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SAFETY OF PIMECROLIMUS AND TACROLIMUS IN THE TOPICAL THERAPY OF ATOPIC DERMATITIS

BEZBEDNOST LOKALNE PRIMENE PIMEKROLIMUSA I TAKROLIMUSA U TERAPIJI ATOPIJSKOG DERMATITISA

Marina JOVANOVIĆ

Atopic dermatitis (AD) as an inflammatory skin condition that commonly follows a chronic course associated with periods of remission and relapse. Being the first step in the "atopic march", it is often associated with other atopic manifestations, such as asthma, allergic rhinitis and food allergies (particularly in infants and children under the age of 2 years) [1–3]. The prevalence of AD is high, estimated to be 15–20% among children, and 1–3% in adults worldwide [1]. Representing a chronic, remitting-relapsing inflammatory dermatitis, AD is often diagnosed and managed by a multidisciplinary group of providers, including allergists, dermatologists, and primary care practitioners. Because the pathogenesis of AD is complex and multifactorial, there are numerous approaches to its therapeutic management. There is still a debate about whether atopy should be distinguished as an important but not required feature for the diagnosis of AD [7, 8]. Although both sides agree that the disease is diagnosed clinically based on the patient's history, characteristic clinical findings mandate the exclusion of other common cutaneous disorders before diagnosis, particularly such as contact dermatitis and cutaneous lymphomas [7, 8], they also recommend against obtaining routine specific IgE serum levels, and point to the lack of specific biomarkers required not only for diagnosis or severity assessment, but also for assessment of therapeutic efficacy [9], results in divergent approaches to the management of AD [10–13].

For many years, topical corticosteroids (TCS) have been the most common treatment for AD, especially if non-pharmacologic interventions have failed [8, 11]; it has been hypothesized that TCS therapy can impede the mechanisms of antigen-processing, thereby inhibiting the release of proinflammatory cytokines [11]. The TCS are effective for both active inflammation and for prophylaxis; however, there are no data to support a specific agent among the TCS classes, and there is limited evidence to recommend an optimal dosing or frequency regimen [8, 11]. Although TCS have long been considered and offered as a first line therapy for treatment of AD, they are associated with serious adverse side effects [5–9].

Due to the chronic nature of AD, effective and safe treatment that can be used as long-term management is of utmost importance [2]. Introduction of the topical calcineurin inhibitors (TCIs), tacrolimus (T) and pimecrolimus (P) almost 15 years ago, was a major breakthrough for the topical anti-inflammatory treatment of AD [14]. TCIs, including T and P, were approved by the Food and Drug Administration (FDA) agency for the treatment of AD in 2000 and 2001, respectively. The TCIs are anti-inflammatory drugs with a lipophilic structure that act by inhibiting the calcineurin phosphatase which disrupts the activation of T cells and mast cells as well as the transcription and release of inflammatory cytokines [15, 16]. Topical T and topical P are the only TCIs in Europe. In 2002, topical T was approved for short or intermittent long-term treatment of moderate to severe AD in Europe. Two concentrations of topical T are available: 0.1% for patients over 16 years of age, and 0.03% for children over 2 years of age. The indication for topical T was extended in 2009 to maintenance treatment of AD. Topical P in a concentration of 1% was approved in 2002 for the treatment of mild-to-moderate AD in patients over 2 years of age. Both drugs are approved for second-line treatment, when other treatments have been ineffective or are contraindicated. [6]. The American Academy of Dermatology

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Abbreviations

AD – atopic dermatitis
TCS – topical corticosteroids
TCI – topical calcineurin inhibitor
T – tacrolimus
P – pimecrolimus
FDA – Food and Drug Administration
AAD – American Academy of Dermatology

(AAD) Guidelines recommend that TCS can be initially used to control a flare, whereas TCI can be applied as maintenance therapy to prevent relapse, although the evidence for this concurrent regimen has been inconsistent. The TCIs are usually offered as a second-line therapy for acute and chronic treatment of AD in patients who have not responded adequately to other topical treatments or when those treatments are not recommended [2]. The guidelines agree that use of TCI, particularly P, at sites of sensitive or thin skin, offers an advantage over use of TCS [8, 11, 14]. A practical algorithm for topical treatment of AD in the Middle East emphasizes the importance of sensitive skin areas [17]. Twice-daily application of either T ointment or P cream is efficacious in treating inflamed AD lesions and resolving pruritus. Unlike TCS, long-term TCI use does not carry the risks of skin atrophy, impaired epidermal barrier function or enhanced percutaneous absorption, and so it is suitable for AD treatment especially in sensitive skin areas [14]. Tacrolimus has been shown to impact Langerhans cells while P does not [12]; in addition to their anti-inflammatory effects, both TCIs have been shown to have additional positive effects on epidermal integrity [18–20]. Murrell et al. reported that patients treated with P saw a reversal of skin thinning of the neck and head, including the eyelids [21]. These treatments provide a safe alternative to TCS, particularly in the treatment of sensitive skin sites such as the head and neck [2, 21]. The most common side effects of TCI are localized site reactions, including burning, stinging, and pruritus, which commonly occur during the first week of treatment; it is important to counsel patients on these potential side effects to prevent premature discontinuation of treatment [10].

There was a temporary decrease in the use of topical T and persistent reduction in topical P in Europe since 2004. Safety warnings issued by regulatory agencies about a potential risk of cancer may have contributed to the reduction in users especially in children, in all countries [6]. In 2006, the FDA agency issued a label change; in the European Union as well as in the United States the labeling of topical T and topical P was updated by adding a warning about cautious use, in order to reduce the potential risk of skin cancer and lymphoma. In 2006, the FDA agency instituted a boxed warning for both TCIs based on a theoretical risk of malignancy (including lymphomas). Currently, P 1% cream is indicated in patients with mild-to-moderate AD aged > 2 years [10]. The age restriction was emphasized in a boxed warning added by the FDA agency in January 2006, which also highlights the lack of long-term safety data and the theoretical risk of skin malignancy and lymphoma [14, 22, 23]. Since then, P has been extensively investigated in short- and long-term studies including over 4000 infants (< 2 years old). These studies showed that P effectively treats AD in infants, with sustained improvement with long-term intermittent use [14, 16]. A decade's worth of clinical experience, epidemiological data, post-marketing surveillance, and adverse event database monitoring failed to demonstrate a causal relationship between TCI use and malignancy [23]. This boxed warning was based on a theoretical increase in risk of malignancy including lymphoma [5]. Prior to its topical use in AD, oral and intravenous T had been used to suppress the systemic immune system in transplant patients. Malignancies have been associated with oral T when used systemically at high concentrations. An animal study with P using a 30 fold greater exposure than seen with topical use, also resulted with associated malignancy development [23]. These findings were used to support the addition of the black box warning. New malignancies have been reported in patients using topical P or T as well. These have been reported by the FDA agency and by the parent drug manufacturer. Independent experts reviewing these cases found no causal association between topical use of TCIs and malignancy. The rate of reported lymphoma in patients exposed to topical T was lower than the expected incidence in age-matched controls [24]. No increased risk of malignancy has been seen in recent meta-analyses or the 10-year Pediatric Eczema Elective Registry as of May 2014 [16, 25].

The American Academy of Allergy, Asthma & Immunology, American College of Allergy, Asthma & Immunology/ the Joint Council of Allergy, Asthma & Immunology, and the AAD Guidelines, advocate for proactive therapy regimen regarding the boxed warning on TCI, although a causal relationship has not been established [10]. For reactive therapy, a twice-daily application of TCS is commonly recommended in the treatment of acute AD. For "proactive" maintenance therapy, the AAD suggests once- to twice-weekly application of TCS in commonly flaring areas to prevent relapses [8, 11]. The Joint Task Force and the AAD Guidelines review large prospective studies that suggest a correlation between increased risks of lymphoma with AD disease severity, without an association with TCI use [10]. Most importantly, the studies of P in infants provided no evidence for systemic immunosuppression, and a comprehensive body of evidence from clinical studies, post-marketing surveillance and epidemiological investigations does not support potential safety concerns [14]. Pimecrolimus 1% is approved for second-line therapy in children (≥ 2 years old) and adults with mild-to-moderate AD [26]. Despite these FDA agency approved indications, clinical trials have shown drug safety in patients as young as 3 months and in long-term use in patients

of all ages [16, 27, 28]. Clinical trials have demonstrated that P is safe for long-term management, as well as effective in achieving clearance of AD lesions and associated symptoms: pruritus was shown to be significantly reduced in as early as 48 hours after initiation of treatment. With photoprotection, TCIs have a favorable safety profile without evidence for increased risk for lymphoma [29].

Although many studies have shown that long-term use of T and P is effective with a favorable safety profile and low systemic absorption, the black box warning has remained in place. A recent systematic review of clinical trials and meta-analyses has shown no significant increased risk of malignancy with TCI use [25]. Systematic analysis has shown efficacy and safety of TCI therapy (at least 6 weeks long), in comparrison with low or mid-potency TCS (hydrocortisone 1% or hydrocortisone butyrate 0.1% cream/ointment), in children with AD aged < 12 years. This comprehensive literature review supports the safety of long-term TCI and intermittent low- to mid-potency TCS therapy in children with AD, with no evidence of cutaneous atrophy or cumulative systemic exposure and no reports of lymphoma [25].

Conclusion

Long-term management of mild-to-moderate atopic dermatitis in infants ≥ 3 to < 12 months old with pimecrolimus or topical corticosteroids has shown that both pimecrolimus and topical corticosteroids had a rapid onset of action with 50% of patients achieving treatment success by week 3; after 5 years, 85% and 95% of patients in each group achieved overall and facial treatment success, respectively. The profile and frequency of adverse events was similar in the 2 groups; there was no evidence for impairment of humoral or cellular immunity in either of groups. The data suggest and support the use of pimecrolimus as a first-line treatment of mild-to moderate atopic dermatitis in infants and children [16]. No firm conclusions can be made on the theoretically increased risk for malignancies associated with a long-term use of pimecrolimus in infants [30].

References

1. Avena-Woods C. Overview of atopic dermatitis. Am J Manag Care. 2017;23(8 Suppl):S115-23.

2. Papier A, Strowd LC. Atopic dermatitis: a review of topical nonsteroid therapy. Drugs Context. 2018;7:212521.

3. Schneider L, Hanifin J, Boguniewicz M, Eichenfield LF, Spergel JM, Dakovic R, et al. Study of the atopic march: development of atopic comorbidities. Pediatr Dermatol. 2016;33(4):388-98.

4. Garg N, Silverberg JI. Epidemiology of childhood atopic dermatitis. Clin Dermatol. 2015;33(3):281-8.

5. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet. 2006;368(9537):733-43.

6. Kuiper JG, van Herk-Sukel MPP, Castellsague J, Pottegård A, Berglind IA, Dedman D, et al. Use of topical tacrolimus and topical pimecrolimus in four European countries: a multicentre database cohort study. Drugs Real World Outcomes. 2018;5(2):109-16.

7. Eichenfield LF, Tom WL, Chamlin SL, Feldman SR, Hanifin JM, Simpson EL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. J Am Acad Dermatol. 2014;70(2):338-51.

8. Schneider L, Tilles S, Lio P, Boguniewicz M, Beck L, LeBovidge J, et al. Atopic dermatitis: a practice parameter update 2012. J Allergy Clin Immunol. 2013;131(2):295-9.e1-27.

9. Guttman-Yassky E, Ungar B, Malik K, Dickstein D, Suprun M, Estrada YD, et al. Molecular signatures order the potency of topically applied anti-inflammatory drugs in atopic dermatitis patients. J Allergy Clin Immunol. 2017;140(4):1032-42.e13.

10. Eichenfield LF, Ahluwalia J, Waldman A, Borok J, Udkoff J, Boguniewicz M. Current guidelines for the evaluation and management of atopic dermatitis: a comparison of the Joint Task Force Practice Parameter and American Academy of Dermatology guidelines. J Allergy Clin Immunol. 2017;139(4S):S49-57.

11. Eichenfield LF, Tom WL, Berger TG, Krol A, Paller AS, Schwarzenberger K, et al. Guidelines of care for the management of atopic dermatitis: section 2. Management and treatment of atopic dermatitis with topical therapies. J Am Acad Dermatol. 2014;71(1):116-32.

12. Jovanović M, Poljački M, Đuran V, Vujanović Lj, Sente R, Stojanović S. Kontaktna senzibilizacija izazvana Compositae biljkama kod obolelih od atopijskog dermatitisa. Med Pregl. 2004;57(5-6):209-18.

13. Jovanović M, Boža P, Karadaglić Dj, Brkić S, Petrović A, Mimica-Dukić N, et al. Contact sensitivity in patients with psoriasis in Vojvodina. Int Arch Allergy Immunol. 2009;148 (4):311-20.

14. Luger T, Boguniewicz M, Carr W, Cork M, Deleuran M, Eichenfield L, et al. Pimecrolimus in atopic dermatitis: consensus on safety and the need to allow use in infants. Pediatr Allergy Immunol. 2015;26(4):306-15.

15. Carr WW. Topical calcineurin inhibitors for atopic dermatitis: review and treatment recommendations. Paediatr Drugs. 2013;15(4):303-10.

16. Sigurgeirsson B, Boznanski A, Todd G, Vertruyen A, Schuttelaar ML, Zhu X, et al. Safety and efficacy of pimecrolimus in atopic dermatitis: a 5-year randomized trial. Pediatrics. 2015;135(4):597-606.

17. Reda AM, Elgendi A, Ebraheem AI, Aldraibi MS, Qari MS, Abdulghani MMR, et al. A practical algorithm for topical treatment of atopic dermatitis in the Middle East emphasizing the importance of sensitive skin areas. J Dermatol Treat. 2018 Nov 21:1-8.

18. Danby SG, Chittock J, Brown K, Albenali LH, Cork MJ. The effect of tacrolimus compared with betamethasone valerate on the skin barrier in volunteers with quiescent atopic dermatitis. Br J Dermatol. 2014;170(4):914-21.

19. Dähnhardt-Pfeiffer S, Dähnhardt D, Buchner M, Walter K, Proksch E, Fölster-Holst R. Comparison of effects of tacrolimus ointment and mometasone furoate cream on the epidermal barrier of patients with atopic dermatitis. J Dtsch Dermatol Ges. 2013;11(5):437-43.

20. Chittock J, Brown K, Cork MJ, Danby SG. Comparing the effect of a twice-weekly tacrolimus and betamethasone valerate dose on the subclinical epidermal barrier defect in atopic dermatitis. Acta Derm Venereol. 2015;95(6):653-8.

21. Murrell DF, Calvieri S, Ortonne JP, Ho VC, Weise-Riccardi S, Barbier N, et al. A randomized controlled trial of pimecrolimus cream 1% in adolescents and adults with head and neck atopic dermatitis and intolerant of, or dependent on, topical corticosteroids. Br J Dermatol. 2007;157(5):954-9.

22. European Medicines Agency. Summary information on referral opinion pursuant to Article 31 of Council Directive 2001/83/EC, as amended, for Elidel and associated names [Internet]. London: EMEA; 2006 [cited 2019 Mar 27]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Elidel_31/WC500012313.pdf.

23. Siegfried EC, Jaworski JC, Hebert AA. Topical calcineurin inhibitors and lymphoma risk: evidence update with implications for daily practice. Am J Clin Dermatol. 2013;14(3):163-78.

24. Ormerod AD. Topical tacrolimus and pimecrolimus and the risk of cancer: how much cause for concern? Br J Dermatol. 2005;153(4):701-5.

25. Siegfried EC, Jaworski JC, Kaiser JD, Hebert AA. Systematic review of published trials: long-term safety of topical corticosteroids and topical calcineurin inhibitors in pediatric patients with atopic dermatitis. BMC Pediatr. 2016;16:75.

26. Elidel (pimecrolimus) cream 1% - prescribing information [Internet]. Wehr: Novartis; 2006 [cited 2019 Mar 26]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/021302s011lbl.pdf.

27. Eichenfield LF, Ho V, Matsunaga J, Leclerc P, Paul C, Hanifin JM. Blood concentrations, tolerability and efficacy of pimecrolimus cream 1% in Japanese infants and children with atopic dermatitis. J Dermatol. 2007;34(4):231-6.

28. Lakhanpaul M, Davies T, Allen BR, Schneider D. Low systemic exposure in infants with atopic dermatitis in a 1-year pharmacokinetic study with pimecrolimus cream 1%*. Exp Dermatol. 2006;15(2):138-41.

29. Jovanović M, Golušin Z. Nonsteroidal topical immunomodulators in allergology and dermatology. Biomed Res Int. 2016;2016:5185303.

30. Weidinger SH, Baurecht H, Schmitt J. A 5-year randomized trial on the safety and efficacy of pimecrolimus in atopic dermatitis: a critical appraisal. Br J Dermatol. 2017;177(4):999-1003.

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LAPAROSCOPIC ASSISTED VAGINAL HYSTERECTOMY AT THE CLINIC OF GYNECOLOGY AND OBSTETRICS IN NOVI SAD

LAPAROSKOPSKI ASISTIRANA VAGINALNA HISTEREKTOMIJA NA KLINICI ZA GINEKOLOGIJU I AKUŠERSTVO U NOVOM SADU

Srđan ĐURĐEVIĆ[1, 2], Aleksandra VEJNOVIĆ[1, 2] and Miloš PANTELIĆ[1, 2]

Summary

Introduction. Laparoscopic assisted vaginal hysterectomy is a surgical procedure with uterine artery ligation followed by vaginal removal of the uterus. The first laparoscopic assisted vaginal hysterectomy was performed by Harry Reich in 1989. **Material and Methods.** The sample included 24 patients who underwent surgery at the Clinic of Gynecology and Obstetrics, Clinical Center of Vojvodina in Novi Sad in the period 2014 - 2017. The most common indications for laparoscopic assisted vaginal hysterectomy included mild uterine prolapse and uterine fibroids (15 patients, 62.5%). The surgery was carried out in two stages: the first, laparoscopic stage and the second, vaginal stage. The laparoscopic stage included mobilization of the bladder, ovaries and uterus to the level of uterine vessels. In the second stage, the cervix was approached vaginally and detached from the urinary bladder, after which the uterus with cervix and adnexa were removed through the vagina. **Results.** The average age of patients was 56.8 years; on average, the surgeries lasted 140 minutes and the mean blood loss was 190 ml. Two (8.3%) patients experienced bladder and ureteral injuries which were resolved by urologists. Laparoscopic assisted vaginal hysterectomy was the only procedure performed in 5 (20.8%) patients, whereas it was combined with anterior and/or posterior colporrhaphy in 14 (58.4%), with pelvic lymphadenectomy in 3 (12.5%) patients, and with uterine morcellation in 2 (8.3%) patients. **Conclusion.** There are no published controlled trials related to the use of laparoscopic assisted vaginal hysterectomy and total laparoscopic hysterectomy in Serbia. This paper presents the preliminary results of the laparoscopic assisted vaginal hysterectomy in 24 patients, comparing them with other techniques of hysterectomy conducted at the Clinic of Gynecology and Obstetrics, Clinical Center of Vojvodina in Novi Sad in the period 2014 - 2017. Laparoscopic assisted vaginal hysterectomy is a good option for surgical treatment of patient with combined pathology of genital organs.

Key words: Hysterectomy, Vaginal; Laparoscopy; Uterine Myomectomy; Leiomyoma; Uterine Prolapse; Gynecologic Surgical Procedures; Intraoperative Complications

Sažetak

Uvod. Laparoskopski asistirana vaginalna histerektomija je hirurška tehnika u toku koje se nakon ligature arterije uterine vrši odstranjenje materice vaginalnim putem. Prvu laparoskopski asistiranu vaginalnu histerektomiju izveo je Heri Rajk (*Harry Reich*) 1989. godine. **Materijal i metode.** Ispitivani uzorak obuhvatio je 24 pacijentkinje koje su operisane u Novom Sadu. Najčešće indikacije za laparoskopski asistiranu vaginalnu histerektomiju bile su početni spad i miomi materice kod 15 (62,5%) pacijentkinja. Laparoskopski asistiranu vaginalnu histerektomiju vršili smo u dva dela: laparoskopskom i vaginalnom. Laparoskopski deo obuhvatio je oslobađanje mokraćne bešike, jajnika i materice do nivoa arterija i vena uterina. U drugom delu operacije vaginalnim putem je oslobođen grlić materice od pripoja sa mokraćnom bešikom i kroz vaginu izvađena materica sa jajnicima i jajovodima. **Rezultati.** Prosečna starost pacijentkinja bila je 56,8 godina, srednje vreme trajanja operacije iznosilo je 140 minuta a prosečan gubitak krvi bio je 190 ml. Kod dve (8,3%) pacijentkinje došlo je do povrede mokraćne bešike i uretera što je zbrinuo urolog. Laparoskopski asistirana vaginalna histerektomija bez dopunskih operacija sprovedena je kod pet (20,8%) pacijentkinja, u zajednici sa prednjom i/ili zadnjom kolporafijom kod 14 (58,4%), sa pelvičnom limfadenektomijom kod tri (12,5 %) i sa intraoperativnim komadanjem materice kod dve pacijentkinje (8,3%). **Zaključak.** U Srbiji još uvek nema publikovanih kontrolisanih istraživanja koja se odnose na primenu laparoskopski asistirane vaginalne i totalne laparoskopske histerektomije. U radu smo prikazali preliminarne rezultate primene laparoskopski asistirane vaginalne histerektiomije kod 24 operisane pacijentkinje i uporedili ih sa drugim tehnikama histerektomije koje su sprovedene na Klinici za ginekologiju i akušerstvo Kliničkog centra Vojvodine u Novom Sadu u periodu od 2014. do 2017. godine.

Ključne reči: vaginalna histerektomija; laparoskopija; miomektomija uterusa; lejomiom; prolaps uterusa; ginekološke hirurške procedure; intraoperativne komplikacije

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Abbreviations

LAVH – laparoscopic assisted vaginal hysterectomy
TLH – total laparoscopic hysterectomy
TAH – total abdominal hysterectomy
VH – vaginal hysterectomy
US – United States

Introduction

Hysterectomy is a surgical removal of the uterus. It can be performed through the abdominal wall – open surgery, total abdominal hysterectomy (TAH) and laparoscopically, total laparoscopic hysterectomy (TLH), or through natural orifice, vaginal hysterectomy (VH), and combined laparoscopic assisted vaginal hysterectomy (LAVH) [1]. Approximately 570.000 hysterectomies are performed in the United States (US) every year, which makes this surgical procedure one of the most common surgeries worldwide. Nowadays, 50% of hysterectomies are performed as open surgery, and another 50% laparoscopically or vaginally [2]. The first vaginal hysterectomy was done in 1813 by Langebeck from Göttingen (Germany), whereas the first successful abdominal hysterectomy was performed in 1853 by Ellis Burnham from US [3]. In 1989, Harry Reich from US was the first to perform LAVH, thus starting a new era of laparoscopic hysterectomy [4]. Vaginal hysterectomy is considered to be a truly minimally invasive approach. Advantages of vaginal approach are related to shorter hospital stay, less blood loss, fewer postoperative complications, lower infection rate and faster return to work [5]. In abdominal hysterectomy, all these parameters are higher, as well as the incidence of febrile morbidity [6]. The learning curve for laparoscopic hysterectomy is greater than for open surgery, and the length of surgery time and risk of intraoperative complications are inversely proportional to the amount of training [7]. After 30 years since the first laparoscopic hysterectomy was performed, laparoscopic surgery in gynecology found its right place. Selection of surgical approach for hysterectomy in individual patient depends on various factors: technical factors, experience and training of the surgeon, clinical factors, size of the uterus, previous operations, number of deliveries etc. [1, 2, 5–7]. LAVH was the first laparoscopic procedure applied to remove the uterus. The surgery has two parts: laparoscopic and vaginal. The laparoscopic part includes ligation of uterine arteries and veins. According to medical indications and request of the patient, tubes and ovaries may be removed together with the uterus and cervix. Colpotomy i. e. vaginal incision, removal of the uterus and vaginal suturing are all performed through the vagina [8]. The aim of this paper is to present a four-year experience in LAVH at the Clinic of Gynecology and Obstetrics of the Clinical Center of Vojvodina in Novi Sad, Serbia.

Material and Methods

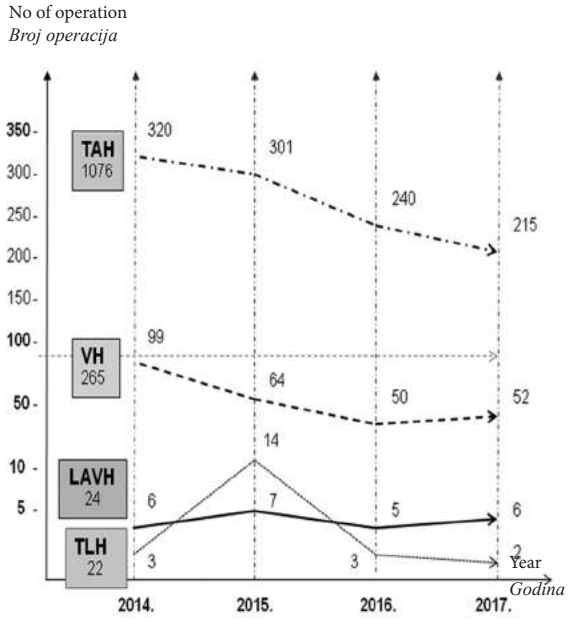
The sample included 24 patients who underwent LAVH at the Clinic of Gynecology and Obstetrics, Clinical Center of Vojvodina in Novi Sad in the period from 2014 – 2017. Preoperative gynecological examination was performed in each patient, as well as transvaginal ultrasound. In patients with irregular genital bleeding, additional diagnostic procedures were carried out: dilation and curettage, cervical biopsy and pathohistology. After estimating the size of the uterus, stage of pelvic organ prolapse and spaciousness of vagina, indications for LAVH were determined. Basic criterion was the size of the uterus, which was not greater than the gravid uterus at the age of 16 gestational weeks. Prior to surgery, each patient underwent standard blood and urine testing, chest X-ray, electrocardiography (ECG) and

Table 1. Characteristics of patients operated using different techniques of hysterectomy at the Clinic of Gynecology and Obstetrics of the Clinical Center of Vojvodina in the period 2014–2017
Tabela 1. *Karakteristike pacijentkinja operisanih primenom različitih tehnika histerektomije na Klinici za ginekologiju i akušerstvo Kliničkog centra Vojvodine u periodu 2014–2017*

Characteristics *Karakteristike*	Number/Range *Broj/Opseg*				Average values/Percentage *Prosečne vrednosti/Procenti*			
	LAVH	TLH	VH	TAH	LAVH	TLH	VH	TAH
Age/*Uzrast*	36 – 70 years *godina*	38-72	37-79	25-81	56,8 years *godina*	43.7	63.5	47.1
Length of surgery/*Operativno vreme*	90 – 185 min. *minuta*	125-190	90-135	80-150	140 min. *minuta*	142	115	121
Blood loss/*Gubitak krvi*	50 – 400 ml. *mililitara*	20-300	50-450	50-1500	190 ml. *mililitara*	150	205	265
Length of stay/*Boravak u bolnici*	5 – 30 days *dana*	3-6	5-10	4-12	10,9 days *dana*	3,9	7.1	6.7
Complications/*Komplikacije*	2	6	17	29	8%	27.2%	6.4%	2.7%

Legend: TAH – total abdominal hysterectomy; VH – vaginal hysterectomy; LAVH – laparoscopic assisted vaginal hysterectomy; TLH – total laparoscopic hysterectomy
Legenda: TAH – totalna abdominalna histerektomija; VH – vaginalna histerektomija; LAVH – laparoskopski asistirana vaginalna histerektomija; TLH – totalna laparoskopska histerektomija

examination by internal medicine specialist and anesthesiologist. If the hemoglobin level was less than 100 g/l, blood reservation was made. Computed tomography (CT) of the chest, abdomen and pelvis was performed in patients with verified cervical or endometrial cancer. If there were some ovarian cysts or tumors, frozen section was performed for intraoperative diagnosis. All patients underwent standard bowel preparation 24 – 48 hours prior to surgery and thromboprophylaxis with elastic stockings or bandages. According to the body mass, prophylactic dose of Fraxiparine or Clexane was administered subcutaneously two hours or night before surgery. Antibiotic prophylaxis (first generation of cephalosporins, 1 – 2 g) was administered within one hour before skin incision. LAVH was performed in two stages: laparoscopic and vaginal. Patients were placed in lithotomy position. Vaginal cleansing, bladder catheterization and disinfection of the operative field were done after endotracheal intubation. The surgery started with placement of 3 – 4 ports. Abdominal entry was performed differently, depending on the surgeon. Uterus manipulator was used to move the uterus to the right position (cranially, laterally etc.). Laparoscopic stage of the surgery included mobilization of the uterus by cutting round and broad ligaments. When indicated, laparoscopic part also included salpingectomy or adnexectomy. After cutting the round ligaments, vesicouterine fold was transected and the bladder was mobilized. The next step was coagulation and cutting of the paracervical tissue together with the uterine vessels. The second part of the LAVH started with incision of the vaginal mucosa to separate it from the cervix and bladder. It continued with sharp transection of the cervicovesical ligament and pushing the bladder upwards. Laterally, paracervical tissue and cardinal ligament were grasped, cut and ligated. Then, the abdominal cavity was open by cutting the peritoneum anteriorly (vesicouterine fold) and posteriorly (rectouterine fold). Thereafter, uterine vessels were grasped, cut and ligated once again. A specimen was sent for pathohistological examination. Pelvic peritonization was done by a purse-string suture. If there was a genital prolapse (cystocele/rectocele), the sur-



Graph 1. Incidence of different techniques of hysterectomy at the Clinic of Gynecology and Obstetrics of the Clinical Center of Vojvodina in the period 2014 – 2017

Grafikon 1. *Učestalost različitih tehnika histerektomije na Klinici za ginekologiju i akušerstvo Kliničkog centra Vojvodine u periodu 2014–2017.*

Legend: TAH – total abdominal hysterectomy; VH – vaginal hysterectomy; LAVH – laparoscopic assisted vaginal hysterectomy; TLH – total laparoscopic hysterectomy

Legenda: TAH – totalna abdominalna histerektomija; VH – vaginalna histerektomija; LAVH – laparoskopski asistirana vaginalna histerektomija; TLH – totalna laparoskopska histerektomija

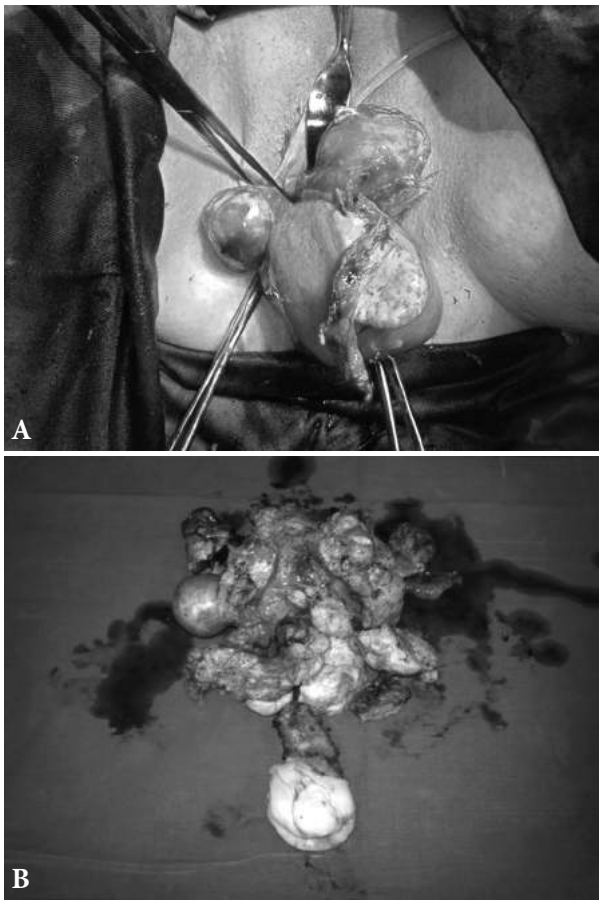


Figure 2. **A** – vaginal part of the laparoscopic assisted vaginal hysterectomy; **B** – specimen of morcellated myomatous uterus with mild uterine prolapse and hypertrophic cervix

Slika 2. ***A*** *– vaginalni deo laparoskopski asistirane vaginalne histerektomije* ***B*** *– preparat materice nakon komadanja u delovima, početni spad materice sa hipertrofijom cerviksa*

Table 2. Indications for laparoscopic assisted vaginal hysterectomy
Tabela 2. *Indikacije za laparoskopski asistiranu vaginalnu histerektomiju*

Indications/*Indikacije*	Number/*Broj*	%
Uterine fibroids/*Miomi materice*	6	25
Mild uterine prolapse/*Početni spad materice*	9	37,5
Ovarian tumors/*Tumori jajnika*	4	16,7
Complex endometrial hyperplasia/*Kompleksna hiperplazija endometrijuma*	2	8,3
Endometrial cancer/*Karcinom endometrijuma*	2	8,3
Cervical cancer/*Karcinom cerviksa*	1	4,2
Total/*Ukupno*	24	100

gery included appropriate correction and suturing of the vaginal wall. Postoperatively, the patients spent 1 – 2 days at the semi-intensive care unit, with early ambulation. The Foley catheter was removed 48 hours after surgery, or 72 hours after surgery if anterior repair and Kelly plication was performed.

Results

The incidence of different hysterectomy techniques performed at the Clinic of Gynecology and Obstetrics of the Clinical Center of Vojvodina in the period 2014 – 2017 is shown in **Graph 1**. About 3403 various gynecological surgeries were performed at the Department of Surgery of the Clinic of Gynecology and Obstetrics, Clinical Center of Vojvodina in Novi Sad, during the period from 2014 to 2017, out of which 1387 (40.7%) were different types of hysterectomy. In the group of 1387 hysterectomy patients, total abdominal hysterectomy (TAH) was done in 1076 (77.6%), vaginal hysterectomy (VH) in 265 (19.1%), LAVH in 24 (1.7%) and total laparoscopic hysterectomy (TLH) in 22 (1.6%) cases. The most frequent surgical technique was TAH. Basic characteristics of patients who underwent different techniques of hysterectomy (LAVH, TLH, VH, TAH) at the Clinic of Gynecology and Obstetrics of the Clinical Center of Vojvodina (age, length of surgery, blood loss, drainage, hospital stay, complications) are presented in **Table 1**. The average age in the LAVH group was 56.8 years, the mean length of surgery was 140 minutes, and 190 ml average blood loss. The hospital stay was between 7 and 30 days, 10.9 days on average. Postoperative complications occurred in 2 (8.3%) cases: urinary bladder injury [1] and thermal ureteral injury [1]. **Table 2** shows the main indications for LAVH: uterine fibroids [6], mild uterine prolapse [9], ovarian tumors [4], complex endometrial hyperplasia [2], endometrial cancer [2] and cervical cancer [1]. Six surgeons, gynecology specialists, performed all the surgeries. LAVH was the only surgical procedure in 5 (20.5%) patients and it was concomitantly performed with anterior or posterior colporrhaphy in 14 (58.4%) patients. Three (12.5%) patients had additional pelvic lymphadenectomy and morcellation of the uterus was performed in 2 (8.3%) patients, shown in **Figure 2**: vaginal part of the LAVH and a specimen of morcellated myomatous uterus.

Discussion

Vaginal hysterectomy is the oldest, safest and minimally invasive type of hysterectomy. Despite this fact, only 25% of all hysterectomies are performed due to benign diseases [1, 3]. This particularly applies to uterine fibroids, which are the most common indication for hysterectomy. In surgical treatment of uterine fibroids, VH accounts only for 4% [5, 9]. In the last 15 years, technological advancement and development of modern endoscopic equipment has led to the significant increase in the number of laparoscopic hysterectomies. Specifically designed instruments and optics are inserted into the abdominal cavity through small abdominal incisions through which the ports are placed. Hysterectomy can be completely done laparoscopically, meaning that all dissections, uterus mobilization and removal are performed in the abdominal cavity [10]. The LAVH is performed when there are contraindications for open surgery, or if the surgery cannot be brought to an end only laparoscopically. Indications for LAVH include benign and malignant diseases of the upper genital tract, when surgical treatment is indicated and the uterus can be removed through the vagina: fibroids, adenomyosis, dysfunctional uterine bleeding, endometriosis, endometrial hyperplasia, cervical dysplasia, endometrial and cervical carcinomas, ovarian cysts, mild genital prolapse etc. LAVH is contraindicated if the vagina is narrow and atrophic, so that the operating field is unapproachable. A large myomatous uterus, that cannot be removed vaginally, is a relative contraindication. LAVH is also contraindicated in large solid ovarian tumors, retroperitoneal and fixed pelvic tumors, extensive and firm adhesions after inflammatory disease or radiotherapy, as well as cardiopulmonary diseases which are incompatible with Trendelenburg position [2, 11]. In our sample, the most common indications for LAVH were mild uterine prolapse and uterine fibroids in 15 (62.5%) patients. The average age of the patients was 56.8 years, surgeries lasted 140 minutes on average, and the mean blood loss was 190 ml. Lee et al. and Chin et al. published similar results [12, 13]. Two (8.3%) patients suffered injuries of the urinary bladder and ureter. Sharp injury of urinary bladder occurred during the vaginal part of the surgery. It was im-

mediately detected and resolved by the urologist. Injury of the ureter was thermal. It occurred during electrocoagulation of uterine artery, but it was detected when the patient became symptomatic six days after surgery. The patient complained about back pain, became febrile and retroperitoneal effusion was detected on ultrasound. She was reoperated and the damaged segment of the ureter was resected and reimplanted into the bladder by urologist. These complications were the reason for longer hospital stay for both patients, 21 and 30 days respectively. These outliers affected the average hospital stay to 10.9 days. LAVH was the only procedure performed in 5 (20.8%) patients, whereas it was combined with anterior and/or posterior colporrhaphy in 14 (58.4%), with pelvic lymphadenectomy in 3 (12.5%) patients and with uterine morcellation in 2 (8.3%) patients. Hong and Young compared two different techniques of LAVH in the period 2010 – 2015 [14]. Classical LAVH technique was performed in 234 patients; myomatous uterus was indicated in the majority of cases (150 patients, 64.1%) and 9 (3.8%) bladder injuries were recorded, which is in accordance with our results. Ferrari et al. published results of randomized study. They compared LAVH and TAH during 24 months [15]. LAVH group comprised 31 patients, average age 48 years. The average length of surgery time was 135 minutes, similar to our results. Conversion from LAVH to open surgery was made in 3 patients without major complications. The length of hospital stay was from 3 to 8 days, which is less than at our clinic.

Conclusion

Although laparoscopic assisted vaginal hysterectomy is in clinical practice since 1989, it is only occasionally performed in health centers in Serbia. There are no published controlled trials related to the use of laparoscopic assisted vaginal and total laparoscopic hysterectomy in Serbia. This paper presents our experience with laparoscopic assisted vaginal hysterectomy. It is a good option for surgical treatment of patients with combined pathology of genital organs.

References

1. Papadopulos A. Laparoscopic assisted vaginal hysterectomy. In: Devaja O, Đurđević S, editors. Laparoscopic surgery in gynecology. Petrovaradin: Futura; 2016. p. 138-45. Serbian.

2. Ramesh B, Dimri PS. Textbook and atlas of laparoscopic hysterectomy. New Delhi, London, Philadelphia, Panama: Jaypee Brothers Medical Publishers; 2016.

3. Senn N. The early history of vaginal hysterectomy. JAMA. 1895;25(12):476-82.

4. Reich H, DeCaprio J, McGlynn F. Laparoscopic hysterectomy. J Gynecol Surg. 1989;5(2):213-6.

5. Johnson N, Barlow D, Lethaby A, Tavender E, Curr L, Garry R. Methods of hysterectomy: systematic review and meta-analysis of randomised controlled trials. BMJ. 2005;330(7506):1478.

6. Shin JW, Lee HH, Lee SP, Park CY. Total laparoscopic hysterectomy and laparoscopy-assisted vaginal hysterectomy. JSLS. 2011;15(2):218-21.

7. Müller A, Thiel FC, Renner SP, Winkler M, Häberle L, Beckmann MW. Hysterectomy - a comparison of approaches. Dtsch Arztebl Int. 2010;107(20):353-9.

8. Horng SG, Huang KG, Lo TS, Soong YK. Bladder injury after LAVH: a prospective, randomized comparison of vaginal and laparoscopic approaches to colpotomy during LAVH. J Am Assoc Gynecol Laparosc. 2004;11(1):42-6.

9. David-Montefiore E, Rouzier R, Chapron C, Darai E. Surgical routes and complications of hysterectomy for benign disorders: a prospective observational study in French university hospitals. Hum Reprod. 2007;22(1):260-5.

10. Song T, Kim TJ, Kang H, Lee YY, Choi CH, Lee JW, et al. A review of the technique and complications from 2012 cases of laparoscopically assisted vaginal hysterectomy at a single institution. Aust N Z J Obstet Gynaecol. 2011;51(3):239-43.

11. Kluivers KB, Hendriks JC, Mol BW, Bongers MY, Bremer GL, de Vet HC, et al. Quality of life and surgical outcome after total laparoscopic hysterectomy versus total abdominal hysterectomy for benign disease: a randomized, controlled trial. J Minim Invasive Gynecol. 2007;14(2):145-52.

12. Lee ET, Wong FW, Lim CE. A modified technique of LAVH with the Biswas uterovaginal elevator. J Minim Invasive Gynecol. 2009;16(6):755-60.

13. Chin K, Ripp ZT, Hamid CA, Balgobin S. Laparoscopic-assisted vaginal hysterectomy (LAVH) re-defined: impact on resident surgical case log reporting. J Minim Invasive Gynecol. 2014;21(2 Suppl):41.

14. Choi HS, Ji YI. Feasibility of modified laparoscopic assisted vaginal hysterectomy for decreasing urinary tract complications. Clin Obstet Gynecol Reprod Med. 2017;3(2):1-5.

15. Ferrari MM, Berlanda N, Mezzopane R, Ragusa G, Cavallo M, Pardi G. Identifying the indications for laparoscopically assisted vaginal hysterectomy: a prospective, randomized comparison with abdominal hysterectomy in patients with symptomatic uterine fibroids. BJOG. 2000;107(5):620-5.

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DETECTION OF ALPHA-1 ANTITRYPSIN GENE MUTATIONS BY POLYMERASE CHAIN REACTION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

DETEKCIJA MUTACIJA GENA ZA ALFA$_1$-ANTITRIPSIN PRIMENOM LANČANE REAKCIJE POLIMERAZE KOD PACIJENATA SA HRONIČNOM OPSTRUKTIVNOM BOLESTI PLUĆA

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Summary

Introduction. The alpha-1 antitrypsin deficiency is the best described genetic cause of chronic obstructive pulmonary disease. The study of the alpha-1 antitrypsin deficiency, as the most important genetic risk factor for chronic obstructive pulmonary disease, is an important step in developing a strategy for the prevention and treatment of this disease. The aim of the study was detection of homozygous and heterozygous deficient gene alleles (protease inhibitor Z and protease inhibitor S) for alpha-1 antitrypsin in the group of patients with chronic obstructive pulmonary disease with the predominance of lung emphysema, as well as determination of a positive correlation between the serum levels of alpha-1 antitrypsin and the corresponding alpha-1 antitrypsin genotype. **Material and Methods.** The study included 90 patients, mutually unrelated individuals, hospitalized due to lung emphysema. The control group included 10 subjects, with no clinical signs of lung emphysema, but with a family history of chronic obstructive pulmonary disease. We attempted to identify the most common deficient alleles (protease inhibitor Z and protease inhibitor S) and the concentration of alpha-1 antitrypsin in the serum of the examinees. The polymorphism between the two allelic forms, protease inhibitor Z and protease inhibitor S, was detected by real-time polymerase chain reaction. **Results.** Protease inhibitor MM genotype alpha-1 antitrypsin was present in all 90 patients with the diagnosis of pulmonary emphysema, and the serum levels of alpha-1 antitrypsin were within the range of reference values. In the control group, there were two cases with mutated protease inhibitor MZ genotype, and in these 2 subjects the serum level of alpha-1 antitrypsin was at the lower limit of reference values. **Conclusion.** In patients diagnosed with lung emphysema, protease inhibitor MM genotype of alpha-1 antitrypsin and normal serum alpha-1 antitrypsin levels, the genetically-determined deficiency of alpha-1 antitrypsin is not responsible for the development of chronic obstructive pulmonary disease.

Key words: Mutation; alpha 1-Antitrypsin; Polymerase Chain Reaction; Pulmonary Disease, Chronic Obstructive; Polymorphism, Genetic; Pulmonary Emphysema; Risk Factors

Sažetak

Uvod. Deficit alfa$_1$-antitripsina je najbolje opisan genetski uzročnik hronične opstruktivne bolesti pluća. Istraživanje deficita alfa$_1$-antitripsina, kao najvažnijeg genetskog faktora rizika za hroničnu opstruktivnu bolest pluća, predstavlja važan korak u razvijanju strategije za prevenciju i lečenje ove bolesti. Cilj istraživanja bio je detekcija homozigotnih i heterozigotnih deficitarnih alela (protein inhibitor Z i protein inhibitor S) gena za alfa$_1$-antitripsin u grupi pacijenata sa hroničnom opstruktivnom bolesti pluća sa predominacijom emfizema pluća, kao i utvrđivanje pozitivne korelacije između nivoa serumskog alfa$_1$-antitripsina i odgovarajućeg alfa$_1$-antitripsin genotipa. **Materijal i metode.** Istraživanjem je bilo obuhvaćeno 90 bolesnika, međusobno nesrodnih osoba, hospitalizovanih zbog emfizema pluća. Kontrolnu grupu je činilo 10 ispitanika, bez kliničkih znakova emfizema pluća, ali sa pozitivnom porodičnom anamnezom hronične opstruktivne bolesti pluća). Svim ispitanicima vršena je identifikacija najčešćih deficitarnih alela (alfa$_1$-antitripsina Z i alfa$_1$-antitripsina S) i određivana koncentracija alfa$_1$-antitripsina u serumu. Polimorfizam između dve alelne forme protein inhibitor Z i protein inhibitor S detektovan je korišćenjem *real-time polymerase chain reaction.* **Rezultati.** Kod svih 90 bolesnika sa dijagnozom emfizema pluća bio je prisutan protein inhibitor MM genotip alfa$_1$-antitripsina, a nivo serumskog alfa$_1$-antitripsina bio je u opsegu referentnih vrednosti. U kontrolnoj grupi identifikovana su dva slučaja sa mutiranim protein inhibitor MZ genotipom, i kod ova dva ispitanika serumski nivo alfa$_1$-antitripsina bio je na donjoj granici referentnih vrednosti. **Zaključak.** Kod bolesnika sa dijagnozom emfizema pluća, protein inhibitor MM genotipom alfa$_1$-antitripsina i nivoom serumskog alfa$_1$-antitripsina u granicama referentnih vrednosti, genetski determinisan nedostatak alfa$_1$-antitripsina nije odgovoran za nastanak hronične opstruktivne bolesti pluća.

Ključne reči: mutacija; alfa 1-antitripsin; PCR; hronična opstruktivna bolest pluća; polimorfizam gena; plućni emfizem; faktori rizika

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Abbreviations

α1-AT	– alpha-1 antitrypsin
COPD	– chronic obstructive pulmonary disease
Real-Time PCR	– real-time polymerase chain reaction
PI (gene)	– Serine (or cysteine) protease inhibitor
Glu	– glutamic acid
Lys	– lysine
Val	– valine
DNA	– deoxyribonucleic acid
6-FAM	– 6-carboxyfluorescein
VIC	– 50-fluorescein
Taq DNA polymerase	– Thermus aquaticus DNA polymerase
SNP	– single-nucleotide polymorphism

Introduction

Chronic obstructive pulmonary disease (COPD) is the leading cause of morbidity and mortality in the world and represents a significant and growing social and economic problem worlwide. According to the World Health Organization, COPD was globally the third on the list of leading causes of death, with 3 million lives lost in 2016 [1].

In recent years, the risk factors for COPD have been in the focus of numerous studies dealing with these issues, given that the identification of risk factors is an important step in developing strategies for the prevention and treatment of any disease. In 80% of cases, COPD is caused by a combination of smoking and genetic predispositions. The best described genetic cause of COPD is the alpha-1 antitrypsin (α1-AT) deficiency [1].

The α_1-AT deficiency is a potentially lethal genetic disorder that leads to the development of a disease primarily with pulmonary and hepatic clinical manifestations. Instructions on the production of α_1-AT protein, which is a protease inhibitor (Pi) from serpin superfamily, are given by the SERPINA1 gene. This gene is located in the long arm of chromosome 14 at position q31-32.3 [1, 2]. The normal gene for α_1-AT (Pi M) possesses extremely high polymorphism, with more than 123 different alleles determined at the protein or genome level [2].

The two most common mutations in the "deficiency" are Pi Z and Pi S, and the rare ones: PiMMalton, PiMPittsburg Mduarte, Pi Null Null and others [2-4].

Protease inhibitor MM (PiMM) phenotype/genotype is characteristic for homozygous persons with a normal Pi M allele who have a normal plasma α_1-AT concentration that provides adequate antiprotease protection. The homozygous persons with PiZZ genotype have a deficient Pi Z allele responsible for 95% of severe α_1-AT deficiency cases [1, 5, 6].

Protease inhibitor Z mutation, which is labeled with glutanic acid (Glu) 342 GAG → lysine (Lys) AAG, occurs in the exon V of the gene for α_1-AT and represents the substitution of glutamine with lysine at position 342, i. e. the transition of guanine to adenine at the nucleotide position g.5297 G >A [5]. This type of mutation provides secretion of only 10 – 15% of the normal amount of α_1-AT from hepatocyte to homozygote. Consequently, α_1-AT polymer formation occurs, resulting in the fact that such large molecules cannot be exported from the liver and remain in it [6–8].

Protease inhibitor S mutation, which is labeled with Glu 264 → valine (Val) GTA GAA, is formed in the exon III inhibitor serpin peptidase (PI gene) and represents substitution of glutamine and valine at amino acid position 264, i. e. the transition of adenine into thymine at the nucleotide position g.2958 A > T [9]. Hepatocytes decompose a great part of the new α_1-AT, which reduces the secretion of α_1-AT, thus creating a state of deficiency [8].

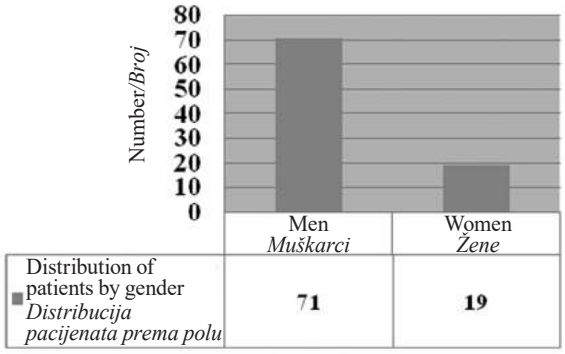
Several studies have shown a correlation between the serum levels of α_1-AT and the corresponding α_1-AT genotype. Thus, the PiZZ genotype of α_1-AT has 10 – 15%, PiSZ 51%, PiMZ 83%, PiSS 93%, PiMS 97% and PiMM 100% normal serum concentration of α_1-AT [9].

The α_1-AT deficiency has been identified in all populations around the world. In the population of our country the most common alleles are Pi M variants with an allelic frequency of 0.9805 (98.05% Pi M). The frequency of Pi S variant is 0.0067 (0.67% Pi S), and Pi Z variant 0.0128 (1.28% Pi Z) [9].

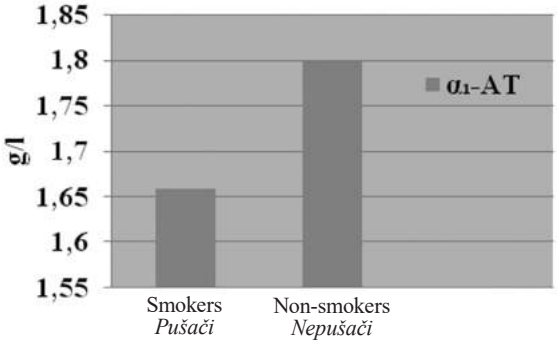
Considering the fact that in our country there are no studies that precisely define the frequency of an allele of the gene for α_1-AT in the group of patients with COPD with a predominance of pulmonary emphysema, the goal of the study was detection of homozygous and heterozygous deficient

Table 1. Single-nucleotide polymorphism (SNP) genotyping Assay AAT E342K or AAT E264K-set of primers and probes specifically designed to a target sequence
Tabela 1. *Test genotipizacije polimorfizma jednog nukelotida (SNP) AAT E342K ili AAT E264K-set prajmera i proba specifično dizajniranih za ciljnu sekvencu*

	Probe sequences (reporter sequence) *Sekvence genskih proba*	Primer sequences *Sekvence prajmera*
SNP Genotyping Assay AAT E342K-TaqMan assay	PiM-5'-VIC-ACCATCGACGAGAAAG-3' PiZ- 5'- FAM-CATCGACAAGAAAG-3'	Forward/*Uzvodni*-5'-GCCTGGGAT-CAGCCTTACAACGT-3' Reverse/*Nizvodni*-5'-CATGGGTATGG CCTCTAAAAACATGG-3'
SNP Genotyping Assay AAT E264K-TaqMan assay	PiM-5'-FAM-GATGATATCGTGGGTGAGTTCATT-TACCAGGTGCTGTAGTTT CCCCTCATC-3' PiS-5'-VIC- GATGATATCGTGGGTGAGTTCATTTTC-CAGGTGCTGTAGTTT CCCCTCATC-3'	Forward/*Uzvodni*-5'-GCCTGGGAT-CAGCCTTACAACGT-3' Reverse/*Nizvodni*-5'-CATGGGTATGG CCTCTAAAAACATGG-3'



Graph 1. Gender distribution of patients with the diagnosis of pulmonary emphysema
Grafikon 1. *Distribucija pacijenata sa dijagnozom emfizema pluća prema polu*



Graph 2. *Average measured values of α1-AT in examined groups of patients with the diagnosis of pulmonary emphysema*
Grafikon 2. *Prosečno izmerene vrednosti alfa 1-antitripsin kod ispitivanih grupa pacijenata sa dijagnozom emfizema pluća*

gene alleles (Pi Z and Pi S) for α_1-AT in patients with COPD, with predominance of pulmonary emphysema, determination of the incidence of polymorphisms Pi S and Pi Z in the gene for α_1-AT, and evaluation of its connection with the risk for COPD in the population of Vojvodina. In addition, in patients with COPD and prevailing emphysema the following aims have also been set: determination of serum concentrations of α_1-AT and establishing a positive correlation between the level of serum α_1-AT and the corresponding α_1-AT genotype.

Material and Methods

The research was conducted at the Institute of Pulmonary Diseases of Vojvodina in Sremska Kamenica as well as in the Deoxyribonucleic acid laboratory of the Institute of Forensic Medicine, Clinical Center of Vojvodina in Novi Sad. The test protocol was approved by the Ethics Committee of the Institute of Pulmonary Diseases of Vojvodina and the Ethics Committee of the Faculty of Medicine in Novi Sad. Selected patients who meet the necessary criteria for inclusion, after being fully informed about the type of examination and detailed introduction to the planned procedure, have confirmed in writing that they voluntarily agreed to participate in the study.

The sample included two groups of subjects, the first group of patients with lung emphysema, and a control group of healthy subjects without clinical signs of emphysema, but with a positive family history of COPD (mother or father were suffering from bullous emphysema and had a α_1-AT deficiency).

Table 2. Distribution of α_1-antitrypsin genotypes in 100 analyzed subjects by using the real-time PCR method
Tabela 2. *Distribuciju α_1-antitripsinskih genotipova za 100 analiziranih ispitanika pomoću real-time polymerase chain reaction metode*

	Number of respondents (n) *Broj ispitanika (n)*	Alph1-antitrypsin genotype *Genotip alfa1-antitripsina*	M/*M* N (%)	M/*Z* N (%)	Z/*Z* N (%)
Group of patients with the diagnosis of lung emphysema/*Grupa pacijenata sa dijagnozom emfizema pluća*	90	Glu 342 Lys Polymorphism *Glutaminska kiselina 342 Lizin polimorfizam*	90 (100%)	0 (0%)	0 (0%)
Group of respondents with a positive family history, without lung emphysema/*Grupa ispitanika sa pozitivnom porodičnom anamnezom, bez emfizema pluća*	10		8 (80%)	2 (20%)	0 (0%)
	Number of respondents (n) *Broj ispitanika (n)*	Alph1-antitrypsin genotype *Genotip alfa1-antitripsina*	M/M N (%)	M/S N (%)	S/S N(%)
Group of patients with the diagnosis of lung emphysema/*Grupa pacijenata sa dijagnozom emfizema pluća*	90	Glu 264Val Polymorphism *Glutaminska kiselina 264 Valin polimorfizam*	0 (0%)	0 (0%)	0 (0%)
Group of respondents with a positive family history, without lung emphysema *Grupa ispitanika sa pozitivnom porodičnom anamnezom, bez emfizema pluća*	10		0 (0%)	0 (0%)	0 (0%)

The study included 90 patients (71 men and 19 women), aged 32 to 83 years, unrelated individuals with their last residence in the territory of Vojvodina, who were hospitalized at the Institute of Pulmonary Diseases in Sremska Kamenica due to lung emphysema. Clinical diagnosis of pulmonary emphysema in all patients was made by functional examination of the respiratory system and radiological or computerized tomography (CT) by verification of pulmonary parenchyma reduction and bullous changes.

The control group included 10 respondents (5 males and 5 females), aged 34 – 51, without clinical signs of emphysema, but with a positive family history of COPD (mother or father suffered from bullous emphysema and had a deficiency of α_1-AT), who presented with difficulty breathing, persistent cough with or without sputum production, repeated respiratory infections, rapid onset of respiratory function problems, or wanted a routine pulmonological examination. All subjects were tested for serum α_1-AT concentration and identification of the most common deficiency alleles (Pi Z and Pi S) of α_1-AT gene was attempted.

The concentration of the serum α_1-AT of patients was determined by the radial immunodiffusion using HUMAN Alpha$_1$-Antitrypsin NL BINDARIDTM Radial Immune Diffusion (RID) kit (Birmingham, UK), to which the surface of the agar added monospecific antibody for the α_1-AT and control serum of human α_1-AT [10].

The polymorphism resulting from the difference in a single nucleotide between two allelic forms Pi S and Pi Z was detected by using a highly specific and precise method of real-time polymerase chain reaction (PCR) that combines conventional PCR amplification and fluorimetry.

The blood samples were taken into the anticoagulant tubes of ethylene diamine tetraacetic acid (EDTA), followed by permanent blood stains on the Flinders Technology Associates (FTA) cards, which were used for further analysis.

After surface disinfection with ultraviolet (UV) radiation and of fittings using 70% ethanol, isolation of nuclear deoxyribonucleic acid (DNA) from individual blood stain samples was performed using the Chelex-100 reagent, supplemented with Proteinase K, according to the proposed protocol [11, 12].

One µl of the diluted sample of isolated nuclear DNA was amplified by real-time PCR method by adding 12.5 µl TaqMan Universal PCR Master Mix (all 4 deoxyribonucleoside triphosphates, AmpliTaq Gold DNA polymerase, Mg^{2+} ions buffer) (Applied Biosystems, Foster City, CA, USA), 1 µl SNP Genotyping Assay set of primer and probe specifically designed for the target sequence (SNP Genotyping Assay AAT E342K or AAT E264K) **(Table 1)** and deionized water, made according to the protocol recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA). During the detection of Pi Z-allele, the TaqMan assay for Pi Z-allel was flagged with the FAM signal molecule (6-carboxyfluorescein), and for the Pi M-allel VIC-signaling molecule (50-fluorescein), while during the detection of Pi S-allele, TaqMan test for Pi S-allel labeled with VIC, and for Pi M-allele FAM. The difference in signal intensity for each tested allele before and after amplification was used to determine the presence of normal and/or mutated alleles. The final PCR mixture volume was 25 µl and contained about 250 ng of genomic DNA.

After the preparation of the reaction mixture for the PCR, the sample plate was placed in the ABI Prism 7000 instrument Sequence Detection System (Applied Biosystems, Foster City, CA). The instrument was programmed using the software so that the initial heating of the mixture at 50° C lasted 2 minutes (activation of AmpliTaq Gold DNA polymerase), complete initial denaturation at 95° C before the first cycle lasted 10 minutes, followed by 45 cycles of PCR, each lasting for 1 minute and 15 seconds, denaturing at 95° C for 15 seconds, and hybridization/elongation at 60° C, for 60 seconds. The instrument read the fluorescence generated during the amplification. By measuring and comparing fluorescence signals, it is possible to determine the presence or absence of certain alleles in each test sample.

The collected data were entered into the computer database, and statistical data processing was performed using the statistical software JMP 7, SAS Institute, Cary, NC. A descriptive and comparative method was used to describe the general characteristics of the subjects as well as of the test results. The correlation between parameters and its presentation and interpretation of significance was done by using the linear correlation coefficient. Continuous variables are shown as mean values ± standard deviation. Student's t-test was used to compare continuous variables with normal distribution. The probability value of $p \leq 0.05$ was considered significant.

Results

Out of the total number of (100) participants, 76 were males and 24 females. **Graph 1** shows the gender distribution of patients with the diagnosis of pulmonary emphysema. Of the total number of patients with pulmonary emphysema, there were more men - 71 (79%), and almost four times fewer women - 19 (21%). The patients were aged from 32 to 83 years.

In regard to smoking, of the total number of patients with pulmonary emphysema, there were 15 women (17%) and 61 men (68%) who were smokers, of the average age of 65.6 (SD = 9.38), while 10 men (11%) and half of the women - 4 (4%) of the average age of 66 (SD = 13.75) years were non-smokers.

The control group included 10 respondents (5 males and 5 females), aged 34 – 51, without clinical signs of emphysema, but with a positive family history of COPD (mother or father suffered from bullous emphysema and had a α_1-antitrypsin deficiency). In this group there were 2 smokers and 8 non-smokers.

The results of our study in the group of patients with pulmonary emphysema indicate that the average measured serum concentration of α_1-AT in

smokers was 1.66 g/l (0.49) and in non-smokers 1.80 g/l (0.43). There was no statistically significant difference in the average values of α_1-AT between smokers and non-smokers ($p > 0.05$) **(Graph 2)**.

Of the 10 examinees from the control group, in three subjects the serum level of α_1-AT was 0.80, 0.81 and 0.93 g/l, and was at the lower limit of reference values. Five subjects had a serum α_1-AT concentration below 2.0 g/l (the average serum concentration of α_1-AT was 1.68 g/l). Only two subjects had the serum level of α1-AT above 2.0 g/l.

The results of the frequency of Pi Z and Pi S polymorphisms in 90 patients diagnosed with pulmonary emphysema, as well as in 10 subjects of the control group **(Table 2)**.

Protease inhibitor MM genotype was present in all 90 (100%) patients with the diagnosis of pulmonary emphysema, while in the control group, 2 cases with mutated heterozygous PiMZ genotype were identified, as well as 8 cases with normal genotype PiMM. It is necessary to point out that, in addition to the heterozygous PiMZ genotype, both subjects had a serum deficiency of α_1-AT. The serum level of α_1-AT was 0.80 and 0.81 g/l, individually and was at the lower limit of the reference values. One of the heterozygous was also a perennial smoker.

Discussion

The only risk factor that comes from the host, i. e. a human, that is well known to be causally related with the development of COPD, is a genetically determined deficiency of α1-AT [13].

In all patients diagnosed with lung emphysema, the PiMM genotype α_1-AT was present and the serum level of α_1-AT was within the limits of the reference values, whereas the genetically-determined deficiency of α_1-AT was not responsible for the development of COPD. PiMM genotype represents individuals who are homozygous with a normal Pi M allele and have a normal concentration of α_1-AT in the plasma to provide adequate protection - antiprotease.

The results of our research confirmed the literature allegations regarding the correlation between the level of α_1-AT in the serum and the corresponding α_1-AT genotype. The level of serum α_1-AT in the subjects with PiMM genotype of α_1-AT was 1.66 g/l (for smokers) and 1.80 g/l (for non-smokers) and was within the limits of the reference values. Floyd et al. reported that in subjects with PiMM genotype α_1-AT with a diagnosed COPD, the average measured values of α_1-AT were 1.39 g/l and 0.127 g/l in PiZZ subjects [14]. The serum α_1-AT in the subjects with PiMZ genotype was 0.80 and 0.81, individually and was at the lower limit of the reference values. According to the literature, individuals with PiMZ genotype are characterized by a serum concentration of α_1-AT of 0.87 g/l (0.5 – 1.2 g/l) [15].

However, there are still controversial opinions about whether people with heterozygous α_1-AT genotype (especially people with PiMZ genotype for α_1-AT) have predispositions for the development of emphysema. Floyd et al. consider that heterozygous individuals with PiMZ, PiSZ, and PiMS genotype for α_1-AT exhibit a tendency to develop and can develop lung emphysema, especially if they are smokers, but do not have a greater risk than the rest of the population. If heterozygotes develop a pulmonary disease, this is because there was an additional impact of another host or environmental factors [14].

According to the recommendations of the most accepted guidelines for the management of COPD, Global Initiative for Chronic Obstructive Pulmonary Disease, it is recommended that in individuals who suffer from COPD before the age of 40 (emphysema in young people) or have a positive family history, α_1-AT should be tested. The values less than 20% of predicted indicate that it is a congenital absence of this enzyme [13, 16].

The study of genetic risk factors for the development of COPD with prevalence of lung emphysema is an important step in developing a strategy for the prevention and treatment of this disease. Genetic discrimination is necessary for the detection of homozygous and heterozygous and the lack of a certain (Pi S and Pi Z) alleles of α_1-AT gene. Based on this concept, a strategy for the prevention and control of COPD will be developed, targeted at the healthy and individuals with an increased risk for COPD predominantly with lung emphysema (positive family history, the level of serum α_1-AT in the lower range of reference values but without clinically developed COPD with prevalence of lung emphysema), as well as those already suffering from COPD who need effective treatment and adequate care. In individuals with an inborn α_1-AT deficiency, nonspecific preventive measures as well as therapeutic procedures, with specific compensation of this enzyme inhibitor, should be applied. The compensation is administered to purified human α_1-AT, which is administered as an intravenous infusion of 60 mg/kg body weight, once weekly or once a month [16, 17].

The main objectives of the strategy are to significantly reduce morbidity and mortality of people with COPD and improve their quality of life. Prevention has the greatest potential to reduce the incidence of COPD. Prevention in the general population is a sustainable strategy in the long run. However, at the same time, it is necessary to implement prevention at the individual and population levels.

By using screening, it is possible to prevent or modify risk factors, prevent the onset or progress of the disease, prevent incompetence, reduce mortality and improve quality of life, provided that effective, affordable and acceptable therapy is available to all who need it. The outcome of the disease can be improved by its early detection, appropriate therapy and effective rehabilitation. Screening with follow-up therapy for people with an increased risk for COPD associated with several risk factors is more cost-effective than focusing on individual risk factors. Appropriate application of knowledge at all levels of health care has multiple benefits for all [13].

Conclusion

Protease inhibitor MM genotype alpha-1 antitrypsin was present in all patients diagnosed with lung emphysema; the serum level of alpha-1 antitrypsin was within the limits of the reference values; the genetically-determined alpha-1 antitrypsin deficiency was not responsible for the development of chronic obstructive pulmonary disease.

In the group of patients with the diagnosis of pulmonary emphysema, there was no statistically significant difference in the average values of alpha-1 antitrypsin between smokers and non-smokers.

The results of our research confirmed the literature results regarding the correlation between the serum level of alpha-1 antitrypsin and the corresponding alpha-1 antitrypsin genotype. Investigations of genetic defects, the alpha-1 antitrypsin deficiency in individuals with a family history of chronic obstructive pulmonary disease will allow the identification of patients with a genetic predisposition to the development of chronic obstructive pulmonary disease, and its prevention and treatment before the onset of symptoms.

References

1. Torres-Durán M, Lopez-Campos JL, Barrecheguren M, Miravitlles M, Martinez-Delgado B, Castillo S, et al. Alpha-1 antitrypsin deficiency: outstanding questions and future directions. Orphanet J Rare Dis. 2018;13(1):114.

2. McNab GL, Ahmad A, Mistry D, Stockley RA. Modification of gene expression and increase in alpha1-antitrypsin secretion after homologous recombination in alpha1-AT-deficient monocytes. Hum Gene Ther. 2007;18(11):1171-7.

3. Fregonese L, Stolk J. Hereditary alpha-1-antitrypsin deficiency and its clinical consequences. Orphanet J Rare Dis. 2008;3:16.

4. Stoller JK, Brantly M. The challenge of detecting alpha-1 antitrypsin deficiency. COPD. 2013;10(Suppl 1):26-34.

5. Beletić A, Dudvarski-Ilić A, Milenković B, Nagorni-Obradović Lj, Ljujić M, Đorđević V, et al. Alpha-1-antitrypsin deficiency – molecular basis, clinical presentation, therapeutic options and an integrative approach in diagnostics. J Med Biochem. 2014;33(1):88-96

6. DeMeo DL, Silverman EK. α1-antitrypsin deficiency-2: genetic aspects of α1-antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk. Thorax. 2004;59(3):259-64.

7. Marciniuk DD, Hernandez P, Balter M, Bourbeau J, Chapman KR, Ford GT, et al. Alpha-1 antitrypsin deficiency targeted testing and augmentation therapy: a Canadian Thoracic Society clinical practice guideline. Can Respir J. 2012;19(2):109-16.

8. Ljujić M, Topić A, Divac A, Nikolić A, Petrović-Stanojević N, Surlan M, et al. Isoelectric focusing phenotyping and denaturing gradient gel electrophoresis genotyping: a comparison of two methods in detection of alpha-1-antitrypsin variants. Transl Res. 2008;151(5):255-9.

9. Zarić B, Stojčević J, Andrijević L, Andrijević I, Kuruc V, Veselinović I, et al. Relation of functional characteristics and serum alpha-1-antitrypsin (AAT) concentration in patients with PiMM phenotype and chronic obstructive pulmonary disease (COPD). Eur J Intern Med. 2011;22(6):603-6.

10. Human alpha-1 antitrypsin bindarid TM radial immunodiffusion kit. Version: 8th January 2003. Birmingham, UK: The Binding Site Group Ltd; 2003.

11. Cone RW, Fairfax MR. Protocol for ultraviolet irradiation of surfaces to reduce PCR contamination. PCR Methods Appl. 1993;3(3):S15-7.

12. Willard JM, Lee DA, Holland MM. Recovery of DNA for PCR amplification from blood and forensic samples using a chelating resin. Methods Mol Biol. 1998;98:9-18.

13. Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS; GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. Risk Factors. NHLBI/ WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) workshop summary. Am J Respir Crit Care Med. 2001;163(5):1256-76.

14. Floyd OC, Carroll TP, O'Connor C, Taggart C, Costello RW, O'Neill SJ, et al. Alpha-1 antitrypsin deficiency ZZ COPD compared to MM COPD. Am J Respir Crit Care Med. 2009;179:A3528.

15. Sitkauskiene B, Serapinas D, Blanco I, Fernández-Bustillo E, Janciauskiene S, Sakalauskas R. Screening for alpha1-antitrypsin deficiency in Lithuanian patients with COPD. Respir Med. 2008;102(11):1654-8.

16. American Thoracic Society; European Respiratory Society. American Thoracic Society/ European Respiratory Society Statement. Standards for the diagnosis and management of individuals with alpha1-antitrypsin deficiency. Am J Respir Crit Care Med. 2003;168(7):818-900.

17. Rebić P. Postavljanje dijagnoze hronične opstruktivne bolesti pluća. In: Micić D, Bošnjak-Petrović V, editors. Hronična opstruktivna bolest pluća. Acta clinica. 2004;4(1):55-64.

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PLAYERS' AND COACHES' ATTITUDES AND KNOWLEDGE OF PROHIBITED DOPING SUBSTANCES

POZNAVANJE I STAV SPORTISTA I TRENERA O ZABRANJENIM DOPING SUPSTANCIJAMA

Miodrag P. DRAPŠIN[1-3], Milana SIMIĆ[1] and Dea KARABA JAKOVLJEVIĆ[1-4]

Summary

Introduction. Doping is an illicit use of illegal substances or substances that the body normally contains, in order to stimulate the competitive ability of athletes, which is in collision with sports ethics as well as the physical and mental integrity of athletes. In 2006, the World Anti-Doping Agency made a list of illicit substances and prohibited their use in different sports. The aim of this research was to evaluate athletes' and coaches' knowledge and attitudes about the use of doping substances. **Material and Methods.** This prospective study included 199 subjects, 164 (82.4%) athletes and 35 (17.6%) coaches. The group of athletes included 88 females and 114 males, with an average age of 24.1 ± 6.4 years, being engaged in sports 9.1±4.7 years on average. The athletes were engaged in the following sports: volleyball, basketball, handball, athletics, wrestling, soccer, and swimming. The coach group included 20 males and 15 females, with an average age of 31.8 ± 8.1 years with a coaching experience of 9.3 ± 3.1 years. **Results.** The differences in the average scores between athletes and coaches were statistically significant ($p = 0.001$; $p < 0.05$), in favor of coaches. The average scores between male and female athletes, and between individual and team coaches showed no significant differences ($p = 0.267$; $p = 0.349$; $p > 0.05$). **Conclusion.** The knowledge on prohibited doping substances was significantly higher in coaches than in athletes, while differences related to gender and collective or individual sports were not found.

Key words: Health Knowledge, Attitudes, Practice; Doping in Sports; Athletes; Mentors; Dietary Supplements; Substance Abuse Detection; Surveys and Questionnaires

Sažetak

Uvod. Doping predstavlja konzumiranje stranih supstancija ili supstancija koje organizam normalno sadrži, s ciljem da se na veštački način stimulišu takmičarske sposobnosti sportista, što je u suprotnosti sa sportskom etikom i fizičkim i mentalnim integritetom sportista. Svetska anti doping agencija je 2006. godine napravila listu nedozvoljenih supstancija i zabranila njhovu primenu u različitim sportovima. Cilj ovog rada bio je da se utvrdi nivo znanja i stavovi sportista i trenera o zloupotrebi zabranjenih i doping supstancija. **Materijal i metode.** Prospektivnom studijom preseka obuhvaćeno je 164 sportista i 35 trenera, od koji je bilo 84 (42,2%) osobe ženskog i 115 (57,8%) muškog pola, prosečne starosti 24,01 ± 6,429 godina. U studiju su bili uključeni glavni, pomoćni i kondicioni treneri koji imaju bar 1 godinu radnog iskustva kao i sportisti koji treniraju bar pet sati nedeljno. Svi ispitanici su popunjavali jedinstvenu anketu od 25 pitanja koja se odnosila na njihovo poznavanje i stav o zabranjenim doping supstancijama. **Rezultati.** Razlike u prosečnim vrednostima skora dobijenog iz Upitnika između aktivnih sportista i trenera bila je statistički značajna u korist trenera ($p = 0,001$; $p < 0,05$). Prosečne vrednosti skora dobijenog iz Upitnika se nisu značajno razlikovale niti u odnosu na pol ispitanika, niti na individualni, ili timski sport ($p = 0,267$; $p = 0,349$; $p > 0,05$). **Zaključak.** Stepen informisanosti i poznavanje zabranjenih doping supstancija značajno je izraženiji kod trenera u odnosu na sportiste, dok razlika u nivou znanja o doping supstancijama nije bila značajna u odnosu na pol ispitanika i individualni, odnosno timski sport kojim se bave.

Ključne reči: znanje o zdravlju, stavovi, praksa; doping u sportu; sportisti; treneri; dijetarni suplementi; otkrivanje zloupotrebe supstanci; istraživanja i upitnici

Introduction

Winning is imperative in modern sports for all the participants, because nowadays sports is a lucrative job. It was not always like this and honor, prestige and personal achievements were the paramount goals. We witness that every point on the scoreboard is bringing the athlete and his team closer both to the victory and financial benefits [1]. Many experts from different fields are involved in creating a winning strategy, successful athletes and coaches, physiotherapists, nutritionists, psychologists [2–4]. While trying to achieve the maximum and win the game, some of them forget about the fair play and sports codex and reach out for solutions to gain edge over the other competitors, forgetting that chemical substances and procedures could put their health at risk and even threaten their lives [5, 6].

The use of performance enhancing substances is not newly discovered and it is well known throughout the history [7]. Five thousand years ago, in ancient China ephedra was used as a stimulant, as well as dried figs, certain types of mushrooms, and strychnine in

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Abbreviations
WADA – World Anti-Doping Agency
TUE – Therapeutic Use Exemption

ancient Greece [8–10]. The World Anti-Doping Agency (WADA) defined doping as use of illegal substances (which could be normally found in the body) and methods in order to improve sports performance and they made a list of banned substances in sports [11–13]**.** Prevention programs are introduced in the sport clubs as efficient tool in motivating athletes to behave according to the rules to preserve their health [14].

In order to develop efficient prevention programs, we need to investigate the level of awareness and attitudes of athletes and their coaches of doping. There were many projects and massive funding to support global and national anti-doping programs, but there is still lack of valid data from the final users [15].

The authors of this article recognized the importance of education of the athletes and others directly involved in competitive sports concerning doping and potential negative impacts on their health.

Literature data provide information about daily use of dietary supplements without previous consultations with the sport nutrition experts from the field and without adequate understanding the pros and cons for the athlete [16, 17]. In his paper, Dascombe reported that among the athletes who took some supplements, most did not even know the active ingredient, the potential side effects, or the way the supplements contributed to their level of fitness. In this group, only 52.4% knew the recommended daily dose, and only 57% wanted to learn more about the supplement [18]. Education of athletes on doping is important and it could easily be done by their coaches and other sports professionals on daily basis [19, 20].

High moral and ethical standards among the athletes should be taught by their coaches, but it has to be supported by knowledge and we wanted to investigate the level of knowledge and attitudes of athletes and their coaches about doping.

Material and Methods

This prospective study included 199 subjects, 164 (82.4%) athletes and 35 (17.6%) coaches. The group of athletes included 88 females and 114 males, with an average age of 24.1 ± 6.4 years, being engaged in sports 9.1±4.7 years on average. The athletes were engaged in the following sports: volleyball, basketball, handball, athletics, wrestling, soccer, and swimming. The coach group included 20 males and 15 females, with an average age of 31.8 ± 8.1 years with a coaching experience of 9.3 ± 3.1 years.

All subjects filled in an anonymous questionnaire of 25 questions referring to knowledge about banned substances in sports, supplements and their attitudes to doping in sports in general**.** The questionnaire on doping was obligatory for every athlete involved in competition and is available on the official web site of Antidoping Agency of the Republic of Serbia (http://www.adas.org.rs/en/tue/tue-obrazac/) **(Questionnaire)**. Data gathered in this research were computed by IBM Statistical package for social sciences (SPSS) 20.0 software using descriptive statistics (mean value, standard deviation, minimum, and maximum), student t-test and χ^2 test. Statistical significance was set at $p < 0.05$.

This research was conducted according to standards of Ethical Committee of the Faculty of Medicine in Novi Sad and all participants were introduced with the aims of the study and gave their written consent for participation.

Results

Our study included 67 (33.7%) volleyball players, 32 (16.1%) basketball players and track and field athletes, 19 (9.5%) handball players, 11 soccer players (5.5%), 3 (1.5%) wrestlers, 2 (1%) judokas, 2 swimmers and biathlon runners (0.5%) and 1 water polo, boxer, triathlon runner, karate player, rower, hockey player and

QUESTIONNAIRE
UPITNIK

General Data/*Opšti podaci*
Gender: M F
Pol M Ž
Date of birth/*Datum rođenja:*_____
Sport/*Sport:*_____
Weekly training hours: (any kind of organized and planned workout):_____
Broj sati treninga nedeljno (bilo koji vid organizovane fizičke aktivnosti)
Years of training/coaching_____
Koliko dugo se bavite sportom/trenerskim poslom:

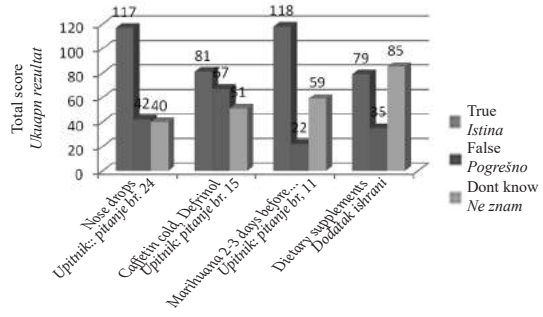
1.	Are you aware of the list of banned substances? *Da li znate spisak zabranjenih supstancija i lekova?*	Yes No Not sure *Da Ne Nisam siguran*
2.	Have you ever taken a prohibited substance? *Da li ste i kada uzeli zabranjenu supstanciju?*	Yes No Not sure *Da Ne Nisam siguran*
3.	Would you ever take a prohibited substance to achieve a sports result? *Da li biste i kada uzeli doping za ostvarenje ličnih rezultata?*	Yes No Not sure *Da Ne Nisam siguran*

4.	Do you think it is possible to cheat a doping test and not get caught? *Mislite li da postoji način da se prevare testovi na doping kontrolu, a da vas ne otkriju?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
5.	The athletes can be tested only during a competition? *Sportista može biti testiran isključivo na takmičenju?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
6.	Therapeutic use exemption (TUE) form is submitted during the competition? *Izuzetak za terapijsku upotrebu (TUE) popunjava se u toku takmičenja?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
7.	Can an athlete test positive only for using a dietetic supplement? *Može li sportista biti pozitivan na doping zbog upotrebe dijetetskog suplementa?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
8.	Do you think that product declaration of dietetic supplements must match the content in the package?/*Deklarisani sastav dijetetskih suplemenata koji se odnosi na stimulanse i steroide mora da odgovara sastavu na kutiji jer je to zakonski regulisano?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
9.	If the dietary supplement is sold at pharmacy, is it safe to use? *Ako je dodatak ishrani iz apoteke, definitivno je dozvoljen u sportu?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
10.	Positive doping tests result is the only reason for an athlete to be sanctioned? *Pozitivan test je jedini način da se sportista sankcioniše?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
11.	If the athlete takes marihuana 2 - 3 days prior to competition, is he/she going to test positive?/*Ako je sportista uzeo marihuanu 2-3 dana pre takmičenja, rezultat testa na takmičenju će biti pozitivan?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
12.	Growth hormones are allowed to use out of the competition? *Hormon rasta i njemu srodne supstancije su dozvoljene izvan takmičenja?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
13.	Cocaine and its metabolites are prohibited only during the competition? *Kokain i njegovi metaboliti zabranjeni su samo na takmičenju?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
14.	If the athlete has asthma and uses Ventolin, which contains active substance beta2agonist-salbutamol, does he/she need TUE? *Sportista ima astmu i koristi pumpicu Ventolin® u kojoj je aktivna supstancija beta2 agonist – salbutamol. Da li sportista mora da traži izuzetak za terapijsku upotrebu?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
15.	Athlete with cold uses ASPIRIN COMPLEX, DEFRINOL and CAFFETIN COLD. Can he test positive? *Sportista koji je prehlađen i koristi ASPIRIN COMPLEX, DEFRINOL, CAFFETIN COLD može biti pozitivan na testiranju?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
16.	Are intravenous infusions or injections containing more than 50 ml over a period of 6 hours allowed?/*Intravenske infuzije ili injekcije u količini većoj od 50 ml u periodu od 6 sati spadaju u zabranjene metode?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
17.	Is Ethanol a banned substance in all sports? *Etanol je zabranjen na takmičenju u svim sportovima?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
18.	Is Propranolol a banned substance in archery, shooting, motor racing and snooker? *Propranolol spada u zabranjene supstancije u streljaštvu, streličarstvu, automobilizmu, bilijaru?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
19.	Is local application of adrenaline (nasal) banned? *Lokalna primena adrenalina (nazalna) je zabranjena?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
20.	Are narcotics (such as morphne, heroin) banned during competition? *Narkotici (morfin, heroin) su zabranjeni za upotrebu u toku takmičenja?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
21.	Is testosterone always banned (both during competition and preparation)? *Testosteron spada u supstancije koje su uvek zabranjene (na takmičenju i izvan njega)?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
22.	Is insulin in the group of substances banned during competition? *Insulin se nalazi u grupi supstancija koje su zabranjene samo na takmičenju?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
23.	Is caffeine banned in all sports? *Kofein je zabranjen u svim sportovima?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
24.	Can an athlete test positive due to use of nasal drops? *Ako sportista koristi kapi za nos može biti pozitivan na testiranju?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*
25.	Is autotransfusion considered doping? *Autotransfuzija se smatra dopingom?*	Yes *Da*	No *Ne*	Not sure *Nisam siguran*

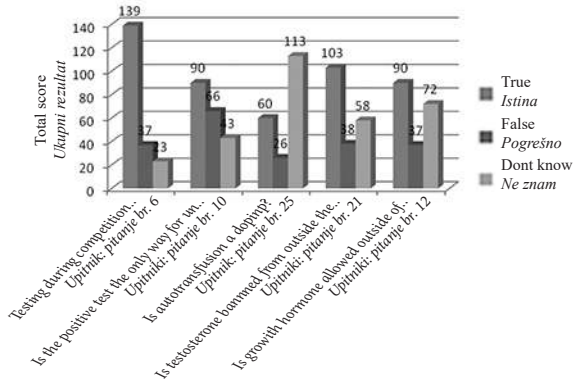
a dancer. Average competitive participation was 10.7 ± 1.1 years with 15.7 ± 8.9 hours of training weekly.

Out of 199 participants in the study, 140 (70%) stated that they were familiar with the content of the list of banned substances, while 59 (30%) gave negative or indiferent replies to the question.

Graph 1 represents the answers to the question: "Is it possible to test positive on doping test only by using



Graph 1. Knowledge about testing positive if using the listed substances
Grafikon 1. *Znanje o pozitivnom doping testu ako se koriste navedene supstancije*



Graph 3. Knowledge about doping tests, sanctions and banned substances
Grafikon 3. *Informisanost o doping kontroli, sankcijama i zabranjenim supstancijama*

nasal drops (117 correct answers), aspirin or defrinol (81 correct answer), marihuana 2 - 3 days prior the competition (118 correct answers) and dietetic supplements (79 correct answers)".

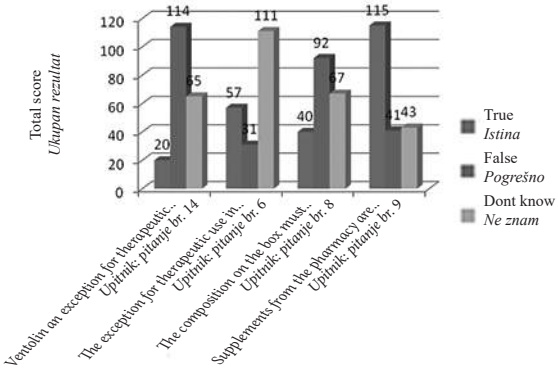
Therapeutic Use Exemption (TUE) and knowledge on the topic is presented in **Graph 2**. Low level of correct answers concerning this topic indicates low level of information.

Graph 3 presents the overall knowledge amongst coaches and athletes about doping tests and sanctions for those with positive test results for using prohibited substances. To the question: "Can the athlete be tested only during competition?" 139 participants answered correctly, while to the question: "Is a positive test result the only way for sanctions against the athelete?" only 90 gave correct answers. To the question about blood doping and autotransfusion, there were only 60 correct answers. To the question about the use of testosterone and growth hormone during competition and during preparations, 103 and 90 out of 199 gave correct answers, respectively.

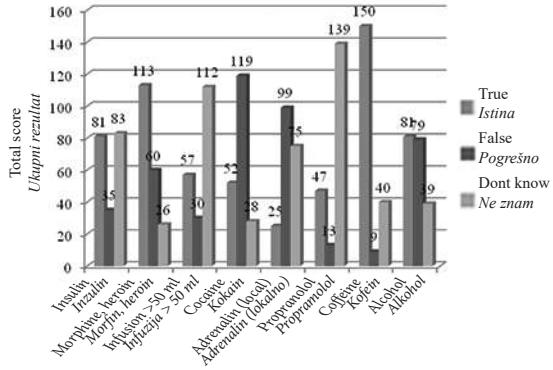
Graph 4 presents knowledge about prohibited substances during competition where it is clear that usage of coffein, morphine and heroin is prohibited, while the atheletes and coaches know little about propranolol (in

Table 1. Differences between average questionnaire scores between coaches and athletes
Tabela 1. *Razlike između prosečnih rezultata upitnika između trenera i sportista*

	No./*Br.*	Average questionnaire scores ($\bar{x}$ ± SD)/*Prosečni rezultati upitnika ($\bar{x}$ ± SD)*
Athletes/*Sportisti*	164	23,65 ± 4,317
Coaches/*Treneri*	35	26,40 ± 4,754
Total/*Ukupno*	199	24,13 ± 4,508



Graph 2. Knowledge about Therapeutic Use Exemption and supplements
Grafikon 2. *Informisanost o izuzeću za terapijsku primenu i suplementima*



Graph 4. Knowledge about using banned substances during competition
Grafikon 4. *Informisanost o upotrebi zabranjenih supstancija tokom takmičenja*

archery, shooting and motor races) and infusions as prohibited.

The **Table 1** presents the average questionnaire scores among active athletes and their coaches with a statistically significant difference between the groups ($p < 0.05$).

Discussion

One of the priorities of WADA is improvement of knowledge about risk factors and better understanding the side effects of doping in sports [1]. Erdman et al. included 582 athletes in their investigation and found that 76.7% (446) of subjects claim to understand anti-doping rules, 89.5% (521) truly believe in those rules, and only 63.2% (368) knows where to find valid information on the topic [21]. In the study which investigated only professional football players, 68% (460) knew about the use of prohibited substances in sports, while 32% (226) did not confirm that they had proper information about anti-doping [16]. Similar studies reported the same results which points out the fact that athletes need to be better informed about doping in sports [1]. In our study, one of the main findings is that the coaches have better knowledge about doping in sports compared to their athletes and the difference is statistically significant ($p < 0.05$). The good side of such results is a possible transfer of knowledge from coaches to their athletes as they are in contact with the athletes on daily basis which could be beneficial in anti-doping efforts. Nevertheless, it is up to the athlete to be diligent about anti-doping, and it is his/her obligation to clearly understand the consequences of malpractice and their professional carriers.

In our paper, we tried to answer the question if there were differences in knowledge about doping between the athletes of different gender. Molobe et al. concluded in their study that knowledge, attitude and practice concerning doping and use of prohibited substances are not good enough, whether we talk about male or female athletes, since the majority of athletes in their study (56%) thought that winning is closely related to use of prohibited substances. This study points out that use of prohibited substances is probably one of the main reasons for doping in sports in both male and female athletes [22]. The results of our investigation show that there is no difference in knowledge of male and female participants.

There were several kinds of sports included in our study and we tried to find out if the attitudes and awareness levels differed between athletes practicing individual sports compared to those in team sports. Dimeo et al. investigated the differences in attitudes towards doping in athletes in team and individual sports. Their findings suggested that in team sports there was a beneficial psychosocial factor between the athletes, since they "rely" on each other and jeopardizing the whole team is a crucial "reject" factor for using prohibited substances [23]. Our results suggest that there is no statistically significant difference in knowledge between the athletes practicing individual compared to team sports.

Conclusion

According to the gathered results, we can conclude as follows: knowledge about banned substances and doping is significantly higher in coaches than in athletes ($p < 0.05$) while there are no gender differences. There is no statistically significant difference in awareness level about banned substances and doping between participants involved in individual and team sports.

There is a necessity to create a continuous learning environment about banned substances and doping for all participants in sports relying on novel scientific data released by the leading authorities like the World Anti-Doping Agency. Future research should consider updating the questionnaire, in order to provide a valid tool for evaluating the efficacy of education in wider sporting community.

References

1. Backhouse S, McKenna J, Robinson S, Atkin A. International literature review: Attitudes, behaviours, knowledge and education. Drugs in sport: past present and future [Internet]. Montreal, Canada: World Anti-Doping Agency; 2007 [cited 2019 Jun 6]. Available from: https://www.wada-ama.org/sites/default/files/resources/files/backhouse_et_al_full_report.pdf.

2. Grujić N. Physiology of sport. Novi Sad: Futura; 2004. Serbian.

3. Rakovac A, Andrić L, Karan V, Bogdan M, Slavić D, Klašnja A. Evaluation of spirometric parameters and maximum oxygen consumption in athletes and non-athletes. Med. Pregl. 2018;71(5-6):157-61.

4. Karan V, Rakovac A, Karan M, Popović M, Klašnja J, Lukač D. Evaluation of body composition and muscular strength in different sports. Med. Pregl. 2017;70(5-6):150-4.

5. Sarović D. Doping in sports. In: Radenović S, Jeremić V. Sports, medicine, bioethic. Bioethic and sports – problems of doping. Belgrade: Bioethic society of Serbia; 2015. p. 39-42. Serbian.

6. Hauw D, Mohamed S. Patterns in the situated activity of substance use in the careers of elite doping athletes. Psychol Sport Exerc. 2015;16(Part 2):156-63.

7. Bloodworth AJ, Petróczi A, Bailey R, Pearce G, McNamee MJ. Doping and supplementation: the attitudes of talented young athletes. Scand J Med Sci Sports. 2012;22(2):293-301.

8. Abourashed EA, El-AlfyAT, Khan IA, Walker L. Ephedra in perspective – a current review. Phytother Res. 2003;17(7):703-12.

9. Calfee R, Fadale P. Popular ergogenic drugs and supplements in young athletes. Pediatrics. 2006;117(3):e577-89.

10. Manouchehri J, Tojari F. Development and validation of instruments to measure doping attitudes and doping beliefs. European Journal of Experimental Biology. 2013;3(2):183-6.

11. Anđelković M, Dikić N. TUE - from medical indications to sport abuse. Sportska medicina. 2007;8(3):1-21.

12. Bowers LD. Anti-dope testing in sport: the history and the science. FASEB J. 2012;26(10):3933-6.

13. Kondric M, Sekulic D, Petróczi A, Ostojic L, Rodek J, Ostojic Z. Is there a danger for myopia in anti-doping education? Comparative analysis of substance use and misuse in Olympic racket sports calls for a broader approach. Subst Abuse Treat Prev Policy. 2011;6:27.

14. Goldberg L, MacKinnon DP, Elliot DL, Moe EL, Clarke GN, Cheong J. The adolescent training and learning to avoid steroid program - preventing drug use and promoting health behaviors. Arch Pediatr Adolesc Med. 2000;154(4):332-8.

15. Ntoumanis N, Ng JY, Barkoukis V, Backhouse S. Personal and psychosocial predictors of doping use in physical activity settings: a meta-analysis. Sports Med. 2014;44(11):1603-24.

16. Maughan RJ, Depiesse F, Geyer H. The use of dietary supplements by athletes. J Sports Sci. 2007;25 Suppl 1:S103-13.

17. Antić D. Evaluation of knowledge on doping in sports among Serbian general practitioners. Med Pregl. 2017;70(1-2):25-31.

18. Dascombe BJ, Karunaratna M, Cartoon J, Fergie B, Goodman C. Nutritional supplementation habits and perceptions of elite athletes within a state-based sporting institute. J Sci Med Sport. 2010;13(2):274-80.

19. Peters C, Schulz T, Oberhoffer R, Michna H. Doping und Dopingprävention: Kenntnisse, Einstellungen und Erwartungen von Athletenund Trainern. Dtsch Z Sportmed. 2009;60(3):73-8.

20. Fung L, Yuan Y. Performance enhancement drugs: knowledge, attitude and intended behavior among community coaches in Hong Kong. Sport J. 2006;9(3):16.

21. Erdman KA, Fung TS, Doyle-Baker PK, Verhoef MJ, Reimer RA. Dietary supplementation of high-performance Canadian athletes by age and gender. Clin J Sport Med. 2007;17(6):458-64.

22. Molobe ID. Knowledge, attitude and practice on drug abuse among sports men and women in Lagos state, Nigeria. International Journal of Medicine and Medical Sciences. 2012;2(3):77-85.

23. Dimeo P, Allen J, Taylor J, Dixon S, Robinson L. Team dynamics and doping in sport: a risk or a protective factor? Report compiled for the World Anti-Doping Agency [Internet]. Stirling: Stirling University; 2011 [cited 2019 Jun 22]. Available from: https://www.wada-ama.org/sites/default/files/resources/files/dimeo_team_dynamics_2011_report_en.pdf.

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SIGNIFICANCE OF POSTERIOR ACOUSTIC ENHANCEMENT ULTRASONOGRAPHIC FINDINGS IN THE DIAGNOSIS OF HEPATOCELLULAR CARCINOMA

ZNAČAJ NALAZA POSTERIORNOG AKUSTIČNOG POJAČANJA ULTRAZVUKA U DIJAGNOSTICI HEPATOCELULARNOG KARCINOMA

Jelena STOŠIĆ[1], Srđan STOŠIĆ[2], Biljana KUKIĆ[3, 4] and Mlađan PROTIĆ[3, 4]

Summary

Introduction. On ultrasound, hepatocellular carcinoma presents with nodular or multinodular lesions of different size and echostructure, sometimes with a surrounding halo, and lateral acoustic shadows or posterior acoustic enhancement. The aim of this study was to determine the incidence of posterior acoustic enhancement in hepatocellular carcinoma. **Material and Methods.** This retrospective study included 120 patients with pathologically verified hepatocellular carcinoma who had undergone ultrasound examination (using real time ultrasounds from different manufacturers, with 3.5 and 5 MHz probes). Ultrasound imaging focused on the size and appearance of the focal lesions, i. e. echostructure and presence or absence of posterior acoustic enhancement as areas of increased echogenicity behind the lesion. **Results.** Posterior acoustic enhancement was observed in 47.3% of all nodular hepatocellular carcinomas, whereas this ultrasound phenomenon was statistically significantly more common in the group of tumors from 3 to 5 cm in size. In the group of multinodular tumors, posterior acoustic enhancement was found in 70% of cases. **Conclusion.** The presence of posterior acoustic enhancement in the detection of focal hepatic lesions may be a significant finding in the diagnosis of hepatocellular carcinoma, especially in patients at risk for developing hepatocellular carcinoma (cirrhosis and chronic liver disease), as well as in monitoring interval growth in size of focal lesions using this ultrasound phenomenon.

Key words: Ultrasonography; Carcinoma, Hepatocellular; Liver Neoplasms; Liver Diseases; Chronic Disease; Liver Cirrhosis; Diagnostic Imaging; Image Processing, Computer-Assisted; Acoustics

Sažetak

Uvod. Ultrasonografski, hepatocelularni karcinom se većinom prikazuje kao multinodularna ili nodularna promena različite veličine i ehostrukture uz moguće postojanje haloa, lateralnih senki, ali i posteriornog pojačanja zvuka poput cista i nekih hemangioma. Cilj rada bio je da se utvrdi sa kolikom se učestalošću fenomen posteriornog pojačanja ultrazvuka nalazi u slučaju postojanja hepatocelularnog karcinoma. **Materijal i metode.** Ispitivanje je sprovedeno kao retrospektivna studija kod 120 pacijenata kod kojih je urađen ultrasonografski pregled (*real time* ultrazvučni aparati različitih proizvođača, sonde 3,5 i 5 MHz) i kod kojih je patohistološki potvrđena dijagnoza hepatocelularnog karcinoma. Ultrasonografskim pregledom su analizirani veličina i izgled fokalnih promena, odnosno ehostruktura, te prisustvo ili odsustvo posteriornog pojačanja ultrazvuka kao zonalno povećanje ehogenosti pozadi u odnosu na fokalne promene. **Rezultati.** Posteriorno pojačanje zvuka bilo je prisutno kod 47,3% svih nodularnih hepatocelularnih karcinoma, pri čemu je ovaj ultrazvučni fenomen bio statistički značajno češće prisutan u grupi tumora veličine 3–5 cm. U grupi multinodularnih tumora posteriorno pojačanje zvuka nađeno je u 70% slučajeva. **Zaključak.** Nalaz posteriornog pojačanja ultrazvuka pri detekciji fokalne promene u jetri ovom metodom, može biti značajan nalaz u dijagnostici hepatocelularnog karcinoma, posebno kod pacijenata u rizičnim grupama za nastanak ovog tumora (ciroza i hronične bolesti jetre), kao i u slučaju intervalnog porasta fokalne promene sa prisutnim ovim ultrazvučnim fenomenom.

Ključne reči: ultrasonografija; hepatocelularni karcinom; neoplazme jetre; bolesti jetre; hronične bolesti; ciroza jetre; dijagnostički imidžing; kompjuterska obrada slike; akustika

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent carcinomas. According to the World Health Organization (WHO) it is the fifth most common carcinomas in the world, with a male to female ratio of 2 : 1 [1–5]. HCC is a neoplasm with a very poor prognosis, unless it is detected in its early stage or adequate state-of-the-art radical treatment is applied, i. e. surgical resection or radiofrequency ablation (RFA) [6, 7]. The five year survival rate among patients diagnosed with HCC is about 15% [8]. The incidence of HCC has been on a constant rise over the last decades not only in undeveloped countries but in the United States as well, which can be attributed primarily to the epidemic of hepatitis C virus [1, 2, 7]. In addition to hepatitis C virus infection, a number of other risk factors have been associated with this tumor, which develops most frequently in patients having chronic liver disease, i. e. cirrhosis [2–6]. Therefore, etiological factors of chronic liver disease overlap with the factors contrib-

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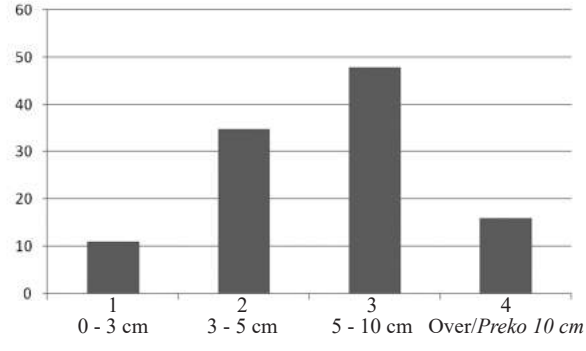
Abbreviations

HCC – hepatocellular carcinoma
RFA – radiofrequency ablation
CT – computed tomography
MRI – magnetic resonance imaging
AFP – alpha fetoprotein

uting to the development of HCC. Apart from hepatitis C and B virus, autoimmune hepatitis and alcohol consumption, the most recent data suggest an association with metabolic diseases – non-alcoholic fatty liver disease (NAFLD) and metabolic syndrome. In different parts of the world other etiological factors have been mentioned (alpha toxin, contraceptives, etc.) as well as the possibility of developing HCC from a subclass of adenoma. HCC rarely develops in a previously intact liver [2, 3, 7, 9–12].

Imaging methods have a crucial role in the diagnosis of HCC. It has become a clinical trend to use characteristic findings, most frequently computed tomography (CT)/magnetic resonance imaging (MRI), i. e. noninvasive criteria, whereas biopsy for the purpose of pathohistological verification is taken only in case of inconclusive imaging findings. Such a decision should be made by a multidisciplinary team [1–3, 6–9, 13–17].

Being non-invasive and widely available in clinical practice, ultrasound examination is the most frequently applied method in surveillance of groups at high risk for developing HCC [4, 5, 15, 18]. Screening protocols include mostly patients with chronic liver disease and cirrhosis, and they usually combine ultrasound monitoring at certain intervals (mostly from 3 to 6 months) with determination of specific biomarkers, most often alpha fetoprotein (AFP) [3, 6, 7, 9]. Generally speaking, AFP seems to be insufficiently reliable, because it is not significantly higher in all patients with HCC, and it can be slightly elevated in chronic liver diseases as well as in some other carcinomas (embryonic, gastric and lung carcinomas) [1, 6]. The American Association for the Study of Liver Diseases (AASLD) recommends ultrasound monitoring at six month intervals with or without determination of AFP [2, 4, 6].



0 - 3 cm 3 - 5 cm 5 - 10 cm over/preko 10 cm

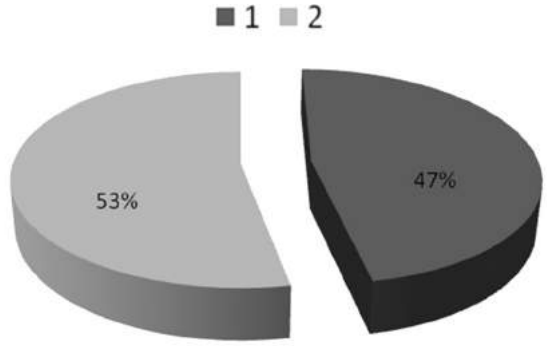
Graph 1. Distribution of HCC according to the tumor size
Grafikon 1*. Distribucija hepatocelularnih karcinoma prema veličini tumora*

On ultrasound, HCC presents with nodular or multinodular lesions of various size and structure, rarely as diffuse lesions. These focal lesions may have different structures, i. e. echogenicity: hypoechogenic, isoechogenic, hyperechogenic, heterogeneous (mosaic) pattern, and they can also have a halo around the lesion, lateral acoustic shadows as well as posterior acoustic enhancement [19]. As it is well known, posterior acoustic enhancement is one of the ultrasound characteristics of cystic focal lesions, and in a certain percentage of liver hemangiomas. However, some authors have found this ultrasound phenomenon to be present in diagnosed HCC [1, 20–22].

The aim of this study was to determine the frequency of posterior acoustic enhancement which was found to be a sonographic feature in some cases of diagnosed HCC. This is even more important because the availability of ultrasound enables detection of various liver lesions, which may often imitate or resemble HCC and vice versa by their ultrasonographic characteristics. The ultrasonographic characteristics of HCC found in the presence of other abnormal lesions in the liver may considerably impede the differential diagnosis and thus hinder early detection of HCC.

Material and Methods

This retrospective study included 120 patients treated at the Oncology Institute of Vojvodina and MC Polyclinic "Simed" in Novi Sad during a 15-year period. These patients had a histopathologically verified HCC. The diagnosis was based on ultrasound guided biopsy or examination of material obtained by surgical resection. The study sample included 88 men and 32 women, whose mean age was 62 years (the youngest and the oldest patients were 28 and 92 years old, respectively). The majority of patients were in the sixth decade of life;



Graph 2. Incidence of posterior acoustic enhancement in the group of histopathologically verified nodular HCC
Grafikon 2*. Učestalost posteriornog pojačanja zvuka u grupi patohistološki dokazanih nodularnih hepatocelularnih karcinoma*

Legend/*Legenda:* 1 - HCC with posterior acoustic enhancement/*hepatocelularnih karcinom sa posteriornim pojačanjem zvuka;* 2 - HCC without posterior acoustic enhancement/*hepatocelularnih karcinom bez poteriornog pojačanja zvuka*

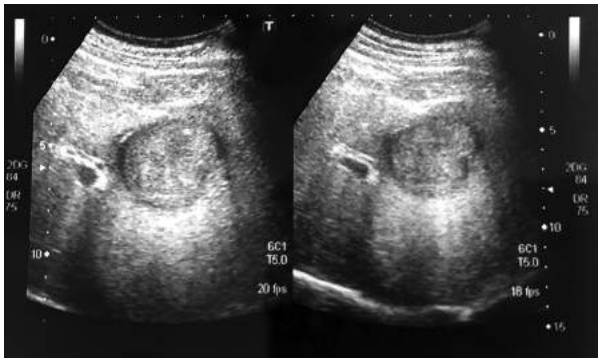


Figure 1. A well defined round focal lesion in the right hepatic lobe showing mosaic echostructure with a hypoechoic halo and posterior acoustic enhancement. A histopathologically verified hepatocellular carcinoma
Slika 1. *Jasno demarkirana okruglasta promena u desnom režnju jetre mozaične ehostrukture sa hipoehogenim haloom i posteriornim pojačanjem zvuka. Patohistološki dokazan hepatocelularni karcinom*

however, it should be noted that female patients were in younger age groups. Histopathological findings indicated a trabecular type of HCC in 91% of cases, whereas the remaining 9% had other types (mixed trabecular-adenoid, adenoid, trabecular-tubular and solid type). Only ¼ of cases (25.5%) presented with HCC in a previously intact liver. In other cases, HCC developed from a histopathologically verified chronic liver lesion, i. e. micronodular, macronodular, mixed (micro-macronodular) cirrhosis, chronic active hepatitis, chronic persistent hepatitis, steatosis as well as hemochromatosis in one patient.

There were no data on the presence of any known etiological factor in about 26.1% of the patients, while in others there was evidence of chronic alcohol consumption, viral hepatitis, and long-term use of contraceptives, antilipemic agents or exposure to pesticides. Hepatitis B virus markers were positive in 73% of the patients (HBsAg was found in 21.8% of the patients, while others had positive Anti-HBc or Anti-HBs). Hepatitis C virus was not included in our study due to the lack of sufficient data available for the period covered by our study.

Ultrasound examinations were performed using real time machines, routinely used in the above mentioned institutions (Siemens Sonoline SL -2, Hitachi EUB-525, Toshiba SSA-770A - Aplio, Toshiba Xario SSA 660A) using probes in the frequency range of 3.5 - 5 MHz. The examiners were experienced in the field of abdominal ultrasound imaging. There was no strict protocol of follow-up, whereas the follow-up interval was determined by the attending doctor-specialist. Ultrasound examinations focused on analyzing the size, location, number and echogenicity of the newly found tumor lesions, as well as the presence or absence of posterior acoustic enhancement, as areas of increased echogenicity behind the lesions.

Results

Out of the 120 histologically verified cases of HCC, ultrasound examination revealed a macroscopic nodular type of HCC (Eggel's classification) in 110 patients, i. e. 91.6% of cases, while the rest were diffuse (infiltrative) and massive tumors. Tumor size could only be determined in the group of nodular lesions, with lesions less than 3 cm being found in 10.2% of cases, tumors ranging from 3 to 5 cm observed in 32.1%, while tumors in the range of 5 to 10 cm were detected in the highest percentage of patients (43.2%). Tumors exceeding 10 cm were found in 14.5% of cases **(Graph 1)**. In 73 patients (60.8%), tumors developed in histologically verified cirrhotic livers, moderately differentiated tumors being considerably more frequent. The multinodular type of HCC was statistically considerably more frequent in cirrhotic livers than solitary forms of HCC. Multinodular type was found in 21.7% of all HCC cases. Posterior acoustic enhancement was observed in 47% of all nodular HCC **(Graph 2),** whereas this ultrasound phenomenon was statistically significantly more often seen in the group of tumors ranging from 3 to 5 cm **(Figures 1 and 2)**. Posterior acoustic enhancement was detected in 70% of multinodular tumors, whereas this phenomenon was absent in the rest of them. In the group of tumors smaller than 3 cm, hypoechogenic solitary tumors were significantly more frequent than hyperechogenic ones. They were most often well differentiated and statistically significantly more common in women than in men. Posterior acoustic enhancement was observed only in one case in this group, that being the case of hyperecho-

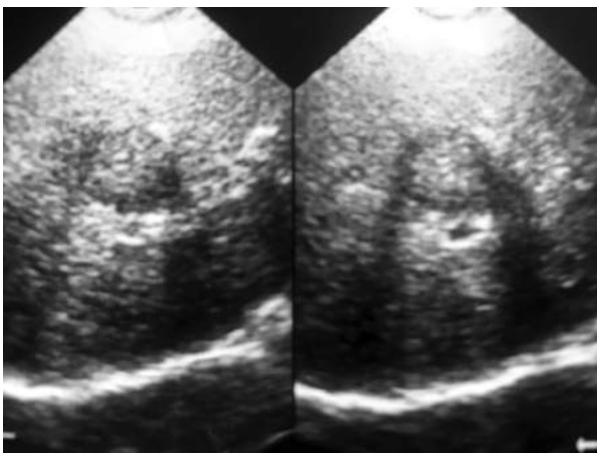


Figure 2. A focal ovoid lesion in the right hepatic lobe, centrally hyperechoic, with a prominent hypoechoic halo and a posterior acoustic enhancement. A histopathologically verified hepatocellular carcinoma
Slika 2. *Ovodina fokalna promena desnog režnja jetre, centralno hiperehogena, sa prominentnim hipoehogenim haloom i posteriornim pojačanjem zvuka. Patohistološki dokazan hepatocelularni karcinom*

genic HCC. Statistically significantly elevated values of AFP were found in 45% of cases.

Discussion

Posterior acoustic enhancement is the result of good transmission of ultrasound waves through a tumor of soft consistency, i. e. acoustic enhancement results from any lesion attenuating the sound less than the surrounding tissue. The intensity of the transmitted ultrasound beam is relatively preserved distal to the lesion.

It is not known for sure which histological substrate leads to the development of this ultrasound phenomenon, but it is assumed that a lesion with a simple structure or homogenous cell population found also in HCC, shows posterior acoustic enhancement in the cirrhotic liver. This phenomenon may result either from tissue characteristics of the lesion or cirrhosis itself.

Posterior acoustic enhancement is usually mentioned in the context of cystic lesions which attenuate sound less than any other soft tissue. This phenomenon is also found in the presence of hemangioma, and this is what we ourselves had observed during ultrasound monitoring of lesions with inconclusive CT/MRI findings until the final diagnosis was established. Maturen et al. [20] observed a typical sonographic image of hemangioma in 31 cases with hyperehogenic lesions with posterior acoustic enhancement which overlapped with HCC.

We observed the highest percentage (almost 50%) of posterior acoustic enhancement in the group of tumors between 3 and 5 cm and less frequently in tumors exceeding 5 cm and only once in a tumor smaller than 3 cm. Khan et al. [1] have also not found posterior acoustic enhancement in a group of tumors (which were mostly hypoechoic) smaller than 3 cm. Maturen et al. [20] found this ultrasound phenomenon in a group of advanced, bigger HCCs, which is in accordance with our results; however, Choi et al. [21] found the same phenomenon much more frequently in smaller tumors (smaller than 3 cm). This difference could be explained by the recognized differences in etiological factors in various regions where these studies were performed, i. e. by the presence or absence of chronic disease, with the majority of cases being underlying cirrhosis [9]. Another reason could be the fact that in various regions of the world with more advanced screening programs, HCC is detected when its dimensions are still rather small, and therefore bigger tumors seem to be less frequent.

The multinodular HCCs, being considerably more common in the cirrhotic liver, were found in 21.7% of our cases, whereas Okuda et al. [23] found them in 19% of cases in the United States and only in 12% of cases in Japan and Africa, which suggests a possible influence of various etiological factors in development of this type of HCC. In the group of multinodular tumors, the ratio of 70% of cases with posterior acoustic enhancement versus 30% of cases without it is considered interesting, because histopathological analysis of tumor tissue in the 70% revealed that the presence of clear (lucidocellulare) cells was smaller (48%), and these cells are thought to be the cause of tumor hyperechogenicity [24, 25]. Multinodular type of HCC without detectable posterior acoustic enhancement had a considerably higher percentage (66.7%) of clear cells. These results contribute to the actuality of the dilemma whether posterior acoustic enhancement occurs as a result of the tumor structure itself or underlying cirrhosis.

In the group of tumors smaller than 3 cm (more common in women) no considerable presence of posterior acoustic enhancement was detected. In this group, there was no statistically significant presence of clear (lucidocellulare) cells. Thus, the question arises regarding the correlation between both, the appearance of the lesion itself (hypo/hyperechogenicity) and the occurrence of a phenomenon such as posterior acoustic enhancement related to gender. There are other various possible etiological factors, as well as the possibility that women in our region go for check-ups earlier/more often so the disease is detected at its early stage, since it is known that early HCC are usually hypoechogenic [1, 19, 21].

Since these small hypoechogenic lesions (smaller than 3 cm) did not present with posterior acoustic enhancement in our results, we came to the conclusion that posterior acoustic enhancement was associated with advanced HCC. We agree with other authors that the changes in the structure of early HCC, when the tumor is growing, may cause the appearance of this ultrasound phenomenon (neovascularization, fatty changes, fibrosis, central necrosis, content of Kupffer cells, sinusoidal dilatation, etc.) [1, 3, 7, 14, 21, 23, 26]. However, in the context of early diagnosis, this fact could diminish the importance of posterior acoustic enhancement, so this could be a limiting factor of the significance of this ultrasound phenomenon in screening, i. e. early detection of HCC. Nevertheless, findings of posterior acoustic enhancement in the detection of focal liver lesions are considered very important since HCCs of bigger dimensions are still more often detected in our population. And last but not least, it is necessary to establish standardized ultrasound examination techniques, given the possible role of the examination technique itself, i. e. the importance of the examination without spatial compounding [21].

Conclusion

Posterior acoustic enhancement may be an important finding in the ultrasound diagnosis of hepatocellular carcinoma. Its occurrence within the monitored groups at risk for the development of hepatocellular carcinoma (cirrhosis, chronic liver disease), or particularly its presence in case of interval increase of a previously observed focal lesion, may be the reason to suggest more frequent ultrasound controls, indicate computed tomography or magnetic resonance imaging, as well as tumor biopsy in case of inconclusive imaging findings.

References

1. Gomaa AI, Khan SA, Leen EL, Waked I, Taylor-Robinson SD. Diagnosis of hepatocellular carcinoma. World J Gastroenterol. 2009;15(11):1301-14.

2. Heimbach JK, Laura MK, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018;67(1):358-80.

3. Bargellini I, Battaglia V, Bozzi E, Lauretti DL, Lorenzoni G, Bartolozzi C. Radiological diagnosis of hepatocellular carcinoma. J Hepatocell Carcinoma. 2014;1:137-48.

4. Bota S, Piscaglia F, Marinelli S, Pecorelli A, Terzi E, Bolondi L. Comparison of international guidelines for noninvasive diagnosis of hepatocellular carcinoma. Liver Cancer. 2012;1(3-4):190-200.

5. Reynolds AR, Furlan A, Fetzer DT, Sasatomi E, Borhani AA, Heller MT, et al. Infiltrative hepatocellular carcinoma: what radiologists need to know. Radiographics. 2015;35(2):371-86.

6. Sato T, Tateishi R, Yoshida H, Ohki T, Masuzaki R, Imamura J, et al. Ultrasound surveillance for early detection of hepatocellular carcinoma among patients with chronic hepatitis C. Hepatol Int. 2009;3(4):544-50.

7. Verslype C, Rosmorduc O, Rougier P. Hepatocellular carcinoma: ESMO-ESDO clinical practice guidelines for diagnosis, treatment and follow up. Ann Oncol. 2012;23 Suppl 7:vii41-8.

8. Bolondi L. State of the art: hepatocellular carcinoma. Future Oncol. 2014;10(15 Suppl):1-6.

9. Noda I, Kitamoto M, Nakahara H, Hayashi R, Okimoto T, Monzen Y, et al. Regular surveillance by imaging for early detection and better prognosis of hepatocellular carcinoma in patients infected with hepatitis C virus. J Gastroenterol. 2010;45(1):105-12.

10. Yau T, Chan P, Epstein R, Poon RT. Management of advanced hepatocellular carcinoma in the era of targeted therapy. Liver Int. 2009;29(1):10-7.

11. Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53(3):1020-2.

12. Yang JD, Harmsen WS, Slettedahl SW, Chaiteerakij R, Enders FT, Therneau TM, et al. Factors that affect risk for hepatocellular carcinoma and effects of surveillance. Clin Gastroenterol Hepatol. 2011;9(7):617-23.e1.

13. Lee JM, Trevisani F, Viligrain V, Wald C. Imaging diagnosis and staging of hepatocellular carcinoma. Liver Transpl. 2011;17(Suppl 2):S34-43.

14. Chen BB, Murakami T, Shih TT, Sakamoto M, Matsui O, Choi BI, et al. Novel imaging diagnosis for hepatocellular carcinoma: Consensus from the 5th Asia-Pacific Primary Liver Cancer Expert Meeting (Apple 2014). Liver Cancer. 2015;4(4):215-27.

15. Choi BI, Lee JM, Kim TK, Dioguardi Burgio M, Vilgrain V. Diagnosing borderline hepatic nodules in hepatocarcinogenesis: imaging performance. AJR Am J Roentgenol. 2015;205(1):10-21.

16. Di Martino M, De Filippis G, De Santis A, Geiger D, Del Monte M, Lombardo CV, et al. Hepatocellular carcinoma in cirrhotic patients: prospective comparison of US, CT and MR imaging. Eur Radiol. 2013;23(4):887-96.

17. Pahwa A, Beckett K, Channual S, Tan N, Lu DS, Raman SS. Efficacy of the American Association for the Study of Liver Disease and Barcelona criteria for the diagnosis of hepatocellular carcinoma. Abdom Imaging. 2014;39(4):753-60.

18. Yu NC, Chaudar V, Raman SS, Lassman C, Tong MJ, Busutil BW. CT and MRI improve detection of hepatocellular carcinoma, compared with ultrasound alone, in patients with cirrhosis. Clin Gastroenterol Hepatol. 2011;9(2):161-7.

19. Sheu JC, Chen DS, Sung JL, Chuang CN, Yang PM, Lin JT, et al. Hepatocellular carcinoma: US evolution in the early stage. Radiology. 1985;155(2):463-7.

20. Maturen KE, Wasnik AP, Bailey JE, Higgins EG, Rubin JM. Posterior acoustic enhancement in hepatocellular carcinoma. J Ultrasound Med. 2011;30(4):495-9.

21. Choi BI, Kim CW, Han MC, Kim CY, Lee HS, Kim ST, et al. Sonographic characteristics of small hepatocellular carcinoma. Gastrointest Radiol. 1989;14(3):255-61.

22. Jacobson D, Coombs B, Schmiedl U, Karani J, Haddad J. Hepatocellular carcinoma imaging. [Internet]. [updated 2016 Apr 8; cited 2019 Apr 20]. Available from: http://emedicine.medscape.com/article/369226-overview.

23. Okuda K, Peters LR, Simson IW. Gross anatomic features of hepatocellular carcinoma from three disparate geographic areas – proposal of new classification. Cancer. 1984;54(10):2165-73.

24. Stošić J. Evaluation of diagnostic models in patients with focal liver lesions [dissertation]. Novi Sad: Faculty of Medicine; 1997. Serbian.

25. Hsu HC, Sheu JC, Lin YH, Chen DS, Lee CS, Hwang LY, et al. Prognostic histologic features of resected small hepatocellular carcinoma (HCC) in Taiwan. A comparison with resected large HCC. Cancer 1985;56(3):672-80.

26. Kutami R, Nakashima Y, Nakashima O, Shiota K, Kojiro M. Pathomorphologic study on the mechanism of fatty change in small hepatocelluar carcinoma of humans. J Hepatol. 2000;33 (2):282-9.

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APPLICATION OF THE EIGHTH EDITION OF THE AMERICAN JOINT COMMITTEE ON CANCER STAGING SYSTEM FOR ORAL CARCINOMA

IMPLEMENTACIJA OSMOG IZDANJA SISTEMA STADIRANJA ORALNOG KARCINOMA AMERIČKOG KOMITETA ZA KARCINOM

Ivana MIJATOV and Saša MIJATOV

Summary

Introduction. Oral squamous cell carcinoma is the sixth most common carcinoma in the world. Annually, it accounts for 5% of all newly discovered cancers. The most important prognostic factor is the stage of the disease. The tumor, node, and metastasis staging system has been the cornerstone for clinical classification of oral squamous cell carcinoma. **Material and Methods**. The study included 65 patients with oral squamous cell carcinoma who underwent surgery at the Clinic of Maxillofacial Surgery of the Clinical Center of Vojvodina in Novi Sad. The tumor, node, and metastasis status was determined according to 7th and 8th edition of the tumor, node, and metastasis classification. **Results**. Statistical differences between the 7th and 8th edition of tumor, node, and metastasis classification were examined. There was also a change in the nodal status; in 20% of patients there was a transition from N1 to N2, as a result of a more precise definition of nodal status in patients with oral carcinoma. **Conclusion**. This research has pointed out the significance of tumor size as a predictive factor in oral squamous cell carcinoma, which indicates the importance of its local control (for surgical and radiological treatment). The 8th edition of the tumor, node, and metastasis classification for oral cavity cancers made a significant shift by clearly defining depth of tumor invasion into the tumor status.

Key words: Mouth Neoplasms; Carcinoma, Squamous Cell; Neoplasm Staging; Prognosis; Neoplasm Invasiveness; Tomography, X-Ray Computed; Predictive Value of Tests; Surgery, Oral

Sažetak

Uvod. Oralni planocelularni karcinom je po učestalosti šesti maligni tumor u svetu. Godišnje oko 5% svih novootkrivenih tumora pripada ovom malignom tumoru. Najvažniji prognostički faktor je stadijum bolesti. Postojeći sistem stadiranja koji uključuje veličinu tumora, nodalni status postojanje udaljenih metastaza je kamen temeljac klasifikacije po kliničkim stadijumima oralnog planocelularnog karcinoma. **Materijal i metode.** Istraživanje je uključilo 65 bolesnika sa oralnim planocelularnim karcinomom koji su lečeni na Klinici za maksilofacijalnu hirurgiju Kliničkog centra Vojvodine u Novom Sadu. Status tumora je određen na osnovu sedmog i osmog izdanja klasifikacije karcinoma Američkog komiteta za karcinom. **Rezultati.** Uočeno je postojanje statistički značajne razlike između sedmog i osmog izdanja kriterijuma za određivanje stadijuma oralnih planocelularnih karcinoma. Postojale su promene i u nodalnom statusu, kod 20% bolesnika uočen je prelaz iz N 1 u N 2 stadijum kao rezultat preciznije definicije N-statusa kod bolesnika sa oralnim karcinomom. **Zaključak.** Istraživanje je pokazalo značaj dimenzije tumora kao prediktivnog faktora kod planocelularnih oralnih karcinoma, čime se naglašava značaj lokalne kontrole kod lečenja oralnog planocelularnog karcinoma tokom hirurškog i radiološkog tretmana. Osmo izdanje klasifikacije za tumore oralne regije dovelo je do značajnih promena uvođenjem dubine invazije u T-status.

Ključne reči: neoplazme usta; skvamozni karcinom; stadiranje neoplazmi; prognoza; invazivnost neoplazmi; CT; prediktivna vrednost testova; oralna hirurgija

Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common carcinoma in the world. Annually, oral carcinoma accounts for 5% of all newly diagnosed tumors, and for 14% of all malignant tumors of the head and neck [1]. In developing countries, oral carcinoma is the third most common carcinoma, after colorectal and cervical carcinoma (Sri Lanka, India, Pakistan, Bangladesh, and Brazil). In some parts of India, oral carcinoma accounts for almost 50% of all malignant tumors. In developed countries, this type of malignant tumor is somewhat less common [1]. It is estimated that this malignant tumor is the 8th most common carcinoma in Europe, although these data vary in relation to the country. The highest incidence of oral carcinoma in Europe is in the Central and Eastern Europe, especially in Hungary, Slovakia and northern France. Not

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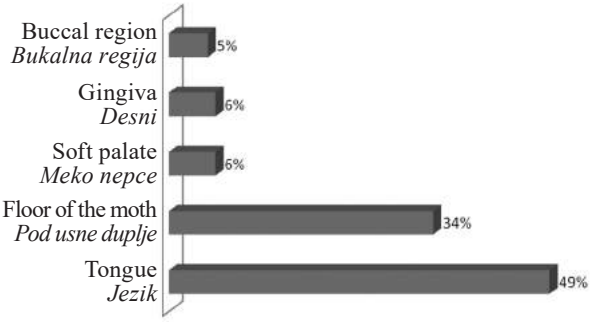
Abbreviations

OSCC – oral squamous cell carcinoma
TNM – tumor, node, and metastasis
HPV – human papillomavirus
AJCC – American Joint Committee on Cancer
DTI – depth of tumor invasion
CT – computed tomography
UICC – Union for International Cancer Control

enough attention has been paid to oral carcinoma, although around 200,000 people are diagnosed annually in the world. According to Globocan data, there were 354.854 newly discovered and registered patients in 2018, 726 in Serbia. The OSCC more commonly affects males than females (male : female ratio is 3 : 1), which is explained by a higher percentage of risky behaviors in men rather than in women. The main etiological factors for the development of oral planocellular carcinoma are smoking and alcohol consumption. These factors act independently as well as synergistically. Poor oral hygiene, inadequate prosthetic restraint, genetic malformations, malnutrition, hypovitaminosis, human papillomavirus (HPV) infections are also etiological factors affecting the development of OSCC [2]. The most common localization of OSCC is the mucosa of the tongue (20 – 40%) and floor of the mouth (15 – 20%), while other localizations of the oral region are affected by a significantly smaller percentage. The average five-year survival of patients is 50% [1, 3]. According to research, African American people have slightly worse five-year survival than Caucasians. The most important prognostic factor is the stage of the disease. The tumor, node, and metastasis (TNM) staging system has been the cornerstone for clinical classification of OSCC. The system itself had drawbacks; it was considered to have low prognostic value especially in the early tumor stage. The 8th edition of TNM staging system used by the American Joint Committee on Cancer (AJCC) brings many changes especially in T status by incorporating of depth of invasion (DTI) in the T category. The aim of this study was to verify the differences in classification of patients according to older 7th and newer 8th TNM classification.

Material and Methods

The study included 65 patients with OSCC who underwent surgery at the Clinic of Maxillofacial Surgery of the Clinical Center of Vojvodina in Novi Sad in the period January 1, 2015 – December 31, 2018. The diagnosis of OSCC was based on medical history, clinical examination and punch biopsies. After pathohistological confirmation of OSCC, the patients were examined by computed tomography (CT) of the head, neck and chest at the Clinic of Radiology of the Clinical Center of Vojvodina. The CT scans were made according to the protocol for CT examination of the head, neck and chest. The patients were operated under general anesthesia. The TNM stage was based on both CT scans and pathohistological findings. The pathological TNM status was determined according to the 7th and 8th editions of the TNM classification. Furthermore, the statistically significant differences between the 7th and 8th edition of TNM classification were examined. The χ^2 test was used with a statistical significance of $p < 0.05$.



Graph 1. Localization of the oral squamous cell carcinoma (No = 65)
Grafikon 1. *Lokalizacija oralnog planocelularnog karcinoma (Br. = 65)*

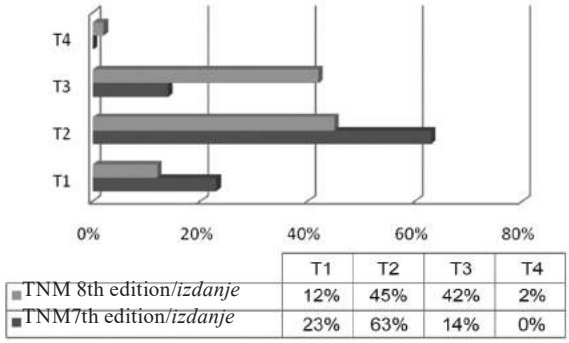
Results

Patients' characteristics

Out of 65 patients, the majority were male (82%) and the average age was 59.65 years (SD ± 9.425). The youngest patient was 38 years old and the oldest 84. Almost half of the subjects had a tumor localized in the mucosa of the tongue and floor of the mouth **(Graph 1)**. Around 83% of patients were smokers (38% smoked 30 cigarettes per day over 30 years), while 67% of patients regularly consumed alcohol.

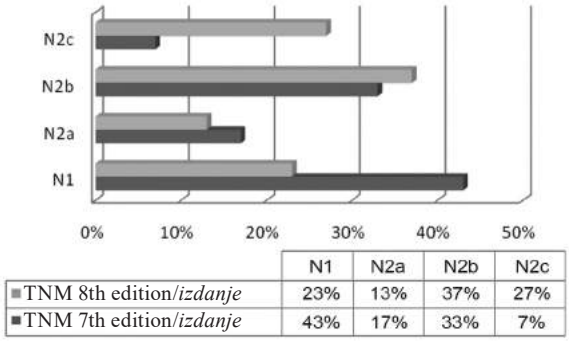
TNM classification of OSCC

Following histological verification of oral OSCC and CT of the head, neck and chest, all patients were operated under general anesthesia. The tumor excision with resection of the lower jaw and the appropriate dissection of the neck was made, depending on the radiological N status. Pathological TNM status was determined according to the 7th and 8th editions of the TNM classification **(Graphs 2 and 3)**. Subsequently,



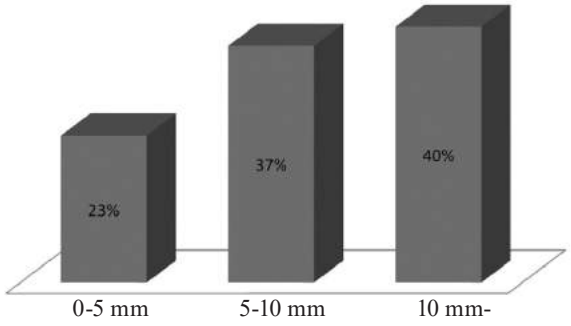
	T1	T2	T3	T4
TNM 8th edition/*izdanje*	12%	45%	42%	2%
TNM7th edition/*izdanje*	23%	63%	14%	0%

Graph 2. Changes in the T status of the examined patients (No = 65)
Grafikon 2. *Promene u T-statusu u uzorku (Br. = 65)*
Legenda: TNM - tumor, nodus i metastaze



	N1	N2a	N2b	N2c
TNM 8th edition/*izdanje*	23%	13%	37%	27%
TNM 7th edition/*izdanje*	43%	17%	33%	7%

Graph 3. Changes in the N status of the examined patients (No = 65)
Grafikon 3. *Razlike u N-statusu u uzorku (Br. = 65)*
Legenda: TNM - tumor, nodus i metastaze



Graph 4. Distribution of the patients according to the depth of tumor invasion (No = 65)
Grafikon 4. *Raspodela bolesnika prema dubini invazije tumora (Br. = 65)*

both T and N categories were compared using these two editions of the TNM classification.

There was a statistically significant difference in the T status between the 7th and 8th editions of TNM classification (Z = -3.921, p = 0.000). In 51% of patients, the T status remained the same, in 42% of patients the T category has increased, while in 8% of patients the T category has decreased. The main reason for changes in T status is implementation of the depth of tumor invasion in the T status. About 37% of the sample (N = 65) had a depth of tumor invasion of 5 – 10 mm, and in 40% the depth was over 10 mm **(Graph 4)**.

There was also a change in the N status; in 20% of patients there was a transition from N1 to N2 category as a result of a more precise definition of N status in patients with oral carcinoma in the 8th edition.

Discussion

The TNM classification of malignant tumors is a cancer staging system developed by Professor Pierre Denoix in the period between 1943 and 1952, which analyzes the size and extent of the primary tumor, the presence of regional lymph node metastases and the presence of distant metastases. The system itself is mostly developed by the Union for International Cancer Control (UICC) in order to establish consensus and standards for the classification of malignant tumors, as well as the AJCC. The UICC and AJCC classification systems were unified in 1987, as a unique TNM system for the classification of malignant tumors [4]. This classification system is used for staging malignant tumors, assessing the response to malignant tumor therapy and analyzing survival. The TNM system describes the malignant tumor stage by an alphanumeric code, describing the three most significant characteristics of malignant tumors [4, 5].

T (TX - T4) stage describes the size and extent of the tumor.

N (NX - N3) stage describes the number and size of lymph node metastasis.

M (M0 - M1) stage describes the presence or absence of distant metastases.

In addition to these three most important characteristics in the TNM classification, the following tumor characteristics are included:

G (1 - 4) degree of tumor cell differentiation
S (0 - 3) serum tumor marker level
R (0 - 2) radicalism of surgical tumor excision
L (0 - 1) invasion of lymph vessels
V (0 - 2) vein invasion (microscopic, macroscopic)
C (1 - 5) reliability modifier of specific parameters.

During guidance on the TNM staging, the following prefixes are used to describe the stage of malignant tumors even more accurately:

c prefix indicates the TNM stage determined by a clinical examination of the patient;

p prefix indicates the TNM stage described after the pathohistological examination of the preparation

y prefix indicates the TNM stage after chemotherapy, radiotherapy or neoadjuvant therapy;

r prefix indicates the TNM stage in recurrent malignant tumors;

a prefix indicates the TNM stage after autopsy;

u prefix indicates the TNM stage determined by ultrasound.

Today, the TNM classification system is used worldwide for staging most primary malignant tumors and carcinomas, but cannot be used in diffuse malignancies such as leukemia. Its use in staging diffuse lymphoma and ovarian carcinoma is also very limited. It should be emphasized that this system has changed and improved over time with the development of technology, new precise diagnostic methods, new biological discoveries and treatment of malignant tumors. So far, AJCC has published 8 revisions of the TNM classification of malignant tumors. Since 2018, the 8th revision of TNM tumor classification has been used. The greatest difference between the 7th and 8th editions of the TNM classification for oral carcinomas is in T status: in the new 8th edition, the T0 status has been removed, while in T1, T2 and T3, the depth of tumor invasion is included as a classification parameter [6]. In this study, when the TNM status was determined in pa-

Table 1. Differences in T status between 7th and 8th edition of TNM classification for oral carcinoma
Tabela 1. *Razlike u T-stadijumu između sedmog i osmog izdanja TNM statusa oralnog karcinoma*

TNM 7th edition from 2010/*TNM 7. izdanje 2010. godina*	TNM 8th edition from 2018/*TNM 8. izdanje 2018. godina*
T0 - no primary tumor/*T0 - nema primarnog tumora*	T0 – removed/*T0 je uklonjeno*
T1 - tumor ≤ 2 cm *T1 - tumor ≤ 2 cm*	T1- tumor ≤ 2 cm or DIT ≤ 5 mm *T1- tumor ≤ 2 cm ili dubina invazije ≤ 5 mm*
T2 - tumor size 2 – 4 cm *T2 - tumor veličine 2-4 cm*	T2 - tumor ≤ 2 cm with DIT 5 – 10 mm or tumor size 2 - 4cm with DIT ≤ 10 mm *T2- tumor ≤ 2 cm sa dubinom invazije 5-10 mm ili tumor veličine 2-4 cm sa dubinom invazije ≤10 mm*
T3 - tumor greater than 4 cm *T3 - tumor veći od 4 cm*	T3 - tumor greater than 4 cm or DIT ≥ 10 mm *T3 - tumor veći od 4 cm ili dubina invazije ≥ 10 mm*
T4 *T4* T4a - moderately advanced tumor with infiltration of the extrinsic muscle of the tongue/*T4a umeren uznaredovali tumor infiltracija ekstrinzičkih mišića jezika* T4b - advanced tumor/*T4b - uznapredovali tumor*	T4a - infiltration of extrinsic muscle of the tongue is excluded, implying invasion of the cortical bone and surrounding tissue as well as the maxillary sinus/*T4a - isključena infiltracija ekstrinzičkih mišića jezika, podrazumeva invaziju kortikalne kosti i okolnog tkiva kao i maksilarnog sinusa* T4b - advanced tumor/*T4b - uznapredovali tumor*

Legenda: TNM - tumor, nodus i metastaze

Table 2. Differences in N status between 7th and 8th edition of TNM classification for oral carcinoma
Tabela 2. *Razlike u N-stadijumu između sedmog i osmog izdanja TNM statusa oralnog karcinoma*

TNM 7th edition of 2010/*TNM 7. izdanje 2010. godina*	TNM 8th edition of 2018/*TNM 8. izdanje 2018. godina*
N0 – no invaded lymph nodes *N0 – nema invadiranih limfnih čvorova*	N0 – no invaded lymph nodes *N0 – nema invadiranih limfnih čvorova*
N1 – one invaded ipsilateral lymph node ≤ 3 cm *N1 – jedan invadiran ipsilateralni limfni čvor ≤ 3 cm*	N1 – one invaded ipsilateral lymph node ≤ 3 cm *N1 – jedan invadiran ipsilateralni limfni čvor ≤ 3 cm*
N2a – one invaded ipsilateral lymph node 3 – 6 cm *N2a – jedan invadirani ipsilateralni limfni čvor 3-6 cm* N2b – multiple invaded ipsilateral lymph nodes ≤ 6 cm *N2b – multipli invadirani ipsilateralni limfni čvorovi ≤6 cm* N2c – ipsi- or contralateral invaded lymph nodes ≤ 6 cm *N2c – ipsi ili kontralateralni invadirani limfni čvorovi ≤ 6 cm*	N2a – metastases in one ipsilateral lymph node > 3 cm but ≤ 6 cm in the largest diameter without extranodal spread or metastases in one lymph node < 3 cm with extranodal spread/*N2a – metastaza u jednom ipsilateralnom limfnom čvoru > 3 cm ali ≤ 6 cm u najvećem dijametru bez ekstranodalnog širenja ili metastaza u jednom limfnom čvoru < 3 cm sa ekstranodalnim širenjem* N2b – ipsilateral multiple metastases in the lymph nodes > 6 cm in the largest diathesis without extranodal spread/*N2b – ipsilateralne multiple metastaze u limfnim čvorovima > 6 cm u najvećem dijametru bez ekstranodalnog širenja* N2c – bilateral or contralateral metastatic altered lymph nodes > 6 cm in the largest diameter without extranodal spread/*N2c – bilateralni ili kontralateralni metastatski izmenjenim limfni čvorovi > 6 cm u najvećem dijametru bez ekstranodalnog širenja*
N3 – any invaded lymph node > 6 cm *N3 – bilo koji invadirani limfni čvor > 6 cm*	N3a – metastasis in the lymph node > 6 cm in the largest diameter without extranodal spread *N3a – metastaza u limfnom čvoru > 6 cm u najvećem dijametru bez ekstranodalnog širenja* N3b – metastases in the lymph node > 3 cm with extranodal expansion, multiple ipsilateral, contralateral and bilateral metastases in lymph nodes with extranodal spread or one contralateral metsatasis in the lymph node less than 3 cm in size with extranodal spread *N3b – metastaza u limfnom čvoru > 3cm sa ekstranodalnim širenjem, multiple ipsilateralne, kontralateralne i bilateralne metastaze u limfnim čvorovima sa ekstranodalnm širenjem ili jedna kontralateralna metastaza u limfnim čvoru manja od 3 cm sa ekstranodalnim širenjem*

Legenda: TNM - tumor, nodus i metastaze

tients with OSCC, the first TNM was determined according to the 7th edition of the TNM classification (since the research took place in 2016 when the 7th edition of the TNM classification was valid). The second TNM was determined according to the 8th edition, after it was published. The largest changes were recorded in T status, with 49% of patients experiencing a change in T status with a transition to a higher T status (42% of patients). This change in T status was statistically significant (Z = -3.921, p = 0.000) and was a result of a more precise definition of the pathological T status and the introduction of new quantitative characteristics. In addition to the macroscopic measurement, the depth of tumor invasion is included as an important characteristic in T1, T2 and T3 stage of the 8th edition of the TNM classification from 2018 **(Table 1)**. According to the 8th edition of the TNM classification (TNM from 2010 and 2018), the highest percentage of patients in the study had T2 (45%) and T3 (42%) stage of tumor size. The depth of invasion (DIT) defines tumor extensions below the surface of the epithelium that indicates a vertical invasion of the tumor from the level of the basal membrane of intact mucosa to the site of the greatest depth of tumor invasion [6, 7]. The interesting fact about this measuring technique is that it can cause practically 'thinner' ulcerous tumors to have a greater depth of invasion, since the DOI is measured from the level of the basal membrane of the intact mucous membrane. In this case, the DOI is greater than the thickness of the tumor [8, 9].

The implementation of the DTI at T status has led to more precise definition of T status of oral squamous cell carcinoma **(Table 1)**. In addition to the T status changes, the new, 8th edition of the TNM classification of oral carcinoma has brought changes in the N status [10, 11]. A qualitative category of presence or absence of extranodal spread has been introduced **(Table 2)**, in addition to already existing quantitative categories of the number and size of invaded lymph nodes [12, 13]. Also, the N3 category is divided into three subcategories. The changes were recorded in tested samples in the N status; 20% of patients who according to TNM classification from 2010 had N1 status, according to the TNM classification from 2018 had N2 status [14]. The main reason is the inclusion of extranodal propagation as one of the characteristics of the N status **(Table 2)** [15].

Conclusion

This study has shown the significance of tumor dimension as a predictive factor in oral squamous cell carcinoma, which indicates the importance of its local control (for surgical and radiological treatment). In recent years, the phenotypic and biological differences of primary tumors have been studied in patients with cervical nodal metastases and in those who did not have symptoms of metastatic cancer because of different responses of tumor cells to therapies with regard to tumor microenvironment. It is believed that the characteristics of the metastatic lymph nodes may be an independent prognostic survival factor, such as the presence of distant metastases. This opens the door to new research in defining the metastasis cascade and parameters significant for the survival of patients suffering from oral squamous cell carcinoma. All these investigations leave room for new revisions of the tumor classification and the introduction of new parameters that are significant predictive factors, both in T and N status of the tumor, node, and metastasis classification. The 8th edition of the tumor, node, and metastasis classification system for tumors of the oral region made a significant shift by more clearly defining depth of tumor invasion in the T status. Future revisions will most likely include some other biological and dimensional characteristics of tumors in the T status, in order to more precisely determine the status itself and therapeutic and predictive potentials.

References

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009;45(4-5):309-16.

2. Scully C, Bagan JV, Hopper C, Epstein JB. Oral cancer: current and future diagnostic techniques. Am J Dent. 2008;21(4):199-209.

3. Gamez ME, Kraus R, Hinni ML, Moore EJ, Ma DJ, Ko SJ, et al. Treatment outcomes of squamous cell carcinoma of the oral cavity in young adults. Oral Oncol. 2018;87:43-8.

4. Lydiatt WM, Patel SG, O'Sullivan B, Brandwein MS, Ridge JA, Migliacci JC, et al. Head and neck cancers - major changes in the American Joint Committee on cancer eighth edition cancer staging manual. CA Cancer J Clin. 2017;67(2):122-37.

5. Brierley J, Byrd D, Bosman F, Kehoe S, Kossary C, Piñeros M, et al. The TNM classification of malignant tumours-towards common understanding and reasonable expectations. Lancet Oncol. 2017;18(7):849-51.

6. Seethala RR, Weinreb I, Bullock MJ, Carlson DL, Ferris RL, Harrison LB, et al. Protocol for the examination of specimens from patients with cancers of the lip and oral cavity [Internet]. 2017 [cited 2019 May 5]. Available from: https://webcache.googleusercontent.com/search?q=cache:PmTUjvRqbhoJ:https://documents.cap.org/protocols/cp-headandneck-lip-oralcavity-17protocol-4001.docx+&cd=1&hl=en&ct=clnk&gl=rs.

7. Ebrahimi A, Gil Z, Amit M, Yen TC, Liao CT, Chaturvedi P, et al. Primary tumor staging for oral cancer and a proposed modification incorporating depth of invasion: an international multicenter retrospective study. JAMA Otolaryngol Head Neck Surg. 2014;140(12):1138-48.

8. Müler S. Update from the 4th edition of the World Health Organization of head and neck tumours: Tumours of the oral cavity and mobile tongue. Head Neck Pathol. 2017;11(1):33-40.

9. Binmadi NO, Basile JR. Perineural invasion in oral squamous cell carcinoma: a discussion of significance and review of literature. Oral Oncol. 2011;47(11):1005-10.

10. Lee CC, Su YC, Hung SK, Chen PC, Huang CI, Huang WL, et al. Recommendation for incorporation of a different lymph node

scoring system in future AJCC N category for oral cancer. Sci Rep. 2017;(1):14117.

11. Wreesmann VB, Katabi N, Palmer FL, Montero PH, Migliacci JC, Gönen M, et al. Influence of extracapsular nodal spread extent on prognosis of oral squamous cell carcinoma. Head Neck. 2015;38(Suppl 1):E1192-9.

12. De Silva RK, Siriwardena BSMS, Samaranayaka A, Abeyasinghe WAMUL, Tilakaratne WM. A model to predict nodal metastasis in patients with oral squamous cell carcinoma. PLoS One. 2018;13(8):e0201755.

13. Pentenero M, Gandolfo S, Carrozzo M. Importance of tumor thickness and depth of invasion in nodal involvement and prognosis of oral squamous cell carcinoma: a review of the literature. Head Neck. 2005;27(12):1080-91.

14. Bernier J, Cooper JS, Pajak TF, van Glabbeke M, Bourhis J, Forastiere A, et al. Defining risk levels in locally advanced head and neck cancers: a comparative analysis of concurrent postoperative radiation plus chemotherapy trials of the EORTC (#22931) and RTOG (# 9501). Head Neck. 2005;27(10):843-50.

15. Fruhwirth GO, Diocou S, Blower PJ, Ng T, Mullen GE. A whole-body dual-modality radionuclide optical strategy for preclinical imaging of metastasis and heterogenous treatment response in different microenvironments. J Nucl Med. 2014;55(4):686-94.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
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EFFECTS OF INTRAVITREAL AFLIBERCEPT (EYLEA) IN THE TREATMENT OF BILATERAL CYSTOID MACULAR EDEMA IN RETINITIS PIGMENTOSA – A CASE REPORT

EFFEKTI INTRAVITREALNOG AFLIBERCEPTA (EYLEA) U LEČENJU BILATERALNOG CISTOIDNOG EDEMA KOD RETINITIS PIGMENTOSAE – PRIKAZ SLUČAJA

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Summary

Introduction. The aim of the study was to evaluate the effects of intravireal injections of aflibercept (Eylea) on bilateral cystoid macular edema in a patient with retinitis pigmentosa. **Material and Methods**. A 17-year-old man presented with a moderate bilateral decrease of visual acuity (0.3) and ocular examination was performed. Optical coherence tomography imaging was performed and cystoid macular edema was detected in both eyes. Due to disease progression in a short period of time, intravitreal repeated injections of aflibercept (Eylea) were initiated according to recent clinical reports. **Results.** The initial values of cystoid macular edema before intravitreal therapy were 248 μm in the right and 237 μm in the left eye; they increased slowly in next several weeks. Four bilateral repeated doses of intravitreal aflibercept injections at 6-week intervals were given in local anesthesia. The patient reported a subjective improvement, and his visual acuity was 4/10 in both eyes. Objectively, the macular edema decreased at week 24, reaching 173 μm in the right and 188 μm in the left eye. **Conclusion.** There are few literature reports on the possible effects of intravitreal aflibercept injections in the treatment of retinitis pigmentosa-related cystoid macular edema. In our study, bilateral macular edema in a patient with retinitis pigmentosa has improved significantly after four consecutive treatments. Further studies are necessary with a larger sample size and longer follow-up period to obtain information on the role and safety of intravitreal drugs for cystoid macular edema in retinitis pigmentosa.

Key words: Macular Edema; Retinitis Pigmentosa; Intravitreal Injections; Recombinant Fusion Proteins; Treatment Outcome

Sažetak

Uvod. Cilj rada je ispitivanje efekta intravitrealnih injekcija aflibercepta (*Elyea*) na bilateralni cistoidni makularni edem kod pacijenta koji boluje od retinitisne pigmentoze. **Materijal i metode.** Sedamnaestogodišnji mladić se u pratnji roditelja javio na oftalmološki pregled zbog umerenog smanjenja vidne oštrine (0,3) na oba oka. Cistoidni edem makule je utvrđen na oba oka optičkom koherentnom tomografijom i pošto se pogoršao u kratkom vremenskom periodu, prema najnovijim kliničkim studijama, uključeni su lekovi nove generacije – aflibercept (*Eylea*) intravitrealne injekcije. **Rezultati.** Početne vrednosti cistoidnog makularnog edema pre intravitrealne terapije bile su na desnom oku 248 μm, a na levom oku 237 μm i postepeno su se povećavale u narednih nekoliko nedelja. Aplikovane su po četiri doze leka, u vidu intravitrealnih injekcija aflibercepta u svako oko, u razmaku od šest nedelja. Nakon toga je postignuto subjektivno poboljšanje, uz poboljšanje vidne oštrine na oba oka, kao i smanjenje makularnog edema. Posle 24 nedelje, centralna debljina makule na desnom oku iznosila je 173 μm, a na levom 188 μm. **Zaključak.** Postoji malo dostupnih podataka iz literature o mogućoj ulozi aflibercepta (*Eylea*) u vidu intravitrealnih injekcija u lečenju cistoidnog makularnog edema kod pacijenata sa retinitisnom pigmentozom. U našoj studiji, došlo je do značajnog poboljšanja nakon četiri konsekutivne intravitrealne injekcije aflibercepta (*Eylea*) u svako oko. Dalje studije su neophodne kako bi se obezbedio veći uzorak i duži period praćenja, te dobile informacije o ulozi i sigurnosti primene intravitrealnih lekova kod ovog naslednog očnog oboljenja.

Ključne reči: makularni edem; retinitis pigmentosa; intravitrealne injekcije; rekombinantni fuzioni proteini; ishod lečenja

Introduction

The term retinitis pigmentosa (RP) refers to a group of hereditary conditions [1] also known as retinal dystrophy. It is caused by the loss of photoreceptors, and is clinically characterized by retinal pigment deposits visible on fundus examination [2]**.** The disease can be inherited as an autosomal-dom-

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Abbreviations

RP – retinitis pigmentosa
CMO – cystoid macular edema
VEGF – vascular endothelial growth factor
BCVA – best corrected visual acuity
CMT – central macular thickness

inant (about 30 – 40% of cases), autosomal-recessive (50 – 60%), or X-linked (5 – 15%) [3]. It is found in approximately 1/4000 individuals, and globally it affects a total of 1 million individuals.

Typical symptoms of RP include functional signs, signs related to visual acuity, and retinal pigment deposits visible on fundus examination and by electroretinography [2]. The signs also include: nyctalopia, photopsia, and progressive visual field loss; however, vision can also be affected by cataracts and/or cystoid macular edema (CMO) [4].

In addition to attenuated retinal vessels, atrophic optic disc changes, bone spicules and pigment clumping, macular cysts have been reported to be associated with RP. The CMO in RP patients is not so common, it has been reported in 11% to 20% of cases, but in some newer studies it varies from 26.9% to even 49% [5–9]. When it develops, CMO may markedly reduce central vision function in RP and lead to severe visual handicap in already visually impaired eyes [10, 11].

The suggested etiological mechanisms include: anti-retinal antibodies, retinal pigment epithelium dysfunction, Muller cell edema, and vitreous traction [12–15].

Various treatments have been attempted to manage CMO in RP, including: systemic, topical and intravitreal corticosteroids, intravitreal or subtenon triamcinolone, topical and oral carbonic anhydrase inhibitors, grid laser photocoagulation of macula, vitrectomy, oral lutein supplementation, etc. However, the use of some types of medications (for example, carbonic anhydrase inhibitors, specifically acetazolamide) is often limited because of their systemic side effects, and most of suggested therapeutic options have only limited and highly variable treatment efficacy [4, 10].

Some studies available in the literature showed that improvement of visual acuity and macular thickness was observed in patients with refractory RP-associated CMO taking oral acetazolamide, followed by intravitreal pegaptanib with no recurrence of CMO. In some cases of RP, like Melo et al. reported, RP-associated CMO did not respond well to intravitreal bevacizumab treatment [16]. On the other hand, certain improvement of macular thickness and visual acuity was confirmed in the study of Yuzbasioglu et al, after use of intravitreal bevacizumab [17]. Similar improvement of CMO was seen in the study of Artunay et al., who were using intravitreal ranibizumab [18].

In a limited number of studies available so far, with recent case reports on CMO associated with RP, the use of intravitreal aflibercept has shown improvement in both visual acuity and macular thickness. Aflibercept has a unique design, different from all the other anti-vascular endothelial growth factor (VEGF) medications, because it acts as a decoy receptor [4]. Aflibercept is a fusion protein (Fc) with constant region of human immunoglobulin G (IgG) and consists of extracellular domains of human VEGF 1 and 2, with improved binding affinity and superior pharmacokinetics. In case report of Mustafa et al., a single unilateral intravitreal injection of aflibercept was given to a patient with RP-associated CMO. Improvement in both visual acuity and macular thickness was seen at 1 month post-injection as well as maintenance of this improvement documented at 6 months [19].

None of the existing therapies has been confirmed so far to have absolute supremacy when it comes to the therapy of RP-related CMO. Despite the fact that pathogenic mechanisms of CMO development in RP have not yet been fully clarified, it is known that VEGF plays a significant role in its development, and can be a potential target of therapy. Aflibercept, as a newer drug, can have a leading role in RP associated CMO therapy, due to all its pharmacological characteristics, as well as the safety profile shown during long-term treatment [20]. More extensive studies, with larger samples of patients and longer-lasting application of intravitreal drugs are needed to confirm improvement of symptoms and clinical aspect, with caution due to potential adverse effects of intravitreal therapy.

Case Report

In 2017, a 17-year-old young man visited the private Eye Center in Novi Sad, Serbia, with his parents. He was previously diagnosed with myopic and astigmatic correction of -3.0 Dsph/ -1.0 Dcylax 0 in both eyes, with best corrected visual acuity (BCVA) of 6/10 ever achieved, suggesting mild bilateral amblyopia. Fundoscopy revealed bilateral dense bone spicules, bilateral CMO, attenuated retinal vessels, and pale optic discs **(Figure 1)**. Spectral domain optical coherence tomography (SDOCT, Zeiss) showed marked bilateral CMO with central macular thickness (CMT) of 248 and 237 μm in the right and left eye, respectively **(Figure 2)**. Intraocular pressure was 17/18 mmHg, and computerized visual field (VF) was performed by Zeiss Humphrey perimeter revealing constricted fields of 10 – 20 degrees in both eyes. He had no history of any previous CMO in earlier 3-year regular ophthalmologic follow-ups, with last CMT of 190 μm in his right, and 182 μm in his left eye. His mother and his brother also have RP, with a milder clinical form, and BCVA of 20/20.

Initially, he received topical dorzolamide (20 mg/ml) eye drops bilaterally twice a day for 3 months, with no significant improvement in the degree or extent of CMO. On further examination, BCVA got worse in a short period of time due to CMO and it was 3/10 in both eyes. Therefore, this young man and his parents were given the information about new clinical study reports and a possible intravitreal treatment of CMO with aflibercept (Eylea).

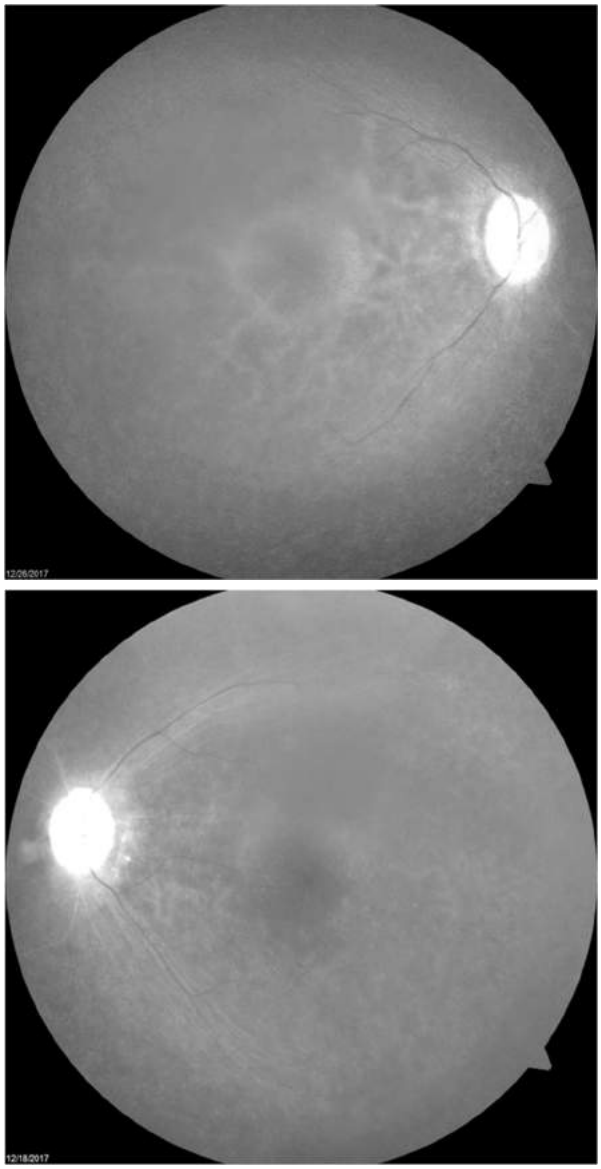

Figure 1. Fundoscopy of a patient with retinitis pigmentosa and associated bilateral cystoid macular edema; the right and left eye
Slika 1. *Slika očnog dna pacijenta sa retinitisnom pigmentozom i udruženim obostranim cistoidnim edemom makule; desno i levo oko*

The anti-VEGF medication selected for this patient was aflibercept (EYLEA; Regeneron Pharmaceuticals, Inc., Tarrytown, New York, USA, and Bayer Healthcare Pharmaceuticals, Berlin, Germany). Risks and benefits of the treatment were discussed with the patient. It was also highlighted that there was a limited evidence base for its usage in RP-associated CMO.

An informed consent was taken and the patient received bilateral intravitreal injections of 0.05 ml aflibercept (40 mg/ml) via standard aseptic technique in the operating theatre. There were no peri- or post-operative complications. A post-operative steroid anti-inflammatory antibiotic and non-steroidal anti-inflammatory drug eye drops were prescribed. Other oral and local medications were immediately discontinued with the start of aflibercept course.

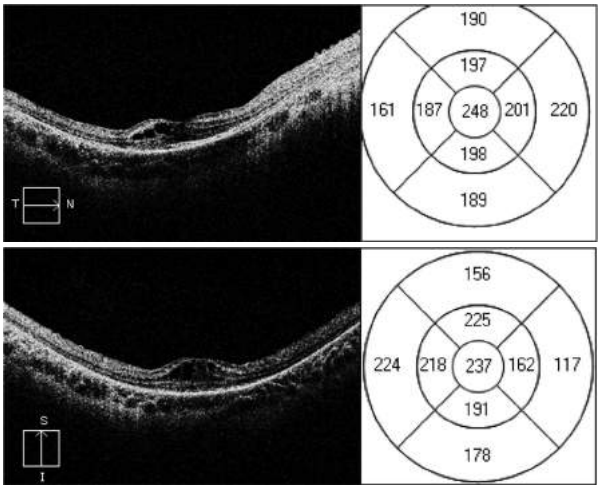

Figure 2. Optical coherence tomography of macula of the right and left eye before intravitreal injections of aflibercept for retinitis pigmentosa associated cystoid macular edema; central macular thickness in microns
Slika 2. *Optička koherentna tomografija makule desnog i levog oka, pre intravitrealne injekcije aflibercepta u lečenju cistoidnog edema makule kod pacijenta sa retinitisnom pigmentozom; centralna debljina makule izražena u mikronima*

Six weeks after the first treatment, BCVA improved to 4/10 in both eyes and the patient noticed subjective improvement. Optical coherence tomography revealed a marked CMO in both eyes, with CMT of 263 and 243 μm in the right and left eye, respectively. Second uncomplicated bilateral intravitreal injection of aflibercept was undertaken.

After 12 weeks from the beginning, his CMT in the right eye was reduced to 53 μm and to 46 μm in the left eye. After 18 weeks, the patient was treated with the third intravitreal aflibercet injection in both eyes, due to persistent CMO and re-accumulation of fluid (increase in CMO was noticed, 20 μm in the right and 35 μm in the left eye compared to CMT right before the third injection). After the third dose, only a minimal reduction of CMO was observed, and there was no major change in BCVA bilaterally. After the fourth injection of aflibercept in both eyes, and after 6 weeks of follow-up, CME decreased by 57 μm in the right and 44 μm in the left eye, compared to values measured before the fourth injection **(Table 1)**. At week 24, the patient's BCVA remained stable, at 4/10 in both eyes, but he had subjective impression of brighter vision, while his CMT returned to 173 μm in the right, and 188 μm in the left eye.

Regular follow-ups were scheduled for this patient and his CMO varied over time. It worsened seriously after the discontinuation of intravitreal aflibercept therapy after a year to almost double in regard to the baseline.

Table 1. Central macular thickness in cystoid macular edema in a patient with retinitis pigmentosa during four consecutive intravitreal injections of aflibercept in both eyes
Tabela 1. *Vrednosti centralne debljine makule kod cistoidnog edema makule kod pacijenta sa retinitis pigmentosom, tokom četiri konsekutivne intravitrealne injekcije aflibercepta u oba oka*

Central macular thickness (μm) *Centralna debljina makule (μm)*	Aflibercept intravitreal injection *Aflibercept intravitrealna injekcija*	Right eye *Desno oko*	Left eye *Levo oko*
3 months before aflibercept therapy/*3 meseca pre početka intravitrealne terapije afliberceptom*		190	182
0 week/*0. nedelja*	1st dose/*1. doza*	248	237
6th week/*6. nedelja*	2nd dose/*2. doza*	263	243
12th week/*12. nedelja*	3rd dose/*3. doza*	210	197
18th week/*18. nedelja*	4th dose/*4. doza*	230	232
24th week/*24. nedelja*	–	173	188

Conclusion

There are only few literature reports on the possible role of intravitreal aflibercept therapy for the treatment of retinitis pigmentosa-related cystoids macular edema. In our patient with retinitis pigmentosa, bilateral macular edema has improved significantly after four consecutive intravitreal treatments with aflibercept. Further studies are necessary with a larger sample size and longer follow-up period, to obtain information on the role and safety of intravitreal drugs for cystoids macular edema in retinitis pigmentosa.

References

1. Marmor MF, Aguirre G, Arden G, Berson E, Birch DG, Boughman JA, et al. Retinitis pigmentosa: a symposium on terminology and methods of examination. Ophthalmology. 1983;90 (2):126-31.

2. Hamel C. Retinitis pigmentosa. Orphanet J Rare Dis. 2006;1:40.

3. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet. 2006;368(9549):1795-809.

4. Strong SA, Gurbaxani A, Michaelides M. Treatment of retinitis pigmentosa-associated cystoid macular oedema using intravitreal aflibercept (eylea) despite minimal response to ranibizumab (lucentis): a case report. Case Rep Ophthalmol. 2016;7(2):389-97.

5. Hajali M, Fishman GA, Anderson RJ. The prevalence of cystoid macular oedema in retinitis pigmentosa patients determined by optical coherence tomography. Br J Ophthalmol. 2008;92(8):1065-8.

6. Pruett RC. Retinitis pigmentosa - clinical observations and correlations. Trans Am Ophthalmol Soc. 1983;81:693-735.

7. Fishman GA, Maggiano JM, Fishman M. Foveal lesions seen in retinitis pigmentosa. Arch Ophthalmol. 1977;95(11):1993-6.

8. Hirakawa H, Iijima H, Gohdo T, Tsukahara S. Optical coherence tomography of cystoid macular edema associated with retinitis pigmentosa. Am J Ophthalmol. 1999;128(2):185-91.

9. Liew G, Strong S, Bradley P, Severn P, Moore AT, Webster AR, et al. Prevalence of cystoid macular oedema, epiretinal membrane and cataract in retinitis pigmentosa. Br J Ophthalmol. 2018 Oct 5:1-4.

10. Ozdemir H, Karacorlu M, Karacorlu S. Intravitreal triamcinolone acetonide for treatment of cystoid macular oedema in patients with retinitis pigmentosa. Acta Ophthalmol Scand. 2005;83(2):248-51.

11. Newsome DA, Blacharski PA. Grid photocoagulation for macular edema in patients with retinitis pigmentosa. Am J Ophthalmol. 1987;103(2):161-6.

12. Heckenlively JR, Jordan BL, Aptsiauri N. Association of antiretinal antibodies and cystoid macular edema in patients with retinitis pigmentosa. Am J Ophthalmol. 1999;127(5):565-73.

13. Strauss O. The retinal pigment epithelium in visual function. Physiol Rev. 2005;85(3):845-81.

14. Reichenbach A, Wurm A, Pannicke T, Iandiev I, Wiedemann P, Bringmann A. Muller cells as players in retinal degeneration and edema. Graefes Arch Clin Exp Ophthalmol 2007;245 (5):627-36.

15. Schepens CL, Avila MP, Jalkh AE, Trempe CL. Role of the vitreous in cystoid macular edema. Surv Ophthalmol. 1984;28(Suppl):499-504.

16. Querques G, Prascina F, Iaculli C, Noci ND. Intravitreal pegaptanib sodium (Macugen) for refractory cystoid macular edema in pericentral retinitis pigmentosa. Int Ophthalmol. 2009;29(2):103-7.

17. Melo GB, Farah ME, Aggio FB. Intravitreal injection of bevacizumab for cystoid macular edema in retinitis pigmentosa. Acta Ophthalmol Scand. 2007;85(4):461-3.

18. Yuzbasioglu E, Artunay O, Rasier R, Sengul A, Bahcecioglu H. Intravitreal bevacizumab (Avastin) injection in retinitis pigmentosa. Curr Eye Res. 2009;34(3):231-7.

19. Artunay O, Yuzbasioglu E, Rasier R, Sengul A, Bahcecioglu H. Intravitreal ranibizumab in the treatment of cystoid macular edema associated with retinitis pigmentosa. J Ocul Pharmacol Ther. 2009;25(6):545-50.

20. Schmidt-Erfurth U, Kaiser PK, Korobelnik JF, Brown DM, Chong V, Nguyen QD, et al. Intravitreal aflibercept injection for neovascular age-related macular degeneration: ninety-six-week results of the VIEW studies. Ophthalmology. 2014;121(1):193-201.

21. Moustafa GA, Moschos MM. Intravitreal aflibercept (Eylea) injection for cystoid macular edema secondary to retinitis pigmentosa - a first case report and short review of the literature. BMC Ophthalmol. 2015;15:44.

22. Miyata M, Oishi A, Oishi M, Hasegawa T, Ikeda HO, Tsujikawa A. Long-term efficacy and safety of anti-VEGF therapy in retinitis pigmentosa: a case report. BMC Ophthalmol. 2018;18(1):248.

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ATYPICAL LOCATION OF ST ELEVATION ACUTE MYOCARDIAL INFARCTION IN RELATION TO THE ELECTROCARDIOGRAM

ATIPIČNA LOKALIZACIJA AKUTNOG INFARKTA MIOKARDA SA ST ELEVACIJOM U ODNOSU NA ELEKTROKARDIOGRAFSKI ZAPIS

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Summary

Introduction. Electrocardiography is an initial non-invasive diagnostic algorithm for ST elevation acute myocardial infarction. Specific electrocardiographic phenomenon is described, when the occlusion of the proximal segment of the right coronary artery or the isolated occlusion of its ventricular branch is presented with ST elevation in the precordial leads. **Case Report.** A 78-year-old woman was admitted as an emergency due to chest pain and electrocardiographically recorded concave elevation in leads V1 – V3. She was diagnosed with ST elevation myocardial infarction of the anterior region and sent to catheterization laboratory for emergency coronary angiography. It showed an occlusion of the proximal-medial right coronary artery. Behind the occlusion, the right coronary artery, posterior descending artery and posterior lateral artery, a hetero-collateral circulation was seen. Two drug-eluting stents were implanted into the proximal segment of the right coronary artery. **Discussion.** The phenomenon of acute myocardial infarction caused by occlusion of the proximal right coronary artery and/or ventricular branches of the right coronary artery, presenting with ST segment elevation in the precordial leads, is a consequence of several anatomical variations: occlusion of non-dominant right coronary artery, isolated occlusion of the ventricular branch of the right coronary artery, and the occlusion of the right coronary artery proximal to the ventricular branch with hetero collateral circulation on the periphery of the right coronary artery, like in our case. Electrocardiographic characteristic pointing to the occlusion of the proximal right coronary artery and/or ventricular branches of the right coronary artery is higher ST elevation in the lead V1 than in the other leads, followed by the absence of Q wave development. This ST elevation is concave. **Conclusion.** It is necessary to emphasize the significance of differential diagnosis of culprit lesion in patients with chest pain and elevation of the ST segment in the precordial leads having in mind further different thera peutic algorithms. Patients with right ventricular myo cardial infarction need to maintain an adequate "preload" and avoid vasodilators in order to maintain the right ventricular stroke volume.

Key words: ST Elevation Myocardial Infarction; Electrocardiography; Coronary Angiography; Heart Ventricles; Stents; Diagnosis, Differential

Sažetak

Uvod. Elektrokardiografija predstavlja inicijalnu neinvazivnu dijagnostičku metodu u algoritmu postavljanja dijagnoze akutnog infarkta miokarda sa ST elevacijom. Opisan je i specifičan elektrokardiografski fenomen kada se okluzija proksimalnog segmenta desne koronarne arterije ili izolovana okluzija ventrikularne grane desne koronarne arterije prezentuje sa ST elevacijom u prekordijalnim odvodima. **Prikaz slučaja.** Bolesnica starosti 78 godina primljena je kao hitan slučaj zbog bolova u grudima i elektrokardiografski registrovane konkavne elevacije u odvodima V1-V3. Pod radnom dijagnozom infarkt miokarda sa ST elevacijom anteriorne regije, upućena je u kateterizacionu labaratoriju radi urgentne koronarografije. Koronarografijom se registruje okludiran proksimalno-medijalni segment desne koronarne arterije. U istom aktu je urađena perkutana koronarna intervencija sa implantacijom dva lekom obložena stenta u proksimalno-medijalni segment desne koronarne arterije. **Diskusija.** Fenomen da se akutni infarkt miokarda uzrokovan okluzijom proksimalnog segmenta desne koronarne arterije i/ili njene ventrikularne grane, manifestuje elevacijom ST segmenta u prekordijalnim odvodima, nastaje kao posledica nekoliko anatomskih varijacija: okluzije nedominantne desne koronarne arterije, izolovane okluzije njene ventrikularne grane i okluzije desne koronarne arterije proksimalnije od odvajanja ventrikularne grane sa kolateralnim prikazom distalnog segmenta i periferije kao u našem slučaju. Elektrokardiografske specifičnosti koje ukazuju na ovaj fenomen su veća ST elevacija u odvodu V1 nego u preostalim odvodima praćena odsustvom Q-zubca. Ovakva ST elevacija ima kupolast oblik. **Zaključak**. Neophodno je naglasiti značaj diferencijalne dijagnoze infarktne arterije kod pacijenata sa anginoznim tegobama i elevacijom ST segmenta u prekordijalnim odvodima imajući u vidu različit terapijski algoritam. Kod pacijenata sa izolovanim infarktom miokarda desne komore neophodno je održati adekvatan *preload* i izbegavati vazodilatatore kako bi se održao adekvatan udarni volumen desne komore.

Ključne reči: STEMI; elektrokardiografija; koronarna angiografija; srčane komore; stentovi; diferencijalna dijagnoza

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Abbreviations

ECG – electrocardiography
NSTEMI – non-ST elevation myocardial infarction
LAD – left anterior descending
RCA – right coronary artery
STEMI – ST elevation myocardial infarction
LBBB – left bundle branch block
PDA – posterior descending artery
PLA – posterior lateral artery
PCI – percutaneous coronary intervention
AVF – augmental voltage foot

Introduction

Electrocardiography (ECG) is an initial non-invasive diagnostic algorithm for acute coronary syndrome. Acute coronary syndrome may be calssified into three categories: acute ST elevation myocardial infarction (STEMI), acute non-ST elevation myocardial infarction (NSTEMI) and unstable angina pectoris (UAP). Electrocardiographic manifestations of NSTEMI and UAP are ST depression and/or negative T waves. The difference between these two entities is in the markers for myocardial necrosis. The NSTEMI is associated with elevated markers of myocardial necrosis. All three entities have the same pathoanatomic substrate - a complicated atherosclerotic plaque. Acute STEMI is the most severe manifestation of acute coronary syndrome.

The ST segment elevation on the ECG must be interpreted in the context of clinical findings. False-positive causes of ST segment elevation are early repolarization, left bundle branch block (LBBB), pre-excitation syndrome, Brugada syndrome, peri/myocarditis, pulmonary embolism, subarachnoid hemorrhage, metabolic disorders (hyperkalemia in the first place), various forms of cardiomyopathy, ECG lead transposition, cholecystitis, persistent juvenile ECG, use of tricyclic antidepressants and phenothiazines [1]. False-negative causes of ST segment elevation are prior myocardial infarction with Q waves and/or persistent ST elevation in paced rhythm and LBBB [1].

The ST segment elevation (measured at the J point) in two or more leads with ST elevation ≥ 2.5 mm in men < 40 years, ≥ 2 mm in men ≥ 40 years and ≥ 1.5 mm in women in V2 - V3 and/or ≥ 1 mm in other leads, indicates acute occlusion of the coronary artery [2].

The ST segment elevation in the precordial leads usually indicates occlusion of the left anterior descending artery (LAD) or one of its branches. There is a description in the literature of a specific electrocardiographic phenomenon where the ST segment elevation in the leads V1 - V3 occurs due to the occlusion of the proximal segment of the right coronary artery (RCA) and/or isolated occlusion of the ventricular RCA branch [3–5].

Several algorithms for electrocardiographic identification of infarct related artery have been established with high accuracy. However, their significance is limited in patients with coronary artery anomalies, LBBB, paced rhythm and patients who had prior myocardial infarction or surgical myocardial revascularization.

We present a patient with acute myocardial infarction caused by occlusion of the proximal-medial segment of RCA, but manifesting with ST segment elevation in the leads V1 - V3, instead in D2, D3, and augmented voltage foot (AVF), which is usual.

Case Report

A 78-year-old woman with arterial hypertension and type 2 diabetes mellitus was admitted as an emergency to the Clinic of Cardiology of the Institute of Cardiovascular Diseases of Vojvodina. The working diagnosis was STEMI in the anterior wall. Chest pain occurred about 3 hours prior to admission. The ECG showed concave ST segment elevations in leads V1 through V3. ST segment changes in the inferior leads were not registered **(Figure 1)** only significant Q waves in derivations D2, D3 and AVF, with negative T wave in D3 and AVF.

Ten years earlier, the patient had an inferior wall STEMI. At that time, she was treated with fibrinolytic therapy (Alteplase). Due to persistent chest pain and ST segment elevation, a rescue percutaneous coronary intervention (PCI) was performed along with implantation of two drug-eluting stents 24 x 3.0 mm and 23 x 3.5 mm in the proximal-medial RCA (overlap technique). At that time, angiography of the left coronary system was without significant stenosis. After the intervention and during the follow-up period, ECG showed: sinus rhythm, Q wave and negative T wave in D2, D3, AVF, V5, and V6 without rhythm disorders.

On admission to our emergency department, the initial blood pressure was 130/80 mmHg and heart rate 60 beats per minute. During the physical examination, the patient presented without cardiac murmur, abnormal breath sounds, swollen jugular vein or edema of lower extremities. Dual antiplatelet therapy with aspirin and ticagrelor was administered along with the analgesic therapy in the emergency room. The patient was immediately transferred to the catheterization laboratory for emergency coronary angiography. As usual, the non infarction artery was performed first, and according to ECG it should have been RCA. We expected LAD to be the infarction artery. However, coronary angiography showed an occlusion of the proximal-medial RCA which was the dominant artery **(Figure 2A)**. Behind the occlusion of the RCA, posterior descending artery (PDA) and posterior lateral artery (PLA), a hetero-collateral circulation was seen **(Figure 2B)**. Angiography of the left coronary system showed a non-significant stenosis.

One drug-eluting stent 38 x 3.00 mm (Resolute Onyx, Medtronic, USA) was implanted into the medial segment of RCA and an "overlap" drug-eluting stent 28 x 3.5 mm (Coroflex-Isar-Neo, Braun, Germany) into the proximal segment of RCA. After implantation of stents and establishment of thrombolysis in myocardial infarction (TIMI) 3 flow

through the occluded blood vessel, two ventricular branches were observed **(Figures 2C and 2D).** The first branch had a small area of vascularization, while the other branch, with a diameter around 1.5 mm, was significantly longer with the significantly larger area of vascularization; the Blush flow was III. Successful PCI resulted in the resolution of the chest pain and ST segment elevation. The post PCI echocardiography showed normal left ventricular ejection fraction of 58. The medial-base segment of the inferior wall was hyperechogenic, non-homogeneous, and fibrotic, which was considered to be the consequence of the prior myocardial infarction. Also, the right ventricle was slightly dilated (tricuspid annular plane systolic excursion (TAPSE) 17 mm, RAVS 81 ml.

The patient was discharged from the hospital 5 days after the intervention without any subjective problems and with residual ST elevation up to + 1 mm in V1 through V3, but without development of Q wave.

Discussion

Occlusion of the RCA commonly manifests with ST segment elevation in the inferior leads. However, an electrocardiographic phenomenon has been described in the literature where the occlusion of the proximal RCA and/or ventricular branches of RCA is associated with the elevation of the precordial ST segment in the V1 - V3 leads [3–5]. Kim et al. reported that the occlusion of the proximal segment of RCA with the collateral circulation in the distal segment and periphery of the RCA manifesting with the elevation of the ST segment in the precordial leads [3]. The same finding was seen at coronary angiography in our patient. An abundant hetero-collateral circulation was found the inferior wall, while the RCA occlusion proximal to the ventricular branches caused myocardial infarction of the right ventricle, and was considered to be the cause of ST elevation in the precordial leads. Geft et al. published an experiment in which the isolated right ventricular myocardial infarction, caused by the infarct model

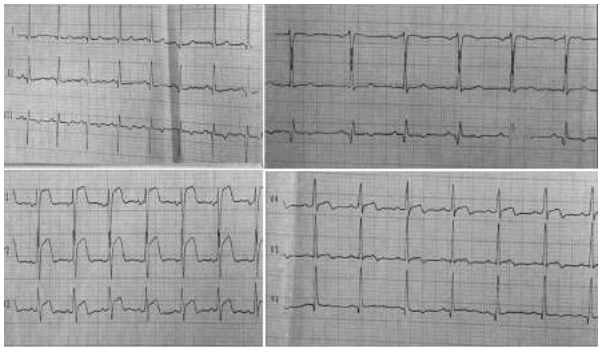

Figure 1. ECG showing concave ST segment elevations in leads V1 through V3
Slika 1. *Elektrokardiogram pokazuje konkavne elevacije ST segmenta u vodovima V1 do V3*

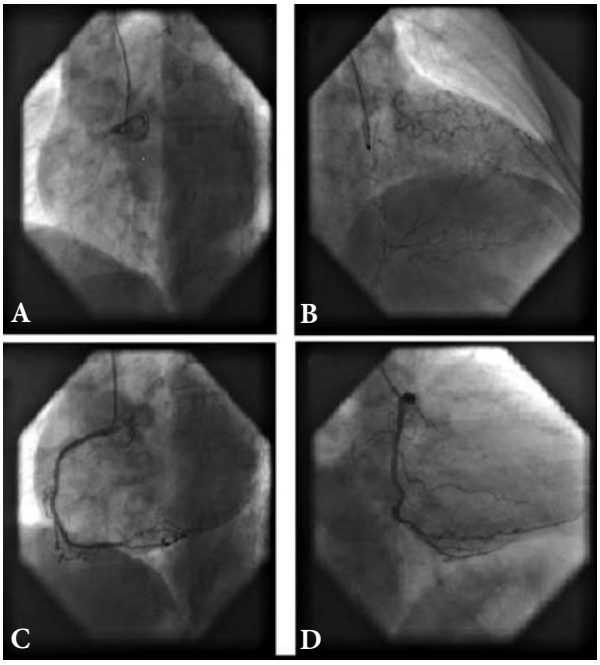

Figure 2A. Coronary angiography shows an occlusion of the proximal-medial RCA, which is a dominant artery; **2B.** Behind the RCA, PDA and PLA occlusion there is a hetero-collateral circulation; **2C and 2D**. After stent implantation and establishment of thrombolysis in myocardial infarction 3 flow through the occluded blood vessel, two ventricular branches are observed
Slika 2A. *Koronarografijom se registruje okluzija proksimalno-medijalnog segmenta desne koronarne arterije koja je dominantna arterija,* ***2B****. Iza okluzije desne koronarne arterije, zadnje lateralne (spoljašnje) grane i zadnje međukomorske grane prikazuju se preko heterokolateralne cirkulacije,* ***2C i 2D****; Nakon implantacije stenta i uspostavljanja thrombolysis in myocardial infarction 3 protoka kroz okludirani krvni sud, dve ventrikularne grane se prikazuju*

in canine, manifested with the elevation of the ST segment in the precordial leads [6]. They came to the conclusion that when the myocardial infarction of the inferior wall and right ventricle coexist, the ST elevation dominates in the inferior leads, and the precordial ST elevation is mostly not registered. Inferior wall ischemia generates dominant electrical forces, while the changes caused by right ventricular ischemia are suppressed. This phenomenon happens because the inferior wall involves a large mass of myocardium compared to the right ventricle.

Acikel et al. reported that the cause of ST segment elevations in leads V1 through V3, while performing primary PCI in patients with acute inferior myocardial infarction, was the occlusion of the ventricular branch of the right ventricle of the RCA [5]. Kida et al. studied 57 patients undergoing PCI for RCA. All patients had angina pectoris, without evidence of myocardial infarction. In 8 patients, ST elevation was registered in the precordial leads when the proximal segment of the RCA was occluded by the

PTCA balloon. These patients either had a functionally dominant left coronary artery, or divided domination between the left and right coronary arteries [7]. The ST elevation was explained as a consequence of the right ventricular ischemia, considering the fact that the inferior wall was vascularized by the left coronary artery.

It can be concluded that isolated right ventricular myocardial infarction, manifesting with the elevation of the ST segment in the precordial leads, occurs in the following anatomical variations: occlusion of non-dominant RCA, isolated occlusion of the ventricular branch of RCA and the occlusion of the RCA proximal to the ventricular branch with hetero-collateral circulation in the periphery of the RCA (as in our case).

The literature describes ECG characteristics that indicate the isolated right ventricular myocardial infarction. First of all, it is a higher ST segment elevation in lead V1 and than in the precordial leads V2 and V3, followed by the absence of Q wave development [8, 9]. Also, this ST segment elevation is concave or dome-like [10]. These characteristics are found in our patient as well. However, these criteria do not have high sensitivity and specificity, because they are derived from a study with a small number of patients. RCA disease is the most common cause of sinus node dysfunction or, in other words, myocardial infarction caused by the occlusion of proximal RCA and/or ventricular branches is often followed by bradycardia [11]. Rhythm disorders were not registered in our patient. Echocardiography can register the slight dilatation of the right ventricle with or without local abnormal wall motion, as in our patient. A slight dilatation of the right ventricle is a predisposing factor for cardiac clockwise rotation, which can explain the appearance of ST segment elevation in the precordial leads. On the other hand, the position of leads V1, V2 and V3 is in the region of the right ventricle. In cases where, for some reason, the posteroinferior wall of the left ventricle is protected from ischemia, electrocardiogram shows ST segment elevation in these leads.

Conclusion

Finally, it is necessary to emphasize the significance of differential diagnosis of culprit lesion in patients with chest pain and elevation of the ST segment in the precordial leads having in mind further different therapeutic algorithms. Patients with right ventricular myocardial infarction need to maintain an adequate "preload" and avoid vasodilators in order to maintain the right ventricular stroke volume.

In the future, along with the progress of primary percutaneous coronary intervention, detection of an isolated myocardial infarction of the right ventricle will also increase. Furthermore, due to the percutaneous coronary intervention of right coronary artery complex lesions with the consequent occlusion of ventricular branches, a higher incidence of isolated right myocardial infarction may be expected.

References

1. Wang K, Asinger RW, Marriott HJ. ST-segment elevation in conditions other than acute myocardial infarction. N Engl J Med. 2003;349(22):2128-35.

2. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. Eur Heart J. 2012;33(20):2551-67.

3. Kim SE, Lee JH, Park DG, Han KR, Oh DJ. Acute myocardial infarction by right coronary artery occlusion presenting as precordial ST elevation on electrocardiography. Korean Circ J. 2010;40(10):536-8.

4. Celik T, Yuksel UC, Kursaklioglu H, Iyisoy A, Kose S, Isik E. Precordial ST-segment elevation in acute occlusion of the proximal right coronary artery. J Electrocardiol. 2006;39(3):301-4.

5. Acikel M, Yilmaz M, Bozkurt E, Gurlertop Y, Kose N. ST segment elevation in leads V1 to V3 due to isolated right ventricular branch occlusion during primary right coronary angioplasty. Catheter Cardiovasc Interv. 2003;60(1):32-5.

6. Geft I, Shah P, Rodriguez L, Hulse S, Maddahi J, Berman D, et al. ST elevation in leads V1 to V5 may be caused by right coronary artery occlusion and acute right ventricular infarction. Am J Cardiol. 1984;53(8):991-6.

7. Kida M, Morishita H, Yokoi H, Yoshinaga M, Yasumoto H, Kimura T, et al. Precordial ST-segment elevation caused by right coronary artery occlusion. J Cardiol. 1987;17(3):455-64.

8. Golovchiner G, Matz I, Iakobishvili Z, Porter A, Strasberg B, Solodky A, et al. Correlation between the electrocardiogram and regional wall motion abnormalities as detected by echocardiography in first inferior acute myocardial infarction. Cardiology. 2002;98(1-2):81-91.

9. Alzand BS, Gorgels AP. Combined anterior and inferior ST-segment elevation electrocardiographic differentiation between right coronary artery occlusion with predominant right ventricular infarction and distal left anterior descending branch occlusion. J Electrocardiol. 2011;44(3):383-8.

10. Grollier G, Scanu P, Gofard M, Lognoné T, Valette B, Bureau G, et al. ST segment elevation in anterior precordial leads and right ventricular infarction. Apropos of 6 cases. Arch Mal Coeur Vaiss. 1992;85(1):67-75.

11. Ando G, Gaspardone A, Proietti I. Acute thrombosis of the sinus node artery: arrhythmological implications. Heart. 2003;89(2):E5.

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KAPOSI SARCOMA IN A NON-IMMUNOCOMPROMISED PATIENT – A POTENTIAL DIAGNOSTIC PITFALL

KAPOŠI SARKOM KOD IMUNOKOMPETENTNOG PACIJENTA - MOGUĆ DIJAGNOSTIČKI PROBLEM

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Summary

Introduction. Kaposi sarcoma is a rare soft tissue tumor that may form masses in the skin, lymph nodes, mucosa and many other organs. It has a strong male predilection and is usually seen in the older population. It is caused by human herpes virus 8. Risk factors include compromised immune system, typically seen in patients with human immunodeficiency virus infection or organ transplant recipients. **Case Report.** We report a 66-year-old Caucasian woman with no previous history of human immunodeficiency virus infection, immunosuppressive therapy or organ transplantation. She was referred to a plastic surgeon by a dermatologist due to a suspected dermatofibroma presenting with one solitary, firm nodule on the dorsal aspect of the foot that she reported to have occurred a year before. A surgery was scheduled in 6 months, as the tumor was assessed as benign. After excisional biopsy and histological evaluation without immunohistochemical staining, that was not available, a diagnosis of benign myofibroblastic tumor was made. Later on, a new similar tumor on the hand appeared and the diagnosis was changed into a malignant tumor. Further pathological examination, using immunohistochemical staining, confirmed Kaposi sarcoma. The malignant cells showed positive immunostaining for CD34, CD31, D2-40, WT1, bcl-2, and human herpes virus 8, but they were CD99 negative. **Conclusion.** Nonspecific clinical presentation and absence of risk factors may mislead the doctors, delay the biopsy and thus delay adequate treatment. In the same time, histological similarities with other disorders and tumors may be challenging for pathologists and lead to wrong diagnosis.

Key words: Sarcoma, Kaposi; Risk Factors; Histiocytoma, Benign Fibrous; Immunity; Diagnosis, Differential; Morphological and Microscopic Findings; Immunohistochemistry

Sažetak

Uvod. Kapoši sarkom je redak mekotkivni tumor koji može formirati tumore u koži, limfnim čvorovima i brojnim drugim organima. Ima izraženu mušku polnu predilekciju i obično se viđa u starijoj populaciji. Izaziva ga humani herpes virus 8. Faktori rizika uključuju kompromitovan imunostatus što se obično viđa kod pacijenata pozitivnih na virus humane imunodeficijencije ili sa transplantiranim organom. **Prikaz slučaja.** Prikazujemo 66 godina staru pacijentkinju bele rase koja je negativna na virus humane imunodeficijencije, nije na imunosupresivnoj terapiji i nije imala transplantaciju. Pacijentkinju je uputio plastičnom hirurgu dermatolog zbog promene suspektne na dermatofibrom, koju ima godinu dana, a koja se prezentovala u vidu solitarnog čvora, čvrste konzistencije, lokalizovanog na dorzumu stopala. Pacijentkinja je zakazana za šest meseci jer je promena klinički procenjena kao benigna. Nakon ekscizione biopsije i histološke analize, bez imunohistohemijskog bojenja koje nije bilo dostupno, promena je dijagnostikovana kao benigni miofibroblastni tumor. U daljem toku sa pojavom recidiva i novog sličnog tumora na šaci, patohistološka dijagnoza je revidirana u tumor koji bi trebalo smatrati malignim. Dalja imunohistohemijska analiza je omogućila postavljanje definitivne dijagnoze Kapošijevog sarkoma. Maligne ćelije su bile pozitivne na CD34, CD31, D2-40, WT1, bcl-2; CD99 negativne i pozitivne na humani herpes virus 8. **Zaključak**. Nespecifična klinička prezentacija i odsustvo faktora rizika mogu zavarati lekara, odložiti biopsiju i samim tim i adekvatno lečenje. Istovremeno histološke sličnosti sa drugim poremećajima i tumorima mogu biti problem za patologa i usloviti postavljanje pogrešne dijagnoze.

Ključne reči: Kapoši sarkom; faktori rizika; dermatofibrom; imunitet; diferencijalna dijagnoza; morfološki i mikroskopski nalazi; imunohistohemija

Introduction

Kaposi sarcoma (KS) was first described by Moritz Kaposi in 1872, but it became well known in the 80's leading to stigmatization of persons with acquired immune deficiency syndrome (AIDS). It is a rare angioproliferative soft tissue tumor that can form masses in the skin, lymph nodes, mucosa and many other organs. KS has strong male predilection (in classic forms with a male to female ratio 17:1) and is usually seen in older population of Mediterranean origin [1]. It is caused by human herpes virus 8 (HHV-8) and it shows the distribution of this virus in the world. Risk factors include compromised im-

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Abbreviations

KS – Kaposi sarcoma
HIV – human immunodeficiency virus
AIDS – acquired immune deficiency syndrome
HHV-8 – human herpes virus 8
PG – pyogenic granuloma

mune system, either as a result of a disease or some medication (iatrogenic form), typically seen in human immunodeficiency virus (HIV) patients or organ transplant recipients. Clinically, there are four subtypes of KS: classic, endemic, epidemic (HIV-related) and immunosuppressive therapy related. The classic form is most common in elderly men, localized on legs and has a slow progression. Endemic is seen in adult men and children in Africa and it can be aggressive with generalized lymph node involvement. Epidemic form occurs in people with AIDS and it usually affects many body parts. Immunosuppressive therapy related KS can be seen in transplant patients and usually involves the skin.

The diagnosis is made by tissue biopsy and the extent of disease can be assessed by different imaging procedures. The therapy depends on the extent of disease, subtype and immunological status of patients and can involve surgery, local radiotherapy and in wide spread forms biologic therapy and chemotherapy. The most aggressive anaplastic forms of KS may have a fatal outcome.

Case Report

We report a 66-year-old Caucasian woman with no previous history of HIV infection, immunosuppressive therapy or organ transplantation. She was referred to a plastic surgeon by a dermatologist due to a suspected dermatofibroma of the skin presenting as a solitary, firm, non-tender nodule on the dorsal aspect of the left foot that she reported to have for a year. Without specific medical history and no specific clinical presentation, KS was not considered as differential diagnosis and the surgeon agreed with the dermatologist's on clinical diagnosis of dermatofibroma. The tumor was a 5 mm nodule of pinkish-brown color with smooth surface and firm consistency **(Figure 1)**. The patient was scheduled for surgery in 6 months, since clinically the tumor was assessed as benign. After excisional biopsy, standard hematoxylin-eosin staining was performed and histological evaluation was done. The pathologist described a small tumor situated in the mid dermis, composed of fascicles of spindle cells, with small number of blood vessels. There was no cytological atypia or tumor necrosis, only a few mitoses, so the lesion was diagnosed as myofibroblastic tumor **(Figure 2)**. Immunohistochemical analysis was not available at that moment. One year later, the patient presented with a recurrent tumor similar to the previous one, on the dorsum of her hand. We performed an excisional biopsy of both tumors. The tumors were CD34 and bcl-2 positive, with some mitotic figures,

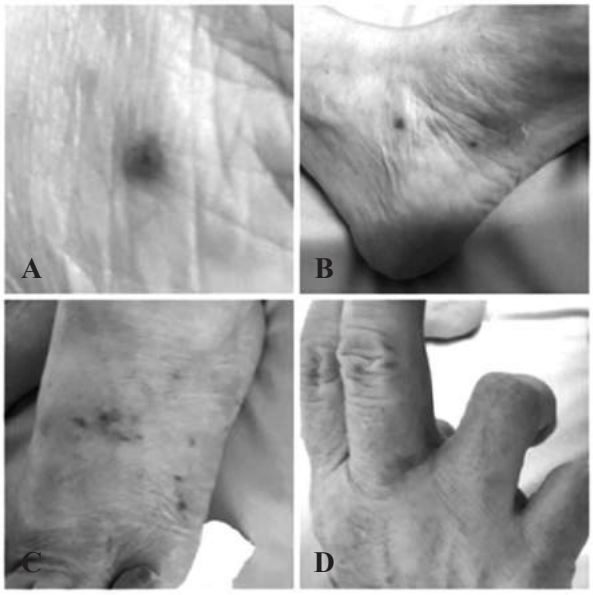

Figure 1. Clinical presentation of tumors in KS patient (A-KS close view, B- KS presentation on plantar side of foot, C- multiple KS tumors on dorsal side of foot, D- KS on hand)
Slika 1. *Klinička prezentacija tumora kod pacijenta sa Kapoši sarkomom (KS) (A-KS uvećano, B- KS na plantarnoj strani stopala, C- višetruki KS tumori na dorzalnoj strani stopala, D- KS na šaci)*

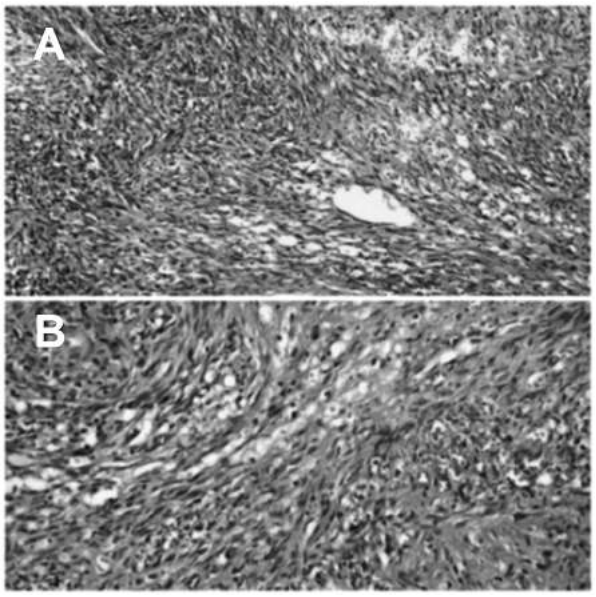

Figure 2. Histopathological presentation of the tumor stage Kaposi sarcoma – first surgery (A-HE x 100, B-HE x 200)
Slika 2. *Patohistološka prezentacija tumora Kapošijevog sarkoma-prva operacija (A-HE x 100, B-HE x 200)*

affecting two separate body parts (foot and hand), so the diagnosis of solitary fibrous tumor was made, presuming that it must be considered as malignant **(Figures 3 and 4)**. In the meantime, the patient developed multiple nodules on both feet in a short pe-

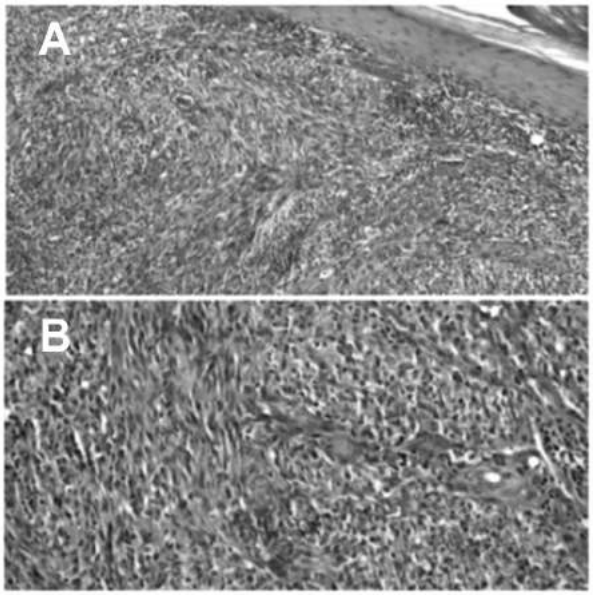

Figure 3. Histopathological presentation of the tumor stage Kaposi sarcoma – second surgery (A-HE x 100, B-HE x 200)
Slika 3. *Patohistološka prezentacija tumora Kapošijevog sarkoma – druga operacija (A-HE x 100, B-HE x 200)*

riod of time. As the diagnosis was changed from a solitary benign to recurrent malignant tumor with multiple similar nodules, we decided to take off all five new nodules on both feet and to consider revision of previous histopathological reports. The final diagnosis was KS of the skin. The tumor was located in the whole dermis, and one of the excised lesions already affected the subcutaneous tissue. The tumor tissue consisted of fascicles of spindle cells and some whorled patterns, with some extravasated red blood cells in the intercellular spaces. The nuclei were oval, elongated, without pleomorphism, but with some visible mitosis. The capillary network was rather prominent with areas of slit-like vascular spaces. The malignant cells were positive for CD34, CD31, D2-40, WT1, bcl-2, and HHV-8, but CD99 negative. The patient was referred for further oncological care. Chest computerized tomography (CT) and abdominal magnetic resonance (MR) were done. No mucosal lesions were detected, no lymph node infiltrations and no visceral manifestations of the disease, so close follow up of the patient was planned without additional therapy for the time being.

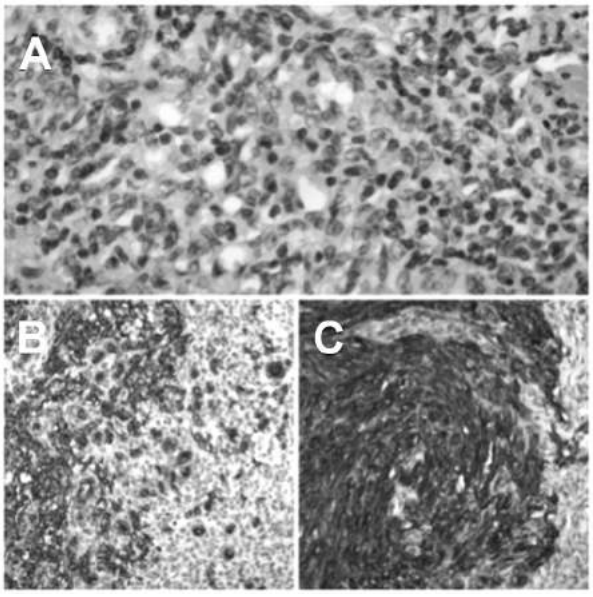

Figure 4. Histopathological presentation of the tumor stage Kaposi sarcoma with immunohistochemical markers: A- HHV8, B-CD34, C-D2-40
Slika 4. *Patohistološka prezentacija tumora Kapoši sarkoma sa imunohistohemijskim bojenjem: A-humani herpes virus-8, B-CD34, C-D2-40*

Discussion

Although the incidence of KS has increased 20-fold during the spread of HIV infection in the 80's and 90's, it is still a rare tumor, especially in general population of non-immunocompromised people. The classic form of KS that was diagnosed in our patient is usually seen in older men presenting as slowly growing lesions that appear in small number and are bilaterally located on the lower limbs. They are usually red-brown macules that slowly progress to plaques and nodules. In more aggressive forms of KS, usually HIV associated, lymph nodes may also be involved.

In this case, the patient had a quite unusual development of the disease with one solitary nodule that persisted for a year as a tumor mimicking dermatofibroma that at one point developed sudden "dissemination" with multiple nodules without macular phase, with both hand and foot involvement. The nonspecific clinical presentation firstly of a solitary, firm nodule deceived the dermatologist and the surgeon and postponed the need for urgent biopsy. If the patient presented with an advanced phase of the disease, with multiple nodules, that might have triggered the doubt that it was something else. The recurrent tumor and occurrence on a remote area (opposite site hand) with very similar histology helped making the diagnosis of malignancy after all. On the other hand, different manifestations of classic KS, such as disseminated lymphadenopathy as described by Jeong and colleagues, can mislead the doctor in other direction, in this case mimicking aggressive lymphoma [2]. Hand involvement, as seen in our patient is rare. Considering the fact that Sbiyaa et al. presented a case with an aggressive form of classic KS with hand bone involvement, we made an X-ray of the affected hand, but no bone involvement was noticed [3].

A similar problem was noticed with pyogenic granuloma (PG) as described by Harmelin A. et al., where KS was mimicking another tumor [4]. As clinical presentations of skin tumors may vary, we expect that histological evaluation will give us straight and clear answers and solve all our dilemmas. Scott PL.

et al. described a case where besides clinical features, similar histological presentations were found in KS and pyogenic granuloma-like KS, and emphasized that histological diagnosis can also be very challenging [5]. Overlapping histological features of PG and PG-like KS, such as epidermal collarette resulting from nodular prominence, ulceration and inflammation, and lobular proliferation of capillaries can create a problem for pathologists [6]. In the early patch stage of KS the histological appearance of the lesion can be close to various inflammatory dermatoses, granulomatous and interstitial skin diseases and tumors. As Grayson W. et al. emphasized, differential diagnosis can be challenging as there are many conditions and diseases varying from completely benign to many different malignant ones, that can have a similar, not only clinical, but also histopathological presentation [7].

Since the waiting list for surgery of presumed benign skin lesions is rather long, and we clinically assumed that this was a dermatofibroma, the histological appearance of spindle cell lesion in a rather uncommon location, with absence of immunohistochemical markers, it postponed the final diagnosis of a malignant disease. Another issue is that doctors tend to think about specific diseases as part of some syndromes, in this case immunosuppressive conditions such as AIDS or a transplant recipient, and thus miss a wider picture that involves absence of risk factors and atypical presentations. Health care providers of any specialization, especially dermatologists and dentists, should think about this disease even in non-risk related groups, in order to reduce underestimation of any suspicious plaque or nodule and thus postpone adequate treatment.

Conclusion

Since Kaposi sarcoma is not widespread in general population, and Europe is a non-endemic territory, we usually do not think about it if the patient is not in typical risk groups. Nonspecific clinical presentation, unusual location and absence of risk factors can mislead the physician, delay the biopsy and thus also delay adequate treatment. At the same time, histological similarities with other disorders and tumors can be challenging for pathologists and sometimes lead to wrong diagnosis. Raising awareness of Kaposi sarcoma is important as it can prevent delay of adequate treatment.

References

1. Bishop BN, Lynch DT. Cancer, Kaposi sarcoma. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 [cited 2019 Jun 19]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK534839/.

2. Jeong EH, Koo DH, Lee SH, Bang KB, Park EH, Seol JS, et al. Aggressive classical Kaposi's sarcoma mimicking malignant lymphoma. Pathol Oncol Res. 2012;18(4):1067-9.

3. Sbiyaa M, El Alaoui A, El Bardai M, Mezzani A, Lahrach K, Marzouki A, et al. Classic and aggressive Kaposi sarcoma with bone involvement. Pan Afr Med J. 2016;23:196.

4. Hamelin A, Bachmeyer C, Moguelet P, Francès C. Kaposi sarcoma mimicking pyogenic granuloma. Ann Dermatol Venereol. 2013;140(10):660-2.

5. Scott PL, Motaparthi K, Krishnan B, Hsu S. Pyogenic granuloma-like Kaposi sarcoma: a diagnostic pitfall. Dermatol Online J. 2012;18(3):4.

6. Grayson W, Pantanowitz L. Histological variants of cutaneous Kaposi sarcoma. Diagn Pathol. 2008;3:31.

7. Grayson W, Landman G. Kaposi sarcoma. In: Elder DE, Massi D, Scolyer RA, Willemze R, editors. WHO classification of skin tumors. 4th ed. Lyon: International Agency for Research on Cancer; 2018. p. 341-3.

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DEVELOPMENT OF THE CITY HOSPITAL IN NOVI SAD – PART I

RAZVOJ GRADSKE BOLNICE U NOVOM SADU – PRVI DEO

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Summary

Introduction. In 1907, the City Council of Novi Sad authorized the building of a new city hospital. The complex of hospital buildings was planned as a modern pavilion-style hospital with a 300 bed capacity. **Foundation.** The Pavilion 1 was intended for Admitting Department, Management and Administrative Departments. The facade of this building was decorated with a monumental mosaic of two angels. The Pavilion 2 was built for Departments of Surgery and Gynecology, whereas the Pavilion 3 included departments for patients with internal diseases, patients with skin and venereal diseases and maternity ward, but occasionally some infectious and neurological patients were treated there as well. In 1912, two new buildings were built: the first was the Antitrachoma Department and the other for patients with tuberculosis. During the First World War, the City Hospital was turned into a military hospital for the wounded, and also for those suffering from abdominal and typhoid fever, as well as from Spanish fever. **Period between the two world wars**. Since 1921, the founder of the hospital and its name have changed, and it has become the General State Hospital. After young physicians, educated at famous European medical centers, were employed, the Novi Sad State Hospital experienced a great advancement, especially in the field of surgery. In 1922, a new building was built, where the Bacteriological Station and the Pasteur Institute were established. The problems that the City Hospital was facing transferred to the General State Hospital, and were mostly financial. Before the Second World War, the State Hospital had a 455 bed capacity. After the Hungarian armed forces occupied Novi Sad in May 1941, the hospital director and all the ward physicians were replaced by Hungarian military doctors who worked there until September 1944.

Key words: History of Medicine; History, 20th Century; Hospitals; Architecture; Facility Design and Construction; Equipment and Supplies, Hospital; Physicians; Yugoslavia;

Sažetak

Uvod. Godine 1907. gradska vlast u Novom Sadu donela je odluku da se zida nova bolnica. Kompleks zgrada bio je planiran po modernom paviljonskom tipu sa kapacitetom 300 postelja. **Osnivanje**. U Paviljonu 1 bilo je Prijemno odeljenje, Uprava i Administracija. Zabat ove zgrade bio je dekorisan kompozicijom u mozaiku koja je predstavljala sliku dva monumentalna anđela. U Paviljonu 2 nalazilo se Odeljenje hirurgije i Odeljenje ginekologije. U Paviljonu 3 bilo je Odeljenje za unutrašnje bolesti ali su tu bile i porodilje, pacijenti sa kožnim i venеričnim bolestima, bolesnici od zaraznih ili nervnih bolesti. Godine 1912. podižu se dva nova objekta – jedan je Antitrahomno odeljenje, a drugi Antibuberkulozno. Tokom Prvog svetskog rata Gradska bolnica je korišćena kao vojna bolnica za ranjenike: tu su ležali i oboleli od trbušnog i pegavog tifusa i oboleli od španske groznice. **Period između dva svetska rata.** Od 1921. godine menja se osnivač bolnice i naziv − postaje Državna bolnica. Angažovanjem mladih lekara edukovanih u poznatim evropskim centrima medicine, bolnica u Novom Sadu doživljava procvat, naročito hirurgija. Godine 1922. podiže se novo zdanje u kojem će biti osnovani Bakteriološka stanica i Pasterov zavod. Problemi koje je imala Gradska bolnica u svom radu preneli su se na Državnu bolnicu i uglavnom su bili finansijske prirode. Pred Drugi svetski rat bolnica ima 455 kreveta. Posle okupacije mađarske vojske, u maju 1941. godine, direktor bolnice i svi šefovi su zamenjeni mađarskim vojnim lekarima koji su tu radili do septembra 1944. godine.

Ključne reči: istorija medicine; istorija, 20. vek; bolnice; arhitektura; projektovanje i izgradnja objekata; oprema i snabdevenost bolnice; lekari; Jugoslavija

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Timeline – Hospitals in Novi Sad before the 20th Century

– 1730: An Orthodox Hospital was situated in the Courtyard of the St. Nicholas Church [1];

– 1754: A Catholic Hospital was built [2, 3];

– 1754: A Jewish Hospital was built [4];

– 1859: "Städtische Spital" (small city hospital) was founded [3];

– 1873: The Main City Hospital was built with a 160 hospital bed capacity and Departments of Surgery and Internal Medicine, but patients with other diseases were also treated there: women during pregnancy and childbirth, women with gynaecologic diseases, patients with eye, ear, skin and venereal diseases, patients with infectious diseases, tuberculosis, as well as patients with mental diseases [2, 5];

– 1876: A Hospital Association was established, for the Merchant Youth [6].

Introduction

At the beginning of the twentieth century, Novi Sad had about 28.000 inhabitants [7] and the need for a new and modern hospital increased significantly. In 1907, the City Council of Novi Sad authorized the building of a new city hospital. The chosen location was on the outskirts of Novi Sad, at the back of the Artesian Bath (later Jodna Spa) and the Calvary. The plans for the new hospital were made by the City Construction Authorities and approved by the Urban Authorities [5] that helped the building donating 200.000 crowns. The project for the hospital complex was made by architect Gyorgy Kopeczek (1864–1920) from Budapest, and the construction was entrusted to the Novi Sad building constructors Vilmos Linarich and Peklo Bela (1867–1960). The building of the hospital began in 1907, and it was completed at the end of 1909 [8].

Foundation

The complex of the City Hospital was planned and built on the corner of Rakòczi Ferenc and Korhaz streets (today Futoški Road and Hajduk Veljkova Street) as a modern pavilion-style hospital with a 300 bed capacity **(Figure 1)** [7]. Firstly, three large and three small objects were built, for the sick and others for technical supplies [7]. Some were built in the years to come **(Scheme 1)** [9].

In the main building, Pavilion 1 was intended for Admitting and Administrative Departments. The Pavilion 2 was built for Departments of Surgery and Gynecology, whereas the Pavilion 3 included departments for patients with internal diseases, patients with skin and venereal diseases and maternity ward, but occasionally some infectious and neurological patients were treated there as well [10].

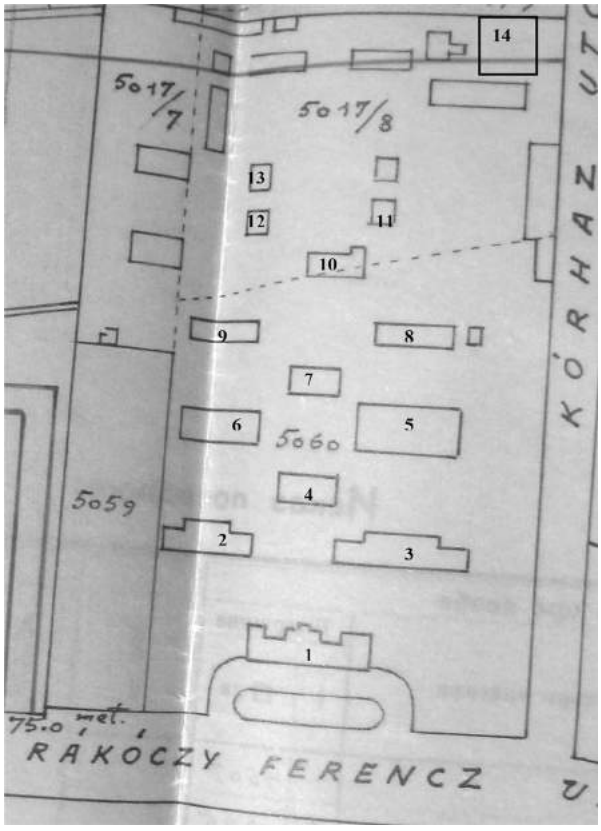
Behind the pavilions, a house for nuns and technical rooms were situated [5, 7] as well as a morgue (at an unknown location, today it does not exist) [11].

The main building was facing the Futoški road with a facade in the Secession architecture style. In the front of Pavilion 1, there was landscaped park with many trees arranged to reduce the traffic noise disturbing the hospital patients [12]. It was reasonable solution, because nearby was the last station of the White tram-line (from 1911–1958) **(Figure 2)**.

The facade of the main building was decorated on the gable: a mosaic connecting Christianity and Antiques, a composition that symbolizes the allegory with two monumental angels. Between them, the year when the hospital was completed, 1909 is written. The female figures of angels are placed in a semicircle, turned to one another with large outspread wings and halos over them. On the left side is Hygeia, the antique goddess of health, as a young girl who carries a glass of remedy to heal the diseased from the disease represented by a snake. The second

Figure 1. Aerial photo of the Hospital and Calvary made in 1926 (Courtesy of Dušan V. Salatić: Novi Sad Chapels. Prometej. Novi Sad, 2013)

Slika 1. *Avionski snimak bolnice i Kalvarije iz 1926. godine (Ljubaznošću Dušana V. Salatića: Novosadske kapele. Prometej. Novi Sad, 2013)*



Scheme 1. A draft of Hospital facilities (probably after 1922), the corner of Rákóczy Ferancz and Kórhaz streets (now Futoška and Hajduk Veljkova Street) in Novi Sad
Shema 1. *Nacrt bolničkih objekata (verovatno posle 1922. godine) ugao ulice Rákóczy Ferancz i Kórhaz (danas Futoška i Hajduk Veljkova ulica) u Novom Sadu*
Legend: **Pavilion 1.** Admitting Department, Management and Administrative Departments, **Pavilion 2.** Surgery and Gynecology Department, **Pavilion 3.** Department of Internal Diseases, Maternity Ward, Department for Skin and Venereal Diseases, **Pavilion 4.** Home for nuns employed in the Hospital, **Pavilion 5.** Antitrachoma Department, **Pavilion 6.** Department of Tuberculosis and Infectious Diseases, **Pavilion 7.** Building for technical eqipment and medical supplies (later Department of Pathology), **Pavilion 8.** Department of other infectios diseases, **Pavilion 9.** Department of Psychiatry, **Pavilion 10.** Bacteriology Station and Pasteur Institute, **Pavilion 11.** The Hospital Pharmacy, **Pavilions 12 and 13.** Department of Hygiene, **Pavilion 14.** Department of Pathology and the Morgue
Legenda: ***Paviljon 1.*** *Glavna zgrada sa prijemnim odeljenjem, upravom i administracijom,* ***Paviljon 2.*** *Odeljenje za hirurške i ginekološke bolesnike,* ***Paviljon 3.*** *za internističke bolesnike, porodiljstvo i kožno-venerične bolesnike,* ***Paviljon 4.*** *Dom časnih sestara zaposlenih u bolnici,* ***Paviljon 5.*** *Odeljenje za lečenje infekcije oka trahomom,* ***Paviljon 6.*** *Odeljenje za bolesnike sa zaraznim bolestima i tuberukulozom,* ***Paviljon 7.*** *Zgrada sa tehničkom opremom i medicinskim materijalom (kasnije Prosektura),* ***Paviljon 8.*** *Odeljenje za lečenje drugih zaraznih bolesti,* ***Paviljon 9.*** *Odeljenje za psihijatriju,* ***Paviljon 10.*** *Bakteriološka stanica i Pasterov Zavod,* ***Paviljon 11.*** *Bolnička apoteka,* ***Paviljoni 12. i 13.*** *Higijenski zavod,* ***Paviljon 14.*** *Prosektura i mrtvačnica*

figure complements the goddess of health and gives a Christian meaning to the composition. In her hands is an hourglass that symbolizes the eternal passage of time and mortality, while the book, the Bible, warns everybody that those who believe will live after death. In the middle of the upper part, the coat of arms of the newly founded Socialist Federal Republic of Yugoslavia was added [8]. Above the central

Figure 2. Old postcard of the State Hospital from 1920 (Courtesy of Prof. Dr. Aleksandra Kapamadžija)
Slika 2. *Stara razglednica Državne bolnice iz 1920. godine (Ljubaznošću prof. dr Aleksandre Kapamadžije)*

part of the building was a clock tower seen in old postcards [11, 12], removed in the middle of the 20th century under unclear circumstances [8].

In the main building, the Admitting and Administrative Departments were originally located: on the ground floor there were waiting rooms, doctors' offices, administration and directors' offices and bathrooms; in the basement there was a pharmacy, histology laboratory [13], bathroom, warehouse, and a kitchen [8].

In the second row, there were two buildings. In Pavilion 2, the Departments of Surgery and Gynecology were settled. The operating theatre was on the first floor, rooms for male and female patients were separated on the ground floor and the first floor. In Pavilion 3, Department for Internal Diseases and the Maternity Department were located. Although the pavilions were intended for certain categories of patients, they were not strictly separated [7]. Many patients with skin problems or venereal diseases were treated as outpatients or they were hospitalized in the Internal Department [7, 10]. The Hospital was equipped with a modern X-ray which was probably in the basement of the Surgery Department.

In the third row of buildings, between Pavilions 2 and 3, Pavilion 4 was located, much smaller than the previous ones and the building was designed symbolizing a cross. It was designed to accommodate nuns who assisted physicians and took care of the patients. The building consisted of a dormitory, a living room, a patient room, a dining room, a bathroom and a chapel [14].

The first hospital director was Dr. Aleksandar Šandor Šosberger, who was also the head of the Gynecologic and Dermatovenereology Department until 1919. He was the first to have performed a Cesarean section (1910) and abdominal hysterectomy (1912) [15]. The head of the Surgery Department was Dr. Ferdinand Nandor Brezovski, who graduated from medicine in Budapest and specialized in surgery and gynecology in Dresden and Berlin [16]. The Head of the Internal Department was Dr. Đura Trifković. At that time, 6 doctors were employed in the hospital, and nursing care was exclusively performed by about 20 nuns [7].

Until the First World War, medical supplies were acquired from private pharmacies through "pharmacy lists" for each patient [17]. It is very likely that the nursing home pharmacy also had basic drugs for nursing patients [5]. The hospital pharmacy was not established until 1922 [5] and its chief was Svetozar Alargić, Master of Pharmacy [18].

In 1912, behind the Pavilion 3, a new building was built, the Pavilion 5 - Antitrachoma Department with a 96 bed capacity. At that time, trachoma eye infection was very widespread in Vojvodina [7] and it was necessary to form a center for the treatment and eradication of this infection. In 1927, a Department for Eye Diseases was established, and today, Eye Clinic is still located in the same building. The first head of the Department was Dr. Jefta Stojaković, a specialist in ophthalmology [7]. In the same year, the Department of Ear, Nose and Throat Diseases was founded, today the Otorhinolaryngology Clinic, and it was located in the basement of the same pavilion. The Department was headed by Dr. Slobodan Matić [7].

Until 1912, several new hospital facilities were built: Pavilion 11, for neurological and psychiatric patients, hospital section for anatomy and pathology (Pavilion 14), and the hospital chapel (unknown location), but none of the two buildings exists any more [5, 7]. Before the beginning of the First World War, the hospital had 8 doctors, about 400 beds, 5 departments and 5 employees [7].

The period between the two world wars

During the First World War, the City Hospital served as a military hospital for wounded and sick Austro-Hungarian soldiers, and even new barracks with a 100 bed capacity were built for those suffering from abdominal and typhoid fever [7, 19]. A new problem arose in 1918/19, when the epidemic of Spanish fever emerged. The capacity of the Department of Infectious Diseases was very small, so patients with infectious diseases were housed in wards around the hospital [7, 19]. Such organization did not provide isolation of the infected patients, so infections were transmitted to other hospitalized patients [19, 20].

After the end of the First World War in 1918, Vojvodina joined the Kingdom of Serbs, Croats and Slovenes. In 1919, the new director of the City Hospital was Dr. Đura Trifković (1919–1932), and after him it was Dr. Risto Miletić Šain (1932–1941). New Heads of Departments were also appointed: Head of the Departments of Internal and Infectious Diseases was Dr. Đura Trifković, Head of the Department of Surgery was Dr. Stanko Matanović (1920), Head of the Department of Gynecology and Obstetrics was Dr. Miladin Veličkov Svinjarev, Head of the Department of Skin and Venereal Diseases was Dr. Jovan Nenadović, Head of the Department of Eye Diseases was Dr. Borislav Mirić, and Head of the Hospital section for anatomy and pathology was Dr. Pavle Strasser (1921) [5]. Since 1921, the founder of the hospital and its name have changed, and it has become the General State Hospital. After young physicians, educated in famous European medical centers, were employed, the Novi Sad General State Hospital experienced a great advancement, especially in the field of surgery.

In 1932, Dr. Vladimir Jakovljević became the Head of the Department of Surgery. He graduated from medicine in Budapest and specialized in surgery in Paris, Berlin, Munich and Heidelberg. He was a very capable general surgeon who first performed duodenopancreatectomy (1935) and was an expert in biliary surgery. In the same year, he also performed the first pulmectomy [16].

Between 1920 and 1922, a building for the treatment of patients with tuberculosis was built (Pavilion 6). The building only had a ground floor, the rooms accommodated a smaller number of beds, and on the south side of the building there was a long hallway for heliotherapy.

At the same time, a new building (Pavilion 10) was built for the Bacteriological Station and the Pasteur Institute, which was founded in 1922 [21–23]. The Bacteriological Station was managed by Dr. Petar Švarc. The Pasteur Institute was founded and managed by Dr. Adolf Hempt (1874–1943) who modified the vaccine against rabies in 1925, and it was accepted by most European countries [24]. These two institutions united in 1924, into the Epidemiological Institute, that became the Institute of Public Health in 1926. Today, the Pasteur Institute is on the list of cultural monuments of the Republic of Serbia and consists of three parts: the main building of the Pasteur Institute, the wooden house where Dr. Hempt and his family lived (the "Deker's Barracks") and the memorial bust of Louis Pasteur in front of the Institute [12, 21, 22]. In 1930, two new buildings (Pavilions 12 and 13) were built in the immediate vicinity of the Pasteur Institute, which are now used by the Clinic of Infectious Diseases [25].

In 1927, the Section of Orthopedics was established within the Surgery Department and was placed in the unused facilities in the basement of the Surgical Pavilion 2. Orthopedic and traumatic patients were treated by general surgeons [5, 26, 27].

In 1921, a Hospital Pathology Department was opened in the General State Hospital with Dr. Pavle Strasser as the Head of the Department (1872–1927). The Department was housed in a small unoccupied building consisting of two rooms, one of which was used for autopsies and the other for the preservation of bodies (Pavilion 14) [13, 28]. During 1933-4, the Department of Pathology was situated in a larger building (probably Pavilion 7). During the occupation, Hungarian military doctors worked at the Department [29].

In 1927, the Department of Ear, Nose and Throat was established in the basement of the Pavilion 5. The Head of the Department was Dr. Slobodan Matić, a well educated doctor and an excellent surgeon [7]. The first operations were performed in 1936 on the ground floor of the Eye Clinic: surgeries of the tonsils, sinuses, ears, endoscopic throat surgeries, esophagus and bronchia were performed [30].

In 1928, the Radiology Department was founded under the leadership of Dr. Dimitrije Nestorović. The department was located in the basement of the Department of Surgery [5, 7, 31].

When the hospital was built, it was a modern and well equipped health institution: bedding capacity, number and quality of staff, equipment and architectural-functional solutions for individual objects were completely in line with the hospital standards of that time. After the First World War, the Department of Infectious Diseases needed expansion, but there were no financial incentives for the construction of new facilities. The problems of the City Hospital continued, and the General City Hospital faced hardships primarily of financial nature [7]. Approximately 20% of patients paid the costs of treatment themselves, while the state paid for the remaining 80% [32].

Before the Second World War, the General State Hospital had a 455 bed capacity [33]. After the Hungarian armed forces occupied Novi Sad in May 1941, the hospital director and all the Head physicians were replaced by Hungarian military doctors and the occupational Military Administration appointed Bela Tesenji and then Antal Džinić the director; all the heads of the department were replaced [18]. They managed the hospital until September 1944, when Novi Sad was liberated.

References

1. Rajković Đ. Iz prošlosti Novog Sada. Javor. 1878 Dec.
2. Stajić V. Građa za političku istoriju Novog Sada. Novi Sad: Matica srpska; 1951.
3. Stajić V. Građa za kulturnu istoriju Novog Sada. Novi Sad: Matica srpska; 1951.
4. O Jevrejskoj bolnici u Novom Sadu. U: Šosberger P. Zbornik članaka. Zbornik naučnog društva istorije kulture Vojvodine 1986. Novi Sad; 1998. p. 148-9.
5. Berić B, Miškov D. Pregled istorije bolnice u Novom Sadu (1730-1980). Med Pregl. 1981;34(5-6):259-65.
6. Beleške o Đoki Mihajloviću, o kužnim bolestima, o Pasterovoj vakcini, o raznim predavanjima, o pojavi meningitisa, o udžbeniku za babice i o bolesničkoj zadruzi Trgovačke omladine. Javor. 1878.
7. Cvejić S. Glavna Pokrajinska bolnica Vojvodine u Novom Sadu. Med Pregl. 1961;14(8):429-32.
8. Brdar V. O zgradama Gradske bolnice u Novom Sadu (1909-1912). Građa za proučavanje spomenika kulture Vojvodine. 2013;26:19-28.
9. Ujvideki all kozkorhaz helyszinrajza [map]. (Located in: Tehnička arhiva Skupštine grada Novog Sada (TAGS-NS)). Hungarian.
10. Dermatovenerologija. In: Popov D, editor. Enciklopedija Novog Sada. Novi Sad: Prometej; 1996;6(gag-deč):323.
11. Gazdajagi epulet. 1913. (Located in: Istorijski arhiv grada Novog Sada f1, kig 24431/1913). Hungarian.
12. Stančić D, Arsić M, Babić S, Bešlin B, Jakšić J, Medović P, et al. Umetnička topografija Novog Sada. Novi Sad: Matica Srpska; 2014. p. 724-5.
13. Medicinski fakultet Novi Sad. Spomenica – Medicinski fakultet Novi Sad 1960-1970. Novi Sad: Medicinski fakultet; 1970.
14. Apacalok. Uslovi rada - pismo časnih sestara. (Located in: Istorijski arhiv grada Novog Sada 24784/1909).
15. Drača P. Razvoj ginekolosko-akušerske službe u Novom Sadu. In: Drača P, editor. Razvoj ginekologije i akušerstva u Vojvodini do 2000. godine. Novi Sad: Medicinski fakultet; 2008. p. 111-50.
16. Breberina M. Istorija hirurgije na teritoriji današnje Vojvodine. In: Pajić VD, Anđelić M, Agošton F, Avramov S, Babović S, Bedov S, et al. Hirurgija – odabrana poglavlja. Knjiga 1. Novi Sad: Simbol; 2009. p. 9-49.
17. Osnovna pravila opšte bolnice slobodne kraljevske varoši Ujvidéka. Novi Sad: Izdanje Srpske knjižare Braće M. Popovića; 1906.
18. Berić B. Bolnica. In: Popov D, editor. Enciklopedija Novog Sada. Novi Sad: Prometej; 1995;4(bob-var):72-5.
19. Čanak G, Vukadinov J, Brkić S, Švarc D, Ružić M, Kovačević N, et al. Razvoj infektološke službe u Novom Sadu. Med Pregl. 2007;60(11-12):625-8.
20. Čanak G. Infektologija. In: Popov D, editor. Enciklopedija Novog Sada. Novi Sad: Prometej; 1997;9(ibr-ješ):149-51.
21. Lalošević D. Devedeset pet godina Pasterovog zavoda u Novom Sadu. In: Spomenica 95 godina Pasterovog zavoda u Novom Sadu. Novi Sad: Pasterov zavod; 2016.
22. Stančić D. The Pasteur Institute complex in Novi Sad as a cultural monument. Med Pregl. 2001;54 Suppl 1:61-3.
23. Godišnji izveštaj o kretanju zaraznih bolesti i nekih masovnih neinfektivnih bolesti u SAP Vojvodini. Novi Sad: Medicinski fakultet, Institut za zdravstvenu zaštitu; 1995-2000.
24. Lalošević D. Klub novosadskih lekara. In: Medici pro arte. 135 godina SLD, 60 godina DLV. Novi Sad: DLV; 2007. p. 31-5.
25. Lalošević D. Pasterov zavod. In: Popov D, editor. Enciklopedija Novog Sada. Novi Sad: Prometej; 2002;19(pad-pet):143-7.
26. Milankov M, Gojkovic Z. Six decades of development. Med Pregl. 2016;69(Suppl 1):7-9.
27. Mikić Ž. Razvoj ortopedije i osnivanje Klinike za ortopedsku hirurgiju i traumatologiju u Novom Sadu. Med Pregl. 1998;51(9-10):457-68.
28. Vučković N. Od Prosekture do Instituta za patologiju – Novi Sad. In: Popović DT, Ajduković N, Berber S, Budakov B, Vapa D, Veselinović I, et al. Patološka anatomija i sudska medicina u Vojvodini: počeci, razvoj i rad. Novi Sad: Matica srpska; 2012. p. 49-52.
29. Mutibarić A. Patologija. In: Popov D, editor. Enciklopedija Novog Sada. Novi Sad: Prometej; 2002;19(pad-pet):157-9.
30. Dobanovački, Dušanka (Univerzitet u Novom Sadu, Medicinski fakultet). Konverzacija sa: Slobodan Mitrović (Klinički centar Vojvodine, Klinika za uho-grlo-nos, Novi Sad, Srb). 2018 dec.
31. Popović L. Radiologija. In: Popov D, editor. Enciklopedija Novog Sada. Novi Sad: Prometej; 2003;22(pra-radno):277-80.
32. Pantelić D. Problemi finansiranja i prodaja Gradske bolnice u Novom Sadu kroz dokumente Istorijskog arhiva Grada Novog Sada. In: Sakač V, editor. 800 godina srpske medicine. Treći Sokolski zbornik. Novi Sad: Naučno društvo za istoriju zdravstvene kulture u Vojvodini; Beograd: Srpsko lekarsko društvo, Sekcija za istoriju medicine; 2016. p. 477-92.
33. Milovanović V, editor. Medicinski godišnjak Kraljevine Jugoslavije. Beograd: Jugoreklam K.D; 1933. p. 257-8.

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1. Uvodnici – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

2. Originalni članci – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

3. Pregledni članci – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

4. Prethodna ili kratka saopštenja – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

5. Stručni članci – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

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7. Članci iz istorije medicine – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

8. Ostali članci – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

Priprema rukopisa

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

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– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

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Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

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Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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.

∗ Bez 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 (urednici) kao autor (autori)

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.

∗ Zbornik radova sa kongresa

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.

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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.

∗ Kompjuterska datoteka

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

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

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– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

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– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

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Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

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), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility 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 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. 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'.

Articles in journals

∗ *A standard article*

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

∗ *An organization 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.

Books and other monographs

∗ *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.

Electronic material

∗ *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.

∗ *A computer file*

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

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.