smeštene ratne i drumske (etapne) bolnice i o tome podneo opširan izveštaj komandantu divizije. Na kraju, organizovao je sanitetske ustanove divizije sa ono malo osoblja koje je imao i u Jovanovcu, Ražnju i Banji aleksinačkoj smestio rezervne bolnice [4]:

1. Divizijsko sanitetsko odeljenje: komandir kapetan dr Mihailo Lazarević, lekarski pomoćnk Karlo Lipold, apotekar Julije Draškoci, apotekarski pomoćnik Karlo Erlih, 94 bolničara;
2. Aleksinačka brigada: komandir sanitetskog odeljenja dr Andrija Janeković i 32 bolničara,
   Poljska bolnica: komandir, kapetan dr Milutin Popović, lekar dr Đorđe Dimitrijević, lekarski pomoćnik Andrija Petrović, apotekar Aurel Kalmar i pomoćnik Nikola Cenić, 53 bolničara;
3. Kruševačka brigada; komandir sanitetskog odeljenja, dr Osvald Hajnc i 47 bolničara.
   Poljska bolnica: komandir, kapetan dr Petar Ostojić, lekarski pomoćnik Đorđe Vidaković, apotekar Dragoslav Kedrović i 53 bolničara;
4. Ćuprijska brigada: komandir sanitetskog odeljenja, dr Andrija Bikl i 47 bolničara,
   Poljska bolnica: komandir, dr Mihalo Hadži-Lazić, apotekarski pomoćnik Pavle Novaković i 48 bolničara.

Po objavljenoj mobilizaciji, Vladan je preko komandanta divizije uputio 13. juna zahtev Ministarstvu za još pet lekara, 10 lekarskih pomoćnika i po dva apotekarska pomoćnika za svaku brigadu, uz upozorenje da će „tek onda sanitetska trupa i zavodi područne mi divizije biti u stanju da vrše poljsku sanitetsku službu...kao što je to propisano“ [4].

Bitka na Mramoru

Uveče, 13. juna 1876. godine, sanitetske trupe divizije bile su raspoređene na položajima u tri borbene jedinice, a 20. juna, u 4 časa izjutra, otpočeo je rat bombardovanjem turskih položaja. U očekivanju ranjenika, dr Vladan je na zavojištu koncentrisao sav raspoloživi sanitetski personal divizije u grupe:

Grupa 1 - pregled i trijaža, kapetan P. Ostojić i M. Hadži-Lazić,
Grupa 2 - hirurške intervencije, dr Vladan i dr L. Stevanović,
Grupa 3 - zavoji i gips, lekarski pomoćnici A. Petrović i Đ. Vidaković,
Grupa 4 - za ponude i okrepljenje ranjenika, apotekari i sveštenik.

Ubrzo, po početku bitke počeli su da pristižu ranjenici - nakupilo ih se 300. Bitka je trajala već 8 sati, ranjenici su slani u II poljsku bolnicu, kada je iznenada vojska počela preko zavojišta da u neredu odstupa, što je demoralisalo osoblje zavojišta „u tom stepenu da ga je samo napereni revolver zadržao na dužnosti, no dok je načelnik saniteta pomagao jednom teškom ranjeniku sve se raspršlalo osim njega, lekarskog pomoćnika Andrije Petrovića i bolničara Stajaće vojske zajedno sa pešadijom, konjicom i artiljerijom“. Tek sutradan su komandanti uspeli da prikupe svoje raštrkane trupe, a dr Vladan svoj sanitet.

Slično se desilo i na istočnom frontu, gde su posle bitke kod Velikog Izvora, 18. jula, napušteni Zaječar i nešto kasnije Knjaževac. U ovoj bici je teže kontuzovan od eksplozije artiljerijske granate načelnik saniteta Timočke divizije major dr Stefan Nedok koji se nalazio u prvoj borbenoj liniji sa svojom ekipom lekara i bolničara radi pružanja hitne pomoći ranjenicima.

Na osnovu iskustva ovih bitaka povećan je broj bolničara po brigadama srpske vojske na 100, dok su na južnom frontu sve bolnice stavljene u Aleksincu pod zajedničku komandu kapetana dr Leonarda Lontkijevića.

Neuspešno ratovanje nastavljeno je na oba fronta, južnom i istočnom, pa je u cilju sprečavanja prodora Turaka u Moravsku dolinu došlo do objedinjavanja pet divizija sa oba fronta (Južnomoravska, Šumadijska, Dunavska, Timočka i kombinovana) pod zajedničkom komandom ruskog generala Mihaila Grigorijeviča Černjajeva pod imenom Moravsko-timočka vojska, preoblikovana u četiri korpusa, na moravskom pravcu dva (Deligradski i Aleksinački) i na istočnom pravcu dva (Banjski i Lukovski) sa istim zadatkom sprečavanja prodora Turaka u Moravsku dolinu. U skladu sa time, 28. jula se reorganizuje sanitet i dr Vladan bude postavljen za načelnika saniteta Vrhovne komande, a za načelnike korpusnih saniteta su tada postavljeni: major dr Stefan Nedok (Lukovski) i kapetani 1. Klase, dr Leonardo Lontkijević (Banjski), dr Aleksandar Verminski (Aleksinački) i dr Pavle Stejić (Deligradski). Ovako objedinjeni sanitet imao je da izdrži velika iskušenja u teškim odbrambenim bitkama vođenim do 20. oktobra, kada je sklopljeno primirje (Šumatovac, Krevet, Veliki Šiljegovac, Deligrad, Đunis) [20].

Nažalost, detalji za rad saniteta u njima su za istoriju izgubljeni, jer su relacije i pisani izveštaji četiri načelnika korpusnih saniteta o njihovom radu podneti dr Vladanu i kod njega ostali neiskorišćeni pošto on, okrenuvši se posle rata 1877/78. drugim, civilnim poslovima, nije stigao da ih uobliči u posebnu knjigu, koja je trebalo da bude sveska 2 njegove druge knjige o istoriji srpskog vojnog saniteta. Stigao je tek 1893. da napiše prvu svesku, jedan članak u novosadskom „Javoru“ 1882. o sanitetu Moravsko-timočke vojske i dve knjige uspomena na srpskoturske ratove koje ne sadrže službene sanitetske pojedinosti. Pred toga, nestale su u kasnijim ratnim vihorima i ratne beleške doktora Dokića, Holeca, Siberta, F. Kopše, I. Kolovića, Svetića, Maržika, Nedoka, Milovanovića, Laze Lazarevića, Katanića, Stojanovića, Sibera i Varhaftiga, medicinara Josifa Handžarlijća i Jeftimija Đorđevića, doktora Guta, Vajsa i Bergera i gospođe Olimpije Noel de Bertije. Sačuvani su jedino memoari medicinara (budućeg doktora) Stevana Ilića i dr Stevana Mačaja.

Ovaj rat, koji se nije srećno završio po Srbiju, ostavivši opustošene istočne i južne njene krajeve koji su bili privremeno okupirani od Turaka, pokazao je da narodna vojska milicijskog tipa, slabo naoružana i obučena, sa malom stajaćom vojskom, izgubljenom u masi mobilisanog naroda, neobučenom za rat, a njen sanitet sa malo sopstvenih kadrova, ne može uspešno da se nosi sa turskim nizamom, obučenim i naoružanim nemačkim oružjem. I da nije bilo velikog broja dobrovoljaca, posebno ruskih, preko 2 500, izgubljenih bitaka bilo bi i više, kao i poslednji boj koji su vodili 1 000 ruskih dobrovoljaca u odbrani Deligradskog šanca i u kome ih je za odbranu Srbije palo 600. Sanitet je, takođe, bio izdašno potpomognut dobrovoljcima iz većeg broja zemalja: Rusije (123), Srba iz Austrougarske (72), Austrijanaca i Švajcaraca (53), Poljaka i Čeha iz Austrije (10), Italijana (6), Engleza (10), Rumuna (4), Bugara (6), Hrvata (2).

U svojoj knjizi II/1 dr Vladan navodi: doktora medicine i hirurgije 59, ruskih lekara i vračeva 49, apotekara i pomoćnika 26, medicinara 147, ruskih feljdšera 25 i nosilaca ranjenika 27 [4]. Gubici Srbije u životima su bili oko 5 000 poginulih i umrlih, oko 1 000 nestalih i oko 9 500 ranjenih; mnogi od njih su ostali invalidi. Jedino istorijski pozitivno bilo je, kako reče u Narodnoj skupštini Jovan Ristić, što se „izmešala bratska krv srpska i braće sa Severa“ i, dodajmo, iskovalo ratno zajedništvo koje će se i u kasnijim ratovima potvrđivati.

Posle jedne briljantne pobede Srba (59 000 vojnika) 23. avgusta, protiv nadmoćnih Turaka (93 000 vojnika) u odbrambenoj bici za Aleksinac kod Velikog Šiljegovca, dr Vladan se našao u grupi unapređenih oficira „pozdravljen“ od generala Černjajeva činom sanitetskog potpukovnika. Zvanično, po vojnom šematizmu, on je unapređen Kneževim ukazom sa rangom od 13. avgusta 1876. Kasnije, posle rata, Knjaz Milan je pokušao da ga 17. oktobra 1876. unapredi u čin pukovnika, što je omela kraljica Natalija, upozoravajući ga da je to protivzakonito, budući da je Zakonom bilo predviđeno da sanitetski oficiri napreduju samo do čina potpukovnika, u kome su se nalazili i sam načelnik srpskog vojnog saniteta, dr Karlo Beloni i njegov pomoćnik dr Filip Taisić. Kao utešnu nagradu, dr Vladan je dobio orden Takovskog krsta o vratu, jedini pored generala Černjajeva [21].

Odmah po sklapanju primirja Vladan je premešten na staru dužnost šefa i lekara Hirurškog odeljenja beogradske vojne bolnice u činu potpukovnika, kako se vidi iz hitno izdatog vojnog šematizma u oktobru mesecu sa novim rasporedima cele vojske.

Između dva rata

Posle rata je dr Vladan otputovao sa generalom Černjajevom u Kišenjev (Vlaška, sada Rumunija), gde se ruska vojska već pripremala za rat sa Turskom. Po povratku, Ukazom Knjaza Milana, dr Vladan je decembra 1876. godine postavljen na mesto načelnika saniteta u Vrhovnoj komandi srpske vojske, sa koga je imao da pripremi sanitet za sledeći rat koji se očekivao [15]. Prvo što je po postavljenju učinio bilo je da uz pomoć načelnika Štaba Vrhovne Komande, pukovnika Ljube Ivanovića, ubedi ministarstvo vojno u potrebu uvođenja u sastav saniteta nosioca ranjenika sa obrazloženjem da se „u prošlom ratu dešavalo da su čitave gomile boraca izlazile iz stroja da bi iznele po gdekojeg ranjenika iz bojnice“, a da je „u izgubljenim bitkama mnogo ranjenika ostajalo na bojištu na milost i nemilost neprijatelju“ .

Na pozitivnim i negativnim iskustvima iz minulog rata radilo se na dva koloseka: prvo, preko ministra vojnog zatraženi su od korpusnih komandi „određeni predlozi“, a u isto vreme je u Sanitetskom odeljenju Ministarstva obrazovana komisija u sastavu: potpukovnik dr Filip Taisić i majori dr Josif Holec i dr Lazar Dokić koja je imala da predloži „sve izmene i dopune ustrojstva vojnog saniteta koje su se pokazale da su nužne usled učinjenog iskustva“, što je komisija i učinila krajem maja 1877. godine [20]. U tome je došlo do zaključenja mira sa Turskom i time je prestao rad Vrhovne komande. Međutim, uskoro je počeo rat Rusije sa Turskom i Vladan se našao u Glavnom đeneralštabu kao načelnik Sanitetskog odeljenja, sa koga mesta je odmah predložio novu „Formaciju saniteta“ od 139 lekara, 165 lekarskih pomoćnika i 75 apotekara i pomoćnika [22]. Kako se ovaj broj nije mogao naći u Srbiji, ministar vojni je odlučio „da se za ovu celj uputi u inostranstvo, pr.A.N./ potpukovnik dr Vladan Đorđević i da se otvori kredit pomenutom zastupniku /srpske Vlade u Beču [23]. U svojoj III knjizi Istorije srpskog vojnog saniteta, dr Vladan opisuje svoje putešestvje po Austriji (Beč-Prag) u cilju angažovanja lekara i drugog osoblja za srpsku vojsku, koje se najvećim delom završilo bezuspešno [22].

Pripremajući materijalna sredstva za rat, Vlada je za vojni sanitet odobrila sumu od 1 350 000 poreskih groša (22 500 zlatnih dukata, što je nažalost bila „samo jedan četvrti deo one sume koja bi bila nužna da sanitet jedne vojske od 5 korpusa bude kako valja opremljen“.

27. jula 1877, Ministarstvo vojno je svima načelnicima korpusnih saniteta poslalo detaljno uputstvo o merama koje se odmah moraju preduzeti, a Glavni Đeneralštab, sa svoje strane, zatražio je od ministarstva pomoć u angažovanju nedostajućeg sanitetskog osoblja, materijala i lekova, pribora i instrumenata, nabavku Esmarhovih paketića prve pomoći za svakog vojnika (oficiri da kupe sami) i predložio izradu „zemunica-baraka“ u koje bi se tokom zime mogle smestiti poljske bolnice „onde, gde u blizini nema ni sela ni kuća“, uz predlog lokacije za svaki korpus posebno [24].

Najzad, tokom novembra meseca izvršena je mobilizacija vojske, ponovo je obrazovana Vrhovna komanda i dr Vladan se vratio na mesto načelnika saniteta. Sa toga mesta on je odmah izdao naređenja svim korpusnim načelnicima o rasporedu svih poljskih bolnica, rezervnih i etapnih bolnica, njihovom kapacitetu i nužnoj opremi [20].

Drugi srpsko-turski rat, 1877-1878. godine

Nažalost, i u ovaj rat Srbija je ušla siromašna, još neoporavljena od prethodnog rata, ali je njena velika prednost u odnosu na Prvi rat bilo da u njemu nije bila sama, jer je Rusija već nekoliko meseci ratovala u severoistočnoj Bugarskoj, privlačeći glavninu turske vojske. Sa druge strane, što se tiče saniteta, Srbija nije mogla računati na pomoć ruskih dobrotvornih misija i pojedinaca, ali je Rusija preuzela obavezu da izdržava celu srpsku vojsku. Na frontove je Srbija izvela 138 000 vojnika, podoficira i oficira, dok je sanitet brojao 64 lekara i magistra hirurgije, 41 lekarskog pomoćnika i 25 apotekara i pomoćnika, tj. jedva jedna trećina formacijskih mesta. Sa kadrom bolničara i nosilaca ranjenika bilo je još gore, zbog tragičnog neshvatanja njihove uloge u ratu, tako da su umesto najzdraviji i najjači, u njih poslati najslabiji, defektni i oni starijih godišta. O odnosu prema sanitetu dr Vladan u svojoj knjizi navodi, kao najrečitiju ocenu, izveštaj jednoga od najsjajnijih srpskih sanitetskih oficira, Poljaka dr Vladislava Jasnjevskog, kasnije poznatog kao „vojnička majka“, kao pouku za sve buduće generacije sanitetskih oficira. Srpska vojska se u ovome ratu striktno pridržavala onovremenih međunarodnih propisa, što najbolje ilustruju podaci da su iz turskih niške i belopalanačke vojne bolnice evakuisani u Sofiju uz pratnju svi zatečeni turski ranjenici i sanitetsko osoblje. Možda još bolji primer je postupak potpukovnika dr Jovana Mašina, načelnika saniteta Šumadijskog korpusa koji je, u želji da svojim prisustvom osigura bezbednost nekoliko stotina turskih ranjenika i brojnog sanitetskog osoblja, među njima i nekoliko nemačkih lekara u turskoj službi, na čelu patrole prvi ujahao u oslobođeni Niš.

Kao rezutat ovoga rata rođena je još jedna značajna i, među najvećima, Velika niška vojna bolnica, čiji je prvi upravnik bio baš dr Vladan Đorđević. Ta bolnica je u svim kasnijim ratovima odigrala veliku ulogu u lečenju srpskih i zarobljenih neprijateljskih vojnika.

Suočen sa teškim ratnim uslovima (hladno zimsko vreme, siromaštvo opreme, nedostatak kadrova), sanitet je u ovome

ratu, relativno kratkih dejstava i dugotrajnog primirja sa polugodišnjim logorovanjem, dao sve od sebe da umanji patnje ranjenika i bolesnika i da spreči pojave epidemija, u čemu je najvećim delom uspeo: u toku borbenih dejstava poginulo je 667 vojnika, dok je u bolnicama umrlo (decembar-februar) 1 716 vojnika. U periodu logorovanja (mart-avgust, 1878) umrlo je 828 vojnika, u poljskim bolnicama lečeno je 6 793 ranjenika (umrlo 185) i 22 262 bolesnika (umrlo 1160) [20]. Usled velikih napora, lekarski kadar pretrpeo je teške gubitke: umrli su tokom rata i primirja majori dr Stefan Nedok i dr Aleksandar Verminski, kapetani doktori Adam Đerman, Radivoje-Rada Petrović, Aleksa Đukić, Periša Šljivić, rezervista dr Andrej Bikl i nekoliko meseci kasnije, od posledica rata, dr Ilija Milijić.

Iz ovoga rata Srbija je izašla uvećana za 4 okruga i sa međunarodno priznatom nezavisošću na Berlinskom kongresu 1878. godine. Njen sanitet, na čijem su čelu bili potpukovnici dr Filip Taisić u Ministarstvu vojnom i dr Vladan Đorđević u Vrhovnoj komandi, časno je izvršio svoju dužnost, oslonjen prvenstveno na domaće medicinske kadrove, uz nešto dobrovoljaca Srba iz Austrougarske.

Pored svoga neposrednog doprinosa tokom rata, dr Vladan je za buduće naraštaje ostavio nezaboravna pisana dela:

1. Istorija srpskog vojnog saniteta, knjiga II, sveska 1, 1893, o ratu 1876. na 343 stranice, u kojoj je, pored svega iznetog o pripremama i početku rata, dao i spisak svih učesnika, što je možda i najvrednije, jer o tome ne postoji nikakav drugi podatak;
2. Istorija srpskog vojnog saniteta, 1880, knjiga III, na 631 stranici. Bez nje danas o svemu što se u ovome ratu događalo ne bi znali ništa, jer sačuvanih dokumenata nema;
3. Dva spisa ličnih uspomena iz rata: „Na granici“ i „Preko granice“, u kojima je osvetlio sve što se dešavalo u Vrhovnoj komandi, oko nje i u ratu uopšte;
4. 25 godina kasnije, 1907, dve knjige uspomena „Srpsko-turski rat“;
5. U novosadskom „Javoru“ 1 882 članka o sanitetu Moravsko-timočke vojske.

Posle Oslobodilačkih ratova

Sa zaključenjem mira prestala je sa radom Vrhovna komanda i dr Vladan napušta aktivnu vojnu službu i prelazi u Ministarstvo unutrašnjih dela za načelnika Sanitetskog odeljenja. Time se istovremeno oprostio od svoga hirurškog delovanja, ali je istovremeno razvio široku organizacionu delatnost od 1878. do 1884. godine, uključujući i temeljne reforme u sanitetu i prvi Zakon o njemu. Potom prelazi na dužnost predsednika beogradske opštine, na kojoj za godinu dana sređuje pitanje vodovoda i kanalizacije i seobu varoškog groblja sa Tašmajdana na Karaburmu.

U avgustu 1885, zbog koncepcijskog sukoba, napušta ovu dužnost i vraća se u vojnu službu, u vreme priprema za rat sa Bugarskom.

Pripreme i rat sa Bugarskom 1885. godine

Samo što se našao na raskršću karijere, dr Vladan Đorđević dobija iz Ministarstva vojnog poziv na sastanak 11. septembra, na kome Kralj Milan njemu i trojici aktivnih pukovnika srpske vojske saopštava da od toga dana neslužbeno funkcionišu kao Vrhovna Komanda. Da je Vladan tada već bio sanitetski pukovnik vidi se iz Šematizma koji je izdalo Ministarstvo vojno juna meseca 1885, u kome se kao datum Ukaza po kome je unapređen u čin rezervnog pukovnika navodi 28. februar 1884. godine. Videvši da se ozbiljno priprema novi rat u kome treba spremiti sanitet za vojsku od 60 000 ljudi, dr Vladan je odmah počeo da prikuplja podatke o sanitetskom osoblju koje se nalazilo u Srbiji: lekara je u Srbiji bilo 104, od toga u vojsci 22, u građanstvu i privatnih 82; lekarskih pomoćnika u vojnoj službi 20, u građanstvu 11 (ukupno 31); apotekara je u vojsci bilo 11, a u građanstvu 38 (ukupno 49) [25].

Pored ovih kadrova, za ratnu službu su se dobrovoljno mogli organizovati i studenti medicine, stariji u činu poručnka, mlađi u činu potporučnika.

Zbog malog broja vojnih lekara, dr Vladan odmah je predložio da se svi građanski lekari unaprede u činove rezervnih oficira, po principu, najistaknutiji u majore, okružni fizikusi u kapetane 1. klase, a sreski, opštinski i privatni, u kapetane 2. klase. Kraljev Ukaz o tome izašao je 14. septembra, a istovremeno i „Raspored sanitetskih aktivnih i rezervnih oficira i ostalog sanitetskog osoblja“. Na Vladanov zahtev glavni apotekar vojske, major Alojz Helih, obavestio ga je 12. septembra „držim da sada imamo u zemlji dovoljno lekova da podmirimo sve potrebe naše vojske i u vanrednom stanju za najmanje dva meseca“, na osnovu čega dr Vladan zaključuje da je „mobilizacija vojske zatekla glavno apotekarsko slagalište prilično spremno... i to se ima zahvaliti jedino uviđavnosti glavnog vojnog apotekara.. što se nažalost ne može kazati ni za upravnika državnog slagališta vojnosanitetskih pribora, ni za one koji su u mirno doba rešavali šta treba da je svagda spremno u magacinu srpskog Društva Crvenog Krsta...“. Naime, popis u glavnom sanitetskom slagalištu pokazao je „dugačku čitulju nepotrebnih stvari koja pre liči na spisak nekakve telalnice sa bit-pazara nego na inventar jednog vojnog depoa“ i to sve posle „onako gorkih iskustava iz dva Srpsko-turska rata“, jer „uprava vojno-državnog saniteta za sedam godina mira ne spremi takoreći ništa za sanitetsku opremu svoje vojske na slučaj rata...“. Slično je bilo i sa magacinima društva Crvenog Krsta u Beogradu, „tu ima svačega, čak i kitajskih lepeza i jedna mašina za sladoled, ali od zavojnog materijala samo 213 Esmarhovih paketića, 4 lekarska etuvija i 8 etuvija za operacije“ [25].

Upoređujući potrebe sa postojećim, dr Vladan kaže „da su se pojedine divizije kretale iz svojih teritorija u mesta koncentracije bez ikakvog sanitetskog pribora“, samo Dunavska divizija beše prilično opremljena, a za ostale divizije trebalo je takoreći tek sve nabavljati“ [25]. Stoga je on, zajedno sa sanitetskim referentima divizija, utvrdio „generalno trebovanje za sanitetsku opremu cele vojske“, na osnovu koga je ministar vojni naložio Sanitetskom odeljenju ministarstva da „u što mogućem kratkom roku nabavi o trošku vladinih kredita za mobilizaciju aktivne vojske sve one zavojne pribore, instrumente i ostale vojnosanitetske pribore koji su pobrojani u priloženom spisku [26]. Nažalost i pored svega došlo je do otezanja i nerazumevanja u Ministarskom savetu oko nabavke pa je „divizija za divizijom kretala iz Niša takoreći bez ikakvih zavojnih priboja“. Tek energičnom reakcijom ministra vojnog, novi sanitetski pribori počeli su stizati u Niš krajem oktobra, a mnogi, kada je rat već počeo.

29. oktobra Štab Vrhovne komande je premešten u Pirot. Problemi se nastavljaju, ovoga puta sa sanitetskom opremom, fijakerima i sanitetskim železničkim vozom, potom sa smeštajem vojske u pozno jesenje doba, bez dovoljno šatora, u nehigijenskim uslovima i teškoćama u pripremanju hrane, pojavom sumnjivih razboljevanja od zaraznih bolesti. Problema je bilo i u organizovanju etapnih bolnica i smeštaja poljskih bolnica [25].

U noći 1/2. novembar 1885. oglašen je rat Bugarskoj. Kralj Milan je preuzeo vrhovnu komandu nad vojskom i vojska je prešla granicu. Tok ovoga nesrećnog i nepotrebnog rata, započet brže-bolje i samo sa aktivnim jedinicama srpske vojske, u toku koga je ona napredovala do Slivnice da tamo bude poražena od ujedinjenih bugarsko-rumelijskih pukova, završio se neslavnim povlačenjem od 8. novembra, privremenim gubitkom Pirota i primirjem, pod pritiskom velikih sila, 16. novembra. Aktivne divizije su pretrpele sledeće gubitke: Drinska divizija - 1 938 na brojno stanje od 8 233 vojnika; Šumadijska – 1 813 od 7 157 vojnika; Dunavska – 1 815 od 8 425 vojnika; Moravska - 626 od 7 559 vojnika i Timočka 505 od 6 002 vojnika, ukupno poginulih, umrlih i nestalih 6 697 od 37 376 ili 18, 5% [25].

Najveći ljudski gubitak u sanitetu je bio mladi major dr Ljubomir Vesović, referent saniteta Drinske divizije koji je kod Slivnice poginuo od topovske granate, a najveći moralni gubitak srpske vojske je bio gubitak ugleda u zemlji i inostranstvu, sve do Balkanskih ratova 1912/13. godine. Ipak, u njemu je bilo, što se tiče saniteta, i pozitivnih primera za budućnost. Prvo, bilo je uspešno organizovanje Niša u glavni bolnički centar, za šta je najveću zaslugu imao major dr Laza Lazarević, drugo, brzo organizovanje sanitetskog voza koji je do Kragujevca i Beograda brzo prenosio ranjenike na dalje lečenje i treće, sistematsko uvođenje u hiruršku praksu antiseptičnog pristupa (kasnije aseptičnog) u obradi i zaštiti rana, što je posle pruske vojske u prusko-francuskom ratu drugi poznati primer. Uz to, pokazalo se da je za kasniji tok izlečenja rane bila odlučujuća primena Ezmarhovog antiseptičnog prvog zavoja.

Na osnovu kratkog, ali nesrećnim događajima bogatog sanitetskog iskustva, dr Vladan je u januara 1886. podneo Kralju Milanu referat „o kompletovanju sanitetske opreme vojske, o manama u samom ustrojstvu poljske vojnosanitetske službe koje bi valjalo blagovremeno popraviti“. Nešto kasnije, 3. februara, referent saniteta Nišavske vojske, potpukovnik dr Mihailo Marković, protivno pravilima službe, zaobišavši svoga neposredno pretpostavljenog dr Vladana, podnosi Kralju svoj referat „po usmenom naređenju Vrhovnog komandanta Kralja“ u kome predlaže projekat preustrojstva vojnog saniteta, između ostalog o uvođenju drugog lekara u puku, o smanjenju poljskih bolnica sa 200 na 100 kreveta i o uvođenju u hirurški rad antisepse, koju je uspešno primenjivao u svojoj diviziji. Na margini referata Kralj stavlja zabelešku: „Misli izložene u gornjem referatu potpukovnika dr Mihaila Markovića usvajam“.

U vreme podnošenja ova dva referata Kralj šalje dr Vladana u inspekciju vojnih bolnica u Timočkoj Krajini i za njegovog zamenika u odsustvu određuje baš dr Markovića, čime priprema tiho uklanjanje dr Vladana sa njegovog položaja. Tako je dr Vladan sa mesta dugogodišnjeg Kraljevog ličnog i porodičnog lekara i ljubimca i ratnog rukovodioca vojnog saniteta uklonjen kraljevskim pučem, sprovedenim tiho, kao kazna za teške sanitetske događaje koji su se tokom vojnih dejstava dešavali tokom povlačenja u zimskim uslovima, kako u vidu pojava neorganizovanog povlačenja, tako i zbog pojave epidemijskih bolesti, pre svega trbušnog tifusa. Na taj način, Kraljica Natalija je najzad dočekala trenutak ispunjenja svojih dugogodišnjih animoziteta prema dr Vladanu.

Kako je u vreme njegovog puta po Krajini, 19. februara 1886. bio zaključen mir, Vrhovna Komanda je samim time bila raspuštena i dr Vladan nije imao gde da se vrati na dužnost, a već 1. marta je izašao u Službenom listu mirnodopski raspored i rang-lista aktivnih oficira u kome je na mestu načelnika saniteta u ministarstvu stajalo ime potpukovnika dr M. Markovića, dok je Vladanovo ime nestalo, slično kao što se 1859. desilo sa dr Emerihom Lindenmajerom po povratku Kneza Miloša iz izganstva na vlast. Kralj Milan, onakav kakav je bio, lako se poigrao i sa njime, kao i sa mnogim drugim svojim trenutnim ljubimcima, iako je dr Vladan njemu bio verni i odani prijatelj i privrženik dinastije Obrenović. Pa i ovoga puta, iako ga je grubo otpustio, dr Vladan je posle nekoliko meseci postao ministar prosvete i vera i na tome mestu se zadržao sve do Kraljeve abdikacije. Kasnije, bio je ministar u više resora i u više Vlada, srpski poslanik u Atini i Carigradu, najzad i predsednik srpske Vlade (1897-1900) koju je sopstvenom ostavkom oborio u znak protesta zbog ženidbe Kralja Aleksandra Obrenovića sa, starijom od sebe, udovicom Dragom Mašin. Taj ostatak njegovog građanskog života i rada zahteva drugu priču.

Posle ratova

Prestankom vojne službe na kraju rata, Vladanova vojnička karijera se definitivno završila. Međutim, on nije zaboravio da ispuni svoj dug prema vojnom sanitetu čiji je ratni načelnik bio u svim dotadanjim ratovima: 1886. godine izlazi „Istorija srpskog vojnog saniteta“, knjiga IV, Srpsko-bugarski rat, a 1893. već odavno iščekivana i zaboravljena prva sveska II knjige „Istorije“ o pripremama, uvodu i početku Prvog srpsko-turskog rata 1876. Pored njih, Vladan je u svome časopisu „Otadžbina“, koji je sa prekidima izdavao od 1875. do 1892, puštajući između ostalog da i oficiri vojničke karijere objavljuju svoja sećanja i analize minulih ratova, već iste godine u članku „Slivnica, treći rat, 1885/86“ izneo vrlo „... golu i i gorku istinu“ o njemu, zbog čega ga je Kralj Milan optužio svojeručno pisanom tužbom „zbog izdavanja državnih tajni“, ali, kako reče dr Vladan „srećom ondašnji sudovi svih istancija nađoše da u Srbiji nema nikakvog zakona koji zabranjuje pisati istoriju, i oslobodiše pisca“.

Vladan, kao vojni pisac, ostavio nam je originalnu, živu i dokumentovanu sliku o razvoju i radu srpskog vojnog saniteta u miru i ratu, sliku svojih muka i posrtanja zbog nemaštine, nekulture i nerazumevanja njegove uloge, njegovih ratnih nevolja i požrtvovanja, i oživeo likove nekolicine sjajnih sanitetskih oficira. Svojim otvorenim karakterom, svojom vulkanskom prirodom i neobuzdanom snagom svoje ličnosti delio je packe onima sa kojima se nije slagao, kritikovao i bio kritikovan, delio i primao udarce, a sa svojom inteligencijom i renesansnim interesovanjem ostao jedan od retkih koji je „znao sve“. Ali, kao pčela koja leti od cveta do cveta, nije imao vremena ni strpljenja da se na nečemu zaustavi, te je tako žrtvovao svoj hirurški dar u korist karijere vojnog i građanskog rukovodioca, kasnije istoričara, književnika, prevodioca, novinara, političara, kao neki srpski Leonardo da Vinči XIX veka. I takav, kakav je, bio je uz dr Lindenmajera i tihog dr Belonija „najkrupnija, najpoznatija i najznačajnija ličnost srpskog vojnog i građanskog saniteta“ XIX veka, njegov predvodnik u ratovima koje je Srbija tada vodila. Lično pošten, bio je i ostao siromah koji je, lišen jedno vreme penzije, morao novinarskim radom da izdržava svoju mnogobrojnu porodicu. U vihoru događaja koji su karakterisali srpsku istoriju, bio je čas na vrhu, čas u ponoru, doživevši čak i da 1906. godine provede šest meseci na robiji, ponovo „zbog odavanja državne tajne“ u njegovom dvotomnom delu „Kraj jedne dinastije“.

Doživevši kao penzioner srpske pobede 1912, 1913. i 1914, srpsku tragediju 1915, konfinaciju u Austriji 1916-1918. i srpsku renesansu 1918, umro je u banji Baden kod Beča 31. avgusta 1930. godine, a sahranjen od države, koju je verno služio u Beogradu, na groblju koje je on stvorio i koje nosi njegovo ime.

Ne gledajući njegove druge velike zasluge za razvoj kako vojnog, tako i civilnog saniteta, on je vojnosanitetsku istoriografiju bezmerno zaslužio svojim delima, jer je tokom sledećih decenija i ratova u kojima je Srbija učestvovala, dobar deo arhivske dokumentacije propao ili se izgubio tako da bi bez njih, kao i knjige prvog srpskog sanitetskog rukovodioca štab-doktora dr Emeriha Lindenmajera, mi o tim događajima danas vrlo malo znali.

Najbolji primer je, upravo, dr Vladan, od koga u Vojnom arhivu postoji samo jedan sačuvani dokument, njegova konduit lista iz 1974. i nekoliko Ukaza u Službenim vojnim listovima.

Koliko je bio cenjen u izemlji i inostrantstvu govori njegovo članstvo u stručnim i naučnim društvima: redovni član Srspskog učenog društva (1869), redovni član lekarske komore u Beču (1870), redovni član Antropološkog društva u Beču (1871), redovni član i osnivač Srpskog lekarskog društva (1872), počasni član Zbora liječnika Hrvatske i Slovenije (1875), dopisni član Društva slavenskih liječnika, Zagreb (1875), dopisni član Španskog higijenskog društva, Madrid (1883), dopisni član belgijskog društa za javnu higijenu, Brisel (1885), dopisni (1882) i redovni (1888) član Srpske Kraljevske Akademija Nauka, redovni član Društva za diplomatsku istoriju, Pariz (1892), počani član Grčkog književnog društva „Parnas“, Atina (1892), počasni član Grčkog književnog društva „Silogos“, Carigrad, (1895), počasni član Ruskog areološkog instituta, Carigrad (1895) i počasni član Srpskog društva Crvenog Krsta (1905).

Nosilac je velikog broja domaćih i stranih odlikovanja koja oslikavaju obe njegove karijere, vojničku i građansku. Od domaćih odlikovanja, to su: 1876 ─ Takovski krst na prsima, Takovski krst o vratu; 1878 ─ Krst Srpskog društva Crvenog krsta; 1883 ─ Takovski krst sa mačevima, Sveti Sava II reda; 1888 ─ Sveti Sava 1. Reda; 1894 ─ Beli orao V reda, Medalja Miloša Velikog, Zlatna medalja za revnosnu službu; 1897 ─ Beli orao IV reda; 1898 ─ Takovski krst 1. reda; 1899 ─ Orden Miloša Velikog II reda; Spomnenice ratova 1876, 1877/78 i 1885 [21].

Strana odlikovanja su: 1871 ─ nemačka Medalja za revnosnu službu u ratu, nemačka Medalja Vilhelma Velikog; 1877 ─ ruski Orden Svete Ane II reda; 1893 ─ grčki orden Svetog Spasitelja 1. Reda; 1894 ─ turski orden Medžidije IV reda; 1897 ─ turski orden Medžidije 1. reda; 1898 ─ turski orden Osmanlije sa brilijantima 1. reda, persijski orden Lava i Sunca 1. reda.

L I T E R A T U R A

1. Konduit-lista iz 1874 godine, Vojni arhiv, pop. 14, kut. 19, reg. 2/1, list 165–166.
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I N M E M O R I A M

Primarijus prof. dr sc. med. DARA STEFANOVIĆ

(1955–2020)

Dana 15. septembra 2020. godine iznenada nas je napustila dugodišnji član kolektiva instituta za radiologiju Vojnomedicinske akdemije (VMA) u Beogradu, profesor Medicinskog fakulteta VMA Univerziteta odbrane i dugogodišnji član Uređivačkog odbora Vojnosanitetskog pregleda (VSP), primarijus prof. dr Dara Stefanović.

Rođena je 22. februara 1955. godine u Kopru. Studije medicine završila je 1980. godine na Medicinskom fakultetu u Beogradu, a specijalizaciju iz radiologije na VMA 1985, kada je i zvanično postala član kolektiva Instituta za radiologiju VMA. Na VMA je odbranila doktorsku disertaciju 2004. godine i iste godine postala docent na Katedri za radiologiju i nuklearnu medicinu VMA, a 2011. godine profesor radiologije na tada već osnovanom Medicinskom fakultetu VMA. Godine 2004. dodeljeno joj je i stručno zvanje primarijus od strane Ministarstva zdravlja Republike Srbije.

U periodu 1988–2011. godine bila je na usavršavanjima u vodećim evropskim centrima iz oblasti primene ultrazvuka i doplersonografije, kao i u oblasti multislajsnog skenera za preglede grudnog koša, abdomena i male karlice. Kao vodeći stručnjak u oblasti
ultrazvuka u zemlji, od 1987. godine bila je stalni predavač u Jugoslovenskoj školi ultrazvuka i predavač po pozivu na mnogim stručnim i naučnim skupovima posvećenim ovoj oblasti.

Bila je učesnik više naučnih projekata realizovanih na klinikama VMA, mentor, komentor i član komisija za ocenu i odbranu većeg broja specijalističkih, magistarskih i doktorskih teza. Objavila je oko 50 stručnih i naučnih radova iz oblasti klasične i interventne nevaskularne radiologije, ultrazvučne dijagnostike površnih tkiva, ultrazvučne dijagnostike abdomena i karlice, doplersonografije parenhimskih struktura abdomena, karlice i površnih organa. Takođe, dala je značajn doprinos kao autor poglavlja u više knjiga i priručnika iz oblasti ultrazvučne dijagnostike koji su prihvaćeni kao zvanični udžbenici na medicinskim fakultetima u Srbiji (Lekarski priručnik SLD, Dijagnostički ultrazvuk u gastroenterologiji i nefrologiji, Ultrazvuk u medicini, Sepsa u abdominalnoj hirurgiji).

Kao član Uređivačkog odbora VSP i recenzent radova iz oblasti radiologije dala je značajan doprinos u podizanju kvaliteta časopisa i njegovom pozicioniranju među vodeće medicinske časopise ne samo u zemlji, već i na globalnom nivou.

Bila je član Srpskog lekarskog društva (Sekcija za radiološku dijagnostiku), Udruženja radiologa Srbije, Udruženja za primenu ultrazvuka u medicini, biologiji i veterini Srbije i Lekarske komore Srbije.

Za svoj dugogodišnji plodni stručni i naučni rad u oblasti ultrazvučne dijagnostike dobila je veći broj priznanja i nagrada, od kojih treba izdvojiti Zlatnu, Platinsku i Dijamantsku povelju Udruženja za primenu ultrazvuka u medicini, biologiji i veterini Srbije za izuzetan doprinos u unapređenju i razvoju medicinske struke i nauke iz oblasti ultrazvuka.

O doktorki Dari mogle bi da se ispišu čitave stranice jer se, kao humanista i vrstan lekar, čitav svoj radni vek predano

i odgovorno posvetila pozivu kojim se bavila. Zbog toga je bila izuzetno cenjena među kolegama i omiljeni nastavnik kod kadeta Medicinskog fakulteta VMA i specijalizanata radiologije koji su svoja prva stručna iskustva sticali na Institutu za radiologiju VMA. Kao lekar, nastavnik i saradnik uvek je svima davala podršku i imala razumevanja za probleme, kako na radnom mestu, tako i privatno. Svima koji su je poznavali, ostaće u sećanju njen optimizam, duhovitost, čovečnost, disciplina i odgovornost koji su plenili. Čuvaćemo uspomenu na nju uz obavezu da kao njene kolege i saradnici nastavimo da ostvarujemo viziju koju je nesebično gajila i negovala – da u službu zdravlja stavimo svoju struku, svoju etičnost i humanost, a, nadasve, ljubav prema lekarskom pozivu i čoveku uopšte.

Neka je večna slava i hvala našoj dragoj i poštovanoj koleginici, prof. dr Dari Stefanović!

Pukovnik dr Dragan Dulović,
Institut za radiologiju VMA

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

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Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

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

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

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