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MAGNETIC RESONANCE IMAGING COMPARISON OF LATERAL COLLATERAL LIGAMENT AND PATELLAR TENDON LENGTH

KOMPARACIJA DUŽINE LATERALNOG KOLATERALNOG LIGAMENTA I PATELARNE TETIVE MAGNETNO-REZONANTNIM IMIDŽINGOM

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Summary

Introduction. The problem of using patellar tendon auto or allografts for lateral collateral ligament reconstruction results in the occurrence of ligaments mismatch. The length of patellar tendon does not match the lateral collateral ligament. **Material and Methods.** Out of 151 patients, who formed the study, 102 were men with the mean age of 30 years (18-54) and 49 women, with the mean age of 34 (18-55), and they all underwent magnetic resonance imaging of the knee. Both patellar tendon and lateral collateral ligament were measured using a three-dimensional isovoxel true-fast-imaging with steady-state precession sequence with water excitation and secondary multiplanar reformations. In order to visualize the lateral collateral ligament insertions precisely, sagittal images were reformatted according to the anatomical, oblique ligament position, in anteriorly tilted, paracoronal plane. The length of the patellar tendon was measured from the patellar apex to the tibial tuberosity insertion site. **Results.** The mean patellar tendon length was 52.88±7.56 mm (37-75) with a significant difference between men and women. The mean lateral collateral ligament length was 61.21±5.77 mm (46-80) with a significant difference between genders. The average differences between lateral collateral ligament and patellar tendon length was 8.38±7.23 mm (-9 to 26) without a significant difference between the genders. In 18 (11.92%) patients, the patellar tendon was longer than the lateral collateral ligament; in 7 patients (4.63%) they were equal; and in 126 patients (83.44%) the patellar tendon was shorter than the lateral collateral ligament. **Conclusion.** The length of patellar tendon does not match the length of lateral collateral ligament. If patellar tendon auto or allograft is used for lateral collateral ligament reconstruction, the lengths of both ligaments must be determined preoperatively in order to avoid intraoperative complications.
Key words: Magnetic Resonance Imaging; Patellar Ligament; Bone-Patellar Tendon-Bone Grafting; Collateral Ligaments; Imaging, Three-Dimensional; Male; Female; Adult; Preoperative Care; Intraoperative Complications.

Sažetak

Uvod. Problem korišćenja patelarne tetive kao autografta ili alografta za rekonstrukciju lateralnog kolateralnog ligamenta dovodi do pojave neusklađenosti dužine navedenih ligamenata. Dužina patelarne tetive ne odgovara dužini lateralnog kolateralnog ligamenta. **Materijal i metode.** Magnetno-rezonantnim imidžingom kolenog zgloba pregledan je 151 pacijent, 102 muškog pola, prosečne starosti 30 godina (18−54), 49 ženskog pola – prosečne starosti 34 godine (18−55). Oba ligamenta, patelarna tetiva i lateralni kolateralni ligament su mereni korišćenjem trodimenzionalne izovokselne sekvence (*eng. true fast imaging with steady-state precession with water excitation*), napravljene su sekundarne multiplanarne reformacije. Kako bi se precizno vizualizovalo mesto insercije lateralnog kolateralnog ligamenta, sagitalni tomogrami su rekonstruisani prema anatomskom toku vlakana – kosa pozicija ligamenta, sa parakoronalnim tiltom ravni. Dužina patelarne tetive merena je od vrha patele do mesta insercije na tuberozitas tibije. **Rezultati.** Prosečna dužina patelarne tetive bila je 52,88 ± 7,56 mm (37−75) sa signifikantnom razlikom između pacijenata muškog i ženskog pola. Prosečna dužina lateralnog kolateralnog ligamenta bila je 61,21 ± 5,77 mm (46−80) sa signifikantnom razlikom između polova. Prosečna razlika u dužini između lateralnog kolateralnog ligamenta i patelarne tetive iznosila je 8,38 ± 7,23 mm (-9 to 26) bez signifikantne razlike među polovima. Kod 18 (11,92%) pacijenata patelarna tetiva je bila duža nego lateralni kolateralni ligament; kod 7 (4,63%) dužina je bila jednaka; a kod 126 (83,44%) pacijenata patelarna tetiva je bila kraća nego lateralni kolateralni ligament. **Zaključak.** Dužina patelarne tetive ne odgovara dužini lateralnog kolateralnog ligamenta. Ukoliko se kao autograft ili alograft koristi patelarni ligament za rekonstrukciju kolateralnog ligamenta, oba ligamenta se preoperativno moraju izmeriti kako bi se izbegle intraoperativne komplikacije.
Klučne reči: Magnetna rezonanca; Patelarni ligament; Kost-tetiva-kost graft; Kolateralni ligament; Trodimenzionalni imidžing; Muško; Žensko; Odrasli; Preoperativna priprema; Intraoperativne komplikacije

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Introduction

The lateral collateral ligament (LCL) is the primary varus stabilizer of the knee [1,2] and its injuries are frequently associated with anterior cruciate ligament (ACL), posterior cruciate ligament (PCL) and posterolateral corner injuries. If these posterolateral injuries remain unrecognized, they may lead to ACL [3] or PCL reconstruction failure. When recognized, in the presence of bony avulsion, the primary repair of posterolateral corner can be successful [4–6]. In the absence of bony avulsion, the primary or secondary reconstruction [6–8] of the LCL and posterolateral corner with a variety of autografts or allografts [9–11] has been recommended with various results.

Noyes and Barber-Westin were the first who described an anatomical graft replacement of the LCL with bone – patellar tendon – bone (BTB) graft together with the reconstruction of the other posterolateral structures [8]. The advantages of using BTB graft for LCL replacement are strong initial fixation, better bony incorporation at the femoral and fibular insertion sites, early knee mobilization which decreases the risk of arthrofibrosis, and the avoidance of prolonged incorporation associated with soft tissue grafts [12]. A potential problem specific to using BTB grafts for LCL reconstructions is the possibility of patellar tendon (PT) graft – LCL mismatch. The goal of this study was to compare and correlate PT and LCL by means of magnetic resonance imaging (MRI). Our hypothesis was that the length of PT graft does not correlate to the length of LCL, and that in the majority of cases is therefore not applicable for LCL reconstruction.

Material and Methods

The study was approved by the Ethical Committee of Institution of Oncology of Vojvodina, which allowed a retrospective review of images and relevant records with waiver of the informed consent. Our sample consisted of 151 patients, 102 men – mean age 30 years (18-54), and 49 women – mean age 34 (18-55), who underwent MRI of the knee at 3-Tesla MR imaging unit (Siemens Trio;

Siemens Medical Solutions, Erlangen, Germany) using a dedicated send/receive 8-channel phased-array knee coil. For examination, a three-dimensional (3D) isovoxel true fast imaging with steady-state precession (FISP) sequence with water excitation and secondary multiplanar reformations (MPRs) was performed. The sequence parameters were (TR/TE=9.44/3.45; turbo factor GRAPPA 2; field-of-view 160x160 mm; matrix size 233x256; pixel size 0.6x0.6 mm; slice thickness 0.6 mm).

MPRs with a 2-mm partition thickness were acquired.

In order to visualize LCL insertions precisely, the sagittal images were reformatted on the system console (Leonardo; Siemens Medical Solutions) according to the anatomical – oblique ligament position, in anteriorly tilted, paracoronal plane.

In this plane, a mid-reference line was adjusted and placed parallel to LCL in order to enable complete ligament visualization from the lateral femoral epicondyle to the head of the fibula. The calipers were also placed centrally inside the ligament (**Figures 1a and 1b**).

The length of the PT was measured in true sagittal plane, from the patellar apex to the tibial tuberosity insertion site. The calipers were placed centrally inside the tendon fibers, at mid-sagittal image, where the tendon was displayed at its maximal thickness [13–15] (**Figure 1c**). In case of presence of constitutionally oblique course of the PT, we checked the tendon position on the reformatted images in coronal plane.

Exclusion criteria included the patients under 18 years of age, conspicuous varus or valgus knee deformity, extensor mechanism abnormalities,
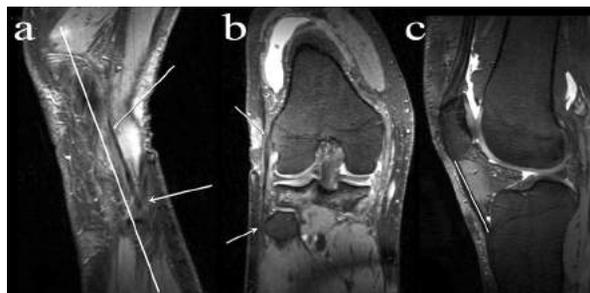
Figure 1. Optimal visualization and measurements
a. 3D true FISP MPR sagittal image – according to the plane of ligament (long white line)
b. 3D true FISP MPR coronal image – visualization of complete LCL, LCL insertions (arrows)
c. 3D true FISP sagittal image – complete visualization of PT (white line)
Slika 1. Optimalna vizualizacija i merenja
a. 3D true FISP MPR sagitalni tomogram – prati pravac ligamenta (duga bela linija)
b. 3D true FISP MPR koronalni tomogra – vizualizacija kompletnog LCL, i insercije LCL (strelice)
c. 3D true FISP sagitalni tomogram – kompletna vizualizacija PT (bela linija)

Table 1. PT and LCL lengths, males and females
Tabela 1. Dužine PT i LCL, muškarci i žene

	102 males/*muškaraca*				49 females/*žena*				
	mean *srednja*	SD	range *raspon*	95% confidence interval/*interval poverenja*	mean *srednja*	SD	range *raspon*	95% confidence interval/*interval poverenja*	Significant difference/*Značajna razlika*
LCL (mm)	62.81	5.61	51-80	61.71-63.92	57.88	4.59	46-66	56.56-59.20	t=5.34 p<0.5
PT (mm)	54.60	7.43	38-75	53.14-56.06	49.31	6.59	37-67	47.41-51.20	t =4.24 p<0.5
LCL-PT (mm)	8.22	7.14	-7 to 26	6.81-9.62	8.71	7.49	-9 to 25	6.56-10.86	t=0.396 p=0.693

knee fracture, joint effusion, cruciate and collateral ligamentous abnormalities and the patients with a prior knee surgery. All MRI measurements and post-processing were performed by a board-certified musculoskeletal radiologist with 10 years experience.

Statistical analysis was obtained through the software Smart Line agency (NS) and included mean, SD and range for all measurement. The comparison between genders was conducted with an unpaired Student's t test. Statistical significance was defined as p < 0.05.

Results

The mean PT length in both genders was 52.88 ± 7.56mm (37-75). The mean PT length was 54.60 ± 7.43 mm (38-75) and 49.31 ± 6.59 (37-67) mm in the men and in the women, respectively. This difference between the men and the women was significant p<0.05 (**Table 1**).

The mean LCL length in both genders was 61.21 ± 5.77 mm (46-80). The mean LCL length was 62.81 ± 5.61 mm (51-80) and 57.88 ± 4.59 (46-66) mm in the men and in women, respectively. This difference between genders was significant p<0.05 (**Table 1**).

The comparison of the differences between LCL and PT length showed that the average difference was 8.38 ± 7.23 mm (-9 to 26). The mean LCL-PT length difference was 8.22±7.14 mm (-7 to 26) and 8.71±7.49 mm (-9 to 25) in the men and in the women, respectively. This difference between genders was not significant p=0.693. In 18

(11,92%) patients, PT was longer than the LCL; it was equal in 7 (4,63%) patients, while in 126 (83,44%) patients PT was shorter than the LCL (**Graph 1**).

Discussion

The LCL is the primary varus stabilizer to the knee. When the knee is in extension the fibular head goes backward, pulled by LCL and femoral biceps tandon, so the ligament produces stability in the extended knee [16]. Coobs et al. [1], LaPrade et al. [9] and Buzzi et al. [17] have shown that only an anatomical LCL reconstruction can restore the near normal knee stability after LCL tears. The anatomic LCL insertion has a semicircular-shaped attachment 3-5 mm posterior and 1-2 mm proximal to the lateral epicondyle of the femur [18, 19] and 8-10 mm posterior to the anterior point of the head of the fibula with the well-defined limits.

The mean LCL length reported in the literature is 63.1 mm (55-71) [20, 21]. Our measurements of the length relate to the middle bundle of the ligament, which is relatively constant along the flexion path [22] and measured an average length of LCL is 61 mm (46–80) and it strongly correlated to the patient's gender.

The average length of the PT in our study was 53 mm (37–75), and there was a significant correlation between the PT length and the patient's gender. Denti et al. [23] measured the PT length intraoperatively and reported the mean PT length of 46 mm. Shaffer et al. [24], who also measured the PT length intraoperatively in 34 patients and La Prade [25] who measured PT length in 50 cadavers, both described the mean PT length of 48 mm (40–63). Although MC Alister et al. [26] recommended lateral radiographs to determine the PT length, MRI is the most frequently used method. Yoo et al. [13] measured PT using MRI in 172 knees and showed the mean PT length of 40 mm (31–52). In the study of Goldstein et al. [14] the mean PT length was 46 mm, and there was a correlation between the patient's height, gender with the PT length. On the other hand, Brown et al. [15] measured the length in 414 knees and reported no correlation between the patient's height and PT length.
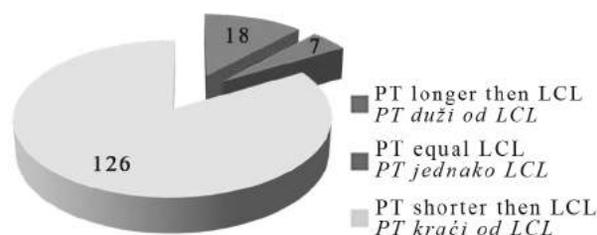
Graph 1. Differences between PT and LCL length
Grafikon 1. Razlike u dužini između PT i LCL

Anteroposteriorly the mean width of LCL at the level of the articular knee joint space is 8.5 mm (5-12 mm) and the mean thickness is 2–4 mm [20]. Having the width of 9–10 mm and the thickness of 3-5 mm [13, 27], BTB graft seems to be a good candidate for LCL reconstruction, but the problem is its inadequate length. Noyes and Barber-Westin [8] were the first to describe the successful use of BTB autograft or allograft for LCL reconstruction in 16 (76%) of 21 knees. Latimer et al. [10] used a large BTB allograft to replace only the LCL. Nine out of ten patients in this series showed an improvement despite addressing only one component of the corner. The authors felt that a 9 mm wide PT allograft, which is wider than LCL, positioned at the isometric point on the lateral femoral condyle, may substitute adequately for the LCL as well as for the popliteofibular and arcuate ligaments. One of the comments to this article was that an increase of 3-5mm in lateral joint line opening may not be an excellent result, meaning that reconstruction of the LCL together with other posterolateral structures would provide better stability [28]. Clinically, < 2 mm of excursion of the graft through the range of motion is thought to be acceptable [29, 30]. It was much later that Noyes and Barber-Westin [12] proposed the preoperative measuring of the distance between the anatomical femoral and fibular insertion site on the lateral radiograph to avoid mismatch between BTB autograft or allografts and LCL. If fixation with interference screw in the femoral tunnel is impossible due to short BTB graft, they propose an inlay technique with a 4-prong staple for grafts.

The limitation of this study is that the measurement of the PT and LCL on MRI are not compared with a cadaver measurement. Further clinical and cadaver research is needed to confirm the MR measurements.

Conclusion

The advantages of using bone - patellar tendon - bone graft for lateral collateral replacement are strong initial fixation, better bony incorporation at the femoral and fibular insertion sites, early knee mobilization which decreases the risk of arthrofibrosis, and the avoidance of prolonged incorporation associated with soft tissue grafts. A potential problem is a mismatch between patellar tendon graft and lateral collateral ligament.

The length of patellar tendon does not match the length of the lateral collateral ligament and, in the majority of cases is therefore not applicable for the lateral collateral ligament reconstruction.

Magnetic resonance imaging is a reliable modality for measurements. It can predict a mismatch and avoid surgery complications.

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AN IMMUNOHISTOCHEMICAL ANALYSIS OF SEX-STEROID RECEPTORS, TUMOR SUPPRESSOR GENE P53 AND KI-67 IN THE NORMAL AND NEOPLASTIC UTERINE CERVIX SQUAMOUS EPITHELIUM

IMUNOHISTOLOŠKA ANALIZA RECEPTORA POLNIH STEROIDA, TUMORSKOG SUPRESORSKOG GENA P53 I KI-67 U NORMALNOM I NEOPLASTIČNOM CERVIKALNOM SKAVOZNOM EPITELU UTERUSA

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Summary

Introduction. Malignant transformation of sex-steroid dependent tissues is associated with the loss of expression of sex steroid receptors as well as of the tumor suppression gene p53. The aim of this study is to evaluate the expression of sex-steroid receptors, p53 and Ki-67 in specimens from pre-malignant and malignant cervical epithelial lesions throughout the menstrual cycle. **Material and Methods.** Immunohistochemical staining was performed on formalin fixed, paraffin embedded tissue sections of normal squamous cervical epithelium, cervical intraepithelial neoplasia and invasive squamous cervical carcinoma, specimens utilizing antibodies against estrogen receptors, progesterone receptors, p53 protein and Ki-67 antigen. **Results.** In the samples taken from the normal cervical tissue, basal cells were usually estrogen receptor-positive, progesterone receptor-negative, p53-negative and Ki-67-negative throughout the menstrual cycle. In contrast, para-basal cells were estrogen receptor-positive and progesterone receptor-negative in the follicular phase, but estrogen receptor-negative and progesterone receptor-positive and Ki-67 positive in the luteal phase. In cervical pre-cancerous and cancer tissue samples (cervical intraepithelial neoplasia and squamous cervical carcinoma), the expression of estrogen receptors decreased. 31.15% of cervical intraepithelial neoplasia and 11.5% of squamous cervical carcinoma were positive for estrogen receptors. However, the expression of progesterone receptors increased. 29.5% of cervical intraepithelial neoplasia and 49.2% of squamous cervical carcinoma were positive for progesterone receptors. Positive staining for p53 was observed in 15 (24.59%) cases of cervical intraepithelial neoplasia and in 39 (64%) of squamous cervical carcinoma. The expression Ki-67 index in squamous cervical carcinoma cases (47.60%) was significantly higher than of cervical intraepithelial neoplasia cases (30.2%) (p=0.041). **Conclusion.** The findings of this study suggest that tumor cervical cells evade normal growth control by sex steroid hormones while synchronously abnormal regulatory mechanisms acquire control of the cell cycle.
Key words: Immunohistochemistry; Receptors, Steroids; Genes, Tumor Suppressor; Cervix Uteri; Uterine Cervical Neoplasms; Cervical Intraepithelial Neoplasia

Sažetak

Uvod. Maligna transformacija onih tkiva koja su zavisna od dejstva polnih steroida (poput grlića materice) najčešće je povezivana sa gubitkom izražavanja receptora koji primaju polne steroide, takođe kao i gubitkom funkcije tumorske supresije gena p53. Cilj ove studije jeste da evaluira izražavanje receptora polnih steroida, p53 i Ki-67 u uzorcima iz premalignih i malignih cervikalnih, epitelnih lezija tokom trajanja menstrualnog ciklusa. **Materijal i metode.** Imunohistohemijsko bojenje je izvedeno na rezovima uzoraka normalnog skvamoznog cervikalnog epitela, cervikalne intraepitelne neoplazije i invazivnog skvamoznog cervikalnog karcinoma koji su fiksirani u formalinu i kalupljeni u parafinu, korišćenjem antitela protiv estrogenskih receptora, progesteronskih receptora, p53 proteina i Ki-67 antigena. **Rezultati.** U uzorcima iz fiziološkog cervikalnog tkiva, bazalne ćelije uglavnom su bile pozitivne na estrogene receptore, a negativne na progesteronske receptore, p53 i Ki-67 tokom trajanja menstrualnog ciklusa. U folikularnoj fazi, parabazalne ćelije su bile pozitivne na estrogene, a negativne na progesteronske receptore, ali u lutealnoj fazi ispoljile su negativnu reakciju na estrogene receptore i pozitivnu na progesterone i Ki-67. U uzorcima prekanceroznih i kanceroznih lezija, reaktivnost estrogenih receptora je znatno snižena. Estrogeni receptori su bili pozitivni kod 31,15% cervikalnih intraepitelnih neoplazija i kod 11,5% cervikalnih karcinoma. Međutim, zabeleženo je povećanje pozitivne reakcije na progesteronske receptore, kod interepitelne neoplazije do 29,5%, a kod karcinoma čak do 49,2%. Pozitivnio bojenje na p53 zabeleženo je u 15 (24,59%) slučajeva cervikalne neoplazije i u 39 (64%) karcinoma. Izraženost Ki-67 indeksa kod cervikalnih karcinoma (47,6% slučajeva) značajno je viša u odnosu na cervikalnu neoplaziju (30,2% slučajeva). **Zaključak.** Nalazi ove studije ukazuju da polni steroidni hormoni nemaju kontrolu rasta tumorskih ćelija cerviksa, dok abnormalni regulatorni mehanizmi kontrolišu ćelijski ciklus.
Ključne reči: Imunohistohemija; Steroidni receptori; Tumorska supresija gena; Grlić materice; Karcinomi grlića materice; Cervikalna intraepitelijalna neoplazija

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Introduction

Cervical cancer (CC) is a leading gynecological cancer and a major public health problem in many parts of the world. Over the past few decades the number of women with cervical cancer has decreased drastically in many countries which have successfully implemented programs of secondary prevention leading to detection of precancerous lesions and early stage CC. However, CC remains the third most commonly diagnosed cancer and the fourth leading cause of cancer deaths worldwide, accounting for 529.800 (9%) of total new cancer cases and 275.100 (8%) of the total cancer deaths among women [1].

Human papilloma virus (HPV) infections play a critical role in the development of CC [2]. More than 90% of cases are associated with HPV infections, particularly with high risk HPV types which can cause oncogenic transformation of cervical cells [3]. However, it has also been documented that only a small fraction of all HPV-infected women will eventually develop CC. In the classic step by step model, it takes at least a 10-year span before persistent dysplasia develops into CC. However, several other cofactors in addition to persistent high risk HPV infection are causally involved to the induction of CC [4]. The most common histological type of CC is the squamous cervical carcinoma (SCC) and the second common type is the adenocarcinoma.

Since the uterine cervix has specific bindings estrogen and progesterone receptors, it belongs to the target tissues for sex steroid hormones. Proliferation of the uterine cervix cells during the menstrual cycle is induced by sex steroid hormones via binding to their receptors [5, 6]. Malignant transformation of sex-steroid dependent tissues such as uterine cervix has been related to the abnormal expression of sex-steroid receptors, suggesting that tumor cells lose the normal growth control by steroid hormones [7].

The tumour suppression gene p53 plays a key role in maintaining genetic cell stability. Its function is to suppress cell proliferation and to direct deoxyribonucleic acid-damaged cells towards apoptosis [8]. The p53 loss of function is probably the most common genetic aberration observed in many different human malignancies and premalignant lesions [9]. In cervical carcinogenesis, inactivation of p53 is a fundamental process occurring either by mutation or more commonly with interaction with HPV-E6-oncoprotein by forming complexes, leading to cell degradation with the mediation of an ubiquitin-dependent proteolysis system [10].

Ki-67 is a cell proliferation-associated nuclear protein that is seemingly associated with epithelial metaplasia, dysplasia and carcinoma [11]. It is expressed throughout all active phases of cell cycle. The assessment of Ki-67 protein expression by immunohistochemistry (IHC) is a predictive factor for growth and proliferation in a variety of tumors [12]. Ki-67 index has been studied extensively as a proliferation and tumor aggressiveness biomarker in SCC. However, the results of previous studies have been conflicting and its prognostic role in CC patients remains controversial [13, 14].

In the present study, we analyzed the presence of sex-steroid hormone receptors, tumour suppression gene p53 and Ki-67 proliferation marker throughout the menstrual cycle in order to investigate a potential correlation of these biomarkers with the cervical carcinogenesis process.

Material and Methods

All histological tissue specimens assessed in this study were taken from women who underwent surgical interventions (hysterectomy, cervical biopsies) performed at the Patras University Hospital. All specimens were examined in our institutions Department of Pathology by three experienced pathologists. Of the 143 cervical specimens, 62 corresponded to cervical intraepithelial neoplasia (CIN), 61 corresponded to invasive squamous cervical carcinoma (SCC) and 20 were the control group (normal squamous epithelium).

The altered expression and relationship between the sex-steroid receptors status estrogen receptors (ER), progesteron receptors (PR), p53 and Ki-67, which potentially correlated with the malignant transformation, was determined by IHC assessment of these markers in tissue samples of uterine cervix.

Immunohistochemistry

This study was conducted by means of IHC staining (biotin-streptabidin indirect method) in selected formalin-fixed paraffin-embedded cervical tissue sections. We used the following antibodies: anti-ERa (Biogenex®) mouse polyclonal in the dilution of 1:100, anti-PR (Biogenex®) mouse polyclonal in the dilution of 1:100, anti-p53 (Dako®) mouse monoclonal in the dilution of 1:200 and Ki-67 (Biogenex®) mouse monoclonal in the dilution of 1:200.

The IHC localization of all protein expression used was nuclear. For the expression of ER and PR receptors, a cut-off of 5% cell positivity was considered to be a positive result. For the tumor suppression gene p53 and proliferation marker Ki-67, the percentage (%) of positive tumor cells was cal-

Table 1. Results of immunohistochemistry (IHC) staining of estrogen receptor (ER) in our sample
Tabela 1. Rezultati imunohistohemijskog (IHC) bojenja estrogen receptora (ER) u našem uzorku

Study population *Proučavana populacija*	Estrogen Receptors (ER)-IHC, % *Estrogenski receptori (ER)-IHC, %*
Normal basal cells of squamous cervical epithelium *Normalne bazalne ćelije skvamatoznog cervikalnog epitela*	ER (+) *ER (+)*
Normal parabasal cells of squamous cervical epithelium *Normalne parabazalne ćelije skvamatoznog cervikalnog epitela*	ER (+) proliferation phase/ ER (-) luteal phase *ER (+) proliferaciona faza/ER (-) lutealna faza*
Cervical intraepithelial neoplasia (CIN) *Cervikalna intraepitelijalna neoplazija (CIN)*	31.15% ER (+)
Squamous cervical carcinoma (SCC) *Skvamatozni cervikalni karcinom (SCK)*	11,15% ER (+)

Table 2. Results of immunohistochemistry (IHC) staining of progesterone receptor (PR) in our samples
Tabela 2. Rezultati imunohistohemijskog (IHC) bojenja progesteronskih receptora (PR) u našim uzorcima

Study population *Proučavana populacija*	Progesterone Receptors (PR)-IHC,% *Progesteronski receptori (ER)-IHC, %*
Normal basal cells of squamous cervical epithelium *Normalne bazalne ćelije skvamatoznog cervikalnog epitela*	PR (-)
Normal parabasal cells of squamous cervical epithelium *Normalne parabazalne ćelije skvamatoznog cervikalnog epitela*	PR (+) proliferation phase / (+) weak positivity in luteal phase *PR (+) proliferaciona faza/ER (+) slaba pozitivnost u lutealnoj fazi*
Cervical intraepithelial neoplasia (CIN) *Cervikalna intraepitelijalna neoplazija (CIN)*	29.5% PR (+)
Squamous cervical carcinoma (SCC) *Skvamatozni cervikalni karcinom (SCK)*	49.2% PR (+)

culated. The statistical analysis of the results was implemented by the Statistical Package for the Social Sciences (SPSS) 14 for windows. Chi-square and t-test were calculated and any p< 0.05 was considered to be statistically significant.

Results

The normal basal cells of the cervical squamous epithelium were in their majority ER-positive and PR-negative throughout the menstrual cycle; the parabasal cells were ER-positive and PR-negative in the follicular phase but ER-negative and PR-positive in the luteal phase. In CIN lesions, 31.15% (19 cases) of the samples were ER-positive and in SCCs lesions, 11.5% (7 cases) were ER-positive **(Table 1)**.

In our control samples, the normal basal and parabasal cells of the cervical epithelium were PR-negative throughout the menstrual cycle, and only a weak and focal PR expression was observed in the parabasal cells during the luteal phase. In CIN lesions, 29.5% (18 cases) were PR-positive and in SCC lesions, 49.2% (30 of 61 cases) were PR-positive **(Table 2)**. The PR expression was significantly different between the three groups (p=0.026).

The IHC staining of p53 was negative in the normal basal and parabasal cells of the normal cervical squamous epithelium. In CIN and SCC lesions, 24.5% and 64% of the samples showed the expression of p53, respectively **(Table 3)**.

The normal basal cells of the cervical epithelium exhibited a very low ratio of Ki-67 positivity (<2%) throughout the menstrual cycle; whereas the

Table 3. Results of immunohistochemistry (IHC) staining of p53 in our samples
Tabela 3. Rezultati imunohistohemijskog (IHC) bojenja p53 u našim uzorcima

Study population *Proučavana populacija*	Tumor suppression gene p53 *Tumor supresorski gen p53*
Normal squamous cells of cervical epithelium *Normalne bazalne ćelije skvamatoznog cervikalnog epitela*	(-) p53
Cervical intraepithelial neoplasia (CIN)/*Cervikalna intraepitelijalna neoplazija (CIN)*	24.59% p53 (+)
Squamous cervical carcinoma (SCC)/*Skvamatozni cervikalni karcinom (SCK)*	64% p53 (+)

Table 4. Results of immunohistochemistry (IHC) staining of Ki-67 in our samples
***Tabela 4.** Rezultati imunohistohemijskog (IHC) bojenja Ki-67 u našim uzorcima*

Study population *Proučavana populacija*	Proliferation marker Ki-67 *Marker proliferacije Ki-67*
Normal basal cells of squamous cervical epithelium *Normalne bazalne ćelije skvamatoznog cervikalnog epitela*	(+) Ki-67 <2%
Normal parabasal cells of squamous cervical epithelium *Normalne parabazalne ćelije skvamatoznog cervikalnog epitela*	(+) Ki-67 luteal phase
Cervical intraepithelial neoplasia (CIN) *Cervikalna intraepitelijalna neoplazija (CIN)*	30.2% Ki-67(+)
Squamous cervical carcinoma (SCC) *Skvamatozni cervikalni karcinom (SCK)*	47.6% Ki-67(+)

parabasal cells showed increased Ki-67-positivity (4%) in the luteal phase. In SCC and CIN samples, the Ki-67 index was positive in 47.6% and 30.2% of cases, respectively **(Table 4)**.

The Ki-67 index in SCC samples was significantly higher than in CIN ones. (p=0.041).

The mean value of Ki-67 was 47.60% (ranging from 30% to 65%) and 30.2% (ranging from 15% to 41%) in SCC and CIN, respectively and this difference was statistically significant (p=0.041).

Discussion

During malignant transformation of cervical epithelium, the expression of the sex-steroid hormonal receptors is significantly altered [7]. However, the presence of sex-steroid hormonal receptors in cervical carcinoma has apparently no prognostic value on the survival time and cannot be influenced by any hormonal therapy in comparison with other target tissues for sex steroid hormones such as breast, endometrial and ovarian carcinomas.

The ER content of the cervical epithelium depends upon the menstrual cycle [15]. As in previous studies, the positive expression of ER in the normal squamous cervical epithelium was observed in the basal and para-basal cells with no significant changes in staining during the menstrual cycle, the only exception being the ER-negativity of parabasal cells in the luteal phase. Various studies showed the presence of a weak reaction to ER in mild dysplasia in comparison with severe dysplasia and invasive SCC where all ER were negative in correlation with the induced by high-risk HPVs changes [7, 16–18]. The expression of ER was found to be decreased in our CIN and SCC samples in comparison with the normal cervical epithelium, which is in accordance with the above reports.

Previous studies reported that the expression of PR was absent in any form of normal squamous cervical epithelium except in the parabasal cells which weak intensity reported in the luteal phase of the cycle [15, 16, 18].

In contrast, PR-positivity was observed in most premalignant and malignant cells containing high-risk HPV. According to published data, the PR expression ranged from 73% to 95.2% and from 59% to 72.7% in CIN and SCC lesions [7, 18]. Our study results concur with those observations. A likely hypothesis could be that increased PR expression may associate with the malignant proliferation of squamous cervical epithelium induced by HPV infection.

Previous studies suggest that p53 function is inactivated in cervical carcinoma either by complex formation with HPV-E6 viral oncoprotein product in HPV-positive cervical carcinoma leading to the degradation of wild-type p53 or rarely (1-6%) by gene mutation in HPV-negative cervical carcinoma [8, 19–20]. However, IHC detection of p53 protein usually fails to distinguish a wild-type p53 from mutated p53 [21].

A wide range of p53 expression among SCC (4.8-77.6%) and CIN (11.4-28.6%) samples has been reported [7, 12, 22–27] in literature. These discrepancies could be attributed to the variety of methods or different cut-offs for positive staining used. Our data are in accordance with the above studies as the expression of p53 was negative in all control cases, but it was expressed and detected in CIN and SCC samples. Furthermore, we found a significant difference of p53 expression in relation to the grade of dysplasia.

Many studies showed that the positivity of KI-67 index strongly correlated with the histological grade of CIN and SCC in comparison to the normal cervical epithelium, the only exception being the parabasal cervical cells during the luteal phase [11, 13, 26–29]. In agreement with the above studies, our findings also demonstrated that the expression of Ki-67 in SCC cases was significantly more pronounced than the corresponding one in CIN (p=0.041). The intensity of Ki-67 expression could be helpful in differentiating various cervical lesions [29, 30]. However, the Ki-67 expression cannot be used as a sole indicator when determining the aggressiveness of premalignant cervical lesions [18, 27, 28].

Conclusions

The loss of estrogen receptor expression during cervical carcinogenesis may play an important role in the malignant transformation, being a strong indicator of cell differentiation and escape from the normal growth control by sex-steroid

hormones. In contrast, the positive expression of progesterone receptors increased and could represent an alternative mechanism for cervical cells to retain their hormonal control and sensitivity.

The expression of p53 was increased throughout more severe grades of dysplasia confirming its central role in the pathogenesis of cervical carcinogenesis. Our findings also demonstrate Ki-67 over-expression in dysplasia and carcinoma, suggesting its significant role in diagnosing and predicting the biologic behavior of a cervical lesion.

Given the apparently inconsistent findings in the results of small studies, larger prospective studies are needed to elucidate and assess the exact role of these markers in cervical carcinogenesis.

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EFFECT OF SERUM LIPID LEVEL CHANGE ON 10-YEAR CORONARY HEART RISK DISTRIBUTION ESTIMATED BY MEANS OF SEVEN DIFFERENT CORONARY RISK SCORES DURING ONE-YEAR TREATMENT

UTICAJ PROMENA KONCENTRACIJE SERUMSKIH LIPIDA TOKOM JEDNOGODIŠNJEG LEČENJA NA PROMENU DISTRIBUCIJE 10-GODIŠNJEG RIZIKA ZA KORONARNU BOLEST SRCA PROCENJENU PRIMENOM SEDAM BODOVNIH SISTEMA

Nevena EREMIĆ KOJIĆ[1], Mirjana ĐERIĆ[1,2] and Jadranka DEJANOVIĆ[2,3]

Summary

Introduction. This study was done in order to evaluate the effect of serum levels of total cholesterol, triglycerides, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol on 10-year coronary heart disease risk distribution change. **Material and Methods.** This study included 110 subjects of both genders (71 female and 39 male), aged 29 to 73, treated at the Outpatient Department of Atherosclerosis Prevention, Centre for Laboratory Medicine, Clinical Centre Vojvodina. The 10-year coronary heart disease risk was estimated on first examination and after one-year treatment by means of Framingham, PROCAM and SCORE coronary risk scores and their modifications (Framingham Adult Treatment Panel III, Framingham Weibul, PROCAM NS and PROCAM Cox Hazards). Age, gender, systolic and diastolic blood pressure, smoking, positive family history and left ventricular hypertrophy are risk factors involved in the estimation of coronary heart disease besides lipid parameters. **Results.** There were no significant differences in nutritional status, smoking habits, systolic and diastolic pressure, and no development of diabetes mellitus or cardiovascular incidents during one-year follow. However, a significant reduction in cholesterol level (p<0.001), triglycerides (p<0.001), low-density lipoprotein cholesterol (p<0.001) and an increase in high-density lipoprotein cholesterol (p<0.02) was present although therapeutic target values were not achieved. In addition, a significant increase was observed in the category of low 10-year coronary heart disease risk (Framingham- p<0.001; Framingham ATP III- p<0.001; Framingham Weibul- p<0.001; PROCAM– p<0.05; PROCAM NS- p<0.05; PROCAM Cox Hazards- p<0.001; SCORE- p<0.001) and a reduction in high-risk category (Framingham- p<0.001; Framingham ATP III- p<0.005; Framingham Weibul- p<0.005; PROCAM– p<0.001; PROCAM NS-p<0.001; PROCAM Cox Hazards- p<0.001; SCORE- p<0.005) in comparison with the risk at the beginning of the study. **Conclusion.** Our results show that the correction of lipid level after one-year treatment leads to a significant redistribution of 10-year coronary heart disease risk estimated by means of seven different coronary risk scores. This should stimulate patients and doctors to persist in prevention measures.

Key words: Coronary Disease; Cardiovascular Diseases; Serum; Lipids; Risk Factors; Male; Female; Adult; Middle Aged; Aged; Risk Assessment; Hyperlipidemias; Treatment Outcome

Sažetak

Uvod. Ovo istraživanje izvedeno je sa ciljem ispitivanja uticaja promena serumskih koncentracija ukupnog holesterola, triglicerida, holesterola u lipoproteinima male gustine i holesterola u proteinima velike gustine na promenu distribucije 10-godišnjeg rizika za koronarnu bolest srca. **Materijal i metode**. U ispitivanje je uključeno 110 ispitanika, 71 žena i 39 muškaraca, starosti 29−73 godine, upućenih u Ambulantu za prevenciju ateroskleroze Centra za laboratorijsku medicinu Kliničkog centra Vojvodine. Procena 10-godišnjeg koronarnog rizika vršena je bazalno i nakon godinu dana lečenja pomoću Framingamskog, PROCAM i SCORE bodovnog sistema i njihovih modifikacija (*Framingham ATP III, Framingham Weibul, PROCAM NS* i *PROCAM Cox Hazards*). Pored parametara lipidskog statusa, u procenu su uključeni: uzrast, pol, sistolni i dijastolni krvni pritisak, pušenje, porodična anamneza i hipertrofija leve komore. **Rezultati.** U posmatranom periodu nije došlo do signifikantnih promena stanja ishranjenosti ispitanika, broja pušača, sistolnog i dijastolnog pritiska, niti do razvoja šećerne bolesti i kardiovaskularnih incidenata. Međutim, ustanovljeno je signifikantno sniženje holesterola (p < 0,001), triglicerida (p < 0,001) i LDL holesterola (p < 0,001), a porast HDL-holesterola (p < 0,02), iako nisu uspostavljene ciljne terapijske vrednosti. Takođe, došlo je do signifikantnog povećanja kategorije niskog 10-godišnjeg koronarnog rizika (*Framingham* – p < 0,001; *Framingham ATP III* – p < 0,001; *Framingham Weibul* – p < 0,001; *PROCAM*– p < 0,05; *PROCAM NS* – p < 0,05; *PROCAM Cox Hazards* – p < 0,001; *SCORE* – p < 0,001) i sniženja kategorije visokog rizika (*Framingham* – p < 0,001; *Framingham ATP III* – p < 0,005; *Framingham Weibul* – p < 0,005; *PROCAM* – p < 0,001; *PROCAM NS* – p < 0,001; *PROCAM Cox Hazards* – p < 0,001; *SCORE* – p < 0,005), u odnosu na rizik na početku praćenja. **Zaključak.** Naši rezultati pokazuju da korekcija nivoa lipida nakon jednogodišnjeg lečenja dovodi do signifikantne preraspodele distribucije 10-godišnjeg rizika za koronarnu bolest srca procenjenog pomoću sedam različitih bodovnih sistema. To bi trebalo da stimuliše i bolesnike i doktore da istraju na merama prevencije.

Ključne reči: Koronarna bolest; Kardiovaskularne bolesti; Serum; Lipidi; Faktori rizika; Muško; Žensko; Odrasli; Odrasli, srednjih godina; Procena rizika; Hiperlipidemije; Ishod lečenja

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Introduction

Cardiovascular diseases (CVD), especially coronary heart disease (CHD) take a very high place among the leading causes of mortality worldwide and particularly in Eastern European countries. The standard mortality rate from cardiovascular diseases was 473.52 per 100.000 inhabitants in Serbia in 2011, thus Serbia is among those countries with high risk factors for CVD with fatal outcome in comparison with the average mortality rate in European Union countries of 212.85 per 100.000 inhabitants [1].

Lipids are among major independent risk factors for development of CHD and still have the central place in the majority of coronary risk scores for 10-year coronary heart disease estimation [2–6].

Lipids are important structural and bioregulatory components of human cell and plasmatic lipoprotein as their transportation circulatory forms. The low-density lipoprotein (LDL) fraction has the greatest atherogenic potential because its contribution to cholesterol accumulation in subendothelial layer of arterial blood vessels is dominant in comparison with other lipoproteins [3, 6]. On the contrary, high-density lipoprotein (HDL) has an antiatherogenic role in reducing cholesterol accumulation in the arterial wall [3] and the potential cardioprotective mechanism can be attributed to its antioxidative, antiinflammatory and antithrombotic activity [6–9]. Numerous studies have shown that triglycerides also have a significant role in atherogenesis and thrombogenesis independently from LDL and HDL levels through the potential of trygliceride-rich-lipoproteins within the reactions of reverse cholesterol transport and through the small, dense LDL (sdLDL) particle formation [6, 10, 11].

The correlation between the rate of atherosclerosis and lipid levels was confirmed in several, large, prospective, epidemiological studies such as Framingham study [12], PROCAM-Munster Heart Study [5,13] and SCORE study [4]. Other non-lipid parameters like age, smoking, hypertension, diabetes mellitus and positive family history for myocardial infarction in first line relatives before 60 years of age were also included in the estimation [4, 5, 12, 13]. Coronary risk scores have been designed as final results of data processing from these studies in order to identify asymptomatic persons at high risk for CHD development [14], and they include certain individual factors whose estimation provides a certain number of scores serving as a basis for expressing 10-year CHD risk in percents in a special table [4, 5, 12, 13]. Another way is to find the field for every single subject which would be closest to his/her age group, level of total cholesterol and systolic blood pressure and which already bears the number representing the percent of 10-year risk [15].

Framingham risk score (FRS) was designed in 1991 and risk factors incorporated in the estimation of risk were age, serum levels of total cholesterol, HDL cholesterol, systolic and diastolic blood pressure, presence of diabetes mellitus and smoking [12, 16]. In 2001, the National Cholesterol Education Program (NCEP) accepted and modified FRS for the guidebook "Adult Treatment Panel III (ATP III)" by changing its age intervals and excluding dyastolic blood pressure (Framingham ATP III risk score). There is a model suitable for use on computer and it is called Framigham Weibul, which assess the presence of the left ventricular hypertrophy, thus being different from other risk score systems [3, 17, 18].

PROCAM risk score scheme includes eight independent risk factors: age, LDL-cholesterol, HDL-cholesterol, triglycerides, systolic blood pressure, smoking, diabetes mellitus and positive family history for myocardial infarction in first line relatives before 60 years of age. This risk score refers only to men. The risk for females in menopause equals the risk for males divided by number four [5, 13, 18]. PROCAM NS modification has resulted from the data obtained from the Multinational MONItoring of trends and determinants in CArdiovascular disease (MONICA) project for the city of Novi Sad, which calculates the 10-year risk by multiplying the total sum of individual scores with the conversion factor 1.37 for men and 1.24 for women [18]. PROCAM *Cox proportional hazards model* is the modification adjusted for the computer use [18].

Risk factors included in the estimation of 10-year CHD risk according to SCORE risk scheme are gender, age, smoking, systolic blood pressure and total cholesterol. There are separate schemes for high- and low-risk regions for CHD as well as for males and females [15].

While the limit for high 10-years CHD risk is set at >20%, medium 10–20%, and low <10% in the majority of score systems, in the SCORE risk system it is >5%, 1-5%, <5%, respectively [4, 15, 19, 20].

Since lipids are in the central place of all coronary risk scores and their levels have a significant influence on a person's position in different risk categories, our investigation was carried out to evaluate the effect of changing lipid level on the distribution of 10-year CHD risk. The estimation was made basally and after one-year treatment by means of seven different risk score systems. These kinds of studies are very rare [21], practically non-existent in our region.

Material and Methods

This prospective, one-year study included 110 subjects of both genders (71 female and 39 male),

aged 29 to 73, without CHD or CHD equivalent disease (diabetes mellitus, peripheral arterial disease, abdominal aortic aneurism or carotid disease), who were examined and treated mainly for lipid metabolism disorder at the Outpatient Department of Atherosclerosis Prevention, Centre for Laboratory Medicine, Clinical Centre Voivodina.

The patients underwent measurements of body mass (kg), body height (cm) and arterial blood pressure (mmHg).

Blood pressure was measured by Riva-Rocci manometer in sitting position after 10-15 minutes rest [22].

Body height was measured by the Martin anthropometer with 0.1 cm precision, the subjects being barefoot, in standing position, their heels together and toes open, with the position of the head such as to make the Frankfurt plane (tragus line that connects the outer ears and ear angle) horizontal [22].

Body mass was measured by decimal scales with mobile weights and precision of 0.1 kg. The subjects were dressed, but 1 kg in summer and 1.5 kg in winter was subtracted on the account of their clothes [22].

Body waist circumference was measured in the middle between the lowest point of rib angle and the uppermost border of the iliac crest by the centimeter tape, with precision of 0.1 cm, the subjects being in standing position [22].

Body mass index (BMI) was calculated from the anthropometric parameters and the nutritional status was assessed according to the criteria of the World Health Organization (WHO) as well as national guideline for obesity [23,24].

In addition, the assessment was also made in relation to the presence of coronary heart disease electrocardiogram (ECG), diabetes mellitus (fasting glucose and glycated hemoglobin A_{1c} – HbA_{1c}) and lipid metabolism disorder (lipid parameters). Coronary heart disease was clinically evaluated and proved by the cardiologist (positive family history of cardiovascular events and/or angina pectoris and/or abnormal coronary angiogram).

The data were analyzed on first examination and after one-year treatment. The risk factors included in the estimation were age, gender, lipid parameters, blood pressure, positive family history, smoking, presence of diabetes mellitus and left ventricular hypertrophy. Each subject had their 10-year CHD risk calculated by applying seven different coronary risk score systems.

Within Framingham risk score, the estimation was done by means of the original, modified according to ATP III, and computer interactive Weibul model. PROCAM risk score system was used as a classical schematic model, a model adapted for computer called *Cox proportional hazards model,* and PROCAM NS. Ten-year CHD risk was estimated according to SCORE risk score system by means of the schemes for European regions at high risk.

Microsoft Excel 2003 was used to process the statistical data. The applied statistical functions included the arithmetic mean, standard deviation, percentage difference and Student T-test [25].

Results

The study sample consisted of 110 participants, of whom 39 (35.5%) were males and 71 (64.5%) were females, without clinical manifestations of atherosclerotic disease. Their average age was 54.5±10.1 years, BMI was 26.7±3.3 kg/m^2 and body waist was 87.5±11.8 cm. The average values of systolic and diastolic pressure were 131.1±15.4 and 82.3±9.1 mmHg, respectively and fasting glucose was 5.3 ± 0.7 mmol/l. Of the total number of participants, 18.2% were smokers and 43% of them had positive family history (**Table 1**).

Regarding the gender, women were significantly more present (p<0.001) and they were older (p<0.02) than men.

As for BMI, all subjects were overweight. There were no significant changes after one-year treatment.

The average values of systolic and diastolic blood pressures in males were 128.3±11.0 and 81.7±7.8 mmHg and in females 132.7±17.3 and 82.6±9.7 mmHg, respectively. The levels of fasting glucose were 5.4 ± 0.7 mmol/l and 5.3 ± 0.7 mmol/l in males and females, respectively. After one- year treatment, there were no significant differences in the levels of systolic and diastolic blood pressures and in fasting glucose levels, either in the whole study sample or in male and female subgroups. There were significantly fewer male smokers than female smokers (15% vs. 20%, p<0.02), with no significant reduction in the number of smokers after one-year treatment.

Positive family history for myocardial infarction in first line relatives was present in 31% of males and 49% of females.

Graphs 1 and 2 show the tested lipid parameters on first examination and after one-year treatment of lipid disorder. On first examination, the levels were as follows: total cholesterol - 7.29±1.41 mmol/l, triglycerides - 2.28±1.05 mmol/l, LDL-cholesterol - 5.07±1.33 mmol/l and HDL-cholesterol -1.23±0.37 mmol/l. After one-year treatment, the levels were: total cholesterol - 5.74±1.12 mmol/l, triglycerides - 1.48±0.73 mmol/l, LDL-cholesterol - 3.70±1.10 mmol/l and HDL-cholesterol - 1.35±0.37 mmol/l. Except HDL-cholesterol, which showed a significant increase after one-year treatment (p<0.02), a significant decrease in concentration of all other tested lipid parameters was recorded (p<0.001).

On first examination, the levels in males were: total cholesterol - 7.05±1.41 mmol/l, triglycerides - 2.30±1.03 mmol, LDL-cholesterol - 4.93±1.37 mmol/l and HDL-cholesterol - 1.08±0.24 mmol/l and in females they were: total cholesterol - 7.43±1.40 mmol/l, triglycerides - 2.25±1.07 mmol, LDL-cholesterol - 5.15±1.31 mmol/l and HDL-cholesterol - 1.31±0.39 mmol/l. After one-year treatment, the levels in males were: total cholesterol - 5.62±1.23

Table 1. Comparison of examined non-lipid risk factors on first examination and after one-year treatment
Tabela 1. Poređenje ispitivanih nelipidskih faktora rizika prilikom prvog pregleda i nakon godinu dana lečenja

	Total/*Ukupna grupa*		Males/*Muškarci*		Females/*Žene*	
	First examination *Prvi pregled*	After one year *Nakon godinu dana*	First examination *Prvi pregled*	After one year *Nakon godinu dana*	First examination *Prvi pregled*	After one year *Nakon godinu dana*
	x±SD	x±SD	x±SD	x±SD	x±SD	x±SD
Age (years)/*Starost*	54.5±10.1	-	51.1±11.4	-	56.2±9.1**	-
BMI (kg/m^2)	26.7±3.3	26.1±3.4	27.0±2.5	26.2±2.6	26.5±3.7	26.0±3.8
Body waist circumference (cm)/*Obim struka*	87.5±11.8	85.9±11.2	95.5±8.0	92.8±7.7	83.1±11.3	82.1±11.0
Systolic BP (mmHg) *Sistolni TA*	131.1±15.4	129.6±15.1	128.3±11.0	128.7±14.2	132.7±17.3	130.1±15.7
Diastolic BP (mmHg) *Dijastolni TA*	82.3±9.1	81.6±8.4	81.7±7.8	82.0±8.3	82.6±9.7	81.4±8.5
Fasting glucose (mmol/l) *Glikemija našte*	5.3±0.7	5.3±0.7	5.4±0.7	5.3±0.7	5.3±0.7	5.3±0.7
Smokers/*Pušači* (%)	18.2	16.4	15.4	15.4	19.7	16.9
Positive family history/ (%)*Pozitivna porodič- na istorija*	43	-	31	-	49	-

Legend: BP - blood pressure/*TA - krvni pritisak*; BMI - body mass index/*indeks telesne mase*; ** p<0.02

mmol/l, triglycerides - 1.50±0.76 mmol/l, LDL-cholesterol - 3.73±1.16 mmol/l and HDL-cholesterol - 1.21±0.31 mmol/l and in females they were: total cholesterol -5.81±1.06 mmol/l, triglycerides - 1.47±0.72 mmol/l, LDL-cholesterol - 3.69±1.08 mmol/l and HDL-cholesterol - 1.43±0.35 mmol/l. All tested lipid parameters, except HDL-cholesterol, were significantly reduced after one-year treatment (p<0.001). The levels of HDL-holesterol rose in males (p<0.05) while in females there were no significant changes. The levels of HDL-cholesterol were higher in females on first examination.

The application of seven different risk score systems for the estimation of 10-year coronary risk in the whole study sample on first examination showed that the percentage of subjects at low risk varied from 14.5% according to Framigham Weibul to 78.2% according to PROCAM; and af-

ter one-year treatment it was 39.1% to 88.2% according to PROCAM and Framingham Weibul, respectively. There was a significant increase in the number of subjects at low risk after one-year treatment according to all tested risk score systems (**Table 2**).

In the intermediate-risk category group, the percentage of subjects was from 11.8% according to PROCAM NS to 45.4% according to Framigham Weibul and after one-year treatment there were 10.0% according to PROCAM and 36.4% according to Framigham Weibul. The reduction in number of persons having intermediate risk was significant only according to Framnigham, Framingham ATP III and PROCAM Cox Hazards (**Table 2**).

On first examination, the percentage of persons at high risk in the whole study sample varied from 8.2% according to PROCAM to 40.0% ac-
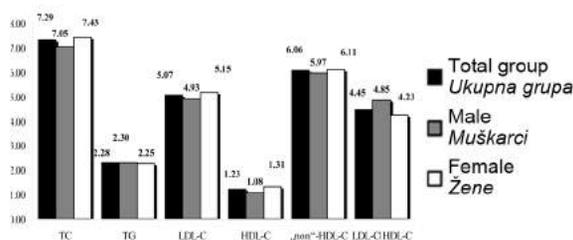
Graph 1. Lipid parameters on first examination
Grafikon 1. Parametri lipidskog statusa pri prvom pregledu
Legend (*legenda*): Total group (*ukupna grupa*), males (*muškarci*), females (*žene*), TC - Total cholesterol (*ukupan holesterol*); TG – triglycerides (*trigliceridi*); C – cholesterol (*holesterol*)
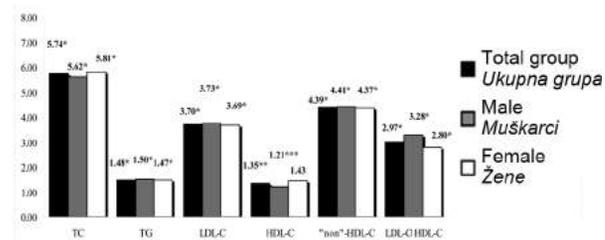
Graph 2. Lipid parameters after one-year treatment
Grafikon 2. Parametri lipidskog statusa nakon godinu dana lečenja
Legend (*legenda*): Total group (*ukupna grupa*), males (*muškarci*), females (*žene*) TC - Total cholesterol (*ukupan holesterol*); TG – triglycerides (*trigliceridi*); C – cholesterol (*holesterol*); * - p<0.001; **- p<0.02; *** - p<0.05

Table 2. Estimation of 10-year CHD risk by means of coronary risk score on first examination and after one-year treatment
Tabela 2. Procena 10-godišnjeg rizika za koronarnu bolest srca prlikom prvog pregleda i nakon godinu dana lečenja

	PP (FE)	NG (AY)		PP (FE)	NG (AY)		PP (FE)	NG (AY)	
	Low risk/*Nizak rizik*			Medium risk/*Srednji rizik*			High risk/*Visok rizik*		
	%	%	p<	%	%	p<	%	%	p<
Framingham	38.2	68.2	0.001	43.6	25.4	0.001	18.2	6.4	0.001
Framingham ATPIII	54.5	78.2	0.001	31.8	15.4	0.001	13.6	6.4	0.005
Framingham Weibul	14.5	39.1	0.001	45.4	36.4	n.s.	40.0	24.5	0.005
PROCAM	78.2	88.2	0.05	13.6	10.0	n.s.	8.2	1.8	0.001
PROCAM NS	74.4	85.5	0.05	11.8	10.9	n.s.	13.6	3.6	0.001
PROCAM Cox Hazards	63.6	83.6	0.001	25.4	13.6	0.005	10.9	2.7	0.001
SCORE	43.6	55.4	0.001	33.6	32.7	n.s.	22.7	11.8	0.005

Legend/*legenda*: FE - first examination/*PP - prvi pregled*, AY - after one year/NG − *nakon godinu dana lečenja*, n.s.− non-significant/*nesignifikantno*

Table 3. Estimation of 10-year CHD risk by means of coronary risk score on first examination and after one-year treatment in males
Tabela 3. Procena 10-godišnjeg rizika za koronarnu bolest srca prlikom prvog pregleda i nakon godinu dana lečenja kod muškaraca

	PP (FE)	NG (AY)		PP (FE)	NG (AY)		PP (FE)	NG (AY)	
	Low risk/*Nizak rizik*			Medium risk/*Srednji rizik*			High risk/*Visok rizik*		
	%	%	p<	%	%	p<	%	%	p<
Framingham	33.3	56.4	0.002	43.6	28.2	n.s.	23.1	15.4	n.s.
Framingham ATPIII	28.2	51.3	0.05	43.6	30.8	n.s	28.2	17.9	n.s.
Framingham Weibul	17.9	41.0	0.025	51.3	35.9	n.s	30.8	23.1	n.s.
PROCAM	38.4	66.6	0.02	38.5	28.2	n.s	23.1	5.1	0.001
PROCAM NS	28.2	59.0	0.01	33.3	30.8	n.s	38.5	10.3	0.001
PROCAM Cox Hazards	25.6	56.4	0.005	43.6	35.9	n.s	30.7	7.7	0.001
SCORE	35.9	46.1	n.s.	25.6	28.2	n.s	38.6	25.6	n.s.

Legend/*legenda*: FE - first examination/*PP - prvi pregled*, AY - after one year/NG − *nakon godinu dana lečenja*, n.s.− non-significant/*nesignifikantno*

Table 4. Estimation of 10-year CHD risk by means of coronary risk score on first examination and after one-year treatment in females
Tabela 4. Procena 10-godišnjeg rizika za koronarnu bolest srca prlikom prvog pregleda i nakon godinu dana lečenja kod žena

	PP (FE)	NG (AY)		PP (FE)	NG (AY)		PP (FE)	NG (AY)	
	Low risk/*Nizak rizik*			Medium risk/*Srednji rizik*			High risk/*Visok rizik*		
	%	%	p<	%	%	p<	%	%	p<
Framingham	49.3	74.6	0.005	35.2	23.9	n.s.	15.5	1.4	0.001
Framingham ATPIII	69.0	93.0	0.001	25.4	7.1	0.001	5.6	0	n.s.
Framingham Weibul	12.7	38.0	0.001	42.2	36.6	n.s.	45.1	25.3	0.005
PROCAM	100	100	n.s.	0	0	n.s.	0	0	n.s.
PROCAM NS	100	100	n.s.	0	0	n.s.	0	0	n.s.
PROCAM Cox Hazards	84.5	98.6	0.005	15.5	1.4	0,001	0	0	n.s.
SCORE	47.9	60.6	n.s.	38.0	35.2	n.s.	14.1	4.2	0.001

Legend/*legenda*: FE - first examination/*PP - prvi pregled*, AY - after one year/NG − *nakon godinu dana lečenja*, n.s.− non-significant/*nesignifikantno*

cording to Framingham Weibul. After one-year treatment, that percentage was 1.8% according to PROCAM and 24.5% according to Framingham Weibul. The reduction was significant according to all seven risk score systems (**Table 2**).

The percentage of male subjects having low risk was from 17.9% according to Framingham Weibul to 38.4% according to PROCAM, and after one-year treatment, it was from 41.0% to 66.6% according to PROCAM and Framingham Weibul, respectively. After one-year treatment, there was a significant rise in the number of male subjects with low risk according to all risk score systems, except SCORE (**Table 3**).

In the intermediate-risk category, the reduction was not significant, while in the high-risk category, a significant reduction was present only according to all PROCAM risk score systems (**Table 3**).

The application of score systems for estimating 10-year coronary risk showed that the low risk category in the female subgroup of the study sample varied from 12.7% according to Framingham Weibul up to 100% according to PROCAM, while after one-year treatment, it was from 38.0% to 100%. Significant differences were not present only when PROCAM, PROCAM NS and SCORE were applied (**Table 4**).

In the intermediate risk category, the significant differences were present only when Framingham ATP III and PROCAM Cox Hazards were applied, while in the high risk category, they were recorded when Framingham, Framigham Weibul model and SCORE were applied as well (**Table 4**).

Discussion

Numerous epidemiological studies have shown that lipids are the most important risk factor and the obligatory parameter of all 10-year coronary risk score systems, which have been or should be included in routine clinical work. Numerous interventional studies have also suggested that the correction of lipid level leads to absolute or relative coronary heart risk reduction. However, until the year of 2009 and *the IMPROVE-dyslipidemia study* conducted by Hatzitolios et al. [21], there were no literature data about the significance of lipid level reduction on the distribution of 10-year CHD risk estimated by means of coronary risk score systems.

The Guideline for Diagnosis and Treatment of Lipid Disorders issued in 2011 recommended the application of SCORE system in our country [26]. However, since none of these score systems is ideal in prediction, the decision was to apply two other mostly used coronary risk scores Framingham (original, modified according to ATP III and computer Weibul model) and PROCAM (original, modified according to MONICA and computer Cox Hazards model) besides SCORE in our research. No literature data are available on such a global estimation of risk score systems. In *the IMPROVE-dyslipidemia study*

[21], Framingham and PROCAM risk scores were applied for the estimation; while in *Co-Laus* study [27], the original Framigham scores and the one recalibrated for Swiss population were used. Similar studies used Framingham and SCORE [28], only Framingham [29] or just PROCAM [30] risk score schemes in our population. Unlike our research, which included subjects with dyslipidemia, other studies performed on our population included randomly selected participants from the general population and the effect of lipid level correction was not estimated.

Our study sample consisted of 110 subjects, 39 males and 71 females, aged 29 to 73 years, the women being older than men as in *the IMPROVE-dyslipidemia* [21] and *Co-Laus* study [27]. Positive family history of myocardial infarction in the first generation before 60 years of age, incorporated as a risk factor in PROCAM risk score, was positive in 45% of subjects in *the IMPROVE-dyslipidemia study* [22] and in 43% of our study sample. The percentage of smokers in our study sample was 18.2%, that being significantly lower than in *the IMPROVE-dyslipidemia study* (45%), and unlike *Co-Laus* study, the number of female smokers was higher than of the male smokers [21, 27].

At the beginning of our research, the serum lipid levels of total cholesterol, triglycerides and LDL-cholesterol were higher than in the previously mentioned studies [27–31], except in *the IMPROVE-dyslipidemia study* because this study was done on dyslipidemic patients and the effect of lipid level correction was estimated after six-month treatment [21]. After one-year treatment, a significant correction of all examined lipid parameters was found in our patients, so the total cholesterol was 5.74 mmol/l, triglycerides - 1.48 mmol/l, LDL-cholesterol - 3.70 mmol/l, while the level of protective HDL-cholesterol rose to 1.35 mmol/l. In *the IMPROVE-dyslipidemia study* the lipid parameters were also significantly corrected after six-month treatment [21].

It can be noticed that at the beginning of our research there were great percentage differences in risk category distribution for 10-year CHD risk calculated by means of seven different CHD risk score systems. Our results suggest that Framingham Weibul model has the most severe classification criteria in female participants. According to it, 45.1% of female subjects are at high risk for CHD, while that percentage is 0-15.5% according to other examined CHD risk score systems. In men, however, SCORE and PROCAM Cox Hazards have the most severe criterion, the percentage distribution of high risk individuals being 38.6% and 38.5%, respectively. It can also be concluded that PROCAM has the mildest criteria in both males and females. The distribution of low CHD risk in males was 38.4% and in females almost 100%, so PROCAM in females is practically useless since it underestimates the risk for CHD due to a very rough approxima-

tion [17]. In *Co-Laus* study [27], *the IMPROVE-dyslipidemia study* [21] as well as in the study conducted by Jovičić et el. [28], the percentage of low risk individuals estimated by means of the applied coronary risk score systmes was significantly higher than in our research.

Although target therapeutic values were not reached [26] after one-year treatment of dyslipidemia, a significant reduction in 10-year CHD risk was achieved according to all score systems applied in the research. Namely, a statistically significant increase in the number of participants at low risk was observed, as well as the decrease in the number of participants having intermediate and high risk. A significant increase in the low risk category was present in the whole study sample and in men according to all score systems, which were applied; whereas, in women it was present in all three Framingham risk scores as well as in PROCAM Cox Hazards. A statistically significant reduction in the high CHD risk category was recorded by all CHD risk score systems in the whole study sample; whereas in men it was recorded by all three PROCAM risk scores and in women by Framingham, Framingham Weibul and SCORE.

Bearing in mind that all CHD risk scores include one or two lipid parameters in their algorithm, these data are in accordance with the correction of tested parameters of lipid status after one-year treatment of our patients. It is similar to *the IMPROVE-dyslipidemia* study, where the total risk reduction was 63% according to Framingham and 45% according to PROCAM risk score [21].

Conclusion

The results of this investigation show that the reduction of total cholesterol, triglycerides, low-density lipoprotein-cholesterol and the increase in levels of high-density lipoprotein-cholesterol have a significant influence on total 10-year coronary heart disease risk reduction. This shows that the application of coronary risk score systems in everyday routine clinical practice would be useful not only for the identification of asymptomatic persons at high risk of developing coronary heart disease and the selection of initial therapy in their treatment but also during the course of treatment, periodically as a confirmation of a positive therapeutic effect. The application of coronary risk score systems could also have a motivational role for patients. It is necessary to correct not only lipids but also other risk factors that can be changed.

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THE RELATIONSHIP BETWEEN POSTERIOR TIBIAL SLOPE AND ANTERIOR CRUCIATE LIGAMENT INJURY

UTICAJ NAGIBA ZGLOBNE POVRŠINE GOLENJAČE NA POVREDE PREDNJEG UKRŠTENOG LIGAMENTA

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Summary

Introduction. The aim of this study was to identify an increased posterior tibial slope as a possible risk factor for anterior cruciate ligament injury. **Material and Methods**. Sixty patients were divided into two groups (with and without anterior cruciate ligament rupture). The posterior tibial slope on the lateral and medial condyles was measured by sagittal magnetic resonance imaging slices by means of computerized method using circles to determine tibial axis. **Results**. The patients with anterior cruciate ligament rupture had a statistically significantly (p=0.06) greater posterior tibial slope on the lateral tibial condyle than the control group (6.68º:5.64º), and a greater slope on the medial condyle (5.49º:4.67º) in comparison to the patients with the intact anterior cruciate ligament. No significant difference in the average values of angles was observed between males and females with anterior cruciate ligament rupture, the average value being 6.23º in men and 5.84º in women on the lateral condyle, and 4.53º in men and 4.53º in women on the medial condyle. **Discussion and Conclusion.** A statistically significant difference between the values of posterior tibial slope was observed between the groups with and without anterior cruciate ligament rupture, the sex having no affect on the value of the posterior tibial slope. The method of measuring angles should be unique.

Key words: Anterior Cruciate Ligament; Risk Factors; Magnetic Resonance Imaging; Anterior Cruciate Ligament Reconstruction; Tibia

Sažetak

Uvod. Cilj studije bio je da utvrdi da li povećan nagib zglobne površine golenjače predstavlja jedan od potencijalnih faktora rizika za povredu prednjeg ukrštenog ligamenta. **Materijal i metode.** Uzorak od 60 pacijenata podelili smo u dve grupe; grupu –sa rupturom i grupu bez rupture prednjeg ukrštenog ligamenta. Na profilnim snimcima dobijenim magnetnom rezonancijom, uz pomoć kompjuterizovane metode koja koristi kružnice za određivanje osovine golenjače, merili smo zadnji golenjačni nagib spoljašnjeg i unutrašnjeg kondila. **Rezultati.** Pacijenti sa rupturom prednjeg ukrštenog ligamenta imali su statistički značajno veći nagib na spoljašnjem kondilu golenjače (p = 0,06) u odnosu na kontrolnu grupu (6,68º naspram 5,64º), uz takođe nešto veći nagib unutrašnjeg kondila (5,49º naspram 4,67º), u odnosu na pacijente sa očuvanim prednjim ukrštenim ligamentom. Među polovima, kod pacijenata sa pokidanim prednjim ukrštenim ligamentom nismo zabeležili značajnu razliku prosečnih vrednosti uglova: na spoljašnjem kondilu golenjače (6,23º kod muškaraca naspram 5,84º kod žena) i unutrašnjem kondilu (4,53º : 4,78º). **Diskusija i zaključak.** Utvrđena je statistički značajna razlika u veličini ugla nagnutosti zglobne površine golenjače između grupe sa pokidanim prednjim ukrštenim ligamentom i kontrolne grupe. Pol ne utiče na veličinu ugla nagnutosti zglobne površine golenjače, ni u grupi sa pokidanim prednjim ukrštenim ligamentom ni u kontrolnoj grupi. Metodologija merenja ugla treba da bude jedinstvena.

Ključne reči: Prednji ukršteni ligament; Faktori rizika; Magnetna rezonanca; Rekonstrukcija prednjeg ukrštenog ligamenta; Tibija

Introduction

The number of registered anterior cruciate ligament (ACL) injuries is on constant rise in the world [1, 2] as well as in the Republic of Serbia [3]. Consequently, the number of performed reconstructions of this structure of knee joint has been also rising [1, 4, 5]. The results of reconstructions are excellent in 80-90% of all cases due to the development of diagnostic methods, such as magnetic resonance imaging (MRI), operative techniques and early rehabilitation [4–9]. However, it is illogical to have a situation where the number of studies dealing with the results and complications of operative treatment [4–9] is incomparably higher than those dealing with the prevention of ACL injuries [1, 3].

The situation changed in the last twenty years so that the interest has increased regarding the necessity to define vulnerable groups, risk factors, causes of ACL injuries [1–3] as well as the possibilities to develop effective preventive trainings [10]. In the last few years the anatomy of knee joint was often high-

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lighted as one of risk factors, such as the influence of anterolateral ligament on knee stability [2], size of intercondylar notch or posterior tibial slope (PTS) as potential factors for ACL rupture [1, 11, 12].

The aim of this study was to identify an increased posterior tibial slope as a possible risk factor for anterior cruciate ligament injury.

Material and Methods

We divided our total specimen of 60 patients into two groups. The first group included 30 patients with complete ACL rupture, 24 men and six women, their average age being 30.41 years (9-59). The second group consisted of 25 men and five women, whose average age was 28.33 years (16-56). This control group did not have ACL rupture on MRI. All of the patients were diagnosed and treated at the Department of Orthopedics and Traumatology of Clinical Centre of Vojvodina in Novi Sad. MRI was performed at the Center for Radiology "VMR" in Novi Sad by a MRI device with 1.0 Tesla magnetic field, manufactured by Philips. All

Figure 2. Determination of tibial axis
Slika 2. *Određivanje osovine golenjače*
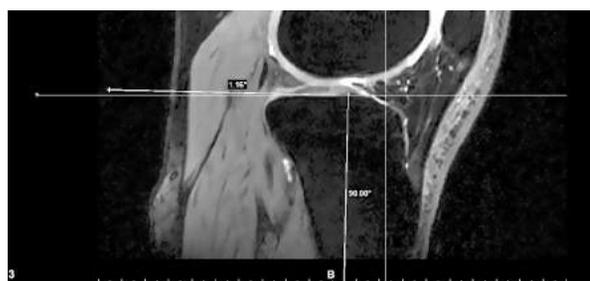
Figure 3. Measuring of posterior tibial slope
Slika 3. *Merenje ugla nagiba zglobne površine golenjače*

of the measurements were performed by the same radiologist. The angles of PTS (**Figure 1**) were measured in the program developed at the Faculty of Technical Sciences in Novi Sad.

The values of PTS were separately measured for the lateral tibial condyles and medial condyles in sagittal slices. The middle of the bone was determined initially in sagittal plane (**Figure 2**). Then two circle lines were made, the smaller distal one reached the tibial cortex on MRI and the proximal one with greater diameter was centered in the proximal part of tibia (**Figure 2**). Finally, a horizontal line was drawn forming a posterior open angle with the vertical line. A new line was drawn on the proximal end of axis under 90 degrees (**Figure 2**). Eventually, a new horizontal line was drawn making a posterior open angle with the previous line in the section plane. The PTS values in degrees were obtained by measuring these angles (**Figure 3).** The average angles were compared between the groups and the hypothesis was tested by Fisher′s test. PTS of medial and lateral condyles were tested separately and the differences between sexes were analyzed.

The exclusion criteria of this study were partial ACL rupture, chondromalacia grade III or IV, osteophyte formations and previous knee surgery.

The statistical analysis included the average values, standard deviation and range of measurements. The results are shown by figures, graphs and tables further in the text.

Figure 1. Posterior tibial slope
Slika 1*. Zadnji nagib golenjače*

Table 1. Group with intact ACL
Tabela 1. Grupa bez pokidanog prednjeg ukrštenog ligamenta

	Mean/*Prosek*	SD	Min.	Max.
Lateral slope/*Lateralni nagib*	5.64	1.90	2.3	9.6
Medial slope/*Medijalni nagib*	4.67	2.36	0.9	10.1

values in degrees, SD - standard deviation/*vrednosti u stepenima, SD - standardna devijacija*

Table 2. Group with ACL rupture
Tabela 2. Grupa sa pokidanim prednjim ukrštenim ligamentom

	Mean/*Prosek*	SD	Min.	Max.
Lateral slope/*Lateralni nagib*	6.68	2.23	3.2	11.1
Medial slope/*Medijalni nagib*	5.49	2.77	1.0	13.2

Table 3. Statistical significance between the groups considering average PTS
Tabela 3. Značajnost razlike između prosečnog unutrašnjeg i spoljašnjeg ugla obe grupe

	F	p
Lateral PTS/*Lateralni nagib*	3.665	.060
Medial PTS/*Medijalni nagib*	1.489	.227

Results

The average angle of PTS on the medial tibial condyle in the control group (without ACL rupture) was 4.67°, while on the lateral condyle it was 5.64° (**Table 1**).

The group with ACL rupture had the average PTS on medial condyle of 5.49° and 6.68° on the lateral one (**Table 2**). The values of measured angles varied from the maximal 13.2° to the minimal 1.0°.

A statistically significant difference was found between the groups (**Table 3**), because the patients with ACL rupture had PTS greater by 1.04° on the lateral tibial condyle than the controls, as well as a somewhat greater slope on the medial condyle by 0.82° in comparison with the patients with intact ACL.

We also analyzed the PTS difference between sexes among the patients with ruptured ACL. The average PTS on the medial tibial condyle in females was 5.84° and 4.78° on the lateral one. The average values of PTS in males were 6.23° and 4.53° on the medial and lateral tibial condyle, respectively (**Graph 1**). The female patients had smaller PTS on the lateral tibial condyle by 0.39°, but it was greater by 0.25° on the medial condyle than in the male patients.

The statistical analysis showed that there was a significant difference between the values of lateral PTS between the groups with and without ACL rupture (F=3.665 p=0.06). We did not find a significant difference between the same groups concerning medial PTS (F=1.489 p=0.227) although the patients with ACL rupture had greater PTS values.

According to Fisher's test there was no difference between PTS concerning the age difference between the groups (F=2.108 p=0.152).

Discussion

The incidence of ACL injury has been on constant rise in the last few decades with the resulting necessity to determine risk factors and causes of injury and to develop preventive measures in order to reduce the incidence of injuries. The risk factors are divided to intrinsic and extrinsic, as well as to changeable and unchangeable [13]. The preventive measures are mainly focused on the correction of changeable conditions during non-contact sports ACL injuries [3, 14]. The extrinsic risk factors include environmental conditions such as playing surface, shoe type, weather conditions and type of sport [3, 13, 15]. The intrinsic risk factors may be divided into anatomic, hormonal, neuromuscular and familial [16]. Numerous anatomic variables include intercondylar notch width, in-
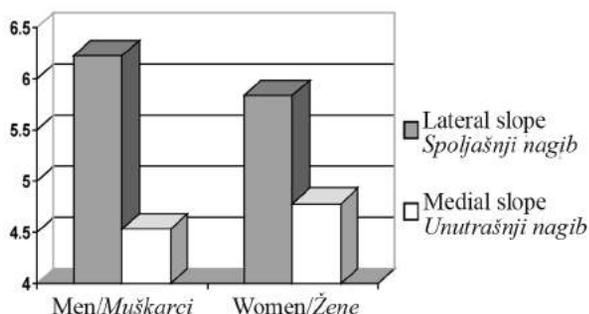
Graph 1. The values of PTS (in degrees) in patients of both sexes with ACL ruptures
Grafikon 1. Vrednosti golenjačnog nagiba (u stepenima) kod pacijenata oba pola sa pokidanom prednjom ukrštenom vezom

creased body mass index, landing kinematics, female sex, anatomic alignment [13, 14]. Anatomic differences between sexes are Q angle, pelvis width, effects of hormones (primarily of estrogen) on the incidence of injury and ligament strength, neuromuscular and biomechanical factors, former knee injuries and age. All of them may be potential risk factors for ACL injury [16]. Aggressive contraction of quadriceps muscle during moderate knee flexion is also a relevant intrinsic factor for noncontact ACL injuries because it leads to increased anterior translation of tibia [17].

There are many inconsistencies regarding the significance of some risk factors; however, there is no doubt that young athletes are the most vulnerable population. Women have 2–10 times higher risk for injury than men depending on the type of sport [13–16]. In our former study performed on 450 operated patients with ACL rupture [3] we concluded that there was no statistical significance of correlation between ACL lesion and footwear type, body mass index, warm up, previous illnesses, everyday therapy and genetic profile. However, it was proved that ACL lesion correlated with the career length, skill level, competitive level, surface quality, and previous injuries. Most of the injuries occurred in a noncontact way, during landing or sudden change of direction.

Recently, anatomic variations are considered as a risk factor for a primary ACL injury, especially the geometry of the proximal tibia, in particular the PTS [1, 11, 12]. Articular surface of tibia in the knee joint consists of lateral and medial condyles which are in contact with the condyles of femur. With tibial axis, this surface makes an angle (PTS) of 7–10 degrees. A greater PTS with axial loading generates a greater anterior translation of tibia [18]. As the ACL is the main stability structure of this movement, this results in its overthightening. This leads to the increased internal tibial rotation and greater force on ACL [19]. Dejour and Bonnin [18] used lateral radiography and found that for every 10 degrees increase in PTS, there was a 6-mm increase in the anterior tibial translation during a single-legged stance. Fening et al. [20] also registered an increased anterior tibial translation but less ACL tightening after high tibial osteotomy. Similar results have been found in cadaveric and computer modeling studies [21,22].

Many studies found a correlation between the PTS and ACL insufficiency [1, 11, 12, 23–30]. However, authors used different diagnostic methods; some used lateral radiography [12, 18, 29, 30], others computerized tomography [24], while the majority, like us, used MRI slices [11, 23, 25, 26, 28]. A common method for determining tibial axis and the way of measuring tibial slope has not been adopted yet [1, 11, 23, 25]. Even the results are interpreted differently, particularly regarding the dilemma whether the medial or lateral PTS has greater effect on ACL injury [1, 11, 23, 25] and if

there is a difference between sexes [12, 29–31]. Authors also disagree about the size of angle that is responsible for risk [1]. Some studies applied the same methods but yielded different results.

Simon et al. [26] used modified Hashemi's method with 3D reconstruction of tibial articular surface. MRI studies, like ours using methods similar to Hudek or Hashemi [23, 25], had similar results of measuring PTS. The difference between groups with and without ACL rupture ranged from 0.87 to 2.64 degrees [25, 27, 28]. We used MRI slices because some mistakes may occur if profile X-rays are used. One of important advantages of MRI is the possibility for visualization of the articular cartilage that is not visible on lateral radiography [11, 24]. Every patient must be in the same position during MRI diagnostics in order to achieve proper sagital slices of the knee joint. This is achieved by fixation of the hip and determination of points at the ends of tibia. For the best bone performance, three-dimensional visualization of articular surface of tibia should be used whenever it is possible. The same standard conditions should be provided [24], although every method has its disadvantages [1].

Since we achieved positive statistical correlations, it can be concluded that there was a significant difference in values of measured angles between the group with ACL rupture and group with intact ACL. Our method relied only on MRI of knee joint, without slices of complete tibia, using circles in tibia for determination of axis. Some authors think that the best method is MRI containing slices of proximal tibia (10-15 cm) or complete tibia [1]. Others believe that the method with circles is a good choice if MRI of complete tibia is not available [23, 25].

A group of Chinese authors [1] applied the meta analysis to systemize the results of 12 studies on PTS and concluded that both lateral and medial PTS were significantly increased in ACL injured groups compared to the controls, the difference being greater in the lateral tibial plateau slope (1.8°) than in the medial slope (1.1°). Our results are similar but with smaller difference (1.04°: 0.82°). In contrast, some Serbian authors [11] think that the patients with greater PTS on lateral condyle but at the same time with lesser PTS on medial condyle are at higher risk for ACL rupture than the controls.

Webb et al. [12] proved that operated patients with increased PTS (of 9.9° on average) more frequently have reruptures of graft and ACL ruptures of contralateral knee in comparison to common PTS (8.5°) in operated patients without the above complications. Since the highest number of ACL reruptures was recorded in the patients having the slope over 12°, it can be concluded that the risk for rerupture is proportional to the increase in the slope angle. Those patients who have the slope over 12° (only 12% of specimen) are at five times

higher risk of further ACL injuries than those having the slope less than 9°.

The average age of our patients was 29 years. This is similar to other studies where the most ACL injured population were in the third decade of life [3, 5–9, 13–16]. The different results may be influenced by different choice of patients [1], because some study samples consisted only of female patients, other samples included only noncontact injuries or patients under 18 years of age [1, 23, 25, 29, 30]. Exclusion criteria may also have influence. Our groups were uniform because there were no statistical differences among our groups considering age and sex structure. Webb et al. as well as Bourke et al. [12, 31] have found that men have greater PTS, while Brandon et al. as well as Todd et al. [29, 30] have observed that PTS is increased in women. They also mentioned that corrective osteotomy should be taken into consideration to reduce the risk of (re)injuries [29, 30]. We did not find a statistically significant difference between sexes so causes for epidemiology of ACL ruptures among women athletes lie in anatomic differences between sexes. Women have increased Q angle and knee valgus, smaller intercondylar notch, wider pelvic ring, influence of hormone (estrogen) on ligament strength and different contraction time of anterior and posterior muscles groups of thigh [16]. Bearing the above in mind, some Swedish authors [10] achieved a significant reduction in ACL injuries in female soccer players: by 15-minute neuromuscular warm-up program, twice a week during the period of three years. It consisted of one-legged and two-legged knee squats, pelvic lifts, the bench, lunges, jumps and proper landings. This program reduced the ACL rate by nearly two-thirds.

The fact that our study was performed on a relatively small sample consisting of only 60 patients and MRI slices of the whole tibia were not available gives the basis to consider our research limited. MRI slices of the whole tibia are necessary for precise determination of tibial axis and for minimizing mistakes during measuring angles, but they are more expensive. Further studies should consider those facts as well as finding new, better and unique methods for visualization of PTS. New studies should create proper standards for connection between PTS and ACL injury as well as to find new prevention methods by neuromuscular trainings.

The question whether corrective osteotomy would reduce the incidence of ACL ruptures and reruptures is still open, as well as the dilemma whether the preventive programs focused on strengthening posterior thigh muscles of athletes with increased PTS can reduce anterior tibial translation.

Conclusion

We found a statistically significant difference in the values of posterior tibial slope between the group with and without anterior cruciate ligament rupture.

The slope on the lateral condyle is greater than on the medial one and it is a more significant parameter.

Sex does not influence values of posterior tibial slope either in the group with ruptured anterior cruciate ligament or in the control group.

Method of measuring angles by magnetic resonance imaging diagnostics should be unique to avoid mistake.

Posterior tibial slope cannot be assumed as an isolated risk factor.

Conditions for prevention of anterior cruciate ligament ruptures can be achieved by knowing risk factors, causes and mechanisms of its injuries.

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REITER'S SYNDROME – DISEASE OF YOUNG MEN - ANALYSIS OF 312 PATIENTS

RAJTEROV SINDROM - BOLEST MLADIH MUŠKARACA - ANALIZA 312 PACIJENATA

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Summary

Introduction. Reiter's syndrome is reactive arthritis occurring after acute urogenital (urethritis, cervicitis) or enterocolitis infections. The associated ophthalmological and/or mucocutaneous changes are full clinical manifestations of this disease. This paper was aimed at analyzing clinical and radiological characteristics and findings of possible etiological factors and protocol for Reiter's syndrome therapy. **Material and Methods.** Of 312 patients included in the study, 279 were men and 33 were women, the ratio between them being 8.5:1. The disease was diagnosed based on clinical evidence of two basic characteristics of Reiter's syndrome: arthritis preceded by acute urogenital or enteral infection. **Results.** Urogenital and enterocolitic form of disease was found in 242 (77.5%) and 52 (16.5%) patients, respectively; whereas the initial cause was not discovered in 18 patients (6%). Three or two main signs of Reiter's syndrome were present in approximately the same number of patients (41.7% and 44.2%), whereas all four signs of disease were present in 14.1% of the patients. Acute or sub-acute form was present in 40.5%, while recurrent and chronic disease was diagnosed in 31% and 28.5% of the patients, respectively. The most frequent clinical manifestation of this disease was on the locomotor system as asymmetrical oligoarthritis localized in lower extremities, present in 69.4% of the patients. Chlamydia trachomatis was found in the synovial fluid in 54% of patients (20/37), ureaplasma or mycoplasma was isolated in the synovial tissue of 73.1% of patients (30/41) and in the peripheral blood mononuclear cells in 93.2% of patients (41/44). Human leukocyte antigen B27 was present in 83.3% of patients. **Conclusion.** Reiter's syndrome is a multisystem disease, predominantly occurring in human leukocyte antigen B27 positive young males. The fact that the causative agents are found in the synovial membrane or synovial fluid is indicative of infectious rather than reactive arthritis.

Key words: Arthritis, Reactive; Synovial Fluid; Diagnosis; Urinary Tract Infections; Enterocolitis; Signs and Symptoms; HLA-B27 Antigen; Therapeutics; Male; Adult

Sažetak

Uvod. Rajterov sindrom reaktivni je artritis koji se ispoljava posle akutne urogenitalne (uretritis, cervicitis) ili enterokolitisne infekcije, a pridružene oftalmološke i/ili mukokutane promene čine kliničku sliku potpunog oblika bolesti. Cilj rada je analiza kliničkih, radioloških i ostalih karakteristika, utvrđivanje mogućih etioloških činilaca i načina lečenja bolesnika sa Rajterovim sindromom. **Materijal i metode.** Studijom je obuhvaćeno 312 bolesnika, 279 osoba muškog i 33 osobe ženskog pola (odnos 8,5 : 1). Dijagnoza bolesti postavljena je na osnovu dve glavne kliničke karakteristike: artritisa kome prethodi akutna urogenitalna ili enteralna infekcija. **Rezultati.** Kod 242 (77,5%) bolesnika utvrđen je urogenitalni oblik bolesti, kod 52 (16,5%) enterokolitisni, a u 18 (6%) ulazno mesto infekcije nije otkriveno. Tri ili dva glavna znaka Rajterovog sindroma bila su prisutna kod približno istog broja obolelih (41,7% odnosno 44,2%), dok je sva četiri znaka bolesti imalo 14,1% bolesnika. Akutni ili subakutni tok imalo je 40,5%, recidivski 31%, a hronični 28,5% bolesnika. Klinički se bolest ispoljila na lokomotornom sistemu najčešće kao asimetrični oligoartritis lokalizovan na donjim ekstremitetima (69,4%). U sinovijskoj tečnosti kod 54% (20/37), bolesnika, izolovana je hlamidija trahomatis, ureaplazma ili genitalna mikoplazma u sinoviji kod 73,1% (30/41), a u perifernim monocitnim ćelijama krvi kod 93,2% (41/44) obolelih. Humani leukocitni antigen B 27 bio je prisutan kod 83,3%. **Zaključak.** Rajterov sindrom je multisistemska bolest koja se predominantno javlja kod mladih muškaraca pozitivnih na humani leukocitni antigen B 27. Nalaz prouzrokovača u sinoviji ili sinovijskoj tečnosti sve više ukazuje da je moguće da se radi o infekcijskom a ne reaktivnom artritisu.

Ključne reči: Reaktivni artritis; Sinovijalna tečnost; Dijagnoza; Infekcije urinarnog trakta; Enterokolitis; Znaci i simptomi; HLA-B27 antigen; Terapija; Muško; Odrasli

Introduction

Reiter's syndrome (RS) is probably as old as the human race. Articular complications following dysentery were known even to Caelius Aurelianus, who lived at the beginning of the fifth century before Christ. This disease was named after the German bacteriologist Hans Reiter, who described it in 1916, although the same disease had been described by the French physicians Feissinger and Leroy a week earlier. The English physician Benjamin Brodie, however, described five patients

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with triad (urethritis, arthritis, conjunctivitis), and two with iritis as far back as 1818 [1].

The synonym for Reiter's syndrome is "reactive arthritis". The term is primarily used to describe spondyloarthritis that occurs after intestinal and urogenital infections mainly in the patients with positive human leukocyte antigen B27 (HLA - B27) [2].

The responsibility for the etiology and pathogenesis of the RS lies with infectious agents, genetic predisposition and immunological mechanisms [3.4]. It is believed that chlamydia (Chlamydia trachomatis - Ct) is the main cause of urogenital Reiter's syndrome, rarely ureaplasma (Ureaplasma urealyticum - Uu) and mycoplasmas (Mycoplasma hominis - Mh), and Shigella enteropathic shape (Shigella), Salmonella (Salmonella), Yersinia and Campylobacter [3–6].

Although Reiter's syndrome was thought to belong to the group of reactive arthritis for years, the growing evidence emerged during last two decades suggesting that RS is infectious arthritis. Immunocytochemical and immunohistochemical methods were applied to detect Chlamydia and its particles in the synovial tissue and/or synovial fluid [5–7]. Polymerization chain reaction (PCR) and hybridization techniques demonstrated the presence of nucleic acid of known microorganisms inducted in the synovial tissue and fluid of patients with post-enterocolitis and post-urethritis reactive arthritis [6–10].

Chlamydia trachomatis is believed to be the main cause of urogenital form of RS in genetically predisposed individuals, but it is not clear how Chlamydia infections lead to the development and relapse of chronic arthritis [10, 11]. There is compelling evidence that bacteria and microbial antigens, which induce reactive arthritis, persist in the joints and other reservoirs as the front door of infection. Due to the intensive immunological and immunogenetic investigations of reactive arthritis over the past decades, current understanding of the mechanism of this disease is mainly based on the possible role of immune response [11, 12]. It is believed that the imbalance of cytokines and the interaction of bacteria and HLA - B27 play a major role in the failure to eliminate bacteria and microbial antigen (inductors) from the body, which leads to the manifestation of disease and chronicity. On the other hand, the microbiological and molecular etiopathogenesis of bacterial persistence is not so well explained although the researchers succeeded in getting the insight into the persistence of Chlamydia on several occasions in previous years [12–16].

The role of HLA - B 27 in the pathogenesis is unclear. Reiter's disease affects about 20% of HLA - B 27 positive cases after acute urogenital or enteric infections. It is assumed that the lower part of the B -27 antigen has the same layout of amino acid as microorganisms that are the drivers of pathological process (the theory of molecular mimicry) [6, 11, 17]. According to the literature, HLA - B27 is found in 60 to 92% of patients, and it is responsible for the slower elimination of pathogens, their antigens, and development of the chronic form of Reiter's syndrome [6, 16, 18, 19].

Clinical manifestations are of the utmost importance for diagnosing this disease. Typical RS starts with urogenital infections, which are more common in men than in women in the third decade of life [6, 11, 17]. General signs and symptoms and other manifestations of disease may occur in the period of 1-4 weeks until the appearance of arthritis. Arthritis begins suddenly; it is usually asymmetrical, oligoarticular, localized in the lower extremities. The acute course of the disease is followed by the sub-acute phase, which lasts from two to 12 months. It may recur in some patients in different time intervals; however, if it persists for more than 12 months, it is considered chronic and usually creates functional problems [6, 17]. The disease can manifest itself in different ways: the affection of the locomotor system as the only clinical manifestation of the disease, called monosymptomatic form and incomplete form by Mladenovic and Arrnet, respectively [20, 21], whereas in a smaller number of patients it is manifested in the complete form: urethritis, arthritis, conjunctivitis / uveitis and mucocutaneous changes [6, 17, 20, 21].

Laboratory tests usually reveal the cause of the initial infection and the presence of inflammation [11, 12, 17]. Radiographic changes in the musculoskeletal system have their topographical and morphological features. The lower extremities (mostly II and III metatarsophalangeal ankle and calcaneus) and sacroiliac joints are most frequently affected, and the spinal cord is rarely affected. Treatment is performed by administering appropriate antibiotics for the initial infection to prevent relapse, and the therapy of chronic arthritis includes non-steroidal anti-inflammatory drugs (NSAIL) and physical procedures. More severe forms of the disease require administration of drugs from the group of basic and / or biological agents. Radiation synovectomy and surgical methods of treatment are sometimes applied (arthroscopic synovectomy) [6, 11, 17, 21]. The aim of this study was to analyze the clinical, radiological and other characteristics, and to identify potential etiologic factors and ways of treatment of patients with RS.

Material and Methods

This study included 312 patients with RS examined and treated at the Department of Rheuma-

tology of Military Medical Academy during the period of 42 years (from 1970 to 2011). There were 279 male and 33 females (the ratio being 8.5:1), whose average age was 27.2 years, ranging from 14 to 65 years.

Reiter's syndrome was diagnosed in our patients according to the applicable criteria [22]: arthritis and/or sacroiliitis, which developed one to four weeks after the previous enteric or urogenital infection. In addition to the two main clinical criteria, arthritis and prior infection, the presence of ophthalmic (acute conjunctivitis, acute anterior uveitistrias) and/or mucocutaneous changes (balanitis, stomatitis, keratodermia-tetrads) were necessary to diagnose the complete form of Reiter's syndrome [17, 22].

The patients who had had reactive arthritis (arthritis of peripheral joints, dactylitis, enthesitis, sacroiliitis) for more than 12 months were classified as having the chronic form of disease [12, 17].

The disease was diagnosed based on the tests and examinations of blood, urine, stool, synovial membrane, synovial fluid and conjunctival swabs, urethra and/or cervix, i.e. prostatic exprimate as well as the determination of antibody titers to Chlamydia, Salmonella and Yersinia and human leukocyte antigen (HLA) typization.

Chlamydia trachomatis was identified in McCoy cell culture treated by cycloheximide and genital mycoplasmas (Ureaplasma urealyticum and Mycoplasma hominis) were determined according to their biochemical properties in liquid selective medium from the swab of the cervix, urethra and prostatic exprimate. The polymerization chain reaction was used for the detection of genital mycoplasma and chlamydia in peripheral blood, synovial fluid and synovial joint of the patient [7–9]. Some patients underwent histological and/or immunofluorescence examination of their biopsy material (skin, affected synovial joints, kidneys), and radiography of affected parts of the musculoskeletal system, ultrasound examination of the heart, joints and tendons (transducer of 3.5 and 7.5 MHz, Diasonix - USA), electrocardiography and bone scintigraphy with Tc99 calculating the index sacroiliac (SI - index). SI - index is the ratio of accumulation of radiolabel in the sacroiliac joints and bones. Normal values in our population amount to 1.38. Statistical analysis included descriptive measurements: the average value, arithmetic mean, standard deviation (SD), range and analytical measurements. General linear model for repeated measures was used to estimate the statistical significance of the parameter data (laboratory Mc Nemar's test was used to evaluate the statistical significance of frequency of positive bacterial findings in the patients before and after therapy). Hi-square test was used to assess the statistical significance of positive findings in different bacterial samples, before and after treatment. The threshold of statistical significance was

set at 0.05. Statistical analyses were performed by the following software: the Statistical Package for the Social Sciences (SPSS) for Windows v. 11.5 (SPSS Inc. Chicago, IL).

Results

Clinical Characteristics

Acute or sub-acute form, recurrent and chronic form of RS was found in 126 (40.5%), 97 (31%), and 89 (28.5%) patients, respectively. The disease with four, three and two main signs was diagnosed in 44 (14.1%), 130 (41.7%) and 138 (44.2%) patients respectively. According to the initial infection, 242 (77.5%) patients had urogenital form, 52 (16.5%) had enterocolitis and the cause was not determined in 18 (6%) patients. Arthritis, often asymmetric, was present in all patients. It was localized only in the lower extremities in 179 (57.4%) patients, in the lower and upper extremities in 130 (41.6%) patients, and in the upper extremities only in 3 (1%) patients. Oligoarticular arthritis was present in 214 patients (69%), polyarticular in 44 (14%), monoarticular in 53 (16.7%) patients. Dactylitis on lower extremitis was found in 43 (17.6%) patients. Out of twelve (3.8%) patients who had dactylitis on the upper extremitis, nine patients had dactylitis of the second and third finger, and three patients had it on the third and fifth finger.

Of 125 (40%) patients who had pain in the spinal column, 110 had radiographic signs of inflammation. Clinical manifestations of the musculoskeletal system by frequency of occurrence are shown in **Table 1.**

The following types of urogenital events were found in 246 (78.8%) patients: the most common was urethritis/uretrocistitis in 71.6% of patients, then prostatitis in 18.8% of the patients, cervicitis in 6.6%, hemorrhagic cystitis in 1.9% and epididymitis in 1.1% of patients. Fifty (16.1%) patients had a prior infection of enterocolitis or enteritis. Eye changes occurred in 139 (44.5%) patients, of which the most frequent was conjunctivitis in 110 (79%), acute iridocyclitis in 21 (15%), and other changes (keratoconjunctivitis, episcleritis) in eight (6%) patients. Changes in the skin, nails and mucous membranes were manifested in 57 (18.2%) patients, of which the most common was balanitis in 42 (13.4%) and five patients had simultaneously two types of such changes. General signs developed in 190 (61.5%) patients: a significant loss in body weight (3-17 kg in a month) in 37 (12%) and subfebrile temperature or fever in 124 (39.7%) patients. As for other manifestations, changes on the heart developed in nine patients (2.8%) and kidney damage was pathologically and histologically verified in four patients beyond doubt.

Laboratory Findings

Human leukocyte antigen typization revealed HLA-B27 in 83.3% of patients. Erythrocyte sedimentation rate was higher in 64.4% of the patients, being over 50 mm/h in 57.7%. Non-specific pro-

Table 1. Clinical characteristics of patients with Ryter's syndrome
Tabela 1. *Kliničke karakteristike Rajterovog sindroma*

Signs of the musculoskeletal system / *Znaci na lokomotornom sistemu*	Number of patients / *Broj pacijenata (%)*	(%) One Localization / *Jednostrana lokalizacija*
Arthritis : number of patients and % / *Artritis: broj pacijenata i %*	312 (100%)	
Knee joints/*Kolenski zglobovi*	228 (73.1%)	149 (79)*
Ankle joints/*Skočni zglobovi*	187 (59.9%)	120 (67)*
Sacroiliac joints †/*Sakroilijačni zglobovi †*	110 (35.0%)	85 (25)*
Wrists/*Ručni zglobovi*	70 (22.4%)	36 (5)*
spondylitis , spondylodiscitis †/*Spondilitis, spondilodiscitis †*	15 (4.8%)	
Metatarsophalangeal/*Metatarzofalangealni*	94 (30.1%)	76 (18)*
PIP joints of the foot/*PIP zglobovi stopala*	44 (14.1%)	36 (8)*
PIP joints of the hand of/*PIP zglobovi šaka*	30 (9.6%)	19 (11)
Metacarpophalangeal/*Metakarpofalangealni*	22 (7.0%)	13 (9)*
The sensitivity of the heel:/*Osetljivost pete:*	113 (36.2%)	70 (43)*
Dactylitis/*Daktilitis:*	55 (17.6%)	
Lower extremities/*Donji ekstremiteti*	43 (17.6%)	38 (5)*
Upper extremities/*Gornji ekstremiteti*	12 (3.8%)	11 (1)*
Bursitis/*Burzitis*	18 (5.7%)	
Elbow/*Lakat*	9 (2.8%)	7 (2)
Knee/*Koleno*	4 (1.2%)	3 (1)
Hip/*Kuk*	4 (1.2%)	2 (2)
Heel/*Peta*	5 (1.6%)	3 (1)

 * Number of patients with bilateral localization/**broj bolesnika sa obostranom lokalizacijom*
 † localization according to radiological findings/*† lokalizacija na osnovu radiološkog nalaza*
 PIP - proximal interphalangeal joint/proksimalni interfalangealni zglob

teins of the acute phase of inflammation were most frequently increased: fibrinogen in 48.3%, C-reactive protein in 40.5%, haptoglobin in 46% of the patients.

The increase in immunoglobulin (IgG, IgA, IgM) was present in 17.6%. Rheumatoid factor (RF) was positive in 2.6%. Anemia was found in 38 patients (12.2%); 26 patients (8.3%) had leukocytosis, 10 (5.3%) patients had thrombocytosis, and an increase in the concentration of uric acid was recorded in 3 (0.9%) patients. Other biochemical tests were within normal limits.

The following pathological findings in urine were recorded in 135 patients: leukocyturia in 81 patients, erytrocyturia in 47 and proteinuria over 150 mg/d in 7 patients.

Urethral, cervical and/or conjunctival as well as prostatic swabs were positive for Chlamydia in 39.7%, for Ureaplasma in 28.5%, and for Mycoplasma in 8% of the patients. Of those patients who were diagnosed to have postenterocolitic form of Reiter's syndrome, Yersinia was isolated in 48%, Salmonella in 36%, Shigella in 12%; whereas, the cause remained undiscovered in other patients. Several types of microorganisms, triggers of the infection, were identified in 8.9% of patients. The analysis of synovial fluid by seeding substrate was performed in 54 patients, and it proved to be positive for Chlamydia and/or genital mycoplasmas in 29.6% (16/54). The polymerase chain reaction method was applied to identify bacteria in blood and/or the target site (synovium and synovial fluid) in 44 patients. All patients had a trigger, the presence of bacteria in at least one of isolated causes. In synovial fluid, one or more bacteria (Chlamydia trachomatis, genital mycoplasmas, Ureaplasma) was isolated in 54% (20/37) of the patients, in the synovium in 731% (30/41) of the patients and in the peripheral blood in 93.2% (41/44) of the patients (**Table 2**).

Radiographic and Other Findings
Radiographic changes in the musculoskeletal system were found in 185 (59.3%) patients. **Table 3** shows the changes commonly seen, whereas 89 (67.9%) patients had findings of Sacroiliitis (SI index exceeding 1.38).

Treatment
The therapeutic approach to patients is focused on the treatment of acute arthritis and the infection. All patients with arthritis were treated with NSAID and followed by physical therapy. Glucocorticoids were administered in the acute phase, in severe forms, systemically and/or locally intra-articularly or in the insertion of the affected tendon. Once

Table 2. PCR analysis of bacterial DNA in the samples of synovial tissues, synovial fluid and peripheral blood mononuclear cells (PBMC), before and after (PBMC samples) treatment with azitomicinom and combined antibiotic therapy in patients with post-urethritis reactive arthritis
Tabela 2. PCR analize bakterijske DNK u uzorcima sinovijalnog tkiva, sinovijske tečnosti i mononuklearnih ćelija periferne krvi(PBMC), pre i posle (PBMC uzorci) azitomicinom i kombinovanom antibiotskom terapijom kod pacijenata sa postveneričnim reaktivnim artritisom

Patients Pacijenti	ST	SF	PBMCb	PBMCa	Patients Pacijenti	ST	SF	PBMCb	PBMCa
	Azithromycin therapy/*Azitromicin*					Combined antibiotic therapy/*Kombinovana antibiotska terapija*			
1	nd	Ct,Uu	Ct ,Uu	-	1	Ct	nd	-	-
2	-	Uu	-	-	2	Ct, Cp	-	Ct	Ct, Cp
3	-	-	Ct, Uu	Ct	3	Ct	Ct, Uu	Ct	Ct
4	-	-	Ct, Mh	Ct, Mh	4	Ct, Mh	nd	Ct, Cp, Mh	-
5	-	Ct	Ct ,Mh	-	5	Ct	Ct, Uu	Ct, Cp, Mh	Ct,
6	Uu	Uu	Uu	-	6	Ct, Mh	Ct	Ct, Mh	-
7	-	-	Ct	Ct,	7	nd	nd	Ct	-
a8	-	-	Mh	Mh	8	nd	nd	Ct	Ct, Cp
9	-	Mh	Mh	-	9	Ct, Cp, Mh	Ct, Cp	Ct, Cp	-
10	Uu	Uu	Uu	-	10	Ct, Cp	Ct	-	Ct, Cp
11	Mh, Uu	-	Mh Uu	, Uu	11	CT	Ct	Ct, Cp, Uu	Ct
12	Uu	Uu	Uu	-	12	CT, Mh	-	Ct, Uu	-
13	Ct	-	Ct, Mh	-	13	-	nd	Ct, Uu	-
14	Uu,Ct	-	Ct,,Uu	-	14	Ct	Ct	Ct, Uu	Ct
15	Mh, Uu	-	Mh,Uu	-	15	Ct	Ct	Ct, Uu	-
16	Mh	-	Mh	Mh	16	Ct, Cp	Ct	Ct	-
17	Mh	-	Mh	-	17	nd	nd	Ct, Uu	Ct
18	Uu	-	Uu	-	18	nd	nd	Uu	-
19	Ct, Cp	-	Ct	Ct	19	Ct, Mh	-	Ct Uu	-
20	Cp, Mh	-	Ct ,Mh	-	20	Ct	Ct	Ct	-
21	Ct ,Uu	Ct	Ct, Uu	-	21	Ct	Ct, Uu	Ct	-
22	Uu	Uu	Uu	-	22	-	-	Ct	Ct

S ST – synovial tissue, SF – synovial fluid; PBMCb - peripheral blood mononuclear cells before therapy; PBMC peripheral blood mononuclear cells after therapy, Ct - *Chlamydia trachomatis*, Cp – *Chlamydia pneumoniae*, Mh – *Mycoplasma hominis*, Uu – *Ureaplasma urealyticum*; ND - not done

ST − sinovijska membrana; SF − sinovijska tečnost; PBMCs − mononuklearne ćelije periferne krvi pre terapije; PBMCa − mononuklearne ćelije periferne krvi posle terapije Ct −Chlamidia trachomatis; Cp − Chlamydia pneumoniae; Mh − Micoplasma hominis; Uu − Ureaplasma urealiticum; nd − nije rađeno

the cause of the initial infection had been identified, antibiotic therapy was introduced. Surgical treatment (arthroscopic synovectomy or front open method) or radiation synovectomy was applied in refractory chronic synovitis. Treatment of 44 patients with chronic post-urethritis Reiter's disease included arthroscopic synovectomy and different antibiotic protocols. The first group of 22 patients underwent arthroscopic synovectomy, using a three-month treatment with azithromycin, which resulted in remission in 77% of the patients (p=0.023). The second group of 22 patients received the combined therapy consisting of arthroscopic synovectomy and triple alternating antibiotic: quinolone 500 mg/d, tetracycline 1 g/d, roxitromicin 300 mg/d for 10 days in the first month then each of the above-mentioned antibiotics for 10 days during the next three months. After the therapy, the remission was achieved in 63.3% of patients (p = 0.437) PCR method was done to identify bacteria in blood in all patients before and after treatment and in the synovium and synovial fluid in the patients with synovitis at the beginning and the end of therapy (**Table 2**). By analyzing the results it was concluded that the frequency of patients with the appearance of bacteria in any sample taken before and after treatment with hemomycin significantly differ (McNemar's test, p=0.002). Before treatment, the finding was positive in 95% of the respondents and negative in 5%, whereas after treatment, it remained positive in 30% and turned negative in 65% out of 19 patients with positive finding before treatment. However, the negative finding before treatment turned positive in 5% of the patients after treatment (**Graph 1**). The frequency of patients with the appearance of

Table 3. The most frequent radiographic changes in 185 (59.3%) patients with RS
Tabela 3. Najčešće radiološke promene u 185 (59,3%) bolesnika sa RS

Localization of Changes *Lokalizacija promena*	Number of changes *Broj promena*
Sacroiliac joints (narrowing, subchondral sclerosis) *Sakroilijačni zglobovi (suženje, suphondralna skleroza)*	110
Heel - (heel spur, Achilles tendon ossification) *Petna kost – (trn petne kosti, osifikacija pripoja Ahilove tetive)*	75
Knee joints - (osteoporosis, joint space narrowing, enthesitis, periostitis) *Kolenski zglobovi – (osteoporoza, suženje zglobnog prostora, entezitis, periostitis*	45
Metatarsophalangeal II, III, and interphalangeal joint of the thumb (osteoporosis, joint space narrowing, cysts, subluxation, periostitis)/*Metatarzofalangealni II, III i međufalangealni zglob palca (osteoporoza, suženje zglobnog prostora, ciste, subluksacije, periostitis)*	23
Ankle joints (osteoporosis, joint space narrowing, periostitis) *Skočni zglobovi (osteoporoza, suženje zglobnog prostora, periostitis)*	9
Spinal cord (T and L/S) - (asymmetric syndesmophytes) *Kičmeni stub (T i L/S) – (asimetrični sindesmofiti)*	16
Total/*Ukupno*	278

bacteria in any sample taken before and after triple antibiotic therapy was statistically significantly different (McNemar's test, p = 0.002). This antibiotic therapy resulted in statistically significant increase in the number of patients with the negative finding. The finding that had been positive in 22 patients before treatment, remained positive in 12 (54.5%) and turned negative in 10 (45.5%) after treatment (**Graph 2**).

In chronic forms of the disease, in which the conventional form of treatment had failed, the basic drugs were applied particularly in patients with cutaneous and nail changes. The drug of choice was metoterxat at a dose of 10 to 15 mg per week. Sulfasalazine was administered to the patients with a more severe form of disease and the intolerance to methotrexate. In recent years, glucocorticoids with prolonged action have been applied intermittently in 128 patients, while the implantation gave a good but transient therapeutic effect in some patients. Four patients received a maximum of colloidal gold to 1250 mg., but with no effect, 26 methotrexate 10-15 mg weekly. Remission was achieved in 12 patients. Sixty-three patients used sulfasalazine 2-3 g daily for 6 months, and the effect was favorable in 23 of them (36%), and one used to take cyclophosphamide 100 mg per day to achieve remission. Radiation synovectomy of the knee was performed with Yttrium 90 (185 MBq) in six (1.9%) patients, with the resulting remission; surgery (arthroscopic or open method) was performed in 61 (19.5%) patients, while knee arthroplasty was done in one patient.

Discussion

Reiter's syndrome is the most common reactive arthritis among younger men caused by microor-
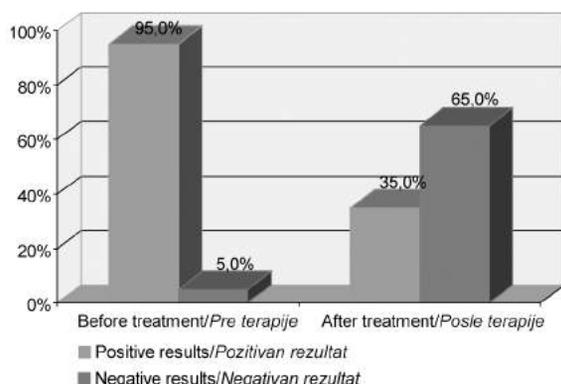
Graph 1. Frequency of patients with bacteria found in any sample before and after treatment with Hemomycin
Grafikon 1. Učestalost ispitanika sa pojavom bakterija u bilo kom uzorku pre i posle terapije hemomicinom
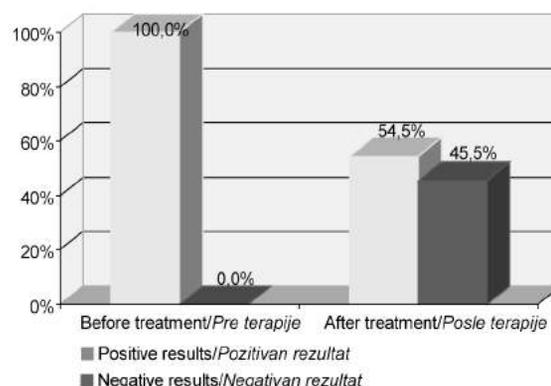
Graph 2. Frequency of patients with bacteria found in any sample before and after triple antibiotic therapy
Grafikon 2. Učestalost ispitanika sa pojavm bakterija u bilo kom uzorku pre i posle trojne antibiotske terapije

ganisms such as urogenital form of Chlamydia, rarely by ureaplasma and Mycoplasma hominis and enterocolitic forms of Shigella (*S. flexneri, S. disenteriae* etc.), Salmonella, Yersinia, Campylobacter, Clostridium difficile [6, 12, 17]. According to the literature, this disease is most common in males in the third decade of life [6, 17, 20]. In our group of 312 patients, 89.4 % were men, their average age being 26.8 years. The highest number of 78.8 % of our patients had urogenital form of disease, which is consistent with the findings of other authors [6, 17]. The most common causes were Chlamydia and Ureaplasma, and Yersinia and Salmonella among enterobacteria. The most frequent infections of urogenital tract were urethritis and urethrocystitis (62.1 %), prostatitis, cervicitis and hemorrhagic cystitis, and rarely epididymitis, which is in accordance with literature [6, 19, 21].

Chlamydia trachomatis is considered the major cause of urogenital form of RS in genetically predisposed individuals, surviving in the synovia in the persistent form [14, 17, 23, 24]. The analysis of synovial fluid by seeding substrate, which had been performed in 54 patients, showed that 29.6% (16/54) of findings were positive for Chlamydia, and/or genital mycoplasmas, that being indisputable evidence of the presence of bacteria in the knee in a certain number of affected persons, which is in accordance with literature data [6–8, 17, 25].

Bacteria in blood and target location (the synovium and synovial fluid) were identified by PCR method in 44 patients. All patients were proved to have "trigger" bacteria in at least one of mentioned samples. In this study, Chlamydia trachomatis was the most common cause (71.4%) of post-urethritis reactive arthritis, which is in accordance with data from the literature [6, 12, 17]. To assess the course of disease and treatment outcomes the following questions had to be answered: what their number was, whether the bacteria were alive (viable) and the extent to which certain genes were active [17, 25, 26-28]. Real-time polymerase chain reaction (RT - PCR) tests [27, 28] were performed to assess the viability of bacteria, which was proved in joints and blood in all patients who were positive for Chlamydia after treatment.

The role of HLA - B 27 in the pathogenesis is unclear [12, 17–19]. HLA - B27 is more important as a predictor of the severity of a disease and the localization of arthritis than as a factor in genetic predisposition to the disease [14, 17, 19]. In this study sample, HLA - B27 was positive in 83.3% of patients, which is consistent with the findings of other authors [6, 14, 27].

The main lesions in Reiter's syndrome are found in the musculoskeletal system [6, 11, 17, 20, 21]. Arthritis is localized in a single joint, usually in the knee, in 10-20% of patients. In our group, arthritis was present in all patients, it was usually asymmetrical, oligoarticular in 69%, polyarticular in 13.7%, monoarticular in 16.7%, and sacroiliitis without peripheral arthritis in one patient. Acute onset and sub-acute course of disease was identified in 41.5%, relapse in 36% and it was chronic in 28.5% of the patients.

Sacroillitis was seen in 71/185 (38.3%) by radiography of sacroiliac joints. According to the literature, sacroiliitis occurs most frequently in HLA - B27 positive patients [6, 17]. In our patients with sacroiliitis, HLA - B27 was positive in 80%. Dactylitis (sausage finger), a diffuse swelling of fingers, was present in 20.4% of patients from this study sample and in the group of 918 patients, who were analyzed by Mladenovic et al., it was described in 26% of patients [17]. Pain in the heel was reported by 36.2 % of our patients. In the literature, there are different data on how many patients had pain in the heel, their number ranging from 5.8% to 65.2% [6, 12, 17, 20]. Conjunctivitis occurs after urethritis, and it usually does not last long. Eye changes developed in 44.5% of our patients, and 79% of them were conjunctivitis, short lasting and in one eye in most cases, and iridocyclitis in 15% of patients. Mladenovic reported the incidence of conjunctivitis to be 35.6% [6, 17, 20]. Mucocutaneous changes are manifested in the skin, mucous membranes of the mouth and the glans penis. Of 18.2% of patients who had changes in the skin, 8.7% had two types of changes. Circinate balanitis and stomatitis was diagnosed in 11.2% and 3.2% of the patients, respectively. The frequency of circinate balanitis in studies with a large number of patients ranges from 17 to 25.6% [6, 17]. Studies conducted in recent years confirm the presence of bacteria or their particles in synovial fluid and/or synovial joints affected by inflammation, bringing into question the view that reactive arthritis is sterile [4, 6, 14, 25, 29-31].

Antibiotics were recommended as the therapy of choice in the patients found to have bacteria, the "trigger" of reactive arthritis. It has been shown that several months (at least 3 to 6) of antibiotic therapy can significantly shorten the time required to achieve remission [29–32].

The patients from both study groups reacted well to the combined surgical and antibiotic therapy. The total number of patients and samples positive for bacteria and bacterial deoxyribonucleic acid (DNA) were lower after treatment, although the eradication of bacteria was not achieved in all cases. Remission of the disease occurred in 77% of patients from the first study group where synovectomy had been performed and in 63.3% of patients (p = 0.437) from the second group of 22 patients treated with synovectomy and triple antibiotic therapy.

In conventional therapy with NSAI, glucocorticoids, basic drugs and short-term antibiotic therapy, remission occurred in 35–45% of patients during the first year [6, 12, 17, 30–32].

Synovectomy contributed significantly to remission in our patients because hypertrophic synovium with infectious agents was removed, thus decreasing the amount of antigenic tissue, and the pathogenicity of bacteria in the joint.

Basic drugs were administered in chronic forms of disease with radiographic changes in the joints and mucocutaneous changes. In the group of patients treated with sulfasalazine, remission was achieved in 36% of patients after 6 months. Such a situation does not give the basis to conclude that remission occurred spontaneously or resulted from the administered therapy (33). Remission was achieved in 58% (14/24) of patients with chronic arthritis and/or keratodermia treated with Methotrexate, which corresponds to the literature data [17, 34].

If the treatment described above fails to give good results, biological therapy, including inhibitors of tumor necrosis factor (TNF-alpha) and interleukins, should be opted for [35].

Conclusion

Reiter's syndrome is a multisystem disease which occurs predominantly in Human Leukocyte Antigen - B27 positive young men. It is most frequently manifested in incomplete form (post-urethritis arthritis). The course of disease is acute or subacute in the majority of patients. Arthritis is usually expressed as oligoarthritis of the lower extremities. The presence of bacterial pathogen genomes and their viability was proved by the modern polymerase chain reaction test for identification of bacteria in the synovium and synovial fluid of the patients having post- urethritis form of Reiter's syndrome

The results of this study showed a statistically significant reduction in the number of patients with positive findings of bacteria in any of the monitored sites and a significant increase in the number of patients who achieved remission after completion of antibiotic therapy and synovectomy.

All this suggests that Reiter's syndrome, although defined as reactive arthritis, has the characteristics of infectious arthritis.

Prolonged antibiotic therapy should be administered in all patients with proven infectious agents at the entry point, whereas diagnostic and therapeutic arthroscopic synovectomy along with nonsteroidal anti-inflammatory drugs, physical therapy and, if necessary corticosteroid therapy, should be applied in patients having arthritis lasting longer than a year as well as proven synovitis.

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REVIEW ARTICLES
PREGLEDNI ČLANCI

THE RELATIONSHIP BETWEEN ATHEROSCLEROSIS AND PULMONARY EMPHYSEMA

POVEZANOST ATEROSKLEROZE I EMFIZEMA PLUĆA

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Summary
Introduction. The etiopathogenesis of atherosclerosis and subsequent pulmonary emphysema has not been fully elucidated. **Experimental Studies** Foam cells are of great importance in the development of these diseases. It is known that local cytokine secretion and modification of native lipoprotein particles, which are internalized by the vascular and alveolar macrophages via the scavenger receptors on the surfaces of these cells, lead to the formation of foam cells. Thus, the exacerbation of local inflammatory process in the vascular and lung tissue ensues due to a generation of reactive oxygen species, resulting in further lipoprotein modification and cytokine production. Accumulating evidence suggests that oxidants may facilitate the inflammatory response by impairing antiprotease function, directly attacking vascular and lung matrix proteins and by inactivating enzymes involved in elastin synthesis and vascular and lung repair. **Clinical Studies** Cigarette smoke is recognized as a rich source of oxidants. Nearly 90% of all patients with chronic obstructive pulmonary disease are smokers. The process of atherogenesis is also influenced by tobacco smoke. **Conclusion** The role of vascular and alveolar macrophages has become increasingly important in understanding the development of atherosclerosis and resulting pulmonary emphysema.
Key words: Atherosclerosis; Pulmonary Emphysema; Smoking; Macrophages; Foam Cells; Cytokines; Lipoproteins; Oxidants; Hypercholesterolemia; Risk Factors

Sažetak
Uvod. Etiopatogeneza ateroskleroze i plućnog emfizema kao posledice nije do kraja razjašnjena. **Eksperimentalna istraživanja.** Penaste ćelije imaju važnu ulogu u patogenezi ovih bolesti. Poznato je da nastanak penastih ćelija određuju lokalna sekrecija citokina i dostupnost modifikovanih lipoproteina koji se vezuju za specifična mesta na površini vaskularnih i alveolarnih makrofaga. Time se naglašava značaj lokalne inflamacijske reakcije u arterijskom zidu i plućnom tkivu, jer reaktivne vrste kiseonika koje se tom prilikom stvaraju, modifikuju kako lipoproteine, tako i produkciju citokina. Oksidansi mogu doprineti rasplamsavanju zapaljenjskog odgovora u vaskularnom i plućnom tkivu slabljenjem antiproteazne funkcije, direktnim uticajem na proteine vaskularnog i plućnog matriksa i inaktivacijom enzima uključenih u sintezu elastina i obnovu vaskularnog i plućnog tkiva. **Klinička istraživanja.** Duvanski dim je značajan izvor oksidanasa. Skoro 90% obolelih od hronične opstruktivne bolesti pluća su pušači. Duvanski dim takođe olakšava nastanak i progresiju ateroskleroznih lezija. **Zaključak.** Izmenjena funkcija alveolarnih i vaskularnih makrofaga bitno doprinosi nastanku ateroskleroze i plućnog emfizema kao posledice.
Ključne reči: Ateroskleroza; Plućni emfizem; Pušenje; Makrofagi; Penaste ćelije; Citokini; Lipoproteini; Oksidanti; Hiperholesterolemija; Faktori rizika

Introduction

Atherosclerosis (AS) (a metabolic, chronic, inflammatory, immune-mediated disease of the arterial walls) is a global health problem. In developed countries, AS and its complications (throm-

bosis, bleeding, ulceration, rupture, calcification, and aneurysms) account for 50% of all deaths [1]. Huge amounts of data have been collected about this disease of civilization: experimental, clinical, functional and ultrastructural, including those that point to common pathogenic mechanisms in the development of AS and pulmonary emphysema (PE), which is also widespread [2-5]. Pulmonary emphysema (abnormal and permanent enlargement of the airspaces distal to the terminal bronchioles accompanied by destruction of the alveo-

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lar walls) is one of the most common causes of premature disability and a very common direct or indirect cause of death, as well [2].

Atherosclerosis is a process that occurs in five phases (endothelial dysfunction, fatty streaks, transient lesions, fibrous plaques and complicated lesions) and leads to hardening, narrowing and occlusion of arteries [6].

Pulmonary emphysema is characterized by vascular lesions with intimal proliferation, additionally narrowing the pulmonary arteries and veins. In severe forms of PE, these changes may also affect the bronchial arteries [7].

The etiopathogenesis of AS and subsequent PE has not been fully understood. Despite the great attention being paid to the study of these chronic, progressive multifactorial diseases, the primary cause of pathological events is still unknown.

Experimental Studies

The use of animal models in the study of AS dates back to 1908, when Ignatowski investigated "the influence of animal food on the organism of rabbits". He demonstrated that herbivores normally never develop AS, except when fed by protein-rich diet. These models were later proved to be excellent for quick achievement of morphological changes and qualitative assessment of altered interaction between blood and blood vessel walls [8].

Rabbits are the most commonly used animals in experimental research of AS. Due to their diet and adequate metabolic constitution, rabbits are susceptible to the development of atherosclerotic changes when fed on hyperlipidemic diet. These changes include macroscopic lesions, fatty streaks, primarily in the aortic arch and thoracic aorta [8].

Rabbits are particularly sensitive to dietary cholesterol. In rabbits, a cholesterol rich atherogenic diet increases the concentration of triglycerides (TG) and low density lipoproteins (LDL), but decreases the concentration of high density lipoproteins (HDL) [9]. In addition, the serum of rabbits, primates, rats, guinea pigs, and birds fed with high cholesterol diet, exhibits cholesterol-rich lipoproteins with β-electrophoretic mobility, the so-called very low density lipoproteins (VLDL) [8].

Foam Cells
Experimental AS induced by high-cholesterol diet is often associated with PE [4, 5, 10–12], increased levels of TG and high concentrations of free fatty acids in the lung tissue [4]. In rabbits, after a month of hypercholesterolemic diet, aortic lesions become microscopically visible. They are characterized by the accumulation of foam cells in the internal elastic membrane, while the endothelial surface is intact. Foam cells are a characteristic feature in the tissue response associated with bronchial obstruction [4, 5, 10–12].

In an experimental model of AS induced by a high-cholesterol diet, monocytes are the first cells adjacent to the endothelium. Then they migrate into the subendothelial space, swallow the oxidized cholesterol, and transform it into foam cells [13]. These cells are believed to play a major role in the pathogenesis of AS and subsequent PE [4, 5, 10–12].

Hypercholesterolemia associated with elevated levels of atherogenic lipoproteins (LDL and VLDL) in the blood leads to chronic presence of LDL in the arterial wall [9]. This condition enhances fatty streaks formation because lipid migration into the subendothelium is greater than its removal from the arterial wall. It is known that local cytokine secretion and modification of native lipoprotein particles, which are internalized by vascular and alveolar macrophages via the scavenger receptors class A (SRA) on the surfaces of these cells, lead to the formation of foam cells. Thus, the exacerbation of local inflammatory process in the vascular and lung tissue ensues due to a generation of reactive oxygen species (ROS), resulting in further lipoprotein modification and cytokine production [13]. It has been shown that mononuclear phagocytic blood cells take part in the phagocytosis of native particles and of modified LDL particles to a lesser i.e. greater extent, respectively after their binding to the SRA. However, these particles may directly migrate into the subendothelium, being subject to phagocytosis, whereas SRA play an important role in the process. These receptors, which mediate the delivery and degradation of modified LDL particles, do not operate on the principle of negative feedback, so even when a large amount of lipid particles is accumulated in the cell, the intake continues, which leads to the formation of foam cells [14]. In a state of continuous inflammation, the concentration of LDL particles in blood in-

creases and through the process of passive diffusion they penetrate the arterial intima, where they are trapped by glycosaminoglycans, and ROS are affected as well. LDL particles become highly sensitive to different stimuli, and may be modified by oxidation, glycosylation, or by incorporation into immune complexes. In addition, LDL particles interact with proteoglycans (biglycan and decorin) and form aggregates, with a catalytic activity of sphingomyelinase, cathepsin D, cathepsin F and lysosomal acid lipase [15].

The ability of oxidized LDL molecules (oxLDL) to induce accumulation of cholesterol in macrophages was their first described proatherogenic property. Other proatherogenic effects of oxLDL particles, referring to endothelial cells, include expression of growth factors affecting smooth muscle cells, generation of superoxide anion (O_2), and endothelial cells apoptosis [14]. The human endothelial receptor that mediates uptake of oxLDL belongs to C-type lecitin family and is referred to as LOX-1 (lecitin-like oxLDL receptor-1) [16].

Foam cell formation is also induced by receptors involved in oxLDL uptake (CD34, macrosialin/ CD68 /) and HDL receptor, which is referred to as SB-1 [16].

It is believed that macrophage-colony stimulating factor (M-CSF), interleukin-3 (IL-3) and granulocyte monocyte colony stimulating factor (GM-CSF) play a key role in the process of foam cell formation [17].

Apart from macrophages, foam cell formation is also promoted by vascular smooth muscle cells with properties of lipophages [13].

Inflammatory Response of the Vessel Wall and the Lung Tissue

Numerous animal studies have shown that activation of endothelial cells and expression of specific molecules, responsible for adhesion, migration and accumulation of monocytes and T-lymphocytes, play a crucial role in AS [8].

There is evidence that a high cholesterol diet soon results in the focal expression of vascular cell adhesion molecule-1 (VCAM-1) at the predilection sites. In addition, lysophosphatidylcholine, a component of modified lipoprotein, activates VCAM-1 gene transcription in endothelial cells. Lipoprotein(a), however, induces a dose-dependent expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, but it does not affect the expression of VCAM-1 and E-selectin. Furthermore, native LDL molecules, binding to the LDL receptors, increase the concentrations of VCAM-1 and E-selectin in human vascular endothelial cells [13]. On the other hand, the expression of these molecules is induced and enhanced by tumor necrosis factor alpha (TNFα) and interleukin-1 beta (IL-1β), which originate from either the circulating blood or the vascular wall. These molecules, which are part of the existing local or systemic inflammatory reaction,

apart from stimulating the expression of adhesion molecules, lead to changes in procoagulant and fibrinolytic endothelial cells, and the surface of the endothelium becomes thrombogenic. By modifying the properties of endothelial morphology, these cytokines affect the production of nitric oxide (NO) and prostacyclin, inducing endothelium to synthesize other cytokines, which enhances their proinflammatory activity. IL-1 and TNFα, endothelial cell activators, induce synthesis of specific membrane glycoproteins, redistributing the cytoskeleton of endothelial cells and increasing the synthesis of platelet-activating factor (PAF). PAF is a phospholipid with potential proinflammatory and thrombogenic properties. Its specific significance in AS is indicated by the fact that components similar to this phospholipid are present in oxLDL, and that blockade of PAF receptors blocks the effects of oxLDL molecules on the peripheral mononuclear cells completely, which refers to the secretion of interferon-γ (IFN-γ) and partly to the secretion of TNFα. IL-1 and TNFα also stimulate endothelial cells to produce interleukin-8 (IL-8) that strongly attracts and activates leukocytes. Migration of leukocytes through the morphologically intact endothelium involves direct migration as a response to other cytokines, so-called chemo-kines. In the early phase of atherogenesis these are monocyte chemotactic protein-1 (MCP-1), localized in atherosclerotic lesions, IL-8, interleukin-16 (IL-16), and C5a-receptor fragment peptide, released in hypercholesterolemia [18].

Inflammatory response has been confirmed to affect the accumulation of lipoproteins in the arterial wall. Inflammatory mediators (TNFα, IL-1 and M-CSF) increase the binding of LDL to the endothelium and smooth muscle cells. After binding to SRA *in vitro*, modified LDL particles initiate a series of intracellular events, among which the activation of urokinase and inflammatory cytokines plays an important role. In this way, due to the presence of lipids, a vicious cycle of inflammation is maintained in the arteries, as well as modification of lipoproteins and continuous inflammation [13, 14]. Thus, LDL molecules enhance the expression of SRA on macrophages and, in synergy with inflammation, facilitate the formation of foam cells [13]. It should also be noted that a large number of activated macrophages undergo apoptosis, after which they release the lipid content into the matrix and form the lipid nucleus of atheromas [17].

Under physiological conditions, human alveolar macrophages (AM) act as a dynamic system composed of phenotypically and functionally diverse subpopulations, whose delicate balance participates in immune regulation. In contrast, the function of AM during the afferent phase of cellular immunity (phagocytosis) is particularly emphasized within the inflammatory and immune response in the lungs, whereas the efferent phase of macrophages is responsible for the activity of

other immune and inflammatory cells through the secretion of soluble mediators, such as prostaglandins, chemotactic factors, PAF, complement components, various enzymes, and others [19]. The activated inflammatory cells in the lungs then stimulate the release of IL-8 from AM, alveolar epithelial cells, and other lung cells, which, afterwards, attracts polymorphonuclear leukocytes (PMN) due to its chemotactic properties. Similar activities are also exhibited by leukotriene B$_4$ (LTB$_4$) [20].

Small blood vessels of the lungs, especially capillaries of intra-alveolar septa, in some animal species exhibit pulmonary intravascular macrophages (PIM). During inflammation, their number increases with inhomogeneous distribution. Studies of the metabolic characteristics of PIM have shown that these cells are much more active than PM [21].

Lysosomal Enzymes of Inflammatory Cells

Alveolar macrophages differ from other tissue macrophages in having a markedly hypertrophic lysosomal apparatus [22]. In contrast to other macrophages, AMs depend on aerobic glycolysis, which is explained by high partial oxygen pressure in the lungs. It has been shown that the activity of AM can be suppressed by the inhibition of oxidative phosphorylation [23], while the suppression of tissue respiration is one of the features of AS. Moreover, AS is associated with a reduced oxygen uptake in the lung tissue and other parenchymal organs [7]. Thus, in AS, PE impairs blood oxygenation, although frequent microembolism in hypercholesterolemia, as well as a decreased oxygen binding capacity due to a cholesterol layer around the red blood cells may contribute to this impairment [23, 24].

Over the last few decades, great importance is given to the role of proteolytic enzymes in the development of PE (**Table 1**). Proteases are constituents of PMN and AM. Phagocytosis increases the release of these enzymes. It is assumed that inflammatory reactions associated with infections and air pollutants may cause the same effect. The release of elastase from neutrophil lysosymes is believed to be mainly responsible for elastin degradation, ultimately resulting in the development of pathological processes in PE. Under normal circumstances, these unwanted events are prevented by anti-proteinase inhibitor enzymes, such as alpha-1 proteinase inhibitor (αPI), secretory leucoprotease inhibitor (SLPI), tissue inhibitors of matrix metalloproteinases (TIMPs), and others, found in serum, tissues, and bronchial secretions [25].

Various studies demonstrate the involvement of immune factors in the etiology of AS, possibly being etiopathogenic mechanisms of AS and PE [2–5, 7, 10–12, 17, 22, 23, 26]. Apart from antigens responsible for immune responses leading to AS, special attention is paid to vascular wall antigens. For example, aging causes the loss of elastin natural properties, and thus it becomes susceptible to deposition of calcium and elastolysis. Enhanced decomposition of insoluble elastin in the vascular wall leads to the appearance of peptides in the blood stream, whereas anti-elastin antibodies are created in response to them [17]. Rabbits with experimental AS and subsequent PE exhibit a progressive titer increase of these antibodies, as well as abnormal accumulation of microfibrils in the elastic tissue, which is closely associated with excessive elasto-lysis of preformed or newly formed elastic fibers during elastic tissue remodeling. Enhanced synthesis of microfibrils may occur in response to elastolysis as a reparative phenomenon, but it also represents a response of the blood vessel wall and pulmonary tissue to elastolysis [27].

Histochemical methods showed an increased non-specific esterase activity of macrophages in the arterial walls during atherogenesis induced by hypercholesterolemic diet. It has also been observed that the amount of esterase is directly proportional to the degree of saturation of the intima with lipoproteins and other macromolecules [28].

Apart from lysosomal non-specific esterases, non-specific esterases have been found on the outer cell membrane of AM. These esterases, also known as ectoenzymes or ectoesterases, play a mediating role in regulation of AM response to various external and internal agents [22]. Some nonspecific esterases exert their activity in the glycocalyx and on the outer side of the macrophage membranes. The most important enzyme with these properties is α-naphthyl acetate esterase (ANAE). The examination of ANAE activities in guinea pig Kurloff cells (mononuclear cells obtained from guinea pigs) showed a membranous and cytoplasmic distribution of its activity [29].

Human AM are reported to have a series of esterases with elastase-like activities. During maturation of these phagocytic cells, the amount of hydrolytic enzymes increases in their lysosomes [22]. In addition, the degree of esterase activity in mononuclear cells correlates with the cell viability and mitotic capacity [28]. It has been established that different local stimuli (immune complexes, antigens, lymphokines, bacterial components, etc.) may trigger the release of hydrolytic lysosomal enzymes into the extracellular space with autodestructive consequences [26].

Extracellular matrix is the principal component of the fibrous plaque. Extracellular matrix degradation is promoted by monocyte-macrophages which release matrix-degrading metalloproteinases (MMP). The activated MMP (gelatinase, collagenase, stromelysin) can degrade all components of extracellular matrix [30]. Apart from MMP, matrix and fibrous cap degradation is also activated by cathepsins and other elastolytic enzymes (**Table 1**) [6]. It should be noted that the imbalance between factors of synthesis and degradation of extracellular matrix plays a major role in the rupture of atherosclerotic plaque and its subsequent consequences [30].

Table 1. Inflammatory cell-derived proteases in atherogenesis and pulmonary emphysema
Tabela 1. Proteaze koje oslobađaju inflamacijske ćelije u aterogenezi i plućnom emfizemu

Protease *Proteaza*	Type of active site *Vrsta aktivnog mesta*	Site of synthesis *Mesto sinteze*	Elastolysis *Elastinoliza*
Elastase	Serine	Neutrophil, macrophage	+
Elastaza	*Serin*	*Neutrofil, makrofag*	+
Proteinase 3/*Proteinaza 3*	Serine/*Serin*	Neutrophil/*Neutrofil*	+
Cathepsin G	Serine	Neutrophil, macrophage	+/-
Katepsin G	*Serin*	Neutrofil, makrofag	+/-
Tryptase/*Triptaza*	Serine/*Serin*	Mastocyte/*Mastocit*	/
Chymase/*Himaza*	Serine/*Serin*	Mastocyte/*Mastocit*	/
Plasminogen activator	Serine	Macrophage	?
Aktivator plazminogena	*Serin*	*Makrofag*	?
Procollagenase	Metal	Neutrophil	/
Prokolagenaza	*Metalo*	*Neutrofil*	/
Gelatinase/*Želatinaza*	Metal/*Metalo*	Neutrophil/*Neutrofil*	/
72 kDa collagenase	Metal	Macrophage	+
72 kDa kolagenaza	*Metalo*	*Makrofag*	+
92 kDa collagenase	Metal	Macrophage	+
92 kDa kolagenaza	*Metalo*	*Makrofag*	+
Stromelysin/*Stromelizin*	Metal/*Metalo*	Macrophage/*Makrofag*	/
Cathepsin B/*Katepsin B*	*Cistein*/Cysteine	Macrophage/*Makrofag*	/
Cathepsin L/*Katepsin L*	Cysteine/*Cistein*	Macrophage/*Makrofag*	/

Clinical Studies

Smoking

Epidemiological studies have shown that smoking plays a principal role in the development of emphysema. It is generally believed that smoking is the most important extrapulmonary etiopathogenic factor of this disease. The development of centriacinar emphysema is believed to be closely related to cigarette smoking [25]. Approximately 90% of patients with chronic obstructive pulmonary disease (COPD) are smokers or former smokers who suffer from frequent bronchopulmonary infections. Chronic airflow limitation, characteristic for these patients, is caused by a mixture of small airway diseases known as obstructive bronchiolitis and parenchymal destruction consistent with emphysema, but the proportion of these components varies from a person to a person. It has been established that an early decrease in lung function in COPD patients correlates with the inflammatory response in peripheral airways, which is similar to inflammatory changes in the central airways (exudation into the airway lumen, goblet and squamous cell metaplasia, inflammatory airway mucosal edema, and increased airway mucosa due to goblet cell metaplasia). However, peripheral airway obstruction is the most common pathological finding in COPD. Cigarette smoke-induced inflammation leads to repeated cycles of repair and damage of the walls of the peripheral airways, which may result in airway wall remodeling (an increase in collagen content, and scar tissue formation). This results in luminal narrowing and permanent airway obstruction [3]. It

has been shown that tobacco smoke stimulates epithelial cells and macrophages to produce TNFα, and other mediators of inflammation. Long-term smoking causes mucous gland hyperplasia and hypertrophy [2]. It is believed that tobacco smoke inhibits antiproteases and stimulates macrophages and PMN to release proteolytic enzymes [20]. Components of tobacco smoke are largely retained in the so-called "transitional zone" (respiratory bronchioles) due to airflow cessation in the respiratory tree. This results in modification of the local microenvironment, whereas respiratory bronchioles of smokers and smokers suffering from AS are sites where large amounts of non-specific lysosomal esterases enter the extracellular space [23]. Then, non-specific esterase, in conjunction with acid phosphatases, elastase, hyaluronidase, cathepsin, collagenases and plasminogen activators, may damage the surrounding tissue [3]. Moreover, the activity of esterase, lysosome, and lactate dehydrogenase was found to be five times higher in AM of smokers with advanced AS than in normal AM. It is assumed that AM and vascular wall macrophages in smokers can upload, modify, and activate PMN-released elastase [28]. Alveolar macrophages of smokers with AS also show accumulation of free fatty acids, phospholipids, and TG [3].

Tobacco smoke is an important source of oxidants. The gas phase of cigarette smoke contains abundant free radicals including nitric oxide [2] that induce oxidative damage to the vascular tissues of smokers, exacerbate the existing inflammatory process and accelerate the progression of atherosclerotic lesions (**Scheme 1**) [19]. Similarly,

smoking stimulates phagocytic activity of lung macrophages and macrophages in COPD patients [2, 20]. An increased number of AM, epithelial cells, lymphocytes, mast cells and other metabolically active lung cells which use oxygen and thereby release ROS, contribute to altering the balance between oxidants and antioxidants in the lungs of patients with COPD (**Scheme 1**) [2, 3, 15, 19, 20].

Comorbidities in Patients with COPD

The first symptoms of COPD include chronic cough and shortness of breath. With the progression of disease, a number of additional symptoms may develop due to multiple organ system damage, which is why comorbidities are quite common in patients having COPD. Since COPD affects long-term smokers and middle-aged people, most patients with COPD may have other diseases associated with smoking (cardiovascular disease, and lung cancer) and with aging (prostate cancer, depression, diabetes mellitus, Parkinson's disease, dementia, arthritis). In addition, COPD may coexist with respiratory tract infections, asthma, osteoporosis and bone fractures, sleep disorders, cataract, glaucoma, gastroesophageal reflux, gastritis, anemia, anxiety disorders and cognitive impairment. Consequently, some coexisting diseases may result from COPD (cardiovascular disease, lung cancer, and osteoporosis). Furthermore, many patients with COPD have multiple comorbid conditions, among which the most common are hypertension, diabetes mellitus, and coronary heart disease [31]. COPD is also associated with systemic (extrapulmonary) conditions, such as systemic inflammation and skeletal muscle dysfunction, which limit physical activities and impair overall health of COPD patients [3].

Lung cancer and cardiovascular diseases (ischemic heart disease, cardiac arrhythmias and heart failure) are reported to be the most common causes of fatal outcome in patients with mild to moderate COPD, while respiratory failure is the leading cause of death in its advanced stage [32].

COPD is an independent risk factor for the development of cardiovascular diseases. The mechanisms associating COPD with AS, ischemic heart disease and stroke are not fully understood. It is believed that chronic systemic inflammation plays a key role in atherosclerotic plaque formation. There is increasing evidence that endothelial function, which is significantly impaired in patients with COPD, may increase the risk of cardiovascular disease [33]. Namely, endothelial dysfunction of pulmonary arteries occurs early in COPD patients, directly induced by tobacco smoke components or indirectly by inflammatory mediators (C-reactive protein /CRP/, fibrinogen, IL-1, TNFα, etc.). Thickening of the intima is the first structural change, followed by proliferation of vascular smooth muscle cells and infiltration of the vessel wall by inflammatory cells. These structural changes are correlated with an increase in pul-
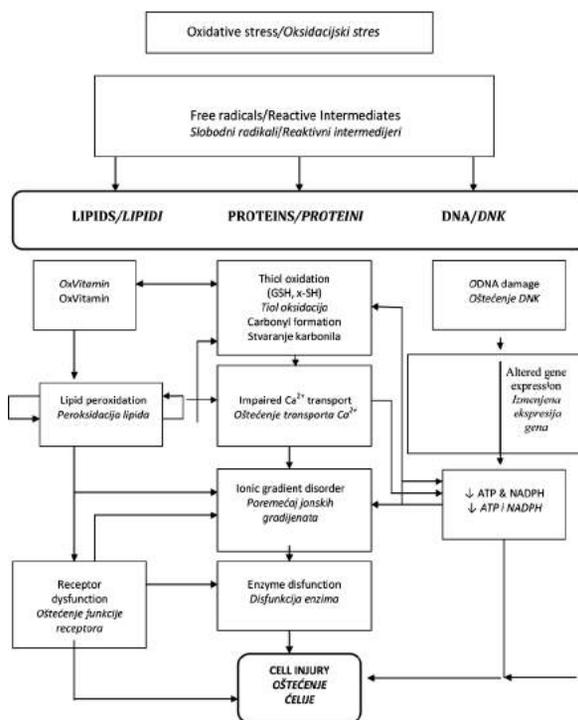
Sheme 1. Oxidative vascular and pulmonary cell injury
Šema 1. *Oksidacijsko oštećenje ćelija vaskularnog i plućnog tkiva*

monary vascular pressure that develops first with exercise and then at rest. As COPD progresses the amount of proteoglycan and collagen increases, which leads to further thickening of the vessel walls. In advanced stages of the disease, changes in the muscular arteries may be associated with emphysematous destruction of the pulmonary capillary bed. Moreover, it was found that thickening of the arterial walls in the systemic circulation correlates with the development of emphysema [7].

Pulmonary hypertension, which develops in severe COPD, is a major cardiovascular complication of the disease. It has been associated with the development of pulmonary heart disease (cor pulmonale), and has a poor prognosis. Pulmonary hypertension and reduction of the vascular network due to emphysema may lead to right ventricular hypertrophy and right heart failure; together with venous stasis and thrombosis, it increases the risk for the development of pulmonary embolism, further compromising the pulmonary circulation [32].

Preventive Measures and Early Detection of Atherosclerosis and COPD

At the present level of medical science, AS is considered to be an inevitable process, i.e. the fatigue of material. Although it inevitably affects the entire human population, clinical treatment includes only its complications. In other words, AS remains undetected until clinical complications arise, and they claim a life every two seconds worldwide [1].

According to statistics, AS and its most common consequences (myocardial infarction and stroke) are the leading cause of death in Serbia [34]. However, it is encouraging that nearly 80% of premature deaths could be prevented by eliminating key risk factors, such as smoking, unhealthy diet and physical inactivity [1]. Therefore, preventative check-ups and healthy habits must be part of everyday life as a natural and essential necessity of everybody [34]. To this end, in the first place, health care professionals should use positive attitude of the population of Serbia to health as the greatest human value, and in their everyday work teach them how to protect and improve their own health and the health of their families. Good family relations are in the second place, as a prerequisite for healthy development of all its members. Bearing that in mind, a family doctor can examine and slow down the development of many diseases associated with aging, including AS and consequent emphysema. At the same time, the doctor does not necessarily have to treat all family members. However, those individuals who decide to be treated by the same doctor are treated as parts of their families. As such, family doctors often know more about their health problems, especially about chronic diseases, at their preclinical stage. Apart from this, the family doctor understands relationships between family members, even unsaid family issues and recognizes family trauma [35].

The worldwide prevalence of COPD is 0.8%, and it is becoming a growing burden on the health care system. The prevalence of this disease is also high in Serbia [36]. According to the Global Initiative for Chronic Obstructive Lung Disease guidelines, more than half of patients with this disease remain undiagnosed [37]. Therefore, patients should be educated that cough, chronic hypersecretion and shortness of breath are not innocent symptoms and that their recognition is very important when it comes to this disease [38]. That is why family doctors play a crucial role in the health care system, because they can integrate physical, psychological, social, cultural and existential characteristics of patients in order to diagnose the disease as early as possible [36].

Conclusion

The pathogenesis of atherosclerosis and subsequent pulmonary emphysema has not been fully elucidated. It is certain though that altered function of alveolar and vascular macrophages is a significant contributing factor in its development. A hope remains that future research on the role of these cells in the pathogenesis of pulmonary emphysema found in atherosclerosis will shed light on yet unknown potential therapeutic modalities.

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PROFESSIONAL ARTICLES
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SURGICAL TREATMENT OF SHOULDER ROTATOR CUFF INJURIES

HIRURŠKO LEČENJE POVREDA ROTATORNE MANŽETNE RAMENA

Srđan NINKOVIĆ, Marko SIMNJANOVSKI, Vladimir HARHAJI, Nemanja KOVAČEV, Nataša JANJIĆ and Mirko OBRADOVIĆ

Summary
Introduction. The rotator cuff is the most important functional structure of the shoulder. The aim of this study was to determine which factors contribute to a rotator cuff injury and to evaluate the results of the surgical treatment at the Department of Orthopedic Surgery and Traumatology in Novi Sad since December 2009 until May 2012. **Material and Methods.** The study sample consisted of 20 patients who had been operated for a shoulder rotator cuff injury. Their mean age was 56.8 ± 9.1. **Results**. According to the Constant Shoulder Score, 75% of the patients had excellent and good results. A statistically significant difference (p<0.05) was found between Constant Shoulder Score of the operated should and the opposite shoulder as well as between the range of external and internal rotation and abduction. After the surgical treatment, 95% of the patients have no limitations in the activities of daily living and they are satisfied with the results of treatment. **Conclusion**. Surgical treatment of a shoulder rotator cuff injury is reliable, time-tested and provides good clinical results especially in patients who were operated within the first three weeks after the injury.
Key words: Shoulder; Surgical Procedures, Operative; Rotator Cuff; Risk Factors; Middle Aged; Range of Motion, Articular; Treatment Outcome; Patient Satisfaction

Sažetak
Uvod. Rotatorna manžetna ramenog zgloba je najvažnija funkcionalna struktura ramena. Cilj istraživanja je bio da ustanovimo koji uzročnici doprinose povredi rotatorne manžetne i da procenimo rezultate hirurškog lečenja na Klinici za ortopediju u Novom Sadu u periodu od decembra 2009. do maja 2012. godine. **Materijal i metode.** Studija je sprovedena na uzorku od 20 ispitanika operisanih zbog povrede tetiva rotatorne manžetne ramena. Prosečna starost ispitanika bila je 56,8 ± 9,1 godina. **Rezultati**. Prema Konstantovoj bodovnoj skali, 75% ispitanika je imalo odličan i dobar rezultat. Statistički značajna razlika (p < 0,05) postojala je između Konstantove bodovne skale operisanog i suprotnog zdravog ramena, kao i između obima pokreta spoljašnje, unutrašnje rotacije i odvođenja. Nakon operacije, 95% ispitanika nije imalo ograničenja u aktivnostima dnevnog života i bilo je zadovoljno rezultatima operacije. **Zaključak.** Hirurški tretman povrede rotatorne manžetne zgloba ramena je pouzdan, vremenski testiran i daje dobre kliničke rezultate − naročito kod pacijenata operisanih u prve tri nedelje od povrede.
Ključne reči: Rame; Operativne hirurške procedure; Rotatorna manžetna; Faktori rizika; Odrasli, srednjih godina; Obim pokreta; Ishod lečenja; Zadovoljstvo pacijenta

Introduction

A shoulder pain is often caused by a rotator cuff injury. The structure of the rotator cuff is very solid. During normal daily activities 140 to 200 N of force is transmitted through these tendons.

The maximum force that an undamaged rotator cuff tendon in elderly people can withstand is 600 N to 800 N [1].

According to the epidemiological data, these injuries account for 9% to even 39% of soft tissue lesions in population over 40 [2, 3], and they most frequently occur due to repeated microtrauma and poor nutrition of the tendon. Non-operative treatment in patients with a chronic rotator cuff injury is recommended when the pain is pronounced but the weakness of the arm is not dramatic and progressive. If there is no significant improvement in the following three to six months, the operative treatment is recommended [4]. Bassett and Cofield [5] have concluded that the most optimal time for operative reparation of a complete tear of the rotator cuff tendon is within the first three weeks after an acute injury. In cases when the weakness is pronounced or progressive, it is necessary to perform the operating procedure as soon as possible because it should be done before retraction of the ten-

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don, loss of its tissue and muscle volume decrease. Codman was the first to describe the technique of rotator cuff reparation in 1911 [6]. Neer perfected the existing open technique in 1972 and established the principals of modern treatment of the rotator cuff injuries [7]. Since then the number of patients satisfied with the results of surgical treatment has ranged from 70% to 95% according to Murray et al. [8]. The next step in treatment was made by applying the shoulder arthroscopy which enabled better overview of the procedure and fewer complications. Today there are different opinions about which technique provides better results but the fact is that the rotator cuff surgery has been yielding ever better results.

The aim of this study was to establish which etiological factors contribute to the rotator cuff injury as well as to assess the results of the performed surgical procedures.

Material and Methods

The study sample consisted of twenty patients, 12 male and eight female patients who had been operated at the Department of Orthopedic Surgery and Traumatology, Clinical Center of Vojvodina in Novi Sad. The data about the treatment were obtained from the medical history forms of the patients treated from 2009 to 2012. The data and measurements were taken during the regular checkups at the Polyclinic of Clinical Center of Vojvodina. The mean age of the participants was 56.8 ± 9.1. Eleven of the patients had been engaged in recreational sports before the injury and four of them continued their recreation after the injury. The causes of injury reported by the patients were as follows: slipping and falling while walking in six cases; an injury while doing sport activities in four cases; while performing a task at work and at home in three cases, each; in a traffic accident in one case and three patients did not report any data regarding

their trauma. The most common mechanism of trauma was fall onto the shoulder in 60%, while lifting a heavy load in 15%, during a quick abduction and flexion movement of the shoulder in 10% and 15% of the patients did not know the exact cause and mechanism of the injury. After the injury, 75% of the patients went to a general practitioner, 10% went to a sports medicine physician and 15% visited a physiatrist.

The same operative technique was applied in all patients. The patient was in the half-sitting position. The upper anterior approach was used to reveal the deltoid muscle which was split between the anterior and medial fascicles. The muscle was split up to 5 cm under the acromion in order to avoid an axillary nerve injury. Then the acromioplasty was done by putting the retractor and chiseling the frontal part of the acromion, which protruded in front of the clavicula and afterwards, the lower surface of the anterior third of the acromion was removed. The stumps of the ruptured rotator cuff tendons were refreshed. The stumps were mobilized by the support sutures (**Figure 1**) and the footprint of the ruptured tendon was refreshed. The articular and bursal side of the tendon were released from the surrounding structures. The tendon of the long head of biceps was surgically treated only if it was damaged or dislocated from the intertubercular groove. In that case, the articular part of the tendon was cut and removed and the part closer to the muscle was fixated under the intertubercular groove or instead a simple tenotomy was done. Then two tunnels were drilled through the greater tubercle of the humerus and the space between the tunnels had to be at least 1 cm in order to provide the high resistance to tearing under load. Afterwards, two metal screws of 3.5 mm in diameter (Grujić & Grujić, Novi Sad, Serbia) (**Figure 2**) were placed into the internal part of the footprint in order to reconstruct its internal part. The Mason-Allen technique [9], which provides a solid and stable form of thread in the tendons and does not damage the tissue, was applied to suture

Figure 1. Mobilized supraspinatus muscle tendon with supported sutures
Slika 1. *Mobilisana tetiva nadgrebenog mišića podržnim šavovima*
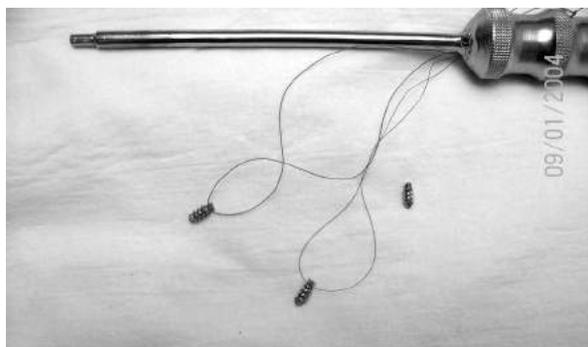
Figure 2. Screws for fixation of the supraspinatus muscle (Grujic-Grujic, Novi Sad, Serbia)
Slika 2. *Zavrtnji za fiksaciju nadgrebenog mišića (Grujić-Grujić, Novi Sad, Srbija)*

the ends of the damaged rotator cuff. Then the threads were put through the bone tunnels and the lateral part of the cuff was sutured. After that, two mattress sutures were placed by means of the threads fixated by previously placed metal screws in order to reconstruct the medial part of the footprint, thus providing the tendon strength along with the optimal contact between the tendon and the bone. After the procedure, the deltoid muscle was reconstructed by transosseous sutures through the acromion.

After the surgery, the patients wore immobilization in a position of 45 degrees abduction of the shoulder and 90 degrees flexion of the elbow in neutral position. The immobilization was removed after six weeks. The patients were given a prophylactic antibiotic dose (first generation cephalosporin 1 g/12 h and Garamycine 240 mg/24 h) preoperatively and during the first two postoperative days to prevent infections. In cooperation with a hemostaseologist, those patients who were at risk of thromboembolism received anticoagulation therapy.

After the immobilization had been removed, a nine-month rehabilitation program was performed at the Outpatient Department for Sports Medicine in Novi Sad. The rehabilitation consisted of passive, active-assisted and active exercises in order to get the full range of motion. The full range of passive motion was achieved in less than six weeks after the immobilization had been removed. Then the period of regaining the range of active motion began and lasted for six months after surgery when the exercises were introduced in order to improve the strength of the shoulder with the full range of motion and to boost the general physical condition. The latter exercises were performed until the ninth month after surgery. After that, the patients returned to their sport and recreational activities.

The results were evaluated by the Constant shoulder score [10].

Results

The average time from being injured to being diagnosed was 10.9 (1 to 36) months. *Magnetic resonance imaging (MRI)* position of 45 degrees abduction of the shoulder was done in all patients after 11.5 (one to 42) months on average after the injury. The average time from the injury to the operation was 14.1 (3 to 45) months. The patients were operated 2.6 months (2 days to 8 months) after MRI. All injuries were unilateral. Twelve patients had the rotator cuff injury on the right side and eight of them had it on the left side. Three of them had a partial tendon tear and 17 had a complete tendon tear. The tear of one tendon was in 14 patients and the tear of two tendons occurred in six cases. The dominant arm was affected in 75% of the patients. A complete tear of supraspinatus muscle occurred in 55% of the cases, a partial tear of supraspinatus muscle happened in 15%, a complete tear of
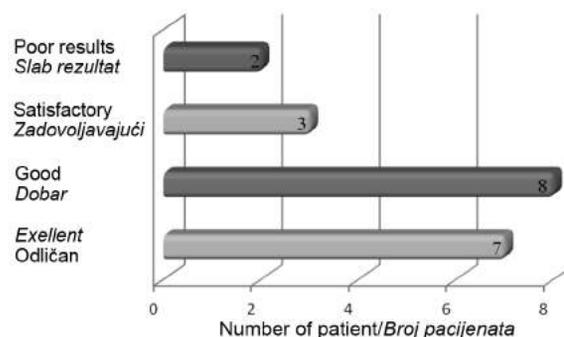
Graph 3. Results of the patients according to the Constant-scoring scale
Grafikon 3. Rezultati ispitanika prema Konstant-ovoj bodovnoj skali

the tendon of supraspinatus and subscapularis muscles was in 20%, and a complete tear of supraspinatus and infraspinatus muscles occurred in 10% of the cases. Postoperative complications occurred in three cases – a partial tear of the sutured deltoid muscle at the acromion, venous stasis and a consecutive phlebitis as well as a deep wound infection in one case each. Twenty percent of the patients did not attend rehabilitation program regularly.

According to the Constant shoulder score, 75% of the patients had excellent and good results, 15% of them had fair results and 10% of the patients had poor results (**Graph 1**). The sum of the Constant shoulder score, range of motion of external and internal rotation of the operated and the opposite healthy shoulder showed a statistically significant difference (p<0.05). By comparing the upper arm abduction, we found a statistically significant difference (p<0.05). The average sum of the Constant score for the operated shoulder was 67±16 and for the opposite healthy shoulder it was 83±7. The sum of the Constant score for the operated shoulder was by 19% less than for the opposite healthy shoulder. The range of external rotation was 73% compared to the healthy side. The range of internal rotation, abduction and flexion was 79%, 88% and 94%, respectively compared to the opposite shoulder (**Tables 1 and 2**).

After the operation, 19 patients are able to perform all activities of daily living, one patient has a permanent restriction in activities of daily living. Ninety five percent of the patients were satisfied with the results of the surgery.

Discussion

The rotator cuff is the most important structure of the shoulder. In the Unitated States of America, 75000 repairs of the rotator cuff are performed annually. By analyzing the age structure, it can be concluded that 85% of patients are between 50 and 65 years of age. These data match the data from other studies where the injury is the most frequent

Table 1. Values of the sum of the Constant scale and range of motion of the patients
Tabela 1. Vrednosti zbira Konstantove skale i obimi pokreta ispitanika

Number of participants *Redni broj ispitanika*	Operated shoulder *Operisano rame*	Opposite shoulder Suprotno rame	Operated shoulder *Operisano rame*	Opposite shoulder Suprotno rame	Operated shoulder *Operisano rame*	Opposite shoulder Suprotno rame
	Sum of the Constant shoulder score *Zbir Konstant-ove skale*		Internal rotation *Unutrašnja rotacija*		External rotation *Spoljašnja rotacija*	
1.	83	92	50°	80°	30°	65°
2.	83	90	55°	70°	60°	60°
3.	34	73	0°	15°	10°	40°
4.	63	77	40°	50°	40°	60°
5.	80	86	45°	50°	50°	45°
6.	78	84	55°	65°	55°	60°
7.	79	83	75°	80°	45°	90°
8.	48	71	20°	70°	55°	55°
9.	78	85	60°	70°	50°	65°
10.	74	79	70°	60°	80°	80°
11.	79	90	65°	70°	30°	50°
12.	63	82	50°	40°	60°	50°
13.	68	88	75°	80°	55°	65°
14.	52	81	60°	70°	75°	95°
15.	75	91	50°	80°	30°	65°
16.	71	84	80°	55°	65°	70°
17.	48	77	60°	70°	45°	70°
18.	30	65	20°	55°	30°	60°
19.	75	91	50°	65°	30°	40°
20.	72	84	35°	65°	30°	85°

in the age group from 40 to 60 with a tendency to increase with each following decade [2, 10–13].

Traumatic injuries of the rotator cuff often remain unrecognized at the first medical examination due to mild symptoms in most cases. When the x-ray imaging excludes fracture or luxation, the patients are often prescribed physical therapy without further diagnostics. The study performed by Sorensen [14] et al. at Denmark's Emergency rooms was aimed at answering the question whether the doctors failed to diagnose the rotator cuff injury. The patients who had sought medical aid for a shoulder injury were clinically examined and the shoulder x-ray was done.

The authors of this study wanted to find out whether the patients were about to be discharged from hospital only based on these diagnostic procedures. After the clinical and x-ray examination, ultrasound of the shoulder was done for the purpose of the research (ultrasound was not a standard diagnostic method). It was found that most of the patients would have been discharged from the hospital and prescribed physical therapy although the additional diagnostics showed a rotator cuff injury. According to this research, special attention should be paid to the patients without clear clinical signs of tendon rupture and, therefore,

early diagnostics must be improved. In our research, 75% of the patients visited the general practitioner after the injury, 10% of them went to a sport medicine physician and 15% visited a physiatrist. The average time from being injured to being diagnosed was 10.9 months, that being a very long period. The explanation of the reason why it took so long to set the diagnosis should be a topic of further research. A late diagnosis delays the treatment and affects the outcome of the treatment [5]. On average the patients were operated three months after having been diagnosed, that being in accordance with the published recommendations for chronic injuries [5].

Yamamoto et al. [15] conducted a research in which they identified risk factors for rotator cuff injuries in general population. The history of shoulder injury of the dominant arm and age were correlated with the frequency of injury. The participants younger than 49 years of age had an injury resulting from a strong force. The results showed that the trauma and degenerative changes contributed to the tendon tear but the trauma had a greater degree of correlation with the tendon tear in younger people. Sorensen et al. [14] reported that 66% of the patients were injured because of the fall onto the shoulder, 19% of them injured the shoulder during

Table 2. Values of the sum of the Constant scale and range of motion of the patients
Tabela 2. Vrednosti zbira Konstantove skale i obimi pokreta ispitanika

Number of participants *Redni broj ispitanika*	Operated shoulder *Operisano rame*	Opposite shoulder *Suprotno rame*	Operated shoulder *Operisano rame*	Opposite shoulder *Suprotno rame*
	Abduction *Abdukacija*		Flexion *fleksija*	
1.	165°	170°	170°	170°
2.	165°	170°	170°	170°
3.	170°	175°	165°	170°
4.	90°	150°	160°	165°
5.	165°	175°	170°	170°
6.	110°	145°	165°	160°
7.	150°	160°	120°	155°
8.	80°	140°	95°	135°
9.	130°	150°	170°	165°
10.	150°	160°	165°	160°
11.	160°	165°	175°	175°
12.	165°	165°	165°	170°
13.	120°	130°	150°	155°
14.	120°	135°	110°	140°
15.	145°	160°	150°	155°
16.	165°	160°	160°	165°
17.	160°	165°	140°	145°
18.	175°	170°	180°	170°
19.	65°	140°	75°	135°
20.	120°	150°	125°	145°

a sudden stretch and 15% of the patients were not aware of the mechanism of injury. The data from their research are not very different from the results of our study, in which two participants younger than 50 said they had been injured while lifting a heavy load and in a traffic accident, which implies that the force which caused the injury was strong. This is in correlation with the observation of Yamamoto and Hattrup [15, 16].

Supraspinatus muscle was affected in 70% of the cases. Namdari and Green [17] got similar results in their research of motion ranges in 345 surgically treated injuries and they reported 62% of isolated injuries of the supraspinatus muscle. Other studies have also confirmed that this muscle is most frequently injured [18–20].

Postoperative complications developed in 15% of the cases. This percentage is somewhat higher than in the study performed by Mansat et al. [21], which included 2948 operated shoulders with postoperative complications in 10.5% of cases.

After surgical treatment, it is necessary to undergo physical treatment in order to increase the muscle strength. According to the results of Gron-

del's study [22], 87% of the participants had excellent and good results measured by the Constant score, and all of them received physical therapy regularly during the 12 months after the operation. In our study, 75% of the participants had excellent and good results according to the Constant score and 20% of the participants did not undergo physical treatment regularly.

A statistically significant difference (p<0.05) was found between the Constant score of the operated shoulder and the opposite healthy shoulder as well as between the range of external and internal rotation and abduction (p<0.05). The period time (10.9 months) between the injury and the diagnosis is one of the negative factors which reduces the ability to regain the pre-injury range of motions. Retraction of the tendon towards the muscle belly increases over time thus preventing successful reparation. In the study performed in 2012, Mayer et al. [23] showed that first the size of muscle decreased and then the length of the injured tendon got shorter. This pathogenetic mechanism causes the decrease in the shoulder motion range and strength after the surgical treatment. Namdari

and Green [17] confirmed that in chronic injuries the affection of the subscapularis muscle led to a decrease in the external rotation, whereas the injury of supraspinatus and infraspinatus muscle led to a decrease in the abduction and the internal rotation.

A year after the surgical treatment, Namdari and Green [17] recorded that the range of flexion was 95% and the range of external rotation was 85% compared to the opposite shoulder. The range of passive internal rotation was 88%. This greater range of internal rotation can be explained by the fact that Namdari and Green [17] measured the passive rotation in their study. In our study, the range of external rotation is smaller because of the higher incidence of injury of both the subscapularis and the supraspinatus muscle.

The study of Kronberg et al. [24] included 37 participants and 80% of them had either none or some occasional limitations in activities of daily living. Romeo et al. [25] followed 72 patients in their study and this percentage was 96. These results do not deviate from the ones obtained in our study.

Several studies have revealed that the function of the shoulder is significantly improved after the rotator cuff repair although a slight reduction of motion range and strength does remain in comparison to the opposite healthy shoulder [17, 24–26]. Our study showed that the range of motion of the operated shoulder compared to the opposite healthy shoulder was 73% to 94%. Despite this, the fact that 95% of the patients can perform all activities of daily living and that 95% of the patients are satisfied with the results of the operative treatment suggests that the results are very good.

In addition to the small sample size, the shortcoming of this study is that the range of motion of the injured shoulder was not measured prior to surgery, which would have provided a better insight in the outcome of the operative treatment. Another possible flaw of this study was our failure to perform postoperative magnetic resonance imaging to assess the condition of the tendon upon the completion of treatment. A control MRI scan would have made it easier to find and interpret the causes of weaker postoperative results. The problem of making a control MRI scan is described in other studies as well. The application of MRI is limited due to a long waiting list and high cost [17, 27].

Conclusion

The rotator cuff injury is most frequent in people between 50 and 65 years of age. Trauma of the shoulder is the most common mechanism of injury, the fall onto the shoulder being the most common cause. The dominant arm is more often affected. The diagnosis is usually made very late, thus affecting the results of treatment. The basic requirement for the positive outcome of the treatment is timely diagnosis and early surgical repair of the torn tendon. Tear of the supraspinatus muscle is the most frequent injury; the second most frequent is the injury of the supraspinatus and subscapularis muscles at the same time. Ninety five percent of the patients are able to perform all activities of daily living and they are satisfied with the results of operative treatment.

Surgical repair of the rotator cuff injury is a reliable, time-tested method, which provides good clinical results particularly in patients without associated bone lesions operated during the first three weeks after the injury.

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CASE REPORTS
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MEDIAL TIBIAL STRESS SYNDROME: CASE REPORT

MEDIJALNI TIBIJALNI STRES-SINDROM: PRIKAZ SLUČAJA

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Summary

Introduction. Although it can be difficult to differentiate pain in lower legs, it is important for clinicians to differentiate medial tibial stress syndrome, which is a rather benign condition, from acute compartment syndrome, which is an emergency, as well as from different types of stress fractures described in this region. The aim of this case report was to present medial tibial stress syndrome as a clinical diagnosis, possible dilemmas in differential diagnosis and the efficacy of rehabilitation treatment. **Case report.** A 25-year old male patient sought medical help complaining of the pain along the distal third of tibia. The pain was present on palpation of the distal two-thirds of the lateral and medial tibial border over the length of 9 cm and on muscle manual testing of foot flexors. The patient underwent physical and exercise treatment for three weeks. The recovery was monitored by visual analogue scale, which measured the lower leg pain, pain on palpation and manual muscle testing. In addition, the patient himself assessed his ability to resume sport activities on the 5–point Likert scale. The final evaluation and measurements showed his complete functional recovery. **Conclusion.** The results obtained in this case show the importance of accurate clinical diagnosis and rehabilitation for medial tibial stress syndrome

Key words: Medial Tibial Stress Syndrome; Diagnosis, Differential; Male; Adult; Signs and Symptoms; Pain; Exercise Therapy; Rehabilitation

Sažetak

Uvod. Diferencijalna dijagnoza bola u potkolenici može biti kompleksna. Za kliničara je važno da razlikuje medijalni tibijalni stres-sindrom što je prilično benigno stanje od akutnog kompartment-sindroma kao urgentnog stanja i različitih vrsta stres-fraktura u ovoj regiji. Cilj je da se prezentuje medijalni tibijalni stres-sindrom kao klinička dijagnoza, moguće dileme u dijagnostici i efekti konkretnog rehabilitacionog tretmana. **Prikaz slučaja.** Pacijent, star 25 godina, javio se na pregled zbog bola duž distalne trećine tibije. Bol je prisutan pri palpaciji medijalne ivice tibije, u distalnoj trećini dužinom od 9 cm kao i pri testiranju plantarnih fleksora stopala. Pacijent je uključen u rehabilitacioni tretman tokom tri nedelje koji je podrazumevao fizikalnu i kineziterapiju. Oporavak je praćen uz pomoć vizuelne analogne skale kojom smo merili bol u potkolenicama, pri palpaciji i pri testiranju plantarnih fleksora stopala kao i uz pomoć petostepene Likertove skale kojom je pacijent subjektivno procenjivao spremnost da se vrati u sportske aktivnosti. Završna ispitivanja i merenja pokazala su potpun funkcionalni oporavak. **Zaključak.** Dobijeni rezultati kroz ovaj prikaz slučaja ukazuju na značaj precizne kliničke dijagnoze i rehabilitacije kod medijalnog tibijalnog stres-sindroma.

Ključne reči: Medijalni tibijalni stres sindrom; Diferencijalna dijagnoza; Muško; Odrasli; Znaci i simptomi; Bol; Vežbe; Rehabilitacija

Introduction

It can be difficult to differentiate the lower leg pain and one of possible diagnosis is medial tibial stress syndrome (MTSS).

In 1966, the American Medical Association defined MTSS as a pain and discomfort in the lower region of the leg resulting from repetitive activity on hard surfaces, or due to forcible, excessive use of the foot flexors, the diagnosis should be limited to musculoskeletal inflammation, excluding stress fractures or ischemic disorders [1].

Medial tibial stress syndrome is characterized by the lower leg pain, in the middle or lower third of the medial edge of the shinbone with tenderness of this region over at least 5 cm [2]. MTSS is a common injury affecting runners either separately or associated with different overuse injuries, and it is also common in other running sports. It is described in gymnasts and ballet dancers [3], as well as in military population [4]. The incidence of MTSS in previously mentioned population ranges from 4 to 35% [2, 5].

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The biomechanical factors associated with this syndrome include foot over-pronation, excessively tight leg muscles and decreased range of motion in subtalar joints [6-8]. Different studies have documented that female gender is a statistically significant risk factor [2,5]. Additional risk factors for athletes are training mistakes, hard surface and fatigue. It is very important for the clinician to differentiate MTSS, which is a rather benign condition, from different types of stress fractures in this region and acute compartment syndrome (ACS) that are much more serious conditions [8]. In addition, the tibial periostitis is described as the beginning of polyarteritis nodosa [10], and some types of tumors may be localized in this region [11].

The patient with MTSS complains of pain at the junction of the middle and distal third of shinbone, the symptoms are mostly bilateral, and they increase with running and decrease after rest [12]. In more severe cases, the patient complains of pain during normal walking and usual activities of daily living, even when resting. The pathophysiology of MTSS and mechanism of pain are not very clear, although the majority of authors agree and report muscle overuse, separation and traction of muscle fibers at the site of junction to the medial edge of the tibia and periostitis [13]. The tibialis posterior muscle was believed to be responsible for the development of MTSS; however, new investigations have found that the soleus and flexor digitorum longus muscles are very important in symptomatology [14].

The metabolism of bone tissue is also important in MTSS. It was found that athletes with periostitis of tibia had lower bone mineral density compared with the healthy control group [15], and after the symptoms had been relieved, the bone mineral density became normal [16].

The purpose of this paper was to present medial tibial stress syndrome as a clinical diagnosis, to evaluate a possible differential diagnosis and to show the efficacy of rehabilitation treatment.

Case Report

A 25-year old male patient sought medical help due to the pain along anterior side of lower legs which lasted five months. He had no previous history of a similar condition. The patient is a football referee and plays football for recreation. Initially, he felt pain while he was running, but later the a pain was present after walking and usual daily activities. The patient's weight and height were 70 kg and 180 cm, respectively, body mass index (BMI) was 21.6 kg/m². The patient reported to have had the left ankle sprain a year before, but denied anything else.

On clinical examination, a sharp pain was provoked by digital palpation of the medial edge of tibia in the distal third as well as by manual muscle testing of foot plantar flexion muscles and walking on toes. Orthopedic and neurological findings were regular. Dorsal pedal and posterior tibial pulses were normal. There was no difference in measurements of lower legs circumferences (40 cm, measured 10 cm below knee joint spot). A decreased hamstrings flexibility and collapse of medial longitudinal arch of feet were noticed. The talocrural ranges of motion were painless, but the range of motion of the left ankle was somewhat decreased compared with the right ankle. The diagnosis was made according to medical history and additional diagnostic test such as plain radiography of distal legs, which was normal.

The rehabilitation (15 therapies during three weeks) consisted of exercises and physical therapy –iontophoresis of anaesthetic (novocain), low level laser therapy (wavelength -780 nm, frequency 2500-HZ, power output-20 mW, spot size-1 cm, energy density-3 J/cm²; duration of treatment- 600s, daily energy dose-6 J, total energy dose-90 J, technique-contact application along the painful medial edge of tibia). Since we did not find the

Figures 1 and 2. Calf muscle stretch
Slike 1 i 2. Vežbe istezanja zadnje lože potkolenice

Table 1. Parameters of recovery monitored during rehabilitation
Tabela 1. Parametri praćenja oporavka tokom rehabilitacije

Parameter	Before therapy *Pre terapije*		5 therapies *5 terapija*		10 therapies *10 terapija*		15 therapies *15 terapija*	
	right/*desno*	left/*levo*	right/*desno*	left/*evo*	right*desno*	left/*levo*	right/*desno*	left/*levo*
Pain when active *Bol pri aktivnosti*	70 mm	70 mm	30 mm	40 mm	30 mm	30 mm	0 mm	0 mm
Pain when passive *Bol u miru*	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm	0 mm
Pain-palpation *Bol-palpacija*	70 mm	90 mm	40 mm	50 mm	10 mm	20 mm	0 mm	0 mm
Pain-walk on toes *Bol-hod na prstma*	50 mm	50 mm	30 mm	40 mm	10 mm	10 mm	0 mm	0 mm
PEK	30°	30°	30°	30°°	15°	20°	15°	15°
DF	25°	15°	25°	15°	25°	20°	25°	20°
PF	50°	40°	50°	40°	50°	45°	50°	45°
MMT	4	4	5	4	5	5	5	5

PEK – passive extension of knee/*pasivna ekstenzija kolena*; DF – dorsal flexion/*dorzalna fleksija*; PF – plantar flexion/*plantarna fleksija*; MMT – manual muscle test/*manuelni mišićni test*

dose recommendations of laser therapy in the treatment of MTSS, the parameters of laser light were in accordance with clinical trials aimed at investigating the efficacy of low level laser therapy in treatment of painful musculoskeletal conditions [17–20].

Treatment included ankle range of motion exercises, hamstrings and Achilles tendon stretching (**Figures 1 and 2**) and the exercises were performed twice a day. The patient was told about his condition and advised to buy orthopedic insoles. The control examinations were done after 5, 10 and 15 therapy sessions. The recovery was monitored by visual analogue scale (VAS) ranging from 0 to 100 mm (0-without pain; 100-unbearable pain), the pain in lower legs was measured when being passive and active, on palpation, during walking on toes (**Table 1**). The 5-point Likert scale was applied to obtain the patient's subjective assessment of his recovery and he was asked whether he was capable to resume his sports activities (**Table 2**).

The hamstrings flexibility was measured by the passive extension of knee (knee flexion was measured by goniometer when the patient was lying on his back with hip flexion of 90 degrees); the muscle strength of plantar flexion muscles was measured by manual muscle test (grade 4 when the patient did the plantar flexion on one foot once or twice and grade 5 when he did the plantar flexion on one

foot five times), talocrural range of motion was measured by gonimeter (degrees) before and after therapy. After one week, a significant alleviation of pain in lower legs was noticed and after three weeks of continual rehabilitation, the symptoms and signs of MTSS disappeared. The complete functional recovery of patient was achieved and he resumed his work and sport activities. No recurring discomforts were reported on the control examination a month later, and the clinical findings of the lower legs were normal.

Discussion

In differential diagnosis, a lower leg pain includes different clinical conditions. Some of them are rather serious and require an urgent surgery, such as ACS, which is characterized by the pain lasting through and after an activity and getting worse by contractions and passive affected muscle stretching and the sensory and motor neurological deficit is a late sign of nerve and muscle ischemia [6]. Stress fracture of tibia causes lower leg pain in sports involving running, and 90 % of patients report pain in medial –posterior part of tibia [21]. In ACS, the clearly localized and sharp pain is felt on palpation, whereas in MTSS the pain is poorly localized and bilateral [13]. In addition, a nocturnal pain and pain on percussion are not characteri-

Table 2. Subjective assessment of recovery
Tabela 2. Subjektivna procena oporavka

Capability to resume sport activities *Sposobnost da se vrati sportu*	Yes, completely *Da, potpuno*	Yes, but not completely *Da, ali ne potpuno*	Yes and no *Da i ne*	No *Ne*	No at all *Ne, uopšte*
5 therapies/*5 terapija*				+	
10 therapies/*10 terapija*			+		
15 therapies/*15 terapija*	+				

stic of MTSS. In MTSS and stress fracture, passive and active talocrural ranges of motion are usually without pain, contrary to the symptomatology of ACS with very painful ranges of motion.

Other clinical conditions include chronic compartment syndrome, tendinitis, muscle distension and/or tear, occlusion of popliteal artery and radiculopathy [21].

In the case report presented hereby, the young man had clinically clear and advanced symptoms of MTSS caused by plantar flexion muscle overuse, and the risk factors included mechanical imbalances of feet-collapse of longitudinal medial arch and over-pronation, which are very well documented in literature, and decreased hamstrings flexibility.

In relation to risk factors of MTSS, investigations show the level of evidence 1 for foot over-pronation and level of evidence 2 for tight calf muscles and higher BMI [21]. A prospective study performed by Plisky et al. has shown that BMI over 20 kg/ m² presents a risk for MTSS in elite runners [22]. BMI of our patient was within normal ranges, but close to the mentioned value that might be in correlation with results of the previous study [22]. In this case, playing football indoors on parquet certainly presents extrinsic risk factors for MTSS. The plain radiography of lower legs was normal. Majority of authors reported normal radiography in MTSS [23, 24], but a small percentage of patients had the signs of periostal reaction [25]. According to these examinations, MTSS is the first clinical diagnosis and magnetic resonance imaging (MRI) and scintigraphy, both having the same sensitivity, are required to solve any dilemmas, if present [25]. An interesting finding was obtained by the authors in the same study, i.e. a great number of abnormal scintigraphies and MRI were found in the asymptomatic control group [25].

A young patient with MTSS is functionally disabled to a great extent in sport activities involving running due to pain, and later, in everyday activities such as walking when the symptoms advance, as it was the case in our patient. The failure to manage MTSS adequately may result in complications such as stress as well as acute, "real" fractures in this region, which would prolong the treatment and abstinence from sport activities [8].

Treatment of MTSS can be difficult and its main goals are alleviation of pain, treatment of the underlying pathophysiological substrate and identification of risk factors in order to eliminate them.

Literature and clinical practice mention and recommend the following treatment methods: stretching of calf muscles, wearing orthopedic insoles to reduce over-pronation [26], massage, electrotherapy [27], acupuncture [28]; however, no clear recommendations are given. Recent research has shown that extracorporeal shock wave therapy (ECSWT) is efficient in treatment of MTSS with promising results [29]. Stretching and strengthening of specific muscles are crucial in correction of muscle imbalances. In addition, education and reduced training schedule are very important in treatment.

Our patient was treated by analgesic and anti-inflammatory procedures of physical therapy and he also started to wear orthopedic insoles to reduce over-pronation. Having received therapy for a week, he reported the reduced pain, so he started with exercises. Three weeks later, he could run without pain and he resumed his sport and work activities.

The complete functional recovery before resuming sport activities is essential in order to prevent recurring discomforts, which may happen if biomechanical imbalances and some training mistakes are not corrected [11].

This paper has some shortcomings that must be considered. Firstly, a great number of different therapy procedures are used in treatment of MTSS [21, 26–30], but there is no precise decision and recommendation in relation to efficacy and advantage of any treatment [30]. In addition, due to differences in definitions and terminology of this syndrome among different authors it is difficult to interpret the results of investigations [21]. Secondly, a single case report cannot suggest a recommendation regarding treatment for the whole population. The main purpose of this case report was to present MTSS as a clinical diagnosis, evaluate possible dilemmas in diagnostic tests and show the efficacy of rehabilitation treatment.

Conclusion

The results of this case report underline the importance of rehabilitation in patients with medial tibial stress syndrome. An accurate and precise diagnosis of lower leg pain provides a more specific rehabilitation and faster recovery. Due to the possibility of recurring discomforts, monitoring and complete functional recovery of patient as well as the elimination of risk factors are crucial before resuming sport activities.

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PARADOXICAL MOVEMENTS OF THE EPIGLOTTIS - A RARE CAUSE OF RESPIRATORY OBSTRUCTION AND ITS SURGICAL TREATMENT

PARADOKSALNI POKRETI EPIGLOTISA - REDAK UZROK RESPIRATORNE OPSTRUKCIJE I NJENO HIRURŠKO LEČENJE

Karol V. ČANJI[1], Slobodan M. MITROVIĆ[1] and Vera M. BELJIN[2]

Summary

Introduction. The aim of this paper was to present a rare disorder of epiglottis function as a cause of breathing disorders and a manner of dealing with this problem. **Case report.** A 59-year-old male patient had breathing disorders in the form of short cessations of breathing two months after a cardiac surgery. He could not tolerate even a slight physical effort. Indirect laryngoscopy and video endoscopy performed with a rigid endoscope indicated paradoxical movements of epiglottis, which closed the entrance to the larynx and caused short cessations of breathing. The patient underwent a subtotal resection of the epiglottis with an argon plasma scalpel. Directoscopy of the larynx was performed under general endotracheal anesthesia. After three weeks, the patient was without any difficulties. The check-up examination after three months showed a small remaining part of the epiglottis. There were no paradoxical movements of the vocal folds; the left vocal fold was shorter, with a loose edge, and the posterior region of the glottis tilted to the left in phonation. The patient reported no breathing disorders three months after the operation. **Discussion.** Airway obstruction can be reduced significantly by surgical treatment of a soft or hanging epiglottis. Subtotal resection by argon plasma scalpel resulted in termination of breathing disorders in the patient described in this paper, and it enabled him to continue his normal everyday activities. **Conclusion.** Paradoxical movements of the epiglottis are a rare cause of breathing disorders. Resection of the epiglottis is a method which gives good therapeutic results.
Key words: Epiglottis; Argon Plasma Coagulation; Male; Middle Aged; Airway Obstruction; Vocal Cord Dysfunction; Laryngectomy; Prolapse

Sažetak

Uvod. Cilj ovog rada je da se prikaže redak poremećaj u funkcionisanju epiglotisa kao uzrok disajnih smetnji i način rešavanja ovog problema. **Prikaz slučaja.** Pacijent, muškarac, star 59 godina, imao je smetnje sa disanjem, dva meseca nakon kardiohirurške intervencije. Smetnje su bile u vidu kratkotrajnog prekida disanja. Nije tolerisao ni male fizičke napore. U indirektnoj laringoskopiji i videoendoskopiji, rigidnim endoskopom uočene su paradoksalne kretnje epiglotisa koji je kao klapna zatvarao ulaz u larinks i dovodio do kratkotrajnih prekida disanja. U opštoj endotrahealnoj anesteziji u direktoskopiji larinksa načinjena je suptotalna resekcija epiglotisa argon-plazma nožem. Nakon tri nedelje pacijent nije imao tegobe. Na kontrolnom pregledu nakon tri meseca, uočen je manji ostatak epiglotisa. Nije bilo paradoksalnih kretnji glasnica, leva glasnica je bila skraćena, mlitave slobodne ivice a u fonaciji je zadnja regija glotisa bila ukošena ulevo. Tri meseca nakon operacije pacijent nije imao disajne smetnje. **Diskusija.** Hirurško lečenje mekog ili visećeg epiglotisa omogućava da se opstrukcija disajnog puta značajno smanji. Suptotalna resekcija argon-plazma nožem, kod pacijenta koji je prikazan u ovom radu, dovela je do prestanka disajnih tegoba i normalnog obavljanja svakodnevnih životnih aktivnosti. **Zaključak.** Paradoksalne kretnje epiglotisa su redak uzrok disajnih smetnji. Resekcija epiglotisa je metoda koja daje dobre terapijske rezultate.
Ključne reči: Epiglotis; Argon plazma koagulacija; Muško; Odrasli, srednjih godina; Opstrukcija disajnih puteva; Disfunkcija glasnih žica; Laringektomija; Prolaps

Introduction

A hanging or soft epiglottis may cause breathing disorders, which are often misinterpreted as asthma attacks or paradoxical vocal fold movement. Misinterpretations of the problem may lead to wrong treatment [1, 2]. Not only the hanging or soft epiglottis but its paradoxical movements can cause breathing disorders as well. The aim of this paper was to present a rare disorder of epiglottis function as a cause of breathing disorder and a manner of its solution.

Case Report

A 59-year-old male patient was referred from the General hospital for breathing disorders in the form of short cessations of breathing, which started two months after a cardiac surgical intervention. He could not tolerate even a slight physical effort.

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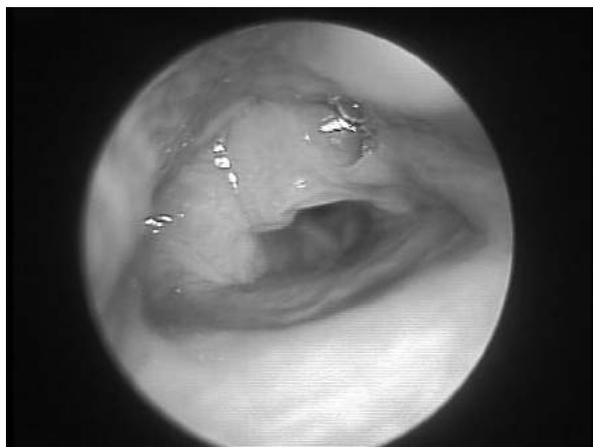
Figure 1. The condition before the operation, showing the arytenoid mucous membrane sinking during respiration
Slika 1. Stanje pre operacije, vidi se uvlačenje sluznice aritenoida koja tone tokom respiracije
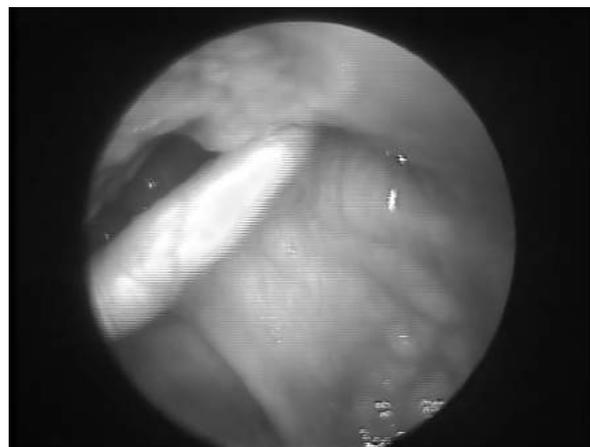
Figure 2. The condition before the operation, showing lingual surface of the epiglottis covering the entrance to the larynx during respiration
Slika 2. Stanje pre operacije, vidi se lingvalna površina epiglotisa koja pokriva ulaz u larinks tokom respiracije

Indirect laryngoscopy and video endoscopy performed with a rigid endoscope indicated that the epiglottis was slightly lowered to the laryngeal inlet. During examination and request for repeated phonation of the vowel "e", the epiglottis descended even more and blocked the view of the larynx. The breathing ceased for a short time, and the arytenoid mucous membrane sank towards the lumen of the larynx (**Figures 1 and 2**). Repeated examinations showed that during respiration and phonation the epiglottis acted as a flap, occasionally closing the lumen of the larynx. It was concluded that it was the paradoxical movements of the epiglottis, but the cause remained unknown. The patient was recommended a surgical treatment. During admission for the operative treatment, the disorders increased.

After admission to the Department, a subtotal resection of the epiglottis was performed with an argon plasma scalpel under general endotracheal anesthesia, using directoscopy of the larynx. In the postoperative course, a minor infection of the resected epiglottis developed, which was treated with antibiotic therapy. At the time of the first check-up, after three weeks, the patient was without any difficulties. Video endoscopy performed by a rigid endoscope was repeated. The examination showed a small remaining part of the epiglottis. There were no paradoxical movements of vocal folds; the left vocal fold was shorter, with a loose edge, and the posterior region of the glottis tilted to the left in phonation (**Figures 3 and 4**). The resulting conclu-

Figure 3. The condition after the operation, showing shortened left vocal fold and the remains of the epiglottis (respiration)
Slika 3. Stanje posle operacije, vidi se skraćena leva glasnica i ostaci epiglotisa (respiracija)

Figure 4. The condition after the operation, showing shortened and loose left vocal fold, and posterior tilting to the left (phonation)
Slika 4. Stanje posle operacije, vidi se skraćena i mlitava leva glasnica i ukošenost zadnje regije u levo (fonacija)

sion was that the cause of the patient's discomfort might have been paresis of the left laryngeal nerve. The patient reported no breathing disorders three months after the operation.

Occasionally, he aspirated a small amount of liquid. The patient continued to perform his everyday activities without any difficulties.

Discussion

Paradoxical movements of the epiglottis probably result from the damaged upper laryngeal nerve. Roy et al. [3] studied the larynx behavior on an in vivo model after transitory unilateral paresis of the outer branch of the upper laryngeal nerve. They identified a deviation of the stalk of the epiglottis and axial rotation of the anterior/posterior commissure in response to the vocal requirements. The hanging epiglottis may be the reason for urgent intubation or tracheostomy [1]. The flat and thin epiglottis may cause complications during intubation by interfering with the vestibule of the larynx [4].

The paradoxical movements of the epiglottis as well as the tilting position of the glottis during phonation in the patient presented in this paper may have resulted from the damage of the upper laryngeal nerve. The exact cause of its damage cannot be established since the disorders occurred two months after intubation and cardiac surgery.

Although the bad general condition of patients, particulary bad respiratory function is contraindication for supraglottic surgery [5] surgical treatment of soft or hanging epiglottis enables significant reduction of airway obstruction. Partial or total resection of the epiglottis and the V resection are reliable methods which provide a good therapeutic result [2, 6]. Subtotal resection by argon plasma scalpel resulted in termination of breathing disorders in the patient described in this paper, and it enabled him to continue his normal everyday activities.

Conclusion

Paradoxical movements of the epiglottis are a rare cause of breathing disorders. Resection of the epiglottis, which was presented in this case study, provides a good therapeutic result.

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BODY COMPOSITION ASSESSMENT IN ATHLETES: A SYSTEMATIC REVIEW

PROCENA TELESNOG SASTAVA KOD SPORTISTA: SISTEMATSKI PREGLED

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Summary

Body composition represents an unbreakable unity of the human body basic structure elements and involves a relative representation of the various constituent elements of the human total body weight. It is well known that body composition changes under the influence of continuous physical activity, and, therefore, it is one of the major components of fitness, and general health of the athletes. Therefore, this topic has become a major field of interest for many exercise and sport scientists as well as clinicians who specialize not only in different training methods but also in the prevention of and rehabilitation from major injuries. To date, having considered issues of accuracy, repeatability and utility, there is no universally applicable criterion or 'gold standard' methodology for body composition assessment in athletes. The main objective of this review was to give a short overview of methods for body composition analysis in athletes and to show and compare the latest data on their usefulness and reliability in order to find the best solution for practical everyday work.

Key words: Athlete, exercise; Body composition

Sažetak

Telesni sastav predstavlja neraskidivo jedinstvo osnovnih elemenata građe ljudskog tela, a podrazumeva relativnu zastupljenost različitih sastavnih elemenata u ukupnoj telesnoj masi čoveka. Telesni sastav se menja pod uticajem fizičke aktivnosti i zato predstavlja jednu od važnih komponenti fitnesa, ali i opšteg zdravstvenog stanja sportiste. Ova tema je postala glavna oblast interesovanja za mnoge lekare i stručnjake iz oblasti sportske medicine izrazito je važno ne samo u primeni na različite metode treninga, već i za prevenciju i rehabilitaciju velikih povreda. Do danas, imajući u vidu pitanje tačnosti, ponovljivosti i jednostavnosti upotrebe, ne postoji univerzalno primenljiv kriterijum ili metodologija koja bi se mogla smatrati „zlatnim standardom" za procenu telesne kompozicije kod sportista. Cilj ovog sistematskog pregleda literature je kratak osvrt na metode za analizu telesnog sastava kod sportista različitih profila, kao i prikaz i poređenje najnovijih podataka o njihovoj primeni i pouzdanosti radi najboljeg rešenja za praktičan svakodnevni rad.

Ključne reči: Sportisti; Vežbanje; Telesni sastav

Introduction

Body composition represents an unbreakable unity of the human body basic structure elements and involves a relative representation of the various constituent elements of the human total body weight [1]. Many studies suggest that body composition is, undoubtedly, significantly associated with physical activity in both sedentary population, and especially in elite athletes. It is known that continuous physical activity has a great influence on body composition, and therefore it is one of the five major components of fitness, and general health of the athletes [2]. Athletes' body composition differs in certain morphological characteristics of persons who are not involved in sports and who are not physically active [3]. Athletes differ among themselves, too. Therefore, a phenomenon known as the "sports morphological optimization" explains that the definitive athletes' body composition depends on sports they do. Because of this, differences in the height and other body features strongly correlated with it are caused by the diversity of requirements that athletes have during the selection process. Differences in body fat and muscle percentage in athletes are caused by the adjustment of body composition to a variety of individual sports [2, 3].

Body composition assessment in elite athletes and everyone who is involved in physical activity is of great importance as a determinant of their performance. Therefore, several highly accurate methods for analyzing the structure of their bod-

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ies have been developed. Laboratory methods include double-energy X-ray absorptiometry (DXA, previously DEXA), densitometry (underwater weighing-UWW), magnetic resonance imaging (MRI), neutron activation analysis and potassium-40 (K) analyses (TBK). Field methods include ultrasound, anthropometry, skinfold thickness (SF) and bioelectric impedance (BIA) [4–6].

However, regardless of the increasing number of technical solutions, the validity of the measured variables is still a key issue in defining and establishing the athlete's body composition structure. Regardless of the fact that we are now able to measure a number of different body composition indicators we do not always register and get relevant information in relation to the effects expected to be achieved. Therefore, a serious problem regarding the choice of appropriate methods, and its accuracy and precision in the analysis of athlete's body composition has arisen.

Bearing the above said in mind, the main objective of this review was to give a short overview of methods for body composition analysis in athletes and to show and compare the latest data on their usefulness and reliability in order to find the best solution for practical everyday work.

Material and Methods

The electronic database MEDLINE was searched from January 1990 to July 2013 and the keywords were: body composition assessment, anthropometry, DXA, BIA, hydrodensitometry, skinfold thick-

ness measurement and athletes. The selected studies had to contain different profile professional athletes, aged 16 to 60 who had constantly been engaged in physical activity for at least 5 years, including the training duration of at least 15 to 20 hours a week. UWW (2C model) was selected as the criterion method for this review because of the fact that only a few studies used 3- or 4- component models. The studies encompassed in this review included one or more of the listed body-composition assessment methods: DXA, hydrodensitometry, inert gas dilution methods, plethysmography, infrared spectrophotometry (NIR), BIA and anthropometric methods. Having in mind that racial differences may potentially affect the results, the studies with black or Native American subjects were excluded. In addition, the studies including the athletes under the age of 16 and over the age of 60 were not taken into consideration.

Results

After preliminary literature search by selected keywords, one hundred and seventy six references were identified in MEDLINE. The preliminary literature scan produced 70 potentially relevant references, which were fully considered and analyzed. These, potentially relevant references were reviewed thoroughly, so that the total number of papers included in this systematic review was 27 (**Scheme 1**).
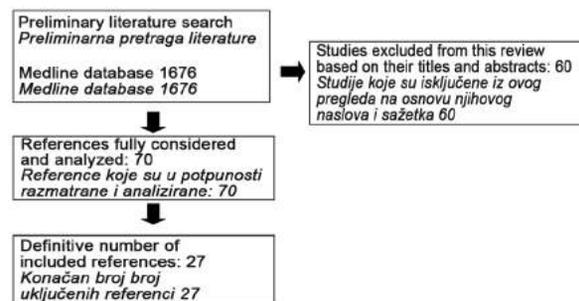
Scheme 1. Selection of studies
Šema 1. Izbor studija

This systematic review included the studies conducted on football players (four studies), volleyball, basketball, and judo players, wrestlers, athletes, body builders, baseball players, triathletes, heptathletes and water polo players (one study), while six papers included different profile athletes.

Anthropometric methods vs. UWW, DXA, NIR, biochemical methods

Fifteen studies included in this systematic review dealt with the comparative analysis of different anthropometric methods for body composition assessment with BodPod method, DXA, hydro-

densitometry, NIR or biochemical method of deuterium dilution. Many of these studies have compared BIA with other methods because it is rapid, noninvasive, and inexpensive; thus its application in the athlete's body composition assessment is in the focus of scientific attention.

In the largest body composition comparative study on BIA and hydrodensitometry Lukaski et al. emphasized that conditions during the athlete's body composition assessment using BIA method must be precisely controlled [4].

Andreoli et al. believe that body fat obtained using BIA and SF measurements is significantly overestimated [5].

The results of Dixon et al. have shown that BIA method underestimates percent body fat (%BF), and therefore it is not supported as a valid method [6].

Portal et al. have shown a positive correlation between %BF measured not only by SF method and BIA, but also by using Bod Pod method [7].

Oppliger et al. suggest the necessity to adapt methods of body composition assessment in order to improve the predictive value and accuracy of measurements in this population [8].

Yet another conducted study included the analysis of hydrodensitometry, SF method, BIA and NIR [9].

Clark et al pointed to SF method as more reliable in assessing %BF than BIA method [10].

Houtkoopr et al. believe that SF method gives more precise %BF estimation compared to NIR [11].

Williams et al. showed high Pearson product moment correlations among hydrodensitometry (HYD), BIA analysis and SF measurements [12].

Segal's reviewing study also emphasized the importance of the strict measurement condition control [13].

Loenneke et al. have concluded that BIA significantly underestimates fat free mass index (FFMI) [2]. Recently Loenneke et al. determined the accuracy of BIA and SF method compared with DXA for estimating %BF [14]. The results of this study do not recommend the use of BIA or SF.

The studies conducted by Stewart et al., De Lorenzo et al. and Claessens et al. made a comparative analysis between DXA, BIA and SF measuring method [15–17]. These studies have yielded fairly uniform results although they were conducted on different athletes.

Moon et al. emphasized the necessity for the introduction of sport-specific adapted BMI [19].

Comparison of different anthropometric methods
The comparative analysis of the application and reliability of various anthropometric body composition assessment methods in athletes was the topic of a separate group of studies with the highlight on the issue of BMI significance as an indicator of body composition of athletes.

Witt et al. showed that BMI method for evaluation of body composition on non-random sample of athletes often classified muscular athletes as obese [25].

Ode et al. had two main goals: to describe the relationship between BMI and %BF, and to define the accuracy of BMI as a measure of %BF not only in athletes but also in sedentary populations [20].

Mazic et al. studied the precision of BMI classification in overweight and obese athletes [21]. Their results showed that overweight in athletes could not be precisely estimated only by BMI >25 kg/m².

Garrido-Chamorro et al. determined the correlation between BMI and body fat, muscle and bone percentage [22]. These results showed that %BF was the only parameter that could affect BMI.

A step further was taken in the study of Neville et al. [23], which compared the %BF assessed by BMI and the SF data from three previously published epidemiological studies.

Huygens et al. suggested SF method as the most reliable one [24].

Only the results obtained by Ostojić differ from the previous facts [25].

Knechtle et al. have concluded that the body composition assessment by means of BIA leads to an overestimation of % BF compared with anthropometric methods [2].

Withers et al. performed a comparative analysis of %BF measured by means of body water (TBW), DXA method, hydrodensitometry and TBK [26].

As for the application and reliability of field methods to assess body composition of athletes, they are very few studies that use ultrasound for this purpose. This method is mainly used to determine the amount of visceral fat in obese humans [27]. Pineau et al. investigated the reliability of %BF measured by ultrasound, compared with DXA as a reference method [28].

Discussion

Most researchers who study the active population (including athletes) now use techniques that provide only an approximation of body composition. It is well known that excess body fat and adiposity have become an aggravating factor of many physical activities, and because of this, these conditions are the reason for concern. On the other hand, the values of body composition can be used as a highly informative predictor for the planning and programming of training and nutrition in physically active population, as one of the most important factors for successful performance in many sports [1, 7].

There is an increasing number of studies that recommend many different technical solutions for body composition assessment in physically active population. However, the key issue in determining the structure of body composition is the validity of the measured results. A major problem occurs

when it is necessary to choose the appropriate methods, regarding its accuracy, validity and precision in comparison with other methods. This systematic review expands and updates recommendations for the body composition assessment in athletes doing different sports.

Because of the importance of the body composition analysis in athletes, as a determinant of their performance, several highly accurate methods have been developed. The laboratory methods include DXA, hydrodensitometry, MRI, X-ray, neutron activation analysis and TBK. The field methods include ultrasound, anthropometry, SF measurement and BIA which are usually normalized and confirmed by standard laboratory methods [6–8].

Hydrodensitometry or UWW is the one of the most precise indirect procedures for body composition assessment. This method is, on one side, very accurate, but on the other, very complex, time consuming and expensive. In addition, it requires special equipment and trained technicians [9–11].

On the other hand, there is a rapid, non-invasive BIA. Sixteen of the above mentioned studies have examined the reliability of BIA method in the estimation of body composition of athletes by performing a comparative analysis of %BF obtained by this and other relevant techniques, such as DXA, hydrodensitometry, deuterium dilution method and plethysmography. Although BIA is a quick, relatively inexpensive and noninvasive method for the body composition evaluation, the results are conflicting. The results obtained in 13 out of 16 studies (81%) indicate the relative unreliability of BIA method in the estimation of body composition in athletes. Five studies (31%) stated that BIA overestimated %BF, while one study (6%) indicated that these values were underestimated. In addition to the studies dealing with the unreliability of the methods, this systematic literature review included two studies giving recommendations for mandatory condition control to improve the accuracy of the results [12–15]. Only three studies (19%) highlighted the relative reliability of this body composition assessment method [16–18].

The results of the study conducted by Moon et al. have once again highlighted the need for the introduction of adapted "sport specific" BMI in order to get the most accurate body composition assessment in physically active population [19]. The most commonly used methods of body composition assessment in athletes, both in field and laboratory conditions, are primarily anthropometric methods. These methods measure the dimensions of the human body (body height, body weight, SF, volume and diameter of the limbs), and use them in the appropriate equations. The indirect assessment of the content of body fat, muscle and bone tissue of athletes by anthropometric methods is relatively easy. The height/weight ratio is used to construct the "reference value" table. Although in theory this is an excellent idea, in

practice, however, there are some barriers - this ratio can be differently interpreted, and, in fact, a greater number of people must be measured in order to generalize the results. In addition, these scales / tables do not take into account individual differences in fat-free mass and relative fat mass (relative amounts of fat mass and lean body mass FM/LBM) in the body, and all this makes them quite unreliable in the body composition assessment in athletes. Anthropometry has some advantages compared to indirect methods such as hydrodensitometry, MRI and DXA. It is relatively inexpensive, non-invasive, fast and reliable and at the same time, does not require a specially trained person to perform it or the athlete's cooperation [20, 21]. However, although it is well known that BMI is a good way to assess the level of nutrition in physically inactive population this method has a number of limitations particularly regarding its application in physically active individuals. Namely, the body composition analysis is based on weight and height, and does not take into account the percentage of body fat and BMI which are often above permitted levels, particularly in this population. If only anthropometric indicators of body composition are taken into account, these results may suggest obesity. However, it is usually the increased body mass that underlies everything since it is much denser than body fat and as such much heavier, whereas body fat percent may be even below the normal range [22]. So, while calculating of BMI is a widely accepted body composition assessing method in the general population, its application in athletes often fails to give the true picture of body structures and therefore many authors recommend the use of body fat as a more accurate parameter for obesity in physically active population [23, 24]. Many studies confirmed the above mentioned facts [25, 26]. A study conducted by Neville et al. even mentions the necessity of adapting and creating new recommendations and protocols specific to the physically active population in order to replace outdated models [23].

This systematic review of literature includes biochemical methods for body composition assessment as well. They are based on biological constants obtained by direct chemical analysis of the body. These methods include the estimation of potassium amount, the total body water and absorption of inert gas. The lean body component has a relatively constant amount of potassium, whose one part is in form of a natural isotope. Gamma rays from the K40 isotope can be detected by Whole Body Scintillation Counter (WBSC) method, which enables the estimation of the total amount of potassium in the body, as well as of the amount of LBM. The results of this method are very similar to those of hydrostatic weighing. Due to the cost and limited availability of WBSC, this method is practically neglected in the process of research and everyday work. Diffusion techniques rely on the characteris-

tics of substances to diffuse into tissues or compartments in the body so they can be detected. Markers, such as tritium and deuterium oxide (heavy water), antipyrine and ethanol, for example, can be used to estimate total body water. It can be difficult to estimate fat-free mass because the body contains nearly constant 73.2% of water, which is almost the total amount of water that is distributed through fat-free mass.

A study conducted by Withers et al. found a significant positive correlation between %BF measured by DXA, TBW and the total amount of potassium measuring. In addition, this study recommends hydrodensitometry for measurement of individual differences in TBW, %BF and bone mass [26].

The development of new technologies went further in 2013. The most recent studies dealing with the precise and reliable body composition analysis of athletes emphasize a new method of subcutaneous adipose tissue topography using Lipometer [28]. This method is presented as a quick, non-invasive, safe and accurate in body composition analysis, the latter being its most important characteristic. It is based on the application of optical devices whose head consists of a set of optical diodes that emit light passing through the skinfolds, and then enters the photodetector. The intensity of the detected light is then converted by computed tomography into the absolute thickness of subcutaneous fat (expressed in millimeters). The results of this pioneering studies suggest the reliability of this new method. However, although the initial results are promising, the accuracy and evaluation remain to be explored and compared with "reference methods" [27, 28].

Conclusion

It is important to determine athletes' body composition not only to monitor the quality of the competition prospects, but also in order to assess the health risk. There are a lot of factors that can limit the validity and possibility to generalize findings of the body composition assessment in athletes. Taking into account, on the one hand, a number of studies which examined and compared the reliability and accuracy of different body composition assessing methods, and on the other hand, the specificity and diversity of the athlete's body composition, not only with each other but also in relation to the sedentary population, it is very difficult to give the accurate estimation of the athlete body composition. Although the main goal of clinical doctors and practitioners is to apply the fastest and easiest methods such as bioelectric impedance analysis, most of the studies emphasize its uncertainties and recommend the mandatory control of measurement condition in order to improve the accuracy of results. On the other hand, anthropometric methods are widely used (especially the calculation of body mass index). Body mass index is often used to assess the obesity level. However, although it is a widely accepted method for assessing the level of nutrition in the general population, its use in athletes often fails to give the true picture of body structures and therefore many authors recommend the use of percent body fat as a more precise parameter in the physically active population. Finally, the rapid development of technology has brought a new method – subcutaneous adipose tissue topography using Lipometer. The pioneering studies seem promising, but further tests and comparisons with a "reference method" are necessary.

It should be noted that this systematic review includes only the articles from Medline database written and published in English, thus studies that could have given a significant contribution to improving the techniques of body composition analysis may have been excluded from the analysis. However, the results of the analyzed papers provide a wide and thorough review that not only can be helpful in finding a standardized criteria and methods for body composition assessment in athletes, but can also serve as a basis for further comprehensive research.

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LETTERS TO EDITORIAL BOARD
PISMA UREDNIŠTVU

Banjari I. Ditch and switch – the question is how much supplements we actually need *(Pitanje je koliko suplemenata nam zapravo treba).*

For the past several months, the question of dietary supplement use among general public has been gaining a lot of attention from the scientific community and the media. This has brought my attention to the article written by Miljković et al. [1] who reported on dietary supplement use among students of the University of Niš. They described the attitudes towards dietary supplements nicely, but also covered all of the hidden traps beneath their use. However, where do we stand from the nutritional point of view?

Nowadays intake of the "white death" (salt, sugar, fats) is a high-risk dietary habit correlated with The Most Wanted list, cardiovascular diseases, cancer, obesity, and diabetes mellitus. They take the first place among death causes globally, affecting the quality of life and life expectancy. Nobody questions the importance of genes, and the environment, but where do we stand in terms of diet predestinating us for certain diseases? The theory of foetal programming made us think of such prerequisites [2]. External factors (diet being the one) during pregnancy determine us with 30%, more than any other determinant (including genes and gender) [3]; this has been thoroughly proved in animal and human studies. Extensive coverage on these aspects can be found in the field of epigenetics [2]. Some time ago, a theory that a diet can alter our genes and predispose us for certain diseases seemed like a science fiction, it is now scientific reality.

The overall burden of The Most Wanted list on the society, economy, and healthcare is overwhelming. Modern way of life, change in lifestyle, psychosocial patterns and dietary habits underlie these pandemics. Simultaneously, the increased awareness of people of prerequisites for diseases and the importance of a balanced diet have led to the use of dietary supplements. For some, supplements are about to improve their overall health, while for others supplements become one of the crucial components in their everyday diet [4]. This has brought us to the point where supplements are abusively used by all people both the healthy and the sick ones. This trend is gaining immense proportions. Supplements are not the magic pill. Where do we draw the line?

Miljković et al. [1] found that 68.1% of all participating students use dietary supplement; 9.76% of them were regular users, with the highest prevalence among female students. Multivitamins were the most popular dietary supplements, but students usually used two or more products in combination. They also found strong impact of media on student's choices, especially among male students. In addition, when asked about reasons for taking supplements, the students stated the strengthening of the immune system and improvement of the nutrition as the two main reasons. The statement made by a significant percentage of respondents, especially among male students (20.29% male *vs.* 6.13% female students) that supplements should not be used must not be neglected. More than a half of the study respondents (51.83%) said that supplements could have detrimental effect on the overall health [1].

The editorial of the Annals of Internal Medicine from December 2013 written by Guallar et al. [5] raised and heavily supported the following, important message: "Stop wasting money on supplements". As extensively argued through several papers published in the same issue of the Annals, there is simply no evidence-basis to justify preventive supplementation.

Study by Fortmann et al. [6] raised a question on how justified community dwelling prevention programmes really are. They emphasized the cost-effectiveness of these highly costly intervention programmes. The authors systematically reviewed databases for primary prevention studies of cardiovascular disease (CVD), cancer or all-cause mortality in adults without chronic disease that used supplements in doses less than the upper tolerable limit.

For multivitamin studies (four studies examined in total), no effect was found for all-cause mortality, or fatal and nonfatal CVD events overall. For cancer incidence, one multivitamin study (Physicians' Health Study II, PHS-II) found reduction in overall incidence after 11.2 years of follow-up, and sex stratification found protective effect only in men for another multivitamin study (Supplementation in Vitamins and Mineral AntioXidants Study, SU.VI.MAX). After combining these two largest studies (n = 27 658), the unadjusted relative risk for all cancer incidence was reduced over 10 years of follow-up (0.93 [CI, 0.87 to 0.99]). Still, for some studies, a harmful effect was found (i.e. increased melanomas among women in SU.VI.MAX) [6]. For single and paired nutrients, 24 studies have been identified (n = 324 653), but with numerous variations between studies (small body of evidence, as noted by the authors possibility of type II error). For the incidence of CVD and cancer, a risk consistent null result was found for β-carotene, while in high-risk subgroups, an increase in lung cancer was found. Vitamin E supplementation had no effect on any of the three observed outcomes, while

the findings were mixed for calcium and vitamin D combination. For example, post-hoc analysis of two trials of calcium supplementation found an increased incidence of colorectal cancer in the calcium group. One trial assessed folic acid supplementation in patients with prior colorectal adenomas and found an increased incidence in prostate cancer in supplemented patients [6].

Are we sobering up on supplementation? Not according to the report published by Euromonitor International in March 2014 [7]. Accordingly, the dietary supplements market is set to grow by 4% annually through to 2018, with the top three biggest sales expected for minerals, combined dietary supplements and omega-3. For the Republic of Serbia (and other surrounding countries), Euromonitor predicts a rise in sales for the dietary supplements market, mainly due to the increased consumer awareness supported by the media. This has been confirmed nicely by Miljković et al.[1]. In addition, their findings are largely in line with the recently published, prospective 5-year report on dietary supplements among Americans by Dickinson et al. [4]. The prevalence of dietary supplements use increased from 64% to 69% among adult Americans between 2007 and 2011, and the number of regular users increased from 28% to 36%. Multivitamins are the most commonly used supplements. The main reason for supplement use stated by 58% of the study's respondents is overall health and wellness, whereas 42% of the respondent mentioned the will to fill in nutrient gaps in the diet [4]. In the meantime, an extensive number of high-quality publications in form of a meta-analysis has been published on the positive sides of variety of dietary supplements. The one examining vitamin D status and breast cancer survival published in Anticancer Research supports supplementation with vitamin D for better survival [8]. Another study examining omega-3 and blood pressure published in the American Journal of Hypertension supports supplementation for hypertensive people [9].

On the other hand, the study by Sayin et al. [10] published in January 2014 in Science Translational Medicine found that supplementation with two antioxidants, N-acetylcysteine (NAC) and vitamin E increases tumour cell proliferation and growth for human lung tumour cells. Bearing in mind their involvement in somatic mutations (in p53, a key tumour suppressor protein) onset in late tumour progression, the authors concluded that these antioxidants may accelerate the growth of early tumours or precancerous lesions in high-risk populations, such as smokers and patients with chronic obstructive pulmonary disease who receive NAC to relieve mucus production. The mice given NAC had 2.8-fold higher tumour burden than the control mice, while in case of vitamin E supplementation the increased tumour burden showed a dose-dependent fashion. After combining these two antioxidants, the antioxidant-treated mice developed more tumours of more advanced histological grade than the controls. Besides, NAC and vitamin E reduced the median and maximal survival by 60 and 50%, respectively. These two antioxidants are highly overlapped; both suppress the expression of genes that participate in the endogenous reactive oxygen species (ROS) defence system [10]. The findings have been replicated and confirmed in human cell lines while using doses usually consumed by healthy humans. As said by the Journal Editor, this study is of high relevance and shows "the dark side of antioxidants".

Miljković et al. [1] give several examples on how active components from dietary supplements interact with medicament therapy and/or our metabolism. Numerous potentially toxic effects, such as intoxication with vitamin D or A, or potentially life-threatening events for herbal supplements have been well documented in literature. A study by Sayin et al. [10] clearly illustrates the danger hidden behind the improper use of supplements. Epigenetic studies also give quite a long list of negative consequences induced by the excessive intake of nutrients due to over-supplementation [2].

Supplementation seems to have sense only in populations living in extreme poverty, with limited or restricted food sources, and fortunately, that is not the case in this part of the world. Of course, specific physiologic and health conditions should be treated separately and on an individual basis as much as possible. For all of the above reasons, I strongly agree with the authors [1]. More efforts should be made to educate consumers and all health workers in order to limit misinformed use, which would diminish the incidence of any related side-effects. In addition, a stronger collaboration with media is needed; they should be encouraged to use scientific evidence and give up-to-date evidence based report on issues of public health significance. A take-home message is "back to nature". Ditch the pill, switch to an apple.

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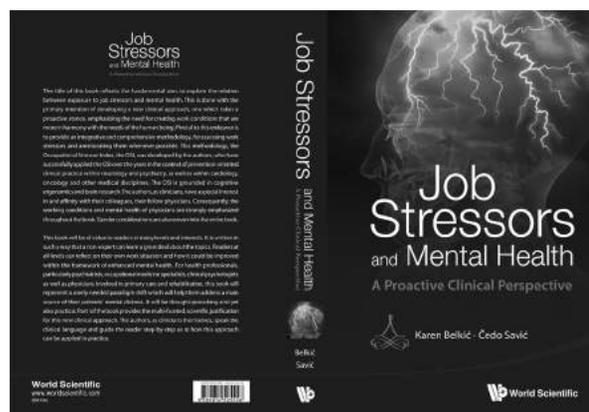
BOOK REVIEWS
PRIKAZI KNJIGA

Belkić K, Savić Č. Job stressors and mental health a proactive clinical perspective.

Dugogodišnji rad autora u traganju za modelom koji bi ušao u svaku „poru" ljudskog rada i radnog okruženja i „izmerio" njegov nepovoljan uticaj na ljudski organizam rezultirao je kreiranjem *Occupational Stressor Index*-a (OSI). Decenijska primena OSI modela u praksi kroz rad samih autora i njihovih saradnika, detaljno prikazana u knjizi, predstavlja dragoceno iskustvo, putokaz, način, opravdanje i potrebu za njegovom širom primenom u kliničkoj praksi. Ne samo zbog objašnjenja same fiziologije procesuiranja svih informacija u ljudskom mozgu do najsitnijih detalja mentalnom hronometrijom i štetnih posledica koje one izazivaju, nego, što je još značajnije, zbog tačnog utvrđivanja nepovoljnih činilaca na radu i načina njihovog štetnog delovanja na ljudski organizam. Ističu su aspekti koje dosadašnji sociološki modeli ne prepoznaju, a koji naročito štetno deluju na mentalno zdravlje ljudi (aspekt pripravnosti − izbegavanje opasnosti, konflikti...). Konkretna primena u svakodnevnom radu, ukazivanje šta tačno treba menjati, na koji način i kako unaprediti rad i radno okruženje za dobrobit radnika u procesu rada jeste suština ove knjige. Detaljno je prikazana klinička primena OSI modela u poboljšanju uslova rada zaposlenih različitih zanimanja sa različitim psihičkim problemima (lekara psihijatra, profesora, programera, medicinske sestre na onkologiji, radnika na videoterminalima, vozača, naučnika, lekara koji odlazi u penziju). Bolja organizacija procesa rada, odnosno harmonizacija rada sa ljudskim mogućnostima i potrebama, suština je obezbeđivanja dobrobiti za sve.

U svetlu savremenog radnog okruženja, naročito razvijenih zemalja, gde psihosocijalni faktori na radu sve više uzimaju primat u odnosu na ranije dominirajuće fizičke štetnosti, mentalnom zdravlju radno aktivne populacije poklanja se sve više pažnje. I u ovoj knjizi je to slučaj − u prvom delu dat je obiman i značajan pregled literature o uticaju uslova rada na pojavu mentalnih i drugih oboljenja, loših životnih navika (bihevioralnih poremećaja) kako kod zaposlenih, tako i onih koji imaju nesiguran posao, koji rade na privremeno-povremenim poslovima, kod nezaposlenih, penzionisa-

nih; ukazano je na to da je stabilan radni odnos važan za psihičku dobrobit zaposlenih. Mentalna oboljenja u vezi sa radom predstavljaju veliki, ne samo zdravstveni, već i socijalno-ekonomski problem koji zahteva integrativni pristup. Upravo je ovo štivo za lekare svih specijalnosti koje izuzetno dobro omogućuje sagledavanje, razumevanje i, samim tim, mogućnost poboljšanja ukupnog radnog okruženja zaposlenih svih profila.

Model OSI je naročito potreban lekarima specijalistima medicine rada koji u svakodnevnom radu ocenjuju radnu spodobnost kandidata za zaposlenje, zaposlenih, obolelih i povređenih, sposobnost za rad na radnom mestu sa povećanim rizikom, vozačku sposbnost i drugo. Sa njima u timu učestvuju i lekari specijalisti drugih grana, naročito neuropsihijatri, psiholozi, kardiolozi i drugi, kojima treba više skrenuti pažnju na značaj uzimanja radne anamneze pacijenata, radi sagledavanja mogućeg uzroka njihovog obolevanja, a isto tako, radi vraćanja na radno mesto posle izlečenja gde uslovi rada nisu poboljšani.

Ova knjiga, odnosno OSI model, jeste upravo ta „karika" koja nedostaje u integrativnom pristupu rešenju problema obolevanja u vezi sa radom, naročito obolevanja od mentalnih bolesti, ali i kardiovaskularnih, malignih, reumatskih i drugih.

Preporučujem je svima kao priručnik u svakodnevnom radu, kako zbog dobrobiti pacijenata o kojima brinemo kao njihovi lekari tako i zbog sopstvene dobroboti na radnom mestu.

Prim. dr sc. med. Olesja Nedić

IN MEMORIAM
IN MEMORIAM

Prof. dr TIBOR LEPEŠ
(1922-2012)

Profesor dr Tibor Lepeš, svetskí poznati medicinski parazitolog, naučni radnik, učitelj generacija studenata i lekara parazitologa u zemlji i celom svetu, erudita i humanista značajan za našu i svetsku medicinu, svojim radom na izučavanju i suzbijanju i eradikaciji parazitarnih bolesti, posebno malarije, član našega Društva, preminuo je 24. januara 2012. u Ženevi gde je živeo.

Rođen je 1922. godine u Subotici u porodici višeg železničkog službenika, pa je mladost proveo u Subotici, Nišu i Beogradu, gde je maturirao i započeo studije medicine koje je nastavio u Segedinu i završio 1945. u Budimpešti. Od 1945. bio je u JNA aktivni sanitetski oficir u Novom Sadu, prvo kao trupni lekar, potom u Vojnoj bolnici. Od 1949. godine bio je u Vojnomedicinskoj akademiji u Beogradu lekar na specijalizaciji.

Specijalistički ispit iz medicinske parazitologije položio je 1952. Zvanje docenta stekao je 1957., a habilitacioni rad – tezu za pukovnički čin o značaju malarije u miru i ratu odbranio je 1962. U toku specijaslizacije je sarađivao sa akademikom Čedomirom Simićem te se smatra jednim od njegovih istaknutih sledbenika. Tih godina se usavršava u centralnim institutima SAD u Vašingtonu i Betezdi i institutima za tropske bolesti u Hamburgu, Amsterdamu, Londonu i Liverpulu. Radi na dijagnostici parazitarnih oboljenja (usavršava metode pregleda), naučnim i organizacionim pitanjima, naročito na problemima tada aktuelne malarije. Uz službu na VMA, od 1952. bio je i koordinator Savezne komisije za malariju a od 1958. i rukovodilac Službe za eradikaciju malarije pri Saveznom zavodu za zdravstvenu zaštitu, kada je u Jugoslaviji i iskorenjena, što je Svetska zdravstvena organizacija potvrdila 1973.

Od 1959. postao je član Komiteta eksperata Svetske zdravstvene organizacije za malariju i naučnih grupa za biologiju malaričnog parazita i hemioterapiju malarije. Godine 1962. po pozivu stupa u službu ŠZO, prvo kao epidemiolog za malariju a od 1964. kao šef Odseka za eradikaciju malarije u Regionalnom uredu za Istočni Mediteran u Aleksandriji. Od 1968. u sedištu SZO u Ženevi bio je šef Odseka za naučnoistraživački rad i informatiku, pri Odeljenju za eradikaciju malarije, čiji direktor postaje 1970. Od 1973. Odeljenje je postalo Program za malariju i ostale parazitarne bolesti čiji je direktor bio do penzionisanja 1982. godine.

Na Medicinskom fakultetu u Novom Sadu je 1972. godine izabran za redovnog profesora po pozivu na Katedri za mikrobiologiju i parazitologiju, te je dolazio povremeno da predaje a 1982. prešao je u Novi Sad i redovno održavao nastavu i objavio udžbenik i praktikum iz parazitologije. Baveći se i problemima medicinske etike i evaluacije procesa nastave i stečenih znanja, uticao je i na neke stavove o tome na Medicinskom fakultetu – jedan je od urednika udžbenika o etici i nekih drugih publikacija. Bio je savetnik u Zavodu za mikrobiologiju i parazitologiju Instituta za zdravstvenu zaštitu, vodio nekoliko naučnih projekata do konačnog penzionisanja 1985. godine.

Osim malarijom bavio se i drugim područjima parazitologije, posebno crevnim parazitima – dijagnostikom amebijaze, tenijaze, helmintima, te se njegove dijagnostičke metode i šire koriste, prenosnicima bolesti – komarcima (u Makedoniji je našao vrstu otpornu na DDT), telesnim vašima. Veliki deo svoga rada je proveo na terenu, te je poznavao celu teritoriju Jugoslavije a radeći u SZO proputo-

vao je svetom stekavši izvanredna saznanja o problemu parazitoza.

Bio je učesnik, inicijator i organizator brojnih međunarodnih akcija i stručnih skupova, inicijator i pisac mnogih uputstava i drugih akata i publikacija SZO. Objavio je preko 60 radova u domaćim i 7 u stranim časopisima. Pisac je i više priloga i redaktor u raznim stručnim i sličnim publikacijama. I kao penzioner je pratio savremenu naučnu i stručnu literaturu, te se povremeno i javljao svojim prilozima.

Bio je dobar predavač, rado slušan. U ophođenju sa saradnicima i drugim ljudima bio je veoma komunikativan, jednostavan, pažljiv prema sagovornicima, srdačan. Veoma širokog obrazovanja, poznavao je mnoge oblasti. Po tim svojim osobinama ostao je u trajnom sećanju ljudi koji su ga poznavali. U mladosti je igrao fudbal, a kasnije je svirao klavir i violinu.

U Društvu lekara Vojvodine Srpskog lekarskog društva bio je aktivan član Redakcijskog odbora ča-

sopisa *Medicinski pregled*. Za počasnoga člana Medicinske akademije SLD izabran je 1988. godine. Bio je počasni član *Association of Military surgeons of the US* (1956), Belgijskog društva za tropsku medicinu (1974), Mađarskog infektološkog društva (1981), redovni član engleskog Kraljevskog društva za tropsku medicinu (1969) i švajcarskog društva za tropsku medicinu (1974, od 1988. počasni član).

Odlikovan je Ordenom rada I reda sa zlatnim vencem (1974) za eradikaciju malarije i Ordenom zasluga za narod. U Hamburgu mu je 1982. godine dodeljena nagrada *Bernard Nocht* za zasluge za izučavanje i suzbijanje tropskih bolesti u svetu.

Svojim celokupnim delom i ljudskim osobinama ostavio je dubok i trajan trag u svetskoj parazitologiji, posebno i u našoj sredini. Njegov humani i plemeniti lik ostaće u trajnoj uspomeni njegovim brojnim saradnicima i sledbenicima, kao i svim poštovaocima njegovoga dela.

Prim. dr Miloš Malenković

Prof. dr ALEKSANDAR MILIČIĆ (1948-2013)

Naš uvaženi član, prof. dr Aleksandar Miličić, posle kraće i teške bolesti, preminuo je 12. septembra 2013. godine.

Prof. dr Aleksandar Miličić je rođen 9.12.1948. u Sarajevu. Osnovnu školu i gimnaziju završio je u Novom Sadu. Medicinski fakultet u Novom Sadu upisao je 1967. a završio 1973. godine. Posle obavljenog lekarskog staža i odsluženja vojnog roka, 1973. godine, Aleksandar Miličić se zaposlio u SŠUP u Sremskoj Kamenici kao lekar opšte prakse i predavač predmeta Prva pomoć i Sudska medicina. Godine 1978. odobrena mu je specijalizacija iz ortopedije, a stalno zaposlenje dobio je u Institutu za hirurgiju u Novom Sadu. Specijalistički ispit iz ortopedije uspešno je položio 1982. godine. Pedagoški kurs položio je 1985. godine.

U zvanje asistenta za predmet Hirurgija sa ratnom hirurgijom na Medicinskom fakultetu u Novom

Sadu Aleksandar Miličić je izabran 1983. godine. Zvanje docenta na Katedri za hirurgiju stekao je 1989. a vanrednog profesora 1995. godine. Februara 2000. godine izabran je u zvanje redovnog profesora Medicinskog fakulteta u Novom Sadu.

Nakon polaganja specijalističkog ispita iz ortopedije, Aleksandar Miličić se aktivno uključio u stručni rad na ortopedskoj klinici Pokrajinske bolnice u Novom Sadu. Posebno se interesovao za patologiju kičmenog stuba. U to vreme, operativno lečenje povreda i oboljenja kičmenog stuba na Ortopedskoj klinici u Novom Sadu nije bilo razvijeno. Višegodišnjim stučnim i naučnim angažovanjem Aleksandar Miličić je uspeo da dodatno približi ovu zahtevnu oblast ortopedske hirurgije kako kolegama na Klinici, tako i drugim ortopedima u Vojvodini a i šire. Svojim doktorskom disertacijom *Rana spondilodeza kao metoda prevencije hronič-*

ne nestabilnosti posle fleksura ili dislokacija cervikalne kičme dodatno je omogućio razumevanje problema posttraumatske nestabilnosti povređene kičme i posebno ukazao na pravce savremenog, pre svega operativnog lečenja ovih povreda. Svojim radom, na temeljima savremene hirurške nauke, spinalnu hirurgiju je uveo na novosadsku ortopedsku kliniku.

Po povratku sa stručnog usavršavanja iz Liona, gde je tokom 1989. godine proveo više meseci u spinalnom centru Bolnice *Eduar Herio*, Aleksandar Miličić je počeo da izvodi i operativne zahvate na kičmenom stubu upotrebom transpedikularnih i transartikularnih tehnika instrumentacije grudnoslabinske i vratne kičme. Ove operativne metode do tada na novosadskoj ortopedskoj klinici nisu rađene. Tada najmodernije, a sada standardne i širokokorišćene, ove operativne tehnike stabilizacije kičme unapredile su ukupni nivo zbrinjavanja povreda kičme u Vojvodini. Uvođenje spinalne hirurgije u rutinsku kliničku praksu na Klinici za ortopediju i u Kliničkom centru Vojvodine uopšte, omogućilo je bolje ukupno zbrinjavanje i onih najteže povređenih pacijenata – politraumatizovanih, čije lečenje često komplikuje i spinalna trauma. Na taj način je povećan i stručni ugled Kliničkog centra Vojvodine kao regionalnog centra za zbrinjavanje povređenih.

Tokom svog stručnog i naučnog rada, Aleksandar Miličić je napisao 123 naučnoistraživačka rada, jednu monografiju (*Povrede kičme*, Visio Mundi Academic Press, Novi Sad, 1996.), 8 samostalnih poglavlja u monografijama i naučnim knjigama, kao i dva poglavlja u zvaničnim udžbenicima za studente medicine (*Hirurgija lokomotornog aparata I i II deo*, Medicinski fakultet u Novom Sadu 1990. i 1992. godine). Učestvovao je kao saradnik u 8 naučnoistraživačkih projekata i bio nosilac teme u 3 naučnoistraživačka projekta.

Doktorske studije započeo je 1984. godine. Doktorsku disertaciju pod naslovom *Rana spondilodeza kao metoda prevencije hronične nestabilnosti posle fraktura ili dislokacija vratne kičme* odbranio je 1988. godine na Medicinskom fakultetu Univerziteta u Novom Sadu.

Više puta je bio predavač na kursevima lekara opšte medicine, medicine rada i stomatologije, kao i na sastancima kontinuirane medicinske edukacije za lekare. Njegova predavanja studentima i lekarima postdiplomcima bila su uvek dobro posećena jer su bila jasna i sažeta, a istovremeno i atraktivna uz interesantne fotografije i slajdove iz bogate lične arhive.

Tokom karijere, profesor Miličić je obavljao i više rukovodećih funkcija − bio je šef Katedre za specijalizacije i uže specijalizacije zdravstvenih radnika i saradnika iz hirurgije od 1999. do 2009. godine, prodekan za naučnoistraživački rad i poslediplomske studije Medicinskog fakulteta u Novom Sadu od 2000. do 2009. godine, koordinator za lekare na specijalizaciji i užoj specijalizaciji na Katedri za hirurgiju Medicinskog fakulteta Novi Sad u periodu 2002–2003. i član Saveta Medicinskog fakulteta Univerziteta u Novom Sadu. Takođe, bio je član Sudskomedicinskog odbora Medicinskog fakulteta Novi Sad, član Upravnog odbora KCV, član Nadzornog odbora KCV, predsednik Stručnog saveta KCV, član Etičkog odbora KCV. Obavljao je funkciju upravnika Instituta za hirurgiju Kliničkog centra Vojvodine u periodu 2000−2006. godine. Funkciju načelnika operativnog odeljenja A na Klinici za ortopediju Kliničkog centra Vojvodine obavljao je od 1998. do 2010. godine.

Aleksandar Miličić je od početka svoje lekarske karijere bio veoma aktivan član Srpskog lekarskog društva − Društva lekara Vojvodine, pre svega u Sekciji za ortopedsku hirurgiju i traumatologiju, i drugih strukovnih organizacija: obavljao je funkciju predsednika vertebrologa Srbije i Crne Gore od 2007. do 2009. godine; bio je član YUOT-a (Jugoslovensko društvo ortopeda i traumatologa), SOTA (Srpska ortopedsko-traumatološka asocijacija) i STA (Srpska traumatološka asocijacija), a od osnivanja, 2003. godine, bio je i član *Spine Experts group* vertebrologa jugoistočne Evrope. U *Medicinskom pregledu* − časopisu Društva lekara Vojvodine Srpskog lekarskog društva objavio je veći broj naučnih radova koji su više puta citirani u zemlji i inostranstvu. Bio je i član Redakcijskog odbora ovog časopisa.

Tih i miran, a ipak vedre prirode, Aleksandar Miličić je bio uvek rado viđen u društvu kolega, medicinskih sestara i drugog osoblja u bolnici. Uvek je bio spreman da druge sasluša i posavetuje. Nebrojeno puta je nesebično pomagao svima koji bi ga zamolili za pomoć.U dugim časovima dežurstava, u trenutcima predaha, profesor je imao običaj da igra šah. Kao majstorski kandidat i dugogodišnji član Šah-kluba *Novi Sad* bio je neprelazna prepreka za sve šahiste. Posebno je voleo da pobeđuje neurohirurge sa kojima bi igrao i simultanke.

Prof. dr Aleksandar Miličić je bio oženjen i otac dve kćerke.

Prof. dr Milan Stanković
Predsednik
Sekcije za ortopedsku hirurgiju i
traumatologiju DLV-SLD

UPUTSTVO AUTORIMA

Časopis objavljuje sledeće kategorije radova:

1. Uvodnici (editorijali) – do 5 stranica. Sadrže mišljenje ili diskusiju o nekoj temi važnoj za Časopis. Uobičajeno ih piše jedan autor *po pozivu*.

2. Originalni naučni radovi – do 12 stranica. Sadrže rezultate sopstvenih originalnih naučnih istraživanja i njihova tumačenja. Originalni naučni radovi treba da sadrže podatke koji omogućavaju proveru dobijenih rezultata i reprodukovanje istraživačkog postupka.

3. Pregledni članci – do 10 strana. Predstavljaju sažet, celovit i kritički pregled nekog problema na osnovu već publikovanog materijala koji se analizira i raspravlja, ilustrujući trenutno stanje u jednoj oblasti istraživanja. Radovi ovog tipa biće prihvaćeni samo ukoliko autori navođenjem najmanje *5 autocitata* potvrde da su eksperti u oblasti o kojoj pišu.

4. Prethodna saopštenja – do 4 stranice. Sadrže naučne rezultate čiji karakter zahteva hitno objavljivanje, ali ne mora da omogući i ponavljanje iznetih rezultata. Donosi nove naučne podatke bez detaljnijeg obrazlaganja metodologije i rezultata. Sadrži sve delove originalnog naučnog rada u skraćenom obliku.

5. Stručni članci – do 10 stranica. Odnose se na proveru ili reprodukciju poznatih istraživanja i predstavljaju koristan materijal u širenju znanja i prilagođavanja izvornih istraživanja potrebama nauke i prakse.

6. Prikazi slučajeva – do 6 stranica. Obrađuju *retku* kazuistiku iz prakse, važnu lekarima koji vode neposrednu brigu o bolesnicima i imaju karakter stručnih radova. Prikazi slučajeva ističu neuobičajene karakteristike i tok neke bolesti, neočekivane reakcije na terapiju, primenu novih dijagnostičkih postupaka ili opisuju retko ili novo oboljenje.

7. Istorija medicine – do 10 stranica. Pišu se na poziv uredništva Medicinskog pregleda i obrađuju podatke iz prošlosti sa ciljem održavanja kontinuiteta medicinske i zdravstvene kulture, a imaju karakter stručnih radova.

8. Druge vrste publikacija (feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih ustanova, područnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novine u medicini, pitanja i odgovori, stručne i staleške vesti i *In memoriam*).

Priprema rukopisa

Propratno pismo

– Mora da sadrži svedočanstvo autora da rad predstavlja originalno delo, kao i da nije objavljivan u drugim časopisima, niti se razmatra za objavljivanje u drugim časopisima.

– Potvrditi da svi autori ispunjavaju kriterijume za autorstvo nad radom, da su potpuno saglasni sa tekstom rada, kao i da ne postoji sukob interesa.

– Navesti u koju kategoriju spada rad koji se šalje (originalni naučni rad, pregledni članak, prethodno saopštenje, stručni članak, prikaz slučaja, istorija medicine).

Rukopis

Za pisanje teksta koristiti *Microsoft Word for Windows*. Tekst treba otkucati koristeći font *Times New Roman*, na stranici formata A4, proredom od 1,5 (i u tabelama), sa marginama od 2,5 cm i veličinom slova od 12 pt. Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana. Naslovna strana treba da sadrži kratak i jasan naslov rada, bez skraćenica, zatim kratki naslov (do 40 karaktera), puna imena i prezimena autora (najviše 6 autora) indeksirana brojkama koje odgovaraju onima kojim se u zaglavlju navode uz pun naziv i mesta ustanova u kojima autori rade. Na dnu ove stranice navesti titulu, punu adresu, e-mail i broj telefona ili faksa autora zaduženog za korespondenciju.

2. Sažetak. Sažetak treba da sadrži do 250 reči, bez skraćenica, sa preciznim prikazom problematike, ciljeva, metodologije, glavnih rezultata i zaključaka. Sažetak treba da ima sledeću strukturu:

– originalni naučni radovi: uvod (sa ciljem rada), materijal i metode, rezultati i zaključak;

– prikaz slučaja: uvod, prikaz slučaja i zaključak;

– pregled rada: uvod, odgovarajući podnaslovi koji odgovaraju onima u tekstu rada i zaključak.

U nastavku navesti do deset ključnih reči iz spiska medicinskih predmetnih naziva (*Medical Subjects Headings, MeSH*) Američke nacionalne medicinske biblioteke.

3. Sažetak na engleskom jeziku. Sažetak na engleskom jeziku treba da bude prevod sažetka na srpskom jeziku, da ima istu strukturu i da sadrži do 250 reči, bez upotrebe skraćenica.

4. Tekst rada

– Tekst originalnih članaka mora da sadrži sledeće celine:

Uvod (sa jasno definisanim ciljem rada), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su korišćene u tekstu) i eventualna zahvalnost autora onima koji su pomogli u istraživanju i izradi rada.

– Tekst prikaza slučaja treba da sadrži sledeće celine: Uvod (sa jasno definisanim ciljem rada), Prikaz slučaja, Diskusija i Zaključak.

– Tekst treba da bude napisan u duhu srpskog jezika, oslobođen suvišnih skraćenica, čija prva upotreba zahteva navođenje punog naziva. Skraćenice ne upotrebljavati u naslovu, sažetku i zaključku. Koristiti samo opšte prihvaćene skraćenice (npr. DNA, MRI, NMR, HIV,...). Spisak skraćenice koje se navode u radu, zajedno sa objašnjenjem njihovog značenja, dostaviti na poslednjoj stranici rukopisa.

– Koristiti mere metričkog sistema prema Internacionalnom sistemu mera (*International System Units – SI*). Temperaturu izražavati u Celzijusovim stepenima (°C), a pritisak u milimetrima živinog stuba (mmHg).

– Ne navoditi imena bolesnika, inicijale ili brojeve istorija bolesti.

Uvod sadrži precizno definisan problem kojim se bavi studija (njegova priroda i značaj), uz navođenje relevantne literature i sa jasno definisanim ciljem istraživanja i hipotezom.

Materijal i metode treba da sadrže podatke o načinu dizajniranja studije (prospektivna/retrospektivna, kriterijumi za uključivanje i isključivanje, trajanje, demografski podaci, dužina praćenja). Statističke metode koje se koriste treba da budu jasne i detaljno opisane.

Rezultati predstavljaju detaljan prikaz podataka dobijenih tokom studije. Sve tabele, grafikoni, sheme i slike moraju da budu citirani u tekstu, a njihova

numeracija treba da odgovara redosledu pominjanja u tekstu.

Diskusija treba da bude koncizna i jasna, sa interpretacijom osnovnih nalaza studije u poređenju sa rezultatima relevantnih studija publikovanim u svetskoj i *domaćoj* literaturi. Navesti da li je hipoteza istraživanja potvrđena ili opovrgnuta. Izneti prednosti i ograničenja studije.

Zaključak u kratkim crtama mora da odbaci ili potvrdi pogled na problem koji je naveden u Uvodu. Zaključci treba da proizilaze samo iz vlastitih rezultata i da ih čvrsto podržavaju. Uzdržati se uopštenih i nepotrebnih zaključivanja. Zaključci u tekstu moraju suštinski odgovarati onima u Sažetku.

5. Literatura. Literatura se u tekstu označava arapskim brojevima u uglastim zagradama, prema redosledu pojavljivanja. Izbegavati veliki broj citata u tekstu. Za naslove koristiti skraćenice prema *Index Medicus*-u (http:// www.nlm.nih.gov/tsd/serials/lji.html). U popisu citirane literature koristiti Vankuverska pravila koja precizno određuju redosled podataka i znake interpunkcije kojima se oni odvajaju, kako je u nastavku dato pojedinim primerima. Navode se svi autori, a ukoliko ih je preko šest, navesti prvih šest i dodati et al.

Članci u časopisima:

∗ *Standardni članak*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *Organizacija kao autor*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *Nisu navedena imena autora*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *Volumen sa suplementom*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *Sveska sa suplementom*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *Sažetak u Časopisu*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije:

∗ *Jedan ili više autora*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Urednik(ci) kao autor*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *Poglavlje u knjizi*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders;1974. p. 457-72.

∗ *Rad u zborniku radova*

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.

∗ *Disertacije i teze*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ *Članak u Časopisu u elektronskoj formi*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monografije u elektronskoj formi*

CDI, clinical dermatology illustrated [monograph on CDROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *Kompjuterski dokument (file)*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computeried Educational Systems; 1993.

6. Prilozi (tabele, grafikoni, sheme i fotografije). *Dozvoljeno je najviše šest priloga!*

– Tabele, grafikoni, sheme i fotografije dostavljaju se na kraju teksta rukopisa, kao posebni dokumenti na posebnim stranicama.

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– Slike pripremiti u JPG, GIF TIFF, EPS i sl. formatu

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– Naslov, tekst u tabelama, grafikonima, shemama i legendama navesti na srpskom i na engleskom jeziku.

– Objasniti sve nestandardne skraćenice u fusnotama koristeći sledeće simbole: *, †, ‡, §, ||, ¶, **, ††, ‡‡.

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Prijem rukopisa vrši se u elektronskoj formi na stranici: **aseestant.ceon.rs/index.php/medpreg/**. Da biste prijavili rad morate se prethodno registrovati. Ako ste već registrovani korisnik, možete odmah da se prijavite i započnete proces prijave priloga u pet koraka.

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Ukoliko autor i svi koautori nisu uplatili članarinu za Medicinski pregled, rad neće biti štampan. Radovi koji nisu napisani u skladu sa pravilima Medicinskog pregleda, neće biti razmatrani. Recenzija će biti obavljena najkasnije u roku od 6 nedelja od prijema rada. Uredništvo zadržava pravo da i pored pozitivne recenzije donese odluku o štampanju rada u skladu sa politikom Medicinskog pregleda. Za sva dodatna obaveštenja obratiti se tehničkom sekretaru:

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E-mail: dlv@neobee.net

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Use Microsoft Word for Windows to type the text. The text must be typed in font Times New Roman, page format A4, space 1.5 (for tables as well), borders of 2.5 cm and font size 12pt. The manuscript should contain the following elements:

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– review papers should have the introduction, subtitles corresponding to those in the paper and conclusion. It is to be followed by up to 10 Key Words from the list of Medical Subject Headings, MeSH of the American National Medical Library.

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– The text should be written in the spirit of Serbian language, without unnecessary abbreviations, whose first mentioning must be explained by the full term they stand for. Abbreviations should not be used in the title, summary and conclusion. Only commonly accepted abbreviations (such as DNA, MRI, NMR, HIV…) should be used. The list of abbreviations used in the text, together with the explanation of their meaning, is to be submitted at the last page of the manuscript.

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and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the problem mentioned in the introduction. Conclusions must be based solely on the author's own results, corroborating them. Avoid generalised and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

5. References. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by et 'al'.

<u>Articles in journals</u>
A standard article
Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

An organisation as the author
Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

No author given
21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

A volume with supplement
Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

An issue with supplement
Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

A summary in a journal
Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

<u>Books and other monographs</u>
One or more authors
Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s) as author(s)
Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

A chapter in a book
Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

A conference paper
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.

A dissertation and theses
Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

<u>Electronic material</u>
A journal article in electronic format
Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

Monographs in electronic format
CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

Acomputer file
Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

6. Attachments (tables, graphs, schemes and photographs). The maximum number of attachments allowed is six!

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