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CASE REPORTS / KAZUISTIKA

Dr Draga Ljočić (Šabac, 1855 – Beograd, 1926) prva je školovana žena – lekar u Srbiji. Posle završene Više ženske škole u Beogradu, započinje studije medicine u Cirihu, Švajcarska, 1872. godine. Nakratko prekida studiranje 1876. kako bi učestvovala u srpsko–turskom ratu u kome stiče čin poručnika. Po završetku rata vraća se u Cirih gde 1878. godine diplomira na Medicinskom fakultetu, i postaje prva Srpkinja sa diplomom lekara.

Osim dr Drage Ljočić, i druge Srpkinje koje su u međuvremenu postale lekari, kao i lekarke – dobrovoljci iz drugih zemalja koje su u Srbiju došle same ili u sklopu medicinskih misija na poziv Crvenog krsta Srbije, učestvovale su, kao pripadnice sanitetske službe, u oslobodilačkim ratovima Srbije 1876–1878. i 1911–1918. Za iskazanu požrtvovanost i hrabrost odlikovane su visokim odličjima (vidi str. 891–2).

Dr. Draga Ljočić (Šabac, 1855 – Belgrade, 1926) was the first woman – medical doctor in Serbia. After graduating at the Higher Women's School in Belgrade, she started studying medicine in Zurich, Switzerland, in 1872. In 1876 she had a short break in studying due to participating in the Serbian – Turkish War in which confered the rank of lieutenant. After the war she went back to Zurich and graduated at the Medical School in 1878, and became the first Serbian woman with medical doctor diploma.

Apart from Dr. Draga Ljočić, the other females from Serbia, becoming in the meantime medical doctors, as well as those from other countries coming in Serbia alone or as part of foreign medical missions at the invitation of the Serbian Red Cross, participated also as members of medical services in the liberation wars of Serbia from 1876 to 1878, and from 1911 to 1918. For their sacrifice and courage in offering health care to wounded soldiers and civilians they were awarded with many orders (see p. 891–2).

ORIGINAL ARTICLES

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Impact of dental implant insertion method on the peri-implant bone tissue – An experimental study

Uticaj hirurške metode ugradnje dentalnih implantata na periimplantatno koštano tkivo

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Abstract

Background/Aim. The function of dental implants depends on their stability in bone tissue over extended period of time, i.e. on osseointegration. The process through which osseointegration is achieved depends on several factors, surgical insertion method being one of them. The aim of this study was to histopathologically compare the impact of the surgical method of implant insertion on the peri-implant bone tissue. **Methods.** The experiment was performed on 9 dogs. Eight weeks following the extraction of lower premolars implants were inserted using the one-stage method on the right mandibular side and two-stage method on the left side. Three months after implantation the animals were sacrificed. Three distinct regions of bone tissue were histopathologically analyzed, the results were scored and compared. **Results.** In the specimens of one-stage implants increased amount of collagen fibers was found in 5 specimens where tissue necrosis was also observed. Only moderate osteoblastic activity was found in 3 sections. The analysis of bone-to-implant contact region revealed statistically significantly better results regarding the amount of collagen tissue fibers for the implants inserted in the two-stage method ($Wa = 59 < 66.5$, $\alpha = 0.05$), but necrosis was found in all specimens, and no osteoblastic activity. Histopathological analysis of bone-implant interface of one-stage implants revealed increased amount of collagen fibers in all specimens, moderate osteoblastic activity and neovascularization in 2 specimens. No inflammation was observed. The analysis of two-stage implants revealed a marked increase of collagen fibers in 5 specimens, inflammation and bone necrosis were found in only one specimen. There were no statistically significant differences between the two methods regarding bone-implant interface region. Histopathological analysis of bone tissue adjacent to the one-stage implant revealed moderate increase of collagen tissue in only 1 specimen, moderate increase of osteoblasts and osteocytes in 3 specimens. No necrotic tissue was found. The analyzed specimens of bone adjacent to two-stage implants revealed a moderate increase in the number of osteocytes in 3 and a marked increase in 6 specimens respectively. This difference was statistically significant ($Wb = 106.5 > 105$, $\alpha = 0.05$). No necrosis and osteoblastic activity were observed. **Conclusion.** Better results were achieved by the two–stage method in bone–to-implant contact region regarding the amount of collagen tissue, while the results were identical regarding the osteoblastic activity and bone tissue necrosis. There was no difference between the methods in the bone–implant interface region. In the bone tissue adjacent to the implant the results were identical regarding the amount of collagen tissue, osteoblastic reaction and bone tissue necrosis, while better results were achieved by the two-stage method regarding the number of osteocytes.

Key words:
dental implants; surgery, oral; dogs, osseointegration.

Apstrakt

Uvod/Cilj. Funkcija dentalnih implantata zavisi od njihove stabilnosti u koštanom tkivu u dužem periodu vremena, odnosno od oseointegracije. Proces uspostavljanja oseointegracije zavisi od nekoliko faktora, od kojih je jedan hirurška metoda ugradnje. Cilj ovog rada bio je da se patohistološki uporedi uticaj hirurške metode ugradnje implantata na periimplantatno koštano tkivo. **Metode.** Eksperiment je urađen na devet pasa. Osam nedelja posle ekstrakcije donjih premolara ugrađeni su implantati jednofaznom metodom na desnoj i dvofaznom na levoj strani donje vilice. Tri meseca posle im-

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plantacije životinje su žrtvovane. Tri određene regije koštanog tkiva su patohistološki analizirane, rezultati ocenjeni i upoređeni. **Rezultati.** U uzorcima jednofaznih implantata nađeno je uvećanje kolagenih vlakana kod pet uzoraka u kojima je, takođe, primećena i nekroza tkiva. Umerena osteoblastična aktivnost je nađena kod tri uzorka. Analizom koštano-implantatne granice utvrđena je statistički značajna razlika u količini kolagenih vlakana kod implantata ugrađenih dvofaznom metodom (Wa = 59 < 66,5, α = 0,05), ali je u svim uzorcima primećena nekroza tkiva bez osteoblastične aktivnosti. Patohistološka analiza koštano-implantatne granice jednofaznih implantata pokazala je povećanu količinu kolagenih vlakana kod svih uzoraka, a umerenu osteoblastičnu aktivnost i neovaskularizaciju kod dva uzorka. Inflamacija nije primećena. Analiza dvofaznih implantata pokazala je izrazito povećanu količinu kolagenih vlakana kod pet uzoraka, inflamacija i nekroza su pronađene kod samo jednog uzorka. Nije bilo statistički značajne razlike između ove dve metode u pogledu koštano-implantatne granice. Patohistološka analiza kosti u blizini jednofaznih implantata pokazala je umereno povećanje količine kolagenih vlakana kod samo jednog uzorka, umereno povećenje broja osteoblasta i osteocita kod tri uzorka. Nije bilo nekrotičnih promena. Analizirani uzorci dvofaznih implantata pokazali su umereno povećanje broja osteocita kod tri uzorka i izraženo povećanje kod šest uzoraka i ova razlika je bila statistički signifikantna (Wb = 106,5 > 105, α = 0,05). **Zaključak.** Bolji rezultati su postignuti dvofaznom metodom u odnosu na jednofaznu u delu kontakta implantata i kosti što se tiče kolagenih vlakana, dok su rezultati bili identični u pogledu osteoblastične aktivnosti i tkivne nekroze. Nije bilo razlike između dve metode u delu koštano-implantatne granice. U koštanom tkivu u blizini implantata rezultati su bili identični u pogledu količine kolagenih vlakana, osteoblastične aktivnosti, i koštanotkivne nekroze, dok su bolji rezultati bili postignuti dvofaznom metodom kad je u pitanju bio broj osteocita.

Ključne reči:
implantati, stomatološki; hirurgija, oralna, procedure; psi; oseointegracija.

Introduction

Replacement of missing teeth with dental implants has become predictable treatment modality over the past several decades. The function of dental implants depends on the process of osseointegration, defined by Brånemark as "direct structural and functional connection between living ordered bone and the surface of load carrying implant". The concept of osseointegration arose from the studies of osseous wound healing that had started in the 1950s by Bränemark. Titanium chambers containing a transillumination system were inserted into the fibulae of rabbits to observe cellular changes during endostael wound healing. At the completion of the study, retrieval of the titanium chamber required the fracture of bone tissue that has integrated with the chamber surface. Bränemark's team found that implants made of commercially pure (c.p.) titanium, careful bone preparation and immobilization of the implant during the initial healing phase were necessary to effect a rigid fixation of the implant to the surrounding bone tissue. Implants that accidentally became exposed to the oral cavity through wound dehiscence exhibited less favorable periimplant healing than the implants that had been submerged under oral mucosa [1]. This concept, which requires a second stage procedure, is still followed today. However, Schroeder and al. [2] demonstrated in the late 1970s that non-submerged or one-stage surgical method allows successful outcome of implant insertion, i.e. successful osseointegration. The fact that only one surgical intervention is necessary allows for soft tissue healing to the transmucosal portion of the implant by primary intention from the moment of implant insertion. It is now recognized that the requirement to submerge the implant during healing is not obligatory, and even has the advantages over sub-merged approach including: 1) the lack of secondary surgical intervention to connect the implant body and transgingival component; 2) a more mature soft tissue healing due to avoiding the second stage surgery; 3) the lack of an interface/microgap between the implant and the abutment at or below the alveolar crest level; 4) healed peri-implant mucosa is not disturbed with second stage procedure for abutment placement or abutment exchanges; 5) during the osseointegration period the implants are accessible for clinical monitoring; 6) cost and time benefit advantage [3]. However, one-stage implantation is not the preferred treatment modality in the cases of: 1) prevention of undesirable implant loading during the osseointegration period when implants are inserted in low-density bone; 2) alveolar ridge augmentation procedures or guided bone regeneration with simultaneous implant placement when the wound has to be closed perfectly to prevent the infection of bone or membrane exposure; 3) integrated implant abutment interference with opposing jaw (in case of one-piece implant design).

For a long time it was considered that implant failure was a result of soft tissue ingrowth in the coronal aspect of bony implant bed, inflammatory infiltrate, granulation tissue, bone resorption and implant mobility that was caused by the implant communication to the oral cavity.

Numerous reports have compared the submerged and non-submerged implant types in animal models [4–13]. To evaluate osseointegration of dental implants by animal studies many methods have been established: radiographic evaluation of bone healing, histopathological and histomorphometric analysis.

Radiographic evaluations obtain the results of peri-implant bone changes, i.e. the differences of peri-implant bone levels. Histomorphometric analysis is the measurement of direct bone-to-implant contact without connective tissue interposition. Such data include no information about functional and structural bone architecture, bone maturity or inflammatory signs. The aim of this study was to histopathologically analyze bone tissue healing in three anatomical regions after insertion of titanium dental implants, determine the differences between them and compare the obtained results using a split mouth design in experimental dog model.

Methods

The study protocol was approved by the Military Medical Academy Ethic Committee. Nine dogs (German shepherds), mean age 4.5 years, mean weight 32 kg, were used in the study. During the experiment the dogs were fed once per day with soft food diet and water *ad libitum* [14].

The study was performed in three phases. In the first phase third and forth premolars were extracted bilaterally in each dog. After a healing period of eight weeks in the second phase the implants were inserted. Using the split-mouth study design 36 titanium dental implants were inserted by the one-stage method on the right mandibular side and two-stage method on the left side, respectively. Three months after implantation the animals were sacrificed. In the third phase pathohistological analysis was performed.

Anesthesia protocol

In premedication, acepromazin (Combelem, Bayer, Germany) was administrated i.v. 0.03 mL/kg and atropin 0.1 mg/kg s.c. Anesthesia was performed using intravenous administration of 5% ketamin chloride (Ketamin chlorid, Hemofarm, Serbia) 0.3 mL/kg. Ketamin chloride is a dissociative anesthetic solution which produces dissociative anesthesia. Third and forth lower premolars were extracted bilaterally. Extraction wounds were sutured with resorbable sutures (Tyco Healthcare group, USA). Following the 8-week healing period implant insertion was performed. Animals were prepared and anesthetized in the same manner as in the first surgical phase. On the left mandibular side two implants were placed by one-stage method (non-submerged) and in the right side in two-stage method (submerged). BCT root form implants were inserted (BCT implant system, Belgrade, Serbia) manufactured of commercially pure titanium, machine surfaced. The intraosseous part of the implant was 13.7 mm long, 4.5 cm wide, with 4 threads.

Insertion of one-stage (non-submerged) implants followed the principals for soft tissue reflection and implant position. Access to the bone was performed by crestal incision followed by elevation of buccal and lingual full thickness flap. Implant socket preparations were performed with bone drill by the speed of 800 rpm and with copious cooling with sterile saline to avoid bone heating and subsequent necrosis. A total of 18 implant sites were prepared and implants placed into the sockets. Soft tissues were closely adapted to the implant necks with interrupted resorbable sutures.

Insertion of implants in two-stage surgical procedure was preformed in the same manner as the contralateral side, but the implants with healing caps were covered with mucoperiosteal flap and sutured.

After three months of healing animals were sacrificed by an overdose of intravenous injection of sodium pentobarbital. The specimens were retrieved after jaws were dissected with hand saw. Individual bone blocks containing implants and surrounding hard tissue were fixed in 4% formaldehyde solution and prepared for decalcified sectioning. The blocks were cut to final sections with the thickness of 5–6 microns in buccolingual direction, stained in PAS, toluidin blue, Von Kossa, Masson trichrom, PAS-diastasis, Vimentin, S-100 protein, citokeratin epithelial membrane antigene (EMA) and neuron specific enolase (NSE). Histological examination was performed in a Leitz microscope (Leica, Heidelberg, Germany) equipped with an image system (Q-500MC, Leica). Three distinct histological regions were examined: bone tissue in contact with the implant (bone-to-implant contact region), bone-implant interface and bone tissue adjacent to the implant. Analyses were performed on 90 specimens from each of the five regions thus comprising 270 specimens. Semi-quantitative analysis was performed for each site (evaluation of inflammatory cell infiltration, tissue necrosis, number of blood vessels, appearance of blood vessel walls, vasodilatation, connective-collagen fibers, osteoblastic reaction, osteocytes) and graded following the grading index: 0–2 (Table 1)

All the analyses were performed 3 months following one- and two-stage implant placement on 90 specimens from each of the three regions (270 specimens). Quantitative analyses of the histopathological findings were performed according to the established grading indices for each evaluated region. Outcomes of two surgical methods were compared using non-parametric Wilcoxon-Mann-Whitney rank-sum test for two small independent samples.

In the process of testing we formed a null hypothesis of equal medians H0 : Me1 = Me2 (there was no difference between the two surgical methods) and research hypothesis H1 : Me1 > Me2 (one stage surgical method was more effective). The null hypothesis was rejected at the significance level $\alpha = 0.05$.

Results

Analysis of bone-to-implant contact region

Bone-to-implant contact region included the analysis of connective–collagen tissue formation, osteoblastic reaction and bone necrosis. Grading indices of bone tissue were assessed according to the established schemes and presented in Table 2.

With regard to the results on the basis of descriptive statistics, better results were achieved by the two-stage method regarding the amount of collagen tissue fibers (Me1 < Me2) and this difference was statistically significant (Wa = 59 < 66, $\alpha = 0.05$) while regarding the osteoblastic activity and bone necrosis the results were identical (Me1 = Me2).

Figure 1 presenting the rank sum values, shows better results of one-stage surgical method regarding the number of osteoblasts (Wa = 99, Wb = 72) and bone tissue necrosis (Wa = 101.5, Wb = 69.5), but not of the amount of collagen fibers (Wa = 59, Wb = 112).

Pathohistological findings

<u>One-stage method</u>

The analysis of bone tissue in the contact with the implant revealed increased amount of connective tissue fibers in five specimens (1, 2, 4, 7 and 9). Necrosis was also observed in the same samples. In the remainder (3, 6 and 8) moderate osteoblastic activity was found (Figure 2).

Table 1

Grading indices of patohistological analyses of the bone-to-implant cobntact region, bone-implant interface and bone tissue adjacent to the implant

Characteristics of bone-to-implant contact region	Grading index			Grading indices of patohistological characteristics of bone- implant interface	Grading index			Characteristics of bone tissue adjacent to the implant	Grading index		
	0	1	2		0	1	2		0	1	2
Connective – collagen tissue fibers	Marked increase	Moderate increase	No increase	Connective – collagen tissue fibers	Marked increase in connective tissue fibers	Moderate increase in connective tissue fibers	No increase in connective tissue fibers	Connective – collagen tissue fibers	Marked increase in connective tissue fibers	Moderate increase in connective tissue fibers	No increase in connective tissue fibers
Osteoblastic activity	Marked osteoblastic activity	Moderate osteoblastic activity	No osteoblastic activity	Osteoblastic activity	Marked osteoblastic activity	Moderate osteoblastic activity	No osteoblastic activity	Osteoblastic activity	Marked osteoblastic activity	Moderate osteoblastic activity	No osteoblastic activity
Bone tissue necrosis	Preserved bone tissue structure	Partial bone tissue necrosis	Marked or complete bone tissue necrosis	Blood vessels	Marked vascular proliferation	Moderate vascular proliferation	No vascular proliferation	Osteocytes	Marked increase in number of osteocytes	Moderate number in osteocytes	Presence of osteocytes
				Inflammatory cell infiltrate	No inflammatory infiltrate	Moderate inflammatory infiltrate	Marked inflammatory infiltrate	Bone tissue necrosis	No necrosis	Partial necrosis	Marked or complete necrosis
				Bone tissue necrosis	No necrosis	Partial necrosis	Complete necrosis				

Table 2

Grading indices of pathohistological characteristics of the bone-to-implant contact region

Specimen number	Connective–collagen tissue fibers		Osteoblastic activity		Bone tissue necrosis	
	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage
1	0	0	2	2	1	1
2	0	0	2	2	1	1
3	2	0	1	2	0	1
4	1	0	2	2	1	1
5	2	0	2	2	0	2
6	2	1	1	2	0	1
7	1	1	2	2	1	1
8	2	0	1	2	0	1
9	1	0	2	2	2	1
Median	1	0	2	2	1	1
Wilcoxon	Wa = 59	Wb = 112	Wa = 99	Wb = 72	Wa = 101,5	Wb=69.5
Wa Statistic for n1 = n2 = 9, α = 0,05	Upper and lower critical values Wa (66, 105)					

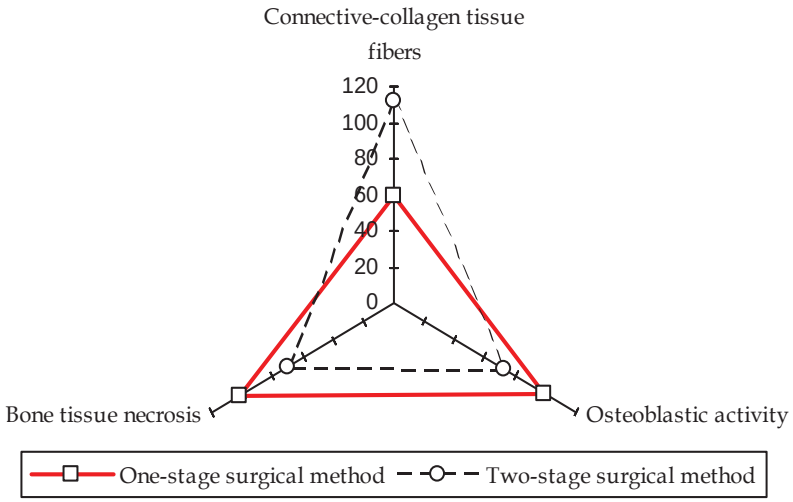

Fig. 1 – Histopathological characteristics of the bone-to-implant contact region – connective-collagen tissue fibers, osteoblastic activity and bone tissue necrosis (rank sum values).

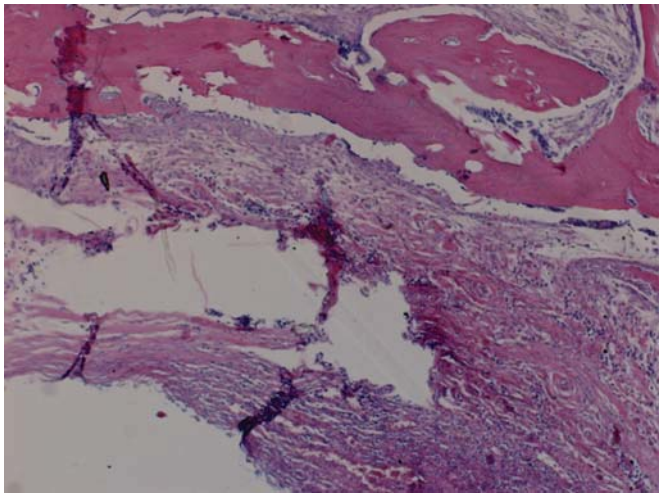

Fig. 2 – Increased amount of connective tissue with a marked chronic inflammatory cell infiltration, partial necrosis and marked vascular proliferation. The presence of compact bone tissue regions with osteoblastic activity is detectable (HE, × 40).

Two-stage method

The analysis of bone tissue in contact with implants revealed increased amount of connective-collagen tissue fibers in all specimens. No osteoblastic activity was observed. Partial bone tissue necrosis was found in eight specimens and in one (5) necrosis was complete (Figure 3).

Analysis of bone-implant interface

The region of bone-implant interface included analysis of connective–collagen tissue fibers, osteoblasts, blood vessels, inflammation and bone tissue necrosis.

Grading indices of the examined characteristics with statistical results are presented in Table 3 and Figure 4.

According to the results presented in Table 3 on the basis of descriptive statistics the results were identical regarding median values of osteoblastic activity, blood vessels, inflammatory cell infiltrate and bone tissue necrosis. Better results were achieved by the two-stage method regarding the

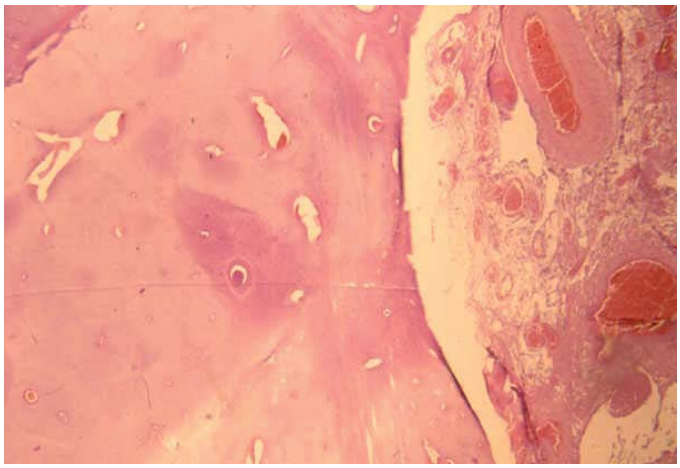

Fig. 3 – On the left: compact lamellar bone. On the right: increased amount of connective–collagen tissue, with numerous dilated blood vessels, marked inflammatory cell infiltrate and partial necrotic lesions (HE, × 40).

Table 3

Grading indices of histopathological characteristics of bone-implant interface

Specimen number	Connective–collagen tissue fibers		Osteoblastic activity		Blood vessels		Inflammatory cell infiltrate		Bone tissue necrosis	
	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage
1	0	2	2	2	2	2	0	0	0	0
2	0	0	2	2	2	2	0	0	0	0
3	1	0	2	1	2	1	0	1	0	1
4	1	0	1	1	1	0	0	0	0	0
5	1	2	1	2	0	2	0	0	1	1
6	1	2	2	2	2	2	0	0	0	0
7	0	0	2	2	2	2	0	0	0	0
8	1	2	2	2	0	2	0	0	0	1
9	0	0	2	2	2	2	0	0	0	0
Median	1	0	2	2	2	2	0	0	0	0
Wilcoxon Wa Statistics for n1 = n2 = 9, α = 0,05	Wa = 91	Wb = 80	Wa = 90	Wb = 81	Wa = 90.5	Wb = 80,5	Wa = 90	Wb = 81	Wa = 94.5	Wb = 76.5
Upper and lower critical values Wa (66, 105)										

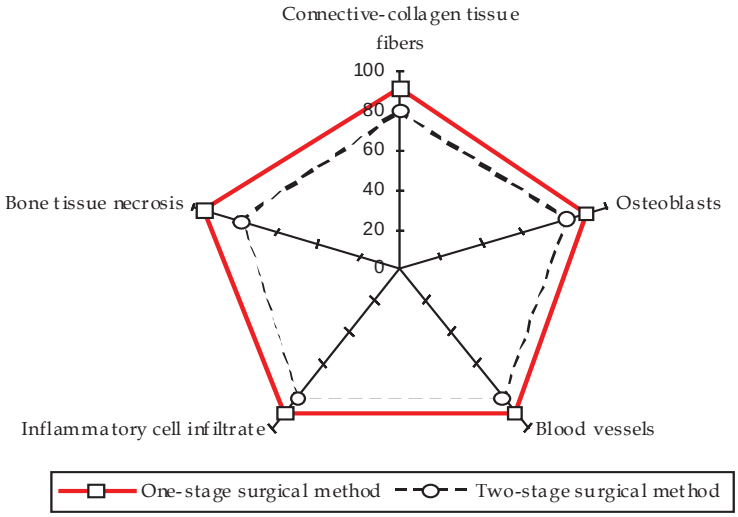

Fig. 4 – Histopathological characteristics of bone implant interface–collagen tissue, osteoblasts, blood vessels, inflammatory cell infiltrate, bone tissue necrosis (rank sum values).

amount of collagen tissue (Me1 < Me2), but there was no statistically significant differences between the methods.

Figure 4 presenting the rank sum values shows better results of one-stage method regarding all the examined characteristics.

Pathohistological findings

One-stage method

The analysis of bone-implant interface revealed increased amount of connective–collagen tissue fibers in all specimens (moderate in five and marked in four specimens), moderate increase in the number of osteoblasts (osteoblastic activity) was observed in two specimens (4 and 5). A marked neovascularization was found in two specimens (5 and 8) and moderate in one (4). Inflammation was absent in all specimens, while necrosis was found in only one specimen (5), (Figure 5).

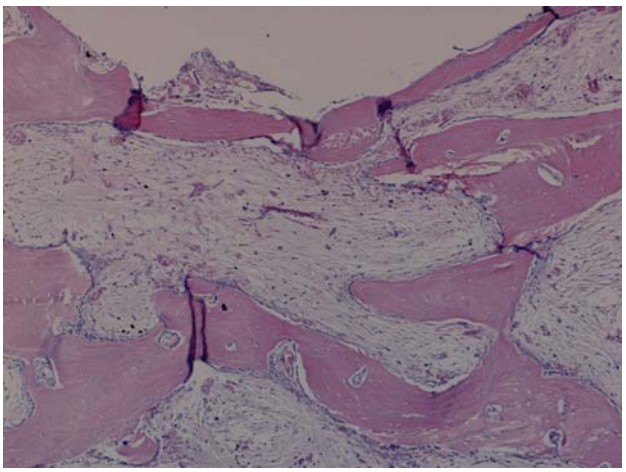

Fig. 5 – A marked amount of connective-collagen tissue fibers between bone lamellae with increased neovascularization (HE, × 40).

Two-stage method

The analysis of bone-implant interface revealed a marked increase in connective tissue fibers in five specimens (2–4, 7 and 9); in the specimen 3 moderate increase in osteoblasts was observed, as well as moderate vascular proliferation. A marked neovascularization was found in specimen 4. Inflammation and bone tissue necrosis were found in only one specimen (3), (Figure 6).

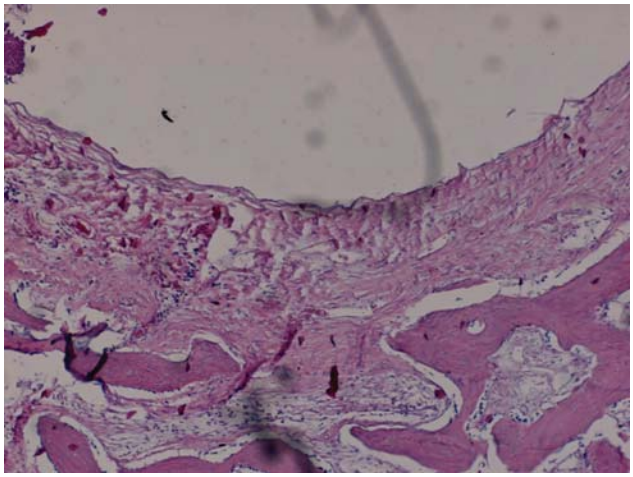

Fig. 6 – Loose subepithelial connective tissue-trabecular bone lattice with collagen tissue proliferation, marked neovascularization, poor osteoblastic activity, moderate inflammatory cell infiltrate and partial bone tissue necrosis (HE, × 40).

Analysis of bone tissue adjacent to the implant

The region of bone-tissue adjacent to the implant included the analyses of connective–collagen tisssue, osteoblasts, osteocytes and bone tissue necrosis (Table 4 and Figure 7).

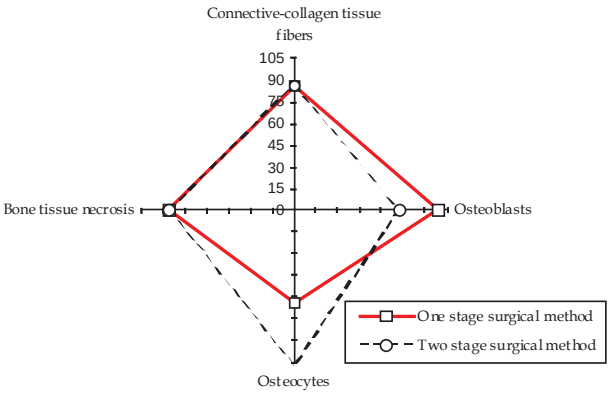

Fig. 7 – Histopathological characteristics of the bone tissue adjacent to the implant–collagen tissue, osteoblastic activity, osteocytes and bone tissue necrosis (rank sum values).

action and bone tissue necrosis (Me1 = Me2), while better results were achieved regarding the number of osteocytes by the two-stage surgical procedure (Me1 < Me2). The latter result was also statistically significant (Wa = 64.5 < 66, Wb = 106.5 > 105, $\alpha = 0.05$).

Figure 7 presenting the rank sum values shows better results regarding osteoblastic activity achieved by the one-stage method, while the results are identical regarding the amount of collagen tissue and bone necrosis.

Histopathological findings

One-stage method

A moderate increase in connective–collagen tissue fibers was found in only one specimen (2) where also no osteoblasts and osteocytes could be observed; a moderate increase in osteoblasts and osteocytes was found in three samples (3, 5 and 6); in regard of the number of osteocytes, a moderate increase was observed in specimens 7 and 9, and a

Table 4

Grading indices of histpathological characteristics of the bone tissue adjacent to the implant

Specimen number	Connective–collagen tissue fibers		Osteoblastic		Osteocytes		Bone tissue necrosis	
	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage	1-stage	2-stage
1	2	2	2	2	2	0	0	0
2	1	1	2	2	2	0	0	0
3	2	2	1	2	1	1	0	0
4	2	2	2	2	0	1	0	0
5	2	2	1	2	1	1	0	0
6	2	2	1	2	1	0	0	0
7	2	2	2	2	1	0	0	0
8	2	2	2	2	0	0	0	0
9	2	2	2	2	1	0	0	0
Median	2	2	2	2	1	0	0	0
Wilcoxon Wa Statistics for n1 = n2 = 9, $\alpha = 0.05$	Wa = 85.5	Wb = 85.5	Wa = 99	Wb = 72	Wa = 64.5	Wb = 106.5	Wa = 85.5	Wb = 85.5
	Upper and lower critical values Wa (66, 105)							

According to the results in Table 4 on the basis of descriptive statistics the results were identical regarding the median values of amount of collagen tissue, osteoblastic remarked increase in specimens 4 and 8. Necrotic bone tissue was not found in any of the analyzed specimens. In the specimen 1 bone structure was completely preserved with no

connective tissue fibers, but with no osteoblasts and osteocytes as well (Figure 8).

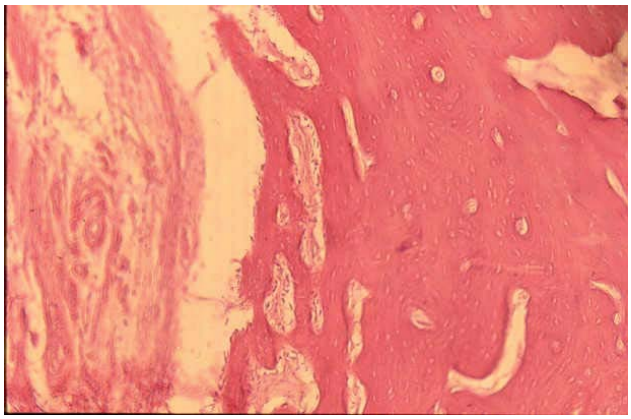

Fig. 8 – On the left: loose subepithelial connective tissue, underneath scattered interconnected bone trabeculae with osteoblastic reaction, collagen tissue proliferation between trabeculae. On the right: reserved lamellar bone tissue with osteocytes in lacunae (HE, × 40).

Two-stage method

A moderate increase in connective tissue fibers was found in only one specimen (2). No osteoblastic activity could be observed in all specimens. A moderate increase in osteocytes was observed in three specimens (3, 4 and 5) and a marked increase in the six remained specimens. Necrotic bone tissue was not found in any of the analyzed specimens, (Figure 9).

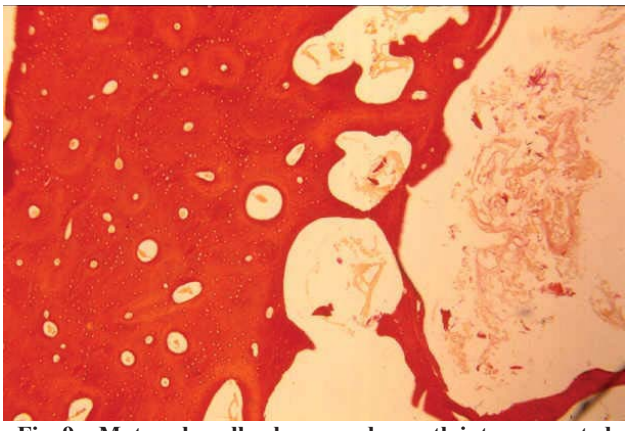

Fig. 9 – Mature lamellar bone, underneath interconnected bone trabeculae and intertrabecular collagen tissue proliferation (HE, × 40).

Discussion

Histopathological analyses that were performed in our study included three bone regions: bone-to-implant contact region, bone-implant interface and bone tissue adjacent to the implant.

In this study the histopathological findings of three distinct peri-implant bone regions were compared following dental implant placement in two different surgical methods: one-stage and two-stage method. Analysis was performed three months after the insertion when osseointegration was assumed to be achieved and the implants to be loaded.

Bone-to-implant contact region analysis revealed that connective–collagen tissue fibers were observed in all specimens with two-stage implants, which was not the case with one-stage implants. A marked proliferation of collagen tissue fibers was found in 7 specimens, but no osteoblastic activity was observed in that region, while necrosis was evident in all specimens. The median values of ostaoblastic activity and bone tissue necrosis were identical for both methods, but the rank sum values showed better results for one–stage procedure. These observations are not in agreement with the findings of Koch et al. [15] who investigated osseointegration of implants of different materials inserted in one- and two-stage surgical procedures in dogs. Healing modalities did not influence the rate of bone-to-implant contact between the implants, among which were the implants of titanium.

Similar results were obtained in the study of Gotfredsen et al. [16] on radiographic bone changes around submerged and non-submerged dental implants in beagle dogs. Namely, these authors performed histological evaluations of tissue reactions to unloaded submerged implants without reopening and unloaded non-submerged TPS implants in six monkeys. After 22 weeks of healing the results indicated that both groups had similar bone levels at the end of healing period and no differences were found in histological analysis of bone-to-implant contact regions between the implant types. They concluded that osseointegration could be established regardless the surgical approach.

Regarding the amount of collagen tissue as a matrix for mineralization and consequent osseointegration, our results are in agreement with the findings of Levy et al. [8] who analyzed osseointegration around porous coated root form implants placed in the canine model in one- and two-stage surgical method. After a 6-week healing, the absolute bone-to-implant contact was greater for submerged implants.

Investigating crestal bone changes around titanium submerged and non-submerged implants in canine mandibles histomorphometrically, Hermann et al. [11] concluded that bone changes were not dependent on surgical technique (one- and two-stage insertion) which is consistent with our results regarding the median values of osteoblastic activity, vascularization, inflammatory cell infiltrate and bone tissue necrosis.

There are two theories of the mechanism responsible for osteogenesis at implant interface in the literature. According to Davies et al. [17] there is no fibrillar material directly at the implant-bone interface. Bone-derived cells deposit calcified accretions to condition the implant surface prior bone formation, thus no collagen fibers directly interface with the implant. The second theory, based on the studies and investigations of Steflik et al. [18], suggests that an unmineralized collagen fiber matrix is deposited at the implant interface and is subsequently mineralized which is in agreement with our investigations showing collagen tissue fibers in the bone-implant interface in all the specimens indicating osteogenesis.

Surgical placement of endosteal implants elicits an osteogenic response largely driven by local factors. The initial healing response is independent on direct mechanical control because bone heals optimally in the absence of functional loading. The vascularly dependent ostegenic process can be easily disrupted by micromotion at a healing bone-implant interface. This is one of the main reasons for some surgeons to advocate two-stage implant placement. Following a maturation phase of about 1 week newly formed osteoid is primarily mineralized when osteoblasts deposit about 70% of the mineral found in mature vital bone. An adequate resistance to loading in humans is achieved in about 18 weeks, but there are no quantitative data [19]. In our experimental study osteoblastic activity was evident in all three bone regions, however there were no statistically significant differences. Regarding the results of rank sum values better results of one-stage implant insertion method were achieved, therefore it may be assumed that experimental model with more samples could provide more precise results.

The region of bone adjacent to the implant has been investigated in not so many studies. This region is considered a key zone for continued osseointegration of the implant. One of the studies investigating the bone tissue supporting an implant is the one conducted by Meenaghan et al. [20] who first described a triple layer of osseous cells in the remodeling process close to blade implant. They suggested that an outer dense cellular layer existed comprised of mesenchymal-like cells interfaced with the implant. A middle layer of highly vascularized osteogenic tissue existed to a third layer of osteoblasts associated with osteoid matrix and bone. Similar findings were reported by Steflik et al. [21] who observed osteoblastic activity in the zone adjacent to implant. In our studies a moderate osteoblastic activity was evident in one-stage implants (3 specimens), but it was not found in two-stage implants, suggesting the advantage of non-submerged implants. But, regarding the number of osteocytes, submerged implants showed better result that was statistically significant.

Conclusion

Within the limits of this animal study better results were achieved by the two–stage method in bone-to-implant contact region regarding amount of collagen tissue, while the results were identical regarding the osteoblastic activity and bone tissue necrosis. There was no difference between the methods in the bone-implant interface region regarding osteoblastic activity, blood vessels, inflammatory cell infiltrate and bone tissue necrosis when the median values were compared, while comparing the amount of collagen tissue two-stage method showed better results. In the bone tissue adjacent to the implant the results were identical regarding the median values of the amount of collagen tissue, osteoblastic reaction and bone tissue necrosis, while better results were achieved by the two-stage method regarding the number of osteocytes. Osseointegration could be established regardless surgical approach.

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REFERENCES

1. *LeGeros, RZ, Craig, RG.* Strategies to Affect Bone Remodeling: Osteointegration. J Bone Miner Res 1993; 8(S2): S583−96.
2. *Schroeder A, van der Zypen E, Stich H, Sutter F.* The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayedsurfaces. J Maxillofac Surg 1981; 9(1): 15−25.
3. *Heydenrijk K, Raghoebar GM, Meijer HJ, van der Reijden WA, van Winkelhoff AJ, Stegenga B.* Two-stage IMZ implants and ITI implants inserted in a single-stage procedure. A prospective comparative study. Clin Oral Implants Res 2002; 13(4): 371−80.
4. *Ericsson I, Nilner K, Klinge B, Glantz PO.* Radiographical and histological characteristics of submerged and nonsubmerged titanium implants. An experimental study in the Labrador dog. Clin Oral Implants Res 1996; 7(1): 20−6.
5. *Weber HP, Buser D, Donath K, Fiorellini JP, Doppalapudi V, Paquette DW,* et al. Comparison of healed tissues adjacent to submerged and non-submerged titanium implants. A histometric study in beagle dogs. Clin Oral Impantsl Res 1996:17(1): 11−9.
6. *Abrahamsson I, Berglundh T, Wennström J, Lindhe J.* The peri-implant hard and soft tissues at different implant systems. A comparative study in the dog. Clin Oral Implants Res 1996; 7(3): 212−9.
7. *Abrahamsson I, Berglundh T, Moon IS, Lindhe J.* Peri-implant tissues at submerged and non-submerged titanium implants. J Clin Periodontol 1999; 26(9): 600−7.
8. *Levy D, Deporter DA, Pilliar RM, Watson PA, Valiquette N.* Initial healing in the dog of submerged versus non-submerged porous-coated endosseous dental implants. Clin Oral Implants Res 1996; 7(2): 101−10.
9. *Fiorellini JP, Buser D, Paquette DW, Williams RC, Haghighi D, Weber HP.* Aradiographic evaluation of bone healing around submerged and non-submerged dental implants in beagle dogs. J Periodontol 1999; 70(3): 248−54.
10. *Moon IS, Berglundh T, Abrahamsson I, Linder E, Lindhe J.* The barrier between the keratinized mucosa and the dental implant. An experimental study in the dog. J Clin Periodontol 1999; 26(10): 658−63.
11. *Hermann JS, Buser D, Schenk RK, Higginbottom FL, Cochran DL.* Biologic width around titanium implants. A physiologically formed and stable dimension over time. Clin Oral Implants Res 2000; 11(1): 1−11.
12. *Berglundh T, Abrahamsson I, Welander M, Lang N, Lindhe J.* Morphogenesis of the peri-implant mucosa: an experimental study in dogs. Clin Oral Implants Res 2007; 18(1): 1−8.
13. *Abrahamsson I, Cardaropoli G.* Peri-implant hard and soft tissue integration to dental implants made of titanium and gold. Clin Oral Implants Res 2007; 18(3): 269−74.

14. *Stamatović N*. Comparative pathohistological analysis of the tissue around endosteal implants inserted in one- and two-stage method. [dissertation]. Belgrade: Military Medical Academy; 2005. (Serbian)
15. *Koch FP, Weng D, Krämer S, Biesterfeld S, Jahn-Eimermacher A, Wagner W*. Osseointegration of one-piece zirconia implants compared with a titanium implant of identical design: a histomorphometric study in the dog. Clin Oral Implants Res 2010; 21(3): 350−6.
16. *Gotfredsen K, Hjorting-Hansen E, Budtz-Jörgensen E*. Clinical and radiographic evaluation of submerged and nonsubmerged implants in monkeys. Int J Prosthodont 1990; 3(5): 463−9.
17. *Davies JE, Chernecky R, Lowenberg B, Shiga A*. Deposition and resorption of calcified matrix in vitro by rat bone marrow cells. Cells Mater 1991; 1: 3–15.
18. *Steflik DE, Corpe RS, Young TR, Sisk AL, Parr GR*. The biologic tissue responses to uncoated and coated implanted biomaterials. Adv Dent Res 1999; 13: 27−33.
19. *Micsh CE*. Contemporary Implant Dentistry. 2nd ed. St. Louis: Mosby Inc; 1999.
20. *Meenaghan MA, Natiella JR, Armitage JE, Wood RH*. Evaluation of the crypt surface adjacent to metal endosseous implants: an electron microscopic study in clinically successful implants. J Prosthet Dent 1974; 31(5): 574−81.
21. *Steflik DE, Parr GR, Sisk AL, Lake FT, Hanes PJ, Berkery DJ,* et al. Osteoblast activity at the dental implant-bone interface: transmission electron microscopic and high voltage electron microscopic observations. J Periodontol 1994; 65(5): 404−13.

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Study of the risk factors associated with the development of malocclusion

Istraživanje faktora rizika od razvoja nepravilnosti vilica i zuba

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Abstract

Background/Aim. Research of the most common causes of irregularities of jaws and teeth is designed in order to find the most efficient mode of their prevention. Frequency of orthodontic malformations (malocclusions) is as high as 60% to 80% in this region with an increasing tendency. Researching the risk factors for orofacial irregularities is designed with the purpose of creating a standardized methodology for risk evaluation, frequency and degree of orthodontic irregularities of face, jaws and teeth. The aim of the study was to identify and analyze the causal factors that lead to forming malocclusions in patients from the Province of Vojvodina and create a uniform methodology for epidemiological research. **Methods.** The research included 127 patients from the current casuistics of the Vojvodina Stomatology Clinic – Department for Jaw Orthopedics. Data for Questionnaire for Epidemiological Surveillance were obtained from medical records of patients, heteroanamnesis, objective findings, functional analysis of stomatognathic system, and additional diagnostic methods. **Results.** The average number of the risk factors was 2.59 per patient, of which 56% were morphological factors, and 44% functional. Acquired risk factors made up 61% of the total number, while congenital risk factors made up 39%, of which 15% were hereditary and 24% were non-hereditary. **Conclusion.** Implementing the Questionnaire for Epidemiological Surveillance, general distribution of anomalies could be presented by the Anomaly index (AI), which dictates the introduction of a standardized questionnaire for epidemiological screening, which would preclude ambiguousness and the differences between the epidemiological research data would be cut to the minimum.

Key words:
malocclusion; risk factors; epidemiologic methods.

Apstrakt

Uvod/Cilj. Istraživanja najčešćih uzroka nastanka poremećaja rasta i razvoja vilica imaju za cilj iznalaženje najefikasnijeg načina sprečavanja njihovog nastanka. Rasprostranjenost ortodontskih nepravilnosti (malokluzija, disgnatija) kreće se na našim prostorima između 60% i 80% sa tendencijom daljeg rasta. Istraživanje faktora rizika od razvoja orofacijalnih nepravilnosti ima za cilj izradu jedinstvene metodologije za procenu rizika, učestalosti i izraženosti ortodontskih nepravilnosti lica, vilica i zuba. Cilj studije bio je da se identifikuju i analiziraju uzroci malokluzije kod pacijenata sa područja Pokrajine Vojvodine i da se uspostavi uniformna metodologija za epidemiološko istraživanje. **Metode.** Istraživanjem je obuhvaćeno 127 pacijenata iz tekuće kazuistike Odeljenja za ortopediju vilica Klinike za stomatologiju Vojvodine. Podaci za Upitnik za epidemiološko praćenje su preuzimani iz kartona bolesnika, detaljne heteroanamneze, objektivnog nalaza, funkcionalne analize stomatognatnog sistema i dodatnih dijagnostičkih metoda. **Rezultati.** Prosečan broj štetnih činilaca iznosio je 2,59 po pacijentu, od čega su 56% predstavljali morfološki činioci, a 44% funkcionalni činioci rizika. Stečeni faktori rizika bili su zastupljeni kod 61% slučajeva ukupnih činilaca, a urođeni faktori rizika kod 39% slučajeva, od čega su 15% nasledni, a 24% nenasledni faktori rizika. **Zaključak.** Upotrebom Upitnika za epidemiološko praćenje opšta rasprostranjenost anomalija može se prikazati primenom indeksa anomalija (AI), koji podrazumeva uvođenje jedinstvenog anketnog lista za epidemiološki nadzor, čime se izbegava uopštenost, a razmimoilaženja u podacima dobijenim epidemiološkim istraživanjima svode na najmanju moguću meru.

Ključne reči:
malokluzija; faktori rizika; epidemiološki metodi.

Introduction

Orofacial irregularities, in addition to dental caries and periodontal desease, are becoming more present both in children and adults, and present a significant percentage of diseases of the orofacial region, and thus a special medical and socioeconomic problem.

Precise data on the prevalence of these irregularities do not exist, and in our region the percentage of these irregularities range between 60% and 80% and have a tendency of

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further growth. In modern societies the prevalence of malocclusions ranges between 40% and 80%, and in the Nordic countries 43% to 79%, with the need for treatment between 30% and 75% [1–4].

As it is impossible to include all the affected by treatment, the solution is in the prevention of developmental disorders of the orofacial structures through daily practice. Orthodontists, in addition to treatment, are dealing with epidemiology and prevention of orthodontic anomalies, but not enough, and there is poor cooperation with other preventive care providers.

According to the American epidemiological study in 1991 class II malocclusion is the most widespread orthodontic anomaly among the North American population. The results of this study showed that class II malocclusion prevalence declines with age. This irregularity is present 25%–30% in the mixed dentition, 20%–25% in early permanent dentition, while in adults its presence is reduced to 15%–20% [5]. Epidemiological surveys carried out in Western and Northern Europe demonstrate a similar prevalence of distal bite in European populations [6–8]. Caucausians in South Africa show a similar representation of class II as does the European population [9]. The prevalence of class II malocclusion was significantly lower in Arab (10%–15%) and Hispanic population (10%–15%), while it was least present in subjects of African-American origin (0%–2%) [10–15].

Upon formation of the National program of preventive dental care, based on the methodology of the World Health Organization (WHO), the study results showed that in our country at the age of 6 between 12% and 32% of children have an orthodontic anomaly, at the age of 12 between 36% and 64 %, and at the age of 15 38% to 60%. Since the WHO form is general, and does not include all the specific disorders of growth and development, in line with our previous findings, we can conclude that the occurrence of orthodontic anomalies in our country is even greater than this type of questionnaire can determine [16–18].

Etiological factors that may lead to irregularities in the face, jaws and teeth can be differently divided and classified, and depending on the author, there are different classifications. It is particularly important to note that there are a number of different etiological factors that may cause disruption of normal growth and development of the orofacial region. They may be biological, chemical, physical, mechanical, nutritional, genetic or psychosomatic.

In the first half of the 20th century, local or external causal factors, among which are functional, were considered the most important, because of the prevalent attitude that malocclusions are a modern civilization disease caused by inadequate function of the jaws in modern life conditions. In the second half of the last century heritage took over the role of the causal factor [19–22].

Today we can surely say the etiology of malocclusion is not simple, and that it is multifactorial and interdependent, which makes classification and systematization of malocclusion even more complex and difficult [23].

Class III malocclusion is widely associated with heredity, which is confirmed in some classic family studies [24]. However, a wide range of non-hereditary factors may contribute to the development of Class III malocclusion - enlarged tonsils, inability to breath through the nose, hormonal disorders, habit of mandibular protrusion, trauma and disease, early loss of first permanent molars and incorrect growth direction of permanent incisors or premature loss of deciduous incisors [25–27].

Open bite has a multifactorial etiology also. It appears that no single factor could be the most frequent and the biggest culprit for the occurrence of open bite. Factor classification is divided into two groups – general and local [28].

Profitt et al. [26] have divided all etiological factors important for the development of diseases and irregularities in the stomatognathic system, into developmental, functional, and traumatic. The most important cause for the malocclusion development is disproportion between the size of the jaws and teeth, and jaws themselves. The reasons for this can be found in a large population migration and mixing of different ethnic groups and nations that is characterized by genetic diversity [29]. These changes influence inherited variations in morphology and function. Inheritance cannot be viewed as a separate causal factor of malocclusions, but only in interaction with other etiological factors. Malocclusions should be observed primarily as a developmental problem, and each developmental phase has its own prevention and activities. Prevention in the infant period referres to introduction of regular diet, prevention of the formation of bad habits and functions as well as their importance for future relationship of the jaws and teeth health [28–30].

The aim of this study was to identify and analize causal factors that lead to forming malocclusions in patients from the Province of Vojvodina and create a uniform methodology for epidemiological research.

Methods

The study included 127 patients of the Department of Orthodontics, Dental Clinic of Vojvodina, examined in the period of one calendar year (January-December). A sample comprised of ethnicaly mixed population from the area of the Province of Vojvodina. Data were abstracted from medical records of patients. Exclusion criteria from the study included: congenital cleft lip, jaw and/or palate, which because of the complexity of the anomalies and the factors that contribute to its development, represent a speciffic set of problems, and thus may blurr the results.

The average age of the patients was 10.70 years, with minimum of 6.68 and maximum of 25.84 years.

In diagnostic examination of the patients in order to better understand the causes of anomalies and causal treatment, we unflaggingly took data through heteroanamnesis, objective findings, functional analysis of stomatognathic system, and additional diagnostic methods specific for orthodontics as a branch of dentistry (dentoalveolar occlusal findings, gnatometric and radiological analysis). Data obtained by systematic examination were entered in table and processed statistically to obtain mean values, standard deviations and percentages. The data provided good insight into

the present population problems, revealing the extent eventual treatment. In order to have clearer insight into the necessary measures, we used statistical list of attending second and fifth grade of elementary school (Table 1).

mouth breathing: no (0), yes (1); orthodontic irregularities in the family: no (0), yes (1)].

Test evaluation was performed regarding: addition of points that shows a total degree of risk: low-risk – less than

Table 1

Statistical list for the second and fifth grade elementary school patients

(No)	Name and surname	With anomaly	With teeth extractions	Cured		Functional disorders	Morphological findings	Therapy		
				OH	6 ± 6			IN	FOR	IN OBS
1										
2										
3										

According to the existing rules and forms, medical check-ups in the second and fifth grade of elementary school are used to implement interceptive and therapeutic measures of already developed anomalies.

Groups of factors are conditionally divided into "morphological" and "functional", in order to diviside the factors that are predominantly of orthodontic anomalies in the narrow sense, and those who represent dysfunction of adjacent soft tissue, which leads to the creation of conditions for the occurrence of anomalies or support the development of already developed anomalies. Of the morphological irregularities the most frequent were: crowding (primary, secondary and combined); spacing (primary and secondary); the loss of permanent teeth in mixed dentition period; congenital disorders of number of teeth (hypodontia and hyperdontia); impaction and retention of teeth; fibrous and proccident frenulum; trauma of facial bones, jaws and teeth and recurrence of similar anomalies in the family – heritability.

Among the oral functions, the standard methods were used to investigate: the position and tone of the tongue; chronic enlargement of the tonsils and adenoid vegetation; chronic and frequent ear, nose and/or throat inflammations; habitual mouth breathing; deviation of the mandible; harmful habits and parafunctions as a separate subgroup (pacifier used over 3 years of age, nail biting, lip biting, finger and tongue sucking, etc.).

In particular, we observed skeletal interjaw relations in sagittal (class), vertical and transversal dimension as an indicator of mutual developmental dis/harmony and position of the upper and lower jaw.

The risk factors registered this way, were statistically analyzed to have better insight into their distribution and relationships.

To assess the risk of orthodontic irregularities we used well-known methods [diseases in pregnancy: no (0), yes (1), rubella (3); delivery: easy/cesarean (0), difficult (1), forceps (2); breastfeeding: 7–9 months (0), 3–6 months (1), less than 2 months (2); supplementary feeding and feeding: spoon (0), bottle(1); type of pacifier: anatomical (0), others (1); bottle position during feeding: regular (0), irregular (1); bad habits: no (0), yes (2); sleeping position: regular (0), irregular (1); hands position during sleep (in relation to the jaws): regular (0), irregular (1); heading height: regular (0), irregular (1);

1/3 of the total possible number of points; medium risk – up to 2/3 of the total possible number of points; high risk – over 2/3 of the total possible number of points.

Maximal number of points was 18: low risk (0–6); medium risk (7–12); high risk (13–18).

Results

Analyzing the total number of potentially harmful factors per patient, we found the following (Figure 1): the number of factors identified ranged between 0 and 6; the most common factor was 3 per patient (29.92%); the rarest registered were the factors 0 (1.57%) and 6 (0.7%); one identified factor had 19.68% of the patients; two factors were registered in 26.77% of the patients; four factors were registered in 14.17% of the patients; five factors were found in 7.1% of the patients.

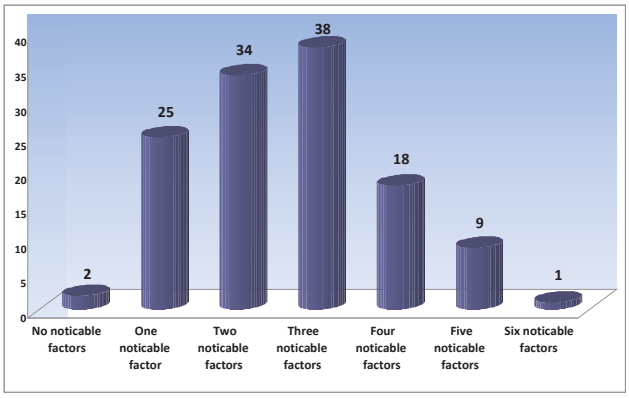

Fig. 1 – The number of patients with risk factors.

An average number of factors per patient was 2.59 with the standard deviation of 1.24. This means that factors distribution follows the Gaussian curve, and includes about two thirds of the sample, which in our case amounts to about 70%.

Of 127 patients, skeletal relationships of the class I was found in 55 (43.3%) patients, class II 57 (44.88%), class III of 15 (11.81%) (Table 2).

Among the classes we found the equal average number of risk factors: class I: 2.47 (SD = 1.23); class II: 2.75 (SD = 1.24); class III: 2.46, (SD = 1.3).

Table 2

The frequency of skeletal class in the sample

Skeletal class	Number of patients
Class I	55
Class II	57
Class III	15
Total	127

Analysis by groups of factors showed that the "morphological" factors made 56% of the identified risk factors and "functional" 44% (Table 3).

Table 3

The presence of basic groups of irregularities

Irregularities	n (%)
Morphological	185 (56)
Functional	145 (44)
Total	330 (100)

Among the morphologically conditioned irregularities the most present were: crowding – 21.5% (16.66% primary, secondary 1.81%, and 3.03% combined); earlier occurrence of similar anomalies in the family – heritage 14.84%; congenital irregularities in number of the teeth – 4.84% (3.63% hypodontia, hyperdontia 1.21%); fibrous and proccident frenulum – 3.93% (frenulum labii superioris proccidens 3.33% and frenulum linguae proccidens 0.6%); trauma of jaw and teeth (3.03%); primary spacing of dental arches (2.72%); impaction and retention of teeth (1.81%); extraction of permanent teeth in mixed dentition period (0.3%) (Table 4).

Table 4

"Morphological" – conditioned irregularities in the sample

Irregularities	n (%)
Primary crowding	55 (29.73)
Similar cases in the family	49 (26.48)
Hypodontio	12 (6.48)
Frenulum labii superioris proccidens	11 (5.94)
Combined crowding	10(5.4)
Acquired spacing	10 (5.4)
Dental trauma	10 (5.4)
Primary spacing	9 (4.86)
Secondary crowding	6 (3.24)
Impactions	6 (3.24)
Hyperdontio	4 (2.16)
Frenulum linguae proccidens	2 (1.08)
Loss of permanent teeth	1 (0.54)
Total	185 (100)

Among the functional irregularities, it was found that bad habits and parafunctions represented 28% of the factors, and improper function about 72% (Table 5).

Table 5

The ratio of irregular functions and bad habits

Irregularities	n (%)
Irregular functions	105 (72)
Bad habits	40 (28)
Total	145 (100)

Irregular functions were present in the following relationship: deviation of the mandible in 45% of the cases; malposition of the tongue and tongue hypotonia in 24% of the cases; enlarged tonsils and adenoid vegetation caused disorder in 20% of the cases; habitual mouth breathing in 6% of the subjects; chronic inflammation of the ear, nose and throat with obstruction of the upper respiratory tract as a harmful factor in 5% of the cases (Table 6).

Table 6

Iregular functions

Bad function	n (%)
Premature contact- habitual bite	47 (44.76)
Irregular position and function of tongue	25 (23.8)
Enlarged tonsils and adenoid vegetations	21 (20)
Chronic otitis, rhinnitis or laryngitis	6 (5.7)
Mouth breathing	6 (5.7)
Total	105 (100)

Harmful habits and parafunctions were further divided into the following most commonly recorded factors: the use of pacifiers (dummies) over the age of 3 in 13% of this group of factors; biting nails in 60% of the cases; biting lips in 15% of the cases; finger sucking in 12% of cases (Table 7).

Table 7

Bad habits

Bad habit	n (%)
Nail biting	24 (60)
Lip biting	6 (15)
Pacifier used over 3 years	5 (12.5)
Finger sucking	5 (12.5)
Total	40 (100)

If the factors were to be viewed as acquired or congenital, the relationship was as followed: acquired factors made 61% of a total member of the factors and congenital factors 39% of the cases.

A more detailed insight into the hereditary factors revealed: discrepancy between a required and the available space for teeth in 20% of the cases (17% primary crowding, and 3% primary spacing); irregularities of the number of the teeth were present in 4% of the cases (3% hypodontia, 1% hyperdontia); recurrence of similar anomalies in the family in 15% (Table 8).

Table 8

Congenital factors in the total sample of subjects

Factors	%
Acquired	61
Inherited factors	
primary crowding	17
similar cases in the family	15
hypodontio	3
primary spacing	3
hyperdontio	1
Total	100

The WHO methodology suggests only recording of the presence or absence of anomalies, which by itself does not provide accurate information. That is why the implementa-

tion of epidemiological studies to determine the presence and types of anomalies in a particular population is important, but also for monitoring the trends of their reduction conducted after the preventive, interceptive and therapeutic measures. Therefore, we suggest the Questionnaire for Epidemiological Surveillance (Table 9).

Researches of experts from multiple disciplines would create the conditions to penetrate deeper into the etiology of orthodontic problems, and to analyze their impact on the general and local development. Based on study of genetic and epigenetic factors, finding of the dominant risk factors and by analyzing the obtained results, it would be possible to make the most appropriate model of primary and secondary preventive care, which would be based on the latest scientific achievements in the field of causes of tooth and jaw irregularities.

Table 9

A sample of Questionnaire for Epidemiological Surveillance

EPIDEMIOLOGICAL QUESTIONNAIRE			1	2	3	4	5	6	.....
SAGGITAL	Occlusal class								
	Crowding in frontal or posterior section								
	Overjet								
	Anterior crossbite								
VERTICAL	Deep overbite over 3mm								
	Anterior openbite	Up to 3 mm							
		Over 3 mm							
	Lateral openbite	Up to 3 mm							
		Over 3 mm							
TRANSVERSAL	Crossbite up to 1/2 of the tooth width								
	Crossbite up to the tooth width								
	Midline discrepancy								
OTHER ANOMALIES	Incisal spacing								
	Rotated lateral teeth								
	Inclined lateral teeth								
	Diasthema	upper frontal teeth							
		lower frontal teeth							
		sum [mm]							
	hypodontia of frontal teeth								
	hypodontia of lateral teeth								
	hyperdontia								
	dental impactions								
	persistency								
	clefts								
	syndroma								
FUNCTIONAL FINDINGS	Incompetent lips	primary							
		secondary							
	dyslalia								
	swallowing								
	chewing								
	forced mandibular posture								
OTHER FINDINGS	dental hygiene								
	number of present teeth								
	premature loss of teeth								
	number of filled teeth								
	number of carious teeth								
	gingival and parodontal disease								
	Hypoplasio	localised							
		generalised							
	Orthodontic treatment	none							
		cured							
		in progress							
		given up							

The general distribution of anomalies can be displayed by the index of anomaly (AI).

This would help avoid the generality and reduce the measures of differences in data obtained by epidemiological studies.

Discussion

Not many research papers have been published on this topic, so we suggested a uniform methodology, in order to obtain the results that are comparable within our region.

For each patient data were collected widely in an otherwise difficult separation of numerous factors that sometimes independently, often in combination, lead to disruption in the growth and development of the stomatognathic system. The combination and multifactoriality as well as overlapping effects of individual factors, are the cause of severe orientation and selection of the right causal treatment of malocclusions. This is more helpful for understanding possible causes of malocclusions, as well as developmental disorders, and step towards preventing these disorders wherever possible.

If we observe the results of analysis of saggital interjaw skeletal relations considering numerous factors that may contribute to the development of irregularities of teeth and jaws, a striking approximation of the average and standard deviation of the factors in all three classes is noticeable, so that we can state that there is no difference between the classes, depending on the number and types of factors that might be contributable.

The opinion that orthodontic anomalies depend on multifactorial etiology is supported by finding that most patients had registered 2, 3 and 4 etiological factors, which was also a finding in an El-Mangoury and Mostafa's research [9]. The fact that there was a minimum number of patients with no registered potentially contributing risk factors can be explained by the eventual presence of some other, less frequent or more hidden factors than those tested routinely, such as inaccurate data obtained from a patient or guardian while taking history, or of the diagnosis failure therapist.

The phenomenon that a certain number of cases had 5 or 6 etiological factors can be partly attributed to the presence of overlapping between the present acting factors in some areas. Such conclusion is in accordance with other authors [27]. Since it is artificial, no matter how detailed it is, replications are possible.

Observing the basic representation of a group of risk factors we found that the "morphological" factors represent 56% of identified risk factors, and "functional" 44%. Similar findings have been presented in other research studies [31–33]. This shows that almost half of the disorders could be influenced on at early age, either by allowing proper function of any preventive eradication of bad habits or reeducated functions of the soft tissue of stomatognathic system. The importance of this fact is even greater if we deeper analyze the functional factors – 72% of these factors in the sample were bad habits (use of a pacifier, nail biting, lip biting, finger and tongue sucking), that can be eradicated by certain measures, after which a significant reduction of expression could be expected, or abnormalities would not develop at all. Sousa et al. [34] confirmed a similar percentage in their study – 65% of functional factors were bad habits. Incorrect functions (deviation of the mandible, abnormal position and hypotonia of the tongue, habitual mouth breathing, etc.), representing 28% of functional depending factors, could be removed by timely interception, functions reeducation, as well as collaboration with specialists in other fields of medicine, that would lead to irregularities of significantly lesser extent. It is therefore important to raise the level of consciousness of wider population and health care providers in other branches of medicine, to achieve a better acquaintance, primarily with health and socioeconomic consequences, of relatively easy to recognize factors and measures of preventive action.

Among the "morphological" factors we can differentiate hereditary congenital and non-hereditary disorders which themselves are an orthodontic anomaly (primary crowding, anomalies of tooth shape and number, undesirable frenulum fixation, diasthema, etc.), and acquired postnatal disorders, which affect the formation or disorders or complicate the existing ones, and they could be promptly removed (secondary crowding, loss of permanent teeth in a period of growth and development, trauma, etc.). The same divison of morphological factors has been revealed by Mossey [19]. Acquired disorders could be the onset prevented or mitigated by improving the knowledge of population about the means of early detection, and easy removal of identifiable risk factors [35, 36]. That would greatly influence spread reduction, or in some cases, where disorders are only a part of the mosaic of factors, the reduction in expression and severity of anomalies. In individuals with an emphasized anomaly it could significantly alleviate the quality of life.

The fact that additionally supports previous statements is that if we examine the relationship between the incidence of hereditary and acquired factors in the sample, the possibility of good prevention is even more obvious: acquired factors represented 61% of the total factors, while genetic factors were present in 39% of the cases.

For risk assesment, it is better to use a standard, known method, and for the epidemiological research, we suggest our Epidemiological Questionnaire (in the results section).

Conclusion

We suggest the use of Questionnaire for Epidemiological Surveillance. As the anomalies are often combined, it is difficult to classify and display a unique index. Evaluation of morphological and functional findings should be registered for each symptom of irregularities in a premade survey list and should always be done in the same sequence in sagittal, vertical and transversal, and other irregularities. In that way errors would be reduced to a minimum extent. Epidemiological surveillance should be performed: for deciduous dentition, at the age of 5 to 6; mixed dentition at the end of the mixed dentition period in the 12th year of life; permanent teeth in high school children at the age of 18, at the end of the period of active growth and development.

REFERENCES

1. *Evensen JP, Ogaard B.* Are malocclusions more prevalent and severe now? A comparative study of medieval skulls from Norway. Am J Orthod 2007; 131(6): 710−6.
2. *Espeland LV, Ivarsson K, Stenvik A.* A new Norwegian index of orthodontic treatment need related to orthodontic concern among 11-year-olds and their parents. Community Dent Oral Epidemiol 1992; 20(5): 274−9.
3. *Thilander B, Myrberg N.* The prevalence of malocclusions in Swedish schoolchildren. Scand J Dent Res 1973; 81(19: 12−21.
4. *Ingervall B, Seeman L, Thilander B.* Frequency of malocclusion and need of orthodontic treatment in 10-year old children in Gothenburg. Swed Dent J 1972; 65(1): 7−21.
5. *Brunelle JA, Bhat M, Lipton JA.* Prevalence and distribution of seleted occlusal sharacteristics in U.S. population, 1988-1991. J Dent Res 1996; 75 Spec No: 706−13.
6. *Laine T, Hausen H.* Occlusal anomalies in Finnish students related to age, sex, absent permanent teeth and orthodontic tretmant. Eur J Orthod 1983; 5(2): 125−31.
7. *Thilander B, Myberg N.* The prevalence of malocclusion in Swedish schoolchildern. Scand J Dent Res 1973; 81(1): 12−21.
8. *Helm S.* Orthodontic treatment priorities in the Danish Child. Dental Health Services. Communitiy Dent Oral Epidemiol 1982; 10(5): 260−3.
9. *El-Mangoury NH, Mostafa YA.* Epidemiologic panorama of malocclusion. Angle Orthod 1990; 60(3): 207−14.
10. *Gardiner JH.* An ortodontic survey of Libyan school-shilden. Br J Orthod 1982; 9(1): 59−61.
11. *Al-Emran S, Wisth PJ, Boe OE.* Prevalence of malocclusion and need for orthodontic treatment in Saudi Arabia. Community Dent Oral Epidemiol 1990; 18(5): 253−5.
12. *de-Muñiz BR.* Epidemiology of malocclusion in Argentine childen. Community Dent Oral Epidemiol 1986; 14(4): 221−4.
13. *Palomino H.* The Aymara of western Bolivia: III. Occlusion, pathology, and characteristics of dentition. J Dent Res 1978; 57(3): 459−67.
14. *Ng'ang'a PM, Ohito F, Ogaard B, Valdehaug J.* The prevalence of malocclusion in 13- to 15-year-old children in Nairobi, Kenya. Acta Odontol Scand 1996; 54(2): 126−30.
15. *Barmes DE.* Dental and nutritional surveys of primitive peoples in the Pacific Islands. Aust Dent J 1967; 12(5): 442−54.
16. *Petrović Dj, Horvat-Banić S.* Electromyographic activity of the temporal and masseter muscles at different occlusal positions. Med Pregl 2007; 60(3−4): 134−9. (Serbian)
17. *Corrucini RS.* How anthropology informs the orthodontics diagnosis of malocclusion's causes. Lewinston, NY: Edwin Mellen Press; 1999.
18. *Marković M, Meškov M, Milosavljević R.* Aetiology of class II division 2 Malocclusion. Bilten UOJ 1994; 27: 5−12. (Serbian)
19. *Mossey PA.* The hereditability of malocclusion: part 2. The influence of genetics in malocclusion. Br J Orthod 1999; 26(3): 195−203.
20. *Varrela J, Varrela TM.* Dental studies of a Finnish skeletal material: a paleopathologic approach. Tandlaegebladet 1991; 96(7): 283−90.
21. *Gottlieb I, Gottlieb O.* Mandibular protrusion in edentulous patients. Oral Surg Oral Med Oral Pathol 1954; 7(8): 813−21.
22. *Gold JK.* A new approach to the treatment of mandibular prognathism. Am J Orthod 1949; 35(12): 893−912, illust.
23. *Pascoe JJ, Hayward JR, Costich ER.* Mandibular prognathism: its etiology and a classification. J Oral Surg Anesth Hosp Dent Serv 1960; 18: 21−4.
24. *Rubbrecht O.* A study of the heredity of the anomalies of the jaws. Am J Orthod Oral Surg 1939; 25: 751−9.
25. *Stojanović L.* Etiological aspects of anterior open bite. Med Pregl 2007; 60(3−4): 151−5. (Serbian)
26. *Profitt WR, Fields HW Jr, Sarver DM.* Contemporary. Orthodontics. 3th ed. St. Louis, Mo: Mosby Elsevier; 2000.
27. *Luz CL, Garib DG, Arouca R.* Association between breastfeeding duration and mandibular retrusion: A cross- sectional study of children in the mixed dentition. Am J Orthod Dentofacial Orthop 2006; 130(4): 531−4.
28. *Góis EG, Ribeiro-Júnior HC, Vale MP, Paiva SM, Serra-Negra JM, Ramos-Jorge ML,* et al. Influence of nonnutritive sucking habits, breathing pattern and adenoid size on the development of malocclusion. Angle Orthod 2008; 78(4): 647−54.
29. *Petrović Dj, Vukić-Ćulafić B, Ivić S.* The influence of the round muscle of the lips on the position of incisors. Glasnik Antropološkog Društva Srbije 2008; 43: 168−76. (Serbian)
30. *Marić D, Čupić S, Vukić-Ćulafić B.* Oral hygiene in children treated orthodonticly. SGS 1982; 5: 349−52. (Serbian)
31. *Peres KG, Barros AJD, Peres MA, Victora CG.* Effects of breastfeeding and sucking habits on malocclusion in a birth cohort study. Rev Saúde Pública 2007; 41(3): 343−50.
32. *Viggiano D, Fasano D, Monaco G, Strohmenger L.* Breast feeding, bottle feeding and non-nutritive sucking; effects on occlusion in deciduous dentition. Arch Dis Child 2004; 89(12): 1121−3.
33. *Berjis N, Sonbolestan M, Jabbarifar E, Farokh KH.* Evaluation the effects of adenoidal hypertrophy on occlusion and indexes of face and jaw in 6–12 years old children. Shiraz E-Medical J 2005; 6(3−4): 1−6.
34. *Sousa RLS, Lima RB, Florencio Filho C, Lima KC, Diogenes AMN.* Prevalence and risk factors of anterior open bite in the complete deciduous dentition in prescholars' children who live in the city of Natal/RN. Rev Dental Press Ortodon Ortop Facial 2007; 12(2): 129–38.
35. *Jena AK, Duggal R, Mathur VP, Parkash H.* Class - III malocclusion: Genetics or environment? A twins study. J Indian Soc Pedod Prev Dent 2005; 2(1)3: 27−30.
36. *Xue F, Wong RW, Rabie AB.* Genes, genetics, and Class III malocclusion. Orthod Craniofac Res 2010; 13(2): 69−74.

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ORIGINAL ARTICLE

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Could application of epinephrine improve hemostatic efficacy of hemoclips for bleeding peptic ulcers? A prospective randomized study

Da li se primenom epinefrina može poboljšati hemostatska efikasnost hemoklipseva kod krvarenja iz peptičkih ulkusa? Prospektivna randomizovana studija

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Abstract

Background/Aim. Bleeding from peptic ulcers can be effectively and safely treated with endoscopic hemoclips therapy. However, due to certain limiting factors of hemoclips, application of combination with another endoscopic method may give better results. The aim of this study was to examine the efficacy and safety of endoscopic hemoclips therapy and to evaluate potential benefits of this therapy combined with epinephrine in the treatment of bleeding peptic ulcers. **Methods.** This prospective randomized study included 70 patients with bleeding gastric or duodenal ulcer. In 34 of the patients endoscopic hemoclips therapy was applied (group I), and in 36 of them a combined therapy of hemoclips and epinephrine (group II). **Results.** Initial hemostasis was achieved in most patients treated with endoscopic hemoclips therapy (94.1%) as well as in the patients treated with combination therapy (97.2%). After initial hemostasis achieved rebleeding occurred in 3 (9.3%) patients treated with hemoclips and in 2 (5.7%) patients treated with combination therapy, but this difference was not statistically significant ($p > 0.05$). The difference in the achieved final hemostasis between the group I (91.1%) and the group II (94.4%) was not statistically significant. Also, the differences between the two groups of patients in the need for blood transfusions, length of hospital stay, need for surgery and mortality were not statistically significant ($p > 0.05$). **Conclusion.** Endoscopic hemoclips therapy is effective and safe in treatment of bleeding peptic ulcers. Combination therapy of hemoclips and epinephrine has no advantage over hemoclips monotherapy.

Key words:
peptic ulcer hemorrhage; endoscopy, gastrointestinal; hemostasis, endoscopic; epinephrine; treatment outcome.

Apstrakt

Uvod/Cilj. Krvarenje iz peptičkog ulkusa može se efikasno i bezbedno lečiti endoskopskom terapijom hemoklipsevima. Ipak, zbog određenih ograničavajućih faktora primene hemoklipseva možda bi kombinacija hemoklipseva sa drugom endoskopskom metodom dala bolje rezultate. Cilj rada bio je da se ispita efikasnost i bezbednost endoskopske terapije hemoklipsevima i procene eventualne prednosti ove terapije kombinovane sa epinefrinom u lečenju krvarenja iz peptičkog ulkusa. **Metode.** Prospektivnom, randomizovanom studijom obuhvaćeno je 70 bolesnika sa krvarenjem iz ulkusa želuca ili duodenuma. Kod 34 bolesnika primenjena je endoskopska terapija hemoklipsevima (grupa I), dok je kod 36 bolesnika primenjena kombinovana terapija hemoklipsevima i epinefrinom (grupa II). **Rezultati.** Inicijalna hemostaza postignuta je kod većine bolesnika lečenih endoskopskom terapijom hemoklipsevima (94.1%) i bolesnika lečenih kombinovanom terapijom (97.2%). Nakon postignute inicijalne hemostaze do ponovnog krvarenja došlo je kod tri (9.3%) bolesnika tretirana hemoklipsevima i kod dva (5.7%) bolesnika lečenih kombinovanom terapijom, ali razlika u učešću rekrvarenja nije bila statistički značajna ($p > 0,05$). Razlika u postignutoj konačnoj hemostazi između grupe I (91.1%) i grupe II bolesnika (94.4%) takođe nije bila statistički značajna, kao ni razlika između ispitivanih grupa bolesnika u potrebama za transfuzijama krvi, dužini hospitalizacije, potrebama za hirurškom intervencijom i u mortalitetu ($p > 0,05$). **Zaključak.** Endoskopska terapija hemoklispevima je efikasna i bezbedna za lečenje krvarenja iz peptičkog ulkusa. Kombinovana terapija hemoklipsevima i epinefrinom nema prednosti nad monoterapijom hemoklipsevima.

Ključne reči:
peptički ulkus, krvarenje; endoskopija, gastrointestinalna; hemostaza, endoskopska; adrenalin; lečenje, ishod.

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Introduction

Endoscopic injection therapy is the most common method for treating bleeding peptic ulcer. Sanation of bleeding is achieved to a high degree using this technique, but the recurrence of bleeding is present in 6%–36% of cases [1–3]. If rebleeding as an important factor of mortality as reduced, one might expect a potential reduction in mortality. It can be difficult to achieve permanent thrombosis of blood vessels in cases of bleeding in jet injections of epinephrine. The application of hemoclips would theoretically be the optimum method in case of visible blood vessels. Hemostasis can be definite if the vessel is adequately ligated by clips [4, 5].

Recent data indicate that the application of endoscopic clips is efficient and safe, than sclerosing and thermal methods, with minor damage to the surrounding tissue, and with a smaller share of rebleeding. Therefore, their application is being increased [6]. However, there are certain limitations in hemoclips applications. In fact, it is difficult to place clips in case of massive bleeding that covers the field of visualization of the lesion or bleeding in case of tangential approach to the lesion. Hemoclips, in combination with other methods such as epinephrine injection, would perhaps show better results. There are scarce literature records on the results of comparison of efficacy of endoscopic hemoclips therapy and a combined hemoclips therapy with epinephrine [7].

The aim of this study was to examine the efficacy and safety of endoscopic hemoclips therapy and evaluate potential benefits of hemoclips therapy combined with epinephrine in treatment of bleeding peptic ulcers.

Methods

This prospective randomized study included 70 patients (47 men and 23 women) hospitalized in the Department of Gastroenterology and Surgery because of acute bleeding from peptic ulcer (hematemesis and/or melena).

All the patients were asked to consent for upper gastrointestinal endoscopy, which was done mostly within 24 h of hospitalization. Endoscopy was performed under local oropharyngeal anesthesia with lidocaine. Bleeding from gastric or duodenal ulcer was diagnosed after endoscopy. The patients were divided into two groups depending on the applied type of endoscopic hemostasis: group I that included 34 patients (23 males and 11 females), average age 60.3 ± 11.19 years (from 29 to 83 years) with endoscopic therapy using hemoclips, and group II with 36 patients (24 males and 12 females), average age 62.3 ± 12.21 years (from 30 to 85 years) with combined therapy of hemoclips and epinephrine.

The examined groups of patients did not differ significantly according to sex, age, use of nonsteroidal anti-inflammatory drugs (NSAIDs), participation of comorbidity, hemoglobin initial values, the localization of the ulcer, ulcer size and affiliation to a certain Forrest group of bleeding ulcer (Table 1). Anamnestic data were obtained by using NSAIDs, in 61.7% patients of the group I and in 61.1% of the group II. NSAIDs diclofenac, nimesulide, indomethacin antiagregation agents, acetylsalicylic acid and less clopidogrel were used. From other diseases, which were present in 50% of the patients of the group I and 52.7% of the group II, were the conditions after myocardial infarction and stent, angina pectoris, hypertension, conditions after stroke, chronic renal failure and chronic obstructive pulmonary disease. The average size of bleeding ulcers in the patients of the group I was 12.6 ± 3.98 mm (7–19 mm) and of the group II 13.1 ± 4.12 mm (8–20 mm). Forrest's classification [8] was used for the classification of bleeding peptic ulcers. The study involved patients with active bleeding in the jet (Forrest Ia), flowing venous bleeding (Forrest Ib) and visible denuded blood vessel without active bleeding (Forrest IIa). The most common, in both groups of patients, was flowing venous bleeding from the ulcer (Forrest Ib).

Table 1

Clinical and endoscopic characteristics of the study groups of patients

Parameter	Hemoclips therapy (n = 34)	Combination therapy (n = 36)	*p*
Male/female, n	23/11	24/12	0.867
Age (years), $\bar{x}$ ± SD	60.3 ± 11.19	62.3 ± 12.21	0.704
NSAID use, n (%)	21 (61.7)	22 (61.1)	0.849
Comorbid diseases, n (%)	17 (50)	19 (52.7)	0.994
Hb level (g/dL), $\bar{x}$ ± SD	7.2 ± 1.4	6.9 ± 1.3	0.914
Stomach ulcer, n (%)	16 (47)	17 (47.2)	0.821
Duodenal ulcer, n (%)	18 (52.9)	19 (52.7)	0.821
Ulcer size (mm), $\bar{x}$ ± SD	12.6 ± 3.98	13.1 ± 4.12	0.508
Forrest group Ia, n (%)	2 (5.9)	5 (13.9)	0.429
Forrest group Ib, n (%)	30 (88.2)	28 (77.8)	0.399
Forrest group IIa, n (%)	2 (5.9)	3 (8.3)	1.000

$\bar{x}$ – mean value; SD – standard deviation; NSAID – nonsteroidal antiflamatory drug; Hb – hemoglobin
Forrest Ia – active bleeding in the jet; Forrest Ib – flowing venous bleeding; Forrest IIa – visible denuded blood vessels without active bleeding

The criteria for excluding patients from the study were: pregnancy, multiple, other, non-ulcus causes of bleeding in the upper gastrointestinal tract, gastric malignancy, the coexistence of acute severe disease, such as septic shock, acute cerebrovascular incident, acute surgical abdomen, systemic condition of increased susceptibility to bleeding – low blood platelet counts below 50,000/mm^3, prolonged prothrombin time with international normalized ratio (INR) over 2 and treatment with anticoagulant drugs.

Hemoclips endoscopic therapy was applied in the group I of the patients with bleeding from peptic ulcer. We used EZ clips of stainless steel material and standard ones curved at the top at the angle of 135° (HX-610-135) and a longer curved at the angle of 90° (HX-610-090L). Clips were placed with the applicator through the accessible channel of 2.8 mm in diameter of standard endoscopes.

The combined hemoclips and epinephrine therapy was applied in the group II of the patients with bleeding from peptic ulcer. The same clips were used as in the group I. Epinephrine diluted in physiological salt solution of 1:10,000 was applied. A standard injection needle was used for application, 23 gauge size, the length of the needles 4 mm. Basically, the first placed were clips (in 25 patients or 69.4%) in a visible bleeding place, immediately after diluted epinephrine was applied, fractionally from 0.5–2 mL in four quadrants around the ulcer at the distance of 2–3 mm and only the bleeding site. The injection was repeated in each quadrant until the mucosa turned to whitish color and until hemostasis was established. In case of more massive bleeding (in 11 patients or 30.5%), when it was not possible to see clearly the bleeding site, the diluted epinephrine was applied immediately after the clip to achieve the reduction of bleeding.

Initial hemostasis was considered successful in case of endoscopically verified cessation of bleeding for at least 5 min after the first endoscopic treatment [7]. In case of continued bleeding, despite endoscopic treatment, urgent surgery is recommended.

Vital functions were monitored in all the patients, while control of bleeding was performed using nasogastric tube drainage. The treatment included partial parenteral nutrition and the use of proton pump inhibitors (PPIs) in the form of intravenous bolus of 80 mg and then infusion of 8 mg/h during 72 h, followed by oral application of PPIs. In case of hemoglobin (Hb) fall below 7 g/dL, a continuation of hematemesis and/or melena, increased heart rate to more than 100 betas per minute or systolic blood pressure fall to less than 100 mmHg, red blood cell transfusions were prescribed.

By recurrent bleeding, we meant the appearance of new hematemesis and/or melena after initial hemostasis, the emergency of fresh blood nasogastric tube aspiration and increasing heart rate over 100 beats per minute with systolic pressure drop over 30 mmHg, as well as a new decline in Hb of at least 2g/dL [7]. Reapplied upper gastrointestinal endoscopy and secondary endoscopic clips hemostasis or clips with epinephrine was used in case of suspicion of recurrent bleeding. Urgent surgery was recommended in case of secondary endoscopic hemostasis failure.

By final hemostasis, we meant the absence of recurrent bleeding 7 days after initial or secondary endoscopic hemostasis. All the patients were clinically followed for 8 weeks after initial endoscopy.

Statistical analysis was performed using descriptive statistics such as measures of central tendency [average value, ($\bar{x}$)] and measures of dispersion [standard deviation (SD)]. Statistical analysis methods that were used in assessing the significance of difference were Student's *t*-test, Mantel-Haenszel's χ^2 test with Yates's correction and Fisher's exact probability test of the null hypothesis. A statistically significant differences between individual characteristics were considered for $p < 0.05$.

Results

Initial hemostasis was achieved in most of the patients treated with endoscopic hemoclips therapy (32/34 or 94.1%) and the patients treated with combination hemoclips and epinephrine therapy (35/36 or 97.2%). The difference in the success of initial hemostasis was not statistically significant ($p > 0.05$).

Rebleeding occurred in 3 (9.3%) patients treated with endoscopic clips therapy and 2 (5.7%) patients treated with combination therapy of clips and epinephrine, after achieving initial hemostasis. However, the difference in the participation of rebleeding was not statistically significant ($p > 0.05$). Also, the difference in the achieved final hemostasis between the patients treated with clips (91.1%) and the patients treated with combination therapy (94.4%) was not statistically significant ($p > 0.05$).

The amount of epinephrine administered in the patients treated with combination therapy of clips and epinephrine, was 11.2 ± 4.56 mL (4–20 mL). The average number of placed clips was slightly higher in the patients treated with clips monotherapy (1.7 ± 0.49, the minimum of 1 and the maximum of 4 clips on bleeding ulcer) than in the patients treated with combination therapy (1.5 ± 0.68, at least 1 clip at the maximum of 3 clips on bleeding ulcer), but the difference was not statistically significant ($p > 0.05$). We had difficulties with placement the clips in 3 patients of the group I and 5 patients of the group II, with ulcer on the posterior wall of the stomach, as well as with ulcer in the duodenal bulb of fibrotic characteristics. Five clips fell off in 3 patients of the group I and 8 clips in 5 patients of the group II. However, replacement of clips was successful. Unsuccessfully placed clips were not counted in the number of clips used to control bleeding.

There were no complications after clips treatment and after combined therapy of clips and epinephrine in the examined groups of patients.

An average of 4.3 ± 1.58 units of blood (from 0 to 9 units) was prescribed to the patients treated with hemoclips. An average of 3.9 ± 1.82 units of blood (from 0 to 8 units) was prescribed to patients whose ulcers were treated with combination therapy. The difference in the number of units of given red blood cell concentrate between the two groups of patients was not statistically significant ($p > 0.05$).

The average length of hospitalization of the group I patients was 9.2 ± 4.54 days (from 4 to 16 days), and of the group II patients 8.9 ± 4.32 days (from 3 to 14 days). The difference in the length of hospitalization was not statistically significant ($p > 0.05$).

One (2.9%) patient, in the group I patients was operated on for recurrent ulcer bleeding on the posterior wall of subcardial area of gastric corpus and secondary failure of endoscopic hemostasis. In the remaining 2 of 3 patients, treated with hemoclips and who had recurrent bleeding ulcers, a

successful secondary endoscopic hemostasis was achieved. In the group treated with combination therapy of epinephrine and hemoclips, 1 (2.7%) patient underwent surgery with recurrent bleeding from duodenal ulcer after the failure of secondary endoscopic hemostasis. A successful secondary endoscopic hemostasis was achieved in another patient with rebleeding from ulcer. The difference in the number of operated patients between the groups was not statistically significant ($p > 0.05$).

Death occurred in 2 (5.8%) patients, in the group treated with hemoclips, with bleeding from ulcer of the stomach corpus and duodenal ulcer after failure of initial hemostasis, continuing bleeding and development of irreversible hemorrhagic shock. Death occurred in 1 (2.7%) patient, in the group treated with combination therapy, with severe bleeding from the stomach ulcer and the inability of establishing initial hemostasis. The presence of significant comorbidity such as angina pectoris, chronic obstructive pulmonary disease or renal insufficiency was established in cases of death in both groups of patients. The difference in mortality was not statistically significant between the two groups of patients (Table 2).

There was no significant difference in the two examined groups of patients, regarding the success of initial hemostasis achieved to a high percentage in both groups. The difference in the participation of rebleeding was not statistically significant. Also, there was no statistically significant difference in achieving final hemostasis in bleeding ulcers, need for blood transfusions, length of hospitalization, needs for surgery and mortality.

According to the results of most studies, combined hemoclips therapy with other agents, such as epinephrine or absolute alcohol has an advantage over epinephrine monotherapy or absolute alcohol in terms of rebleeding, need for surgery and in some cases in terms of mortality [15, 16]. However, there have been a few studies dealing with comparative analysis of hemoclips efficiency and hemoclips combined with other hemostatic agent. One study investigated the clips effectiveness with clips combined with absolute alcohol [17], while the other study investigated the clips effectiveness with clips combined with epinephrine [18]. The results of both studies, similar to our results, showed no statistically significant difference in the participation of rebleeding, need for surgery and mortality. Therefore, the combined treatment is not favored in comparison with clips monotherapy.

Table 2

Treatment results in the compared groups of patients

Parameter	Hemoclips therapy (n = 34)	Combination therapy (n = 36)	*p*
Initial hemostasis, n (%)	32 (94.1)	35 (97.2)	0.608
Recurrent bleeding, n (%)	3/32 (9.3)	2/35 (5.7)	0.663
Final hemostasis, n (%)	31 (91.1)	34 (94.4)	0.669
Amount of epinephrine administered (mL), $\bar{x} \pm SD$	0	11.2 ± 4.56	
Number of hemoclips applied, $\bar{x} \pm SD$	1.7 ± 0.49	1.5 ± 0.68	1.397
Transfusion requirement (number of units), $\bar{x} \pm SD$	4.3 ± 1.58	3.9 ± 1.82	0.969
Hospital stay (days), $\bar{x} \pm SD$	9.2 ± 4.54	8.9 ± 4.32	0.278
Surgery, n (%)	1 (2.9)	1 (2.7)	1.000
Mortality, n (%)	2 (5.8)	1 (2.7)	0.608

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

Discussion

Epinephrine monotherapy is considered to be suboptimal due to the high participation of rebleeding [9–11]. Thermal methods such as electrocoagulation, photocoagulation and argon plasma coagulation were effective but the potential damage to tissues is possible [1, 12, 13].

Hemoclips are new therapeutic alternatives with the effect identical to surgical ligature. The advantages of hemoclips application are direct application on the blood vessel, no damage to bleeding lesion, no limit to the number of applied clips, interventions can be repeated and in case of failure of hemostasis they are a useful marker for radiologists in the application of transcatheter embolization [14]. Due to the existence of some limiting factors of hemoclips applications such as chronic ulcer with a fibrous base, precise identification of bleeding lesion and lesion location, we wanted to examine whether application of combined methods of hemoclips and epinephrine could give better results.

In the group of patients treated with combination therapy, clips were first placed in a visible bleeding place and immediately after it diluted epinephrine was applied. In case of more massive bleeding, when it was not possible to see clearly the bleeding site, diluted epinephrine was first applied to achieve the reduction of bleeding and immediately after that clips were used. Theoretically, local injection therapy applied before placing clips can lead to tissue edema, and there can appear difficulties in placing a blood vessel clip. Gevers et al. [19] first applied epinephrine in all patients and then clips. They showed that the combined therapy of epinephrine and clips was even less efficient in comparison with the epinephrine monotherapy. The study of Lo et al. [4] had an identical approach to epinephrine and hemoclips, as in this study injection. They compared combined hemoclips and epinephrine therapy with epinephrine monotherapy, showing the superiority of combination therapy.

According to some authors, a higher volume of epinephrine, such as 13–20 mL or even 35–45 mL, can be significantly more effective in achieving hemostasis, reducing the need for urgent surgery, reducing hospitalization time and mortality. The higher amount of applied epinephrine proved to be safe, not leading to the occurrence of cardiac arrhythmias [20, 21].

The size of ulcers and the presence of active bleeding from ulcers, as well as Forrest I bleeding lesions, could have an impact on the success of endoscopic hemostasis. It is difficult to achieve hemostasis with larger ulcers over 20 mm [22]. There was no statistically significant difference in the size of ulcer, and all ulcers were less than 20 mm in both groups of patients. Also, there was no statistically significant difference between the two groups of patients regarding the Forrest group of bleeding ulcers. Therefore, it can be concluded that for the success of endoscopic hemostasis, the kind of therapy (hemoclips or hemoclips with epinephrine) was responsible first, while the size of ulcer bleeding and the Forrest group had no effect in our examines.

Addition of epinephrine to clips had no significant effect on reducing the average number of clips placed on bleeding ulcers in our patients. Specifically, the average number of placed clips was ranked slightly higher in the patients treated with monotherapy clips than in the patients treated with combination therapy, but the difference was not statistically significant. We had difficulties in placing clips in ulcers of fibrous characteristics and the ulcers localized subcardially on the back wall of the stomach. Five clips fell off in 3 patients of the group I and 8 clips in 5 patients of the group II. However, replacement of clips was successful. Similar data were presented by Lo et al. [4] who had failed at placing 6 clips in 4 patients. According to some authors [14, 17], implementation of a transparent cylinder on top of an endoscope could help or the use of endoscopes with optics side in cases of difficult access to a lesion such as subcardial region.

We prescribed PPIs to all the patients parenterally within 72 h and then orally, before endoscopic intervention and/or immediately after the initial endoscopic hemostasis was achieved. It was proven that a significant suppression of acid secretion with PPIs improves the success of endoscopic hemostasis while the benefit is missing when applying H2 receptor antagonists. Achieving pH values above 6 in the stomach, under the influence of IPPs makes stabilization of blood clot possible [23–25].

Complications after endoscopic clips therapy and epinephrine injection therapy are rare. We have to be cautious with deeper ulcerations due to the possibility of perforation in applying clips. Also, it is possible to cause formation of active bleeding in the lesions that are not actively bleeding, such as a visible vessel or clot in the ulcer [14]. Epinephrine is considered to be the safest of injectable agents that are used in repair of bleeding from peptic ulcers. The possibility of cardiac arrhythmia is described, but as a very rare complication [2]. There were no complications following endoscopic application of clips and application of clips with epinephrine in the studied patients.

Conclusion

This study showed that endoscopic hemoclip therapy is efficient and safe in treatment of bleeding peptic ulcers. Combination therapy of hemoclips and epinephrine has no advantage over hemoclips monotherapy. Therefore, the combination therapy is not currently recommended until obtaining the eventual more relevant data from larger studies.

REFERENCES

1. *DiMaio CJ, Stevens PD*. Nonvariceal upper gastrointestinal bleeding. Gastrointest Endosc Clin N Am 2007; 17(2): 253−72, v.
2. *Park WG, Yeh RW, Triadafilopoulos G*. Injection therapies for nonvariceal bleeding disorders of the GI tract. Gastrointest Endosc 2007; 66(2): 343−54.
3. *Hsu PI, Lin XZ, Chan SH, Lin CY, Chang TT, Shin JS*, et al. Bleeding peptic ulcer: risk factors for rebleeding and sequential change in endoscopic findings. Gut 1994; 35(6): 746−9.
4. *Lo CC, Hsu PI, Lo GH, Lin CK, Chan HH, Tsai WL*, et al. Comparison of hemostatic efficacy for epinephrine injection alone and injection combined with hemoclip therapy in treating high-risk bleeding ulcers. Gastrointest Endosc 2006; 63(6): 767−73.
5. *Hepworth CC, Kadirkamannathan SS, Gong F, Swain CP*. A randomized controlled comparison of injection, thermal and mechanical endoscopic methods of hemostasis on mesenteric vessels. Gut 1998; 42(4): 462−9.
6. *Cipolletta L, Bianco MA, Marmo R, Rotondano G, Piscopo R, Vingiani AM*, et al. Endoclips versus heater probe in preventing early recurrent bleeding from peptic ulcer: a prospective and randomized trial. Gastrointest Endosc 2001; 53(2): 147−51.
7. *Chou YC, Hsu PI, Lai KH, Lo CC, Chan HH, Lin CP*, et al. A prospective, randomized trial of endoscopic hemoclip placement and distilled water injection for treatment of high-risk bleeding ulcers. Gastrointest Endosc 2003; 57(3): 324−8.
8. *Laine L, Peterson WL*. Bleeding peptic ulcer. N Engl J Med 1994; 331(11): 717–27.
9. *Barkun AN, Bardou M, Kuipers EJ, Sung J, Hunt RH, Martel M,* et.al. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. Ann Intern Med 2010; 152(2): 101−13.
10. *Laine L, McQuaid KR*. Endoscopic therapy for bleeding ulcers: an evidence-based approach based on meta-analyses of randomized controlled trials. Clin Gastroenterol Hepatol 2009; 7(1): 33−47.
11. *Vergara M, Calvet X, Gisbert JP*. Epinephrine injection versus epinephrine injection and a second endoscopic method in high risk bleeding ulcers. Cochrane Database Syst Rev 2007; (2): CD005584.
12. *Sung JJ, Tsoi KK, Lai LH, Wu JC, Lau JY*. Endoscopic clipping versus injection and thermo-coagulation in the treatment of non-variceal upper gastrointestinal bleeding: a meta analysis. Gut 2007; 56(10): 1364−73.
13. *Ferguson CB, Mitchell RM*. Nonvariceal upper gastrointestinal bleeding: standard and new treatment. Gastroenterol Clin North Am 2005; 34(4): 607−21.
14. *Cipolletta L, Rotondano G, Bianco MA, Piscopo* R. Mechanical modalities of endoscopic therapy: clips, loops, and beyond. Tech Gastrointest Endosc 2005; 7: 132-8.

15. *Calvet X, Vergara M, Brullet E, Gisbert JP, Campo R.* Addition of a second endoscopic treatment following epinephrine injection improves outcome in high-risk bleeding ulcers. Gastroenterology 2004; 126(2): 441−50.
16. *Barkun AN, Martel M, Toubouti Y, Rahme E, Bardou M.* Endoscopic hemostasis in peptic ulcer bleeding for patients with high-risk lesions: a series of meta-analyses. Gastrointest Endosc 2009; 69(4): 786−99.
17. *Shimoda R, Iwakiri R, Sakata H, Ogata S, Kikkawa A, Ootani H*, et al. Evaluation of endoscopic hemostasis with metallic hemoclips for bleeding gastric ulcer: comparison with endoscopic injection of absolute ethanol in a prospective, randomized study. Am J Gastroenterol 2003; 98(10): 2198−202.
18. *Chung IK, Ham JS, Kim HS, Park SH, Lee MH, Kim SJ.* Comparison of the hemostatic efficacy of the endoscopic hemoclip method with hypertonic saline-epinephrine injection and a combination of the two for the management of bleeding peptic ulcers. Gastrointest Endosc 1999; 49(1): 13−8.
19. *Gevers AM, De Goede E, Simoens M, Hiele M, Rutgeerts P.* A randomized trial comparing injection therapy with hemoclip and with injection combined with hemoclip for bleeding ulcers. Gastrointest Endosc 2002; 55(4): 466-9.
20. *Lin HJ, Hsieh YH, Tseng GY, Perng CL, Chang FY, Lee SD.* A prospective, randomized trial of large- versus small-volume endoscopic injection of epinephrine for peptic ulcer bleeding. Gastrointest Endosc 2002; 55(6): 615−9.
21. *Park CH, Lee SJ, Park JH, Lee WS, JooYE, Kim HS*, et al. Optimal injection volume of epinephrine for endoscopic prevention of recurrent peptic ulcer bleeding. Gastrointest Endosc 2004; 60(6): 875−80.
22. *Park CH, Joo YE, Kim SH, Choi SK, Rew JS, Kim SJ.* A prospective, randomized trial comparing mechanical methods of hemostasis plus epinephrine injection to epinephrine injection alone for bleeding peptic ulcer. Gastrointest Endosc 2004; 60(2): 173−9.
23. *Bardou M, Toubouti Y, Benhaberou-Brun D, Rahme E, Barkun AN.* Meta-analysis: proton-pump inhibition in high-risk patients with acute peptic ulcer bleeding. Aliment Pharmacol Ther 2005; 21(6): 677−86.
24. *Leontiadis GI, Sharma VK, Howden CW.* Proton pump inhibitor therapy for peptic ulcer bleeding: Cochrane collaboration meta-analysis of randomized controlled trials. Mayo Clin Proc 2007; 82(3): 286−96.
25. *Andriulli A, Loperfido S, Focareta R, Leo P, Fornari F, Garripoli A,* et al. High-versus low-dose proton pump inhibitors after endoscopic hemostasis in patients with peptic ulcer bleeding: A multicentre, randomized study. Am J Gastroenterol 2008; 103(12): 3011−8.

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The variable Jung as a predictor of mortality in patients with pulmonary edema

Varijabla Jung kao prediktor mortaliteta kod bolesnika sa plućnim edemom

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Abstract

Background/Aim. In our Intensive Coronary Care Unit (CCU) a specific scoring system named the AMIS_NS was developed both for prediction of mortality in patients with acute myocardial infarction and for evaluation of the quality of work. One of the most important variables of the AMIS_NS system is the variable Jung which stands for the interrelationship unified mortality predictors. The variable includes all the values of systolic blood pressure, heart rate and age, without limiting values for any of these. The cut-off value is 2.08. The patients with the lower variable value account for a significantly higher mortality. Data on the actual infarction are not necessitated now for this variable. The aim of this study was to assess the significance of the variable Jung in non-infarction patients with acute pulmonary edema. **Methods.** In a 24-month period out of 2,223 patients there were 1,087 and 1,136 patients with and without acute myocardial infarction, respectively. There was the subgroup without myocardial infarction of 312 (84.1%) patients admitted with the diagnosis of pulmonary edema. The subgroup with myocardial infarction consisted of 59 (15.9%) patients who were admitted for acute myocardial infarction and pulmonary edema which developed immediately after admission or during hospitalization in the CCU. For all the patients a uniform questionnaire was fulfilled on admission. Data were put into the personal computer. The variable "Jung" was used: (systolic bloog pressure/heart rate × age) × 100. **Results.** Regarding sex, there was no difference in mortality, so that males and females were regarded as a whole. Previous myocardial infarction was equally registered in both groups. The investigated persons had less percent of mortality and a significantly higher systemic pressure as well as higher value of the variable Jung. There was no statistically significant difference in the heart rate between the two groups. In both groups of deceased patients the variable Jung (1.5 *vs* 1.6) was significantly lower in respect to the survived patients (2.3 *vs* 2.1). **Conclusion.** The variable Jung is simple, highly reliable and can absolutely be used as a significant indicator of clinical status also in non-infarction patients with the acute pulmonary edema, no matter if it is caused by acute myocardial infarction or not.

Key words:
pulmonary edema; mortality; heart rate; age groups; blood presure; prognosis.

Apstrakt

Uvod/Cilj. U koronarnoj jedinici Instituta za kardiovaskularne bolesti urađen je sopstveni sistem ocenjivanja nazvan AMIS_NS, za predviđanje mortaliteta bolesnika sa akutnim infarktom miokarda i procenu kvaliteteta rada. Taj sistem je primenljiv u svim koronarnim jedinicama. Jedna od najvažnijih varijabli AMIS_NS sistema je varijabla Jung koja predstavlja međuodnos sistemskog sistolnog krvnog pritiska i proizvoda srčane frekvence i životnog doba. Granična vrednost varijable Jung je 2.08. Bolesnici sa nižom vrednošću varijable imaju značajno viši mortalitet. Za ovu varijablu nisu nužni podaci o aktuelnom infarktu. Cilj rada bio je da se proveri značaj varijable Jung kod neinfarktnih bolesnika sa akutnim plućnim edemom. **Metode.** U periodu ispitivanja od 24 meseca, od ukupno 2 223 bolesnika bilo je 1 087 bolesnika sa, a 1 136 bez akutnog infarkta miokarda. Grupu ispitanika činilo je 312 (84.1%) bolesnika sa dijagnozom plućnog edema na prijemu. Kontrolnu grupu predstavljalo je 59 (15.9%) bolesnika sa akutnim infarktom i plućnim edemom koji se razvio neposredno po prijemu ili tokom hospitalizacije u koronarnoj jedinici. Za sve bolesnike pri prijemu popunjavao se jednoobrazni upitnik. Podaci su se unosili u personalni računar. Varijabla Jung je izračunavana prema formuli: (sistolni krvni pritisak/srčana frekvencija × životno doba) × 100. **Rezultati.** U odnosu na pol, nije bilo razlike u mortalitetu tako da su muškarci i žene bili posmatrani kao celina. Pret-

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hodni infarkt miokarda podjednako je bio zastupljen u obe grupe. Ispitanici su imali manji mortalitet i značajno viši sistemski krvni pritisak, kao i veću vrednost varijable Jung. Nije bilo statistički značajne razlike u srčanoj frekvenciji između dve grupe. U obe grupe kod umrlih bolesnika varijabla Jung (1.5 *vs* 1.6) bila je značajno niža nego kod preživelih (2.3 *vs* 2.1). **Zaključak.** Varijabla Jung se, apsolutno, može primeniti kao značajan prediktor mortaliteta kod bolesnika bez akutnog infarkta miokarda koji imaju plućni edem.

Ključne reči:
pluća, edem; mortalitet; srce, frekvencija; životno doba, grupe; krvni pritisak; prognoza.

Introduction

Out of the necessity for accurate evaluation of the patient's current clinical state, necessary diagnostic procedures, rational treatment, assessment of the final outcome and financial costs – numerous mathematically derived scoring systems have been developed worldwide.

Practically, so far all scoring systems refer to the intensive care units (ICUs), and not to the coronary care units (CCUs). Literature indicates that there were attempts of applying various scoring systems also in CCUs for patients with acute myocardial infarction (AMI).

The most famous ICU scoring systems were: APACHE III and SAPS II. Comparison of these two scoring systems (APACHE III *vs* SAPSII) [1] shows that their characteristics were: specificity 75.9% *vs* 72.2%; sensitivity – 39% vs.75.9% and total prediction – 84.0% *vs* 75.4%, respectively. Area under the ROC curve for SAPS II was 0.908 [2]. There was no data about the Hosmer and Lemeshow goodnes off-life test. They proved their applicability, but they were very complicated and unpractical.

Precisely, because of the fact that there is no specific score system for CCUs, a unique scoring system was created in the CCUs of the Institute of Cardiovascular Disease of Vojvodina in Sremska Kamenica, Serbia. The scoring system was developed on 505 patients with AMI. It was used for the evaluation of outcomes in patients with AMI immediately after the admission to the CCU. We named this scoring system the AMIS_NS score – acronym for **a**cute **m**yocardial **i**nfarction **s**core_**N**ovi **S**ad. All mathematical models in creation of AMIS_NS have specificity of over 95%, sensitivity of over 40% and overall accurate prediction of over 90%. These mathematical results show that the presented models rank in the top of prediction models. The area under the ROC curve was 0.85. This scoring system is simple, but highly reliable from the aspect of mathematics and statistics (Hosmer and Lemeshow goodnes off-life test = 0.78) [3].

The first use of the mathematical models AMIS_NS score gave only relatively acceptable results. The number of patients with the lower score coefficient was noticed, who according to the mathematical model, were supposed to survive but they did not. The sensitivity and specificity were low. Separate analysis of the deceased patients' records showed certain patterns among them. All the patients were elder, with low systemic blood pressure (SBP) (but not less than 80 mmHg), and higher heart rate (HR) (but not over 140/min). We found data in the literature that confirm a correlation between lower blood pressure (< 120 mm Hg) and higher heart rate (> or = 90/min) which appear to be predictors of mortality not only in cardiac patients, but also in non-cardiac patients [4–6]. A combination of heart rate, blood pressure and age was not found as a single variable. We combined these variables (SBP, age and heart rate) together to form a single variable. It includes all the values of age, heart rate and SBP. We named the new variable "the variable Jung" [3].

Univariate and multifactorial analysis proved the variable Jung to be an important predictor of mortality in the AMIS_NS score system with the referential value of 2.08 for patients with AMI. It also gives the best proportion between sensitivity and specificity for mortality prediction. Therefore, it is one of the most significant variables of the AMIS_NS scoring system.

$$\text{The Variable Jung} = \frac{\text{Systolic systemic pressure}}{\text{Heart rate} \times \text{Age}} \times 100$$

The lower value of the variable, the greater mortality risk, while the higher value means higher survival rate. Logically, the variable as a predictor of mortality is not enough by itself. It is necessary to combine it with other risk factors.

The use of this variable significantly improves the possibility of predicting mortality with the increase of sensitivity, specificity and surface under the ROC curve [3].

The aim of this paper was to examine the applicability and significance of the variable Jung in the prediction of mortality among the patients who were hospitalized for the life-threatening condition – acute pulmonary edema whether or not they had AMI, as well as to prove its applicability for patients without AMI, based on sensitivity, specificity and the surface under the ROC curve.

Methods

In a 24-month period out of 2,223 admitted patients hospitalized at the CCU, 1,087 (48%) patients were treated for AMI. There were 1,136 (52%) non-infarction patients.

Out of 1,087 (48.9%) patients with AMI, 59 (5.43%) patients also had pulmonary edema.

Out of 1,136 patients without myocardial infarction 312 (27.5%) were diagnosed with acute pulmonary edema.

The target group of 371 patients with pulmonary edema were divided into two subgroups. The subgroup I without AMI consisted of 312 (84.1%) non-infarction patients with the diagnosis of pulmonary edema. The subgroup II with AMI consisted of 59 (15.9%) patients who were admitted for the AMI, and the pulmonary edema which developed immediately after the admission or during the hospitalization at the CCU. Clinical characteristics of study participants are shown in Table 1.

As measures of central tendency the arithmetic mean was calculated as well as the standard deviation and the range of dispersion of parameters (measurements). The relations were analyzed by the suitable parametric and non-parametric tests. In order to find cut-point values, and sensitivity and specificity, ROC analyses were performed.

The basis and data processing were done using the EPI 5 and SPSS statistical program.

Results

The subgroup of patients without AMI included 312 patients with the confirmed pulmonary edema. Ischemic edema was the most frequent one (43.0%), followed by edema as a part of hypertensive crisis (38.2%), dilatated cardiomyopathy (8.5%), valvular diseases (6.9%) and edema with undefined etiology in (3.4%).

The subgroup of patients with AMI contained all edemas that were solely of ischemic origin.

Previous myocardial infarction was equally represented in both groups. In the group without AMI it was 106 (37.2%) and in the group with AMI 20 (37.3%).

The main characteristics of patients and components of the variable Jung in all groups and in cohorts with pulmonary edema with and without AMI, are shown in Table 1.

In all the patients without AMI, and in those with AMI there was no difference in age between the survivors and deceased patients ($p = 0.746$).

There was no statistically significant difference in age in deceased patients between two subgroups without AMI, and with AMI (70.15 years *vs* 67.28 years, respectively; $p = 0.267$). In the group without AMI, according to age, deceased patients were significantly older (70.3 years *vs* 67.1 years; $p = 0.013$). In the group with AMI the deceased patients were older (67.28 years *vs* 66.23 years; $p = 0.623$), but the difference was not significant.

In deceased patients according gender there was no difference in age in the group with edema ($p = 0.08$) without AMI ($p = 0.209$) and with AMI ($p = 0.209$).

In all the patients without AMI and with AMI, admission SBP was significantly lower in deceased patients ($p < 0.001$).

The subgroup without AMI had a statistically significantly higher SBP than the patients from the group with AMI (161.6 mmHg *vs* 140.4 mmHg; $p = 0.001$). In both subgroups the admission SBP in deceased patients was significantly lower than in the survivors ($p = 0.0001$ and $p = 0.00245$, respectively).

In all the patients without AMI and with AMI, the heart rate is significantly lower in deceased patients ($p = 0.004$).

There was no significant difference in heart rate on admission in deceased patients between the two subgroups (97.89 beat/min *vs* 104.54 beat/min; $p = 0.370$). In the group without AMI of heart rate deceased patients was significantly lower ($p = 0.037$). In the group with AMI it was not statistically different ($p = 0.0817$).

The value of the variable Jung in all the patients without AMI, and with AMI was statistically significantly ($p < 0.001$) higher in the survived patients than in the deceased patients (2.28 *vs* 1.53, respectively).

Table 1

The main characteristics of the patients and the components of the variable Jung in all groups and in cohorts with pulmonary edema with and without acute myocardial infarction (AMI)

Parameters	All the patients			The patients with pulmonary edema and without AMI			The patients with pulmonary edema and AMI		
	Alive n = 290	Died n = 81	*p*	Alive n = 256	Died n = 56	*p*	Alive n = 34	Died n = 25	*p*
Age (years), $\bar{x} \pm SD$	67 ± 9	69 ± 9	0.057	67 ± 9	70 ± 8	0.013	66 ± 9	67 ± 10	ns
Female, n (%)	152 (52.4)	35 (43.2)	ns	140 (54.7)	27 (48.2)	ns	12 (35.3)	8 (32.0)	ns
Male, n (%)	138 (47.6)	46 (56.8)		116 (45.3)	29 (51.8)		22 (64.7)	17 (68/0)	
Systolic arterial pressure at admission (mmHg) $\bar{x} \pm SD$	160 ± 45	102 ± 63	< 0.001	162 ± 45	100 ± 70	< 0.001	140 ± 43	106 ± 49	0.001
Heart rate (beats/minute) $\bar{x} \pm SD$	111 ± 25	100 ± 37	0.004	112 ± 26	98 ± 41	0.004	108 ± 23	104 ± 27	ns
Jung's variable, $\bar{x} \pm SD$	2.28 ± 0.91	1.53 ± 1.13	< 0.001	2.31 ± 0.93	1.49 ± 1.24	< 0.001	2.09 ± 0.71	1.63 ± 0.9	0.028

Statistically, in the survived patients, there was no significant difference in the numeric value of the variable Jung (without AMI 2.31 and with AMI group 2.09; $p = 0.201$).

Statistically, in the deceased patients, there was no significant difference in the numeric value of the variable Jung (the group without AMI 1.49 and the group with AMI 1.63; $p = 0.383$).

Out of patients with pulmonary edema 16.6% died. Mortality was frequently higher in the group with edema and myocardial infarction (39.2% *vs* 12.3%).

In order to evaluate values of the created variable Jung, standard statistics indicators were used: specificity, sensitivity and surface under the ROC curve (Figures 1–3).

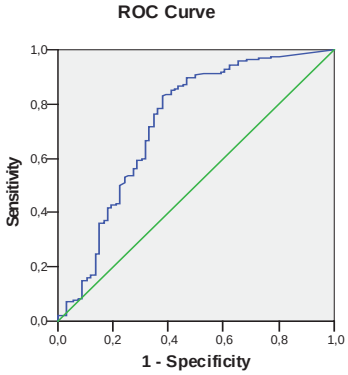

Fig. 1 – The standard statistics indicators for all the patients.
In all the patients: cut-off = 1.58; the surface under the ROC curve 0.727; sensitivity 62.1% and specificity 83.2%; positive prediction value = 47%; negative prediction value = 90%.

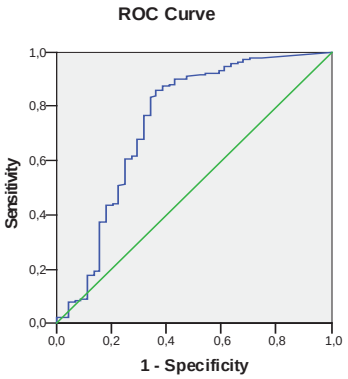

Fig. 2 – The standard statistics indicators for the patients with pulmonary edema without acute myocardial infarction (AMI).
In the subgroup without AMI: cut-off = 1.53; the surface under the ROC curve 0.739; sensitivity 63.6% and specificity 85.9%; positive prediction value = 45%; negative prediction value = 93%.

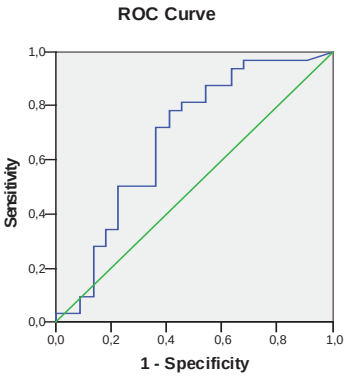

Fig. 3 – The standard statistics indicators for the patients with pulmonary edema and acute myocardial infarction (AMI).
In the subgroup with AMI: cut-off = 1.65; the surface under the ROC curve 0.678;
Sensitivity 59.1% and specificity; 78.1%; positive prediction value = 65%, negative prediction value = 74%.

For ST-elevation myocardial infarction (STEMI) patients in AMIS_NS scoring system the cut-off value was: alive > 2.08 > deceased.

In this research for pulmonary edema patients the cut-off value was: alive > 1.58 > deceased.

Discussion

Acute cardiogenic pulmonary edema is a common medical emergency with high early mortality. Initial clinical assessment would benefit from accurate mortality prediction.

Fifty percent of patients admitted with acute heart failure syndromes will present with acute cardiogenic pulmonary edema as the principal finding. Clinical management pathways may be improved by rapid and accurate estimation of early mortality [7].

Results in the incidents of death vary in the literature. Data from 3CPO score are from 1.069 patients (78 ± 10 years; 43% men; 7-day mortality 9.6%) [7].

In PEPS score, 276 consecutive patients hospitalized with acute pulmonary edema from 1998 to 2000 were retrospectively studied. During the initial hospitalization, 58 (21%) patients died and 218 (79%) patients were discharged [8].

In our study mortality rate in patients who died of pulmonary edema was 16.6%. Mortality was frequently higher in the subgroup with edema and AMI (39.2%) *vs* 12.3% without AMI.

Of particular interest is the strong independent predictive value of systolic blood pressure, which has been described in numerous trial data [9–13].

Lee et al. [9] developed a risk score for 30-day and 1-year mortality rate in community-based patients presenting with heart failure and found that age and systolic blood pressure were independent predictors, alongside respiratory rate, blood urea nitrogen level, hyponatremia, and a number of comorbidities.

Gray et al. [7] developed a simple risk score, 3CPO, to predict early outcome in severe acute cardiogenic pulmonary edema. Patients were elderly (78 ± 10 years), predominantly women (57%), and unwell with a marked tachycardia (112 ± 22 beats per minute), tachypnoea (32 ± 7 respiratory rate per minute), hypertension (SBP 161 ± 36 mm Hg), acidosis (pH 7.22 ± 0.09), and hypercapnia (pCO2, 7 ± 2.3 kPa) [7].

Fiutowski et al. [8] in their score system (PEPS) for pulmonary edema used calculation of common clinical diagnostic tests (electrocardiogram, blood pressure, heart rate, and white cell count) to determine in-hospital mortality risk in patients with an acute episode of cardiogenic pulmonary edema. Statistical analyses revealed that the most significant predictors of in-hospital mortality were acute myocardial infarction, heart rate greater than 115/beats/min, SBP of 130 mmHg or less, and white blood cell count greater than 11,500/mm^3 on presentation [8].

Fonarow et al. [10] reported that blood pressure above 115 mmHg was the best cut-point to indicate lower risk.

The literature proves the significance of low SBP, especially when followed with high heart rate. This combination was proved by multifactorial analysis to be an independent

mortality predictor, both for intra-hospital and long term follow-up [14, 15].

In our study, SBP was less than 110 mmHg within all the groups of deceased patients, while heart rate was over 100 beat/min. These facts correlate with the literature. Measurement of SBP, apart of heart rate, is one of the basic examinations of each patient, especially a life threatening patient. SBP gives information about left myocardial pump function. This is of vital essence for patients not only with AMI but also with heart failure. Together with heart rate it is one of the key elements that determine: level of urgency, type of required intervention and adequate medication. SBP lower than 80 mmHg leads to cardiac shock with all its fatal implications [3].

In almost all studies, predictors of mortality are: SBP (overall accepted as the most important), age and heart rate. In the literature all data confirms the importance of age as risk factor of both morbidity and mortality. Old age is a significant risk factor of atherosclerosis and its complications.

In 1989 Assmann and Schulte [16] stated: "With today's level of knowledge, we have to treat the age as an independent and unchanged variable, but as we learn more about impact of genetic on risk of early appearance of atherosclerosis, maybe it will be proved that significance of age actually varies depending on genetic constitution".

Although it is accessed as an independent risk factor, age of life is in correlation with other risk factors, including hyperlipoproteinemia, hypertension and diabetes which prevalence increase with aging [17].

Univariant analysis results in AMIS_NS score system prove age to be an important and reliable predictor of mortality (p = 0.011). Mortality significantly increases with the age of over 59 disregarding gender [3].

Sinus tachycardia exists with most of hospitalized patients. It is a consequence of enlarged tonus of sympathicus, caused by: pain, fear, increased temperature or myocardial failure. Persistent sinus tachycardia increases oxygen consumption and it is a bad prognostic sign [18].

Combination of heart rate (> or = 90/min) and SBP (< 120 mmHg) immediately after hospitalization is a very important predictor of intra-hospital mortality. Multivariant analysis shows that heart rate after admission is independent predictor for intra-hospital as well as one-year mortality [14].

A 22-year study showed that heart rate is a risk factor of mortality, caused not only by coronary and cardio-vascular disease, but also by all other causes of death among young and middle aged males and females [19]. In that 22- year study it was concluded that cardiovascular mortality of patients with increased heart rate is consequence of combination of both increased heart rate and high SBP. Increased heart rate is simple for clinical assessment and important although unspecific predictor of mortality [5].

While creating the AMIS_NS scoring system, it was found that heart rate of over 140 beat/min causes a significant increase of mortality, 33.3% (p = 0.05) [3].

In our study, the patients with pulmonary edema had heart rate over 100 beat/min. A difference was significant (p = 0.004) but it was lower within diseased than survived patients. In this research, evaluation of only heart rate did not confirm it as a predictor of mortality, opposite to data from the literature.

Predictors of mortality: SBP, age of life and heart rate are quoted as sole or in combination of two factors of prediction. However, until this day these factors have not been unified together as mortality predictor.

We unified the mentioned predictors, not limiting values for any of these. The formula includes all the values of: age, heart rate and SBP. Age of life represents not only mathematical but also biological age of patient. In this way it includes not only genetic predispositions but also social and health changes gained through time. SBP represents pump function of the left ventricle – the ability of the heart muscle to sustain the volume of strike. Together with heart rate, it represents the compensatory mechanism.

Applying the variable Jung also in the patients with pulmonary edema gives acceptable values of sensitivity 62.1%, specificity 83.2% and surface under the ROC curve 0.727, the positive prediction value = 47% and negative prediction value = 90%; cut-off is 1.58.

The presented statistical parameters prove applicability of the variable Jung as a valid indicator of clinical status of patients with pulmonary edema and without AMI, immediately after admission.

Within the AMIS_NS score the cut-off variable Jung was 2.08. The patients with lower variable value had higher risk of mortality, while the patients with a higher variable value were more likely to survive.

In this research the cut-off value was 1.58, which precisely shows its limitations. One cut-off cannot be considered as universal – its value will most likely be different for each disease. Therefore it is necessary to calculate the benchmark cut-off for a specific disease and after that it can be used in routine practice. The variable is not a score for a specific disease, but rather an important parameter of patient's clinical state in the given moment in time.

The variable alone is not enough to be used as an exact and independent mortality predictor. In order to be used for prediction of mortality it should be combined with other risk factors or predictors of mortality.

This variable can be used to provide an immediate assessment of the patients' risk for mortality and guide initial management.

Conclusion

The variable Jung is simple, highly reliable and can absolutely be used as a significant indicator of clinical status also in non-infarction patients with acute pulmonary edema, no matter if it is caused by AMI or not.

REFERENCES

1. *Reina A, Vázquez G, Aguayo E, Bravo I, Colmenero M, Bravo M.* Mortality discrimination in acute myocardial infarction: comparison between APACHE III and SAPS II prognosis systems. PAEEC Group. Intensive Care Med 1997; 23(3): 326−30.
2. *Schuster HP, Schuster FP, Ritschel P, Wilts S, Bodmann KF.* The ability of the Simplified Acute Physiology Score (SAPS II) to predict outcome in coronary care patients. Intensive Care Med 1997; 23(10): 1056−61.
3. *Jung. R.* Evaluation of the results in patients with myocardial infarction treated in a coronary care unit based on stratifying their condition at admission [dissertation]. Novi Sad: Faculty of Medicine, University of Novi Sad; 2001. (Serbian)
4. *Desegni E, Goldbourt U, Reicher – Reiss H, Kaplinsky E, Zion M, Boyko V,* et al. The predictive value of admission heart rate on mortality in patiens with acute myocardial infarction. J Clin Epidemiol 1995; 48(10) : 1197–205.
5. *Reunanen A, Karjalainen J, Ristola P, Heliövaara M, Knekt P, Aromaa A.* Heart rate and mortality. J Intern Med 2000; 247(2): 231−9.
6. *Kikuya M, Hozawa A, Ohokobo T, Tsuji I, Michimata M, Masubara M,* et al. Prognostic significance of blood pressure and heart rate viriabilities: the Ohasama study. Hypertension 2000; 36(5): 901−6
7. *Gray A, Goodacre S, Nicholl J, Masson M, Sampson F, Elliott M,*et al. The development of a simple risk score to predict early outcome in severe acute acidotic cardiogenic pulmonary edema: the 3CPO score. Circ Heart Fail 2010; 3(1): 111−7.
8. *Fiutowski M, Waszyrowski T, Krzemińska-Pakula M, Kasprzak JD.* Pulmonary edema prognostic score predicts in-hospital mortality risk in patients with acute cardiogenic pulmonary edema. Heart Lung 2008; 37(1): 46−53.
9. *Lee DS, Austin PC, Rouleau JL, Liu PP, Naimark D, Tu JV.* Predicting mortality among patients hospitalized for heart failure: derivation and validation of a clinical model. JAMA 2003; 290(19): 2581–7.
10. *Fonarow GC, Adams KF Jr, Abraham WT, Yancy CW, Boscardin WJ.* Risk stratification for in-hospital mortality in acutely decompensated heart failure: classification and regression tree analysis. JAMA 2005; 293(5): 572–80.
11. *Auble TE, Hsieh M, Gardner W, Cooper GF, Stone RA, McCausland JB,* et al. A prediction rule to identify low-risk patients with heart failure. Acad Emerg Med 2005; 12(6): 514–21.
12. *Collins S, Storrow SB, Kirk JD, Pang PS, Diercks DB, Gheorghiade M.* Beyond pulmonary edema: diagnostic, risk stratification, and treatment challenges of acute heart failure management in the emergency department. Ann Emerg Med. 2008; 51(1): 45–57.
13. *Fonarow GC.* Epidemiology and risk stratification in acute heart failure. Am Heart J 2008; 155(2): 200–7.
14. *Disegni E, Goldbourt U, Reicher-Reiss H, Kaplinsky E, Zion M, Boyko V*, et al. The predictive value of admission heart rate on mortality in patients with acute myocardial infarction. J Clin Epidemiol 1995; 48(10): 1197−205.
15. *Kikuya M, Hozawa A, Ohokubo T, Tsuji I, Michimata M, Matsubara M*, et al. Prognostic significance of blood pressure and heart rate variabilities: the ohasama study. Hypertension 2000; 36(5): 901−6.
16. *Assmann G, Schulte H.* Diabetes mellitus and hypertension in the eldery: concomitant hyperlipidemia and coronary heart disease risk. Am J Cardiol 1989; 63(16): 33H−7H.
17. *Jung R.* Who survives myocardial infarction? Beograd: Zadužbina Andrejević; 2003. (Serbian)
18. *Panić G.* The effects of fibrinolytic treatment of myocardial infartion by the use of the fibrinolytic method [dissertation]. Novi Sad: Faculty of Medicine, University of Novi Sad; 1994. (Serbian)
19. *Greenland P, Daviglus ML, Dyer AR, Liu K, Huang CF, Goldberger JJ*, et al. Resting heart rate is a risk factor for cardiovascular and noncardiovascular mortality: the Chicago Heart Association Detection Project in Industry. Am J Epidemiol 1999; 149(9): 853−62.

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Distal tibial pilon fractures (AO/OTA type B, and C) treated with the external skeletal and minimal internal fixation method

Zbrinjavanje preloma distalnog pilona tibije (AO/OTA tipa B, C) metodom spoljašnje skeletne i minimalne unutrašnje fiksacije

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Abstract

Background/Aim. Distal tibial pilon fractures include extra-articular fractures of the tibial metaphysis and the more severe intra-articular tibial pilon fractures. There is no universal method for treating distal tibial pilon fractures. These fractures are treated by means of open reduction, internal fixation (ORIF) and external skeletal fixation. The high rate of soft-tissue complications associated with primary ORIF of pilon fractures led to the use of external skeletal fixation, with limited internal fixation as an alternative technique for definitive management. The aim of this study was to estimate efficacy of distal tibial pilon fratures treatment using the external skeletal and minimal internal fixation method. **Methods.** We presented a series of 31 operated patients with tibial pilon fractures. The patients were operated on using the method of external skeletal fixation with a minimal internal fixation. According to the AO/OTA classification, 17 patients had type B fracture and 14 patients type C fractures. The rigid external skeletal fixation was transformed into a dynamic external skeletal fixation 6 weeks post-surgery. **Results.** This retrospective study involved 31 patients with tibial pilon fractures, average age 41.81 (from 21 to 60) years. The average follow-up was 21.86 (from 12 to 48) months. The percentage of union was 90.32%, nonunion 3.22% and malunion 6.45%. The mean to fracture union was 14 (range 12–20) weeks. There were 4 (12.19%) infections around the pins of the external skeletal fixator and one (3.22%) deep infections. The ankle joint arthrosis as a late complication appeared in 4 (12.90%) patients. All arthroses appeared in patients who had type C fractures. The final functional results based on the AOFAS score were excellent in 51.61%, good in 32.25%, average in 12.90% and bad in 3.22% of the patients. **Conclusion.** External skeletal fixation and minimal internal fixation of distal tibial pilon fractures is a good method for treating all types of inta-articular pilon fractures. In fractures types B and C dynamic external skeletal fixation allows early mobility in the ankle joint.

Key words:
tibial fractures; orthopedic procedures; external fixators; internal fixators; treatment outocme.

Apstrakt

Uvod/Cilj. Prelomi distalnog pilona tibije podrazumevaju spoljašnje artikularne prelome metafize tibije i teže unutrašnje artikularne prelome pilona tibije. Ne postoji univerzalni metod za lečenje preloma distalnog pilona tibije. Ovi prelomi se leče metodom otvorene redukcije i stabilne fiksacije (ORIF) i spoljašnjom skeletnom fiksacijom. Visok procenat komplikacija na mekom tkivu udružen nakon primarne ORIF preloma pilona, nameće upotrebu metode spoljašnje skeletne fiksacije sa minimalnom unutrašnjom fiksacijom, kao alternativnu tehniku za konačno izlečenje. Cilj rada bio je da se utvrdi efikasnost lečenja distalnog pilona tibije primenom metode spoljašnje skeletne i minimalne unutrašnje fiksacije. **Metode.** Prikazali smo seriju od 31 operisanog bolesnika sa prelomima pilona tibije. Bolesnici su operisani metodom spoljašnje skeletne fiksacije sa minimalnom unutrašnjom fiksacijom. Prema AO/OTA klasifikaciji 17 bolesnika imalo je prelom tipa B, a 14 prelom tipa C. Kruta spoljašnja skeletna fiksacija je transformisana u dinamičku spoljašnju skeletnu fiksaciju šest nedelja posle operacije. **Rezultati.** Retrospektivnom studijom analiziran je 31 bolesnik sa prelomima pilona tibije, prosečne starosti 41,81 (21–60) godina. Prosečno vreme praćenja iznosilo je 21,86 (12–48) meseci. Procenat zarastanja preloma iznosio je 90,32%, nezarastanja 3,22% i lošeg zarastanja 6,45%. Prosečno trajanje zarastanja preloma iznosilo je 14 (12–20) nedelja. Bilo je 4 (12,19%) in-

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fekcija oko klinova spoljašnjeg skeletnog fiksatora i 1 (3,22%) duboka infekcije. Artroza skočnog zgloba kao kasna komplikacija, pojavila se kod 4 (12,90%) bolesnika. Sve artroze su nastale kod bolesnika koji su imali prelom tipa C. Krajnji funkcionalni rezultati na osnovu AOFAS skora bili su odlični kod 51,61%, dobri kod 32,25%, umereni kod 12,90% i loši kod 3,22% bolesnika. **Zaključak.** Spoljašnja skeletna fiksacija i minimalna unutrašnja fiksacija preloma distalnog pilona tibije dobra je metoda za lečenje svih tipova intraartikularnih preloma pilona. Kod preloma tipa B i C, dinamička spoljašnja skeletna fiksacija dozvoljava rane pokrete u skočnom zglobu.

Ključne reči:
tibija, prelomi; ortopedske procedure; fiksatori, spoljni; fiksatori, unutrašnji; lečenje, ishod.

Introduction

In contrast to the rotational mechanisms that result in malleolar fractures and fracture-dislocations of the ankle, distal tibial pilon fractures typically result from high-energy axial-loading mechanisms. Distal tibial pilon fractures include extra-articular fractures of the tibial metaphysis and the more severe intraarticular tibial plafond or pilon fractures. The clinical manifestation of this fractures difference is the generation of osteochondral fracturing, comminution and displacement of the weight-bearing articular portion of the tibial plafond and distal tibial metaphysis, as well as the development of marked swelling, blistering and devitalization of the surrounding soft-tissue envelope typically identified in tibial pilon fractures. These fractures are estimated to comprise 3% to 10% of all tibia fractures and less than 1% of lower extremity fractures. These high energy injuries, usually caused by falls from heights or motor vehicle accidens, are often open fractures and they are frequently associated with additional trauma in other areas of the body [1–3]. They are one of the most challenging injuries in orthopaedic traumatology [4]. Several treatment methods are recommended for the treatment of these injuries including external skeletal fixation, intramedullary nailing, and plate fixation [5–8]. The aim of this study was to estimate efficacy of a treatment of a distal tibial fracture (AO type B and C) using the method of external skeletal fixation combined with minimal internal fixation.

The high rate of soft-tissue complications associated with primary open reduction, internal fixation (ORIF) of distal tibial pilon fractures led to use of external skeletal fixation, with limited internal fixation as an alternative technique for definitive management. Our aim was to analyze original results of distal tibial pilon fractures treatment using the external skeletal and minimal internal fixation method.

Methods

The patients with distal tibial fracture were operated on at the University Orthopedic and Traumatology Clinic, Niš. The patients with intra-articular fractures (AO/OTA types B and C) were operated on using the method of external skeletal fixation and minimal internal fixation. To perform minimal internal fixation, screws and K-wires were used. In patients with types B and C fractures, rigid external skeletal fixation was transformed into dynamic external skeletal fixation 1.5 month later. All fractures were classified according to the AO/OTA classification [9]. The Gustilo-Anderson classification system was used for all open fractures [10]. To perform external skeletal fixation, a Mitkovic's unilateral external skeletal fixator was used. To analyze the final functional results, the AOFAS scoring system was used [11].

Results

This retrospective study involved 31 patients with distal tibial pilon fractures. According to the AO/OTA classification, 17 patients had fractures type B and 14 patients fractures type C (Figures 1–5 and 6–9 respectively). The average age was 41.81 (21–60) years, and there were 20 male patients and 11 female patients. Open fractures appeared in 11 (35.48%) patients. A total of 10 (32.25%) fractures were caused by car accidents, 14 (45.16%) by falls from heights, whereas 7 (22.58%) fractures appeared under different circumstances, such as in accidents at work, falls from stairs, or as a result of slip and fall accidents on an even surface. The average follow-up of the patients was 21.86 (12–48) months. There were 28 (90.32%) unions, 1 (3.22%) nonunions and 2 (6.45%) malunions. The mean to fracture union was 14 (range 12–20) weeks. As regards complications, infection around the pins of the external skeletal fixator appeared in 4 (12.19%) and deep infections appeared in 1 (3.22%) patients. Ankle joint arthrosis as late complication appeared in 4 (12.90%) patients. All arthroses appeared in patients with fractures type C. According to AOFAS, the final functional results were excellent in 16 (51.61%) patients, good in 10 (32.25%), average in 4 (12.90%) patietns and bad in 1 (3.22%) cases. All the patients were operated on as urgent cases, immediately after hospitalization.

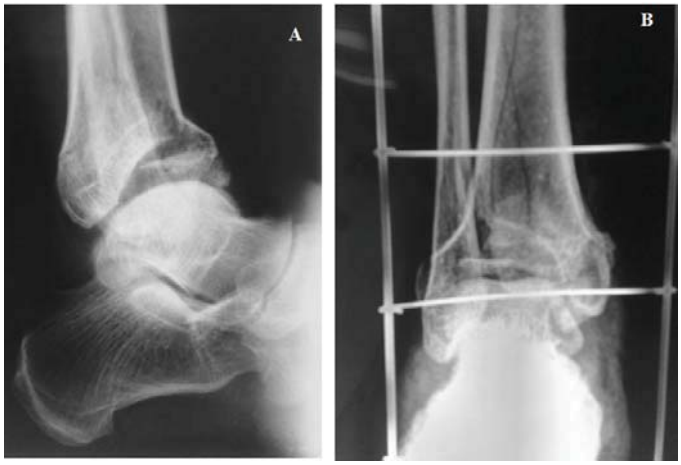

Fig. 1 (A and B) – Radiographs of distal tibial pilon fracture (AO/OTA type C) after the injury.

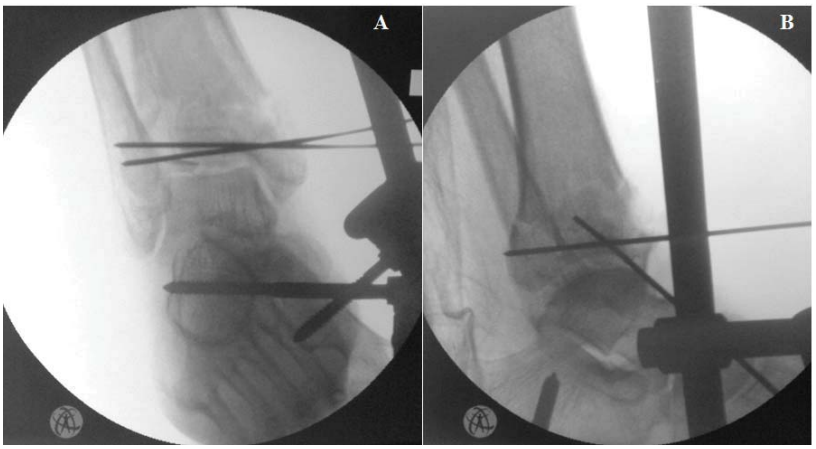

Fig. 2 (A and B) – Radioscopic views after external skeletal fixation and minimal internal K-wires fixation.

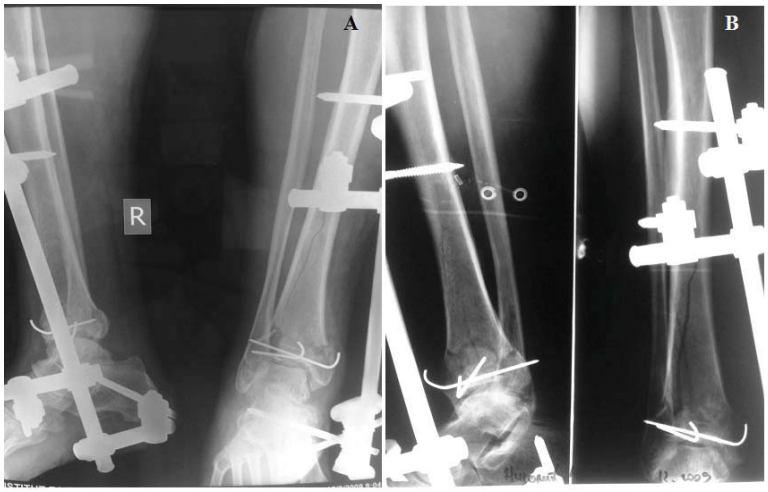

Fig. 3 – Radiopgraphs views after the surgery (A), and after 1 month (B).

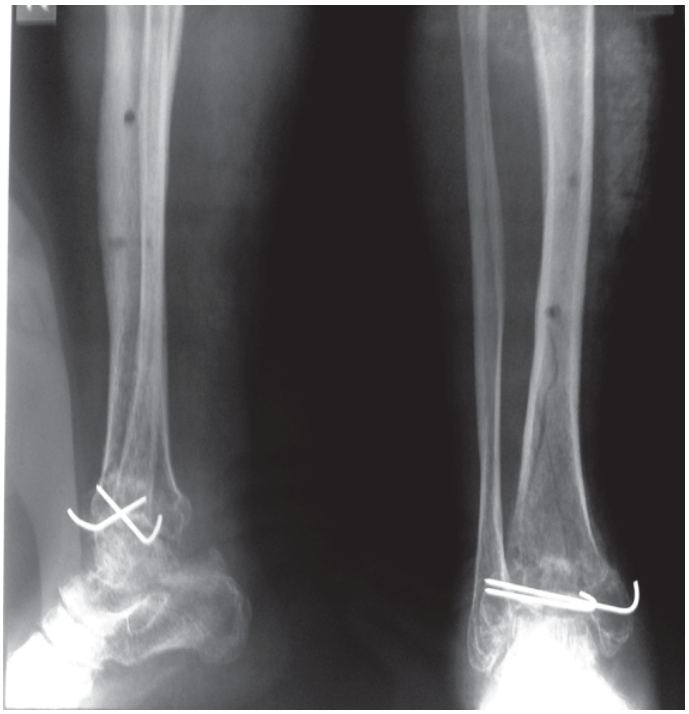

Fig. 4 – Radiographs after external skeletal fixator removal, 14 weeks after the injury.

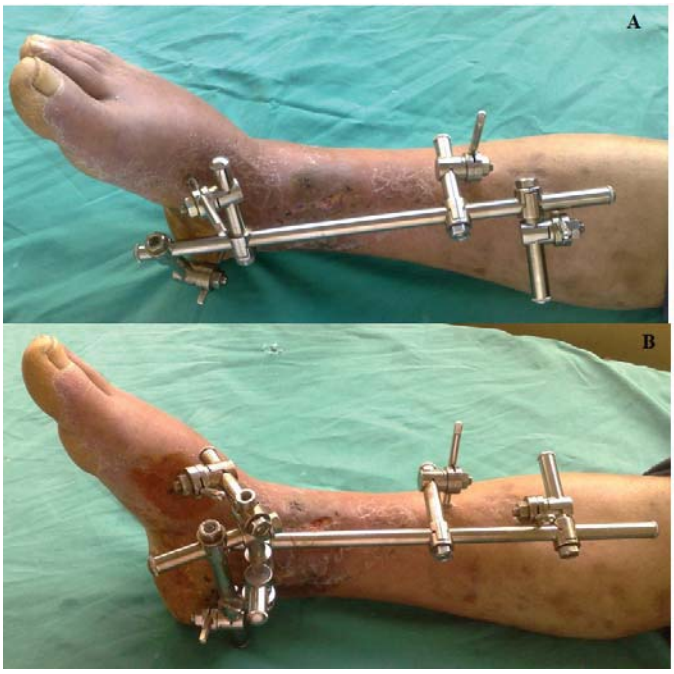

Fig. 5 – A) Rigid external skeletal fixation of distal tibial pilon fracture (ligamentotaxis); B) Dynamic external skeletal fixation (the same patient 6 weeks after the surgery).

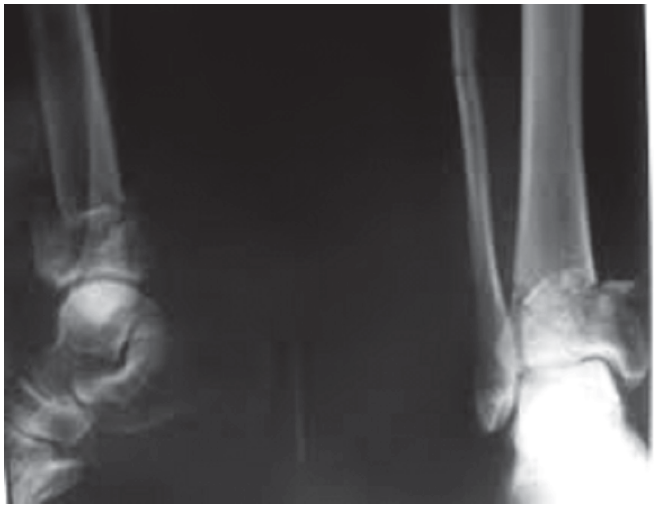

Fig. 6 – Radiographs of distal tibial pilon fracture (AO/OTA type C) after the injury.

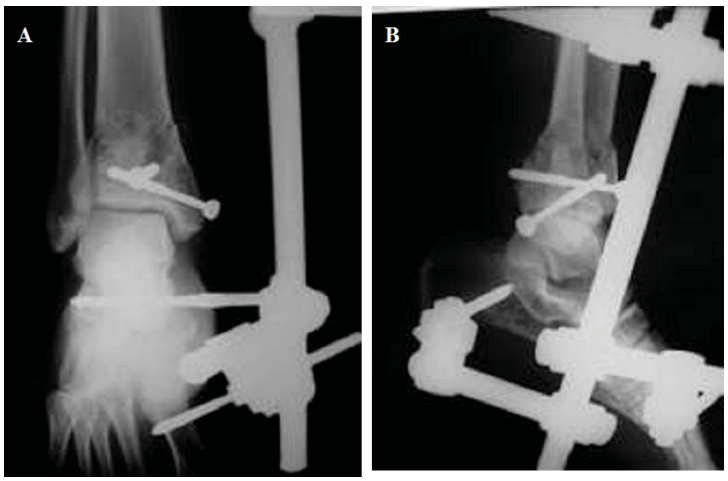

Fig. 7 (A and B) – Radiographs after external skeletal fixation and minimal internal screws fixation.

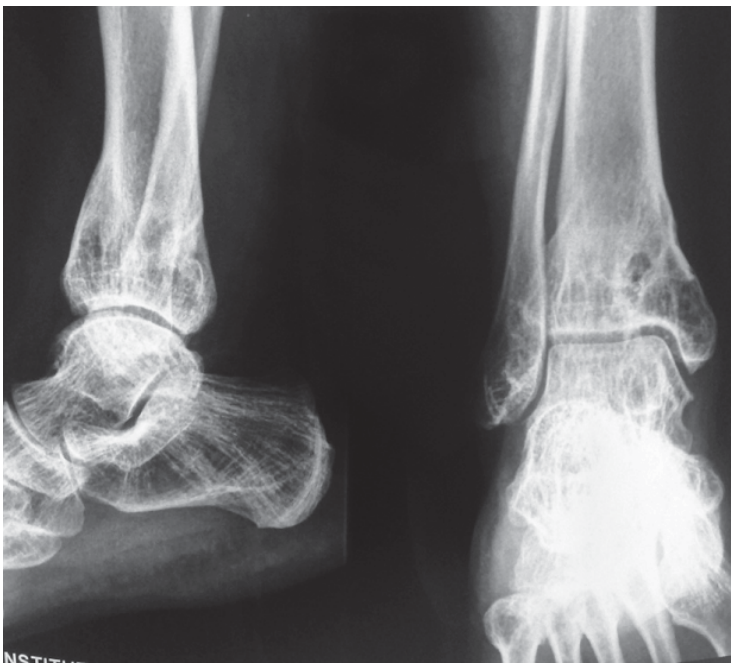

Fig. 8 – Radiographs after external skeletal fixator and screws removal.

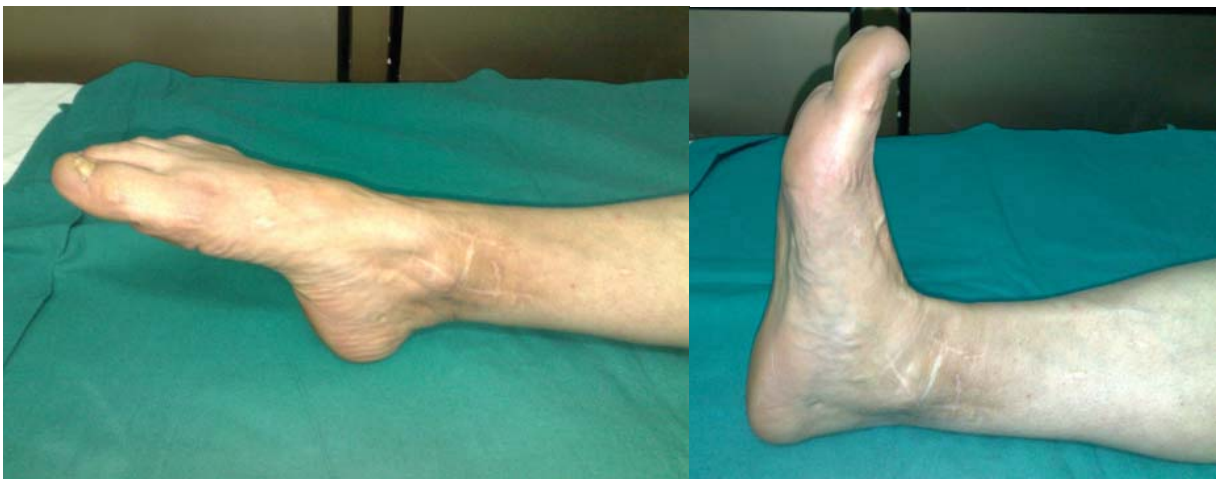

Fig. 9 – The final functional result 4 months after the injury.

Discussion

In the decade 1980 to 1990 numerous publications favoured the approach to distal tibial fractures that included external skeletal fixation as primary stabilization, with or without some form of limited internal fixation. This was in reaction to numerous complications that were observed previously following ORIF [12]. High-energy distal tibial fracture with soft tissue compromise remains a treatment dilemma. Clinical series from the 1980 and 1990 using primary ORIF had complications rates of greater than 50%, most related to soft-tissue complications and infections, including amputation rates as high as 17% [13,14]. The high rate of soft-tissue complications associated with primary ORIF of pilon fractures led to use of external skeletal fixation, with limited internal fixation as an alternative technique for definitive management. Hybrid external skeletal fixation with limited open reduction has proved to be a safe, reproducible, and effective treatment modality for this complex fracture [15]. Distal tibial fractures are serious injuries which most frequently appear in car accidents or in falls from heights. There is no universal method in treating these fractures. The most frequent methods are operation, open reduction and internal fixation, intramedullary fixation, plate fixation, external skeletal fixation. Some authors recommend a two-step procedure. After applying the external skeletal fixation, an internal plate fixation is performed [6–18]. We used the external skeletal fixation as one-step procedure in the treatment of distal tibial pilon fracture. We presented the results of distal tibia fracture treatment using the method of external skeletal fixation combined with minimal internal fixation (AO/OTA fractures types B and C). Studies show that minimal internal fixation and external skeletal fixation achieve good results in the treatment of these fractures. A higher percentage of superfi-

cial infections around the pins does not affect the final outcome of the treatment [19]. Bone [1] also describes satisfactory results in the application of this method. In fractures type B and C, it is necessary to achieve fracture reduction and articular tibial surface reconstruction. Fixation by means of screws and K-wires is open and minimal. External skeletal fixator pins are placed, 2 in the proximal fragment, and 2 in the foot. One pin is placed in the calcaneus, the other in the I metatarsal bone. After that, the external skeletal fixator frame with clamps and carriers of the clamp placed. In this way, rigid fracture fixation is achieved, and it transforms into dynamic fixation 6-week post-surgery, which allows early ankle joint mobility [20]. A dynamic external skeletal fixation is placed on an already existing external skeletal fixator construction with additional carriers of the clamp and clamps. This system for external skeletal fixation is suitable for additional interventions, such as fracture position correction while the apparatus is carried. Studies describe this method of treatment as definitive or temporary method, after which intramedullary or plate fixation of fracture will be performed [21]. Our experience in the treatment of these fractures as definitive method and our results are very encouraging, giving us right to consider this method suitable for treating all types of distal tibial pilon fractures. It is important to emphasize that these fractures are considered as urgent, and they should be treated urgently. Urgent surgical intervention reduces the possibility of complications.

Conclusion

External skeletal fixation of distal tibial and pilon fractures as one-step procedure is a good method for treating all types of fractures. In fractures types B and C, dynamic external skeletal fixation allows early mobility in the ankle joint.

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R E F E R E N C E S

1. *Bone LB.* Fractures of the tibial plafond. The pilon fracture. Orthop Clin North Am 1987; 18(1): 95−104.
2. *Mandracchia VJ, Evans RD, Nelson SC, Smith KM.* Pilon fractures of the distal tibia. Clin Podiatr Med Surg 1999; 16(4): 743−67.
3. *Burgess AR, Dischinger PC, O'Quinn TD, Schmidhauser CB.* Lower extremity injures in drivers of airbag-equipped automobiles:clinical and crash reconstruction correlations. J Trauma1995; 38(4): 509−16.
4. *Pollak AN, McCarthy ML, Bess RS, Agel J, Swiontkowski MF.* Outcomes after treatment of high-energy tibial plafond fractures. J Bone Joint Surg Am 2003; 85-A(10): 1893−900.
5. *Mosheiff R, Safran O, Segal D, Liebergall M.* The unreamed tibial nail in the treatment of distal metaphyseal fractures. Injury 1999; 30(2): 83−90.
6. *Khoury A, Liebergall M, London E, Mosheiff R.* Percutaneous plating of distal tibial fractures. Foot Ankle Int 2002; 23(9): 818−24..
7. *Anglen JO.* Early outcome of hybrid external fixation for fracture of the distal tibia. J Orthop Trauma 1999; 13(2): 92−7.
8. *Babis GC, Vayanos ED, Papaioannou N, Pantazopoulos T.* Results of surgical treatment of tibial plafond fractures. Clin Orthop Relat Res 1997; (341): 99−105.
9. *Ruedi T, Murphy WM.* AO Principles of Fracture Management. Vol. 1. Stuttgart-New York: Thieme; 2000.
10. *Gustilo RB, Anderson JT.* Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. J Bone Joint Surg Am 1976; 58(4): 453−8.
11. *Kitaoka HB, Patzer GL.* Analysis of clinical grading scales for the foot and ankle. Foot Ankle Int 1997; 18(7): 443−6.
12. *Barbieri R, Schenk R, Koval K, Aurori K, Aurori B.* Hybrid external fixation in the treatment of tibial plafond fractures. Clin Orthop Relat Res 1996; (332): 16−22.
13. *McFerran MA, Smith SW, Boulas HJ, Schwartz HS.* Complications encountered in the treatment of pilon fractures. J Orthop Trauma 1992; 6(2): 195−200.
14. *Wyrsch B, McFerran MA, McAndrew M, Limbird TJ, Harper MC, Johnson KD*, et al. Operative treatment of fractures of the tibial plafond: A randomized, prospective study. J Bone Joint Surg Am 1996; 78(11): 1646−57.
15. *French B, Tornetta P 3rd.* Hybrid external fixation of tibial pilon fractures. Foot Ankle Clin 2000; 5(4): 853−71.
16. *Blauth M, Bastian L, Krettek C, Knop C, Evans S.* Surgical options for the treatment of severe tibial pilon fractures: a study of three techniques. J Orthop Trauma 2001; 15(3): 153−60.
17. *Dickson KF, Montgomery S, Field J.* High energy plafond fractures treated by a spanning external fixator initially and followed by a second stage open reduction internal fixation of the articular surface-preliminary report. Injury 2001; 32(Suppl 4): SD92−8.
18. *Patterson MJ, Cole JD.* Two-staged delayed open reduction and internal fixation of severe pilon fractures. J Orthop Trauma 1999; 13(2): 85−91.
19. *El-Shazly M, Dalby-Ball J, Burton M, Saleh M.* The use of transarticular and extra-articular external fixation for management of distal tibial intra-articular fractures. Injury. 2001; 32(Suppl 4): SD99−106.
20. *Mitkovic M, Bumbasirevic M, Lesic A, Golubovic Z.* Dynamic external fixation of comminuted intra-articular fractures of the distal tibia (type C pilon fractures). Acta Orthop Belg 2002; 68(5): 508−14.
21. *Hontzsch D, Karnatz N, Jansen T.* One-or two-step management (with external fixator) of severe pilon-tibial fractures.Aktuelle Traumatol 1990; 20(4): 199−204. (German)

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Health-related quality of life and depression in Rett syndrome caregivers

Kvalitet života i depresija kod roditelja dece obolele od Retovog sindroma

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Abstract

Background/Aim. Rett syndrome (RTT) is a severe neurodevelopmental disorder primarily affecting females with an estimated incidence of 1 : 10,000–15,000 female births. Currently, there is no specific treatment that halts or reverses the progression of RTT. Therefore, management was mainly symptomatic, focussed on optimising patient's abilities. The aim of this study was to investigate factors influencing health-related quality of life (HRQoL) and depression in mothers who care for children with Rett syndrome (RTT) in Serbia. **Methods.** The cross-sectional study was conducted on 49 mothers giving care to females with RTT. Caregivers` HRQoL was assessed by using the SF-36 questionnaire. Clinical severity score (CSS) of RTT patients and Beck Depression Inventory II (BDI -II) scale were used to quantify RTT severity and mothers` depression, respectively. Statistical assessment included descriptive statistics, *t*-test, Pearson correlation coefficient and multiple logistic regression. **Results.** The age of mothers ranged from 22 to 55 years and of their affected children from 3 to 29 years. Severe depression was observed in 15 (30.6%) participants. CSS and BDI – II scores correlated negatively with all SF-36 domains and composite scores. Lowest scoring domains of HRQoL in mothers giving care to RTT children were mental health, vitality and role functioning emotional. Multiple linear regression analysis revealed that severity of RTT patients` disability (CSS) and caregivers` age are factors with strongest influence to HRQoL and depression in care giving mothers. **Conclusion.** Mothers giving care to children with RTT are at high risk of severe depression and lower HRQoL scores of domains that reflect mental well-being. Results of this study can help in planning subsequent interventions directed at families dealing with Rett syndrome.

Key words:
rett syndrome; caregivers; mothers; depression; quality of life.

Apstrakt

Uvod/Cilj. Retov sindrom (RTT) je težak neurorazvojni poremećaj koji prvenstveno pogađa devojčice. Incidencija se procenjuje na 1 : 10 000–15 000 živorođene dece ženskog pola. Trenutno ne postoji specifična terapija koja bi mogla da promeni tok ove bolesti. Stoga je tretman uglavnom simptomatski sa naglaskom na unapređenje pojedinih sposobnosti bolesnika. Cilj ove studije bio je ispitivanje faktora koji utiču na kvalitet života (HRQoL) i depresiju majki koje brinu o deci oboleloj od RTT u Srbiji. **Metode.** Studija preseka je obuhvatila 49 majki koje brinu o deci oboleloj od RTT. Kvalitet života je ispitivan pomoću SF-36 upitnika. Skor težine kliničke slike (CSS) bolesnika sa RTT i Bekova skala depresije II (BDI – II) upotrebljeni su u proceni težine bolesti kod dece, odnosno stepena depresije kod majki. Statistička analiza je uključila deskriptivne metode, *t*-test, Pirsonov koeficijent korelacije i multiplu linearnu regresiju. **Rezultati.** Starost majki kretala se u rasponu od 22 do 55 godina, a uzrast bolesnika od 3 do 29 godina. Teška depresija je zapažena kod 15 (30,6%) učesnica u studiji. Skorovi CSS i BDI – II negativno su korelisali sa svim SF-36 dimenzijama i zbirnim skorovima. Najniže ocenjene dimenzije kvaliteta života kod majki koje brinu o deci sa Retovim sindromom su mentalno zdravlje, vitalnost i emocionalno funkcionisanje. Multipla linearna regresija pokazala je da godine majke i težina kliničke slike deteta imaju najsnažnije dejstvo u pravcu pojave depresije i lošijeg kvaliteta života u ovoj populaciji. **Zaključak.** Majke koje brinu o deci sa RTT imaju visok rizik za pojavu teške depresije i nižih skorova HRQoL u domenima koji odražavaju mentalno stanje. Rezultati ove studije mogu pomoći u planiranju adekvatne podrške porodicama koje imaju članove obolele od RTT.

Ključne reči:
retov sindrom; staratelji; majke; depresija; kvalitet života.

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Introduction

Rett syndrome (RTT) is a severe neurodevelopmental disorder primarily affecting females with an estimated incidence of 1 : 10,000–15,000 female births [1, 2]. Mutations in the X-linked gene methyl CpG-binding protein 2 (MECP2) have been found in the majority of patients [3, 4]. However, diagnosis of RTT remains a clinical one, by usage of established criteria [5]. Main clinical features of RTT include progressive psychomotor deterioration, autism, stereotypic movements of the hands, loss of acquired language and decreased cranial growth. The identification of a MECP2 mutation can support a clinical diagnosis but it is not a basis for diagnosis [5, 6]. RTT has a wide clinical variability in terms of its severity, and phenotype-genotype correlation has become more elucidated in recent large studies [7].

Currently, there is no specific treatment that halts or reverses the progression of RTT, and management is mainly symptomatic, focussed on optimising patient's abilities. Among RTT patients 50–80% develops epilepsy at a median age of 3 years [8], so anticonvulsant drugs are the mainstay of pharmacological approach to these patients.

Plethora of evidence from worldwide studies indicates proneness for depression and lower health-related quality of life in mothers caring for children with disabilities [9–12]. Apart from child disease characteristics, some sociodemographic factors (family income, marital status, mother's age etc.) were also recognized for having significant influence on these outcomes [13, 14]. However, studies addressing depression, health-related quality of life (HRQoL) and social issues in RTT caregivers remain sparse [15–17].

The aim of this study was to investigate HRQoL and depression in mothers caring for children with RTT in Serbia. A specific aim of our investigation was to assess the influence of sociodemographic factors and clinical severity of child disease to HRQoL and depression in care giving mothers.

Methods

The cross-sectional study was conducted on 49 mothers giving care to females with RTT. The study was performed during the period from January 1, 2010 to July 31, 2010 in Mother and Child Health Care Institute of Serbia in Belgrade, with a set of questionnaires being sent to a total of 60 mothers caring for RTT children regularly controlled and treated in this institution. Approval by the institution`s ethics committee was obtained. Mother and Child Health Care Institute of Serbia is a tertiary care paediatric center and represents referent hospital for RTT syndrome in Serbia. Inclusion criteria were that the diagnosis of RTT in child is established on the basis of "The Rett Syndrome Diagnostic Criteria Work Group" criteria [7], and that the residency of investigated family is in Serbia. Mothers diagnosed with major medical or psychiatric condition were excluded from the study. A set of applied questionnaires was comprised of three parts. Part 1 consisted of a sociodemographic questionnaire that addressed mothers' age, marital status, education level, employment status (employed, unemployed), family income (combined family income measuring above or below two average salaries) and the place of residency (urban/rural). Serbian translation of SF-36 [18], a generic HRQoL instrument, comprised part 2 of a questionnaires set. SF-36 measures eight domains of HRQoL calculated within eight scales: physical functioning (PF), role functioning physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role functioning emotional (RE), and mental health (MH). The domains of SF-36 are used to calculate composite scores - physical health composite score (PCS) and mental health composite score (MCS), as well as SF-36 total composite score (TCS). PCS is calculated as an average value of PF, RP, BP, GH and VT domains. The MCS is also calculated out of five domains: VT, SF, RE, MH and GH. The scores for the SF-36 are based on a 0 to 100 scale; zero represents the lowest possible score, and 100 represents the highest possible score. In general population, used as a norm-based reference group, 50 represents the mean score. HRQoL scales were presented as T-scores (mean 50, SD 10) by linear transformation of raw scores that optimize comparisons across the different scales of the SF-36. Higher values meant better domains of HRQoL. Scoring and calculation of SF-36 scales were performed according to Ware's survey manual recommendations. Part 3 measured depression with a Serbian translation of Beck Depression Inventory – II (BDI-II) [19]. Scores of BDI-II from 0 to 13 were considered as minimal, 14–20 as mild, 20–28 as moderate and 29–63 as severe depression [20]. Completed questionnaires were retrieved from 49 subjects with the response rate of 81.7%.

Severity of RTT was determined by using a Clinical Severity Score (CSS) developed specifically for this disease [21]. The CSS is a composite score based on thirteen individual categories measuring clinical features of RTT. All the scores range from 0 to 4 or 0 to 5 with 0 representing the least severe and 4 or 5 representing the most severe finding, while a total score ranges from 1 to 58. The CSS score was assigned and evaluated by the paediatric neurologist.

Descriptive statistics, such as mean ± standard deviation (SD) on the collected data were calculated. Pearson correlation coefficients were used to examine the relation between SF-36 domains, composite and total scores to scores of BDI-II, CSS and age of mothers giving care to children with RTT. We used *t*-test to compare SF-36 domains, composite and total scores of the studied group to general population. Assessing the difference of SF-36 scores and BDI-II score between the group of mothers giving care to less severely affected children with RTT (CSS $\leq$ 20) and the group with more severely affected children (CSS > 20) was also performed by *t*-test. This cut-off value for CSS was used since it represents median CSS in our group of patients.

We used multiple linear regression to investigate the influence of sociodemographic factors of care giving mothers and the presence of epilepsy in RTT patients on SF-36 composite scores (PCS, MCS and TCS) and BDI-II scores in our study group. Mothers educational, employment and marital status, family income, place of residency (village or city), number of children in family and the presence of epilepsy in RTT children were the factors selected for testing. The statistically significant level was set at $p < 0.05$.

Results

The demographic characteristics of 49 mothers caring for children with RTT are presented in Table 1, while clinical features of 49 female children with RTT are summarized in Table 2. Age of mothers ranged from 22 to 55 years and of their affected children from 3 to 29. In RTT patients, mean CSS was 21.5 (range from 10 to 39) with 23 (46.9%) patients scoring ≤ 20 on CSS.

Table 1
Characteristics of the participant mothers giving care to children with Rett syndrome (N = 49)

Variable	Values
Age (years), $\bar{x}$ ± SD	37.5 ± 7.5
Marital status, n (%)	
married	41 (83.7)
divorced	8 (16.3)
Education, n (%)	
elementary school	4 (8.2)
high school	33 (67.3)
university	12 (24.5)
Place of residency, n (%)	
urban	37 (75.5)
rural	12 (24.5)
Employment status, n (%)	
employed	35 (71.4)
unemployed	14 (28.6)
Family income, n (%)	
below average	19 (38.8)
above average	30 (61.2)
Number of children in family, n (%)	
1	10 (20.4)
≥ 2	39 (79.6)

Table 2
Characteristics of the children with Rett syndrome (N = 49)

Variable	Values
Age (years), $\bar{x}$ ± SD	12.2 ± 6.7
Clinical Severity Score (CSS)), $\bar{x}$ ± SD	21.5 ± 7.9
Epilepsy, n (%)	
present	32 (65.3)
absent	17 (34.7)

Our study revealed that a slight majority of mothers had minimal scores of BDI-II (53.2%), 8 (16.4%) of them had mild to moderate depression, while severe depression was observed in 15 (30.6%) of the investigated participants. Furthermore, we found statisticly significant correlation between CSS, BDI-II, mother's age and all domains of SF-36 (Table 3.). Patients` age did not show a significant correlation with CSS scores. The CSS scores had significantly negative correlation with all SF-36 domains and composite scores with highest correlation coefficients found for VT, GH and all composite scorres. We demonstrated a high statistical significance of negative correlation between BDI-II and all SF-36 domains with highest correlation coefficients for SF, VT and PCS domains. Mother`s age correlated negatively to all SF-36 domains and composite scores with high statistical significance, particularly for SF, PF, VT and PCS (Table 3).

We found that the lowest scoring domains of HRQoL in mothers giving care to RTT children were mental health (47.3 ± 29.6), vitality (43.6 ± 27.8) and role functioning emotional (42.1 ± 42.4), but none of domains differed significantly to general population norms. However, when we compared HRQoL scores between two groups of mothers divided on the basis of CSS (≤ 20 and > 20) we found significantly lower values of MH, PF, PCS and TCS in the group caring for more severely affected children (Table 4). Other

Table 3
Correlation between each of 8 domains and 3 composite scores of SF-36 health-related quality of life instrument and Clinical Severity Score (CSS), maternal depression (measured by Beck Depression Inventory-II – BDI-II) and maternal age (AoM)

Variable	PF	RP	BP	GH	VT	SF	RE	MH	MCS	PCS	TCS
CSS	-0.354[a*]	-0.398[**]	-0.343[*]	-0.423[**]	-0.519[**]	-0.335[*]	-0.408[**]	-0.478[**]	-439[**]	-463[**]	-441[**]
BDI-II	-0.744[**]	-0.687[**]	-0.836[**]	-0.832[**]	-0.891[**]	-0.903[**]	-0.728[**]	-0.862[**]	-855[**]	-900[**]	-880[**]
AoM	-0.526[**]	-0.339[*]	-0.408[**]	-0.441[**]	-0.485[**]	-0.519[**]	-0.407[**]	-0.446[**]	-464[**]	-492[**]	-482[**]

The values presented as Pearson correlation coefficients. CSS – Clinical Severity Score; BDI-II – Beck Depression Inventory-II; AoM – age of mothers; PF – physical functioning; RP – role functioning physical; BP – bodily pain; GH – general health; VT – vitality; SF – social functioning; RE – role functioning emotional; MH – mental health; MCS – mental composite score; PCS – physical composite score; TCS – total composite SF-36 score; *$p < 0.05$; **$p < 0.01$

Table 4
Mean scores for SF-36 health-related quality of life domains and composite scores and Beck Depression Inventory-II (BDI-II) in the mothers giving care to the children with Rett syndrome

Variable	Total group ($\bar{x}$ ± SD)	Clinical severity score ($\bar{x}$ ± SD)		*t*-test (*p*-value)
		≤ 20 (n = 23)	> 20 (n = 26)	
BDI-II	17.0 ± 13.3*	14.1 ± 10.7	19.6 ± 14.9	0.001
PF	73.1 ± 27.4	79.1 ± 20.9	67.7 ± 31.5	0.001
RP	52.0 ± 37.4	61.9 ± 32.7	43.3 ± 39.7	0.104
BP	51.5 ± 32.9	57.1 ± 29.9	46.5 ± 35.2	0.421
GH	49.7 ± 27.7	57.9 ± 23.8	42.3 ± 29.3	0.06
VT	43.6 ± 27.8	53.5 ± 22.8	34.8 ± 29.3	0.089
SF	47.9 ± 32.1	52.2 ± 30.5	44.2 ± 33.6	0.369
RE	42.1 ± 42.4	55.0 ± 40.9	30.7 ± 41.0	0.73
MH	47.3 ± 29.6	56.5 ± 22.6	39.2 ± 32.9	0.008
MCS	46.1 ± 29.6	55.04 ± 24.9	38.2 ± 31.6	0.064
PCS	54.2 ± 28.4	62.1 ± 22.7	47.1 ± 31.4	0.009
TCS	50.9 ± 29.4	59.1 ± 24.2	43.7 ± 32.2	0.021

PF – physical functioning; RP – role functioning physical; BP – bodily pain; GH – general health; VT – vitality; SF – social functioning; RE – role functioning emotional; MH – mental health; MCS – mental composite score; PCS – physical composite score; TCS – total composite SF-36 score.

HRQoL domains showed reduced values in the group caring for children with CSS > 20, but theres was no statistically significant difference. A significant statistical difference was found between these two groups in BDI-II scores (Table 4).

Multiple regression analysis identified CSS and mothers` age as factors significantly influencing depression level and all HRQoL composite scores. Multivariate model showed also that employment status significantly affected mothers` depression level (Table 5).

Table 5

Multiple linear regression model using significant values to predict health-related quality of life and depression of caregivers

Variable	β coefficient	*p*-value
PCS		
age of mothers	-1.803	0.001
CSS	-1.164	0.040
MCS		
age of mothers	-1.738	0.002
CSS	-1.205	0.041
TCS		
age of mothers	-1.817	0.002
CSS	-1.171	0.049
BDI-II		
age of mothers	1.034	0.000
employment status	10.13	0.019
CSS	0.509	0.034

PCS – physical composite score; MCS – mental composite score; TCS – total composite SF-36 score; BDI-II – Beck Depression Inventory-II; CSS – Clinical Severity Score.

Discussion

Challenges of caring for children with RTT are only sparsely reported in the literature. A substantial number of studies have found that HRQoL is significantly worse in mothers caring for a disabled child compared with mothers of children without disability [22–24]. Rett syndrome is a severe neurodevelopmental disorder, so our study aimed to confirm impact of debilitating disease on psychological and the physical functioning of care giving mothers. We assessed HRQoL and presence of depression in 49 mothers caring for children with RTT. We also analyzed possible correlations of HRQoL, depression level, clinical severity of RTT and sociodemographic characteristics of mothers. Clinical severity and BDI-II scores were found to be significantly related to all the domains and composite scores of SF-36. These findings are in accordance with investigations of caregivers for patients with different chronic diseases [25–27]. Thus, more severe clinical manifestations of RTT in children were correlated to higher level of depression and lower HRQoL of their mothers. Also, significantly lower BDI-II, MH, PF, PCS and TCS in group caring for more severely affected children (CSS ≤ 20) further pointed out the impact of clinical severity of child`s disease on parental well-being.

The presence of severe depression (BDI-II score ≥ 29) in 30.6% of care giving mothers is similar to findings of studies involving primary caregivers of children with disabilities [15, 28]. Most of studies investigating mental health of parents with disabled children have found higher scores for maternal depression compared to control groups [29]. We decided to address only maternal HRQoL and depression since a number of research consistently reported that fathers of children with disabilities show normal depression scores [30, 31]. Observation that mothers experience more distress than fathers could be caused by the fact that mothers take on a larger part of care and practical work that children with disabilities require. More proper burden measures could substantiate this hypothesis for RTT caregivers in future studies.

A significant negative correlation of BDI-II scores to CSS that we proved in our study also corresponds to findings that severity of clinical manifestations in children with disability is closely related to parental psychosocial stress [32–34]. However, some studies that addressed depression in parents with children affected with cerebral palsy did not find a significant correlation of depression and clinical severity of child's disease [27, 34].

Bahi-Buisson et al. [17] indicated that the presence of epileptic and non-epileptic seizures in RTT patients had a significant impact on their family's quality of life. Multivariate regression analysis that we performed showed no significant influence of seizure presence to HRQoL domains or depression level. On the other hand, CSS of RTT patients was identified as significant factor that adversely affect all SF-36 composite scores and BDI-II score in their mothers. Calculating CSS includes the presence of epilepsy among the variety of other signs and symptoms encountered in RTT.

A recent Turkish study pointed out a significant negative correlation between BDI scores and all domains of HRQoL tested by Nottingham Health Profile with maternal educational level having strongest impact on HRQoL [35]. Maternal education was recognized as a predictor of maternal depression and lower domain scores of HRQoL in other studies [36]. Our study did not show any significant influence of maternal education to HRQoL and depression level. The most probable reason is our small study sample with only 4 mothers with college education.

Studies conducted in patients with different neurologic diseases or their caregivers (muscular dystrophies, multiple sclerosis) showed a significant negative correlation between depression and HRQoL in tested subjects [18, 37]. Similar results were obtained in our study. These findings indicate that depression associated with chronic disease significantly affects HRQoL, both in patients and their caregivers.

The largest study to date involving HRQoL in RTT caregivers observed lowest score for MCS among composite HRQoL scores [15], similarly to our study. A high prevalence of severe depression in our group could be related to lower scores in the mental health domain of SF-36. Laurvick et al. [15] also identified family income and behaviour problems in RTT affected children as the strong predictors of lower mental health scores, while age of mothers did not affect mental or physical health [15]. In our study, family income was not proved as a significant "buffer" of psychosocial stress. This finding does not correspond to a number of studies dealing with caregivers of children with disabilities [15, 36]. There are, however, researchers who, similarly to our results, did not prove significant influence of family income on care-

giver well-being [10, 12, 16]. Our study showed that age of mothers had significant impact on investigated outcomes (BDI-II score and all SF-36 composite scores), while unemployment was a significant predictor of higher depression level.

The domains of HRQoL mainly affected in our study group were RE, MH and VT scores. Other HRQoL studies with caregivers of children with disability reported similar experience [35, 38]. Dividing the study group on the basis of children`s CSS, showed significantly lower HRQoL scores and higher depression level in mothers with more severely affected children. This result strongly contributes to finding that clinical severity of the childs` disease is one one of the strongest factors influencing HRQoL and depression level. This is in accordance to the conclusions of a large Canadian study that identified care giving demands and child behaviour as significant influencing factors on emotional and physical well-being of caregivers for children with cerebral palsy [38].

Our study has few considerable limitations. A cross-sectional design limits the possibility of discerning causal relationships and relatively small number of participants implies the need for multicentric study. Future studies should include prospective repeated measurements of HRQoL and depression in order to obtain more accurate conclusions. Also, the genetic profile of patients was not analyzed as possible predictor of investigated outcomes, since only 53% of patients had been established with molecular diagnosis. However, a study with RTT caregivers did not show a correlation of genotype (MECP mutations) and caregiver HRQoL [21].

Conclusion

Our study showed a high prevalence of depression among mothers caring for children with Rett syndrome. Mostly affected domains of HRQoL in this population were role functioning emotional, vitality and mental health, all significantly influenced by maternal age and clinical severity of their children`s disease. The results of this study can help in planning subsequent interventions directed at families dealing with Rett syndrome. On the basis of our findings, future interventions should include early recognition of depression symptoms, providing better employment possibilities for mothers giving care to children with RTT and improvement of specific medical measures to alleviate clinical severity of affected children.

R E F E R E N C E S

1. *Weaving LS, Ellaway CJ, Gecz J, Christodoulou J.* Rett syndrome: clinical review and genetic update. J Med Genet 2005; 42(1): 1–7.
2. *Bienvenu T, Philippe C, De Roux N, Raynaud M, Bonnefond JP, Pasquier L,* et al. The incidence of Rett syndrome in France. Pediatr Neurol 2006; 34(5): 372−5.
3. *Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY.* Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature Genet 1999; 23(2): 185–8.
4. *Huppke P, Laccone F, Kramer N, Engel W, Hanefeld F.* Rett syndrome: analysis of MECP2 and clinical characterization of 31 patients. Hum Mol Genet 2000; 9(9): 1369-75.
5. *Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahi-Buisson N,* et al. Rett Syndrome: Revised Diagnostic Criteria and Nomenclature. Ann Neurol 2010; 68(6): 944−50.
6. *Williamson S, Christodoulou J.* Rett syndrome: new clinical and molecular insights. Eur J Hum Genet 2006; 14(8): 896–903.
7. *Bebbington A, Anderson A, Ravine D, Fyfe S, Pineda M, de Klerk N.* Investigating genotype–phenotype relationships in Rett syndrome using an international data set. Neurology 2008; 70(11): 868−75.
8. *Glaze DG, Percy AK, Skinner S, Motil KJ, Neul JL, Barrish JO.* Epilepsy and the natural history of Rett syndrome. Neurology 2010; 74(11): 909−12.
9. *Breslau N, Staruch KS, Mortimer EA.* Psychological distress in mothers of disabled children. Am J Dis Child 1982; 136(8): 682−6.
10. *Blacher J, Shapiro J, Lopez S, Diaz L.* Depression in Latina mothers of children with mental retardation: a neglected concern. Am J Ment Retard 1997; 101(5): 483−96.
11. *Brehaut JC, Kohen DE, Raina P, Walter SD, Russell DJ, Swinton M.* The Health of Primary Caregivers of Children With Cerebral Palsy: How Does It Compare With That of Other Canadian Caregivers? Pediatrics 2004; 114(2): 182−91.
12. *Bourke J, Ricciardo B, Bebbington A, Aiberti K, Jacoby P, Dyke P.* Maternal physical and mental health in children with Down syndrome. J Pediatr 2008; 153(3): 320–6.
13. *Mobarak R. Khan N, Munir S, Zaman S, McConachie H.* Predictors of Stress in Mothers of Children With Cerebral Palsy in Bangladesh. J Pediatr Psychol 2000; 25(6): 427−33.
14. *Manuel J, Naughton M, Balkrishnan R, Smith BP, Koman LA.* Stress and Adaptation in Mothers of Children With Cerebral Palsy. J Pediatr Psychol 2003; 28(3): 197−201.
15. *Laurvick CL, Msall ME, Silburn S, Bower C, de Klerk N, Leonard H.* Physical and Mental Health of Mothers Caring for a Child With Rett Syndrome. Pediatrics 2006; 118(4): 1152−64.
16. *Moore H, Leonard H, de Klerk N, Robertson I, Fyfe S, Christodoulou J.* Health Service Use in Rett Syndrome. J Child Neurol 2005; 20(1): 42−50.
17. *Bahi-Buisson N, Guellec I, Nabbout R, Guet A, Nguyen G, Dulac O,* et al. Parental view of epilepsy in Rett Syndrome. Brain Dev 2008; 30(2): 126−30.
18. *Drulovic J, Pekmezovic T, Matejic B, Mesaros S, Manigoda M, Dujmovic I,* et al. Quality of life in patients with multiple sclerosis in Serbia. Acta Neurol Scand 2007; 115(3): 147–52.
19. *Tovilović S, Novović Z, Mihić Lj. Jovanović V.* The role of trait anxiety in induction of state anxiety. Psihologija 2009; 42(4): 491−504.
20. *Beck AT, Steer RA, Brown, GK.* Beck Depression Inventory. 2nd ed. San Antonio, TX: The Psychological Corporation; 1996.
21. *Neul JL, Fang P, Barrish J, Lane J, Caeg EB, Smith EO,* et al. Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome. Neurology 2008; 70(16): 1313−21.
22. *Cadman D, Rosenbaum P, Boyle M, Offord DR.* Children with chronic illness: family and parent demographic characteristics and psychosocial adjustment. Pediatrics 1991; 87(6): 884 –9.
23. *Dyson LL.* Response to the presence of a child with disabilities: parental stress and family functioning over time. Am J Ment Retard 1993; 98(2): 207–18.

24. *Friedrich WN, Friedrich WL.* Psychosocial assets of parents of handicapped and nonhandicapped children. Am J Ment Defic 1981; 85(5): 551–3.
25. *Cushner-Weinstein S, Dassoulas K, Salpekar JA, Henderson SE, Pearl PL, Gaillard WD,* et al. Parenting stress and childhood epilepsy: the impact of depression, learning, and seizure-related factors. Epilepsy Behav 2008; 13(1): 109−14.
26. *Shaligram D, Girimaji SC, Chaturvedi SK.* Quality of life issues in caregivers of youngsters with thalassemia. Indian J Pediatr 2007; 74(3): 275−8.
27. *Sajedi F, Alizad V, Malekkhosravi G, Karimlou M, Vameghi R.* Depression in Mothers of Children with Cerebral Palsy and Its Relation to Severity and Type of Cerebral Palsy. Acta Med Iranica 2010; 48(4): 250−4.
28. *Ones K, Yilmaz E, Cetinkaya B, Caglar N.* Assessment of the quality of life of mothers of children with cerebral palsy (primary caregivers). Neurorehabil Neural Repair 2005; 19(3): 232−7.
29. *Veisson M.* Depression symptoms and emotional states in parents of disabled and non-disabled children. Soc Behav Personal 1999; 271(11): 87−98.
30. *Wolf L, Noh S, Fisman S, Speechley M.* Psychological effects of parenting stress on parents of autistic children. J Autism Dev Disord 1989; 19(1): 157−66.
31. *Dagenais L, Hall N, Majnemer A, Birnbaum R, Dumas F, Gosselin J,* et al. Communicating a diagnosis of cerebral palsy: caregiver satisfaction and stress. Pediatr Neurol 2006; 35(6): 408−14.
32. *Altindag O, Iskan A, Akcan S, Koksal S, Ercin M, Ege L.* Anxiety and Depression Levels in Mothers of Children with Cerebral Palsy. Turk J Phys Med Rehab 2007; 53: 22−4.
33. *Blacher J, McIntyre LL.* Syndrome specificity and behavioural disorders in young adults with intellectual disability: cultural differences in family impact. J Intellect Disabil Res 2006; 50 (Pt 3): 184−98.
34. *Lambrenos K, Weindling AM, Calam R, Cox AD.* The effect of a child's disability on mother's mental health. Arch Dis Child 1996; 74(2): 115−20.
35. *Bumin G, Günal A. Tükel F.* Anxiety, depression and quality of life in mothers of disabled children. SDÜ Týp Fak Derg 2008; 15(1): 6−11.
36. *Fávero-Nunes MA, dos Santos MA.* Depression and quality of life in mothers of children with pervasive developmental disorders. Rev Lat Am Enfermagem 2010; 18(1): 33−40.
37. *Im SH, Lee SC, Moon JH, Park ES, Park YG.* Quality of life for primary caregivers of muscular dystrophy patients in South Korea. Chinese Med J 2010; 123(4): 452−7.
38. *Raina P, O'Donnell M, Rosenbaum P, Brehaut J, Walter SD, Russell D.* The Health and Well-Being of Caregivers of Children With Cerebral Palsy. Pediatrics 2005; 115(6): 626−36.

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Conversion from calcineurin inhibitors to sirolimus of recipients with chronic kidney graft disease grade III for a period 2003–2011

Konverzija sa kalcineurinskih inhibitora na sirolimus kod primalaca sa hroničnom insuficijencijom bubrežnog grafta trećeg stepena u periodu 2003–2011.

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Abstract

Background/Aim. Tremendous breakthrough in solid organ transplantation was made with the introduction of calcineurin inhibitors (CNI). At the same time, they are potentially nephrotoxic drugs with influence on onset and progression of renal graft failure. The aim of this study was to evaluate the outcome of a conversion from CNI-based immunosuppressive protocol to sirolimus (SRL) in recipients with graft in chronic kidney disease (CKD) grade III and proteinuria below 500 mg/day. **Methods.** In the period 2003–2011 24 patients (6 famale and 18 male), mean age 41 ± 12.2 years, on triple immunosuppressive therapy: steroids, antiproliferative drug [mycophenolate mofetil (MMF) or azathiopirine (AZA)] and CNI were switched from CNI to SRL and followe-up for 76 ± 13 months. Nine patients (the group I) had early postransplant conversion after 4 ± 3 months and 15 patients (the group II) late conversion after 46 ± 29 months. During the regular outpatient controls we followed graft function through the serum creatinine and glomerular filtration rate (GFR), proteinuria, lipidemia and side effects. **Results.** Thirty days after conversion, in all the patients GFR, proteinuria and lipidemia were insignificantly increased. In the first two post-conversion months all the patients had at least one urinary or respiratory infection, and 10 patients reactivated cytomegalovirus (CMV) infection or disease, and they were successfully treated with standard therapy. After 21 ± 11 months 15 patients from both groups discontinued SRL therapy due to reconversion to CNI (10 patients) and double immunosuppressive therapy (3 patients), return to hemodialysis (1 patient) and death (1 patient). Nine patients were still on SRL therapy. By the end of the follow-up they significantly improved GFR (from 53.2 ± 12.7 to 69 ± 15 mL/min), while the increase in proteinuria (from 265 ± 239 to 530.6 ± 416.7 mg/day) and lipidemia (cholesterol from 4.71 ± 0.98 to 5.61 ± 1.6 mmol/L and triglycerides from 2.04 ± 1.18 to 2.1 ± 0.72 mmol/L) were not significant. They were stable during the whole follow-up period. Ten patients were reconverted from SRL to CNI due to the abrupt increase of proteinuria (from 298 ± 232 to 1639 ± 1641/mg day in 7 patients), rapid growth of multiple ovarian cysts (2 patients) and operative treatment of persisted hematoma (1 patient). Thirty days after reconversion they were stable with an insignificant decrease in GFR (from 56.10 ± 28.09 to 47 ± 21 mL/min) and significantly improved proteinuria (from 1639 ± 1641 to 529 ± 688 mg/day). By the end of the follow-up these patients showed nonsignificant increase in the serum creatinine (from 172 ± 88 to 202 ± 91 mmol/L), decrease in GFR (from 56.10 ± 28.09 to 47 ± 21 mL/day) and increased proteinuria (from 528.9 ± 688 to 850 ± 1083 mg/min). **Conclusion.** In this small descriptive study, conversion from CNI to SRL was followed by an increased incidence of infections and consecutive 25–50% dose reduction in the second antiproliferative agent (AZA, MMF), with a possible influence on the development of glomerulopathy in some patients, which was the major reason for discontinuation of SRL therapy in the 7 (29%) patients. Nine (37.5%) of the patients experienced the greatest benefit of CIN to SRL conversion without serious post-conversion complications.

Key words:
kidney transplantation; graft survival; kidney failure, chronic; calcineurin; sirolimus; disease progression.

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Apstrakt

Uvod/Cilj. Značajan prodor u transplantaciji solidnih organa postignut je uvođenjem kalcineurinskih inhibitora (KNI). Istovremeno, njihovi potencijalno nefrotoksični efekti mogu da doprinesu nastanku i progresiji insuficijencije bubrežnog grafta. Cilj ispitivanja bio je da se utvrdi ishod konverzije sa imunosupresivnih protokola baziranih na KNI na sirolimus (SRL) kod primalaca sa trećim stepenom hronične bubrežne slabosti grafta i proteinurijom manjom od 500 mg/dan. **Metode.** U periodu od 2003. do 2011. 24 bolesnika (6 žena i 18 muškaraca), prosečne starosti 41 ± 12,2 godine, na trostrukoj imunosupresivnoj terapiji: steroidi, antiproliferativni lek [mekofenolat mofetil (MMF)/ azatioprin (AZA)] i KNI prevedeno je sa KNI na SRL i praćeno 76 ± 13 meseci. Devet bolesnika (I grupa) prevedeno je rano, tokom prve postransplantacione godine (4 ± 3 meseca) i 15 kasno, nakon prve godine (46 ± 29 meseci). Tokom redovnih ambulantnih kontrola pratili smo funkciju grafta praćenjem vrednosti serumskog kreatinina, jačine glomerulske filtarcije (JGF), proteinurije i lipidemije. **Rezultati.** Tridesetog dana nakon konverzije kod svih bolesnika vrednosti JGF, proteinurije i lipidemije bile su neznato povećane. Tokom prva dva meseca svi bolesnici imali su makar jednu urinarnu ili respiratornu infekciju, a kod 10 bolesnika se reaktivirala citomegalovirusna infekcija/bolest, Bolesnici su uspešno izlečeni standardnom terapijom. U preriodu od 21 ± 11 meseci kod 15 bolesnika iz obe grupe obustavljena je terapija SRL zbog: rekonverzije na KNI (10 bolesnika) ili dvostruke imunosupresivne terapije (3 bolesnika), vraćanja na hemodijalizu (1 bolesnik) i smrti (1 bolesnik). Devet bolesnika bilo je i dalje na terapiji SRL. Do kraja praćenja oni su znatno popravili JGF (sa 53,2 ± 12,7 na 69 ± 15 mL/min), a neznatno povećali proteinuriju (sa 265 ± 239 na 530,6 ± 416,7 mg/dan) i lipidemiju (holesterol sa 4,71 ± 0,98 na 5,61 ± 1,6 mmol/L i trigliceride sa 2,04 ± 1,18 na 2,1 ± 0,72 mmol/L). Svi su bili stabilni tokom praćenja. Deset bolesnika vraćeno je na KNI zbog naglog povećanja proteinurije, sa 298 ± 232 na 1639 ± 1641 mg/dan (7 bolesnika), brzog rasta multiplih ovarijalnih cista (2 bolesnika) i operativnog lečenja perzistentnog hematoma (1 bolesnik). Od rekonverzije do kraja praćenja bili su stabilni, ali sa neznatnim sniženjem JGF (sa 56,10 ± 28,09 na 47 ± 21 mL/min) i značajno nižom proteinurijom (sa 1639 ± 1641 na 529 ± 688 mg/dan). Do kraja praćenja kod njih se neznatno povećala vrednost serumskog kreatinina (sa 172 ± 88 na 202 ± 91 mmol/L), smanjila vrednost JGF (sa 56,10 ± 28,09 na 47 ± 21 mL/min) i povećala proteinurija (sa 528,9 ± 688 na 850 ± 1083 mg/dan). **Zaključak.** U ovom malom deskriptivnom ispitivanju prevođenje sa KNI na SRL bilo je praćeno većom incidencijom infektivnih komplikacija, što je uslovilo sniženje doze drugog antiproliferativnog leka (AZA ili MMF) za 25–50% i moguće imalo uticaj na pojavu glomerulopatije, koja je bila razlog za prekid terapije sirolimusom kod sedam (29%) bolesnika. Najveću korist od konverzije sa CNI na SRL imalo je devet (37,5%) bolesnika koji nisu ispoljili značajne komplikacije nakon konverzije.

Ključne reči:
transplantacija bubrega; graft, preživljavanje; bubreg, hronična insuficijencija; kalcineurin; sirolimus; bolest, progresija.

Introduction

Possible beneficial effects of early postransplant conversion from calcineurin inhibitors (CNI) to sirolimus (SRL) in patients with normal renal graft function and proteinuria have been well documented in recently published papers [1–4]. There are only a few papers with a small number of patients dealing with conversion from CNI to SRL-based protocol in patients with graft chronic kidney disease [5–11]. The main idea of this conversion was to prolong the duration of graft chronic kidney disease (CKD) and postpone the dialysis. It is well-known that patients who are on these less toxic protocols are at a higher risk for subclinical, acute and chronic rejection if, through SRL concentration was lower than 10–15 ng/mL in the first 6 months [11]. In the same time the risk for malignancies [12–14] and cardiovascular events should be decreased [15, 16].

The aim of the study was to investigate the outcome of conversion from immunosuppressive protocol based on CNI to SRL, measuring glomerular filtration rate (GFR) estimated by the Cockcroft-Gault equation, proteinuria, lipidemia and resistive index of interlobar arteries, as well as side effects in patients with graft CKD grade III and proteinuria below 500 mg/day.

Methods

From March 2003 to December 2011, 24 patients (6 females and 18 males) were switched from immunosuppressive protocols based on CNI (15 cyclosporine A and 9 tacrolimus) with steroids and antiproliferative agent [azathioprine (AZA) or mycophenolate mofetil (MMP)] to SRI and prospectively followed 76 ± 13 months.

Before transplantation, all the patients had slowly progressive chronic kidney disease that lasted more than 10 years: chronic glomerulonephritis (21 patients), endemic nephropathy (1 patient) and congenital anomalies (2 patients). The patients with glomerulonephritis presented mostly with daily proteinuria below 1 or 2 gr, some of them with intermittent or persistent microhematuria and mild, easily controlled hypertension (with ACE inhibitor and/or diuretics). Only four patients were histologically verified: three with IgA nephropathy and one with rapidly progressive glomerulonephritis (anti-glomerular basement membrane positive with isolated renal disease).

Eighteen patients received a graft from living related donors, and six from deceased donors. HLA matching was 50 ± 12,5%, panel reactive antibody (PRA) was zero and pretransplant lymphocytotoxic cross match was negative. All the patients had increased blood pressure, kept in target range (120–130/70–80 mmHg) with a low dose of beta blocker in combination with an ACE inhibitor or a calcium channel blocker.

Before conversion from CNI to SRL all the patients had graft CKD grade III estimated by the Cockcroft-Gault equation and daily proteinuria below 500 mg. Graft function

in these patients was suboptimal initially, due to older donor age and nephroangiosclerosis, or as a consequence of an ischemic-reperfusion injury. Although most of these patients, as expected, had histology verification for mild or moderate tubulointerstitial fibrosis, the aim of this study was to determine clinical parameters, particularly GFR and proteinuria, as markers of efficient conversion from CNI to SRL.

Two groups of patients were formed based on timing of conversion: the early and the late. The early converted group had 9 patients converted before the end of the first postransplant year (mean 4 ± 3 months post-transplant), with proteinuria below 150 mg/day in two patients and from 150–500 mg/day (379 ± 232 mg/day) in seven patients. The late converted group included 15 patients that were switched after the first postransplant year (mean 46 ± 26 months), with proteinuria below 150 mg/day in eight patients and 150–500 mg/day in remaining seven patients (mean 215 ± 207 mg/day).

Before switching to SRL, basic clinical examination was performed. It consisted of physical examination and evaluation of morphology and hemodynamics of renal graft by color Doppler sonography in the level of interlobar arteries. Basic laboratory analysis, blood cell count and standard biochemical parameters were checked (serum creatinine, cholesterol and triglycerides). Graft function was evaluated by serum creatinine and GFR estimated by the Cockcroft – Gault equation. Proteinuria was measured in daily urine samples using the biuret method.

Conversion from CNI to SRL was abrupt. After a night dose of CNI, next morning the first dose of SRL was introduced. First C0 SRL concentration was monitored the third day with target levels of 7–10 ng/mL from 6th to 12th posttransplant months and 5–10 ng/mL after first post transplant year. When SRL reached the target range, the doses of antiproliferative drugs were decreased by 25–50% (MMF from 2 g to maximum 1.5 g, and AZA from 125 or 150 mg to maximum 100 mg). Steroids were kept at maintenance doses (5–10 mg daily).

After conversion, check-ups were on day 3, 6 and 30, then switched to weekly between 3 to 6 months, bi-weekly between 6 to 9 months, monthly between 9 to 12 months and every three months after one year. Follow-up on each visit included serum creatinine, GFR calculated using the Cockcroft-Gault equation and side effects (worsening of hypertension, lipidemia and proteinuria, new episodes of acute rejection, infections, acute cardiovascular incidents, patients and graft survival and new onset of malignancies). Worsening of proteinuria above 1 g/day was a marker of glomerulopathy development in the course of graft CKD.

All blood analyses were done in our central laboratory using standard procedures. Hematologic analyses were performed on autoanalyzer (Bayer). Creatinine concentration was measured colorimetrically with alkaline picrate (Dimension RXL Dade Behring).

The Cockcroft–Gault equation allows creatinine clearance to be estimated from the serum creatinine:

$$\text{CCr (mL/min)} = \frac{(140 - age) \times \text{lean body weight (kg)}}{\text{CrS [mg/dL] H} \times 72} \times 0.85 \text{ (if female)}$$

SRL C0 concentration was measured with Imx Sirolimus Assay based on MEIA (micro particular enzyme immunoassay) technology (Abbot). Reactivation of cytomegalovirus infection was detected with PCR method on Amplicor Hoffman La Roche with positive test above 400 copies/L.

For statistical analysis we used *t*-test in Excel on standard personal computers. The results of analysis were presented in tables as mean value ± 1 SD (standard deviation) and probability was considered significant if $p < 0.05$.

Results

Initially, all the patients showed a benefit of conversion from CNI to SRL. One month after conversion, the patients from both groups improved graft function and increased lipidemia and proteinuria. Graft hemodynamics, expressed as measured resistive index in interlobar arteries, although significantly increased in the early group, stayed within the referent range (Tables 1 and 2).

Table 1

The results of early conversion from calcincurin inhibitors (CNI) to sirolimus (SRL) in 9 patients

Parameters	CNI ($\bar{x}$ ± SD)	SRL (30th day) ($\bar{x}$ ± SD)	*p*
Creatinine S (μmol/L)	204 ± 74	166 ± 74	ns
GFR (mL/min)	48.9 ± 16	65.6 ± 23.5	ns
Proteinuria 24 h (mg)	379 ± 22	979 ± 1956.	ns
Cholesterol (mmol/L)	4.8 ± 1.5	7.63 ± 0.84	0.001
Triglycerides (mmol/L)	2.63 ± 1.1	4.8 ± 1.74	ns
RI ILA	0.63 ± 0.05	0.68 ± 0.03	0.038

S – serum; GFR – glomerular filtration rate; RI ILA – resistive index in interlobar arteries; ns – non significant.

During the 8-year follow-up, 15 patients (out of 24) discontinued sirolimus therapy for different reasons (Table 3).

Nine patients that remained on sirolimus therapy, followed for 65 ± 20 months, significantly improved hemoglobin and graft function. Proteinuria and lipidemia increased insignificantly and hemodynamic parameters of renal allograft were unchanged (Table 4).

Ten patients were reconverted to CNI after 21 ± 11 months. Initially, upon conversion they insignificantly im-

Table 2

The results of late conversion from calcineurin inhibitors (CNI) to sirolimus (SRL) in 15 patients

Parameters	CNI ($\bar{x}$ ± SD)	SRL (30th day) ($\bar{x}$ ± SD)	*p*
Creatinine S (μmol/L)	202 ± 45	167 ± 35	ns
GFR (mL/min)	52.4 ± 11.4	61.8 ± 19	ns
Proteinuria 24 h (mg)	215 ± 207	1051 ± 1920	ns
Cholesterol (mmol/L)	6.25 ± 4.2	5.63 ± 3.12	ns
Triglycerides (mmol/L)	2.24 ± 1.32	3.18 ± 1.57	ns
RI ILA	0.68 ± 0.06	0.68 ± 0.05	ns

S – serum; GFR – glomerular filtration rate; RI ILA – resistive index in interlobar arteries; ns – non significant.

Table 3

The outcome of conversion from calcineurin inhibitors (CNI) to sirolimus (SRL)

Group	SRL	reCNI	ST + AP	Hd	Death
I	3	4	1	0	1
II	6	6	2	1	0
Total	9	10	3	1	1

The group I – converted from CIN to SRL before the end of the first posttransplant years; the group II – converted from CIN to SRL after the first post-transplant years; reCNI – reconversion to calcineurin inhibitors; ST – steroids; AP – antiproliferative agent; Hd-hemodialysis.

Table 4

The patients with sirolimus (SRL) therapy – initial (start) and final (end) results

Parameters	CNI start ($\bar{x}$ ± SD)	SRL end ($\bar{x}$ ± SD)	*p*
Creatinine S (μmol/L)	203 ± 31	167 ± 28	0.01
GFR (mL/min)	53.2 ± 12.7	69 ± 15	0.014
Proteinuria (mg/day)	265 ± 239	530.6 ± 416.7	0.061
Cholesterol (mmol/L)	4.71 ± 0.98	5.6 ± 1.6	0.116
Triglycerides (mmol/L)	2.04 ± 1.18	2.1 ± 0.72	0.45
Hemoglobin (g/L)	124.2 ± 13.1	140.4 ± 14.7	0.013
RI ILA	0.66 ± 0.05	0.66 ± 0.05	0.37

CNI – celcineurine inhibitors; S – Serum; GFR – glomerular filtration rate; RI ILA – resistive index in interlobar arteries.

proved graft function, but later showed a considerable increase in proteinuria, lipidemia and worsened hemodynamic parameters in the level of kidney interlobar arteries. They were reconverted to CNI due to development of glomerulopathy, which presented with abrupt onset and progressive increase in proteinuria, mostly in subnephrotic range (7 patients), newly formed multiple ovarian cysts with consecutive serious lower extremity edema (2 patients) and an operative treatment (1 patient). The result of reconversion was a nonsignificant decrease in proteinuria and worsening of renal function, lipidemia and resistive index without hemodynamic consequences (Table 5). By the end of the total follow-up, 76 ± 13 months, these patients were still in renal failure grade III, with slowly progressive nonsignificant increase in serum creatinine, proteinuria and decrease in glomerular filtration rate.

Early after the conversion two of the patients developed serious crural edema and multiple ovarian cysts with oligomenorrhea. After reconversion to CNI they lost edema and ovarian cysts and returned to a regular period.

Initially, most of our patients had low C0 SRL concentration (5–7 ng/mL) and showed early infective complications in spite of dose correction of the second antiproliferative agent. All the converted patients had acute pyelonephritis caused by *E. coli* or *Enterobacter*, and two of them had pneumonia caused by *Hemophillus influence*. Ten patients had symptomatic reactivation of cytomegalovirus (CMV) infection, successfully treated with ganciclovir or valganciclovir. Three of the patients with recurrent bacterial or viral infections stopped SRL therapy, and by the end of the follow-up had double immunosuppressive therapy with steroid and a second antiproliferative agent (AZA or MMF). In these patients GFR and serum creatinine remained in the same range as at the time of SRL discontinution.

One of the patients during this period progressed to end-stage renal failure and started dialysis. In the meantime he had a second transplantation.

One of the patients with apparently inadequate compliance and blood pressure control died after acute massive intracranial bleeding.

No new onset of malignancies was noticed in any of the followed patients.

In all the followed patients antihypertensive therapy was unchanged, but the dose of hypolipemic agents was increased.

Table 5

The patients converted from calcineurin inhibitors (CNI) to sirolimus (SRL) and reconverted to CNI (reCIN) on day 30, and at the end of follow-up (after 76 ± 13months)

Parameters	CNI start ($\bar{x}$ ± SD)	SRL end ($\bar{x}$ ± SD)	*p* CNI start : SRLend	reCNI, 30th day ($\bar{x}$ ± SD)	CNI end ($\bar{x}$ ± SD)	*p* reCNI:CNI end
CreatinineS (μmol/L)	185 ± 76	167 ± 61	0.28	172 ± 88	202 ± 91	
GFR (mL/min)	49.45 ± 13,54	55.82 ± 22.67	0.23	56.10 ± 28.09	47 ± 21	
Proteinuria (mg/day)	298 ± 232.13	1639 ± 1641*	0.015	528.9 ± 688*	850 ± 1083	ns* / ns
Cholesterol (mmol/L)	5.6 ± 1.6	7.22 ± 0.95	0.011			
Triglycerides (mmol/L)	2.39 ± 0.68	4.37 ± 1.42	0.001			
RI ILA	0.66 ± 0.06	0.69 ± 0.04	0.177			

*** – Compared values at the end of SRL therapy to those 30 days after reconversion to CNI; GFR – glomerular filtration rate; RI ILA – resistive index in interlobar arteries; ns – non significant.**

Discussion

Some authors proposed less toxic immunosuppression for all renal allograft recipients to minimize the risk for development of chronic allograft nephropathy, decrease the incidence of cardiovascular deaths and new onset of malignancies [12–16]. The main idea for patients who already had graft dysfunction was avoiding calcineurine inhibitors and their toxic effects on graft hemodynamics, which potentially could accelerate progression of renal failure [16]. The question remains when the right time is for conversion from CNI to SRL and who should be converted.

It appears that the timing of conversion is not important, while the initial level of renal graft damage is. The CONVERT trial showed that the best results of conversion from calcineurin inhibitors to sirolimus showed patients with GFR above 40 mL/min and urine protein/creatinine ratio less than 0.11 [1, 2].

The effect of sirolimus on growth factor inhibition resulted in a specific profile of side effects: increased incidence of postoperative liquid collection, decelerated wound healing and function recovery of primary non-functioning grafts [17, 18]. Some authors believe that SRL special profile of side effects is dose-dependent [18].

Sirolimus should not be introduced immediately in the postoperative period and should be avoided in marginal grafts, patients with long cold ischemia time and high-risk patients. The best results were seen in patients with low immunological risk and without graft dysfunction. Potentially good candidates may also be patients with stent in renal artery and newly discovered skin malignancies.

The conversion can be abrupt, but with good C0 SRL concentration, especially in the first six postransplant months (10–15 ng/mL) to ensure the minimal risk for subclinical, acute and chronic rejection. Our patients had lower target concentrations in the first six months due to serious and recurrent infective complications. None of our patients in the first post-conversion year had a clinical episode of acute rejection, or worsening of chronic graft dysfunction.

Introducing SRL in immunosuppressive protocol since 2003, we converted all those stable, low-risk graft recipients in grade III renal failure in the first post-transplant year and later, with normal or slightly increased proteinuria, for which we thought that they could benefit from CNI withdrawal.

High doses of two antiproliferative immunosuppressive agents acting in different phases of T and B lymphocyte cell cycles may potentially result in increase in infectious complication, or facilitate myelotoxic effects or digestive symptoms [9].

Ten (42%) of the patients in the period of 21 ± 11 months after the initial conversion, reconverted to CNI after the increase of 24 h proteinuria above 2 g. The development of glomerulopathy worsened the course of pre-exisiting chronic graft dysfunction, but without a significant change in urinary sediment, serum creatinine or GFR. Histological examination showed focal and segmental glomerulosclerosis in all the patients. Thirty days after reconversion to CNI, proteinuria was below 0.8 g daily and slowly increased to 1.8 g by the end of the follow-up. Graft function was almost the same as at the beginning of the treatment. Color Doppler evaluation of kidney hemodynamics through resistive indices in interlobar arteries of renal graft stayed in the normal range (below 0.7), which additionally confirmed the stability of graft function and good potential for future graft survival. The reason for such increase in proteinuria could be explained partly by low total dose of immunosuppressive therapy (low C0 SRL concentration combined with additional decrease in the dose of the second antiproliferative agent due to serious infectious complications). Notwithstanding with these findings, some authors reported development of glomerulopathy in the patients with high C0 SRL concentrations [19]. An explanation for this could be that it represents the progression of pre-existing disease, or that it is a consequence of specific SRL side-effect profile with impact on podocytopathy [20].

Almost all the papers about conversion from CNI to SRL stressed the worsening of lipidemia in spite of the correction of hypolipemic therapy. Our patients showed insignificant increase in serum lipids. They were not using hypolipemic therapy regularly because they could not afford or refund this expensive therapy. The patients with SRL therapy, in spite of the persistent dyslipidemia, did not show deteriorating effect on graft function.

Conclusion

In this small prospective and descriptive study CNI to SRL conversion was followed by the increased incidence of infections and consecutive 25–50% dose reduction of the se-

cond antiproliferative agent (AZA, MMF), with a possible influence on the development of glomerulopathy in some patients, which was the major reason for discontinuation of SRL therapy in 7 (29%) of the patients. Nine (37.5%) of the patients experienced the greatest benefit of CNI to SRL conversion without serious post-conversion complications.

Worsened lipidemia could be corrected with regular use and proper dose of hypolipemic agents and did not influence GFR in patients on SRL therapy. Patients reconverted to calcineurin inhibitors showed slow and progressive chronic kidney graft disease.

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R E F E R E N C E S

1. *Schena FP, Pascoe MD, Alberu J, del Carmen Rial M, Oberbauer R, Brennan DC,* et al. Conversion from calcineurin inhibitors to sirolimus maintenance therapy in renal allograft recipients: 24-month efficacy and safety results from the CONVERT trial. Transplantation 2009; 87(2): 233−42.
2. *Hanto DW, Chudzinski R.* What does the CONVERT trial really tell us about conversion from calcineurin inhibitors to sirolimus? Transplantation 2009; 87(2): 164−5.
3. *Flechner SM.* Sirolimus in kidney transplantation indications and practical guidelines: de novo sirolimus-based therapy without calcineurin inhibitors. Transplantation 2009; 87(8 Suppl): S1−6.
4. *Larson TS, Dean PG, Stegall MD, Griffin MD, Textor SC, Schwab TR,* et al. Complete avoidance of calcineurin inhibitors in renal transplantation: a randomized trial comparing sirolimus and tacrolimus. Am J Transplant 2006; 6(3): 514−22.
5. *Saurina A, Campistol JM, Piera C, Diekmann F, Campos B, Campos N,* et al. Conversion from calcineurin inhibitors to sirolimus in chronic allograft dysfunction: changes in glomerular haemodynamics and proteinuria. Nephrol Dial Transplant 2006; 21(2): 488−93.
6. *Weir MR, Blahut S, Drachenburg C, Young C, Papademitriou J, Klassen DK,* et al. Late calcineurin inhibitor withdrawal as a strategy to prevent graft loss in patients with suboptimal kidney transplant function. Am J Nephrol 2004; 24(4): 379−86.
7. *Abramowicz D, Hadaya K, Hazzan M, Broeders N, Hoang AD, Ghisdal L,* et al. Conversion to sirolimus for chronic renal allograft dysfunction: risk factors for graft loss and severe side effects. Nephrol Dial Transplant 2008; 23(11): 3727−9.
8. *Diekmann F, Budde K, Oppenheimer F, Fritsche L, Neumayer HH, Campistol JM.* Predictors of success in conversion from calcineurin inhibitor to sirolimus in chronic allograft dysfunction. Am J Transplant 2004; 4(11): 1869−75.
9. *Diekmann F, Campistol JM.* Conversion from calcineurin inhibitors to sirolimus in chronic allograft nephropathy: benefits and risks. Nephrol Dial Transplant 2006; 21(3): 562−8.
10. *Wali RK, Mohanlal V, Ramos E, Blahut S, Drachenberg C, Papadimitriou J,* et al. Early withdrawal of calcineurin inhibitors and rescue immunosuppression with sirolimus-based therapy in renal transplant recipients with moderate to severe renal dysfunction. Am J Transplant 2007; 7(6): 1572−83.
11. *Amer H, Cosio FG.* Significance and management of proteinuria in kidney transplant recipients. J Am Soc Nephrol 2009; 20(12): 2490−2.
12. *Webster AC, Lee VW, Chapman JR, Craig JC.* Target of rapamycin inhibitors (sirolimus and everolimus) for primary immunosuppression of kidney transplant recipients: a systematic review and meta-analysis of randomized trials. Transplantation 2006; 81(9): 1234−48.
13. *Salgo R, Gossmann J, Schöfer H, Kachel HG, Kuck J, Geiger H,* et al. Switch to a sirolimus-based immunosuppression in long-term renal transplant recipients: reduced rate of (pre-)malignancies and nonmelanoma skin cancer in a prospective, randomized, assessor-blinded, controlled clinical trial. Am J Transplant 2010; 10(6): 1385−93.
14. *Alberú J, Pascoe MD, Campistol JM, Schena FP, Rial Mdel C, Polinsky M,*et al. Lower malignancy rates in renal allograft recipients converted to sirolimus-based, calcineurine inhibitor-free immunotherapy: 24-month results from the CONVERT trial. Transplantation 2011; 92(3): 303−10.
15. *Monaco AP.* The role of mTOR inhibitors in the management of posttransplant malignancy. Transplantation 2009; 87(2): 157−63.
16. *Kasiske BL, Guijarro C, Massy ZA, Wiederkehr MR, Ma JZ.* Cardiovascular disease after renal transplantation. J Am Soc Nephrol 1996; 7(1): 158−65.
17. *Weir MR, Wali RK.* Minimizing the risk of chronic allograft nephropathy. Transplantation 2009; 87(8 Suppl): S14−8.
18. *Ruiz JC, Campistol JM, Sánchez-Fructuoso A, Rivera C, Oliver J, Ramos D,*et al. Increase of proteinuria after conversion from calcineurin inhibitor to sirolimus-based treatment in kidney transplant patients with chronic allograft dysfunction. Nephrol Dial Transplant 2006; 21(11): 3252−7.
19. *Weir MR, Diekmann F, Flechner SM, Lebranchu Y, Mandelbrot DA, Oberbauer R,* et al. mTOR inhibition: the learning curve in kidney transplantation. Transpl Int 2010; 23(5): 447−60.
20. *Letavernier E, Bruneval P, Mandet C, Duong Van Huyen JP, Péraldi MN, Helal I,* et al. High sirolimus levels may induce focal segmental glomerulosclerosis de novo. Clin J Am Soc Nephrol 2007; 2(2): 326−33.

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GENERAL REVIEW

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Insect repellents – transmissive disease vectors prevention

Repelenti – zaštita od vektora transmisivnih oboljenja

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Ključne reči:
repelenti; bolest, prenosioci; preventivno-medicinska zaštita.

Introduction

In the course of evolution, a number of animal species has come into conflict with humans over food and habitat. This conflict is still ongoing, but not in its original form. Through evolution some species had to change their diet and habitat, leading to changes in the structure of the orifice and organs of movement. A number of arthropods got close to people and used all of their omissions for their own survival, without significant morphological and anatomical changes.

By conquering new areas, the man has, through his activities, eradicated a number of species, disturbing the ecological food chains of other species and diverting the flow circulation with infectious agents from the enzootic to the epizootic status leading to the appearance of numerous outbreaks. As a consequence, these shifts and changes lead to the appearance of new diseases in the form of pandemics. Faced with this problem, the man had to change his tactics and make use of all achievements in order to deal with harmful arthropods. However, the target species are becoming resistant, changing their behaviour, and occasionally migrating. In these situations, direct chemical treatments would not yield expected results. Thus, in the last decades there has been an emphasis on the development of various preventive measures, particularly emphasizing the individual protection of people by means of repellents [1].

The testing of substances' repellent properties was initiated in the fifties of the twentieth century and is still ongoing [2–5] . There were different approaches to defining repellents, but in our opinion the definition given by WHO (2006) is complete: repellents are products that are applied to the skin or clothing to prevent or deter arthropods from attacking humans and other animals. They are used in particular against outdoor biting insects and in situations where individual protection is a priority but treated mosquito nets, vaporizing mats and the chemical control methods are not appropriate [6]. The property of repellents to, with their scent, discourage insects from plants, animals or humans was used in healthcare, veterinary and agriculture to protect humans, animals and plants from the attacks and bites of harmful species of arthropods, which, in the process of taking food, take over, maintain and transmit numerous pathogen transmissible infectious-diseases. Mosquitoes serve as vectors responsible for transmitting several forms of viral encephalitis, yellow fever, Dengue fever, Bancroftian filariasis and epidemic polyarthritis to humans; more than 700,000,000 people are infected yearly [2]. Malaria, which is transmitted by the bite of a mosquito infected with the single-celled protozoa of the genus *Plasmodium*, is responsible for 3,000,000 deaths annually . In 1999, West Nile virus was detected for the first time in the Western hemisphere [7–10]. Infected ticks can transmit Lyme disease, Rocky Mountain spotted fever, ehrlichiosis, Q fever, babesiosis, tularemia, STARI (Southern tick-associated rash illness) and tick paralysis [11–14]. Flies are the vectors responsible for transmitting African trypanosomiasis, leishmaniasis, onchocerciasis and loiasis to humans. Flea bites may transmit plague and, in South America, kissing bugs transmit Chagas disease [15].

In this region, apart from the Lyme disease, which was discovered in 1987 and transmitted primarily by ticks, we have seen recently the occurrence of tularemia and the increased number of patients with hemorrhagic fever, where ticks are also primary vectors, or one of the possible means of transmitting infectious agents [16–19].

The infectious potential of mosquitoes in our environment has not been sufficiently investigated. Although it is known that there are potential vectors for malaria pathogens in Serbia, so far there have been no conditions for the occurrence of indigenous cases. In our country there are also reg-

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istered cases of patients with dirofilaria, which is caused by *Dirofilaria immitis* and *Dirofilaria repens* [20, 21]. Vectors of parasites (*D. repens* and *D. imitis*) are the types of mosquitoes that exist in our environment, belonging to the genera Anopheles, Aedes and Culex.

It is known from the literature that some species of mosquitoes in this region (*Aedes vexans*) carry Tahyna transmitted virus, while the two unknown etiological agents were isolated from anofelic mosquitoes. In recent years, there has been an increasing number of meningitis epidemics in our surroundings (Romania), and relevant vectors in our country, too [22] .

These data indicate a need to combat harmful arthropods, to monitor them and research their infectious potential, habitat, abundance, movement and the diet-related behaviour [23, 24].

Preventing people from being bitten by vectors and molesters, involves a series of activities that undermine conditions for their development and appearance of the habitat (draining fields, controlled reclamation, frequent changes of water in catchments), use of nets, impregnated clothing and use of repellents [25–29].

Repellent properties and the mechanism of action

Repellents are by their physical and chemical properties liquid substances, with a characteristic odour that evaporates at room temperature. The relation between chemical structure of substances and repellent efficacy is still unclear, because repellents belong to various groups of chemical compounds. Table 1 shows the structure of the most commonly represented repellents on the market.

It is believed that the demonstration of biological efficacy requires the presence of amides, imides, alcoholic or phenol groups in the molecule. Another important parameter for assessing the effectiveness of a repellent is the boiling point, on which evaporation and the effect substances have on insect olfactory cells (some substances acting mechanically, through contact or on insect's sense of taste) [30, 31].

Volatile repellent molecules behave according to the Fick's diffusion model and evaporate into the atmosphere and penetrate into the skin. Due to this process, there is a decline in its concentration, "sink conditions" on the skin surface and the weakening of the biological response [32].

Understanding the mechanism of repellent's loss from the skin surface is important both from the entomological and the toxicological point of view. If a repellent evaporates faster from the application site than it is absorbed, it will be more effective, and the duration of its response depends on many external and internal factors. Absorbed through the skin, the repellent reaches the systemic circulation and further on all organs and tissues, which can lead to adverse effects to user's body [33, 34].

Studies on insects' neurophysiology show different classes of chemoreceptors on their bodies, which are differently sensitive to certain chemical groups in molecules of repellent substances. According to their irritating effect, chemical dosage groups are classified in the following order: amides > imides > alcohols > phenols [35].

Repellents may act differently on insects' chemoreceptors: inhibit a response of normally sensitive neuron to lactic acid (time depends on repellent concentration); cause weak stimulation of neurons, as an attractant for insects (DEET – diethyl toluamide in low concentrations attracts insects); simultaneously activate different types of receptors, causing insects to behave irritably, disoriented, tending to flee as soon as possible away from the zone of action of repellent evaporations [35].

It is unclear if a repellent works by common mechanisms in different arthropods, thus there is confliting evidence in the literature. So, ticks detect repellent on the first tarsi pair of legs (Haller's organ) and insects detect the same supstances on the antennae.

The widely accepted hypothesis that DEET interferes with the detection of lactic acid has been challenged by the demostrated DEET induced repellence in the absence of lactic acid. A recent investigation on *Anopheles gambiae* has suggested that the olfactory receptor neurons (ORNs) for 1-octen-3-ol, a component of human sweat, that in a combination with CO_2 acts as an attractant for this species, is blocked by DEET.

Biological efficacy of repellents

Two parameters are vital for assessing the biological efficacy of repellents in terms of a minimum effective dose and the time of protection (t) from the bites of insects and ticks. The minimum effective dose (MED) is the smallest amount of repellent that is sufficient to prevent insect bites. Its values are different for different repellents [36]. Also, the same repellent can have different values depending on the type of insect. Maibach et al. [37] define MED as the minimum effective dose of repellent that protects 99% of the body from the bites of hematofage arthropods for 30 min. It is expressed in mg/cm^2.

Figure 1 shows relationship of the time of protection and concentrations of repellents on the skin surface. Immediately after the application of a repellent at the initial time (t_o)

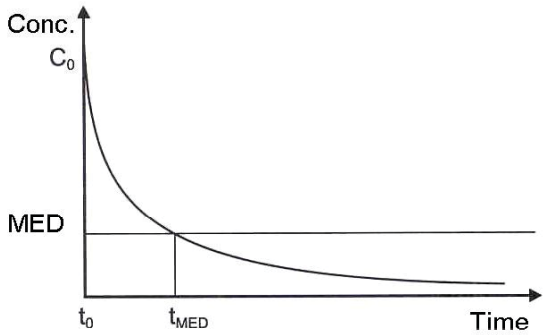


Fig. 1 – The time of protection (t) is the change in concentration of repellent of the skin surface over time (t) = t_{med}-t_0 [37]
t_0 – the initial time (immediately after the application of a repellent on the skin surface); C_0 – the initial concentration of a repellent (immediately after the application on the skin surface); t_{med} – the time during which the concentration of repellent decreases to the minimum effective dose (MED)

its concentration (C_o) is highest. Over time, its concentration decreases reaching in time (t_{MED}) a concentration (Ctmed) equal to the minimum effective dose. The period in which the concentration of C_o is reduced to Ctmed (Ctmed = MED) is called the time of repellent's protection [37]. In addition to determining the minimum effective dose and the time of protection afforded by concentrated substance when applied to the skin, it is more realistic to assess these parameters for the preparations of those substances, which, apart from the repellent substance, contain excipients that significantly influence the behaviour of agents in course of application.

To assess the biological efficacy of repellents, many factors should be taken into account: environmental factors (temperature, time of day, wind speed, light intensity, humidity); factors related to characteristics of the host (individual susceptibility, diet, movement, absorption through the skin, sweating); product characteristics (skin retention time, the ability to form coating, smell, the type of formulation, the concentration of repellent); factors related to the zones or areas for protection (skin characteristics: thickness, age, attrition, the degree of vascularization, injuries).

Categories of insect repellents

Commercially available insect repellents can be divided into two categories: synthetic chemical repellents and plant-derived essential oils (Table 1).

Diethyl toluamide

DEET serves as an effective repellent against mosquitoes, ticks and other arthropods when used on the skin and clothing [38, 39]. DEET is available in repellent preparation concentrations ranging from 5% to 100% although most products contain less than 40%, as the concentration increases with a plateau at 50% [40, 41] (Table 2). In most situations a concentration of 10% to 35% DEET will provide adequate protection. Higher concentrations may be indicated if a high-risk exposure is anticipated. DEET is safe for use on cotton, wool and nylon, although it has been found to damage spandex, rayon, acetate and pigmented leather. DEET may dissolve plastic and vinyl. With proper application the safety record of DEET remains excellent. There have been 43 case reports on DEET toxicity during the past 5 decades including 25 cases with central nervous system symptoms, one case with cardiovascular involvement, and 17 with cutaneous/allergic reactions [42]. Reported central nervous system symptoms include lethargy, confusion, acute manic psychosis, headaches, ataxia, disorientation, acute encephalopathy, convulsions, tremors and seizures [43, 44]. Cardiovascular symptoms include bradycardia and hypotension. Cutaneous and allergic symptoms include anaphylaxis, urticaria, hemorrhagic bullae and erosions [45]. The safety of DEET for children has often been questioned. Of 6 reported deaths involving DEET, 3 were caused by intentional ingestion of

Table 1

The structure of the most commonly represented repellents on the market

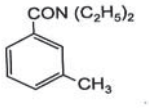
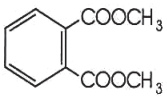
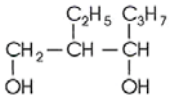
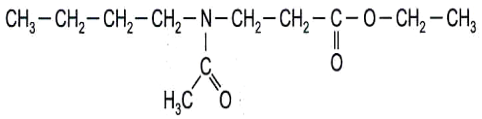
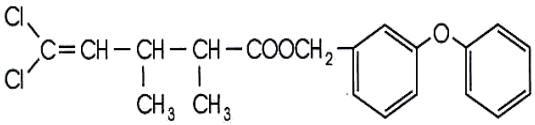
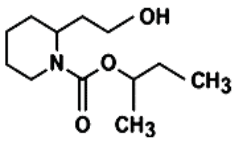
Substances (INCI name)	Structural formula
Diethyl toluamide (DEET)	
Dimethyl phtalate	
Ethyl hexanediol	
Ethyl butylacetylaminopropionate (IR 3535)	
Permethrin	
Hydroxyethyl isobutyl piperidine carboxylate (Piperidin)	

Table 2

Protection time of insect repellents [40, 41]

Active ingredient concentration	Protection time (h)	Formulation
Diethyl toluamide (DEET) < 10%	1–3	Pump spray, aerosol gel, lotion, stick
Diethyl toluamide (DEET) < 10–33%	4–6	Pump spray, aerosol gel, lotion, stick, emulsion
Diethyl toluamide (DEET) 15% Permethrin 0.2%	6–7	Lotion, stick
Ethyl butylacetylaminopropionate (IR 3535) 18%	5–6	Pump spray, stick
Hydroxyethyl isobutyl piperidine carboxylate (Piperidin) 7%	3–4	Pump spray
Hydroxyethyl isobutyl piperidine carboxylate (Piperidin) 15–20%	6–8	Aerosol
Citronella oil 5–15%	20–30 min	Pump spray, lotion, oil, towelette
Lemon eucalyptus oil 10–30%	2–5	Lotion

DEET, one involved a child with ornithine carbamoyltransferase deficiency, and 2 were in children who had central nervous system symptoms after overuse of DEET [46]. Most reported cases of adverse or lethal events involve overuse or incorrect use of the product [47]. Interestingly, increased systemic absorption of DEET has been described with a concurrent use of sunscreen of DEET-containing repellents. A mouse was reported at high dose and included neurotoxic effects such as tremors, loss of coordination, hyperactivity, paralysis, and an increase in body temperature. Other side effects included eye and skin irritation, reproductive effects, mutagenicity, and alterations in the immune system [48, 49].

One cross-sectional survey evaluating parental use of insect repellents indicated that an educational outreach to improve parents' use of insect repellents may prove beneficial [50]. The authors found that education plays a key role, as parents often do not read labels and many left repellents on their children's skin overnight theoretically increasing potential chemical exposure. In addition, some parents treated the child's face directly, a method discouraged in this population because of warnings from poison control centres that eye contact may be associated with a high rate of adverse symptoms.

Effects of DEET on pregnant and lactating women have also been examined [51]. In a study investigating effects of DEET on fetuses of pregnant women, no adverse effects on survival of growth and development at birth and at 1 year of age were detected after maternal exposure during the second or third trimester. In lactating women, no evidence exists that using DEET while breast-feeding causes toxicity to infants.

Hydroxyethyl isobutyl piperidine carboxylate (picaridin, piperidin)

Picardin or piperidin has many characteristics of the ideal insect repellent as it is odourless, does not feel sticky or greasy on application, is less likely to irritate the skin, and will not damage plastics or fabrics. In Europe, solutions with concentrations up to 20% have been demonstrated as protective for up to 8 to 10 hours. Like DEET, picaridin's mechanism of action is unknown, but is thought to provide a vapour barrier that works to deter the insect from biting. Picaridin is effective against mosquitoes, biting flies, and ticks. In 2000, the World Health Organization proclaimed that, because of its safety, effectiveness, and cosmetic properties, picaridin was the recommended product for repelling the mosquitoes that carry malaria noting that, under some circumstances, it was more effective than DEET [52].

Permethrin

Permethrin is a synthetic pyrethroid, acts as a repellent and an insecticide that is highly effective against ticks, mosquitoes, and other arthropods. Permethrin's mechanism of action requires direct contact with the insect, making this compound poorly suited for skin application. Permethrin acts on the nervous system of insects. It interferes with sodium channeles to discrupt the function on neurons, and causes muscules to spasm, culminating in paralysis and death [53, 54]. Permethrin has low mammalian toxicity, is poorly absorbed by the skin, and rapidly by skin and blood esterases. Permethrin may be used on clothing, shoes, bed nets, and camping gear and requires reapplication after every 5 washings. Permethrin insecticide increases the effect of DEET, and this combination is currently mostly used for impregnating clothing and other materials. The application of impregnated materials is common in malaria endemic areas and other areas, to protect military operational units [55].

Ethyl butylacetylaminopropionate (IR3535)

IR3535 is a new alternative to DEET. It has been available in Europe for 20 years and has been sold in the United States since 1999 [56]. In our country IR3535 appeared in 2001 in Pest off (stick and spray) preparation at a concentration of 18%. IR 3535 is labelled for use against mosquitoes, ticks, and biting flies [57, 58]. IR 3535 fulfils all the following requirements for the outstanding properties of a repellent: effective protection of the skin from insects, long-lasting repellent action for several hours, also under difficult climatic conditions, maximum skin and mucous membrane tolerance without toxic allergic or sensitising properties, high chemical stability under use conditions, cosmetic properties, good formulability with common cosmetic and pharmaceutical basic formulations, acceptable costs per use/application of the

final product [59, 60]. On the basis of toxicological studies the US Euvironmental Protection Agency (EPA) has concluded that the IR3535 is practically non-toxic to mammals, including infants and children.

Plant-based repellents

Thousands of plants have been tested as sources of insect repellents. Although none of the plant-derived chemicals tested to date demonstrates the broad effectiveness and duration of the protection of DEET, a few do show repellent activity [61, 62]. Plants whose essential oils reportedly have repellent activity include citronella, neem, tansy, cedar, verbena, pennyroyal, geranium, lavender, pine, cajuput, catnip, cinnamon, rosemary, basil, thyme, allspice, garlic and peppermint. Unlike synthetic insect repellents, plant-derived ones have been relatively poorly studied. When tested, most of the essential oils yielded short-lasting protection, lasting from a few minutes to as long as 2 hours [63, 64].

Relief from insect bites

Cutaneous responses to arthropod bites are polymorphic and range from the common localized wheal-and-flare reactions (type I hypersensitivity) to the delayed bite lesions ((type IV hypersensitivity). Rarely, systemic arthus-type reactions and even anaphylaxis may occur.

Bite reactions are the result of sensitization to salivary antigens, which leads to the formation of both specific immunoglobulin E (IgE) and immunoglobulin G (IgG) antibodies. Immediate-type reactions are mediated by IgE, IgG and histamine, while cell-mediated immunity is responsible for the delayed reactions. Several modalities exist to alleviate the itch of insect bites.

Topical corticosteroids are useful to reduce the associated erythema, itching, and induration of insect bites. In cases of extensive and intensely itching bites, a short and rapidly tapered course of oral prednisone (or its equivalent) is effective in reducing the uncomfortable symptoms of extensive bite reactions. Application of diphenhydramine or benzocaine (an ester-type topical anaesthetic) should be avoided because of the risk of these compounds inducing allergic contact sensitivity. Pramoxine-containing lotions can also help reduce itching.

Oral antihistamines, such as cetirizine and levocetirizine, are effective in reducing itching and swelling associated with insect bites [65, 66]. In individuals who are highly sensitive, nonsedating antihistamines may be successfully taken prophylactically to reduce the subsequent cutaneous reactions to arthropod bites. After Bite (an over-the-counter solution containing ammonium) has been found to relieve the type I hypersensitivity symptoms associated with mosquito bites [67].

Recommendations for the use of insect repellents products are: the product should be applied in a thin layer on the skin; the product is applied only on the skin surface, clothing, or both; it should not be applied under clothing; for the use on face, the product should be first applied on the hand and then carefully spread on the face; contact with the eyes and mouth should be avoided; the person who is applying the product to a child, should carefully apply it on a child's skin, except around the eyes and mouth; children younger than ten years, should not be allowed to independently apply a repellent product; insect repellents should be kept away from children; repellent preparations should not be used for children under two years; a mosquito net should be used for their protection; products that contain DEET in concentrations of less than 10% should be used for the protection of children; after applying repellent products, hands should be wiped (cleaned) to avoid contact with eyes and lips; repellent product should never be applied to cuts, wounds or irritated skin; manufacturer's instructions should be read before the use of a repellent product.

Conclusion

Vector repellent is one element in the prevention of vector-borne diseases. Use of skin repellents can reduce the risk of insect bites and thus infection.

R E F E R E N C E S

1. U.S. EPA. Active Ingradients Found in Insect Repellents. Available from: http://www.epa.gov/pesticides/helath/mosquitoes/ai-insectr.htm [cited 2010 Jannury 28].
2. *Brown M, Hebert AA.* Insect repellents: an overview. J Am Acad Dermatol 1997; 36(2 Pt 1): 243−9.
3. *Kimps NW, Bissinger BW, Apperson CS, Sonenshine DE, Roe RM.* First report of the repellency of 2-tridecanone against ticks. Med Vet Entomol 2011; 25(2): 202−8.
4. *World Health Organization.* Guidelines for efficacy testing of mosquto repelents for human skin. Geneva: World Health Organization; 2009.
5. *Peterson C, Coats J.* Insect repellents-past, present and future. Pest Outlook 2001; 12: 154-158.
6. *World Health Organization.* Personal and household protection. In: *World Health Organization.* Pesticides and their application. 6th ed. Geneva: World Health Organization; 2006. p. 98−103.
7. *Byass P.* Making sense of long-term changes in malaria. Lancet 2008; 372(9649): 1523−5.
8. *World Health Organization.* World Malaria Report 2008. Geneva: World Health Organization; 2008.
9. *World Health Organization.* Dengue and dengue hemorragic fever 2009. Geneva: World Health Organization; 2009.
10. *Neilsen CF, Armijos MV, Wheler S, Carpenter TE, Boyce WM, Kelley K*, et al. Risk factors associated with human infection during the 2006 West Nile virus outbreak in Davis, a residential community in northern California. Am J Trop Med Hyg 2008; 78(1): 53−62.
11. *Stanek G, Fingerle V, Hunfeld KP, Jaulhac B, Kaiser R, Krause A*, et al. Lyme borreliosis: clinical case defination for diagnosis and menagment in Europa. Clin Microbiol Infect 2011; 17(1): 69−79.
12. *Stricker BR, Johnson L.* Lyme disease: the next decade. Infect Drug Resist 2011; 4: 1−9.

13. *World Health Organization*. The vector-borne human infections of Europa their distribution and burden of public health. Geneva: World Health Organization; 2004.
14. *Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T*. Tick-borne encephalitis virus a review of an emerging zoonosis. J Gen Virol 2009; 90(Pt 8): 1781−94.
15. *Joshi A, Narain JP, Prasittisuk C, Bhatia R, Hasim G, Jorge A*, et al. Can visceral leishmaniasis be eliminated from Asia. J Vector Borne Dis 2008; 45(2): 105−11.
16. *Krstić M, Stajković N*. Risik of infection by Lyme disease cause in green surfaces maintance workers in Belgrade. Vojnosanit Pregl 2007; 64(5): 313−9. (Serbian)
17. *Cekanac R, Pavlovic N, Gledovic Z, Grgurevic A, Stajkovic N, Lepsanovic Z*, et al. Prevalence of Borrelia burgdorferi in Ixodes ricinus ticks in Belgrade area. Vector Borne Zoonotic Dis 2010; 10(5): 447−52.
18. *Dakić Z, Kulišić Z, Stajković N, Pelemiš M, Čobeljić M, Stanimirović Z*, et al. Ecology of Anopheles mosquitoes in Belgrade Area, estimating vector potential for malaria retransmission. Acta Vet 2008; 58(5−6): 603−15.
19. *Kuljić-Kapulica N, Tasić D, Stajković N, Krstić M*. Detection of antibodies to West Nile (WNH) in human sera. Med Rev 2009; 2: 9−12.
20. *Mišić S, Stajković N, Tešić M, Mišić Z, Lesić Lj*. Human dirofilariasis: Report of three cases of Dirophilaria repens infection. Int J Parasitol 1996; 38(1): 360.
21. *Jelenek T, Schulte-Hillen J, Loscher T*. Human dirofilariasis. Int J Dermatol 1996; 35(12): 872−5.
22. *Gligić A, Adamović Ž*. Isolation of Tahyna virus from Aedes vexans mosquitoes in Serbia. Microbiol 1976; 13(2): 119−23 (Serbian).
23. *Stajković N*. Vector distribution and control. Arch Tox Kin Xenob Metab 1997; 52: 63−7.
24. *Milutinović R, Stajković N*. Repellents-protection from insects and ticks. Beograd: NNK international; 2004. (Serbian)
25. *Stafford SK*. Tick Menagement Handbook. New Haven, CT: Connecticut Agricultural Experiment Station; 2007.
26. *Rendi-Wagner P*. Risk and prevention of tick-borne encephalitis in travelers. J Travel Med 2004; 11(5): 307–12.
27. *Centers for Disease Control and Prevention*. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevetion, and Control. Available from: www.cdc.gov/.../westnile/.../wnv-guidelines-apr [updated 2013 June 11].
28. *Carney MR, Husted S, Jean C, Glaser C, Kramer V*. Effficacy of Aerial Spraying of Mosquiro Adulticide in Reducing Incidence of West Nile Virus, California, 2005. Emer Infect Dis 2008; 14 (5): 747−55.
29. *Kintchen W, Lawrence LK, Coleman ER*. The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets. J Vector Ecol 2009; 34(1): 50−61.
30. *Ditzen M, Pellegrino M Vosshall L*. Insect odorant receptors are molecular targets of the insect repellent DEET. Science 2008; 319(5871): 1838−42.
31. *Stanczyk NM, Brookfield JF, Ignell R, Logan JG, Field LM*. Behavioral insensitivity to DEET in Aedes aegypti is a genetically determined trait residing in changes in sensillum function. Proc Natl Acad Sci U S A 2010; 107(19): 8575−80.
32. *Patel DR*. Assessment of insect repellents. London: The Physical Pharmacy University Press; 1995.
33. *Miller JD*. Anaphylaxis associated with insect repellent. N Engl J Med 1982; 307(21): 1341−2.
34. *DeGarbino JP, Laborde A*. Toxicity of an insect repellent: N, N-diethyl-m-toluamide. Vet Hum Toxicol 1983; 23: 422−3.
35. *Davis EE*. Insect repellents: concepts of their mode of action relative to potential sensory mechanisms in mosquitoes. J Med Entomol 1985; 22(3): 237−43.
36. *Barnard D, Xue R*. Laboratory evaluation of mosquito repellents against Aedes albopictus, Culex nigripalpus and Ochlerotatus triseriatus (Diptera: Culicide). J Med Entomol 2004; 41(4): 726−30.
37. *Maibach HI, Khan AA, Akers W*. Use of insect repellents for maximum efficacy. Arch Dermatol 1974; 109(1): 32−5.
38. *Milutinovic R, Vuleta G, Milić J, Stajković N*. Assessment of efficiency of repellent formulations with N,N-diethyl-m-toluamide in Laboratory Conditions. Inter J Cosm Sci 1999; 21(1): 7-14.
39. *Milutinović R, Stajković N*. Assessment of efficiency of repelent preparations. Acta Dermatovenerol Iugosl 1996; 19: 52−4. (Serbian)
40. *Stajkovic N*. Prophylactic importance of repellents. Profilaksa 1997; 1(4): 8−15.(Serbian)
41. *Katz MT, Jason H, Miller MD, Adelaide A, Hebert MD*. Insect repellents: historical perspectives and new developments. J Am Acad Dermatol 2008; 58(5): 865−71.
42. *Briassoulis G, Narlioglou M, Hatzis T*. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. Hum Exp Toxicol. 2001; 20(1): 8−14.
43. *Osimitz TG, Murphy JV*. Neurological effects associated with use of the insect repellent N,N-diethyl-m-toluamide (DEET). J Toxicol Clin Toxicol 1997; 35(5): 435−41.
44. *Qiu H, Jun HW, McCall JW*. Pharmacokinetics, formulation, and safety of insect repellent N,N-diethyl-3-methylbenzamide (deet): a review. J Am Mosq Control Assoc 1998; 14(1): 12−27.
45. *Osimitz TG, Grothaus RH*. The present safety assessment of deet. J Am Mosq Control Assoc 1995; 11(2 Pt 2): 274−8.
46. *Roland EH, Jan JE, Rigg JM*. Toxic encephalopathy in a child after brief exposure to insect repellents. Can Med Assoc J 1985; 132(2): 155−6.
47. *Wantke F, Focke M, Hemmer W, Gotz M, Jarisch R*. Generalized urticaria induced by diethyltoluamide-containing insect repellent in a child. Contact Dermatitis 1996; 35(3): 186−7.
48. *Vozmediano JM, Armario J, Gonzales-Cabrerizo A*. Immunologic contact urticaria from diethyltoluamide. Int J Dermatol 2000; 39(11): 876−7.
49. *Ross EA, Savage KA, Utley LJ, Tebbett IR*. Insect repellent [correction of repellant] interactions: sunscreens enhance DEET (N,N-diethyl-m-toluamide) absorption. Drug Metab Dispos 2004; 32(8): 783−5.
50. *Menon KS, Brown AE*. Exposure of children to DEET and other topically applied insect repellents. Am J Ind Med 2005; 47(1): 91−7.
51. *McGready R, Hamilton KA, Simpson JA, Cho T, Luxemburger C, Edwards R*, et al. Safety of the insect repellent N,N-diethyl-M-toluamide (DEET) in pregnancy. Am J Trop Med Hyg 2001; 65(4): 285−9.
52. *Badolo A, Ilboudo-Sanogo E, Ouedraogo AP, Costantini C*. Evaluation of the sensitivity of Aedes aegypti and Anopheles gambiae complex mosquitoes to two insect repellents: DEET and KBR 3023. Trop Med Int Health 2004; 9(3): 330−4.
53. *Djelić N, Soldatović B, Andjelković M, Milutinović R*. The rate of sister-chromatid exchanges in cultured human peripheral blood lymphocytes treated with permethrin. Genetika 1997; 29(2): 97−102.
54. *Taplin D, Meinking TL*. Pyrethrins and pyrethroids in dermatology. Arch Dermatol 1990; 126(2): 213−21.
55. *Debboun M, Strickman DA, Klun JA*. Repellents and the military: our first line of defense. J Am Mosq Control Assoc 2005; 21(4 Suppl): 4−6.
56. *Kitchen LW, Lawrence KL, Coleman RE*. The role of the United States military in the development of vector control products, including insect repellents, insecticides, and bed nets. J Vector Ecol 2009; 34(1): 50−61.

57. *Cilek JE, Petersen JL, Hallmon CE*. Comparative efficacy of IR3535 and deet as repellents against adult Aedes aegypti and Culex quinquefasciatus. J Am Mosq Control Assoc 2004; 20(3): 299−304.
58. *Milutinović R. Stajković N, Milić J*. Assessment of efficiency of repellent preparations containing ethylbutylaminopropionate. SOFW J 2002; 128(11): 14−6.
59. *Milić J, Milutinović R, Simović S*. The effect of the insect repellent type on physical stability and rheological properties of emulsions based on polymeric emulssifier/surfactant mixtures. SOFW 2002; 128(3): 38−43.
60. *Fradin MS, Day JF*. Comparative efficacy of insect repellents against mosquito bites. N Engl J Med 2002; 347(1): 13−8.
61. *Sukumar K, Perich MJ, Boobar LR*. Botanical derivatives in mosquito control: a review. J Am Mosq Control Assoc 1991; 7(2): 210−37.
62. *Trongtokit Y, Rongsriyam Y, Komalamisra N, Apiwathnasorn C*. Comparative repellency of 38 essential oils against mosquito bites. Phytother Res 2005; 19(4): 303−9.
63. *Noosidum A, Prabaripai A, Chareonviriyaphap T, Chandrapatya A*. Excito-repellency properties of essential oils from Melaleuca leucadendron L., Litsea cubeba (Lour.) Persoon, and Litsea salicifolia (Nees) on Aedes aegypti (L.) mosquitoes.. J Vector Ecol 2008; 33(2): 305−12.
64. *Zhu J, Zeng X, Yanma, Liu T, Qian K, Han Y,* et al. Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. J Am Mosq Control Assoc 2006; 22(3): 515−22.
65. *Karppinen A, Brummer-Korvenkontio H, Petman L, Kautiainen H, Herve JP, Reunala T*. Levocetirizine for treatment of immediate and delayed mosquito bite reactions. Acta Derm Venereol 2006; 86(4): 329−31.
66. *Reunala T, Brummer-Korvenkontio H, Karppinen A, Coulie P, Palosuo T*. Treatment of mosquito bites with cetirizine. Clin Exp Allergy 1993; 23(1): 72−5.
67. *Zhai H, Packman EW, Maibach HI*. Effectiveness of ammonium solution in relieving type I mosquito bite symptoms: a double-blind, placebo-controlled study. Acta Derm Venereol 1998; 78(4): 297−8.

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CURRENT TOPIC

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Laparoscopy in gynecologic oncology: A review of literature

Laparoskopija u ginekološkoj onkologiji – pregled literature

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Introduction

Introducing laparoscopy in gynecology during the 1960s in Europe placed gynecology as pioneer in this less invasive approach in surgery. At that time, gynecological laparoscopy was predominantly used in diagnostics by means of direct visualization of *pelvis minor*. During the 1970s the gynecologists shifted the use of endoscopic technology from diagnostic to surgical purposes such as tubal ligation. From that time, laparoscopy has become a leading technique in diagnostics and surgical treatment of benign lesions in gynecology [1]. By using laparoscopy in detection of bladder and prostate carcinoma spreading into the lymph nodes urologists have become the pioneers of the use of laparoscopy in the field of oncology [2]. During the late 1980s, the initiators of endoscopic oncology began with laparoscopic evaluations of the condition and spreading of the malignant disease. The first reports were available at the beginning of 1990s, and with them came the controversial opinions about the usefulness of laparoscopy and minimal invasive surgery in gynecologic oncology. Disagreements were mainly associated with medicolegal aspects of possible consequences resulted from inadequate surgical treatment of malignant diseases.

The trends of laparoscopy use in gynecologic surgery

There has been an increasing trend in the use of minimal invasive techniques for resection and/or staging of malignancies in gynecology during the last ten years. Many studies report the advantages of these procedures, their efficacy, safety and adequacy in surgical treatment of gynecologic malignancies [3–6]. A survey conducted in 2004 and 2007 among the members of the Society of Gynecologic Oncologist (SGO) in the USA showed a significant increase in the use of laparoscopy. Forty-six percent of 850 SGO members responded to the survey. In 2004 survey, laparoscopic surgery was indicated in 84% of cases while in 2007 survey it was increased to 91%. The following laparoscopic procedures were most often indicated [7]: laparoscopically assisted vaginal hysterectomy (LAVH) or total laparoscopic hysterectomy (TLH) and staging of endometrial carcinoma (43%); diagnostics of adnexal masses (39%); prophylactic salpingo-oophorectomy in case of women at high risk for ovarian cancer (11%).

These procedures are accepted as the most convenient for use in endoscopic surgery. The question regarding the conversion from laparoscopy to laparotomy was answered by 90% of the survey participants. In 2004, 25% of them did the conversion, while in 2007 only 3% of conversions were reported by 94% of surveyed SGO members [7]. The mentioned data and numerous papers published in Europe and Asia point to the increasing trend of using laparoscopy in gynecologic oncology (Table 1).

The launch of minimally invasive surgery reduces the operation-induced trauma, provides a faster recovery, shortens the hospitalization and lowers the total costs of treatment. The purpose of using laparoscopy in gynecologic surgery is to confirm therapeutic efficacy compared with standard surgical procedures and to reduce the appearance of side effects. Still open are dilemmas regarding the results of treatment after laparoscopic surgery in oncology and intraoperative complications such as injuries of intestines, larger blood vessels and tumor cells dissemination.

Small incisions suitable for ports and laparoscopic instruments do not make possible the removal of large solid tumors. Tearing the tumor into pieces and its rough excision from pelvis and abdomen may cause spreading and expansion of tumor cells in the abdomen and development of metastases in the area of port incisions. The basic aim of treat-

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Table 1

Laparoscopic procedures that are frequently applied in gynecological oncology

Localization of a malignant tumor	Type of laparoscopic surgery
Cervix uteri carcinoma	Laparoscopically assisted radical vaginal hysterectomy (*Schauta-Amreich* and *Schauta-Stoeckel*) Laparoscopically assisted radical vaginal trachelectomy Laparoscopic radical abdominal hysterectomy Laparoscopic lymphadenectomy (pelvic and para-aortic) Laparoscopic evaluation of the stage of the disease
Endometrial carcinoma	Laparoscopic evaluation of the stage of the disease Laparoscopically assisted vaginal hysterectomy with bilateral adnexectomy Laparoscopic hysterectomy with bilateral adnexectomy Laparoscopic lymphadenectomy (pelvic and para-aortic)
Ovarian carcinoma	Diagnostic laparoscopy Second look surgeries

ment in oncology is the complete removal of malignant tumor and interruption of its spreading, and control and alleviation of disease symptoms. If the aim can be achieved by minimally invasive laparoscopic procedures than their use is justified but not at any price and if they are harmful to patients' health [8]. Nevertheless, laparoscopic surgery in gynecologic oncology has become a standard procedure in the majority of medical institutions in developed countries. Modern medical technology, acquired experience, and better surgical training with modern endoscopy equipment have been the main reasons for that.

The surgeons have agreed that laparoscopic techniques are associated with extremely gradual process of learning, which starts with small and simple procedures and goes up to more complex and comprehensive laparoscopic operations. It should be mentioned that laparoscopic operations in gynecologic oncology could be performed only by surgeons who have already mastered the techniques of classic surgery and are skilled to manage the complications. The learning curve starts with classic surgery procedures in gynecologic oncology and continues with learning the basics of laparoscopic operations and skills under supervision. The next step is to have sufficiently enough training after which come the actual performance of laparoscopic procedures and operations in the treatment of gynecologic oncology patients [8].

The use of robotics in laparoscopy

Robotic assisted surgery is a new aspect in gynecologic oncology which eliminates the basic ergonomic problem for a surgeon and the most important long learning curve. In addition, it gives a 3-D vision and magnification: the surgeon controls the camera, the image is directly projected, the movements are intuitive, the instruments are articulated and ergonomic, the tremor is eliminated. The first surgical robots were presented during the 1980s. The development of robotic surgery made possible broader applications for surgical indications. ROBODOC was the first surgical robot approved by the United States Food and Drug Administration FDA. The next were Automatic Endoscopic System for Optimal Positioning (AESOP) in 1994 and ZEUS, a second-generation robotic system in 1998 [9]. The da Vinci surgical system is the most sophisticated of the surgical robotic systems. Based upon the first reports made by Advincula and Reynolds on the use of robot for myomectomies, FDA approved the use of the da Vinci in gynecologic procedures in April 2005 [10–13]. At the annual SGO meeting in February 2006, Boggess [14] did a live demonstration of radical hysterectomy and reported on 13 previously performed operations. Since then, the use of robotic surgery in gynecologic oncology has constantly been improved in world centers.

Laparoscopy in endometrial carcinoma

Laparoscopic approach in treatment of endometrial carcinoma implies laparoscopic determination of the stage of the disease combined with laparoscopically assisted vaginal (LAVH) or laparoscopic hysterectomy and bilateral adnexectomy. In the initial FIGO stage I of endometrial carcinoma, which is limited only to the uterus, laparoscopically assisted vaginal hysterectomy with bilateral adnexectomy should be applied whenever it is technically possible [15, 16]. Zullo et al. [17] conducted a randomized study to compare laparoscopy *vs* laparotomy in patients with early stages of endometrial carcinoma. The authors showed that the safety and efficiency of laparoscopy was the same as in the open approach, pointing out the benefit of laparoscopy in relation to the quality of life during the first 6 months after the surgery. Tozzi et al. [18] reported the first results of the survival of patients with endometrial carcinoma who were operated laparoscopically in comparison to those patients who underwent open surgery. Based on the average follow-up of 44 months of patients with endometrial carcinoma FIGO stage I, they found that a disease-free interval among laparoscopically operated patients was 91% compared to 94% among patients treated with classic surgery. Overall survival was 86% compared to 90% in patients with laparotomy. Malur et al. [19] presented 70 patients with stage I-III of endometrial carcinoma: 37 patients had laparoscopically assisted vaginal hysterectomy and 33 underwent open surgery. Comparative analysis of the removed lymphatic nodes and duration of surgery did not show a statistically significant difference. The recurrence-free interval did not show statistically significant difference between the laparoscopy group (97%) and

the laparotomy group (93%). Similar results were presented in relation to overall survival, 84% in the laparoscopic group, and 91% in the laparotomy group.

Ju et al. [20] included 5 prospective and 8 retrospective studies in a meta-analysis. The comparison of the laparoscopic approach to open surgery in endometrial carcinoma did not confirm a statistically significant difference for the overall survival and the recurrence of the disease, while the number of complications was lower in the group with the laparoscopic approach. Furthermore, the analysis of 5 studies dealing with the number of lymphatic nodes in tested groups did not confirm any statistically significant difference. In the study of Janda et al. [21], the quality of life after total laparoscopic hysterectomy (TLH), n = 190, and total abdominal hysterectomy (TAH), n = 142, was examined. In the early phase of the recovery period, the improvement of the quality of life was more pronounced in patients with TLH. Better quality of life continued its trend in the TLH group even 6 months after the surgery. Longer duration of the surgery was statistically significant in the TLH group (138 ± 43 min), when compared with the TAH group (109 ± 34 min; p = 0.001). Intraoperative complications were similarly present in both of the groups (TAH 8/142, 5.6%, and TLH 14/190, 7.4%; p = 0.55). During the postoperative period, two times more adverse events occurred in the TAH group than in the TLH patients (33/142, 23.2%, and 22/190, 11.6%, respectively; p = 0.004). Serious postoperative complications were more frequent in the TAH patients (27/142, 19.0%) than in the TLH group (15/190, 7.9%; p = 0.002). The advantages of the laparoscopic approach, together with the vaginal hysterectomy imply less percentage of postoperative complications, shorter recovery period at the hospital, even in the group of obese patients [22].

Laparoscopy in cervix uteri carcinoma

Vaginal radical hysterectomy as a method of choice in the treatment of cervix uteri carcinoma was the initial idea for performance of the first laparoscopic lymphadenectomy in the treatment of early invasive cervix uteri carcinoma. The greatest advantage of the vaginal operative procedure is the possibility of closure of the cervix at the very beginning of the surgery and the reduction of chances for tumor dissemination. Dargent performed the laparoscopic lymphadenectomy after the Schauta's vaginal radical hysterectomy in 1986 in Lion, and that was the first step of implementation of laparoscopy in combination with already familiar radical vaginal surgery. Dargent published the first results after the surgeries of 51 patients, where a three-year long survival was registered in 95%. At the beginning, laparoscopy had extraperitoneal approach, but since 1992, Querleu has been promoting a transumbilical transperitoneal laparoscopic dissection of lymphatic nodes [23]. After more than 10 years of implementation in surgical practice, the analysis showed that the laparoscopic lymphadenectomy is an equally safe and reliable method as laparotomy, with the great advantage of the minimally invasive approach. Laparoscopic lymphadenectomy can also be performed as a diagnostic procedure in patients with the early stage of the cervix uteri carcinoma before making the decision on the selection of the therapeutic procedure [24, 25]. More frequent implementation of laparoscopy created conditions for performance of laparoscopic radical abdominal hysterectomy and laparoscopically assisted radical vaginal trachelectomy (LVRT) [26–29]. In 1994, Daniel Dargent presented the concept of radical vaginal trachelectomy, where the body of uterus, ovaries, and the fallopian tubes were preserved and the vaginal approach radically removed the cervix uteri, the upper third of the vagina and a part of parametrium. This procedure is combined with the laparoscopically assisted lymphadenectomy in an identical way as in the case of laparoscopically assisted radical vaginal hysterectomy [23]. These surgeries are reserved only for the early stages of the cervix uteri carcinoma with the aim of preservation of the patient's fertility [30]. Implementation of laparoscopy in the advanced disease is limited to lymphadenectomy, which is performed in some centers, because it was proved that the patients who had their bulky lymph nodes removed before the therapy had better survival [8]. Introduction of robotic surgery has also widened the indication of laparoscopic robotic surgery implementation in the treatment of cervical carcinoma [31]. Magrina et al. [32] presented the comparison of robotic laparoscopic radical hysterectomy with classical laparoscopic approach and laparotomy. The average durations of robotic procedure, laparoscopy and laparotomy were 189.6 min, 220.4 min and 166.8 min, respectively, with loss of blood of 133 mL, 208 mL, and 443.6 mL, respectively. The average number of removed lymphatic nodes was 25.9 in robotic laparoscopy, 25.9 in laparoscopy and 27.7 in laparotomy with hospital stay of 1.7, 2.4 and 3.6 days, respectively. There were no differences in intra- and postoperative complications among the tested groups. During the follow-up of all the three groups in duration of 31.1 months, no disease recurrence was registered [32].

Laparoscopy in ovarian carcinoma

There is a general consensus that implementation of laparoscopy as a surgical approach in treatment of benign adnexal masses is entirely justified due to reduced loss of blood, shorter hospital stay, less complications and pain and reduced treatment expenses in comparison to the open approach [33].

The role of laparoscopy in the treatment of ovarian carcinoma remains in the domain of discussion and insufficiently clear directives. In the majority of cases, laparoscopy imposed itself as a method of surgical staging of the disease and deciding on further treatment of ovarian carcinoma.

When compared, the results of laparoscopic surgical staging, in relation to laparotomy, showed reduced loss of blood, shorter hospital stay and less complications with longer operative time. The numbers of lymphatic glands and survival were not significantly statistically different [34, 35].

However, there are pending questions related to implementation of laparoscopy, and now even robotic laparoscopy, in cytoreductive surgery of the advanced ovarian carcinoma, which is still diagnosed in about 75% of cases.

Conclusion

Implementation of laparoscopy, *ie* minimally invasive surgery (MIS) in gynecological oncology represents today a significant therapeutic modality of treatment without compromising basic oncological principles. Using such a medical technology in oncology patients in Serbia imposes discussion on a new approach in education and organization of centers with adequate equipment where this type of surgery could be implemented with a sufficient number of cases.

R E F E R E N C E S

1. *Gordon AG, Magos AL*. The development of laparoscopic surgery. Bailliere's Clin Obstet Gynaecol 1989; 3(3): 429–49.
2. *Hald T, Rasmussen F*. Extraperitoneal pelvioscopy: a new aid in staging of lower urinary tract tumors. A preliminary report. J Urol 1980; 124(2): 245–8.
3. *Walker JL, Piedmonte M, Spiros N*. Phase III trial of laparoscopy versus laparotomy for surgical resection and comprehensive surgical staging of uterine cancer: a Gynecologic Oncology Group study funded by the National Cancer Institute. Gynecol Oncol 2006; 101(suppl 1): S11–2.
4. *Frumovitz M, dos Reis R, Sun CC, Milam MR, BeversMW, Brown J*, et al. Comparison of total laparoscopic and abdominal radical hysterectomy for patients with early stage cervical cancer. Obstet Gynecol 2007; 110(1): 96–102.
5. *Leblanc E, Querleu D, Narducci F, Occelli B, Papageorgiou T, Sonoda Y*. Laparoscopic restaging of early stage invasive adnexal tumors: a 10-year experience. Gynecol Oncol 2004; 94(3): 624−9.
6. *Abu-Rustum NR, Sonoda Y*. Transperitoneal laparoscopic staging with aortic and pelvic lymph node dissection for gynecologic malignancies. Gynecol Oncol 2007; 104(2) :5–8.
7. *Mabrouk M, Frumovitz M, Greer M, Sharma S, Schmeler KM, Soliman PT,* et al. Trends in laparoscopic and robotic surgery among gynecologic oncologists: A survey update. Gynecologic Oncology 2009; 112(3): 501–5.
8. *Đurđević S, Vidaković S*. Primena laparoskopije u ginekološkoj onkologiji,. In*: Đurđević S, Kesić V,* editors. Ginekološka onkologija. Novi Sad: Faculty of Medicine; 2009. p. 336−40.
9. *Mendivil A, Holloway RW, Boggess JF*. Invited Review: Emergence of robotic assisted surgery in gynecologic oncology: American perspective. Gynecol Oncol 2009; 114(2): S24−S31.
10. *Advincula AP, Reynolds RK*. The use of robot-assisted laparoscopic hysterectomy in the patient with a scarred or obliterated anterior cul-de-sac. JSLS 2005; 9(3): 287–91.
11. *Advincula AP, Song A, BurkeW, ReynoldsRK*. Preliminaryexperiencewithrobot-assisted laparoscopic myomectomy. J Am Assoc Gynecol Laparosc 2004; 11(4): 511–8.
12. *Reynolds RK, Advincula AP*. Robot-assisted laparoscopic hysterectomy: technique and initial experience. Am J Surg 2006; 191(4): 555–60.
13. *Reynolds RK, Burke WM, Advincula AP*. Preliminary experience with robot-assisted laparoscopic staging of gynecologic malignancies. JSLS 2005; 9(2): 149–58.
14. *Boggess JF*. Robotic-assisted radical hysterectomy for cervical cancer: National Library of Medicine Archives. 2006 [updated 2006; cited 2008 August 11]. Available from: http://www.nlm.nih.gov/medlineplus/surgeryvideos.html.
15. *Cho YH, Kim DY, Kim JH, Kim YM, Kim YT, Nam JH*. Laparoscopic management of early uterine cancer: 10-year experience in Asan Medical Center. Gynecol Oncol 2007; 106(3): 585–90.
16. *Barakat RR, Lev G, Hummer AJ, Sonoda Y, Chi DS, Alektiar KM*, et al. Twelve-year experience in the management of endometrial cancer: a change in surgical and postoperative radiation approaches. Gynecol Oncol 2007; 105(1): 150–6.
17. *Zullo F, Palomba S, Russo T, Falbo A, Costantino M, Tolino A*, et al. A prospective and randomized comparison between laparoscopic and laparotomic approaches in women with early stage endometrial cancer: a focus on the quality of life. Am J Obstet Gynecol 2005; 193: 1344–52.
18. *Tozzi R, Malur S, Koehler C, Schneider A*. Laparoscopy versus laparotomy in endometrial cancer: first analysis of survival of a randomized prospective study. J Minim Invasive Gynecol 2005; 12(2): 130–6.
19. *Malur S, Possover M, Michels W, Schneider A*. Laparoscopic-assisted vaginal versus abdominal surgery in patients with endometrial cancer-a prospective randomized trial. Gynecol Oncol 2001; 80(2): 239−44.
20. *Ju W, Myung SK, Kim Y, Choi HJ, Kim SC*. Comparison of Laparoscopy and Laparotomy for Management of Endometrial Carcinoma. A Meta-analysis. Int J Gynecol Cancer 2009; 19(3): 400−6.
21. *Janda M, Gebski V, Brand A, Hogg R, Jobling TW, Land R*, et al. Quality of life after total laparoscopic hysterectomy versus total abdominal hysterectomy for stage I endometrial cancer (LACE): a randomised trial. Lancet Oncol 2010; 11(8): 772−80.
22. *Devaja O, Samara I, Papadopoulos AJ*. Laparoscopically Assisted Vaginal Hysterectomy (LAVH) Versus Total Abdominal Hysterectomy (TAH) in Endometrial Carcinoma Prospective Cohort Study. Int J Gynecol Cancer 2010; 20(4): 570−5.
23. *Mandic A, Novakovic P, Nincic D*. Surgical approaches towards fertility preservation in young patients with early invasive cervical carcinoma. J BUON 2009; 14(4): 581−6.
24. *Leblanc F, Narducci F, Chevalier A, Taieb S, Castelain B, Querleu D*. Pretherapeutic laparoscopic staging of locally advanced cervical carcinomas: technique and results. Gynecol Oncol 2005; 99(3 Suppl 1): S157–8.
25. *Fagotti A, Fanfani F, Longo R, Legge F, Mari A, Gagliardi ML*, et al. Which role for pre-treatment laparoscopic staging? Gynecol Oncol 2007; 107(1 Suppl 1): S101−5.
26. *Chen DM, Lim PC, Spirtos NM*. Laparoscopic radical hysterectomy. CME J Gynecol Oncol 2001; 6(1): 117−28.
27. *Dargent D*. Laparoscopic assisted vaginal radical hysterectomy – evolution of a concept. CME J Gynecol Oncol 2001; 6(1): 102−9.
28. *Kadar N, Reich H*. Laparoscopically asisted radical Schauta hysterectomy and bilateral laparoscopic pelvic lymphadenectomy for the treatment og bulky stage I B carcinoma of the cervix. Gynecol Endosc 1993; 2: 135−42.
29. *Canis M, Mage G, Wattiez A, Pouly JL, Manhes H, Bruhat MA*. Does endoscopic surgery have a role in radical surgery of cancer of the cervix uteri? J Gynecol Obstet Biol Reprod (Paris) 1990; 19(7): 921. (French)
30. *Leblanc E, Narducci F, Ferron G, Querleu D*. Indications and teaching fertility preservation in the surgical management of gynecologic malignancies: European perspective. Gynecol Oncol 2009; 114(1): 32–6.
31. *Yim GW, Kim SW, Nam Eji, Kim YT*. Role of Robot-Assisted Surgery in Cervical Cancer. Int J Gynecol Cancer 2011; 21(1): 173−81.

32. *Magrina JF, Kho RM, Weaver AL, Montero RP, Magtibay PM.* Robotic radical hysterectomy: Comparison with laparoscopy and laparotomy. Gynecol Oncol 2008; 109(1): 86–91.
33. *Medeiros LR, Stein AT, Fachel J, Garry R, Furness S*. Laparoscopy versus laparotomy for benign ovarian tumor: a systematic review and meta-analysis. Int J Gynecol Cancer 2008; 18(3): 387–99.
34. *Ghezzi F, Cromi A, Uccella S, Bergamini V, Tomera S, Franchi M,* et al. Laparoscopy versus laparotomy for the surgical management of apparent early stage ovarian cancer. Gynecol Oncol May 2007; 105(2): 409–13.
35. *Park JY, Bae J, Lim MC, Lim SY, Seo SS, Kang S*, et al. Laparoscopic and laparotomic staging in stage I epithelial ovarian cancer: a comparison of feasibility and safety. Int J Gynecol Cancer 2008; 18(6): 1202–9.

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CASE REPORTS

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Telemedicine consulting in the patient preparation and planning of prosthetic tooth replacement

Telemedicinska konsultacija u pripremi pacijenta i planiranju protetske nadoknade zuba

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Abstract

Introduction. In the management of edentulous spaces, there is a permanent need of a dentist-prosthetician in charge to consult other specialists. Modern telemedicine, based on powerful computer and telecomunication systems, offers an adequate answer to these challenges, being able to transfer and obtain clinical data and consultation information over large distances. Using smartphone or a computer, the teleconsultant acces the system, downloads and review the data and photographs and gave suggestions. The system then enables direct, real time contact with the consultant, chat, or directs them to contact each other by phone. **Case report.** We presented telemedicine consulting in the patient preparation and planning of prosthetic tooth replacement in 3 cases with different teleconsultation requirements: the first case for prosthetic rehabilitation of his upper teeth, the second one for prosthetic management of his partial edentulousness and "a growth on his gums" in the vestibular region of the frontal teeth and the third one for prosthetic management of total edentulousness of her upper jaw. We used the system of telemedicine in dentistry, established at the Faculty of Medicine in Kosovska Mitrovica. The operation was based on the computer application system XPA3 Online, computer networking and mobile smartphone network. All consultations were succefull with no need for further procedures in regional center. **Conclusion.** The use of a mobile smartphone has brought about the mobility and availability of teleconsultant specialists in an extent never seen before. Prostheticians are thus able to offer better service to their patients and improve the quality of management of partially or totally edentulous patients, especially in rural areas.

Key words:
telemedicine; prosthodontics; surgery, oral; oral surgical procedures, preprosthetic; diagnosis; jaw, edentulous, partially; mouth, edentulous; treatment outcome.

Apstrakt

Uvod. Prilikom zbrinjavanja bezubih prostora, postoji stalna potreba stomatologa protetičara za konsultacijama lekara drugih specijalnosti. Moderna telemedicina bazirana na moćnim kompjuterskim i telekomunikacionim sistemima pruža adekvatan odgovor na ove izazove i u stanju je da prenese i donese kliničke podatke i konsultacione informacije na velike daljine. Koristeći *smartphone* ili računar telekolsuntant pristupa sisetemu, preuzima i analizira podatke i slike i daje sugestije. Sistem, na taj način omogućava diretan kontakt sa konsultantom u radno vreme. **Prikaz bolesnika.** Prikazana je primena telemedicinske konsultacije u pripremi pacijenta i planiranju protetske nadoknade zuba kroz tri telemedicinske konsultacije vezane za različite proteske probleme: protetska rehabilitacija zuba gornje vilice, protetsko zbrinjavanje parcijalne bezubosti i „izrasline desni" u vestibularnom predelu frontalnih zuba, protetsko zbrinjavanje totalne bezubosti gornje vilice. Korišćen je sistem telemedicinskog centra Medicinskog fakulteta Univerziteta u Prištini (Kosovska Mitrovica) čiji rad je zasnovan na kompjutersko-medicinskom aplikacionom sistemu *XPA3 Online* i širokoj dostupnosti računarske mreže i mreže mobilnih pametnih telefona (*smartphone*). Sve telekonsultacije su završene uspešno, bez potrebe za daljim lečenjem bolesnika u višim centrima. **Zaključak.** Korišćenjem mobilnih *smart* telefona, mobilnost i pristupačnost telekonsultanata specijalista podignuta je do samog maksimuma. Na ovaj način protetičari mogu da pruže bolju uslugu pacijentu i poboljšaju kvalitet zdravstvenog zbrinjavanja parcijalno ili totalno bezubih pacijenata.

Ključne reči:
telemedicina; protetika; hirurgija, oralna; hirurgija, oralna, preprotetske procedure; dijagnoza; vilica, parcijalna bezubost; bezubost; lečenje, ishod.

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Introduction

Nowadays, in the era of intense development of medicine, the need for the opinion of other experts about the patient problem at hand is an everyday necessity. More people (and more eyes) involved, focused experience of a larger body of experts, and problem solving from the point of view of different specialties, enable realization of long desired simpler and high quality patient management. This is especially the case in insufficiently clear or ambiguous situations. Based on the use of sophisticated computer and communication technology, it is able to offer an effective specialist consultation, to reduce overall costs, and to broaden the scope of dentistry health care to encompass the whole world, including inaccessible regions [1].

On the other hand, in spite of ever growing needs for such methods in dentistry, they are being utilized much less compared to medicine [2]. The reason for such a situation is the absence of an adequate regional or national center for processing and coordination of consultations.

Our system of telemedicine in dentistry is based on the formation of a telemedicine center at the Faculty of Medicine University of Priština Kosovska Mitrovica. The operation is based on the computer-medicine application system *XPA3* Online, and a wide availability of computer networking and mobile smartphone network. The procedure implies permanent availability of professionals of almost all specialties in the telemedicine center of this University. Always alert specialists carry with them their smartphone devices or they have Internet access in their immediate surroundings. If a dentist need some specialist consultation, he/her collects relevant clinical and patient history information according to the adopted procedure, takes digital photographs, targeted radiographs, and orthopan tomography, and logging onto the system site uploads the information to the central server. He/her can choose the consultant personally or requests specialist consultation of any available teleconsultants. Emergency of the request can also be determined as low, normal, or high urgency consultation. The central server looks for the available specialists and place calls or sends them SMS information about the received request for teleconsultation, stating the topic of the request and number of accompanying digital files. Using smartphone or a computer access point, the teleconsultant logs on the system, downloads and review the data and photographs. Immediate review is also possible, as well as suggestions and counseling of the dentist requesting consultation, and the reply is posted on the central server. The system then informs the requester of teleconsultation about the received reply, enabling also a direct, real time contact with the consultant, chat, or directs them to contact each other by phone. The requester receives the reply, possibly with schemes and photographs.

The aim of this paper was to present telemedicine consulting in the patient preparation and planning of prosthetic tooth replacement.

Case reports

The following cases could illustrate the use of this technology.

Case 1

A 67-year-old male patient contacted his dentist. in Belgrade for prosthetic rehabilitation of his upper teeth. Since the observed status of the teeth was not clear enough, the dentist decided to request a specialist consultation with the specialists in dentistry prosthetics, oral surgery, and endodontics, *via* the Teledentistry Center of the University of Priština Kosovska Mitrovica. Digital intraoral photographs were taken from different angles and aspects, and already made retroalveolar radiographs were digitalized. After accessing the Internet and opening of www.XPA3.com domain, authentication and authorization of the dentist were made by the XPA3 Online system, enabling the formation of a new request for teleconsultation. The request was filled in, available clinical and anamnestic data about the patient and his disease were described, and accompanying files were uploaded to the system. The request was then sent. The system instantly informed available teleconsultants, who had 24 h at their disposal to give their opinion and suggestions. Teleconsultants reviewed the received request on their laptops, analyzed the problem and send their reply *via* the system (Figures 1 and 2). The dentist received the responses, and based on the teleconsultation advice and suggestions, successfully managed his patient both preprosthetically and prosthetically.

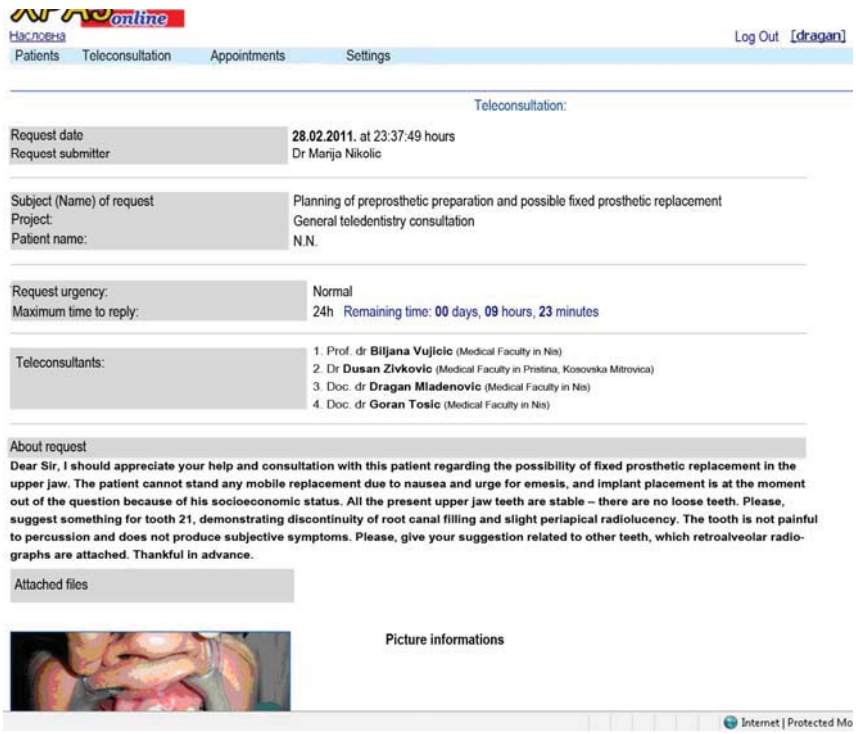

Fig. 1 – Review of teledentistry request by a teleconsultant.

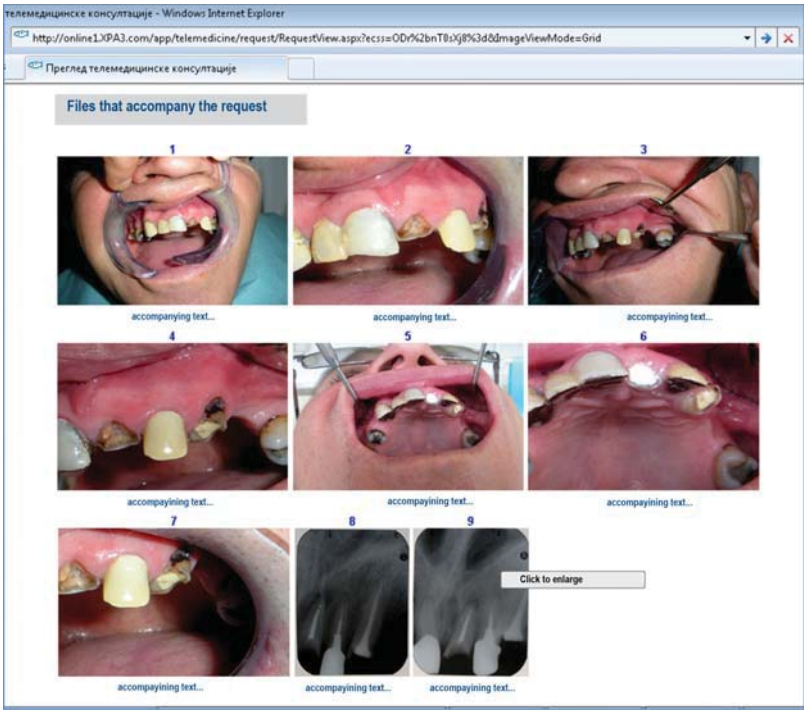

Fig. 2 – Network view of the files attached to teledentistry request.

Case 2

A 52-year-old, male patient, visited his dentist in the Serbian enclave in Kosovo and Metochia for prosthetic management of his partial edentulousness and "a growth on his gums" in the vestibular region of the frontal teeth. The patient was partially immobile due to his right leg fracture and very concerned about the appearance of this tumor. Since the transport to the appropriate specialist was additionally complicated by bad weather, the dentist sent an emergency request for teleconsultation via the XPA3 Online system of the telemedicine center, using a dial-up Internet connection. After the receipt of the request, the application software of the system effectuated teleconsultation with the adequate specialist (in oral surgery) for instant data review. The teleconsultant, using his smartphone and General Packet Radio Serbice (GPRS) Internet connection, downloaded the consultation data, and based on the available information, photographs, and radiographs, made a distant clinical diagnosis of *epulis* (a benign lesion situated on the gingiva) (Figures 3 and 4). The consultation was completed in the requested period (4 h), including re-consultations with another three specialists of oral surgery who achieved a full agreement about the diagnosis of Giant cell *epulis* (K06.8) and suggested surgical removal. The patient was completely managed in the period to come, with histopathology confirming the distant clinical diagnosis.

Fig. 3 – A teleconsultant receives the request on his smartphone and initiates access to the online center.

Fig. 4 – Smartphone review of downloaded files via the General Packet Radio Service (GPRS) connection and access to the Teledentistry Center.

Case 3

A female patient, aged 48 years, came to the Department of Dentistry Prosthetics of the Dentistry Clinic in Niš for prosthetic management of total edentulousness of her upper jaw. Since the presence of atypical uneven spots, such as osseous and soft tissue exostoses and plicae, was significant, the therapist required specialist consultation of another specialist in dentistry prosthetics and a specialist in oral surgery. Fifteen digital photographs were taken, out of which twelve showed intraoral status of the ridge in vestibular, occlusal, and palatinal views, three photographs showed the face en face, left, and right profile. The photographs, with the accompanying comments, clinical finding, description of the required suggestions, and digital orthopan file, were posted on the central server (Figure 5). The application algorithm found the available specialists in the relevant fields and immediately contacted them. Based on this telemedicine consultation, the patient was successfully managed with minimal surgical preprosthetic preparation.

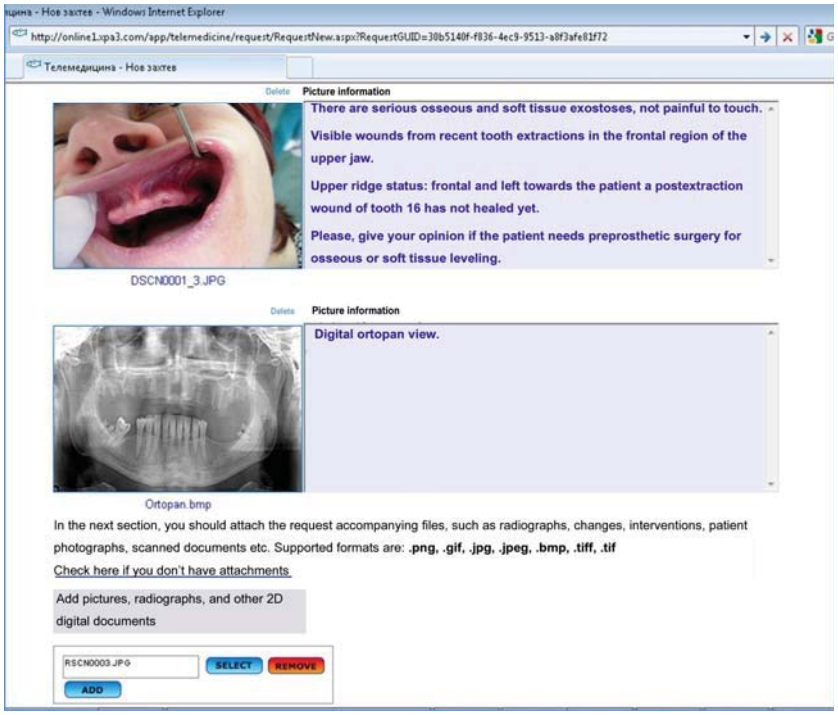

Fig. 5 – Data input, preparation of telemedicine consultation and input of 16 digital pictures showing serious osseous and soft tissue exostoses of upper alveolar ridge.

Discussion

A huge advance has been made in the sphere of telecommunication technology and computers, enabling rapid and effective medical consultations at large distances. The presence of wireless data transfer at high speeds, WCDMA and HSPA technologies with standard speeds of 7.0 Mbps within mobile phone network providers, as well as widespread use of smartphone devices with powerful operation systems and rapid processors, enabled patient review and examination at almost any place and at any time. Pretreatment consultation, consultation during the treatment, and post-treatment consultation are becoming a standard for any patient in an insufficiently clear situation.

It has been a customary practice so far that such consultations are initiated and effectuated through e-mail in case of Store and Forward technology or through direct streaming of binary information in case of Real Time consultation [3]. However, e-mail transfer of information has an important flaw if photographs or accompanying files are numerous and have to be downloaded and reviewed one by one; in order to eliminate the flaw, Aziz and Ziccardi [4] have suggested that files should be merged into one PowerPoint presentation and transferred as a single file to be opened and read by smartphone devices or computers. The finding of ours was that these shortcomings could be completely eliminated by the use of telemedicine central application XPA3 Online as an intermediary between the dentists and their teleconsultants. The application itself performs the listing and clinet download of the files to smartphone devices or computer Internet browsers.

A dentist-prosthetician, in the planning of an intervention usually consults an endodontist or oral surgeon in insufficiently clear clinical situations. In these cases, a phone call has been the traditional method of requesting and starting a consultation; patient referral and sending of medical documentation to both specialists then usually followed. If the patient could not be transported, the conclusion has been based only on the verbal description of the case. The University Center of Telemedicine in Kosovska Mitrovica here provides prostheticians and patients with a means of accurate data transfer and review of the data by the requested specialists, enabling them too to make an instant patient appointment for the necessary intervention. The method is conditioned only by having a personal computer or smartphone

device at disposal, both with Internet access. Not only that this enables appropriate specialist consultation to be made, but also the requesting of teleconsultant opinion regardless of his whereabouts. In oral surgery, telemedical solutions have been demonstrated in the field of diagnosis and treatment planning for impacted third molars based on a similar principle of using a central server which coordinates teleoral surgeons [5], and the issue of chronic periapical processes can be elucidated using teleendodontics without any statistically significant difference between the actual clinical conditions and via the Store and Forward method at a distance [6–9].

Apart from the role in pretreatment period, distant consultations in dentistry prosthetics have a vital role in the manufacturing of fixed or mobile replacements, with distant planning of the architecture of skeletonized prosthesis, superstructure of dental implants, design of inlays, onlays, and mini-bridges using the CAD/CAM technology, and correction of the angle of tooth ablation [7–8].

In the posttreatment period, such a telemedicine method enables a dentist-prosthetician to get an insight into the possible problems in patients who are no longer present at the dental clinic. A prosthetician is able to inspect the mouth cavity for possible changes reviewing the relevant photographs, to detect changes or damage to the replacement surface, esthetic or functional disorders or shortcomings, occlusal problems, and may also examine posttreatment radiographs. A patient himself can contact his prosthetician and explain to him visually the posttreatment situation which causes discomfort or dissatisfaction (e.g., a diastema between the frontal teeth) [4]. A patients may himself take photographs of the spot of irritation with his prosthetic replacement and send them to the dentist using video teleconsultation service [10].

The first author of this paper had received from a previously unseen patient the photograph of irritation as the result of inadequate upper total prosthesis, representing *epulis fisuratum*, after which the diagnosis was confirmed and clinically managed.

Conclusion

The utilization of telemedicine methods in dentistry prosthetics is a rapid and effective way of getting a necessary specialist consultation. These methods should be widely available and appropriately supported both technically and technologically by the system for management and coordination. The necessary preconditions for that are already in place, above all the technology of digital photography, radiography, and widely spread Internet access. The expansion of mobile smartphone technology significantly contributes to the mobility and wide availability of the methods. Our own task is to work to improve the education of both professionals and their patients and to offer appropriate training. In this way we can all contribute to the improvement of dental care of our patients in the domain of dental prosthetics.

REFERENCES

1. *Jevtović I.* Telemedicine – the future that has already begun. Kragujevac: School of Medicine; 2008. (Serbian)
2. *Kopycka-Kedzierawski DT, Billings RJ.* Teledentistry in inner-city child-care centres. J Telemed Telecare 2006; 12(4): 176−81.
3. *Torres-Pereira C, Possebon RS, Simões A, Bortoluzzi MC, Leão JC, Giovanini AF,* et al. Email for distance diagnosis of oral diseases: a preliminary study of teledentistry. J Telemed Telecare 2008; 14(8): 435−8.
4. *Aziz SR, Ziccardi VB.* Telemedicine using smartphones for oral and maxillofacial surgery consultation, communication, and treatment planning. J Oral Maxillofac Surg 2009; 67(11): 2505−9.
5. *Duka M, Mihailović B, Miladinović M, Janković A, Vujicić B.* Evaluation of telemedicine systems for impacted third molars diagnosis. Vojnosanit Pregl 2009; 66(12): 985−91. (Serbian)
6. *Baba M, Seçkin D, Kapdağli S.* A comparison of teledermatology using store-and-forward methodology alone, and in combination with Web camera videoconferencing. J Telemed Telecare 2005; 11(7): 354−60.
7. *Mihailović B, Miladinović M, Mladenović D, Lazić Z, Janković A, Živković D,* et al. Computerized dentistry. Beograd: Obeležja, 2009. (Serbian)
8. *Ogodescu SA, Sinescu C, Ogodescu AE, Negrutiu M, Bratu E.* Digital Tools in the Interdisciplinary Orthodontic Treatment of Adult Patients. Int J Biol Biomed Eng 2010 ; 4(4):97–105.
9. *Kyriacou E, Pavlopoulos S, Berler A, Neophytou M, Bourka A, Georgoulas A,* et al. Multi-purpose HealthCare Telemedicine Systems with mobile communication link support. Biomed Eng OnLine 2003; 2: 7.doi:10.1186/1475-925X-2-7
10. *Visser JJ, Bloo JK, Grobbe FA, Vollenbroek-Hutten MM.* Video teleconsultation service: who is needed to do what, to get it implemented in daily care? Telemed J E Health 2010; 16(4): 439−45.

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CASE REPORT

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Drug-related pityriasis rubra pilaris with acantholysis

Pityriasis rubra pilaris sa akantalizom izazvana lekom

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Abstract

Introduction. Acantholysis is rarely reported histological feature of Pityriasis rubra pilaris (PRP), recently recognized as having diagnostic specificity for differentiating PRP from psoriasis. **Case report.** Adult male patient one week after the introduction of simvastatin had experienced pruritic erythemo-squamous eruption on head and upper trunk that in a month progressed to erythrodermia, with islands of sparing. Histological picture combined pemphigus-like acantholysis with alternating hyper- and parakeratosis, follicular plugs and dermal inflammation, and confirmed the clinical diagnosis of classic adult type 1 PRP. Acitretin therapy resulted in a resolution of skin disease. Patch test with simvastatin was negative, scratch test was positive, and it was estimated that potential risk of oral challenge with simvastatin outweighed actual need for it. Drug triggering PRP episode is the most likely explanation for temporal relation between the start of simvastatin treatment and skin eruption. **Conclusion.** In management of rare inflammatory skin disease, such as PRP, we have to carefully observe and evaluate not only diagnostic features but possible external influences on its course also.

Key words:
pityriasis rubra pilaris; simvastatin; diagnosis; drug therapy; treatment outcome.

Apstrakt

Uvod. Akantoliza je retko prikazivana histološka karakteristika *Pityriasis rubra pilaris* (PRP) čiji značaj u diferencijalnoj dijagnozi prema psorijazi je nedavno prepoznat. **Prikaz bolesnika.** Prikazali smo odraslog bolesnika sa eritemoskvamoznom erupcijom po glavi i gornjem delu trupa praćenom svrabom koji je počeo nedelju dana nakon uvođenja simvastatina. Tokom mesec dana razvila se eritrodermija sa ostrvcima pošteđene kože. Histološki nalaz suprabazalne akantolize sa naizmeničnim zonama hiperkeratoze i parakeratoze u epidermisu i inflamatornog infiltrata u dermisu potvrdio je kliničku dijagnozu klasičnog adultnog oblika PRP. Terapija acitretinom dovela je do izlečenja kožnih promena. Epikutani *patch* test sa simvastatinom bio je negativan, *scratch* test bio je pozitivan, a test ekspozicije nije urađen jer je procenjeno da u tom momentu nosi veliki rizik. Epizoda PRP pokrenuta lekom je najverovatnije objašnjenje za vremensku povezanost kožnih promena sa početkom uzimanja simvastatina. **Zaključak.** U lečenju retkih zapaljenskih oboljenja kože treba pažljivo da tumačimo značaj kako dijagnostičkih parametara, tako i mogućih spoljašnjih uticaja na tok oboljenja.

Ključne reči:
pitirijazis rubra pilaris; simvastatin; dijagnoza; lečenje lekovima; lečenje ishod.

Introduction

Pityriasis rubra pilaris (PRP) is a rare inflammatory skin disease, with 6 distinct forms that differ in the age of onset, clinical features, behavior, and prognosis: adult-onset forms (classical and atypical), juvenile forms (classic, circumscribed and atypical), and human immunodeficiency virus-associated one. Pathogenesis, etiology and triggering factors of PRP are not well characterized, and dilemmas about the diagnosis, associated diseases or therapeutic approach are frequent. Psoriasis is one of the most important differential diagnostic considerations, more in clinical than histological aspects. Acantholysis is a rarely reported histological feature of PRP that is recently recognized to have diagnostic specificity for differentiating PRP from psoriasis.

Case report

A 61-year-old male patient, experienced pruritic erythematous scaling eruption on head and upper trunk one week after the introduction of simvastatin 10 mg daily (Simvor® tbl 10 mg, Ranbaxy lab). The patient had discontinued simvastatin in five days, pruritus subsided, but during the next month the eruption spread to erythrodermia with pal-

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moplantar keratoderma, eyelid ectropion and small sharply demarcated “islands of sparing” (Figure 1). The clinical diagnosis was classical adult PRP type 1. The past medical

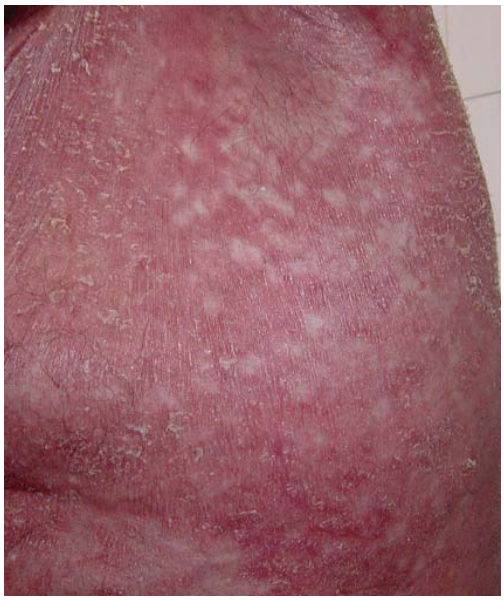

Fig. 1 – Erythrodermia with sharply demarcated islands of unaffected skin, typical adult form of Pityriasys rubra pilaris (PRP).

history of the patient was unremarkable: the patient did not currently use other medication, nor had reported any previous drug reaction. Apart from moderate hyperlipidemia, all the findings were within normal limits, including temperature, blood count and basic biochemistry profile, tests of thyroid function, autoimmune disorders, muscle enzymes, malignancy screening and direct skin immunofluorescence. Suprabasilar acantholysis suggestive for pemphigus vulgaris was the most prominent histological pattern in biopsy of recent papula (Figure 2), and features more usual for PRP (al-

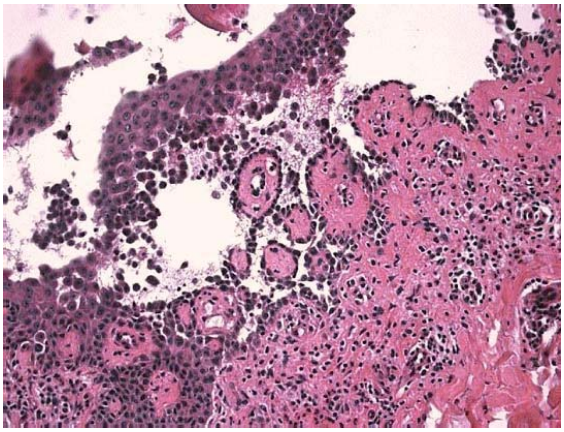

Fig. 2 – Skin histology: suprabasal acantholysis suggestive of pemphigus vulgaris (haematoxylin-eosin, original magnification × 200).

ternating hyper- and parakeratosis, dyskeratosis, follicular plugs, perivascular inflammation in upper dermis) were present in the biopsy of older plaque (Figure 3). Methylprednisolone therapy (initially 80 mg daily, tapered during 4 weeks) was without response. Acitretin, 35 mg daily brought a complete clearance in 3 months, following an inverse pattern of the initial cranio-caudal spread. It was discontinued after five months, without recurrence of PRP for the next 4 years. Nine months after clearance skin tests were performed with 2% simvastatin solution in water for injection; patch test was negative, scratch skin test was positive, revealed wheal along scratch line through simvastatin solution (Figure 4). Oral exposition test was not performed because it was estimated that a potential risk outweighed the actual need for simvastatin.

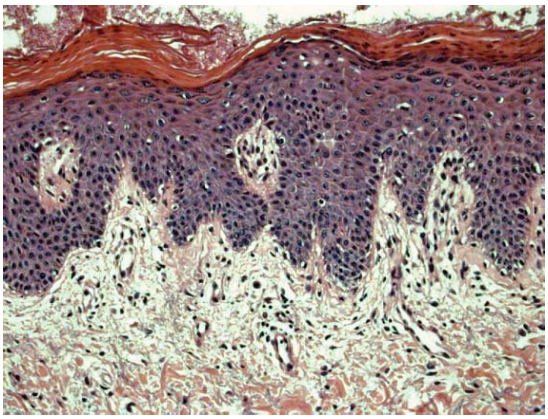

Fig. 3 – Skin histology: alternating hyper- and parakeratosis, dyskeratosis (haematoxylin-eosin, original magnification × 200).

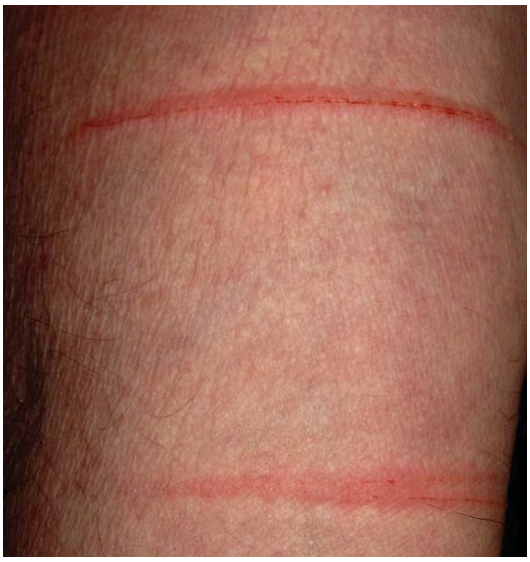

Fig. 4 – Positive scratch skin test with simvastatin: the upper line is a 3 mm wide wheal along the scratch line with aqueous simvastatin solution; the lower scratch line, surrounded with flare, represents negative control with water for injection.

Discussion

There is no single histological characteristic unique for PRP. The diagnosis combines several features (alternating hyper- and parakeratosis, follicular plugs, follicular lip parakeratosis, dermal perivascular infiltrate) and exclusion of

other differentials [1]. Early reports about acantholysis in PRP histology treated it as rare or incident finding [2, 3]. One large retrospective analysis of PRP histology detected small foci of acantholysis in about 70% of specimens [1]. Types of epidermal clefts in PRP were described as Darier-like, Hailey-Hailey-like, pemphigus-like, visible as solitary or combined patterns, or having features of epidermolytic hyperkeratosis [1–4]. With hypergranulosis and follicular plugs, acantholysis was of help in distinguishing histological findings favouring PRP towards others that are more psoriatic (capillary dilatations, hypogranulosis and epidermal pustules) [1]. Although the term acantholytic PRP was proposed for cases clinically and histologically suggestive of blistering disease, further reports of cases or case series are sparse and acantholysis is still a frequent cause of diagnostic dilemma [5]. All reported acantholytic PRP cases were typical adult erythrodermic form (type 1 PRP), but it is not conclusive whether acantholysis is a feature of solely type 1, or this form allows easier clinical recognition in spite of the unusual histology [1–5].

Intriguing is a relation between the start of new drug use and particular PRP episode, especially role of immediate hypersensitivity to simvastatin (as suggested by positive cutaneous scratch test), but can only be hypothesized. Oral challenge is the only way to sufficiently prove causal role of simvastatin, but due to severity of erythrodermia and positive skin tests, the risk of oral challenge was estimated to be unacceptable. Scratch test, when positive, is confirmatory test for immediate type hypersensitivity (urticaria – anaphylaxis), and presented case of PRP had no elements of such reaction pattern. Patch skin test when positive is confirmatory test for delayed type hypersensitivity (i.e. drug induced exanthemata), but patch test with simvastatin was negative in the presented patient, therefore excluding drug eruption with features of PRP. Drug triggering PRP is the most likely explanation for temporal association between the introduction of drug and occurrence of skin eruption, and quite long period of several months for clearance of PRP after drug discontinuation.

Pathogenesis of PRP is not resolved: apart from post-infectious forms of mostly juvenile and HIV-associated PRP, triggering events are not characterized also. Drug treatment as a trigger of PRP has only exceptionally rarely been evaluated. In the literature, one unique case of PRP induced with labetalol had been confirmed with oral challenge [6]. In the same period of the seventies of the 20th century beta blockers were recognized as triggers of psoriasis, skin disease that share some similarities with PRP. Later on drug-related PRP was hypothesized in few cases in the retrospective analysis, but were not evaluated by appropriate challenges in particular patients; incriminated drugs were anticonvulsants, antihistamines, diltiazem [1]. Aggravation of PRP has been described with topical imiquimod, suggesting that an imiquimod induced shift towards Th1 cytokine profile could be proinflammatory stimulus for PRP [7]. Statins have numerous pleiotropic (cholesterol-independent) effects on the immune system, and in clinical setting statins exert both anti- and proinflammatory properties. Cases of autoimmune diseases (lupus, dermatomyositis) closely related to statin treatments were described in the literature [8]. Also, cases of acquired ichtyosis caused by pravastatin treatment [9], or psoriasis relapsing upon treatment with different statins [10] suggest that statin effects on epidermal lipid homeostasis can have clinical implications in predisposed individuals.

Conclusion

Acantholysis is unexpected and underreported histological finding that should be recognized in diagnostic management of PRP. We should be careful to observe and to further investigate possible external influences on the entire course of rare inflammatory diseases, such as PRP. More extensive knowledge about the pathogenesis sequence of PRP is needed, to be able to estimate the role of external factors possibly influencing a cascade of skin inflammation and the course of PRP.

REFERENCES

1. *Magro CM, Crowson AN.* The clinical and histomorphological features of pityriasis rubra pilaris. A comparative analysis with psoriasis. J Cutan Pathol 1997; 24(7): 416−24.
2. *Kao GF, Sulica VI.* Focal acantholytic dyskeratosis occurring in pityriasis rubra pilaris. Am J Dermatopathol 1989;11(2): 172−6.
3. *Howe K, Foresman P, Griffin T, Johnson W.* Pityriasis rubra pilaris with acantholysis. J Cutan Pathol 1996; 23(3): 270−4.
4. *Tannenbaum CB, Billick RC, Srolovitz H.* Multiple cutaneous malignancies in a patient with pityriasis rubra pilaris and focal acantholytic dyskeratosis. J Am Acad Dermatol 1996; 35((5 Pt 1): 781−2.
5. *Sebastian A, Koff AB, Goldberg LJ.* PRP with subcorneal acantholysis: case report and review. J Cutan Pathol 2010; 37(1): 99−101.
6. *Finlay AY, Waddington E.* Cutaneous reactions to labetalol. Br Med J 1978; 1(6118): 987.
7. *Yang FC, Jessup C, Dahiya M, Reynolds R.* Pityriasis rubra pilaris exacerbation with topical use of imiquimod. Int J Dermatol 2008; 47(10): 1076–8.
8. *Noel B.* Lupus erythematosus and other autoimmune diseases related to statin therapy. J Eur Acad Dermatol Venereol 2007; 21(1): 17−24.
9. *Sparsa A, Boulinguez S, le Brun V, Roux C, Bonnetblanc JM, Bedane C.* Acquired ichtyosis with pravastatin. J Eur Acad Dermatol Venereol 2007; 21(4): 549−50.
10. *Jacobi TC, Highet A.* A clinical dilemma while treating hypercholesterolaemia in psoriasis. Br J Dermatol 2003; 149(6): 1305−6.

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CASE REPORT

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Surgical treatment of penetrating atherosclerotic ulcer of the descending aorta

Hirurško lečenje penetrantnog aterosklerotskog ulkusa descendentne aorte

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Abstract

Introduction. The term "penetrating atherosclerotic ulcer" (PAU) of the aorta describes the condition in which ulceration of an aortic atherosclerotic lesion penetrates the internal elastic lamina into media. PAU is a high-risk lesion due to its deleterious effects on the integrity of aortic wall, with potentially fatal outcome. **Case report.** A patient with intensive, sharp chest pain irradiating to the back but with no signs of myocardial ischemia on an electrocardiogram was referred to our hospital. Transthoracic echocardiography showed no pathological changes of the ascending aorta. However, multislice computed tomography (CT) showed an aortic ulcer with varying degree of the subadventitial hemorrhage in the region of the thoracic aorta at the level of Th 8–9. Due to imminent rupture of the penetrating aortic ulcer, the patient was promptly prepared for surgery. A 15 cm long subadventitial hematoma was found intraoperatively in the right posterolateral aspect of the descending aorta, 5 cm above the diaphragm and 7 cm below the origin of the left subclavial artery. The affected segment of the aorta was resected, followed by an inlay aortic reconstruction with a Dacron tube graft of 24 mm. Control CT revealed satisfactory reconstruction of the descending aorta. **Conclusion.** PAU is a rare, but potentially fatal disease. Open surgery in patients with PAU is an effective treatment strategy, although endovascular treatment options are emerging.

Key words:
aorta, thoracic; aortic rupture; atherosclerosis; ulcer; diagnostic techniques and procedures; cardiovascular surgical procedures; transplants.

Apstrakt

Uvod. Penetrantni aterosklerotski ulkus (PAU) aorte predstavlja progresivno prodiranje aterosklerotskog procesa u unutrašnji i središnji sloj zida aorte. PAU je lezija visokog rizika zbog štetnog uticaja na integritet zida aorte sa mogućim smrtnim ishodom. **Prikaz bolesnika.** Bolesnik sa jakim i oštrim bolom u grudima koji se širio u leđa, ali bez znakova ishemije miokarda na elektrokardiogramu, primljen je u našu kliniku. Transtorakalna ehokardiografija nije pokazala patološku promenu na ascedentnoj aorti. Međutim, kompjuterizovana tomografija (KT) pokazala je postojanje aortnog ulkusa sa različitim stepenom subadventicijalnog krvarenja u regionu Th 8–9 torakalne aorte. Usled neposredne rupture PAU, bolesnik je upućen na operaciju. Intraoperativno, nađen je subadventicijalni hematom dužine 15 cm u posterolateralnom delu descedentne aorte, 5 cm iznad dijafragme i 7 cm ispod odvajanja leve potključne arterije. Posle odstranjivanja zahvaćenog dela aorte, urađena je rekonstrukcija aorte Dacron-skim graftom prečnika 24 mm. Kontrolna KT pokazala je zadovoljavajuću rekonstrukciju descedentne aorte. **Zaključak.** Pored toga što je retko, PAU je potencijalno fatalno oboljenje. Otvorena hirurgija je efikasna metoda lečenja PAU, premda se u novije vreme razvijaju endovaskularne metode rešavanja ovog problema.

Ključne reči:
aorta, torakalna; aorta, ruptura; arterioskleroza; ulceracija; dijagnostičke tehnike i procedure; hirurgija, kardiovaskularna, procedure; graftovi.

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Introduction

The term "penetrating atherosclerotic ulcer" (PAU) of the aorta describes the condition in which ulceration of an aortic atherosclerotic lesion penetrates the internal elastic lamina into media [1]. The progression of aneurysmal dilatation is usually slow. Aortic ulcers may break through into the adventitia to form a pseudoaneurysm [2]. In this situation the hematoma is contained by the overlaying adventitia and some authors consider this type of aortic lesion a contained aortic rupture [3]. With regard to the new aortic dissection classification, PAU is considered a form of aortic dissection [4]. Mixed clinical presentation, varied patient population, and data limited to small numbers of retrospectively reviewed patients have made a data-driven algorithm for the surgical treatment of PAUs difficult to construct. Most authors believe that PAU is a high-risk lesion with potentially fatal outcome [5].

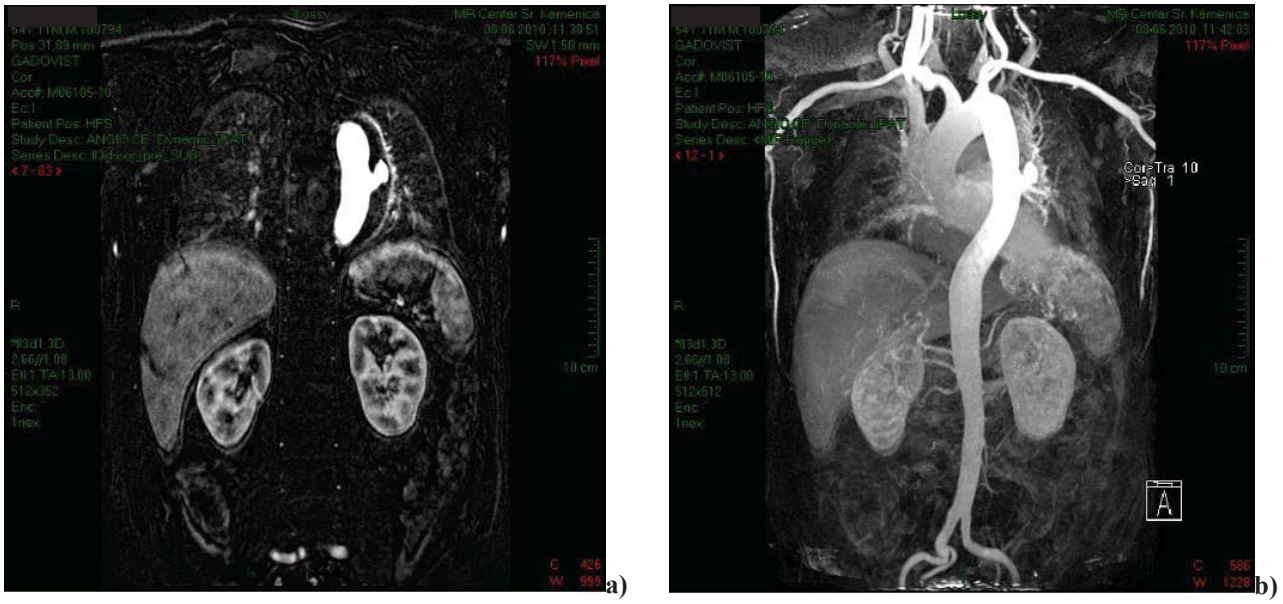

Fig. 1(a, b) – Contrast-enhanced magnetic resonance angiography (MRA) images show a penetrating ulcer in the descending thoracic aorta.

Cace report

A 55-year-old man, with a history of hypertension and smoking, that suffered a sudden onset of severe chest pain was admitted to our hospital. Intensive, and sharp chest pain with irradiation to the back had appeared suddenly during the night. On admission, chest radiography was performed showing enlarged cardiac shadow mainly due to elongated and enlarged descending thoracic aorta. There was no evidence of myocardial ischemia on an electrocardiogram. Transthoracic echocardiography (TTE) registered no pathological changes of the descending aorta. Multislice computed tomography (CT) showed an aortic ulcer with varying degree of subadventitial hemorrhage in the region of the thoracic aorta at the level of Th 8–9. Magnetic resonance imaging (MRI) was performed subsequently in order to delineate the process extension, showing saccular aortic aneurysm (2 × 2 cm) with surrounding hematoma and left pleural hemorrhagic effusion, which in fact was PAU. Aortography revealed irregular edge of the aortic defect which corresponded to described aortic exulceration on CT (Figure 1). After the diagnosis of imminent PAU rupture was established, the patient was urgently transferred to the operating room for emergency surgery.

Cerebrospinal fluid (CSF) drainage was initiated at the L 2–3 level in order to obtain CSF pressure < 10 mmHg. A selective double-lumen endotracheal intubation was performed using a Carlen's tube. Posterolateral thoracotomy was performed in the 5th left intercostal space. There was no need for phrenotomy neither extra- nor transperitoneal approach to the upper parts of the abdominal aorta. A 15 cm long subadventitial hematoma was found in the right posterolateral aspect of the descending aorta, 5 cm above the diaphragm and 7 cm below the origin of the left subclavial artery. Systemic heparinization was achieved with 5,000 IU of heparin. Operation was further conducted on partial extracorporeal circulation (ECC) – 2.5 L/min – with the left femoral artery and the inferior vena cava cannulation. In the upper parts of the body we obtained a controlled hypotension with maximal systolic arterial pressure between 80 and 100 mmHg. The patient was not cooled down in order to avoid fibrillation of the heart. Aortic clamping was performed above and under the level of the PAU. The affected segment of the aorta was resected, followed by an inlay aortic reconstruction with a Dacron tube graft 24 mm along with three intercostal arteries included in the distal anastomosis through a separate anastomosis (Figure 2). The patient was subsequently weaned from partial ECC. Partial ECC time was 71 minutes. Drainage of the left pleural space was achieved with two tube drains. The postoperative period was uneventful. Control CT revealed a satisfactory reconstruction of the de-

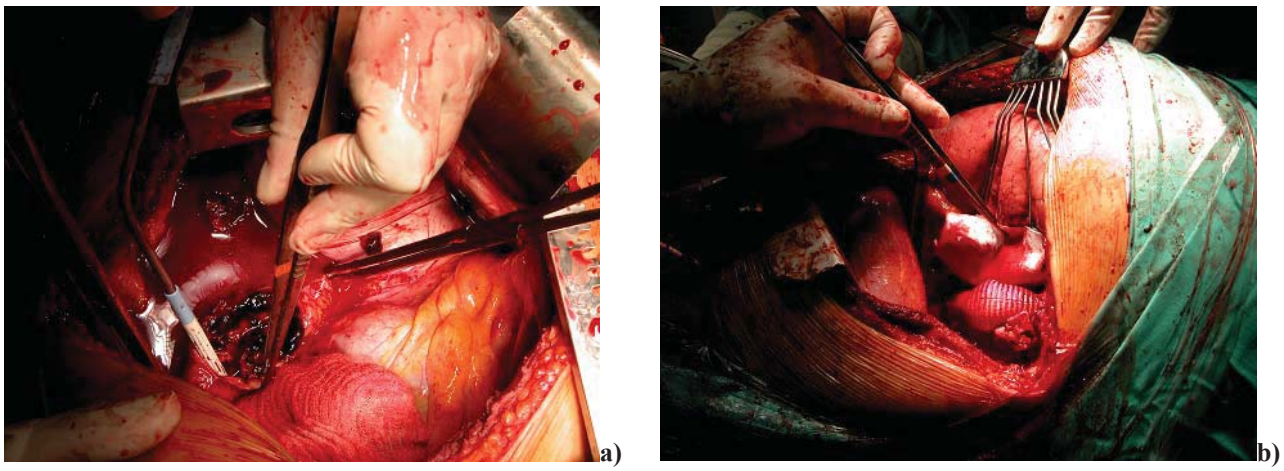

Fig. 2 – a) Intraoperative findings of penetrating ulcer of the descending aorta; b) Dacron graft reconstruction

scending aorta. The patient was discharged in good postoperative condition with no postoperative complications, on the 20th postoperative day.

Discussion

The correct initial diagnosis and immediate appropriate management of PAU is essential. It is often very difficult for the attending physician to establish the diagnosis of PAU because the pathophysiology and diagnostic algorithm for PAU have not been fully understood, and the amount of comprehensive literature description is still lacking [5].

PAU can lead to the development of intramural hematoma, aortic dissection, aortic aneurysm or rupture. In a retrospective study of 198 patients in whom aortic dissection was initially diagnosed, 7.6% of patients were found to have PAU [6]. In some cases, hematoma extension causes stretching of the weakened aortic wall, leading to the formation of a saccular aortic aneurysm [6]. Yokoyama et al. [7] suspected that some spontaneous aortic rupture due to atheromatous plaque as previously reported might have been due to the perforation of PAU. Therefore, we believe that PAU might be recognized as a cause of aortic rupture with increasing frequency in the future by sensitive imaging techniques such as CT, MRI and transesophageal echocardiography. In our case CT scan showed PAU with varying degree of surrounding hemorrhage located in the thoracic aorta with left pleural hemorrhagic effusion. In emergency situation, CT is the imaging modality of choice to identify and locate the PAU [5].

Haris et al. [2] reported that the disease progression is slow, with a low prevalence of acute rupture or other life-threatening complications. Coady et al. [8] reported that the risk of aortic rupture in patients with penetrating aortic ulcer is 40% compared to patients with Stanford type A or type B dissection where the risk is 7% and 3.6%, respectively.

Draining CSF from the lumbar region may reduce CSF pressure, improve blood flow to the spinal cord and reduce the risk of ischemic spinal cord injury [9]. Partial ECC is also a very useful approach to avoid visceral and spinal ischemia during aorta cross clamping. In our patient no neurological deficit has been observed during postoperative period. Persistent or recurrent pain, hemodynamic instability and a rapidly expanding aortic diameter have been considered as indications for surgical treatment. The authors emphasized that most patients with PAU are at high risk for surgical intervention because of their advanced age and poor general health [5, 8].

Murgo et al. [10] emphasized that surgical repair of the descending thoracic aorta is frequently complicated by respiratory disease, renal insufficiency, or spinal ischemia and recommended the transluminal placement of endovascular stent-grafts for PAUs. In patients with PAUs initially managed with medical therapy, Cho et al. [11] reported that one-third of these patients required surgical repair during follow-up for the progression of PAUs to aneurysms, dissections, or perforations. Despite improvements in surgical techniques and postoperative care, conventional operative repair of the descending thoracic aorta for PAUs is still associated with high morbidity and mortality rates [12]. Endovascular repair seems to be a promising option showing lower morbidity and mortality rates with respect to open surgical repair [13]. Specifically, there are no prospective randomized studies comparing open and endovascular treatment of PAU. Reports in the literature primarily include single-center experiences and non-randomized studies of open and endovascular stent graft procedures with limited follow-up [13].

Conclusion

PAU is a rare, but potentially fatal disease. It is typically seen in elderly individuals with hypertension and atherosclerosis and usually involves the descending thoracic aorta. With onset of acute chest pain in a patient with no evidence of myocardial ischemia, aortic dissection or rupture, PAU should be considered. In emergency situation, CT is the imaging modality of choice to identify PAU. Open surgery in patients with PAU is an effective treatment strategy, although endovascular treatment options are emerging.

R E F E R E N C E S

1. *Vilacosta I, San Roman JA, Aragoncillo P, Ferreiros J, Mendey R, Graupner C*, et al. Penetrating atherosclerotic aortic ulcer: documentation by transesophageal echocardiography. J Am Coll Cardiol 1998; 32(1): 83–9.
2. *Haris JA, Bis KG, Glover JL, Bendick PJ, Shetty A, Brown OW*. Penetrating atherosclerotic ulcers of the aorta. J Vasc Surg 1994; 19(1): 90–8; discussion 98–9.
3. *Ando Y, Minami H, Muramoto H, Narita M, Sakai S*. Rupture og the thoracic aorta caused by penetrating aortic ulcer. Chest 1994; 106(2): 624–6.
4. *Hayashi H, Matsuoka Y, Sakamoto I, Sueyoshi E, Okimoto T, Hayashi K,* et al. Penetrating atherosclerotic ulcer of the aorta: imaging peatures and disease concept. Radiographics 2000; 20(4): 995–1005.
5. *Ganaha F, Miller DC, Sugimoto K, Do YS, Minamiguchi H, Saito H*, et al. Prognosis of aortic intramural hematoma with and without penetrating atherosclerotic ulcer: a clinical and radiological analysis. Circulation 2002; 106(3): 342–8.
6. *Karthikeyan D, Vijay S, Kumar T*. Penetrating atherosclerotic ulcer of aorta - a case report. Indian J Radiol Imaging 2003; 13: 191–4.
7. *Yokoyama H, Ohmi M, Sadahiro M, Shoji Y, Tabayashi K, Moizumi Y*. Spontaneous rupture of the thoracic aorta. Ann Thorac Surg 2000; 70(2): 683–9.
8. *Coady MA, Rizzo JA, Hammond GL, Pierce JG, Kopf GS, Elefteriades JA*. Penetrating ulcer of the thoracic aorta: what is it? How do we recognize it? How do we manage it?. J Vasc Surg 1998; 27(6): 1006–6.
9. *Khan SN, Stansby GP*. Cerebrospinal fluid drainage for thoracic and thoracoabdominal aortic aneurysm surgery. Cochrane Database Syst Rev 2004; (1): CD003635.
10. *Murgo S, Dussaussois L, Golzrian J, Cavenaile JC, Abada HT, Ferreira J,* et al. Penetrating atherosclerotic ulcer of the descending thoracic aorta: treatment by endovascular stent-graft. Cardiovasc Intervent Radiol 1998; 21(6): 454–8.
11. *Cho KR, Stanson AW, Potter DD, Cherry KJ, Schaff HV, Sundt TM 3rd*. Penetrating atherosclerotic ulcer of the descending thoracic aorta and arch. J Thorac Cardiovasc Surg 2004; 127(5): 1393–1401.
12. *Demers P, Miller DC, Mitchell RS, Kee ST, Chagonjian L, Dake MD*. Stent-graft repair of penetrating atherosclerotic ulcers in the descending thoracic aorta: midterm results. Ann Thorac Surg 2004; 77(1): 81–6.
13. *Kouchoukos NT, Masetti P, Rokkas CK, Murphy SF*. Hypothermic cardiopulmonary bypass and circulatory arrest for operations on the descending thoracic and thoracoabdominal aorta. Ann Thorac Surg 2002; 74(5): S1885–7; discussion S1892–8.

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CASE REPORT

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Rapidly vanishing lung pseudotumor in a patient with acute bilateral bronchopneumonia

Brzo nestajući pseudotumor pluća kod bolesnika sa akutnom bilateralnom bronhopneumonijom

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Abstract

Introduction. Rapidly vanishing lung pseudotumor (phantom tumor) refers to the transient well-demarcated accumulation of pleural fluid in the interlobar pulmonary fissures. Most frequently their appearance is associated with congestive heart failure, but also other disorders like hypoalbuminemia, renal insufficiency or pleuritis. Its rapid disappearance in response to the treatment of the underlying disorder is a classical feature of this clinical entity. **Case report.** A 47-year-old woman, chronic smoker with symptoms of shortness of breath, orthopnea, chills, cough, weakness and the temperature of 39.2°C was admitted to our hospital. A posteroanterior chest X-ray revealed cardiomegaly with the cardiothoracic ratio of > 0.5, blunting of both costophrenic angles and an adjacent 6 × 5 cm well-defined, rounded opacity in the right interlobar fissure. Transthoracic 2-dimensional echocardiography demonstrated left ventricular hyperthrophy with a systolic ejection fraction of 25% and moderate mitral regurgitation. The patient's symptoms resolved rapidly after diuresis, and repeated chest X-ray four days later showed that the right lung opacity and pleural effusions had vanished. **Conclusion.** The presented case underlines the importance of the possibility of vanishing lung tumor in patients with left ventricular failure and a sharp oval lung mass on the chest X-ray. This is the way to avoid incorrect interpretation of this finding causing additional, unnecessary, costly or invasive imaging, interventions and drugs.

Key words:
lung; radiography; diagnosis, differential; heart failure; bronchopneumonia.

Apstrakt

Uvod. Brzo nestajući pseudotumor pluća (fantom tumor) predstavlja prolaznu, jasno ograničenu kolekciju pleuralne tečnosti u interlobarnim pulmonalnim fisurama. Najčešći uzrok njihovog nastanka je zastojna srčana slabost, ali, takođe, i drugi poremećaji kao što su hipoalbuminemija, bubrežna insuficijencija i pleuritis. Tipična karakteristika ovog kliničkog entiteta je njegovo brzo nestajanje pri lečenju osnovnog, uzročnog oboljenja. **Prikaz bolesnika.** Bolesnica, stara 47 godina, hronični pušač, primljena je u našu bolnicu zbog simptoma gušenja, ortopneje, jeze i drhtavice, kašlja, opšte slabosti i povišene telesne temperature od 39.2 °C. Radiografija pluća i srca u posteroanteriornoj projekciji je pokazala postojanje kardiomegalije sa kardiotorakalnim indeksom > 0,5, zasenčenje oba kostofrenična sinusa i dobro ograničenu, kružnu infiltraciju veličine 6 × 5 cm u predelu desne interlobarne fissure. Dvodimenzionalni transtorakalni ehokardiogram je pokazao postojanje hipertrofije leve komore sa sistolnom ejekcionom frakcijom od 25% i umerenom mitralnom regurgitacijom. Vrlo brzo nakon započinjanja diuretske terapije simptomi su se povukli i četvrtog dana hospitalizacije pri ponovljenoj radiografiji pluća došlo je do kompletnog povlačenja pleuralnih izliva kao i znakova zasenčenja na plućima. **Zaključak.** Prikaz naše bolesnice naglašava značaj razmatranja mogućnosti nestajućeg pseudotumora pluća kod bolesnika sa levostranom srčanom slabošću i oštro ograničenom ovalnom masom na radiografiji pluća. Na ovaj način može se izbeći pogrešna interpretacija radiografskog nalaza i dalje nepotrebno izlaganje bolesnika dodatnim, skupim i invazivnim ispitivanjima, intervencijama i lekovima.

Ključne reči:
pluća; radiografija; dijagnoza, diferencijalna; srce, insuficijencija; bronhopneumonija.

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Introduction

Vanishing lung pseudotumor refers to the transient well demarcated accumulation of pleural fluid in the interlobar pulmonary fissures [1]. Most frequently their appearance is associated with congestive heart failure, but also other disorders like hypoalbuminemia, renal insufficiency and pleuritis [2]. Awareness of this form of pleural effusion is important for the differential diagnosis of pulmonary mass on radiography. Making the correct diagnosis is crucial in order to prevent further inappropriate and possibly harmful investigations and treatment (e.g. lung biopsy and/or surgery). Its rapid disappearance in response to underlying disorder treatment is a classical feature of this clinical entity [3].

Case report

A 47-year-old woman, chronic smoker with symptoms of shortness of breath, orthopnea, chills, cough, weakness and the temperature of 39.2 °C was referred to our pulmology ward by her physician. The symptoms started five days before admission to our hospital, and she had been treated ambulatory with penicillin and paracetamol for two days before the referral. Physical examination revealed extreme obesity (body mass index 35.4 kg/m^2), high blood pressure (170/100 mmHg), sinus tachycardia with systolic murmur on the apex, tachypnea and extensive bilateral crackles over both lung fields. There was no pedal oedema or elevated jugular venous pulse. Laboratory analyses revealed leucocytosis (18.3 × 10^9/L) with neuthrophilia and highly elevated markers of acute inflammation [erytrocyte sedimentation rate (ESR) 90, C-reactive protein (CRP) 257mg/L, fibrinogen > 10g/L)]. Other biochemical analyses including serum creatinine and albumin gave normal result. Three series of sputum culture were aseptic, as well as blood cultures.

On admission, posteroanterior chest X-ray revealed cardiomegaly with cardiothoracic ratio of > 0.5, blunting of both costophrenic angles and an adjacent 6 × 5 cm well-defined, rounded opacity in the right interlobar fissure (Figure 1). Transthoracic 2-dimensional echocardiography demonstrated left ventricular hyperthrophy with a systolic ejection fraction of 25% and a moderate mitral regurgitation. These findings were consistent with the diagnosis of acute bilateral bronchopneumonia and left ventricular failure. The treatment with two antibiotics (ceftriaxone, ciprofloxacin), diuretics (furosemide, spironolactone) and angiotensin-converting enzyme inhibitor (ramipril) was promptly instituted. The patient's symptoms resolved rapidly after extensive diuresis, and repeated chest X-ray four days later showed that the right lung opacity and pleural effusions had vanished (Figure 2). The patient was discharged 8 days after admission.

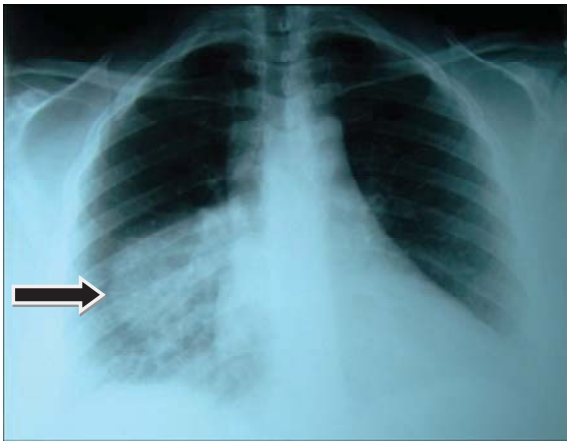

Fig. 1 - Posteroanterior chest X-ray on admission – bilateral pleural effusions and a sharp 6 × 5 cm well-defined, rounded opacity in the right interlobar fissure (arrow).

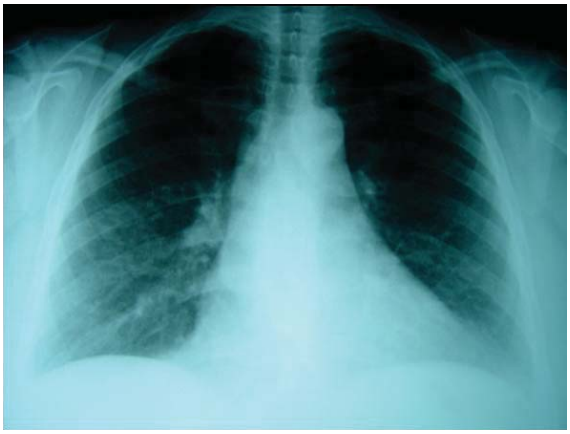

Fig. 2 – Posteroanterior chest X-ray 4 days after institution of heart failure and antibiotics therapy – right lung opacity and pleural effusions had vanished.

Discussion

We reported an extremely rare case of vanishing lung pseudotumor in a patient with acute bilateral bronchopneumonia and previously unknown heart failure. In fact, the vanishing lung pseudotumor was the first clinical manifestation of heart failure in our patient and probably the associated bilateral bronchopneumonia promoted its outbreak.

The descriptors "vanishing", "pseudotumor" or "phantom tumor" (most frequently in Serbian literature) are used interchangeably and they refer to the transient localized collection of pleural fluid in the interlobar fissures which is typically transudative [1]. The most frequent cause of lung pseudotumor is congestive heart failure from various causes [4–6], but it may also be caused by hypoalbuminamia, renal insufficiency and pleural infections [2]. Noteworthy, the presence of an interlobar pleural effusion does not necessarily correspond to the severity of the left heart failure. In fact, it may be the first or even the only sign of the left ventricular failure [5, 6].

Although the congestive heart failure with pleural effusion is very often encountered in clinical practice, vanishing lung pseudotumors as its complication can be seen extremely rare so as the exact incidence of this entity is difficult to assess due to the small number of reported cases.

Vanishing pseudotumor is a phenomenon predominantly occurring in the right lung, especially along the right

horizontal fissure. Infrequently, it can occur in the horizontal and oblique fissures simultaneously [7].

Regarding pathogenesis of vanishing lung pseudotumors several authors [3, 6] attempted to explain it, and all of them presumed that pleuritis with subsequent adhesions resulting in symphysis is responsible for the localization of the fluid in the pleural space. Thereafter several predisposing factors contribute for accumulation of fluid in the interlobar space such as the anatomy of the pleural venous system, the right recumbent position assumed by many cardiac patients and finally the pulmonary lymphatic drainage.

Differential diagnosis of interlobar pleural effusions includes transudates from congestive heart failure and renal failure, exudates from an asbestosis-related disorder or from a parapneumonic process, hemothorax, chylothorax, malignant effusions and pulmonary thromboembolism [8–10].

Vanishing lung pseudotumors rapidly disappear with underlying cause treatment. In fact, it is the landmark of this phenomenon.

Conclusion

The presented case underlines the importance of the possibility of vanishing lung pseudotumor in patients with left ventricular failure and a sharp oval lung mass on chest X-ray. This may help to avoid incorrect interpretation of this finding causing additional, unnecessary, costly or invasive imaging, interventions and drugs. Treatment of this condition involves underlying disorder managing, like congestive heart failure in the presented case, leading to the rapid disappearance of the lung mass on control chest radiography.

R E F E R E N C E S

1. *Light RW*. Pleural diseases. 4th ed. Philadelphia, PA: Lippincott, Williams & Wilkins; 2001.
2. *Van Gelderen WF*. Vanishing pleural fluid collections in cardiac failure simulating lung tumors. Australas Radiol 1994; 38(2): 93–6.
3. *Millard CE*. Vanishing or phantom tumor of the lung; localized interlobar effusion in congestive heart disease. Chest 1971; 59(6): 675–7.
4. *Athappan G, Ariyamuthu VK, Rajamani V*. Phantom tumor of the lung. Internet J Cardiol 2007; 5(1): 7.
5. *Đurić O, Stanić B*. Vanishing lung tumor. Pneumon 1994; 32:167–71.
6. *Kabnick EM, Sobo S, Cooper C, Alexander LL*. Vanishing lung tumor. J Natl Med Assoc 1985; 77(3): 229–30.
7. *Ardic I, Yarlioglues M, Celik A, Kaya MG*. Vanishing or phantom tumor of the lung. Tex Heart Inst J 2010; 37(6): 730–1.
8. *Stark P, Leung A*. Effects of lobar atelectasis on the distribution of pleural effusion and pneumothorax. J Thorac Imaging 1996; 11(2): 145–9.
9. *Buch KP, Morehead RS*. Multiple left-sided vanishing tumors. Chest 2000; 118(5): 1486–9.
10. *Haus BM, Stark P, Shofer SL, Kuschner WG*. Massive pulmonary pseudotumor. Chest 2003; 124(2): 758–60.

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CASE REPORT

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Rare locations of metastastatic renal cell carcinoma: A presentation of three cases

Retka mesta metastatskog karcinoma bubrežnog parenhima

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Abstract

Introduction. Metastatic renal cell carcinoma (RCC) frequently spreads not only to neighboring lymph nodes, but also to distant organs, including the lungs, liver, bones and brain. **Case report.** We presented three cases of RCC with colon metastasis. In the first, 63-year-old patient, after left nephrectomy followed with lyphadenectomy in paraaortic lymph node, left hemicolectomy was done due to RCC metastasis in rectosigmoid colon. In the second, 35-year-old patient, left radical nephrectomy was followed two years later with partial right nephrectomy, lung metastasectomy, small bowel and coecum resection and right orchiectomy all as separate procedures in different time intervals. The patient died from brain and bone metastases two years after the first surgery. The third, 35-year-old patient, had right nephrectomy followed by repeted lymphadenectomies after 6, 12 and 24 months. Four years later RCC spreaded to coecum and right hemicolectomy was performed. **Conclusion.** RCC treated with nephrectomy should be carefully followed up with imaging methods as a proper treatment of RCC metastases to distant organs could be important for a patient survival.

Key words:
carcinoma, renal cell; neoplasm metastasis; urologic surgical procedures; treatment outcome.

Apstrakt

Uvod. Tumori bubrežnog parenhima (*renal cell carcinoma* – RCC) čine 3% maligniteta odraslih osoba. Najčešća lokalizacija metastaza RCC je u plućima, limfnim žlezdama, jetri i kostima, ali se u retkim slučajevima nalaze i u drugim organima. **Prikaz bolesnika.** Dve godine nakon radikalne levostrane nefrektomije zbog RCC, 63-godišnjem bolesniku je učinjena limfadenektomija zbog metastaze RCC u paraaortalnu limfnu žlezdu. Deset meseci kasnije, učinjena je levostrana hemikolektomija zbog metstaze RCC u rektosigmoidni kolon. Kod drugog bolesnika, starog 37 godina, dve godine nakon levostrane radikalne nefrektomije zbog RCC, urađena je parcijalna desnostrana nefrektomija. U daljem periodu praćenja bolesnika, urađeno je više hirurških zahvata u cilju uklanjanja metastaza iz različitih organa: metastazektomija pluća, resekcija tankih creva i cekuma, i desnostrana orhiektomija. Dve godine nakon primarnog hirurškog zahvata došlo je do letalnog ishoda usled širenja bolesti na koštani sistem i mozak. Treći bolesnik, star 35 godina, nakon desnostrane nefrektomije zbog metastatskih promena RCC u limfnim žlezdama prošao je kroz limfadenektomije 6, 12 i 24 meseca nakon primarne operacije. Kod istog bolesnika četiri godine nakon primarne operacije, učinjena je desnostrana hemikolektomija zbog metastatske promene RCC u cekumu. **Zaključak.** Praćenje bolesnika nakon nefrektomije zbog RCC uz korišćenje metoda snimanja u određenim vremenskim intervalima doprinosi pravovremenom otkrivanju metastaza. Primena odgovarajućeg hirurškog zahvata u ranoj fazi poboljšava produžavanja i popravlja kvalitet života bolesnika.

Ključne reči:
karcinomi bubrežnog parenhima; neoplazme, metastaze; hirurgija, urološka, procedure; lečenje, ishod.

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Introduction

Renal cell carcinoma (RCC) is the third most common urogenital malignancy and present 3% of all malignancies in adults [1]. Although 50% of all RCC cases are diagnosed by imaging techniques in an early, asymptomatic phase, 20–30% of patients already have metastatic disease [2]. Moreover, 20–40% patients with localized RCC will develop local RCC recurrence or distant metastasis [3]. RCC metastases are usually located in the lungs, lymph nodes, liver, bones and suprarenal gland, but they can rarely be found in other organs. We presented three cases with RCC metastasis in different locations.

Case 1

A 63-year-old man was admitted to the hospital after a mass of 72 × 36 mm was detected in his left renal region. Two years earlier, the patient had been submitted to left radical nephrectomy, and pathology had confirmed RCC. Multislice computed tomography (MSCT) had not revealed any changes in the thorax and abdomen. A mass localized behind the tail of the pancreas, near the aorta was removed through a subcostal incision (in the same line as the scar from the previous surgery). Pathology confirmed paraaortic lymph node RCC metastatic disease, but did not reveal any malignant cells in other lymph nodes. Ten months later, the patient returned with irregular stools and bloating, and an ultrasound study showed a hyperechogenic structure localized in the left paraumbilical region just under the skin. Although abdominal MSCT excluded local tumor recurrence or organ and abdominal wall changes, colonoscopy revealed a vegetative 2 cm tumor, localized in the sigmoid colon 30 cm from the anus, and the patient went through hemicolectomy. Intraoperative findings included infiltration of the sigmoid colon wall and serosa by tumor, and enlarged mesenteric lymph nodes (Figure 1), and pathology confirmed RCC metastasis to the colon. Comparative histological and immunohistochemical analysis showed identical type of RCC in the kidney, lymph nodes and colon.

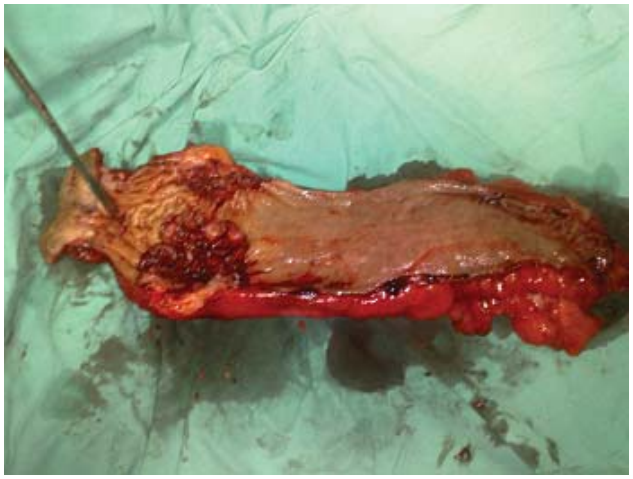

Fig. 1 – A resected sigmoid colon with intraluminal renal cell carcinoma (RCC) metastasis.

Case 2

A 35-year-old man with the history of left radical nephrectomy for RCC followed by chemotherapy (interferon alfa for 12 months and vinblastine for 11 months) was admitted in our hospital because of solid mass near the lower pole of the right kidney discovered in the follow-up study. Diagnostic evaluation before nephrectomy included MSCT which had shown large (10 × 13 × 14 cm) left kidney tumor and bilateral lung nodular metastasis up to 35 mm in diameter (Figure 2). Pathology revealed RCC stage III, nodular gland (NG) 3 with fields of bleeding and necrosis, with light granular cells and tumor infiltrated renal capsula, lymph nodes and blood vessels (pT3a Nx Mx). Postoperatively, during chemotherapy, the follow-up study including MSCT of abdomen and thorax were performed. One year after the surgery, control MSCT showed just one nodular change without progression in the left posterobasal lung which was recorded two months after the surgery.

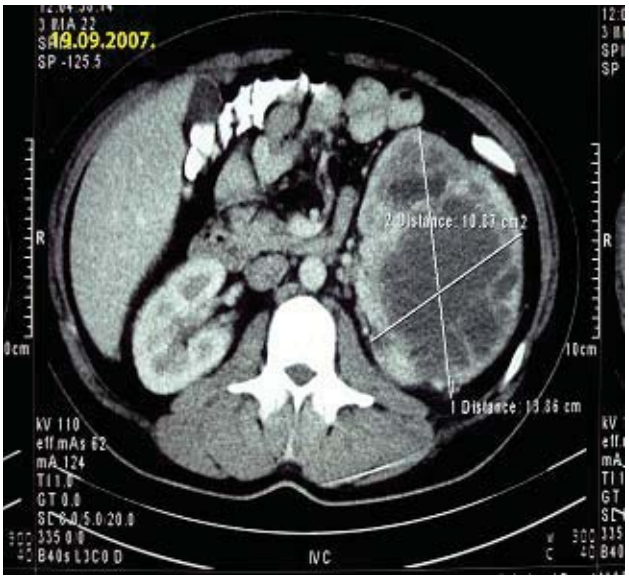

Fig. 2 – A large left kidney tumor.

Fifteen months after the surgery, MSCT revealed a 30 mm nodular metastasis in the left lower lung (Figure 3) and a 3 cm solid mass near the lower pole of the right kidney, below the hilus (Figure 4). Because of these findings, MSCT performed before nephrectomy was reviewed again, and reassessment showed that this solid mass was present in the initial study, but it was smaller and was diagnosed as a cyst (Figure 5). In response to these findings, the patient underwent right partial nephrectomy, and pathology revealed clear cell RCC Fuhrman grade II (pT1a, L0V0). One month later, the patient underwent left lung metastasectomy which included removal of four different lesions of different dimensions (60 mm, 45 mm, 30 mm and 25 mm). Pathology examination of these lesions confirmed that they were metastatic of RCC origin.

However, two and half months after the last operation, the patient came to the Emergency Department for nausea

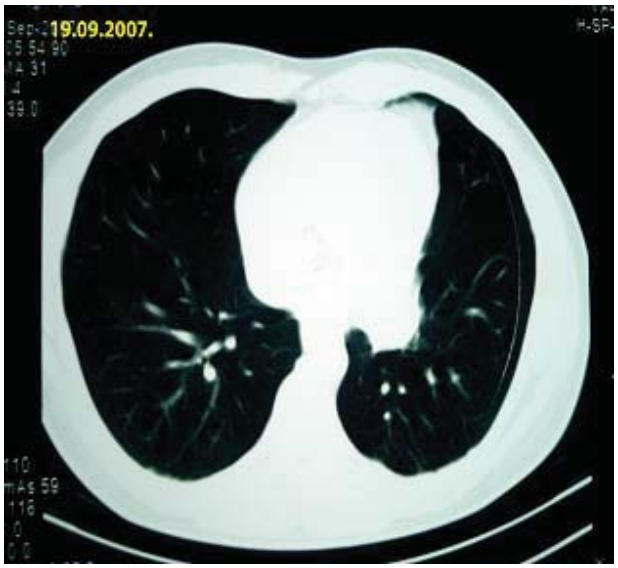

Fig. 3 – Nodular renal cell carcinoma (RCC) metastases in the lung.

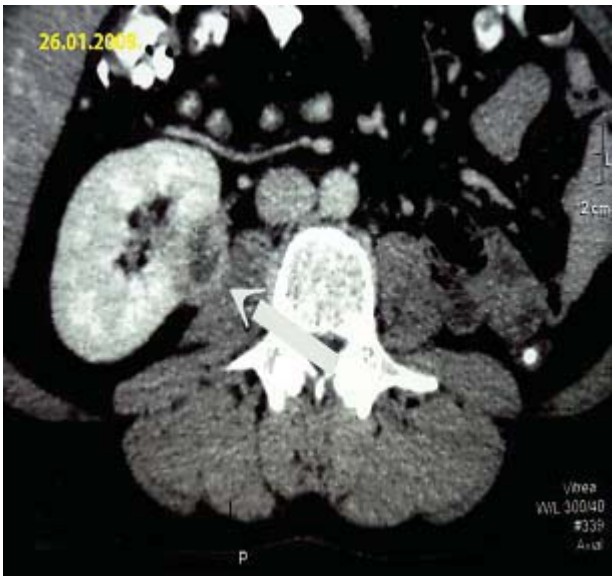

Fig. 4 – A 3 cm renal cell carcinoma (RCC) at the solitary right kidney, previously considered as a small simplex cyst (see Fig. 5).

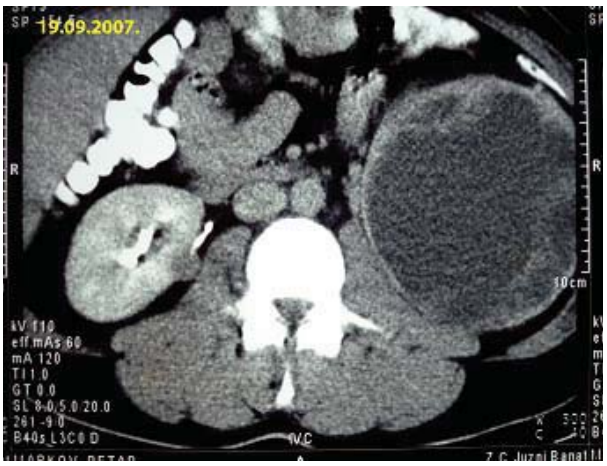

Fig. 5 – Initial MSCT before any surgery shows a large left kidney tumor and a small cystic formation on the right kidney considered at this moment as a simplex cyst.

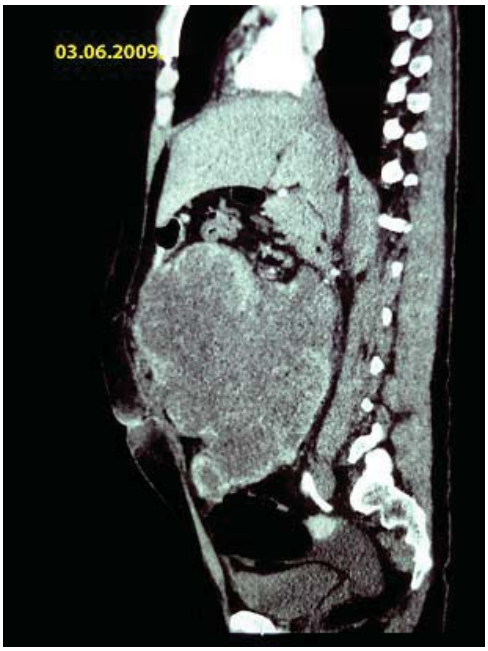

Fig. 6 – MSCT shows a big intaperitoneal lobular mass originating from the abdominal wall, involving the rectus abdominis muscle.

and vomiting. His abdomen was distended and tender to palpation, and laboratory studies showed anemia (hemoglobin 88 g/L^{-1}, hematocrit 28%). Abdominal ultrasound examination showed a cystic formation, and MSCT revealed a solid hyperdense mass (38 × 35 mm) in the left lung and a big intaperitoneal lobular mass originating from the abdominal wall and involving the rectus abdominis muscle (Figure 6). The patient underwent surgery that included tumor mass extirpation, small bowel and cecum resection and termino-lateral ileocolonic anastomosis (Figure 7). Pathology examination showed a high grade RCC metastasis in the large bowel and fat tissue. Postoperatively, the patient also complained of painful right testis, and physical examination revealed a palpable 15 mm mass in the scrotum.

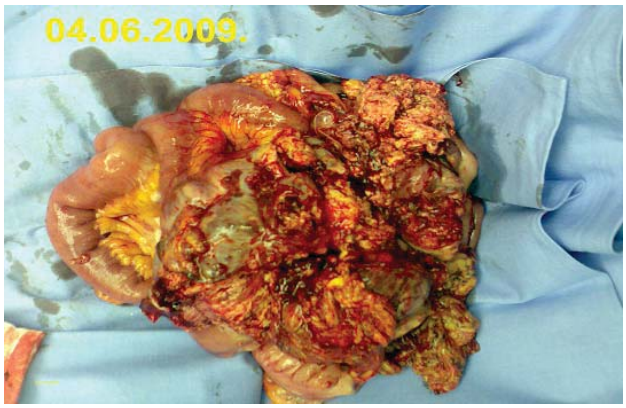

Fig. 7 – Tumor mass extirpation, small bowel and cecum resection and termino-lateral ileocolonic anastomosis [pathology examination showed a high grade renal cell carcinoma (RCC) metastasis in the large bowel and fat tissue].

Although biochemical markers, alpha-fetoprotein (AFP) and beta human chorionic gonadotropin ([Beta HCG]) were normal, ultrasound examination showed a 21 mm echoheterogeneous mass that was clearly differentiated from other tissue in the right testis (Figure 8). The patient then had right orchectomy, and pathology confirmed that this testicular mass was metastatic RCC. Then, one month later, bone scintigraphy detected a left parieto-temporal bone metastasis (Figure 9), and head MSCT detected multiple brain metastases. At this point the patient received three cycles of chemotherapy with temsirolimus. From this point on, the patient's condition continued to deteriorate, and the patient died two and a half years after his primary operation.

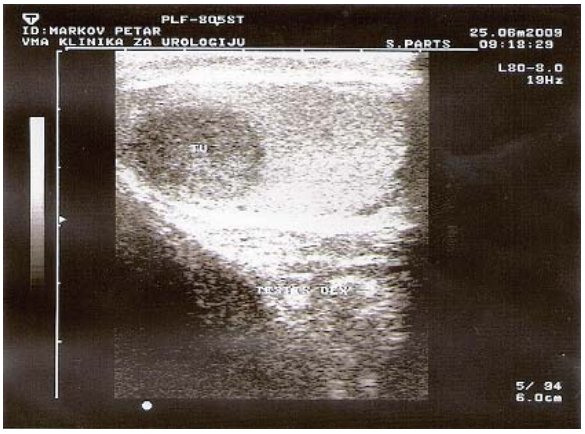

Fig. 8 – Ultrasound examination showed a 21 mm echoheterogeneous mass [renal cell carcinoma (RCC) metastasis] in the right testis.

metastasis in three stages 6, 12 and 24 months after the primary surgery, was admitted in our hospital for primary disease evaluation. His main symptoms were pain in the right thigh and frequent liquid stools alternating with constipation. Although biochemical parameters and abdominal CT were normal, colonoscopy revealed submucosal tumorous 3 × 3 cm lesion across the ileocecal valve, and the patient underwent right hemicolectomy. Pathology revealed a large (5 cm) colon metastasis of RCC, but analysis of 13 lymph nodes showed reactive inflammation without evidence of malignancy. Chemotherapy consisted of interferon alpha (IFN-alpha) and 5-fluorouracil for three months. Follow-up evaluation (including imaging) nine months later did nor reveal any RCC recurrence, and the patient was sent back to his referring hospital for the regular follow-up care.

Disscusion

Despite modern diagnostic techniques, 20–30% of renal cancer patients have metastasis at the time of diagnosis, and 20–40% of patients who undergo nephrectomy for the treatment of RCC will develop distant metastasis [3]. Without treatment, the five-years survival rate in patients with metastatic RCC is less than 10%, and the average survival is 7–12 months [4]. Computed tomography (CT, MSCT) has an unreplaceable role in the follow-up of patients with high risk of metastasis. The aim of these methods is early detection of solitary metastasis that can be urgently surgically removed [5]. All three patients in this report were followed up after nephrectomy in accordance with the European Association of Urology (EAU) guidelines.

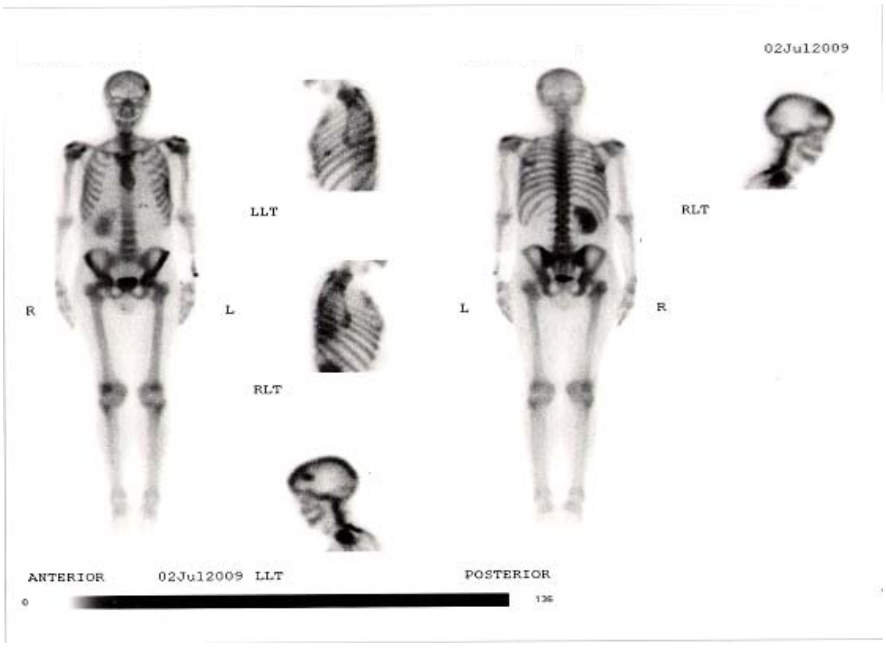

Fig. 9 – Bone scintigraphy detected left parieto-temporal bone metastasis.

Case 3

A 39-year-old man with the history of right radical nephrectomy for the stage G2, pT1, Nx, Mx RCC, who later required surgical removal of retroperitoneal RCC lymph nodes

RCC can give metastasis in the lymph nodes or any other organ, but the most frequent metastasis are dicovered in the lungs, lymph nodes, liver, bone and adrenal gland [5]. Surgical removal of solitary metastasis is considered the best therapeutic option, and studies have shown that the five year

survival was up to 35% higher in patient who had surgery, compared to those who did not [6]. Today surgical removement of metastasis is frequently combined with tyrosin kinase inhibitors and "target" therapy, and this therapy may confer some benefit, and increase survival by several months.

Up to 3.3% of patients have lymph node involvement at the time of nephrectomy. These patients have poor prognosis. Some data suggest that lymph node dissection does not improve survival [7], but one recent retrospective study from the USA on 900 patients showed that lymph node dissection may prolong survival by five months [8]. The first patient in our report had the evidence of paraaortic lymph node RCC metastasis two years after nephrectomy, and was treated with surgery. The second patient initialy underwent regional lymph node dissection and had no evidence of further lymph node involvement throughout the follow-up period. The third patient, soon after the nephrectomy, six months later, underwent regional paracaval lymph node dissection due to metastasis and the same operation was repeated on two other occasions in the next two years.

Patients with solitary surgically resectable RCC pulmonary metastasis have better prognosis than patients with metastasis in other organs, and a five-year survival rate after lung metastasectomy is up to 54% [9]. A five-year survival of patients with complete resection of multiple pulmonary metastasis is up to 29%. Immunotherapy with interferon-alpha in cases of metastatic disease involving the lungs is beneficial for a small number of patients 7–15% [10]. RCC develops from the proximal tubules who have a high level of P-glycoprotein expresion that leads to resistance for known types of chemotherapy. Chemotherapy with 5 flurouracil has better results if combined with interferon immunotherapy; this is the treatment offered, in accordance to EAU protocols, to the second patient in this report and resulted in regression of lung metastasis and remission of the disease [5]. Temsirolimus has somewhat better results regarding survival in patients with metastatic RCC when administered as first line therapy [5], and this is why it was used in our second patient.

Prognosis is poor in patients with bone metastasis, especially when metastasis involves the axial skeleton. A five-year survival is up to 38% in patients who undergo resection of solitary bone metastasis from the pelvis or limbs, but it is only 7% in patients with multiple bone metastasis [11]. Similarly, data show that surgical treatment of solitary bone metastasis results in a prolonged survival and better quality of life [12]. The second patient in our report did not have surgery or therapy with biphosphonates, after bone scan revealed metastasis to the scull, because of his poor general condition.

RCC metastasis in the gastrointestinal tract, excluding the liver, are rare, and are seen in 0.2–0.7% of patients [13]. Such metastatic lesions are most frequently localized in the pancreas, whereas RCC metastases in the stomach, small bowel or colon are very rare. All the three patients in our case report had RCC metastassis to the colon, and bowel resection was the only treatment option. Although gastrointestinal metastasis is rare, all the three reported patients had diagnostic workup of the abdomen, including colonoscopy because of gastrointestinal symptoms, such as abdominal pain, flatulence, diarrhea or constipation. Indeed, colonoscopy confirmed the presence of tumor metastasis in the colon in two patients. Although published data show that RCC metastatic lesions in the intestinal tract most frequently manifest as gastrointestinal bleeding or occlusion, that was not the case in our report. The third reported patient underwent immuno-chemotherapy, including interferon alpha and 5-flourouracil, because the target therapy for metastatic RCC was not available at our hospital in 2007.

Cerebral RCC metastasis occurs in 4–10% of patients [6]. Radiotherapy has no effect on cerebral RCC metastasis, but surgical excision of solitary metastasis may prolong survival by12.1 months on average [14], whereas radiosurgery using the gamma knife may prolong survival by up to 15 months in selected cases [15]. In the second patient, surgical treatment for multifocal brain lesions was not possible, and radiotherapy of the metastases was not an option because cerebral RCC metastasis is resistant to radiation therapy.

RCC patients with nephrectomy need a prolonged follow-up, because delayed metastasis may occur even 20 years after nephrectomy. Surgical resection of metastatic lesions, especially solitary lesions, has a place, and a multidisciplinary approach gives the best results [5, 6].

Conclusion

Early detection of organ RCC metastasis, while a lesion is still surgically treatable, and the surveillance of oncologic patients in accordance to the EAU guidelines, adjusted as needed to each individual patient, could provide good results. A multidisciplinary approach to the treatment is recommended in patients with metastatic RCC, since it might result in better quality of life and longer survival.

REFERENCES

1. *Garcia JA, Cowey CL, Godley PA.* Renal cell carcinoma. Curr Opin Oncol 2009; 21(39): 266–71.
2. *Cooperberg MR, Mallin K, Ritchey J, Villata JD, Carol PR, Kane CJ.* Decreasing size at diagnosis of stage 1 renal cell carcinoma: analysis from the National Cancer Data Base, 1993 to 2004. J Urol 2008; 179(6): 2131−5.
3. *Lam JS, Shvarts O, Leppert JT, Figlin RA, Belldegrun AS.* Renal cell carcinoma 2005: new frontiers in staging, prognostication and targeted molecular therapy. J Urol 2005; 173(6): 1853−62.
4. *Ljungberg B.* Prognostic factors in renal cell carcinoma. Scand J Surg 2004; 93(2): 118−25.
5. *Ljungberg B, Cowan NC, Hanbury DC, Hora M, Kuczyk MA, Merseburger AS,* et al. EAU guidelines on renal cell carcinoma: the 2010 update. Eur Urol 2010; 58(3): 398−406.
6. Ruutu M, Bono P, Taari K. Resection of renal cell carcer metastases:Where do we stand in 2008? Eur Urol Suppl 2008; 7(5): 436−42.
7. *Blom JH, van Poppel H, Marechal JM, Jacqmin D, Sylvester R, Schröder FH,* et al. Radical nephrectomy with and without lymph node dissection: preliminary results of the EORTC randomized phase III protocol 30881. EORTC Genitourinary Group. Eur Urol 1999; 36(6): 570−5.

8. *Pantuck AJ, Zisman A, Dorey F, Chao DH, Han KR, Said J,* et al. Renal cell carcinoma with retroperitoneal lymph nodes: role of lymph node dissection. J Urol 2003; 169(6): 2076−83.
9. *Kavolius JP, Mastorakos DP, Pavlovich C Russo P, Burt ME, Brady MS*. Resection of metastatic renal cell carcinoma. J Clin Oncol 1998; 16(6): 2261−6.
10. *Coppin C, Porzsolt F, Awa A, Kumpf J, Coldman A, Wilt T*. Immunotherapy for advanced renal cell cancer. Cochrane Database Syst Rev 2005; (1): CD001425.
11. *Jung ST, Ghert MA, Harrelson JM, Scully SP*. Treatment of osseous metastases in patients with renal cell carcinoma. Clin Orthop Relat Res 2003; (409): 223−31.
12. *Lin PP, Mirza AN, Lewis VO, Cannon CP, Tu SM, Tannir NM,* et al. Patient survival after surgery for osseous metastases from renal cell carcinoma. J Bone Joint Surg Am 2007; 89(8): 1794−801.
13. *Senadhi V, Jani N, Erlich R*. Metastatic Renal Cell Cancer and a Gastric Mass: An Unusual Finding. Case Rep Gastroenterol 2010; 4(3): 421−8.
14. *Wroński M, Arbit E, Russo P, Galicich JH*. Surgical resection of brain metastases from renal cell carcinoma in 50 patients. Urology 1996; 47(2): 187−93.
15. *Sheehan JP, Sun MH, Kondziolka D, Flickinger J, Lunsford LD*. Radiosurgery in patients with renal cell carcinoma metastasis to the brain: long-term outcomes and prognostic factors influencing survival and local tumor control. J Neurosurg 2003; 98(2): 342−9.

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CASE REPORT

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Fulminant Wegener's granulomatosis: A case report

Fulminantna Wegener-ova granulomatoza

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Abstract

Introduction. Granulomatosis Wegener is anti-neutrophil cytoplasmic antibodies (ANCAs)-associated systemic vasculitis of unknown etiology. It is manifested as granulomatous necrotizing inflammation of the upper and lower parts of the respiratory tract, glomerulonephritis and systemic vasculitis involving most frequently the skin and oral mucous membrane. Sera markers of this disease are c-ANCA and p-ANCA. **Case report**. We presented a female patient aged 52 years with purpuric spots that had appeared on the lower legs ten months before admission to our hospital. The disease ran an aggressive course, and a month before admission hemorrhagic bullae, skin ulcers, hoarseness, dyspnea, generalized arthralgia, fatigue and fever had rapidly developed. Histopathological examination of a skin sample revealed necrotizing vasculitis, so that sera markers concentrations were elevated (c-ANCA, p-ANCA). There was a perforation of the nasal septum found on rhinoscopy. During hospitalization acute abdominal pain occurred, a possible tumor in the small intestine and possible granulomas in the liver were seen by multislice computed tomography (MSCT) examination, with normal findings on the lungs and kidneys. The treatment started with methylprednisolone: 500 mg/d i.v. infusion for consecutive 3 days, then 60 mg/d. On exploratory laparotomy small bowel perforation and diffuse peritonitis were found. Unstable in the postoperative period, the patient died on the day 12 of hospitalization. **Conclusion**. The reported patient was with fulminant Wegener's granulomatosis, dominantly with skin changes and with gastrointestinal manifestation. This case accents the need for rapid systemic clinical evaluation in a severely ill patient with unclear diagnosis.

Key words:
wegener granulomatosis; diagnosis; purpura fulminans; gastrointestinal diseases; histological techniques.

Apstrakt

Uvod. Wegener-ova granulomatoza je sistemski vaskulitis nepoznate etiologije povezan sa prisustvom antineutrofilnih citoplazma antigen antitela (ANCA). Manifestuje se nekrotizujućom granulomskom upalom gornjih i donjih delova disajnih puteva, glomerulonefritisom i sistemskim vaskulitisom koji zahvata kožu i sluznicu usne duplje. Seromarkeri oboljenja su c-ANCA i p-ANCA. **Prikaz bolesnika.** Prikazana je bolesnica stara 52 godine, kojoj su se 10 meseci pre prijema u Kliniku pojavile purpurične mrlje na potkolenicama, a mesec dana pre prijema hemoragičke bule i ulceracije na koži, uz promuklost, dispnoju, bol u zglobovima, malaksalost i febrilnost. Histopatološkim pregledom uzorka izmenjene kože verifikovan je nekrotizujući vaskulitis, a koncentracije seromarkera su bile povišene: c-ANCA 18 IU/mL; p-ANCA 30 IU/mL. Uočena je i perforacija nosnog septuma. Tokom hospitalizacije javio se i bol u trbuhu, a pregledom multislajsnom kompjuterizovanom tomografijom (MSCT) viđen je mogući tumor na tankom crevu, kao i mogući granulomi u jetri, uz uredan nalaz na plućima i bubrezima. Započeto je lečenje metilprednizolonom 500 mg/d *iv* infuzijom tokom 3 dana, potom 60 mg/d. Eksplorativnom laparotomijom nađena je perforacija tankog creva uz difuzni peritonitis. Nestabilna u postoperativnom toku, bolesnica je preminula 12. dana hospitalizacije **Zaključak.** Prikazana je bolesnica sa Wegener-ovom granulomatozom fulminantnog toka sa dominantnim promenama na koži i gastrointestinalnom traktu. Ovaj slučaj naglašava potrebu za brzom sistemskom kliničkom procenom kod teškog bolesnika sa nejasnom dijagnozom.

Ključne reči:
vegenerova granulomatoza; dijagnoza; purpura, fulminantna; gastrointestinalne bolesti; histološke tehnike.

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Introduction

Wegener granulomatosis (WG) is anti-neutrophil cytoplasmic antibodies (ANCAs)-associated systemic vasculitis of small arteries and veins of unknown etiology. It is manifested as granulomatous necrotizing inflammation of the respiratory tract, glomerulonephritis and systemic vasculitis involving most frequently the skin and oral mucous membrane. In the lungs, paranasal sinuses and nasopharynx nodular infiltrates can be found, with fever, weakness, sinus pain, or bloody or purulent discharge from the nose, cough, hemoptysis and dyspnoea. Skin lesions are found in 50% of patients; only 13% with initial skin changes, usually on the legs: pyoderma gangrenosum-resembling lesions, papules, vesicles, palpable purpura, subcutaneous nodules, plaques and noduloulcerous lesions resembling polyarteritis nodosa. Oral or nasal ulcers and/or perforation of the nasal septum can be presented on visible mucous membranes. The disease also affects the joints, kidneys, eyes and central nervous system (CNS) [1–4].

Case report

A female, aged 52 years, was admitted to the Clinic for Dermatology, Military Medical Academy in Belgrade, with a 10 month history of purpuric spots on the lower legs. One month before admission hemorrhagic bullae on the lower extremities and hands, skin ulcers of the right lower leg and thigh, abdomen and buttocks, hoarseness, dyspnea, generalized arthralgias, fatigue and fever up to 38°C had rapidly developed (Figures 1–4). By chest X-ray, performed few days before admission, nodular infiltrates were observed. There was a perforation of the nasal septum at the time of admission as seen by rhinoscopy. On the third day of hospitalization abdominal pain was reported. In laboratory analyses elevation of nonspecific inflammatory factors was evident [erythrocyte sedimentation rate (ESR) 63 mm/h, C-reactive protein (CRP) 263 mg/L], with a significant leukocytosis (33.2×10^9/L) and neutrophilia (93.5%). Other blood count parameters, blood biochemistry, complement component 4 (C4) and liver enzymes serum activity were within normal range. C3 was decreased (0.6 g/L). In urinalysis hemoglobin and leukocytes were evident, with lot of sediment erythrocytes, leukocytes and bacteria, but urine culture was sterile, such as blood culture was. Concentration of 24-h urine protein was slightly elevated: 0.234 g (upper limit 0.15 g). ELISA human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) tests were negative. In peripheral blood smear increased number of leukocytes was evident (55.7×10^9/L), as well as elevated percentage of neutrophiles (95%). Immunologig analysis revealed negative antinuclear antibodies (ANA) (HEp2 cell's substrate) and ENA screening tests, anticentromere antibodies (ACA) were within the physiologic range, but ANCAs were elevated (c-ANCA 18 IU/mL, p-ANCA 30 IU/mL). In direct immunofluorescence (DIF) of purpuric papule specimen IgM deposits in the basement membrane zone, fine- and coarse-grained deposits of C3 in the walls of blood vessels of the dermis were found.

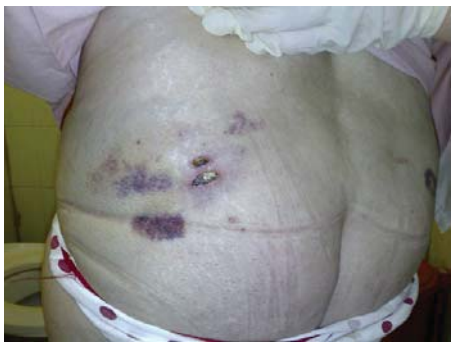

Fig. 1 – Hemorrhagic bullae on the back.

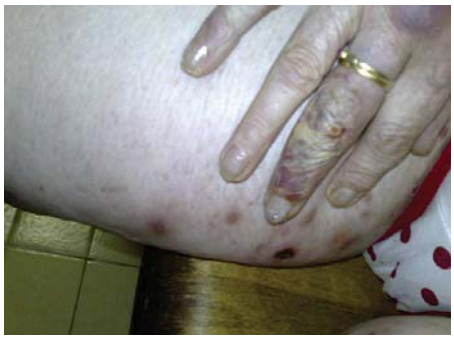

Fig. 2 – Hemorrhagic bullae on the hands and skin ulcers on the right thigh.

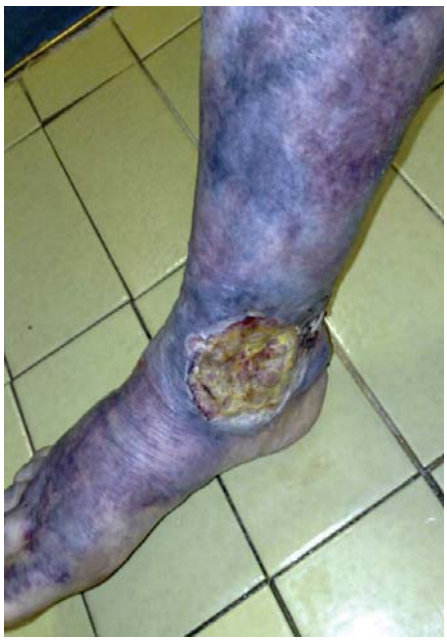

Fig. 3 – Leg ulcers.

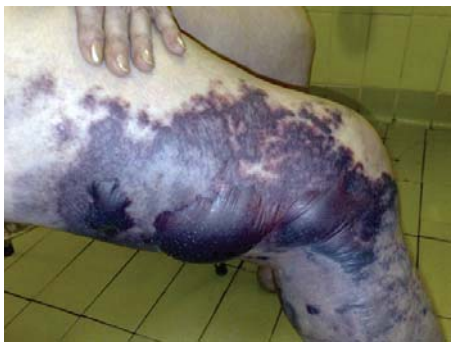

Fig. 4 – Hemorrhagic bullae on the thigh.

Histopathologic examination of the purpuric papule revealed necrotizing vasculitis of the skin (prominent extravascular neutrophilia and foci of suppuration in the dermo-subcutaneous border) (Figure 5). Histopathologic examination of nasal squamous mucosa revealed an acute inflammation. Ultrasound examination of the abdomen revealed dilated intestinal convolutions with proper findings on liver, gallbladder, spleen and kidneys, but certain amount of ascites. Esophagogastroduodenoscopy revealed ulceration on a small curve diameter of 5 mm, the bottom covered with white patches of scum from the edge dark crust (biopsy was not performed). MSCT of the chest and abdomen revealed right lung bullous formation of 2 cm in diameter at the top of the upper lobe, a number of possible granulomas in the liver, abdominal cavity was dominated by the free gas and the presence of free intraabdominal fluid, with a finding on the small bowel which may correspond to tumor formation or small intestine curve in volvulus. Based on skin changes, nasal perforation and positive c- and p-ANCA, in consultation with other specialists (rheumatologist, pulmologist, otolaryngologist) diagnosis of WG was established and the therapy with a pulse dose of methylprednisolone (500 mg/d i.v., for 3 consecutive days) and 60 mg/d i.v. after pulse dose was administred. Other therapy included: systemic antibiotics (metronidazole, ciprofloxacine), analgesics, antipyretics, fluconazole and pantoprazole gastroprotective therapy. Because of a possible tumor in the small intestine revealed by MSCT examination and the possible development of surgical abdomen (strong abdominal pain) exploratory laparotomy was performed on the day 6 of hospitalization, in which a small bowel perforation was found with diffuse peritonitis. Unstable in the postoperative period, the patient died on the day 12 of hospitalization due to peritonitis.

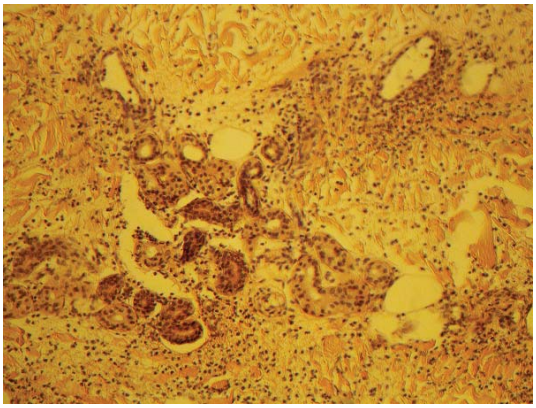

Fig. 5 – Histopathological finding of the purpuric papule – prominent extravascular neutrophilia, and foci of suppuration in the dermo-subcutaneous border (N&E, ×20).

Discussion

In 1930s, Wegener described 3 patients in their 30s with granulomatous vasculitis affecting the nose and throat, lungs and kidneys. The syndrome became known as 'Wegener's granulomatosis [1]. Untill 1980s, the significance of the association of c-ANCA with WG was not recognized. The incidence of WG is estimated to be 5 to 12 cases per million, with a slight female predominance [2]. Classic WG is the triad of granulomatous inflammation of the upper and lower respiratory tracts, systemic necrotizing small vessel vasculitis and immune glomerulonephritis. Patients with classic WG have a high mortality rate if left untreated, whereas patients with limited forms have isolated features of the triad and less severe involvement [3]. Mucocutaneous involvement occurs in approximately 40% of patients with WG, and it can be the presenting sign in 10% [4]. The most common lesions are palpable purpura, followed by oral ulcers. Skin changes that resemble pyoderma gangrenosum can also be seen. The upper or lower respiratory tracts are involved in up to 90% of patients with WG. Nasal involvement is responsible for presenting complaints in greater than 70% of cases. Suggestive symptoms and signs include recurrent epistaxis, mucosal ulcerations and nasal septal perforation. Patients with pulmonary involvement present with dyspnea, cough, hemoptysis or pleuritis, and chest X-rays demonstrate infiltrates or nodules. Approximately 75% of patients eventually develop glomerulonephritis [5]. Other organ systems commonly affected in WG include musculoskeletal (70%), ocular (30–60%), neurologic (20–50%) and gastrointestinal (5–10%). Most common gastrointestinal manifestations include: abdominal pain, nausea/vomiting, diarrhea, hematochezia or melena. Elevated c-ANCA occurs in approximately 80% of patients with classic or severe WG, but only in 60% of patients with limited disease, and p-ANCA occurs in approximately 10% of WG patients [3]. The majority of skin biopsy specimens show nonspecific histopathologic changes, but up to 50% demonstrate leukocytoclastic vasculitis and/or granulomatous inflammation. The mainstay treatment for patients with classic WG is systemic corticosteroids in conjunction with cyclophosphamide, resulting in a remission in up to 75% of patients [5]. In our patient, skin changes, nasal septum perforation, small bowell perforation due to vasculitis and occuring of ANCAs established the diagnosis of WG. Lethal outcome occurred due to diffuse peritonitis. Most common symptoms and signs of a small-vessel vasculitis of the gastrointestinal tract are abdominal pain, diarrhea and hematochezia. The frequency of gastrointestinal manifestations in WG is actually uncertain, because of small number of documented severe disease case reports [6, 7]. A study involving 45 patients found abdominal symptoms in 4 of them [6]. Severe gastrointestinal involvement manifested during the early stages of WG, particulary without concomitant renal disease is very rare, just few cases were previously reported, but without lethal outcome [7, 8]. In a study of Pagnoux et al. [9], presentation and outcome of gastrointestinal (GI) involvement in systemic necrotizing vasculitis were evaluated – a group of 62 patients with systemic small and medium sized vessel vasculitis and GI involvement: polyarteritis nodosa (total of 38), Churg Strauss syndrome (total of 11), WG (total of 6), microscopic polyangiitis (total of 4) and rheumatoid arthritis-associated vasculitis (total of 3). GI manifestations were present at or occured within 3 months of establishing diagnosis in 81% of the patients: abdominal pain (97%), nausea or vomiting (34%), diarrhea (27%) and hematochezia or melena (16%).

Further, 34% patients devoloped surgical abdomen, 18% peritonitis, 15% bowel perforation, 16% bowel ischemia/infarction and 6% intestinal occlusion. Peritonitis, bowel perforations, GI ischemia/infarctions and intestinal occlusion were the only GI manifestations significantly associated with increased mortality (for this group of patients 6-month and 5-year survival rates were 60% and 46%, respectively). But, in the subgroup of WG patients none of severe GI manifestations related with increased mortality were evident. The most severe manifestations in WG (total of 6) were esophageal (total of 1), gastroduodenal (total of 2) and colorectal (total of 2) ulcerations, without lethal outcome due to GI manifestations.

Conclusion

Our patient showed severe gastrointestinal and skin features, fulminant course and lethal outcome. This confirms the necessity for rapid systemic clinical evaluation in any critically ill patients with unclear diagnosis.

R E F E R E N C E S

1. *Crissey JT, Parish LC*. Vasculitis: the historical development of the concept. Clin Dermatol 1999; 17(5): 493−7.
2. *González-Gay MA, García-Porrúa C*. Systemic vasculitides. Best Pract Res Clin Rheumatol 2002; 16(5): 833−45.
3. *Chung L, Kea B, Fiorentino DF*. Cutaneous vasculitis. In: *Bolognia JL, Jorizzo JL, Rapini RP*, editors. Dermatology. 2nd ed. Philadelphia, PA: Mosby Elsevier; 2008. p. 360−1.
4. *Fiorentino DF*. Cutaneous vasculitis. J Am Acad Dermatol 2003; 48(3): 311−40.
5. *Hoffman GS, Kerr GS, Leavitt RY, Hallahan CW, Lebovics RS, Travis WD*, et al. Wegener granulomatosis: an analysis of 158 patients. Ann Intern Med 1992; 116(6): 488−98.
6. *Haworth SJ, Pusey CD*. Severe intestinal involvement in Wegener's granulomatosis. Gut 1984; 25(11): 1296−300.
7. *Pickhardt PJ, Curran VW*. Fulminant vasculitis in wegener's granulomatosis: CT findings with pathologic correlation. AJR Am J Rheum 2001; 177(6): 1335−7.
8. *Storesund B, Gran JT, Koldingsnes W*. Severe intestinal involvement in Wegener's granulomatosis: report of two cases and review of the literature. Br J Rheumatol 1998; 37(4): 387−90
9. *Pagnoux C, Mahr A, Cohen P, Guillevin L*. Presentation and outcome of gastrointestinal involvement in systemic necrotizing vasculitides: analysis of 62 patients with polyarteritis nodosa, microscopic polyangiitis, Wegener granulomatosis, Churg-Strauss syndrome, or rheumatoid arthritis-associ. Medicine (Baltimore) 2005; 84(2): 115−28.

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LETTER TO THE EDITOR

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Female doctors awarded in Serbian liberation wars during 1876–1878 and 1912–1918

Odlikovane žene lekari učesnice ratova za oslobođenje Srbije od 1876. do 1878. i od 1912. do 1918. godine

To the Editor:

In the absence of a Serbian doctors before the outbreak of the Balkan Wars, military service included female doctors [1]. This practice continued in the First World War. It is understandable that the female doctors did not participate directly in military operations, but they occupied important positions in the reserve military hospitals, often working simultaneously in civilian health care facilities [2]. After all, treating soldiers suffering from cholera or typhus fever was dangerous as participation in medical unitsint the front.

Dr. Draginja Babić and Dr. Zorka Brkić Popović died from typhus during the First World War [3].

Besides female doctors from Serbia, in these wars were also involved physicians, volunteers mainly from Slavic countries, whether they came alone or as part of foreign medical missions at the invitation of the Serbian Red Cross.

It is particularly interesting figure of Dr. Marija Zibold, German woman from Riga. As a young doctor, with medical student Draga Ljočić, she participated as a volunteer in the Serbian-Turkish wars from 1876 until 1878 and gained the rank of Reserve Ambulance Lieutenant. After the war Dr. Marija Zibold stayed in Serbia, and as wittily remarked by one of her biographers, when not participating in the wars that Serbia waged later, she had a private practice in Belgrade. Dr. Marija Zibold died in 1939, in the rank of Major as a reserve medical holder of numerous medals [2, 4].

According to our records 25 female doctors from Serbia were honored for participation in the wars of liberation.

The grateful fatherland rewarded with appropriate decorations those who particulary destinguished.

The largest number of female doctors was awarded with the Cross of St. Sava and Cross of Charity, and some were promoted with foreign medals. Dr. Marija Zibold was a holder of Takovo Cross, the most important medal during the Obrenović dynasty [5].

Data on decorations can be found in publications on participation of physicians in the wars of liberation, but the most reliable are those published in the Official Military Gazette and Serbian Newspapers.

Awarded female doctors were:

1. **Dr. Draginja Babić,** Order of St. Sava*, 4th class, in 1913, and Cross of Mercy in 1914 [5, 6]
2. **Dr. Boginja Barjaktarević,** Cross of Mercy† in 1914 [6]
3. **Dr. Božana Bartoš Mihailović,** Order of St. Sava, 5th class, in 1913, and 4th class in 1915 [7–9].
4. **Dr. Ana Brkić Milijanović,** Order of St. Sava, 5th and 4th class. [5].
5. **Dr. Zorka Brkić Popović,** Order of St. Sava, 5th class in 1913, Cross of Mercy in 1913, and Order of St. Sava, 4th class in 1914 [6, 7, 9].
6. **Dr. Ljubica Brkić,** Order of Red Cross‡ in 1914 [10].
7. **Dr. Ljubica Djurić Goćevac,** Order of St. Sava, 5th class in 1910 [11].
8. **Dr. Marija Zibold,** The Order of the Cross of Takovo§ 5th class, Order of White Eagle**, Order of St. Sava, Order of Red Cross and Order for Honor [5].

* **Order of St. Sava** was decoration instituted in 1883 by the order of King Milan Obrenović (King Milan I of Serbia). It had five rows, and was esatblished to recognize civilian achievements until 1914 when change was made permitting military personnel to receive it for military merit. Since 1945. the highest award presented by the Serbian Orthodox Church is called the Order of St. Sava and it has three rows.

† **Order of the Cross of Mercy** was decoration instituted in 1913. It was awarded for nurturing the sick and wounded during and after the war. From 1993 the Republic of Srpska assigns it as it's decoration.

‡ **Order of the Red Cross** was established by the Serbian Red Cross Society in 1877. It was established to recognize outstanding merits in war and peace, for care and welfare for old, wounded, and sick by the S.R.C.S. with consent from Chancellor of Royal Orders.

§ **Order of the Cross of Takovo** was a decoration instituted in the Principality of Serbia in 1865 to mark the 50th anniversary of the Second Serbian Uprising started in Takovo. It had five rows, and was established to recognize military and civilian achievements. The order was suppressed in 1903. after dynasty Karađorđević came to throne.

** **Order of the White Eagle** was decoration instituted in 1882. As Royal Order in the Kingdom of Serbia, Kingdom of SHS, and Kingdom of Yugoslavia was conferred on Serbian and Yugoslav citizens for achivements in peace or war, or for special merits to the Crown, to the state and nation. It had five orders. The order was reestablished in the Republic of Serbia in 2009.

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9. **Dr. Andjela Jakšić,** Order of St. Sava 5th class in 1913, Order of Red Cross, French Gold Medal War Merit [7, 9, 12].
10. **Dr. Katarina Jakšić Radulaški,** Order of St. Sava, 4th class [5].
11. **Dr. Neda Jovanović,** Order of St. Sava, 4th class in 1915 [8].
12. **Dr. Darinka Krstić,** Order of St. Sava, 5th and 4th class [5], Cross of Mercy in 1914 [5, 6.]
13. **Dr. Sofija Lješević,** Silver Medal for Diligent Service* in 1914, Order of Red Cross, and Order of St. Sava, 5th class [13–15].
14. **Dr. Draga Ljočić Milošević,** Order of St. Sava, 4th class, in 1904, and Gold Medal for Diligent Service in 1913 [7, 9, 16].
15. **Dr. Pravda Marković**, Cross of Mercy in 1914 [17].
16. **Dr. Radmila Milošević Lazarević,** Gold Medal for Diligent Service in 1914, and Order of St. Sava, 4th class, in 1915 [8, 18].
17. **Dr. Slavka Mihajlović Klisić,** Cross of Mercy in 1914, Order of St. Sava, 5th class, in 1914, and 4th class in 1915 [8, 9, 19].
18. **Dr. Natalija Nikolajević Davidović**, Order of St. Sava, 5th class in 1913, and 4th class in 1914, Silver Medal for Diligent Service in 1914, Czechoslovak Order of the White Lion [5, 7–9, 13].
19. **Dr. Marija Pavlovićeva**, Order of St. Sava, 5th class, in 1913, and Cross of Mercy in 1914 [7, 9, 19].
20. **Dr. Jelena Popadić,** Order of St. Sava, 5th class, in 1913, and 4th class in 1915, Cross of Mercy in 1914, and the Gold Medal of the French [5, 7–9, 17].
21. **Dr. Marija Prita Vučetić,** Order of St. Sava, 4th class, in 1913, and Cross of Mercy in 1914 [9, 17].
22. **Dr. Nadežda Stanojević,** Order of St. Sava, 5th and 4th class [5, 8, 9].
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25. **Dr. Eva Haljecka**, Order of St. Sava, 5th class, in 1913, Cross of Mercy in 1914, and Order of St. Sava, 4th class, in 1915 [6–9].
26. **Dr. Staka Čubrilović,** Order of St. Sava, 4th class [5].

REFERENCES

1. *Stanojević V*. Serbian medical society and its members during Serbian Liberation wars 1876-1878. and 1912-1918. Belgrade: Serbian Medical Society Memorial. 1972. p. 114−24. (Serbian)
2. *Gavrilović V*. Female doctors in wars 1876. 1945. on Yugoslavian ground. Belgrade: Scientific Society For Yugoslavian Health Education; 1976. p. 15−30. (Serbian)
3. *Subotić MV*. Memorial to doctors and medics who were killed and deceased during the wars between 1912-1918. regular, correspondent, honorary members and founders, benefactors and donors 1872–1922. Belgrade; Serbian Medical Society; 1922. (Serbian)
4. *Lazovic J, Sujic R*. Women Doctors In the Serbian Sanitary Service During the Balkan Wars. Acta Med Hist Adriat 2007; 5(1): 71−82. (Serbian)
5. *Milanović M*. Biographical lexicon, Known serbian doctors, Belgrade, Toronto. Belgrade: Vojna štamparija; 2005. (Serbian)
6. Serbian Newspapers LXXXI. 1914. p. 229. (Serbian)
7. Serbian Newspapers LXXX. 1913. p. 425. (Serbian)
8. Serbian newspapers LXXXII. 1915. p. 446. (Serbian)
9. Official Military Gazette XXXIII. 1913. p. 186. (Serbian)
10. Yugoslavian Archive 39, f-16. (Serbian)
11. Official Military Gazette XXVII. 1910. p. 46. (Serbian)
12. Yugoslavian Archive 39, f-57. (Serbian)
13. Serbian Newspapers LXXXI. 1914. p. 921. (Serbian)
14. Degree number 1405. (Serbian)
15. Degree number 893. (Serbian)
16. Serbian Newspapers LXXI. 1904. p. 919. (Serbian)
17. Serbian Newspapers LXXXI. 1914. p. 225. (Serbian)
18. Serbian Newspapers LXXXI. 1914. p. 337. (Serbian)
19. Serbian Newspapers LXXXI. 1914. p. 217. (Serbian)

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* **Medal for Diligent Service** was established in 1913. It had two degrees: gold and silver. Allocated to military and civilian personnel for the faithful and diligent service, as well for the help to the Serbian army during the war. In year 1922 the range of merit was expanded, and it was also given for excellent and dedicated service in times of peace.

E R R A T U M

Nedok A. Sećanje povodom Dana sanitetske službe Vojske Srbije: štab-doktor Emerih P. Lindenmajer (1806–1883) [Remabrance on the occasion of the Day of Armed Foeces Medical Services of Serbia: Staff Doctor Emmerich Lindenmayer (1806–1883)], Vojnosanit Pregl 2013; 70(8): 794–5.

Na str. 795, referenca broj 9 / On page 795, a reference No. 9 listed as:
9. *Novaković S.* Kancelarija Kneževih ordena: Šematizam odlikovanih lica u Kneževini/Kraljevini Srbiji. knj. I (1865–1894). Beograd: Arhiv Srbije; 1894.

treba da glasi/ should read as:

9. Kancelarija Kneževih ordena: Šematizam odlikovanih lica u Kneževini/Kraljevini Srbiji. knj. I (1865–1894). Beograd: Arhiv Srbije; 1894.

I N M E M O R I A M

dr stom. med. MOMČILO ŽIVANČEVIĆ potpukovnik u penziji (1949–2013)

Posle kraće i teške bolesti, 30. jula 2013. godine napustio nas je potpukovnik u penziji, dr stom. med. Momčilo Živančević, dugogodišnji član kolektiva Instituta za naučne informacije Vojnomedicinske akademije (VMA) u Beogradu, gde je vršio dužnost načelnika Odeljenja za obradu medicinskih naučnih informacija.

Dr Živančević rođen je 5. marta 1949. godine u Beogradu gde je završio Stomatološki fakultet. Ubrzo posle stupanja u aktivnu vojnu službu 1981. godine, zapošljava se u Institutu za naučne informacije VMA na poslovima obrade medicinskih naučnih informacija, gde ostaje sve do penzionisanja 2005. godine.

U vreme njegovog dolaska u Institut, medicinska naučna informatika, kao nova naučna disciplina, bila je tek u začetku na prostoru bivše SFRJ, pa dr Živančević 1986–1987. godine odlazi na specijalizaciju iz te oblasti u Amsterdam (Holandija). Po povratku sa specijalizacije, aktivno učestvuje u organizaciji službe za selektivnu diseminaciju podataka za korisnike iz VMA, kao i iz drugih medicinskih naučnih i akademskih institucija u zemlji i održava seminare i kurseve o korišćenju elektronskih baza naučne publicistike koje su se tek tada počele koristiti u naučnom i stručnom radu. Kao stručnjak iz ove oblasti učestvovao je u izradi kataloga strane biomedicinske periodike u Republici Srbiji (period 1991–1996), a godinama je držao i nastavu iz predmeta Informatika u biomedicini na poslediplomskim i specijalističkim studijama u VMA. Osim toga, učestvovao je u pripremi „Referativnog biltena“ i „Informativnog biltena“, publikacija Instituta za naučne informacije preko kojih su se brojne generacije zdravstvenih radnika iz vojnosanitetskih ustanova širom zemlje informisale o najnovijim kretanjima u biomedicinskoj nauci i struci. Istovremeno, bio je angažovan i na poslovima stručne redakcije radova za „Vojnosanitetski pregled“ i svojim radom značajno je doprineo njegovom sadašnjem statusu kao jednom od retkih domaćih časopisa sa međunarodnom reputacijom.

Bio je veliki stručnjak, izuzetan radnik, uvažavan i od kolega, kao i najbližih saradnika. Za svoj predan i odgovaran rad više puta je pohvaljivan i nagrađivan, a 2002. godine odlikovan je i Ordenom za zasluge u oblastima odbrane i bezbednosti trećeg stepena.

Mi, njegove kolege i saradnici iz Instituta za naučne informacije pamtićemo ga kao velikog profesionalca koji je u oblasti medicinske naučne informatike bio uzor mnogima. Ostavio je neizbrisiv trag u pozicioniranju Instituta kao značajne potpore svim delatnostima VMA. Zbog toga, neka mu je večna slava i hvala!

prof. dr Silva Dobrić,
načelnik Instituta za naučne informacije VMA i
glavni i odgovorni urednik „Vojnosanitetskog pregleda“

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

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

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

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

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

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

Tabele

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

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

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

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Examples of references:

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

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

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

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Tables

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

Illustrations

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

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

Abbreviations and symbols

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

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

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Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

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

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