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Academician Kosta Todorović [5 July (22 June according to the old calendar) 1887 to 19 September 1975] was a famous Serbian infectologist, professor of the Faculty of Medicine in Belgrade, a regular member of the Serbian Academy of Sciences and Arts and a member of the National Academy of Medicine in Paris, France (see pp. 653–660).

Akademik Kosta Todorović [5. jul (22. jun po starom kalendaru) 1887. do 19. septembra 1975] je bio čuveni srpski infektolog, profesor Medicinskog fakulteta u Beogradu, redovni član Srpske akademije nauka i umetnosti i član Nacionalne akademije za medicinu u Parizu, Francuska (vidi str. 653–660).

ORIGINAL ARTICLES

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Development and validation of a questionnaire for measuring drug-induced nausea

Razvoj i ispitivanje punovažnosti upitnika za merenje mučnine izazvane lekovima

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Abstract

Background/Aim. There are several questionnaires for measuring intensity of nausea after drug administration, but they are either too settings specific (like those measuring chemotherapy-induced nausea), or they were not properly tested for reliability and validity. The aim of this study was to develop and validate a reliable instrument that can measure drug-induced nausea. **Methods.** The cross-sectional study for assessing reliability and validity of a questionnaire was performed. The questionnaire with 5 items and answers according to the Likert's scale was developed during two brainstorming sessions of the research team. Its reliability, validity and temporal stability were tested on the sample of 128 outpatients taking iron salts orally. **Results.** The final version of the Drug-Induced Nausea Scale (DINS) with 5 items showed excellent reliability, both when rated by the investigators (Cronbach's alpha 0.892) and by the patients themselves (Cronbach's alpha 0.897). It was temporally stable, and both divergent and convergent validity tests had very good results. Factorial analysis revealed only one factor, which means that the whole scale is measuring only one phenomenon, intensity of nausea, as was originally intended. **Conclusion.** The DINS is reliable and valid instrument for measuring intensity of drug-induced nausea. Identification of patients with high intensity of drug-induced nausea by this questionnaire will help prescribers to decide whether the therapy should be stopped or the patient switched to less emetogenic therapy.

Key words:
drug therapy; iron; nausea; pharmaceutical preparations; psychometrics; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Do sada je objavljeno nekoliko upitnika za merenje intenziteta mučnine posle primene lekova, ali su oni ili suviše specifični za određenu grupu lekova (npr. oni koji mere mučninu posle primene hemioterapije) ili njihova pouzdanost i punovažnost nisu propisno ispitani. Cilj ove studije je bio da se razvije upitnik za merenje mučnine izazvane lekovima i ispitaju njegova pouzdanost i punovažnost. **Metode.** Studija je bila dizajnirana kao studija preseka za procenu pouzdanosti i validnosti upitnika. Upitnik sa pet pitanja i ponuđenim odgovorima po Likertovoj skali bio je razvijen na dva nestrukturirana sastanka istraživačkog tima. Pouzdanost, punovažnost i stabilnost u vremenu ovog upitnika su ispitani na uzorku od 128 vanbolničkih bolesnika koji su uzimali oralne preparate gvožđa. **Rezultati.** Krajnja verzija Upitnika za mučninu izazvanu lekovima (UMIL) sa pet pitanja je pokazala odličnu pouzdanost, kako kada su upitnike popunjavali istraživači za vreme intervjua sa bolesnicima (Kronbahov alfa koeficijent 0,892), tako i kada su upitnike popunjavali samo bolesnici (Kronbahov alfa koeficijent 0,897). Upitnik je bio stabilan u vremenu, a testovi konvergentne i divergentne punovažnosti su dali vrlo dobre rezultate. Faktorska analiza je otkrila samo jedan faktor, što znači da ceo upitnik meri samo jedan fenomen, intenzitet mučnine, kako je originalno i nemeravano. **Zaključak.** Upitnik UMIL je pouzdan i punovažan instrument za merenje intenziteta mučnine izazvane lekovima. Otkrivanje bolesnika sa visokim stepenom mučnine izazvane lekovima pomoći će propisivačima da odluče da li terapiju treba prekinuti ili preći na manje emetogene lekove.

Ključne reči:
lečenje lekovima; gvožđe; mučnina; lekovi; psihometrija; ankete i upitnici.

Introduction

Drugs have varying potential to induce nausea and/or vomiting. Center for vomiting in medulla oblongata is under the influence of substances from blood, stimulation of nerve endings in gastrointestinal tract and impulses from chemioreceptor zone. Neurotransmitters with significant effect on the center are histamine, acetylcholine, dopamine, 5-hy-

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droxytriptamine, substance P and endogenous cannabinoids [1]. Cytostatic drugs cause nausea in as much as 10% (drugs with low emetogenic potential) to 90% (drugs with high emetogenic potential) patients [2], while opioids cause nausea in 48% of patients when used for treatment of cancer pain and in 27% when used for postoperative pain [3]. Nausea rate after oral administration of iron salts amounts to 11% [4], and it is probably caused by the accumulation of free radicals in gastrointestinal mucosa [5]. Drug-induced nausea is big problem in everyday clinical practice, as many patients are not compliant to the prescribed therapy or discontinue the therapy due to nausea.

There are several questionnaires for measurement of nausea intensity after drug administration, usually developed specifically for certan drug groups, like the Chemotherapy-Induced Nausea and Emesis Quality of Life (CINI QOL) questionnaire [6] or the Gastrointestinal Symptom Questionnaire (GSQ) designed to measure nausea after oral drug intake [7] and tested in the patients taking iron salts. Within its program of developing standardized set of the patient-reported outcomes (Patient-Reported Outcomes Measurment Information System – PROMIS) the National Institute of Health in the USA created also the Gastrointestinal Symptom Scales (GSS), and one of them measures nausea caused by either disease or drug [8]. However, these scales are either too settings specific (like CINI QOL), or were not properly tested for reliability and validity after drug administration (like GSQ, or PROMIS GSS-nausea). The reliable and valid questionnaire for measurement of drug-induced nausea as general phenomenon could be an important clinical tool for assesing tolerability of emetogenic drugs and necessity to discontinue therapy or switch to less emetogenic one. If drug-induced nausea is mild, a prescriber could further decrease it through timing intake of the drug with food or giving only one daily dose before going to bed, and in this way preserve potentially very efficient drug for the patient instead of switching to other drugs (which could cause nausea, too). Besides, after adequate explanation and rating of nausea, the patients with a mild form will be more compliant to the prescribed therapy.

The aim of our study was to develop a questionnaire for measurement of intensity of drug-induced nausea and test its reliability and validity on a sample of adult patients taking iron salts orally.

Methods

Design

The study was of a cross-sectional type, and assessed reliability and validity of newly developed questionnaire for measurement of drug-induced nausea (Drug-Induced Nausea Scale – DINS) among outpatients taking iron salts orally.

Construction of the new questionnaire

Developing of the new questionnaire was done according to the guidelines set by Robert F. DeVellis [9], through 8 steps. In the first step (determining object of measurement), drug-induced nausea was chosen as an object of measurement, being one of the most frequent causes of discontinuation of effective drug therapy [10]. The second step, generating an item pool, was conducted through two brainstorming sessions of the authors, one week apart. In the third step (determining format for measurement) each item was constructed in the form of positive statement which should reflect certain element of nausea. Five possible answers were offered for each statement, in the form of Likert's scale: "never", "rarely", "sometimes", "often", and "always". The answers were rated from 1 ("never") to 5 ("always"). Total score of the questionnaire was calculated by summation of answers to individual items. The patients with the total score from 1 to 10 had mild nausea, those from 11 to 20 moderate nausea, and the patients with the score from 21 to 25 severe nausea. The fourth step (revision and correction of the initial pool of items) was made by the three-member expert committee composed of a psychiatrist, a gastroenterologist and a clinical pharmacology specialist employed by the Clinical Center Kragujevac, Serbia. Within the fifth step, one validation item for discovering socially desirable behavior of respondents was included in the questionnaire: "I always try to help other people. "In the sixth step the initial pool of the DINS items was tested on 5 PhD students (at Faculty of Medical Sciences, University of Kragujevac, Serbia) for clarity and comprehension. After the pilot a few minor changes were made, and then the final Serbian version of the DINS was copied and prepared for the reliability testing on the sample of 128 outpatients. The seventh (evaluating the items) and eighth (optimizing the questionnaire length) steps are described below.

Translation and cultural adaptationof supplementary questionnaire for validation purposes of the DINS instrument

The translation and cultural adaptation of the PROMIS-GSS-nausea questionnaire (4 items) was made according to the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) guidelines [11]. Permission for translation of PROMIS-GSS-nausea (version with 4 items) from English to Serbian was granted by the National Institutes of Health Patient Reported Outcomes Measurment Information System. The original scale was first translated into Serbian by two investigators who were Serbian native language speakers (S. Janković and A. Prokić). They translated the scale independently of each other, and then the translations were harmonized to one Serbian version at the meeting of the study investigators. The harmonized Serbian version was then translated back to English by Dr Zan Friscic, native English speaker, citizen of Australia. When translated back to English, Dr Friscic was not aware of the original English version of the PROMIS-GSS-nausea. The back-translation to English was then compared with the original English version by the study investigators and at the new meeting of investigators the final Serbian version of the PROMIS-GSS-nausea was agreed on. The final translation of PROMIS-GSS-nausea into Serbian was then tested on 5 PhD students (at Faculty of

Medical Sciences, University of Kragujevac, Serbia) for clarity and comprehension. After the pilot, a few minor changes were made, and then the final Serbian version of PROMIS-GSS-nausea was copied and prepared for the reliability testing.

Data collection – population and the sample

The final Serbian versions of the both new (DINS) and translated (PROMIS-GSS-nausea) questionnaires were tested for reliability on the outpatients who visited community pharmacies in Osečina, western Serbia. The visits took place during the year 2016. The inclusion criteria were the oral intake of iron salts for at least two weeks, literacy and age over 18. The exclusion criteria were previous gastrectomy, cognitive disorders (score at Mini-Mental State Examination below 24), mood disorders and mental retardation. The sample of the patients was of consecutive nature, i.e., all patients who visited community pharmacies during the study period (and satisfied inclusion and exclusion criteria) were offered the questionnaire. During the first encounter, the questionnaires were completed in two ways: at first, by the investigators who were questioning the patients, and second, by the patients themselves. At the second encounter, two weeks later, the patients were repeatedly interviewed by the study investigators who completed the same questionnaires again. The study was approved by the Ethics Committee of Clinical Center Kragujevac, Serbia. The patients were treated with due respect and care, according to the principles stated in Declaration of Helsinki.

Data analysis

Reliability testing

Reliability of the questionnaire was tested by three methods. First, internal consistency was determined through calculation of Cronbach's alpha for the questionnaire as a whole. Second, the questionnaire was divided by split-half method to two parts with the same number of questions, and Cronbach's alpha was calculated for each of the parts. Using the alphas for both parts, number of questions in each part and average correlation between the questions in both parts of the original questionnaire, the Spearman-Brown coefficient for the questionnaire as a whole was calculated by the Spearman-Brown "prediction" formula [12]. Third, for each question mean score and their variances were calculated in order to check their suitability for measurement of whole extent of nausea severity.

Factorial analysis

Principal component analysis of the questionnaire was made in order to discover principal factors [13]. The principal component analysis groups the items of a scale to a smaller number of principal components which describe most of the variance of the responses to the scale items. Each of the principal components identified covers part of the variance in the data, and they are not correlated between themselves. The components (factors) covering maximal variance are kept, while the otherswith small amount of variance are discarded. The amount of variance covered by each component is measured by its eigenvalue. First, suitability of the questionnaire and sample for factorial analysis was tested by the Kaiser-Meyer-Olkin measure of sampling adequacy and by the Bartlett's test of sphericity. Then, the factors were extracted at first without rotation, with conditions that the eigenvalues had to be greater than 1.0, and using the Scree-plot (the extracted factors were above the "elbow" of the graph). Second, referent axes were rotated orthogonally, by the Varimax method, and another extraction of the factors was made, using the same criteria as for the unrotated solution. The following was reported for the extracted factors: loadings, eigenvalues, and percentage of variance explained. The extracted factors were then named accordingly. All calculations were performed by the SPSS statistical software, version 18.0.

Validity

The content validity of the questionnaire was evaluated by an independent panel of three experienced clinicians at the Clinical Center Kragujevac, Serbia: psychiatrist, gastroenterologist and clinical pharmacology specialist.

The criterion validity was tested by three methods: comparison of the DINS scores when the questionnaire was completed by the investigators and by the patients themselves, convergent validity testing by comparison of the DINS score with the PROMIS-GSS-nausea score, and the divergent validity testing by comparison of the DINS score with the score of the Intolerance of Uncertainty (IU) questionnaire. The permission to use the Intolerance of Uncertainty questionnaire in Serbian language (which measures intolerance of uncertainty in everyday life and was previously validated in Serbian population) was granted by the Associated Professor, Ljiljana Mihić, psychologist, the University of Novi Sad, Serbia [14]. The correlations between scores on the questionnaires were calculated and presented in Multimethod, multi-trait matrix. All calculations were performed by the SPSS statistical software, version 18.0.

Temporal stability

Temporal stability of the DINS and the PROMIS-GSS-nausea results was tested by second completion of the questionnaires by the investigators who repeatedly interviewed the patients two weeks after the first encounter. The patients were invited to the second encounter by phone.

Results

The first version of the DINS questionnaire contained 5 questions, which after the pilot and minor adjustments was tested on the sample of 128 outpatients: mean age 45.8 ± 13.5 years, male/female ratio 16/112 (12.5%/87.5%), education elementary school/high school/university = 26.6%/51.6%/21.6%, place of residence, urban/rural = 83/45 (64.8%/35.2%), and all patients except 2 (1.6%) were prescribed with oral iron for treatment of anemia. Thirty-eight patients (29.7%) were taking iron salts before meal, 7 (5.5%) during meal, 68 (53.1%) after meal and remaining 15

(11.7%) did not take care about the timing of drug intake. Seventy patients (54.7%) were previously introduced with the gastrointestinal adverse effects of iron preparations, and the remaining 58 patients (45.3%) were not. Sixteen patients (12.5%) did have previous experience with nausea after oral drug intake, and the remaining 112 (87.5%) did not. Finally, 53 (41.4%) patients suffered from at least one chronic non-contagious disease, and 75 (58.6%) did not.

Mean score of the DINS was 8.6 ± 5.1 (range from 5 to 25). There were no significant differences in severity of nausea (the DINS score) according to the sex (females 8.6 ± 5.1, males 8.3 ± 4.7, $p = 0.781$), education (elementary school 8.9 ± 4.5, high school 8.7 ± 5.3, higher education 8.3 ± 4.4, $p = 0.910$) or place of living (urban 8.6 ± 5.4, rural 8.5 ± 4.4, $p = 0.962$) of the study participants.

Reliability testing

After testing the original 5 items from the questionnaire, and examining results of correlation matrix, mean values, variance, skewness and kurtosis of distributions of responses for each of the items, none of the items was removed, leaving final version of the DINS questionnaire with 5 items. Criteria for removing the items were extreme means, near zero variances and correlation coefficients with a majority of other items below 0.2. Cronbach's alpha of the final version with 5 items was 0.892, when the scale was rated by the investigators. The mean values of responses, standard deviations, skewness and kurtosis for each item of the DINS are shown in Table 1. After division of the DINS questionnaire by the split-half method, the Spearman-Brown coefficient for the questionnaire as a whole was calculated by the Spearman-Brown "prediction" formula, and its value was 0.834. When the scale was rated by the patients themselves, Cronbach's alpha was 0.897.

Cronbach's alpha of the PROMIS-GSS-nausea questionnaire with 4 items was 0.739, when the scale was rated by the investigators. After division of the PROMIS-GSS-nausea questionnaire by the split-half method the Spearman-Brown coefficient for the questionnaire as a whole was calculated by the Spearman-Brown "prediction" formula, and its value was 0.662. When the scale was rated by the patients themselves, Cronbach's alpha was 0.737.

Factorial analysis

Factorial analysis of the DINS was made by the principal components method. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.815 and the Bartlett's test of sphericity was significant ($p = 0.000$). Only one factor was extracted, explaining in total 70.1% of variance and with eigenvalue 3.503.

Factorial analysis of the PROMIS-GSS-nausea questionnaire was made also by the principal components method. The Kaiser-Meyer-Olkin measure of sampling adequacy was 0.614 and the Bartlett's test of sphericity was significant ($p = 0.000$). Only one factor was extracted, explaining in total 56.22% of variance and with eigenvalue 2.249.

Validity

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

Divergent criterion validity was tested through non-parametric correlation between scores of the DINS scale (when it was rated by an investigator and by the patients themselves) and scores of the IU scale (when it was rated by investigator and by patients themselves). The convergent criterion validity was tested through the non-parametric correlation between the scores of the DINS scale (when it was rated by an investigator and by the patients themselves), the scores of the PROMIS-GSS-nausea scale (when it was rated by an investigator and by the patients themselves). The correlation coefficients between the DINS and the IU scales and between the PROMIS-GSS-nausea and the IU scales were below 0.2 and were statistically insignificant. The non-parametric correlation was chosen due to the non-normal distribution of some of the scores. The Spearman's correlation coefficients are shown in the Multi-trait, multi-method matrix (Table 2).

Table 1

Mean values, standard deviation, skewness and kurtosis of responses to the items of DINS questionnaire (the responses are rated from 1 to 5 on a Likert scale).

Item	Mean response	Standard deviation	Skewness	Kurtosis
Did you feel nausea during drug therapy?	1.97	1.386	1.157	-0.084
During drug therapy, did you feel nausea always in the same time during a day?	1.89	1.399	1.338	0.319
During drug therapy, how often you could not perform your daily activities due to nausea?	1.71	1.243	1.641	1.404
Was your appetite decreased due to nausea during drug therapy?	1.36	.858	2.651	6.599
Did you feel an urge to vomit during drug therapy?	1.63	1.100	1.746	2.083

DINS – drug-induced nausea scale.

Table 2

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

Parameter	DINS score, rated by an investigator	DINS score, rated by a patient	PROMIS-GSS-nausea score, rated by an investigator	PROMIS-GSS-nausea score, rated by a patient	IU score, rated by an investigator	IU score, rated by a patient
DINS score, rated by an investigator	1	0.956**	0.765**	0.765**	0.131	0.126
DINS score, rated by a patient	0.956**	1	0.757**	0.759**	0.127	0.123
PROMIS-GSS-nausea score, rated by an investigator	0.765**	0.757**	1	0.961**	0.052	0.037
PROMIS-GSS-nausea score, rated by a patient	0.765**	0.759**	0.961**	1	0.018	0.013
IU score, rated by an investigator	0.131	0.127	0.052	0.018	1	0.972**
IU score, rated by a patient	0.126	0.123	0.037	0.013	0.972**	1

****significant correlation at $p < 0.001$.**
DINS – Drug Induced Nausea Scale. PRMIS-GSS – Patient-Reported Outcomes Measurement Information System Gastrointestinal Symptom Scale; IU – Intolerance of Uncertainty.

Temporal stability

The DINS scale showed excellent temporal stability: when rating (by an investigator) was repeated on the same patients two weeks later, the correlation between the scores (Spearman's coefficient) was 0.965 ($p < 0.001$). Cronbach's alpha after the repeated rating was 0.901.

The PROMIS-GSS-nausea scale also showed excellent temporal stability: when rating (by an investigator) was repeated on the same patients two weeks later, the correlation between the scores (Spearman's coefficient) was 0.947 ($p < 0.001$). Cronbach's alpha after the repeated rating was 0.742.

Discussion

The final version of the DINS scale with 5 items showed excellent reliability, both when rated by the investigators and the patients themselves. It was temporally stable, and both divergent and convergent validity tests had very good results. The factorial analysis revealed only one factor, which means that the whole scale was measuring only one phenomenon, intensity of nausea, as was originally intended. The DINS scale was also more reliable than the previously validated PROMIS-GSS-nausea scale.

Although the PROMIS-GSS-nausea scale was used for measuring intensity of nausea in a variety of gastronitestinal diseases, showing high ability to discriminate between the subtle changes in the nausea intensity [15], it was not previously used to measure drug-induced nausea. In our study, it showed necessary level of reliability for this purpose, but the DINS surpassed it by far with its high Cronbach's alpha around 0.9.

Since nausea and vomiting are particularly severe in the patients receiving chemotherapy, it is not surprising that the largest number of instruments for measuring drug-induced vomiting was specifically developed in this area. Recent systematic review has found seven instruments for measuring chemotherapy-induced nausea, retching and vomiting [16]. A majority of these instruments cover three key domains (nausea, vomiting and retching) and are prepared in several forms which are adjusted for three different phases of nausea-vomiting-retching phenomenon: anticipatory, acute and delayed. Our instrument DINS was focused on nausea domain, which is usually the only one present when the patients take less emetogenic drugs other than cytostatics [17]. Therefore, the DINS should not be used for the measurement of chemotherapy induced nausea, retching and vomiting, but for estimation of nausea caused by less emetogenic drugs prescribed to outpatients.

Although limited to the measurement of nausea, the items from the DINS instrument cover essential aspects of this phenomenon, which could be also applied to vomiting and retching: occurrence (item 2), duration (item 1) and severity (items 3,4 and 5) [17]. The Gastrointestinal Symptom Questionnaire by Pereira et al. [7] also covered these aspects of nausea, but the answers to questions had only three modalities, "mild", "moderate" and "severe", limiting discriminative power of the scale. Although in their study, Pereira et al. [7] did not measure internal consistency of their questionnaire, most likely it would not be too high, since the questionnaire related only to condition of a patient on the day of rating, and misses chronicity as important aspect of drug-induced nausea. We also would like to point out that the second question (During drug therapy, did you feel nausea always in the same time during a day?) could be better formulated in a way which would take into account timing of a drug intake during the day (e.g., During drug

therapy, did you feel nausea always after its administration?) in order to capture causality between intake of a drug and emergence of nausea. However, this new formulation would have to be tested in a future study.

Main limitations of this study were non-homogenous nature of the study sample, i.e., some of the patients had previous experience with nausea after oral drug intake, some did not, and female sex was largely predominant, due to higher incidence of iron-deficiency anemia. This non-homogeneity could be responsible for somewhat wider dispersion of the patients' responses. Besides, the patients were taking only one drug (iron salts) which causes nausea, so the results could be drug type – specific, and may not apply to nausea caused by other drugs. Future studies with the same questionnaire should be conducted on several patient subgroups which are taking other emetogenic drugs in order to get complete insight into its functionality.

Conclusion

In conclusion, the DINS is a reliable and valid instrument for measuring intensity of drug-induced nausea. Identification of patients with high intensity of drug-induced nausea by this questionnaire will help prescribers to decide whether the therapy should be stopped or the patient switched to less emetogenic therapy.

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R E F E R E N C E S

1. *Hendren G, Aponte-Feliciano A, Kovac A*. Safety and efficacy of commonly used antiemetics. Expert Opin Drug MetabToxicol 2015; 11(11): 1753–67.
2. *Turini M, Piovesana V, Ruffo P, Ripellino C, Cataldo N*. An assessment of chemotherapy-induced nausea and vomiting direct costs in three EU countries. Drugs Context 2015; 4: 212285.
3. *Leppert W*. Emerging therapies for patients with symptoms of opioid-induced bowel dysfunction. Drug Des Devel Ther 2015; 9: 2215–31.
4. *Tolkien Z, Stecher L, Mander AP, Pereira DI, Powell JJ*. Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. PLoS One 2015; 10(2): e0117383.
5. *Geisser P, Burckhardt S*. The pharmacokinetics and pharmacodynamics of iron preparations. Pharmaceutics 2011; 3(1): 12–33.
6. *Martin CG, Rubenstein EB, Elting LS, Kim YJ, Osoba D*. Measuring chemotherapy-induced nausea and emesis. Cancer 2003; 98(3): 645–55.
7. *Pereira DI, Couto Irving SS, Lomer MC, Powell JJ*. A rapid, simple-questionnaire to assess gastrointestinal symptoms after oral ferrous sulphate supplementation. BMC Gastroenterol 2014; 14: 103.
8. *Spiegel BM, Hays RD, Bolus R, Melmed GY, Chang L, Whitman C*, et al. Development of the NIH Patient-Reported Outcomes Measurement Information System (PROMIS) gastrointestinal symptom scales. Am J Gastroenterol 2014; 109(11): 1804-14.
9. *DeVellis RF*. Scale Development, Theory and Applications. 2nd ed. Newbury Park: SAGE Publications; 2003.
10. *Morrow GR, Navari RM, Rugo HS*. Clinical roundtable monograph: new data in emerging treatment options for chemotherapy-induced nausea and vomiting. Clin Adv Hematol Oncol 2014; 12(3 Suppl 9): 1–14; quiz 15.
11. *Wild D, Grove A, Martin M, Eremenco S, McElroy S, Verjee-Lorenz A,* et al. ISPOR Task Force for Translation and Cultural Adaptation. Principles of Good Practice for the Translation and Cultural Adaptation Process for Patient-Reported Outcomes (PRO) Measures: report of the ISPOR Task Force for Translation and Cultural Adaptation. Value Health 2005; 8(2): 94–104.
12. *Streiner DL, Norman GR*. Health Measurement Scales - a practical guide to their development and use. 4st ed. Oxford: Oxford University Press; 2008.
13. *Badia X, Arribas F, Ormaetxe JM, Peinado R, de Los Terreros MS*. Development of a questionnaire to measure health-related quality of life (HRQoL) in patients with atrial fibrillation (AF-QoL). Health Qual Life Outcomes 2007; (5): 37.
14. *Mihić Lj, Sokić J, Samac N, Ignjatović I*. Serbian adaptation and validation of the Intolerance of Uncertainty Scale. Primenjena psihologija 2014; 7(Suppl): 347–70. (Serbian)
15. *Khanna D, Hays RD, Shreiner AB, Melmed GY, Chang L, Khanna PP,* et al. Responsiveness to Change and Minimally Important Differences of the Patient-Reported Outcomes Measurement Information System Gastrointestinal Symptoms Scales. Dig Dis Sci 2017; 62(5): 1186–92.
16. *Brearley SG, Clements CV, Molassiotis A*. A review of patient self-report tools for chemotherapy-induced nausea and vomiting. Support Care Cancer 2008; 16(11): 1213–29.
17. *Phillips RS, Friend AJ, Gibson F, Houghton E, Gopaul S, Craig JV,* et al. Antiemetic medication for prevention and treatment of chemotherapy-induced nausea and vomiting in childhood. Cochrane Database Syst Rev 2016; 2: CD007786.

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Retroperitoneoscopic nephrectomy for a nonfunctioning kidney

Retroperitoneoskopska nefrektomija afunkcionalnog bubrega

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Abstract

Background/Aim. The minimally invasive laparoscopic nephrectomy was first performed in 1991. The objective of this paper was to present the surgical technique of retroperitoneoscopic nephrectomy and to our experience with this procedure in removal of non-functioning kidneys. **Methods.** This retrospective study enrolled 55 patients who underwent retroperitoneoscopic nephrectomy at our institution during the period from January 2011 to November 2016. All patients had a unilateral non-functioning kidney confirmed by intravenous or computed tomography (CT)-urography and renal scintigram. Their medical records were analyzed for demographic data, duration of surgery, average blood loss, duration of hospital stay as well as time to return to normal life activities. **Results.** The mean age of patients was 43 years (range 23–78). Perioperative or early postoperative mortality was not recorded. Mean operative time was 82 minutes (range 45–210). The average blood loss was 90 mL (40–450). The average hospital stay was 4 days (3–7). Return to life activity was in average after 12 days (9–15). **Conclusions.** Retroperitoneoscopic nephrectomy for a non-functioning kidney is a feasible, safe, and effective minimally invasive method. The length of hospital stay and convalescence was shorter than after open nephrectomy.

Key words:
nephrectomy; minimally invasive surgical procedures; retroperitoneal space.

Apstrakt

Uvod/Cilj. Minimalno invazivna laparoskopska nefrektomija izvedena je prvi put 1991. godine. Cilj ovog rada je prikaz hirurške tehnike retroperitoneoskopske nefrektomije i naših iskustava sa primenom ove metode u lečenju bolesnika sa afunkcijom bubrega. **Metode.** Ova retrospektivna studija obuhvatila je 55 bolesnika kojima je urađena retroperitoneoskopska nefrektomija u periodu od januara 2011. do novembra 2016. Kod svih bolesnika afunkcija bubrega dijagnostikovana je intravenskom ili kompjuterizovanom tomografijom (CT)-urografijom i potvrđena scintigrafijom bubrega. Demografski podaci, trajanje operacije, prosečan gubitak krvi, trajanje bolničkog lečenja, kao i vreme do povratka uobičajenim aktivnostima su analizirani na osnovu medicinske dokumentacije. **Rezultati.** Prosečna starost bolesnika bila je 43 godine (raspon 23–78). Perioperativni i rani postoperativni mortalitet nisu zabeleženi. Prosečno trajanje operacije bilo je 82 minuta (raspon 45–210). Prosečan gubitak krvi bio je 90 mL (raspon 40–450). Prosečno trajanje hospitalizacije bilo je 4 dana (raspon 3–7). Bolesnici su se vratili uobičajenim aktivnostima u proseku nakon 12 dana (raspon 9–15). **Zaključak.** Retroperitoneoskopska nefrektomija afunkcionalnog bubrega je izvodljiva, sigurna i efikasna minimalno invazivna metoda. Hospitalizacija i period oporavka kraći su u odnosu na otvorenu nefrektomiju.

Ključne reči:
nefrektomija; hirurgija, minimalno invazivne procedure; retroperitonealni prostor.

Introduction

The first minimally invasive laparoscopic nephrectomy was reported by Clayman et al. [1] in 1991. Removal of the right kidney with renal mass measuring 3 cm lasted for 7 hours. The same group attempted laparoscopic nephrectomy using a retroperitoneal approach, but they found it less comfortable and more hazardous for the development of pneumothorax [2].

The retroperitoneoscopic approach was further popularized by Gaur [3] with his innovative creation of retroperitoneal space using balloon dilatation. With improvements in technical equipment and experience gained, this approach was used for more complex procedures like heminephrectomy, pyeloplasty, ureterolithotomy and partial nephrectomy for malignancies [4].

The objective of this paper was to present the surgical technique of retroperitoneoscopic nephrectomy for a non-

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functioning kidney and to review our experience of the first 55 cases.

Methods

This retrospective study is based on the review of medical records of 55 patients who underwent retroperitoneoscopic nephrectomy for a nonfunctioning kidney during the period January 2011 to November 2016. Diagnosis of a nonfunctioning kidney was made by intravenous urography (IVU) or computed tomography (CT) urography and confirmed with renal scintigram. Morphology of nonfunctioning renal units was assessed by retrograde and/or antegrade pyelography, when necessary. Each renal unit with less than 8% of functional parenchyma was considered to be nonfunctional. Removal of the nonfunctioning kidney was indicated in the presence of recurrent pyelonephritis, stone disease, or hypertension that can not be controlled by medications.

Following the standard preoperative evaluation, patients were subjected to surgical treatment. All procedures were performed by the single surgeon (V.S.) under general anesthesia.

Surgical technique

After being introduced into general anesthesia, each patient received a urinary catheter. Thereafter the patient was placed in an adequate (left or right) lateral flank position. The operative field was prepared in a standard way. Three trocars were placed by open technique. A 2 cm incision is made above the iliac crest and after an opening of lumbodorsal fascia, blunt finger dissection technique is used for the creation of retroperitoneal space (Figure 1a). The posterior port was placed under tactile control 2 cm beneath the 12th rib (Figure 1b), close to the paraspinal musculature, taking care to avoid injury to neurovascular structures. The anterior port was placed after blunt dissection of the peritoneum. At the beginning of the operation, the anterior port was placed under visual control, but with experience, it was found that blunt dissection of the peritoneum, using the index finger, was sufficient for safe placement of trocar under tactile control (Figure 1c). The anterior port was placed beneath the tip of the12th rib, on the anterior axillary line, taking care to avoid injury of a peritoneal sac. Finally, a 12 mm medial port was placed through the initial incision (Figure 1d) and a camera was introduced. Leakage of gas was prevented by two stitches around the trocar. Our 5 mm and two 12 mm trocar were used for the retroperitoneoscopy. The 12 mm was placed on the dominant surgeon's hand (anterior port for the right sided procedure or posterior port for the left sided procedure).

Gas was insufflated to create pneumoretroperitoneum to the pressure of 12 mmHg. The next step was an intraoperative orientation and identification of Gerota fascia that was incised posteriorly (Figures 2a and 2b). The lower pole of a kidney was identified and dissected. Thereafter, the ureter (Figure 3a) and gonadal vessels were identified and dissected. The ureter was clipped and transected (Figure 3b). Proximal ureteral stump was pulled laterally in order to lift the lower pole of the kidney and proceed with dissection of the renal hilum. The artery was identified and meticulously dissected as well as the renal vein subsequently.

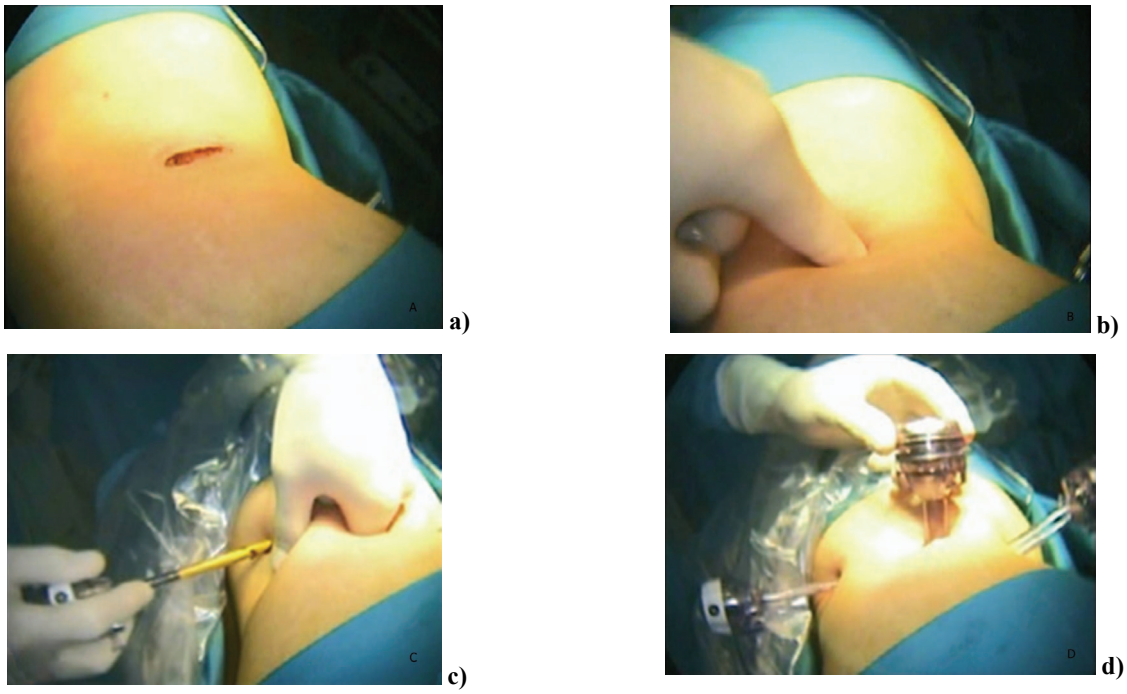

Fig. 1 – Surgical technique of retroperitoneoscopic nephrectomy. a) retroperitoneoscopic approach: primary incision; b) blunt finger dissection of retroperitoneal space; c) placement of anterior 5 mm trocar under tactile control; d) placement of medial 12 mm (camera) port.

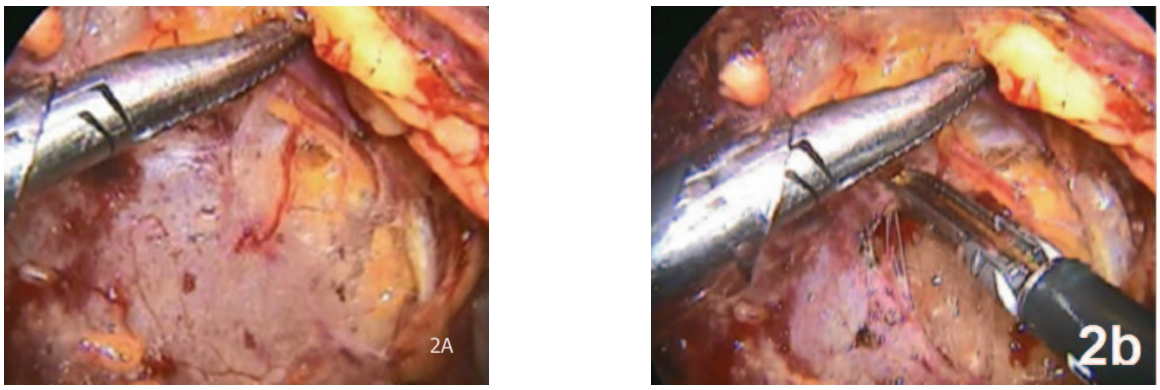

Fig. 2 – a) Dissection, and b) incision of Gerota fascia.

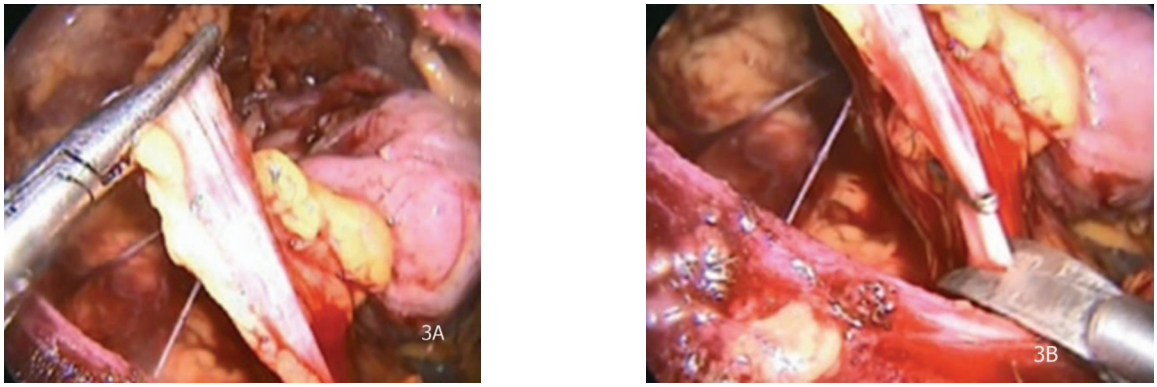

Fig. 3 – a) Dissection of the ureter and b) transaction of the previously clipped ureter.

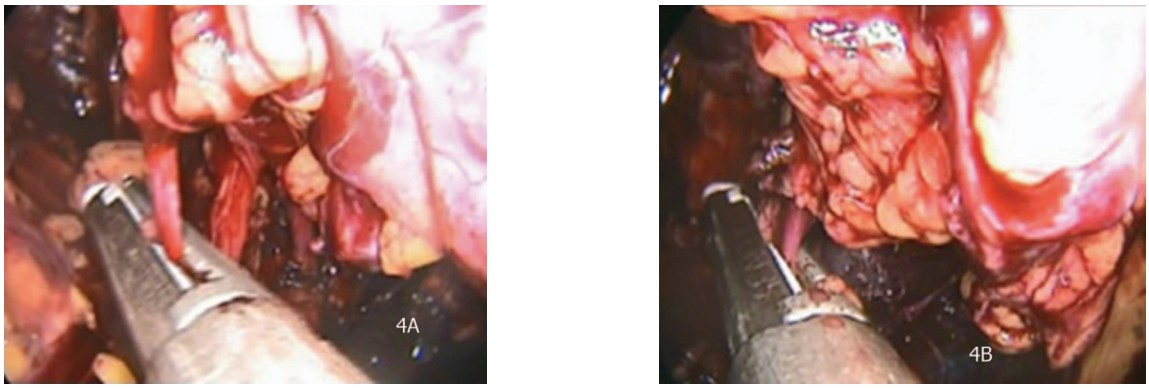

Fig. 4 – Control of a) dissected renal vein and b) dissected renal artery using ENDO Gia stapler.

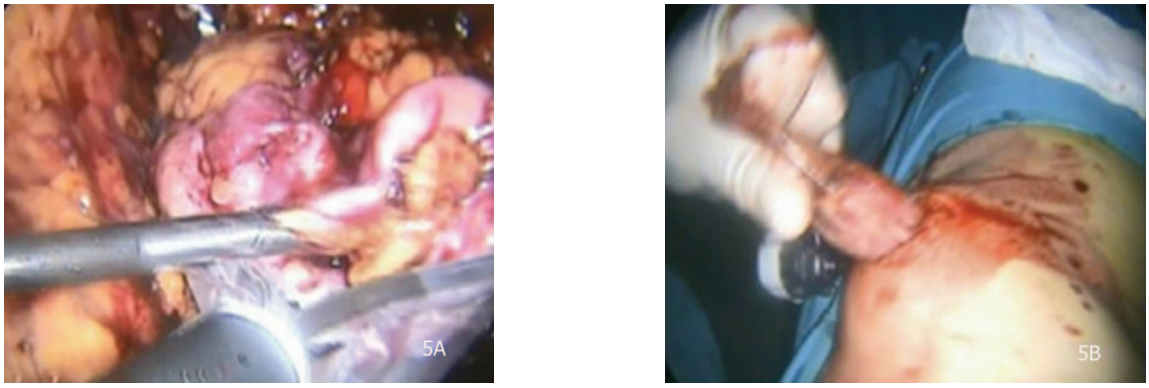

Fig. 5 – a) Entrapment of kidney in Endobag; b) Removal of the surgical specimen.

The ENDO Gia vascular stapler was used to secure renal artery (Figure 4a) and vein (Figure 4b). The procedure was continued cephalad using a harmonic scalpel for dissection of attachments of the upper pole of the kidney. Removal of the surgical specimen depended on the size of the kidney. Small hypoplastic kidneys were removed using specimen retrieval bags (Figures 5a and 5b), but a removal of bigger sized specimen required widening of the initial incision. The procedure was completed with control of hemostasis and leaving the drain in the retroperitoneum. Finally, port sites were sutured.

On the first postoperative day, the urinary catheter was removed. A drainage tube was removed when an amount of the drained liquid was less than 20 mL/24 hours. Patients were discharged upon removal of the drain. Routine check-up visits were scheduled 2 weeks after surgery at outpatient department, and thereafter annually.

Statistical analysis was made using an Excel program. Parametric data were analyzed using descriptive methods, mean value and range.

The study was approved by the Ethical Committee of Clinical Center of Vojvodina.

Results

A total of 55 patients (38 males and 17 females) underwent retroperitoneoscopic nephrectomy for a nonfunctioning kidney. The mean age of the patient was 43 years (range 23–78). Etiology of nonfunctioning kidneys is presented in Table 1.

Table 1

Etiology of nonfunctioning kidneys

Etiology	Number (%) of patients
Ureteral calculi	11 (20)
Uretero-pelvic junction stricture	10 (18.2)
Ureteral stricture	12 (21.8)
Renal atrophy/hypotrophy	20 (36.4)
Stenosis *a. renalis*	2 (3.6)
Total	55 (100.0)

The average duration of surgery was 82 minutes (range 45–210), Mean blood loss was 90 mL (range 40–450). Four (7.3%) patients underwent conversion to open surgery: two cases due to severe fibrosis and another two due to the size of the kidney. Postoperative complications were noted in 5 (9.1%) patients: paralytic ileus, fever, and prolonged drainage. The average duration of hospital stay was 4 days. Patients returned to normal life activities after 12 days (range 10–15). There was no perioperative and early postoperative mortality. Our results are comparable with reported series of retroperitoneoscopic nephrectomies (Table 2).

Discussion

Traditionally, urologists have used open retroperitoneal approach for renal surgery more frequently. Surprisingly, the majority of laparoscopic surgeons dealing with renal pathology are not familiar with retroperitoneoscopy. The main disadvantage of retroperitoneoscopy seems to be reduced working space that can cause problems with trocar placement, intraoperative orientation in a surgical field, and entrapment of the organ. On the other hand, posterior access enables easier and faster identification of anatomical structures of the upper urinary tract and keeps peritoneal cavity isolated, reducing the risk of inadvertent organ injuries.

Indications for retroperitoneoscopic nephrectomy include chronic pyelonephritis, obstructive or reflux uropathies, renovascular hypertension, nephrosclerosis, dysplastic kidney, acquired renal cystic disease, polycystic kidneys, renal tuberculosis and end-stage-kidney disease before transplantation [5].

This procedure is contraindicated in the case of uncontrolled coagulopathy and untreated infection associated with hemodynamic instability. Morbid obesity and previous retroperitoneal surgery have been considered relative contraindications for retroperitoneoscopy. Inexperienced surgeons should refrain from performing retroperitoneoscopy in cases with xanthogranulomatous pyelonephritis and renal tuberculosis because these conditions are associated with severe perirenal scarring and the higher rate of conversions to open surgery [5, 6].

Complications associated with the retroperitoneoscopic approach are possible at each step of the procedure. Access related complications are lesions of abdominal wall vessels, lesions of peritoneum or pleura, injuries of solid and hollow organs. Bleeding is the most unpleasant complication during surgery. It should be carefully inspected at the end of the procedure after lowering of gas pressure in the operative field [4, 7].

Table 2

Results of reported series of retroperitoneoscopic nephrectomies

Author	Year	Number of patients (n)	Mean duration of surgery (min)	Blood loss (mL)	Complication rate n (%)	Conversion rate (%)	Hospital stay (days)
Quintela et al. [10]	2006	43	160	200	4 (9.3)	13.9	2.1
Gupta and Gautam [5]	2005	351	98	65	22 (6.3)	13.3	3
Gaur [11]	2000	38	132	84	6 (4.4)	na	na
Hemal et al. [12]	1999	43	114	na	2 (4.7)	5	3.4
Gill [13]	1998	36	263	117	2 (5.6)	na	5.4
Rassweiler et al. [14]	1998	17	188	na	2(11.8)	5.9	6

na – not available data.

Rassweiler et al. [8] have found a significant advantage of the laparoscopic and retroperitoneoscopic over the open approach for nephrectomy in terms of duration of surgery, consumption of analgesics and duration of hospital stay. Also, they found that retroperitoneoscopic nephrectomy was more favorable than laparoscopic nephrectomy in terms of lower transfusion rate (5.9% vs. 16.7%), lower conversion rate (5.9% vs. 11.1%) and lower complication rate (29.4% vs. 38.9) [8]. Garg et al. [9] have published recently similar findings. They found a significantly higher visual analog score in nephrectomized patients who were treated by laparoscopic approach than those who received retroperitoneoscopy (4.9 vs. 2.7 on day 1 and 3.2 vs. 1.1 on day 2).

Table 2 shows the summarized characteristics of previously reported series [5, 10–14]. Certainly, with experience gained and technical improvements of instrumentaria, the duration of surgery, complication and conversion rates are minimized. However, patients should be warned of possible conversions to open surgery, particularly in cases of pyonephrosis or severe perirenal adhesions.

Conclusion

Retroperitoneoscopic nephrectomy for a nonfunctioning kidney is a feasible, safe and effective minimally invasive method. The length of hospital stay and convalescence was relatively short. Results obtained with retroperitoneoscopic nephrectomy in this study are comparable with reported series.

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REFERENCES

1. *Clayman RV, Kavoussi LR, Soper NJ, Dierks SM, Merety KS, Darcy MD,* et al. Laparoscopic nephrectomy. N Engl J Med 1991; 324(19): 1370–1.
2. *Kerbl K, Figenshau RS, Clayman RV, Chandhoke PS, Kavoussi LR, Albala DM,* et al. Retroperitoneal laparoscopic nephrectomy: laboratory and clinical experience. J Endourol 1993; 7(1): 23–6.
3. *Gaur DD*. Laparoscopic operative retroperitoneoscopy: use of a new device. J Urol 1992; 148: 1137–9.
4. *Liapis D, de la Taille A, Ploussard G, Robert G, Bastien L, Hoznek A,* et al. Analysis of complications from 600 retroperitoneoscopic procedures of the upper urinary tract during the last 10 years. World J Urol 2008; 26(6): 523–30.
5. *Gupta NP, Gautam G*. Laparoscopic nephrectomy for benign non functioning kidneys. J Minim Access Surg 2005; 1(4): 149–54
6. *Traxer O, Pearle MS*. Laparoscopic nephrectomy for benign disease. Semin Laparosc Surg 2000; 7(3): 176–84.
7. *Rassweiler J, Seemann O, Frede T, Henkel TO, Alken P*. Retroperitoneoscopy: experience with 200 cases. J Urol 1998; 160(4): 1265–9.
8. *Rassweiler J, Fornara P, Weber M, Janetschek G, Fahlenkamp D, Henkel TO, et al.* Laparoscopic nephrectomy: the experience of the laparoscopy working group of the German Urologic Association. J Urol 1998; 160(1): 18–21.
9. *Garg M, Singh V, Sinha RJ, Sharma P*. Prospective randomized comparison of transperitoneal vs retroperitoneal laparoscopic simplenephrectomy. Urology 2014; 84(2): 335–9.
10. *Quintela RS, Cotta LR, Neves MF, Abelha DL Jr, Tavora JE*. Retroperitoneoscopic nephrectomy in benign pathology. Int Braz J Urol 2006; 32(5): 521–8.
11. *Gaur DD*. Simple nephrectomy: retroperitoneal approach. J Endourol J Endourol 2000; 14(10): 787–90; discussion 791.
12. *Hemal AK, Talwar M, Wadhwa SN, Gupta NP*. Retroperitoneoscopic nephrectomy for benign diseases of the kidney: prospective nonrandomized comparison with open surgical nephrectomy. J Endourol 1999; 13(6): 425–31.
13. *Gill IS*. Retroperitoneal laparoscopic nephrectomy. Urol Clin North Am 1998; 25(2): 343–60.
14. *Rassweiler J, Frede T, Henkel TO, Stock C, Alken P*. Nephrectomy: A comparative study between the transperitoneal and retroperitoneal laparoscopic versus the open approach. Eur Urol 1998; 33(5): 489–96.

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The significance of smoking as a risk factor for the disorder of the obstructive pulmonary pattern in the patients with systemic sclerosis

Značaj pušenja kao faktora rizika od poremećaja plućne funkcije kod bolesnika sa sistemskom sklerozom

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Abstract

Background/Aim. Systemic sclerosis (SSc) is a chronic systemic disease of the connective tissue. It is characterized by diffuse microangiopathy, increased activity and creating deposits of collagen in the skin and internal organs. Involvement of the lung function disturbances in SSc is a bad prognostic sign. The aim of our study was to investigate the association between smoking habits and lung function disorder in the SSc patients. **Methods.** The testing was conducted at the Clinic for Rheumatology and Immunology of the Military Medical Academy in 2016. In this study, we included 42 patients with the newly diagnosed SSc and the patients whose disease had been diagnosed earlier. **Results.** The patients were classified according to the smoking habits, 14 (33.3%) patients were nonsmokers, while 28 (66.7%) patients were current (23 patients) or ex-smokers (5 patients). We found no significant differences in examined parameters among smokers and nonsmokers. In addition, distribution of the patients with the obstructive pulmonary pattern revealed by spirometry was uniform between smokers and nonsmokers. The concentrations of C reactive protein (CRP) were significantly higher in the SSc patients with the obstructive pulmonary pattern. The patients with the obstructive pattern on spirometry had significantly lower values of forced vital capacity (FVC), forced expiratory volume in the first second (FEV1), FEV1/FVC ratio, diffusing lung capacity for carbon monoxide (DLCO) and FVC/DLCO ratio. **Conclusion.** In our study, we concluded that in the SSc patients with the obstructive pulmonary pattern revealed by spirometry, there were no significant differences between smokers and nonsmokers. CRP is a significant predictor of the lung involvement existence in the SSc patients.

Key words:
scleroderma, systemic; lung diseases, obstructive; risk factors; smoking.

Apstrakt

Uvod/Cilj. Sistemska skleroza (SSc) je hronična sistemska bolest vezivnog tkiva. Karakteriše se difuznom mikroangiopatijom, povećanom aktivnošću i stvaranjem depozita kolagena u koži i unutrašnjim organima. Zahvatanje pluća u SSc je loš prognostički znak. Cilj rada je bio da se ispita povezanost navike pušenja i nalaza plućne funkcije kod bolesnika sa SSc. **Metode.** Ispitivanje je sprovedeno u Klinici za reumatologiju Vojnomedicinske akademije tokom 2016. godine. Bilo je uključeno 42 bolesnika oba pola sa novootkrivenom sistemskom sklerozom, kao i bolesnici kod kojih je bolest ranije dijagnostikovana. **Rezultati.** Bolesnici su bili podeljeni u grupe u pogledu navike pušenja: 14 (33,3%) bolesnika bili su pušači, a 28 (66,7%) bolesnika nepušači (23 bolesnika) ili bivši pušači (5 bolesnika). Nisu nađene značajne razlike ispitivanih parametara između pušača i nepušača. Distribucija bolesnika sa opstruktivnom nalazom na plućima, registrovanim spirometrijom, bila je jednoobrazna između pušača i nepušača. Koncentracije C reaktivnog proteina (CRP) su bili značajno više kod bolesnika sa SSc i opstruktivnim nalazom na plućima. Bolesnici sa opstruktivnim nalazom na plućima, registrovanim spirometrijom, imali su značajno niže vrednosti forsiranog vitalnog kapaciteta (FVC), forsiranog ekspirijumskog volumena tokom prve sekunde (FEV1), FEV1/FVC odnosa, difuzijskog kapaciteta za ugljen monoksid (DLCO) i FVC/DLCO odnosa. **Zaključak.** Kod bolesnika sa SSc i opstruktivnom nalazom na plućima, registrovanim spirometrijom, nema značajne razlike između pušača i nepušača. CRP je značajan prediktor za postojanje poremećaja plućne funkcije kod bolesnika sa SSc.

Ključne reči:
sklerodermija, sistemska; pluća, opstruktivne bolesti; faktori rizika; pušenje.

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Introduction

Systemic sclerosis (SSc) is a chronic systemic disease of the connective tissue. It is characterized by diffuse microangiopathy, increased activity and creation of deposits of collagen in the skin and internal organs [1]. Disease severity depends on the degree of damage to important visceral organs [2].

The affected respiratory system manifests events such as serositis, interstitial lung disease (ILD), pulmonary vascular disease, muscular weakness and infection [3]. Involvement of the lungs in SSc is a bad prognostic sign.

In systemic sclerosis, ILD can be seen in most patients [4]. As the first manifestation of the disease in SSc, ILD is very rare, and is associated with the presence of antibodies against topoisomerase I (anti-SCL-70 antibodies) [5, 6].

In systemic sclerosis, involvement of the lungs is evident in the late stages of the disease [4, 7].

A restrictive ventilatory defect is typical for patients with ILD. Static lung volumes are usually reduced in these patients [8].

The determination of pulmonary function tests is important for diagnosis in the patients with ILD and SSc. These patients have decreased forced volume vital capacity (FVC) and diffusing lung capacity for carbon monoxide (DLCO).

After the perceived widespread bronchiectasis and peribronchial fibrosis during the autopsy in the patients with progressive SSc, it was concluded that there was a possible obstruction of the small airways in these patients [8].

The aim of our study was to investigate whether there was an impact of smoking on the lung function disorder in the patients with SSc.

The hypothesis was that smoking was a leading cause that led to pulmonary function disorder involvement, but there were many factors that could cause the restrictive disturbances in the patients with SSc.

Methods

In our study, we tested the pulmonary function in two groups of patients – current or ex-smokers and non-smokers. The testing was conducted at the Clinic for Rheumatology and Immunology of the Military Medical Academy in 2016.

We included 42 patients with the newly diagnosed SSc and the patients whose disease had been diagnosed earlier. All patients met the American Rheumatism Association (ARA) criteria for diagnosis of SSc [9].

The exclusion criteria were asthma, hypersensitivity pneumonitis and exposure to organic dusts.

Spirometry is performed using the spirometer Cardinal Health, Jaeger (Germany).

The pulmonary function testing included a measurement of FVC, forced expiratory volume in first second (FEV1), FEV1 /FVC ratio, DLCO and FVC/DLCO ratio.The patients were classified according to the smoking habits: 14 (33.3%) patients were nonsmokers, while 28 (66.7%) patients were current (23 patients), or ex-smokers (5 patients). The patients were also compared on the basis of the presence or absence of an obstructive pattern on spirometry (obstructive: FEV1/FVC ratio < 80% + FEV1 < 80%, $n = 11$, nonobstructive: $n = 31$).

Statistical analysis

Normally distributed variables are presented as mean ± standard deviation (SD) and categorical variables are presented as relative frequencies. Concentrations of C-reactive protein (CRP) were log-transformed prior to analyses in order to obtain a normal distribution. The data were compared by the Student's *t* test for continuous variables and by the χ^2-test – contingency tables, for categorical variables. The correlations were assessed by the Pearson's correlation analysis. The logistic regression analysis was performed in order to explore the independent predictors for the development of obstructive pulmonary pattern in the patients with SSc. All statistical analyses were performed using the PASW Statistics version 18.0 and MedCalc Software version 11.4. The differences with $p < 0.05$ were considered to be statistically significant.

Results

Table 1 presents the general characteristics, concentrations of CRP as a marker of SSc and parameters of pulmonary function in the patients clustered by the smoking status. We found no significant differences in the analyzed parameters among the smokers and nonsmokers.

Table 1

General characteristics, C-reactive protein (CRP) and parameters of pulmonary function according to the smoking status in the systemic sclerosis patients

Patients	Smokers (n = 28)	Nonsmokers (n = 14)	*p*
Age (years)	51.57 ± 11.06	51.43 ± 10.13	0.968
Gender, male (%)	10.7	21.4	0.383
FVC (% predicted)	97.95 ± 23.00	90.61 ± 15.31	0.287
FEV1 (% predicted)	98.41 ± 20.61	91.49 ± 15.88	0.277
FEV1/FVC (% predicted)	96.91 ± 13.80	94.69 ± 11.84	0.610
DLCO (% predicted)	66.59 ± 16.99	59.07 ± 21.75	0.226
FVC/DLCO (% predicted)	73.45 ± 16.49	66.14 ± 25.30	0.265
Obstructive pattern on spirometry (%)	25.0	28.6	0.541
CRP (mg/L)*	2.30 (1.30–4.08)	2.80 (1.10–7.11)	0.709

Data are presented as mean ± SD for continuous variables, or as relative frequencies for categorical variables and compared by the Student's *t*-test, or by the χ^2-test, respectively. The values are presented as geometric mean (confidence interval) and log-transformed prior to analysis.

FVC – forced vital capacity; FEV1 – forced expiratory volume in first second; DLCO – diffusing lung capacity for carbon monoxide.

Table 2

General characteristics, C reactive protein (CRP) and parameters of pulmonary function according to the obstructive pattern on spirometry

Patients	Obstructive pattern (n = 11), mean ± SD	Nonobstructive pattern (n = 31), mean ± SD	*p*
Age (years)	57.27 ± 9.81	51.97 ± 11.03	0.665
Gender, male (%)	36.4	6.5	0.032
Smoking status (%)	63.6	67.7	0,804
FVC (% predicted)	83.12 ± 27.16	99.90 ± 16.50	0.020
FEV1 (% predicted)	82.99 ± 25.08	100.76 ± 14.54	0.007
FEV1/FVC (% predicted)	88.08 ± 14.26	99.04 ± 11.55	0.015
DLCO (% predicted)	52.91 ± 18.12	68.05 ± 17.64	0.020
FVC/DLCO (% predicted)	58.13 ± 23.99	75.58 ± 16.26	0.010
CRP (mg/L)*	4.79 (2.54–8.99)	1.95 (1.09–3.46)	0.031

For abbreviations see under Table 1.
Data are presented as mean ± SD for continuous variables, or as relative frequencies for categorical variables and compared by the Student's *t*-test or by the χ^2-test respectively. The values are presented as geometric mean (confidence interval) and log-transformed prior to analysis.

In addition, the distribution of patients with the obstructive pulmonary pattern revealed by spirometry was uniform between the smokers and nonsmokers.

We analyzed the same parameters as previously in our patients in two groups (smokers or ex-smokers and non-smokers), divided according to the presence or absence of the obstructive pulmonary pattern revealed by spirometry. The obtained results are presented in Table 2.

In the group of smokers, there were 7 (25%) patients with the obstructive pattern on spirometry and 21 (75%) without it. In the group of non-smokers were 4 (29%) patients with the obstructive pattern on spirometry and 10 without it.

Expectedly, the patients with the obstructive pattern on spirometry (7 smokers or ex-smokers and 4 non-smokers) had significantly lower values of FVC, FEV1, FEV1/FVC ratio, DLCO and FVC/DLCO ratio. Both subgroups were uniform by age, but male sex was more prevalent among the carriers of the obstructive pattern.

Additionally, the concentrations of CRP were significantly higher in the SSc patients with the obstructive pulmonary pattern (smokers or ex-smokers and 4 non-smokers).

In order to achieve more in-depth insight into the associations of CRP levels with the parameters of pulmonary functions, we performed the correlation analysis. We found that concentrations of CRP were in the significant negative correlations with FVC, FEV1 and DLCO. Also, the CRP levels were in reciprocal relationships with FEV1/FVC and FVC/DLCO ratios, although the statistical significance was not reached. The above-mentioned results are presented in Table 3.

Table 3

Correlations of concentrations of C reactive protein (CRP) with the parameters of pulmonary function in the systemic sclerosis patients

Parameters	Pearson's correlation coefficient	*p*
FVC (% predicted)	-0.358	0.030
FEV1 (% predicted)	-0.445	0.006
FEV1/FVC (% predicted)	0.183	0.278
DLCO (% predicted)	-0.413	0.011
FVC/DLCO (% predicted)	-0.313	0.059

For abbreviations see under Table 1.

Finally, we tried to find some independent predictors of obstructive pulmonary pattern development on spirometry. The multivariate logistic regression analysis was employed for this purpose. The results are presented in Table 4. Apart from the CRP concentration, the age and smoking status were also included in the model. The concentration of CRP was revealed as a significant predictor of development of lung function disorder involvement in the SSc patients. Neither smoking nor age of patients were recognized as the independent associates with the obstructive pattern assessed by spirometry.

Table 4

Multivariate logistic regression analysis of independent predictors of the obstructive pulmonary pattern in the sclerosis patients patients

Variables in model	OR	95 % CI	*p*
CRP	9.043	(1.006–81.282)	0.049
Age	0.944	(0.864–1.031)	0.198
Smoking habits (0 - no, 1 - yes)	1.548	(0.371–6.468)	0.549

CRP – C reactive protein; OR – odds ratio; CI – confidence interval.

Discussion

Lung involvement in SSc is inflammatory consequence of the underlying disease and characterized by the activation of alveolar macrophage, fibroblast proliferation and extracellular matrix, probably under the influence of uncontrolled cytokine. ILD is usually evaluated after the appearance of the respiratory symptoms [3].

The degree of the lung parenchyma involvement in the patients with SSc can be estimated by the use of the pulmonary function tests.

The pulmonary function parameters that provide the best information are total lung capacity (TLC), FVC, FEV1, and DLCO [3].

Monitoring of the FVC with the newly diagnosed SSc is useful, because its reduction indicates the occurrence of lung lesions and progression of the underlying disease [10].

Greenvald et al. [11] proved that the non-smokers had greater decrease in TLC and static lung compliance compared to the current and ex-smokers.

Individual variability is important in disorder of the pulmonary function and it is not enough to known how smoking affects the pulmonary function.

Cherniack at al. [12] described that in the patients with idiopathic pulmonary fibrosis, which are non-smokers and former smokers, diffusing capacity per liter of lung volume (DLCO/VA) and FEV1/FVC ratio were significantly lower than in the patients who were smokers, while TLC and FVC were higher than in the non-smokers.

Our study shows that distribution of the patients with the obstructive pulmonary pattern, revealed by spirometry, is uniform between the smokers and nonsmokers, which can be explained in the way that the smoking habit in the patients with SSc is not the only reason for airway involvement. There are many factors that make the pulmonary function abnormal.

Steen et al. [13] showed that in the patients with scleroderma, who smoked more frequently, severe obstructive changes were revealed. Compared to the non-smokers, the patients who smoked and had restrictive lung disease had more severe disease. DLCO was significantly decreased in the patients-smokers compared with the nonsmokers.

A study showed that the pulmonary function in the patents nonsmokers with SSc was not different compared with the nonsmoking reference population [14].

The patients with the obstructive pattern on spirometry had the significantly lower values of FVC, FEV1, FEV1/FVC ratio, DLCO and FVC/DLCO ratio. Both subgroups were uniform by age, but male sex was more prevalent among the carriers of the obstructive pattern. Also, in our study it was proven that the concentrations of CRP were significantly higher in the SSc patients with the obstructive pulmonary pattern.

Our study confirmed the findings of a large Canadian study [5] reporting that the elevated baseline CRP levels were associated with the concomitant diffuse cutaneous involvement and severity of skin and lung involvement. In our study, the concentrations of CRP were the significant predictors of development of lung involvement in the SSc patients.

Conclusion

We concluded that in the SSc patients with the obstructive pulmonary pattern revealed by spirometry, there were no significant differences between smokers and non-smokers. CRP is a significant predictor of the lung involvement existence in the SSc patients, which can be explained in the way that the smoking habit in the patients with SSc is not the only reason for airway involvement. There are many factors that make the pulmonary function abnormal.

In the future, further research on the possible causes of the lung involvement in the patients with systemic sclerosis should be explored in some larger studies.

REFERENCES

1. *Ferri C, Valentini G, Cozzi F, Sebastiani M, Michelassi C, La Montagna G,* et al. Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. Medicine (Baltimore) 2002; 81(2): 139–53.
2. *Kowal-Bielecka O, Fransen J, Avouac J, Becker M, Kulak A, Allanore Y*, et al. Update of EULAR recommendations for the treatment of systemic sclerosis. Ann Rheum Dis 2017; 76(8): 1327–39.
3. *Plavec G, Tomić I, Bihorac S, Kovacević G, Pavlica L, Cvetković G,* et al. Lung involvement in systemic connective tissue diseases. Vojnosanit Pregl 2008; 65(9): 688–91.
4. *Minai OA, Dweik RA, Arroliga AC.* Manifestations of scleroderma pulmonary disease. Clin Chest Med 1998; 19(4): 713–31, viii–ix.
5. *Muangchan C, Harding S, Khimdas S, Bonner A, Canadian Scleroderma Research Group, Baron M,* et al. Association of C-reactive protein with high disease activity in systemic sclerosis: results from the Canadian Scleroderma Research Group. Arthritis Care Res (Hoboken) 2012; 64(9): 1405–14.
6. *McNearney TA, Reveille JD, Fischbach M, Friedman AW, Lisse JR, Goel N,* et al. Pulmonary involvement in systemic sclerosis: associations with genetic, serologic, sociodemographic, and behavioral factors. Arthritis Rheum 2007; 57(2): 318–26.
7. *Warrick JH, Bhalla M, Schabel SI, Silver RM.* High resolution computed tomography in early scleroderma lung disease. J Rheumatol 1991; 18(10): 1520–8.
8. *D'Angelo WA, Fries JF, Masi AT, Shulman LE.* Pathologic observations in systemic sclerosis (scleroderma). A study of fifty-eight autopsy cases and fifty-eight matched controls. Am J Med 1969; 46(3): 428–40.
9. *Valentini G, Iudici M, Walker UA, Jaeger VK, Baron M, Carreira P*, et al. The European Scleroderma Trials and Research group (EUSTAR) task force for the development of revised activity criteria for systemic sclerosis: derivation and validation of a preliminarily revised EUSTAR activity index. Ann Rheum Dis 2017; 76(1): 270–6.
10. *Steen VD, Conte C, Owens GR, Medsger TA Jr.* Severe restrictive lung disease in systemic sclerosis. Arthritis Rheum 1994; 37(9): 1283–9.
11. *Greenwald GI, Tashkin DP, Gong H, Simmons M, Duann S, Furst D*E, et al. Longitudinal changes in lung function and respira-

tory symptoms in progressive systemic sclerosis. Am J Med 1987; 83(1): 83–92.

12. *Cherniack RM, Colby TV, Flint A, Thurlbeck WM, Waldron JA Jr, Ackerson L*, et al. Correlation of structure and function in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1995; 151(4): 1180–8.
13. *Steen VD, Owens GR, Fino GJ, Rodnan GP, Medsger TA Jr.* Pulmonary involvement in systemic sclerosis (scleroderma). Arthritis Rheum 1985; 28(7): 759–67.
14. *Schneider PD, Wise RA, Hochberg MC, Wigley FM.* Serial pulmonary function in systemic sclerosis. Am J Med 1982; 73: 385–94.

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The use of complementary serological and molecular testing for blood-borne pathogens and evaluation of socio-demographic characteristics of intravenous drug users on substitution therapy from Šumadia district of Serbia

Komplementarno serološko i molekularno testiranje krvno-prenosivih patogena i procena sociodemografskih karakteristika kod korisnika intravenskih droga na supstitucionoj terapiji u Šumadijskom okrugu Srbije

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Abstract

Background/Aim. Intravenous drug users (IDUs) are still a high risk-group for cross-reacting blood-borne infections, for vertical pathogen transmission as well as for potentially blood/plasma donation (especially as "paid" donors). The aim of our study was to establish the profile of opiate addict and prevalence of blood-borne pathogens – Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Human Immunodeficiency Virus (HIV) among 99 patients on substitution therapy with methadone and buprenorphine from Šumadia District. **Methods**. The Treatment Demand Indicator (TDI) of Pompidou-questionnaire was used to assess the history of drug abuse and risk behavior. All blood samples were tested for HBV surface antigen (HBsAg), anti-HCV antibody (anti-HCV) and HIV antigen/antibody (HIV-Ag/Ab) by Enzyme-Linked ImmunoSorbent Assay (ELISA) or Chemiluminescent Immuno-Assay (CIA). Investigations were also performed for HBV, HCV and HIV by molecular testing – Polymerase Chain Reaction (PCR) method. **Results.** The majority of patients were males (81.8%), median age 32 (19–57) years, lived in a city (99%), unemployed (58.6%), with finished secondary school (67.7%), unsafe injecting practices (34.3%) and never previously tested for HBV (39.4%), HCV (36.4%) nor HIV (28.3%); only 4% of them previously got HBV-vaccine. The complementary testing resulted with following results: HBV ELISA/CIA and PCR negativity for 66 patients and positive results (by ELISA/CIA and PCR) for 19 patients. However, a difference was observed in the ELISA/CIA-negative/PCR-positive result for 12 and ELISA/CIA-positive/PCR-negative for two patients respectively. Further, the negative results for HCV (ELISA/CIA and PCR testing) were found in 15 IDUs and positive results (using both methods) were found in 58 patients. Different results for ELISA/CIA-negative / PCR-positive results were found in 11 IDUs and ELISA/CIA-positive/PCR – negative results were found in 15 patients. All investigated IDUs were negative for HIV (ELISA/CIA and PCR testing) and for pathogens of opportunistic infection (*Cryptococcus neoformans*; *Pneumocystis carinii*; PCR testing), as well as for West Nile Virus (PCR testing). Just one IDU was positive for syphilis (ELISA and confirmatory testing). **Conclusion.** Our study demonstrated that the positivity for HBV and HCV is still very high (33.4% and 84.8%, respectively) in IDUs. Thus, we suggest that drug users have to be periodically screened using a complementary serological/molecular testing – concerning differences/discrepancies in the results obtained using these methods.

Key words:
opioid-related disorders; blood-borne pathogens; methadone; hepatitis b virus; hepatitis c virus; hiv; serology; demography; serbia.

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Apstrakt

Uvod/Cilj. Korisnici intravenskih droga predstavljaju visokorizičnu grupu zbog međusobnih infekcija krvno prenosivim bolestima, vertikalne transmisije patogena, kao i zbog mogućnosti da budu potencijalni donori krvi/produkata plazme (naročito kao plaćeni donori). Cilj našeg istraživanja bio je utvrđivanje demografsko-sociološkog profila 99 opijatnih zavisnika Šumadijskog okruga lečenih u Kliničkom centru Kragujevac supstitucionom terapijom metadonom i buprenorfinom, kao i određivanje prevalencije infekcija krvno prenosivim bolestima: virusom hepatitisa B (HBV), virusom hepatitisa C (HCV) i virusom stečene imunodeficijencije (HIV). **Metode.** Ispitanici su odgovarali na pitanja iz Pompidu upitnika i podaci iz ovog upitnika korišćeni su za analizu osnovnih socio-demografskih karakteristika. Svi uzorci su prvo testirani ELISA (*Enzyme-Linked ImmunoSorbent Assay) i CIA (Chemiluminescent Immuno-Assay*) metodom, a zatim PCR-om (*Polymerase Chain Reaction*). **Rezultati.** Najveći broj ispitanika bilo je muškog pola (81,8%), starosti 32 (19–57) godine, 99% ispitanika živelo je u gradu, nezaposlenih je bilo 58,6%, sa završenom srednjom školom 67,7%, a korisnika neadekvatne primene igala bilo je 34,3%. Netestiranih na HBV bilo je 39,4%, na HCV 36,4%, HIV 28,3% a samo njih 4 (4%) primilo je vakcinu protiv HBV. Što se tiče analiza na prisusustvo HBV infekcije, ELISA/CIA i PCR negativnih je bilo 66, HBV ELISA/CIA i PCR pozitivnih bilo je 19, HBV ELISA/CIA-negativnih/PCR-pozitivnih 12 i HBV ELISA/CIA-pozitivnih/PCR-negativnih 2 ispitanika. Testiranje na HCV infekciju je pokazalo sledeće: ELISA/CIA i PCR negativnih ispitanika je bilo 15, HCV ELISA/CIA i PCR pozitivnih bilo je 58, HCV ELISA/CIA-negativnih/PCR-pozitivnih 11, a HCV ELISA/CIA-pozitivnih/PCR-negativnih 15. Svi ispitanici bili su negativni na HIV (ELISA/CIA i PCR testiranje), kao i na patogene oportunističkih infekcija (*Cryptococcus neoformans*; *Pneumocystis carinii*; PCR testiranje) i na prisustvo virusa zapadnog Nila (West Nile Virus; PCR testiranje). Jedan ispitanik bio je pozitivan na sifilis (ELISA testiranje). **Zaključak.** Naši rezultati pokazali su da je pozitivnost na prisustvo patogena krvno prenosivih bolesti HBV i HCV visoka u ispitivanoj grupi korisnika intravenskih droga i iznosi 33,4% i 84,8%, respektivno. Preporuka bi bila da oni budu periodično testirani na prisustvo HBV, HCV i HIV infekcije komplementarnim ELISA/CIA testovima, kao i PCR testovima, obzirom na izvestan stepen diskrepance u dobijenim rezultatima serološkog i molekularnog testiranja.

Ključne reči:
poremećaji izazvani opioidima; krvno-prenosivi patogeni; metadon; hepatitis b, virus; hepacivirus; hiv; serologija; demografija; srbija.

Introduction

The integrative approach is the necessity in the modern treatment of addiction, considering especially an early detection and additional treatment of somatic states in intravenous drug users (IDUs) [1]. The "drug-use-disorder" show to be very frequent in European countries (approximately 0.5% of population or about two million people), with relatively more problems of diseases caused by "drug-use-disorder" in Western Europe and especially high rates of hepatitis B virus (HBV), hepatitis C virus (HCV) and humane immunodeficiency virus (HIV) infection in this vulnerable population [2, 3].

The intravenous drug users face stigma not only due to psychological and behavioral aspects of their functioning, but also because of significantly higher rates of blood-borne and/or sexually transmitted infections due to unsafe injecting practices and risky sexual behaviors [4]. The pharmacological substitution programs of methadone and buprenorphine are the "harm avoidance" programs that are also useful in prevention of blood-borne infections [5]. The opiate addiction treatment in Serbia is conducted in four clinical centers by supervision of the Ministry of Health of the Republic of Serbia – including more than 4,000 patients on substitution therapy, but objective, precise and longitudinal data about opiate addiction and infectious disease "co-occurrence" is still missing [6].

The serological testing, like routine screening of blood donors, are performed by the anti-HCV Enzyme Linked ImmunoSorbent Assay (ELISA) or Chemiluminescent Immuno-Assays (CIA) methods. In the last years of the 20th century, two more tests were initiated to detect the presence of HCV: HCV Ag/Ab (antigen/antibody), and HCV Nucleic Acid Testing (NAT) or Polymerase Chain Reaction (PCR) assays [7, 8].

The period of the "window" (the time from entering the virus in the body until the moment when it is detectable by the available techniques) before the introduction of PCR was about 70 days. By introducing the PCR individual testing, this period is reduced to 15 days [2], while the window period for HCV Ag/Ab is 40 days [7]. The window period is 16 days for HIV Ag/Ab, while in HIV PCR it is reduced to 9 days [7]. The actuality of comparisons of test results by the ELISA and PCR methods lies in determining the infectivity of the samples or the phase of infection in which there are dependents on psychoactive substances in this case [3]. It is necessary to determine the infectivity of the tested addicts and in terms of delineation of the test results: they did not come into contact with these viruses, the start of the infection – the "window" period, an infection, and past active infection.

The study aim was to evaluate the profile of an opiate addict and above all the prevalence of blood-borne infections such as HBV, HCV and HIV among IDUs on substitution therapy with methadone and buprenorphine in Šumadia District of Serbia. The results of complementary ELISA or CIA and PCR testing were also compared in attempt to improve the pathogen monitoring system as well as the diagnostic algorithm for this vulnerable population.

Methods

Patients

In this study the patients on substitution therapy with methadone or buprenorphine at the Department of Addicti-

ons, Psychiatric Clinic, Clinical Centre Kragujevac – as a regional addiction treatment center – were included. This centre is managing the drug dependence treatment using the Treatment Demand Indicator (TDI) approach. The TDI was formulated in 2000 by the European Monitoring Centre for Drugs and Drug Addiction – EMCDDA/Pompidou Group, aiming to collect comparable and reliable data about the number and characteristics of drug addicts in EU countries [9].

TDI evaluates the treatment needs and assesses the history of drug abuse and risk behavior. Collected data from this questionnaire was used for the socio-demographic and injection practice analysis. Diagnoses of opioid related disorders (F11) or other psychoactive substance related disorders (F19) were established using the International Statistical Classification of Diseases and Related Health Problems.

The protocol of this "cross-sectional" study was approved by the Ethics Committee of the Clinical Centre Kragujevac and conducted in accordance with all the ethical principles of the Declaration of Helsinki. Informed consent was obtained from all of the patients before starting any study procedure.

Pathogen investigation methods

Total of 99 IDUs were tested using the complementary serological and molecular testing. All samples were taken into 9 mL tubes with K2EDTA (Bio-One Vacuette, Greiner), then the tubes were centrifuged at 3500 rpm, 30 minutes and plasma samples were analyzed. The samples were initially tested by ELISA or CIA systems (Evolis, Biorad; Architect i2000 SR, Abbott) and afterward with s201 system (COBAS Ampliprep/ COBAS Taqman, Roche). The preliminary positive specimens were analyzed using ID (Individual Donation) PCR. The preliminary negative samples were tested by mini-pool (MP) PCR technique (6 samples).

HCV ELISA/CIA-negative/PCR-positive samples were tested using the confirmatory test (n = 11; Innolia Innogenetics) [7]. In addition, all 99 IDUs were tested on pathogens of opportunistic infections, such as *Cryptococcus neoformans* and *Pneumocystis carinii*. Finally, the patients were also investigated for syphilis by ELISA or CIA method and West Nile Virus (WNV) by the PCR testing.

The pathogen investigations were performed in the Institute of Transfusiology and Hemobiology of the Military Medical Academy (MMA) and in the Institute of Microbiology of the MMA in Belgrade – during the period from July to August 2015.

Statistical analysis

The data was presented as absolute numbers, median and percentage. The tables were used to present the socio-demographic characteristics of IDUs as well as the complementary serological and molecular investigations. The 2 × 2 contingency table was used to compare the results of serological and molecular testing. The statistical analyses were performed using the SPSS 20.0 software. Differences were considered as statistically significant if the *p* value was less than 0.05.

Results

The majority of IDUs were males, city residents and prevailingly had completed the secondary school (their characteristics are presented in Table 1).

Table 1

The socio-demographic characteristics of IDUs

Parameters	Values
Age (years)	
median	32
range	19–57
Gender, n (%)	
male	88 (81.8)
female	11 (18.2)
Residents of the city, n (%)	98 (98.9)
Unemployed, n (%)	58 (58.6)
Education level – secondary school, n (%)	67 (67.7)
Unsafe injecting practices, n (%)	34 (34.3)

IDUs – intravenous drug users.

Amongst all 99 IDUs, some of them were never tested on virus infections – exactly, on HBV 39, on HCV 36, on HIV 28; in addition, just 4 IDUs got a vaccine against HBV.

The complementary ELISA or CIA and PCR testing of the IDUs demonstrated the predominant concordance between the serological and molecular analysis. The results confirmed that more than 80% of IDUs had HCV positivity, proved by both testing, comparing to more than 30% proved HBV infection. The concordance and discordance between the different methods (ELISA or CIA vs. PCR) are shown in Table 2.

Absolute numbers of patients – analyzed by the comparative serological and molecular testing for HCV vs. HBV in this study – are summarized in Table 3 and Figure 1.

Table 2

Complementary ELISA or CIA and PCR investigations for viruses

Pathogen type	ELISA or CIA and PCR testing	IDUs n (%)
HBV	ELISA/CIA-negative/PCR-negative	66 (66.7)
	ELISA/CIA-positive/PCR-positive	19 (19.2)
	ELISA/CIA-negative/PCR-positive	12 (12.1)
	ELISA/CIA-positive/PCR-negative	2 (2.1)
HCV	ELISA/CIA-negative/PCR-negative	15 (15.1)
	ELISA/CIA-positive/PCR-positive	58 (58.6)
	ELISA/CIA-negative/PCR-positive	11 (11.1)
	ELISA/CIA-positive/PCR-negative	15 (15.1)
HIV	ELISA/CIA-negative/PCR-negative	99 (100)

HIV – human immunodeficiency virus.
For other abbreviations see under Figure 1.

Table 3

The HBV and HCV presence investigated by ELISA or CIA and PCR

ELISA or CIA testing (absolute numbers)			PCR testing (absolute numbers)		
Pathogen type	HCV		Pathogen type	HCV	
	negative	positive		negative	positive
HBV			HBV		
negative	25	56*	negative	18	50*
positive	1*	17*	positive	12*	19*

***significant difference in the HBV and HCV positivity (1 + 17 vs. 56+17; 12+19 vs. 50 + 19); $p < 0.01$.**
For abbreviations see under Figure 1.

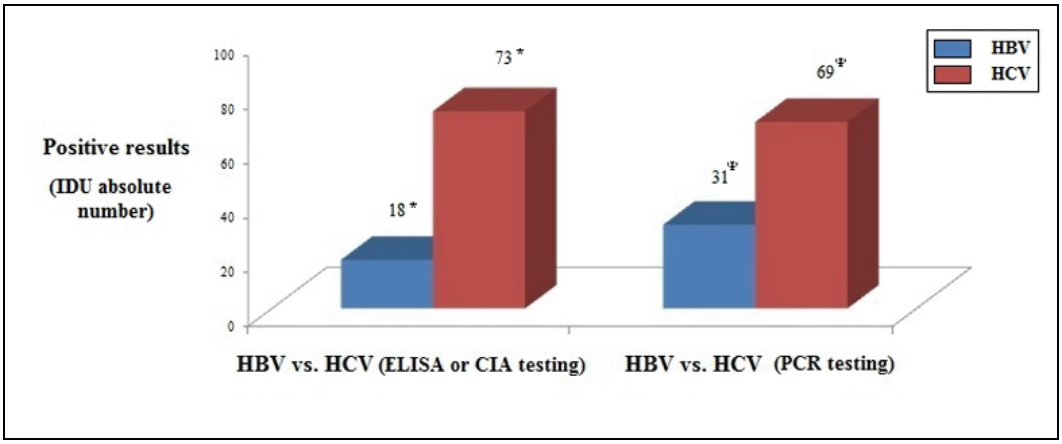

Fig. 1 – Comparative HCV and HBV values determined by ELISA/CIA and PCR.
***,Ψ significant differences between HBV and HCV positivity by both, serological and molecular testing ($p < 0.01$).**
IDU – intravenous drug users; HCV – hepatitis C virus; HBV – hepatitis B virus; ELISA/CIA – Enzyme-linked Immunosorbent Assay/Chemiluminiscent Immuno Assay; PCR – Polymerase Chain Reaction.

Table 4

The results of ELISA or CIA and PCR testing for other pathogens

Pathogen type	ELISA or CIA and PCR testing	IDU n (%)
Treponema pallidum	ELISA/CIA-negative	98 (99)
	ELISA-positive *VDRL-positive **TPHA-positive Confirmatory test-positive	1 (1)
Cryptococcus neoformans	PCR-negative	99 (100)
Pneumocystis carinii	PCR-negative	99 (100)
West Nile Virus	PCR-negative	99 (100)

IDU – intravenous drug users; VDRL – Venereal Disease Research Laboratory testing; TPHA – *Treponema pallidum* Hemagglutination test.
For other abbreviations see under Figure 1.

As presented, the number of HCV vs. HBV positive IDUs was significantly ($p < 0.01$) higher in our study group, using both ELISA or CIA and PCR techniques.

Regarding the opportunistic infections, all 99 IDUs were negative (PCR testing) for the most common pathogens: *Cryptococcus neoformans*, *Pneumocystis carinii* and WNV. Only one patient was positive on syphilis by ELISA, VDRL and TPHA. These results are presented in Table 4.

The presented negativity for the opportunistic infection pathogens can be explain because of no severe compromised immune system – since all IDUs were negative on HIV.

Discussion

Several risk factors make IDUs vulnerable to HCV, HBV, HIV, syphilis and opportunistic infection caused by *Cryptococcus neoformans*, *Pneumocystis carini* and WNV [10]. Hazardous behaviors include the use of non-sterilized needles and unprotected sexual activities, unsafe tattooing, cupping, blood transfusion or dental procedures in both IDUs and non-IDUs. Besides, a lack of access to health services, low socio-educational level, homelessness, history of imprisonment, social exclusion, unemployment, alcohol addiction

and presence of other diseases complicate the features of infection by HCV, HBV and HIV viruses and their related outcomes in many IDUs.

The global prevalence of HCV infection among IDUs in 2010 was 46.7%, implicating that some 7.4 million of the 16 million IDUs worldwide are infected with the HCV. The HBV infection rate among IDUs is about 14.6%, that is 2.3 million IDU are infected with mentioned virus, and 18.9% or 3 million of IDUs are living with HIV worldwide [11].

Despite the higher prevalence and "transmissibility" and the equal or higher economic costs of HCV compared to HIV infection, especially among IDUs, viral hepatitis received far less attention than HIV related disease. Worldwide, the prevalence of HIV infection amongst IDUs was calculated as 17.9% in 2009 and 18.9% in 2010 [3].

In our study there is no HIV infection among IDUs and that is similar as prevalence in Iran (0.7%) and among blood donors tested earlier in MMA (0.005%) [8, 12]. Prevalence of HBV (33.4%) was lower than in Italy (where the prevalence is 60.7%), while in Mexico it is 85%, similar as in Greece and Portugal, but significantly higher than prevalence in Uruguay (20%), Iran (0.7%) and among blood donors (0.20%) [13–15]. IDUs had a much higher probability of acquiring infection than non-injectors, confirming the role of intravenous transmission. The prevalence was highest for HCV infection (84.8%) and that is lower than prevalence observed in Estonia and Latvia (about 90%), Romania and Portugal, and similar as in Russia (73%). The HCV prevalence is higher than in Hungary (23%) and among blood donors (0.12%) [12–16].

The difference between results of ELISA/CIA and PCR testing can be explained in two ways. Firstly, ELISA/CIA negative, but PCR positive results show that infection with HBV or HCV is in the "window" period – that means that the concentration of viral antigen or antibodies against them are too low that they cannot be measured by ELISA/CIA [8]. On the other hand, the PCR negative ELISA/CIA positive results are common when we have cases of old HCV infections. The number of these results can be even 20% among blood donors, so the prevalence of 15.1% in our study is in that range [17, 18]. The number of two HBV PCR negative ELISA/CIA positive results show that the HBV DNA levels in the HBsAg-positive samples can be extremely low. About 6% of donations would be negative by the current MP HBV PCR methods. About 3% of donations would remain undetected by sensitive single-donor PCR. These results indicate caution in any consideration of dropping the HBsAg screening [18].

IDUs in Serbia have similar social-demographic characteristics as in Italy: 88% vs. 84% are males, median age is 32 vs. 35 years, mostly unemployed. However, IDUs in this investigation were not well-educated because only 4% of patients received HBV vaccine – while 29% in Italy [13]. This fact could be a consequence of the suboptimal medical care system in investigated region.

Conclusion

The majority of IDUs were males (aged 19–57 years), city residents and predominantly with completed secondary school. This study undoubtedly demonstrated improved safety of originally designed the complementary (ELISA/CIA and PCR) pathogen monitoring system. Our results confirmed that injecting drug practice continues to be an important risk factor for blood-borne infections; the positivity for HBV and HCV was still very high – 33.4% and 84.8%, respectively. Thus, drug users have to be periodically screened by the complementary serological/molecular testing – concerning differences/discrepancies in the results obtained by using these methods. Finally, we speculate that HBV vaccination should be actively obtainable/offered to all HBV-negative IDUs.

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REFERENCES

1. *Wiessing L, Ferri M, Grady B, Kantzanou M, Sperle I, Cullen KJ,* et al. Hepatitis C virus infection epidemiology among people who inject drugs in Europe: a systematic review of data for scaling up treatment and prevention. PLoS One 2014; 9(7): e103345.
2. *Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B*, et al. The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur Neuropsychopharmacol 2011; 21(9): 655–79.
3. *Wales N.* A review of viral hepatitis in injecting drug users and assessment of priorities for future activates. Geneva: WHO; 2009.
4. *Gyarmathy VA, Neaigus A, Li N, Ujhelyi E, Caplinskiene I, Caplinskas S,* et al. Infection disclosure in the injecting dyads of Hungarian and Lithuanian injecting drug users who self-reported being infected with hepatitis C virus or human immunodeficiency virus. Scand J Infect Dis 2011; 43(1): 32–42.
5. *Ruan Y, Liang S, Zhu J, Li X, Pan SW, Liu Q*, et al. Evaluation of harm reduction programs on seroincidence of HIV, hepatitis B and C, and syphilis among intravenous drug users in southwest China. Sex Transm Dis 2013; 40(4): 323–8.
6. *Djukić Dejanović S, Borovčanin M.* Principes of treatment of opiate dependents in Serbia. In: *Djukić Dejanović S, Nastasić P,* editors. Dependence diseases: Modern achivements in Prevention, treatment and rehabilitation Beograd: ECPD; 2015. p. 10–2. (Serbian)
7. *Trkuljić M, Borovčanin N, Vučetić D, Jovičić D*. Transmissible diseases – Etiopathogenesis, markers testing, inactivation of pathogenes. In: *Balint B, Trkuljić M, Todorović M,* editors. Basic principles of chemotherapy. Beograd: Čigoja štampa; 2010. p. 421–505. (Serbian)
8. *Vučetić D, Kecman G, Ilić V, Balint B.* Blood donors' positivity for transfusion-transmissible infections: the Serbian Military Medical Academy experience. Blood Transfus 2015; 13(4): 569–75.

9. *Simon R, Donmall M, Hartnoll R, Kokkevi A, Ouwehand AW, Stauffacher M*, et al. The EMCDDA/Pompidou Group treatment demand indicator protocol: a European core item set for treatment monitoring and reporting. Eur Addict Res 1999; 5(4): 197–207.
10. *Allain PJ*. Emerging Viruses in Transfusion. In: *Barbara JA, Regan FA, Contreras MC,* editors. Transfusion Microbiology. New York: Cambridge University Press; 2008. p. 75–86.
11. *Honarvar B, Odoomi N, Moghadami M, Kazerooni PA, Hassanabadi A, Dolatabadi PZ,* et al. Blood-Borne Hepatitis in Opiate Users in Iran: A Poor Outlook and Urgent Need to Change Nationwide Screening Policy. PLoS One 2013; 8(12): e82230.
12. *Zamania S, Radfarb R, Nematollahic P, Fadaiec R, Meshkatic M, Mortazavia S,* et al. Prevalence of HIV/HCV/HBV infections and drug-related risk behaviours amongst IDUs recruited through peer-driven sampling in Iran. Int J Drug Policy 2010; 21(6): 493–500.
13. *Camoni L, Regine V, Salfa MC, Nicoletti G, Canuzzi P, Magliocchetti N,* et al. Continued high prevalence of HIV, HBV and HCV among injecting and noninjecting drug users in Italy. Ann Ist Super Sanità 2010; 46(1): 59–65.
14. *Goulao J, Götz W*. European Monitoring Centre for Drugs and Drug Addiction. Annual Report 2012: The state of the drug problem in Europe. Lisbon: EMCDDA; 2012.
15. *Amon JJ*. Hepatitis in drug users: time for attention, time for action. Lancet 2011; 378 (9791): 543–4.
16. *Balint B, Vucetic D, Todorovic-Balint M, Borovcanin N, Jovanovic-Cupic S, Mandusic V*. Safety improving by complementary serological and molecular testing combined with pathogen reduction of the donated blood in window period (Letter). Transfus Apher Sci 2013; 49(1): 103–4.
17. *Busch M, Glynn S, Stramer S, Orland J, Murphy E, Wright D*, et al. Correlates of hepatitis C virus (HCV) RNA negativity among HCV- seropositive blood donors. Transfusion 2006; 46(3): 469–75.
18. *Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP*. Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. Transfusion 2004; 44(9): 1332–9.

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Characteristics of gastric and duodenal mucosa in the patients with primary biliary cholangitis

Karakteristike mukoze želuca i duodenuma kod bolesnika sa primarnim bilijarnim holangitisom

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Abstract

Background/Aim. Primary biliary cholangitis (PBC) is an immune-mediated chronic cholestatic disease of liver, with a slow progression. The aim of our study was to determine the correlation of PBC, atrophic gastritis (AG) and gluten-sensitive enteropathy (GSE), to identify the macroscopic and histopathological modifications of gastric and duodenal mucosa which occur in PBC and to analyze the frequency of these changes compared to a control group. **Methods.** This study included 50 patients with PBC and 46 control subjects with the dyspeptic symptoms, without liver disease. All of the examined subjects underwent esophagogastroduodenoscopy. Macroscopic and histopathological findings of the gastric and duodenal mucosal samples were recorded and analyzed. **Results.** There was no statistically significant association between the PBC and AG, or between the PBC and *Helicobacter pylori* infection. There was a highly significant difference in the frequency of *Helicobacter pylori* infection and the presence of GSE in the patients in the control group compared to those with PBC. **Conclusions.** The patients with PBC are at a lower risk for *Helicobacter pylori* infectionand atrophic gastritis. Testing for GSE in the PBC patients may be beneficial, considering the higher incidence of GSE amongst these patients. GSE represents a risk factor for the presence of PBC and the patients with GSE are nearly four times more likely to have PBC.

Key words:
liver cirrhosis, biliary; gastritis, atrophic; glutens; celiac disease; comorbidity; histology; helicobacter pylori.

Apstrakt

Uvod/Cilj. Primarni bilijarni holangitis (PBC) je imunski posredovana hronična holestatska bolest jetre sa sporom progresijom. Cilj našeg istraživanja bio je da se utvrdi korelacija između PBC, atrofičnog gastritisa (AG) i gluten-senzitivne enteropatije (GSE), da se identifikuju makroskopske i histopatološke promene mukoze želuca i duodenuma kod PBC i analizira učestalost ovih promena u poređenju sa kontrolnom grupom. **Metode.** U studiju je bilo uključeno 50 bolesnika sa PBC i 46 kontrolnih bolesnika sa dispeptičnim tegobama, bez bolesti jetre. Svi ispitanici su bili podvrgnuti ezofagogastroduodenoskopiji. Makroskopski i histopatološki nalaz i uzorak mukoze želuca i duodenuma su snimljeni i analizirani. **Rezultati.** Nije registrovana statistički iznačajna povezanost između PBC i AG, između PBC i *Helicobacter pylori* infekcije. Uočena je visokostatistički značajna razlika u učestalosti *Helicobacter pylori* infekcije i postojanja GSE kod bolesnika u kontrolnoj grupi u odnosu na one sa PBC. **Zaključak.** Bolesnici sa PBC imaju manji rizik za *Helicobacter pylori* infekciju i AG. Testiranje za GSE kod PBC bolesnika može biti korisno, s obzirom na veću učestalost GSE među ovim bolesnicima. GSE predstavlja faktor rizika od prisustva PBC i bolesnici sa GSE imaju skoro četiri puta veću predispoziciju za PBC.

Ključne reči:
jetra, bilijarna ciroza; gastritis, atrofijski; gluten; celijakija; komorbiditet; histologija; helicobacter pylori.

Introduction

Primary biliary cholangitis (PBC) is an immune-mediated chronic cholestatic disease of the liver, with a slow progression. Biochemical analysis classicaly demonstrates persistently higher levels of alkaline phosphatase and gamma-glutamyltransferase [1]. Immunological analysis usually shows the presence of anti-mitochondrial antibodies (AMA), while anti-nuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA) are present in approximately one third of all

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PBC patients. Diagnosis is confirmed by histopathology acquired through liver biopsy. Although a number of studies have suggested immunological, genetic, infective and ecological factors, the exact mechanisms of the etiopathogenesis of PBC are not fully understood. Autoimmune features of PBC (infiltration of biliary epithelial cells with Th1 lymphocytes; expression of adhesion molecules (ICAM1, VCAM1, MHC molecules, IL2 receptors, TNF alpha, IFN gamma), including humoral and cellular immunity disorders, indicate potential overlap with other autoimmune diseases including the Sjörgen's syndrome, scleroderma, hypothyroidism and celiac disease [2–4].

Gluten sensitive enteropathy (GSE), also known as celiac disease, is a chronic disease typically affecting the proximal small intestine of genetically predisposed individuals with an inadequate immune response to gluten and similar proteins found in oat, rye and barley. GSE is diagnosed based on the histopathological findings of biopsy of the duodenal and/or jejunal mucosa, and by determining the serum levels of anti-gliadin, anti-endomysial (AEMA) and anti-transglutaminase antibodies [3]. According to the Marsh classification, there are five stages of the disease [5]. Celiac disease-associated autoimmune diseases of the other organ-systems have already been described in the literature (liver, kidneys, skin, cardiovascular, nervous, endocrine and reproductive system) [6]. The best documented are GSE-associated autoimmune diseases of the liver: PBC (with incidence 3 to 7%), autoimmune hepatitis (3% to 6%), and primary sclerosing cholangitis (2% to 3%) [4, 6].

According to the data obtained from the available literature, there is significantly less evidence about the overlap of atrophic gastritis (AG) and PBC. Two forms of chronic AG were described: type A autoimmune gastritis, with the presence of anti-parietal autoantibodies (APA) and type B gastritis associated with persistent *Helicobacter pylori* (*H. pylori*) infection. These two types have different etiologies, topographic distributions and histopathological features [7, 8]. The association between type A, AG and autoimmune hepatic diseases remains controversial and, in the literature, small number of data examining the relationship between *H. pylori* gastritis and PBC is presented [7].

The group of autoimmune "overlap" syndromes includes syndromes which contain characteristics of at least two diseases.

The aim of this study was to investigate the correlations between PBC and AG, and GSE, to determine the difference in the presence of atrophic gastritis and GSE in the PBC patients in relation to the controls and finally, to identify other macroscopic and histopathological changes of gastric and duodenal mucosa present in the patients with PBC (*H. pylori* infection).

Methods

This retrospective study included 50 patients with PBC, treated at the tertiary health center in Serbia, from 2009 to 2013. The control group consisted of 46 persons who were examined because of dyspeptic complaints.This study was approved by the Ethics Committee of our hospital. All patients gave informed written consent prior to participation in this study.

The recorded demographic data (age, gender) were analyzed. The diagnosis of PBC was based on laboratory and immunological analysis as well as the histopathological findings of liver biopsy, performed wherever possible. Exclusion criteria were blood coagulation disorders [international normalized ratio (INR) > 1.5] and the presence of ascites.

A complete immunological work-up was performed, which included ANA IgG in rodent tissue, ANA HEp2 in human cells, AMA, ASMA, APA, and AEMA, using the immunofluorescence technique. Titres more than 1 : 80 were considered as clinically significant. The titre of anti-transglutaminase antibodies (TGA) was determined using ELISA, and expressed in U/mL. Where possible, percutaneous biopsy of the right lobe of the liver was performed and the liver tissue samples were sent for histopathological analysis. According to the Scheuer's classification, a histopathological stage of PBC was expressed in four categories (Table 1) [9]. In our study, the PBC patients were categorized into two groups: the patients in the early stage of the disease (mild and moderate fibrosis) and the patients with advanced liver disease (severe liver fibrosis and cirrhosis). As a part of the routine diagnostic algorithm, esophagogastroduodenoscopy was performed.

Table 1

Scheuer's classification for grading and staging of chronic hepatitis

Scheuer's classification	Portal/perioral activity	Lobular activity
Grade		
0	None	None
1	Portal inflammation	Inflammation, no necrosis
2	Mild piecemeal necrosis	Focal necrosis /acidophil bodies
3	Moderate piecemeal necrosis	Severe focal cell damage
4	Severe piecemeal necrosis	Damage with bridging necrosis
Stage		Fibrosis
0	None	
1	Enlarged, fibrotic portal tracts	
2	Perioral or portal-portal septa, but intact architecture	
3	Fibrosis with architectural distortion, no obvious cirrhosis	
4	Probable or definite cirrhosis	

The macroscopic findings of endoscopy were recorded and analyzed, while biopsy samples of duodenal and gastric mucosa were taken and sent for processing. A record was taken of the following: the presence and degree of atrophy (graded in three stages, according to the Sydney-Houston classification); *H. pylori* status (graded in three stages according to the degree of colonization); the presence and degree of GSE according to the Marsh classification (Table 2) [8, 10, 5]. In the control group, a record of demographic data and gastrointestinal medical history was taken. Esophagogastroduodenoscopy was performed with biopsies of the duodenal and gastric mucosa, using the same criteria as in the study group.

Table 2

Modified Marsh classification of gluten-sensitive enteropathy (GSE)

Marsh type	Intraepithelial lymphocytes *per* 100 enterocytes	Crypts	Villi
0	< 40	Normal	Normal
1	> 40	Normal	Normal
2	> 40	Increased	Normal
3a	> 40	Increased	Mild atrophy
3b	> 40	Increased	Marked atrophy
3c	> 40	Increased	Absent

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, Version 20.0 (IBM Corp., Armonk, New York, USA). Categorical variables were shown by frequencies and relative numbers (percentages). Basic descriptive statistics included means, standard deviations, ranges and percentages. The χ^2-test was used to verify significant differences in the frequency of *H. pylori* infection, mucosal atrophy and GSE in the PBC and control groups and it was followed by the logistic regression analysis – univariante model including the factors marked as significant by the χ^2-test. The values less than 0.05 for the type 1 statistical error (alpha) were considered being statistically significant.

Results

The total number of patients diagnosed with PBC was 50, with the mean age of 56 ± 10 years (age range 29–79 years). The control group consisted of 46 patients, mean age 60 ± 13 years (age range 36–84 years). In the study group, 98% of patients were females and 2% were males, while the gender distribution among controls was: 47.8% of females and 52.2% of males.

All patients within the study group were tested for the presence of AMA in the serum, with positive results in 76% of the cases. No statistically significant difference in the presence of AMA was found between the group of patients with the early-stage, and those with the advanced stage of the disease ($p > 0.05$). ANAs were positive in 22.5% of the PBC patients. In cases where it was possible, the patients were also tested for the presence of APA, which were positive in 7.1% of the patients with PBC, and AEMA, which were positive in 12.5% of the patients with PBC.

A biopsy of the liver was performed in most of the patients in the study group (47 of 50 patients): 60% of the subjects had mild, 2% had moderate and 16% had severe liver fibrosis, while cirrhosis was confirmed in 22% of the patients. The patients were divided into two groups based on a stage: 61.7% of the patients with the early-stage and 38.8% with the advanced disease of the liver.

The correlation between the PBC/PBC stage and gastric mucosal atrophy, *H. pylori* infection, and GSE was analyzed, and no statistical significance was found (Tables 3 and 4).

Table 3

The association between PBC, AG and *H. pylori*

Parameters	Present in PBC patients (%)	*p*
AG	40	0.475
H. pylori	20	0.916

PBC – primary biliary cholangitis; AG – atrophic gastritis; *H. pylori – Helicobacter pylori*.

Table 4

The association between stage of PBC, AG, *H. pylori* and GSE

Parameters	PBC gr I and gr II (%)	PBC gr III and IV (%)	*p*
AG	50	50	0.531
H. pylori	55.60	44.40	1.000
GSE	42.90	57.10	0.680

PBC – primary biliary cholangitis; AG – atrophic gastritis; *H. pylori – Helicobacter pylori*; GSE – gluten-sensitive enteropathy.

In our study, we have analyzed the frequency of AG, *H. pylori* gastritis and GSE among the patients in the study group and the subjects in the control group. We found no statistically significant difference in presence of AG between these two groups. Further, we found a highly significant statistical difference in presence of *H. pylori* infection in the control group compared to the PBC group and the frequency of GSE in the patients with PBC (Table 5).

Table 5

The difference in presence/absence of the GA, *H. pylori* infection and GSE between the study group and the control group

Parameters	PBC (%)	Control group (%)	*p* value
AG	40	50	$p = 0.338$
H. pylori	20	47.8	$p = 0.005$
GSE	20.9	6.5	$p = 0.047$

PBC – primary biliary cholangitis; AG – atrophic gastritis; *H. pylori – Helicobacter pylori*; GSE – gluten-sensitive enteropathy.

Table 6

Logistic regression analysis for *H. pylori and* GSE

	Parameter	B	S.E.	Wald	df	Sig	EXP(B)	95% CI EXP (B)	
								lower	upper
Step 1a	*H. pylori*	1.299	0.475	7.469	1	0.006	0.273	0.107	0.692
	Constant	0.405	0.264	2.367	1	0.124	0.124		
	GSE	1.333	0.705	3.577	1	0.059	3.794	0.953	15.11
	Constant	0.235	0.229	1.047	1	0.306	0.791		

***H. pylori* – *Helicobacter pylori*; GSE – gluten sensitive enteropathy; Sig – significance; CI – confidence interval.**

Applying the univariante logistic regression, it was proven that the presence of *H. pylori* infection was a significant protective factor and that individuals with *H. pylori* infection were less likely to have PBC [odds ration (OR) = 0.27; 95% confidence interval (CI) = 0.11–0.69; $p < 0.01$]. The presence of GSE, however, was shown to be a risk factor for the presence of PBC, and the patients with GSE were almost four times more likely to have PBC (OR = 3.79; 95% CI = 0.95–15.11; $p = 0.059$) (Table 6).

Discussion

This study was conducted in order to find out more about connection of autoimmune disease of the liver, duodenum and gaster. In our study, the study group consisted of patients with PBC. Most of the patients were females, with the mean age of 56. The results obtained in our study matched findings from available literature. The presented studies suggested that PBC has a female predominance and in some studies it is suggested that female-to-male ratio is about 8 : 1 [11]. Also, most of the patients with PBC are over 40 years of age [1].

Regarding the immunological analysis, most of the PBC patients in our study had the AMA positive disease. Our findings are in compliance with the results from available literature. Sakauchi et al. [11] conducted a cross-sectional study of PBC in Japan and included 5,805 patients. Among them, 86.6% had AMA. Joshita et al. [12] reported that a great majority of patients in their study (369 of 395, 93.4%) had positive AMA.

The data on the correlation between the GSE and PBC is rather controversial. Results of our study revealed that GSE was present in 20.9% of the patients with PBC, with statistically significant difference to the control group (6.5% of the subjects with GSE in the control group; $p = 0.047$). Further analysis showed that GSE was a risk factor for presence of PBC, meaning that the GSE patients had more than four times risk of having PBC.

Some studies did not suggest that GSE occurs frequently with the liver diseases [6, 13–15]. However, studies which included a larger number of patients indicated the positive correlation between the PBC and GSE. Lawson et al. [15] conducted study that included 4,732 patients diagnosed with GSE and 23,620 control subjects within the General Medical Services [16]. The results from this study suggest that the patients with PBC are three times more likely to develop GSE than the general population. Further, a large study conducted in Sweden and Denmark, including the patients diagnosed with GSE from the National Registers, indicates a higher risk for developing PBC in the patients with GSE [16].

It is suggested within the present literature, that the prevalence of AG is up to 10.9%, annually, while about 50% of the world population is infected with *H. pylori* [17–19]. It was challenging task to try to find out more about correlation between PBC and gastritis, both atrophic and *H. pylori*, because there is a small number of information related to this topic within the presented literature and the results are rather controversial. The results from our study did not show a statistically significant difference in the presence of AG in the patients with PBC compared to the control group. Furthermore, we found that *H. pylori* infection occurs less frequently in the patients with PBC than in the control group, with a statistically significant difference. The results showed in the studies from present literature provided similar correlation [18, 20].

On the other hand, a positive correlation of PBC and pernicious anemia was found mostly as case reports.The relationship between PBC and pernicious anemia was first described by Renoux et al. [21] in 1980. Chung et al. [22] presented the case of a 46-year-old woman who, three years after being diagnosed with PBC, was also diagnosed with pernicious anemia. Abenavoli et al. [23] presented the case of a 36-year-old woman diagnosed with GSE, PBC and *H. pylori* infection.

In addition to the case reports presented in the available literature, there was also a large prospective study including 289 patients divided into three groups (control group, patients with cirrhosis and patients with portal hypertension without cirrhosis). The patients were tested for the presence of gastric mucosal atrophy, metaplasia, dysplasia and the presence of *H. pylori* infection. The results obtained in this study indicated that the frequency of gastric mucosal atrophy was higher in the group of patients with cirrhosis than in the control group [24].

Conclusion

Our study showed no statistically significant difference in the presence of gastric mucosal atrophy between the PBC patients and controls. A highly significant statistical difference in the presence of *H. pylori* infection in the control group compared to the PBC group and the frequency of GSE in the patients with PBC was also confirmed. The analysis of the results obtained in our study showed that GSE represented a risk factor for the presence of PBC, meaning that the

individuals with GSE have almost four times more chance to develop PBC. This indicates that the testing of the PBC patients for the presence of GSE would be prudent so as to ensure proper treatment. Additionally, for those with GSE, it is necessary to exclude the presence of PBC within the "overlap" syndrome. Based on the results of our study, it may be concluded that the patients with PBC, are at a lower risk for *H. pylori* infection. Future studies are expected to identify the complex pathogenetic mechanisms of this phenomenon.

REFERENCES

1. *Hohenester S, Oude-Elferink RP, Beuers U*. Primary biliary cirrhosis. Semin Immunopathol 2009; 31(3): 283–307.
2. *Watt FE, James OF, Jones DE*. Patterns of autoimmunity in primary biliary cirrhosis patients and their families: A population-based cohort study. QJM 2004; 97(7): 397–406.
3. *Jones DE, Donaldson PT*. Genetic factors in the pathogenesis of primary biliary cirrhosis. Clin Liver Dis 2003; 7(4): 841–64.
4. *Drastich P, Honsová E, Lodererová A, Jarešová M, Pekáriková A, Hoffmanová I*, et al. Celiac disease markers in patients with liver diseases: A single center large scale screening study. World J Gastroenterol 2012; 18(43): 6255–62.
5. *Marsh MN*. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiac sprue'). Gastroenterology 1992; 102(1): 330–54.
6. *Mirzaagha F, Azali SH, Islami F, Zamani F, Khalilipour E, Khatibian M*, et al. Coeliac disease in autoimmune liver disease: A cross-sectional study and a systematic review. Dig Liver Dis 2010; 42(9): 620–3.
7. *Antico A, Tampoia M, Villalta D, Tonutti E, Tozzoli R, Bizzaro N*. Clinical usefulness of the serological gastric biopsy for the diagnosis of chronic autoimmune gastritis. Clin Dev Immunol 2012; 2012: 520970.
8. *Dixon MF, Genta RM, Yardley JH, Correa P*. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J SurgPathol 1996; 20(10): 1161–81.
9. *Scheuer PJ*. Primary biliary cirrhosis: diagnosis, pathology and pathogenesis. Postgrad Med J 1983; 59 Suppl 4: 106–15.
10. *Price AB*. The Sydney system: Histological division. J Gastroenterol Hepatol 1991; 6(3): 209–22.
11. *Sakauchi F, Mori M, Zeniya M, Toda G*. A cross-sectional study of primary biliary cirrhosis in Japan: Utilization of clinical data when patients applied to receive public financial aid. J Epidemiol 2005; 15(1): 24–8.
12. *Joshita S, Umemura T, Nakamura M, Katsuyama Y, Shibata S, Kimura T,* et al. STAT4 gene polymorphisms are associated with susceptibility and ANA status in primary biliary cirrhosis. Dis Markers 2014; 2014: 727393.
13. *Bardella MT, Quatrini M, Zuin M, Podda M, Cesarini L, Velio P,* et al. Screening patients with celiac disease for primary biliary cirrhosis and vice versa. Am J Gastroenterol 1997; 92(9): 1524–6.
14. *Chatzicostas C, Roussomoustakaki M, Drygiannakis D, Niniraki M, Tzardi M, Koulentaki M,* et al. Primary biliary cirrhosis and autoimmune cholangitis are not associated with coeliac disease in Crete. BMC Gastroenterol 2002; 2: 5.
15. *Lawson A, West J, Aithal GP, Logan RF*. Autoimmune cholestatic liver disease in people with coeliac disease: a population-based study of their association. Aliment Pharmacol Ther 2005; 21(4): 401–5.
16. *Sorensen HT, Thulstrup AM, Blomqvist P, Nørgaard B, Fonager K, Ekbom A*. Risk of primary biliary liver cirrhosis in patients with coeliac disease: Danish and Swedish cohort data. Gut 1999; 44(5): 736–8.
17. *Huang J, Cui J*. Evaluation of Helicobacter pylori Infection in Patients with Chronic Hepatic Disease. Chin Med J 2017; 130(2): 149–54.
18. *Dohmen K, Shigematsu H, Miyamoto Y, Yamasaki F, Irie K, Ishibashi H*. Digestive diseases and sciences 2002; 47(1): 162–9.
19. *Brown ML*. Helicobacter pylori: Epidemiology and Routes of Transmission. Epidemiol Rev 2000; 22(2): 283–97.
20. *Floreani A, Biagini MR, Zappalà F, Farinati F, Plebani M, Rugge M,* et al. Chronic atrophic gastritis and Helicobacter pylori infection in primary biliary cirrhosis: a cross-sectional study with matching. Ital J Gastroenterol Hepatol 1997; 29(1): 13–7.
21. *Renoux M, Beaugrand M, Lévy VG, Bernard JF, Boivin P*. Primary biliary cirrhosis andperniciousanemia. A fortuitous association. Gastroenterol Clin Biol 1980; 4(2): 109–13.
22. *Chung CS, Hsu YC, Huang SY, Jeng YM, Chen CH*. Primary biliary cirrhosis associated with pernicious anemia. Can Fam Physician 2010; 56(9): 889–91.
23. *Abenavoli L, Arena V, Giancotti F, Vecchio FM, Abenavoli S*. Celiac disease, primary biliary cirrhosis and helicobacter pylori infection: one link for three diseases. Int J Immunopathol Pharmacol 2010; 23(4): 1261–5.
24. *Ibrişim D, Cevikbaş U, Akyüz F, Poturoğlu S, Ahishali E, Güllüoğlu M,* et al. Intestinal metaplasia in portal hypertensive gastropathy: a frequent pathology. Eur J Gastroenterol Hepatol 2008; 20(9): 874–80.

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The impact of socioeconomic factors on quality of life and functional impairment in patients treated for oropharyngeal carcinoma

Uticaj socioekonomskih faktora na kvalitet života i funkcionalno oštećenje bolesnika lečenih od orofaringealnog karcinoma

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Abstract

Backround/Aim. Considering the distinct increase in the incidence of oropharyngeal cancer over oral cavity cancers and changing epidemiology with human papilloma virus (HPV) infection emerging as an important risk factor, there is a need to establish better treatment choices in specific groups of patients with oropharyngeal cancer. The aim of this study was to assess the quality of life (QOL) and functional performance and the impact of different demographical data, stage of disease, and treatment type on these parameters in patients with oropharyngeal cancer with successfully achieved locoregional control a year after the treatment. **Methods.** Study included 87 patients who underwent QOL and functional impairment assessment 12 to 14 months after finished oncological treatment with the following questionnaires: the European Organization for Research and Treatment of Cancer Quality-of Life-Questionnaire-C30 (EORTC QLQ-C30), European Organization for Research and Treatment of Cancer Quality of- Life Questionnaire-Head and Neck 35 (EORTC QLQ-H&N35) and The Karnofsky Performance Scale (KPS). **Results.** Specific groups of patients had significantly different post-treatment QOL scores. The factors associated with the worse QOL scores were female gender, not being in a partnership, level of education and HPV status. **Conclusion.** Clinicians should consider socioeconomic factors and HPV status in planning the recovery after treatment of patients with oropharyngeal carcinoma. Gender, education level and employment are the variables that form a certain risk profiles associated with the lower QOL.

Key words:
papillomaviridae; socioeconomic factors; pharyngeal neoplasms; quality of life; treatment outcome.

Apstrakt

Uvod/Cilj. Incidencija orofaringealnih karcinoma se povećavala tokom poslednje decenije, a epidemiologija promenila sa pojavom humanog papiloma virusa (HPV) kao bitnog faktora rizika od ovih karcinoma. Potrebno je ustanoviti bolje terapeutske izbore za specifične grupe bolesnika koji se leče od orofaringealnog karcinoma. Cilj ove studije bio je da se procene kvalitet života i funkcionalne performanse, kao i uticaj različitih demografskih faktora, stadijuma bolesti i tipa terapija na te parametre kod bolesnika sa orofaringealnim karcinomom kod kojih je postignuta uspešna lokoregionalna kontrola, godinu dana posle sprovedene terapije. **Metode.** Studija je uključila 87 bolesnika koji su odgovorili na upitnike o kvalitetu života i funkcionalnim performansama: *European Organization for Research and Treatment of Cancer Quality-of Life-Questionnaire*-C30 – EORTC QLQ-C30), *European Organization for Research and Treatment of Cancer Quality of- Life Questionnaire-Head and Neck* 35 (EORTC QLQ-H&N35) i *Karnofsky Performance Scale* (KPS), 12 do 14 meseci posle završenog onkološkog tretmana. **Rezultati.** Specifične grupe bolesnika značajno su se razlikovale u skorovima na upitnicima za kvalitet života posle lečenja. Faktori koji su bili povezani sa slabijim rezultatima su bili ženski pol, život bez partnera, nivo obrazovanja, zaposlenost i HPV status. **Zaključak.** Kliničari bi trebali da uzmu u obzir socioekonomske faktore i HPV status u planiranju postoperativnog

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oporavka kod bolesnika lečenih od orofaringealnog karcinoma. Pol bolesnika, nivo obrazovanja i zaposlenost su faktori koji nose određen nivo rizika koji je povezan sa nižim nivoom kvaliteta života kod ovih bolesnika.

Ključne reči:
papillomaviridae; socijalno-ekonomski faktori; farinks neoplazme; kvalitet života; lečenje, ishod.

Introduction

It is estimated that oropharyngeal cancer makes up to 3% of all newly diagnosed carcinomas, with majority of cases occurring in developing countries [1, 2]. Although common risk factors are preventable and most of the cases are easily diagnosed by a standard oral exam, due to a huge lack of awareness, disease is usually detected in the advance stages [3].

In the past decade, patient's quality of life (QOL) and functioning after the treatment became an important additional tool for assessing the treatment outcome of oral cavity and oropharyngeal cancer [4]. A number of recent studies assessed quality of life in patients with both entities combined, but it should be considered that oropharynx and oral cavity are two different anatomical sites, each with its own specific anatomy and functions. Oropharyngeal region includes following sub-sites: base of tongue, tonsil, and oropharynx, opposing to oral cavity region which includes lip, oral tongue, floor of mouth and gums, palate or other sections of the mouth. This distinctions became more important in light of the new patterns noticed in etiology and incidence trends. First, there is a distinct increase in the incidence of oropharyngeal cancer with the decrease in the incidence of oral cavity cancers [5, 6]. In the United States, tonsillar cancer showed to be most frequent diagnosed oropharyngeal cancer. Second most frequent diagnosed site was base of the tongue. Both sites showed increasing incidence during a period from 2000 to 2010 comparing to the trends for other anatomic sites of the oral cavity and oropharynx.[6] Secondly, a shift in age of diagnosis has happened, making 6th and 7th decade of life high risk period for oropharyngeal cancer compared to oral cavity cancer [6, 7]. Thirdly, epidemiology of oropharyngeal cancer changed, with risk factors like smoking and alcohol replaced with human papilloma virus (HPV) infection. Oropharyngeal cancer caused by HPV occurs in different population to that commonly associated with head and neck cancers, with significantly better prognosis than the HPV negative cancers [8]. These trends are forcing us to further narrow our focus on better treatment choices for oropharyngeal cancer and post-treatment quality of life in specific groups of patients. The patients with oropharyngeal cancer confront the substantial QOL issues after successful cancer management [9]. Depending on the sociodemographic characteristics, choice of the treatment and stage of the disease, going back to regular diet, performing usual everyday tasks and professional duties require a significant effort in these patients.

The aim of this study was to assess the impact of different demographic data, HPV status, stage of disease, and treatment type on QOL and functional performance in the patients with oropharyngeal cancer with successfully achieved locoregional control a year after the treatment.

Methods

This cross-sectional study included 87 patients diagnosed with carcinoma of the oropharynx in the Clinic for Otorhinolaryngology and Maxillofacial Surgery of the Clinical Centre of Serbia in Belgrade in one-year period (from January 2009 to January 2010). This study was approved by the Institutional Ethics Committee (440/IX-3/09), and all patients signed informed consent form prior to their inclusion into the study. The patients were treated in the period from undergoing necessary diagnostic procedures (clinical exam, tumor biopsy and histopathology verification, radiological diagnostics). The modality of treatment for every patient was decided on the Oncological Board (consisting of radiotherapist, head and neck surgeons, oncologist and histopathologist). The HPV positivity was confirmed with HPV16 *in situ* hybridisation and the positive p16 immunohistochemical staining of the tissue samples [10, 11]. The surgical therapy involved resection of the tumor (local resection or hemiglossectomy) with some form of neck dissection in case of cervical lymphadenopathy. Radiotherapy consisted of external radiotherapy with a total dose of 60 to 70 Gy in 30–35 fractions for 6–7 weeks. The patients received chemotherapy concurrently with radiotherapy; three courses of cisplatin (CDDP) intravenously, on 1st, 4th and 7th week of radiotherapy. In the patients who were disease-free, QOL and functional impairment assessment was conducted 12 to 14 months after finished oncological treatment. The patients with recurrent disease were excluded from the study.

For assessing the QOL, two types of questionnaires were used: the European Organization for Research and Treatment of Cancer Quality - of Life - Questionnaire-C30 (EORTC QLQ-C30) and the European Organization for Research and Treatment of Cancer Quality of- Life Questionnaire - Head and Neck 35 (EORTC QLQ-H&N35) [12]. The questionnaires were translated into Serbian. The EORTC QLQ-C30 is a cancer-specific questionnaire, divided into 5 functioning scales (physical, role, emotional, cognitive and social), 3 symptom scales (fatigue, nausea/emesis and pain), 6 single items (dyspnea, insomnia, appetite loss, constipation, diarrhea and financial impact) and one global health and QOL scale. The scores were given as a 0–100 scale. The higher scores for the global QOL scale and for a functional scale indicated a higher level of functioning, and the higher scores for a symptom scale or a single-item scale indicated more severe symptoms and worse QOL. The EORTC-H&N35 is a site-specific questionnaire designed to assess QOL in the head and neck cancer patients made of 7 symptom scales (pain, swallowing, sense, speech, social eating, social contact and sexuality) and 11 single items associated with the location, symptoms of the disease and treatment

(teeth problems, mouth opening, dry mouth, sticky saliva, coughing, feeling ill, painkiller intake, nutritional supplements, feeding tube, weight loss and weight gain). The highest scores represented the highest level of symptoms. The scores were interpreted into the scoring guidelines established by the EORTC manuals. The Karnofsky Performance Scale (KPS) Index was used to classify the patients' functional impairment. The scores range from 0 to 100; the higher score, the patient is more able to carry out daily activities [13]. The differences in EORTC QLQ-C30, EORTC QLQ-H&N35 and KPS Index scores were compared depending on age, gender, place of living, level of education, living arrangement/marital status, employment position, HPV status, the American Joint Committee on Cancer (AJCC) stage of the disease and treatment choices of the patients.

Statistical analysis was performed using the SPSS v20 (SPSS Inc., Chicago, IL). To determine differences between examined groups of patients, depending on the investigated parameters, the *t*-test and ANOVA were used. The Pearson's correlation test was used to determine the correlation between EORTC QLQ-C30, EORTC QLQ-H&N35 and KPS Index scores and other parameters. The *P*-value less than 0.05 was considered statistically significant.

Results

The study included 87 patients (69 males and 18 females) of an average age of 59.6, years. The patients were diagnosed and treated for oropharyngeal carcinoma between October 2009 and October 2011 in the Clinic for Otorhinolaryngology and Maxillofacial Surgery of the Clinical Centre of Serbia in Belgrade. Basic demographic characteristics of the patients were given in Table 1. The patients are predominantly male, living in urban areas, in partnerships or married, laborers with secondary high school education. Out of all patients included in the study, 39 (44.8%) were HPV positive. Most of the patients (47.1%) were diagnosed with stage IV oropharyngeal cancer. The treatment modalities differed; most of the patients were treated operatively with postoperative radiotherapy (31%) or with radio/chemotherapy (31%).

The mean value and standard deviation of EORTC QLQ-C30, EORTC QLQ-H&N35 and KPS Index scores are given in Tables 2 and 3. Regarding EORTC QLQ-C30 and KPS Index, women had significantly worse physical, emotional, cognitive and social functioning, and felt more fatigued, had more frequent dyspnea, insomnia, and appetite loss than men (Table 4). Emotional and cognitive functioning was significantly worse in the patients who were single ($p = 0.048$ and $p = 0.046$ respectively), than in those living in marriage or partnership. There was significantly higher global quality of life in the patients with higher education (faculty and PhD) ($p = 0.039$). The unemployed suffer more from insomnia that the patients working in managerial positions ($p = 0.046$). The HPV positive patients were complaining significantly less of pain and dyspnea comparing to the HPV negative patients ($p = 0.024$ and $p = 0.043$ respectively). Physical functioning was significantly better in the patients in the stage I of the disease comparing to the patients in the stages III and IV of the disease ($p = 0.2$ and $p = 0.008$ respectively). Social functioning was significantly better in the patients who underwent surgery comparing to the patients who underwent radio/chemotherapy and the patients who underwent surgery with radio/chemotherapy ($p = 0.033$ and $p = 0.025$ respectively). In the EORTC QLQ-H&N35 questionnaire, the women had significantly higher scores than the men regarding senses, contact, sexuality and felling ill ($p < 0.05$). The patients living in a partnership or in marriage had significantly less complaints about their sexual life ($p = 0.008$), felt less ill ($p = 0.049$) and used less painkillers ($p = 0.006$) than the patients who were single. The patients with the stage I carcinoma complained about the senses problem significantly less than the patients with the stages III and IV of carcinomas ($p = 0.221$ and $p = 0.25$ respectively). The patients treated with radio/chemotherapy felt significantly more pain than those treated operatively with postoperative radio/chemotherapy ($p = 0.017$).

Table 1

Demographic characteristics of the patients included in the study

Characteristics	n (%)
Gender	
male	69 (79.3)
female	18 (20.7)
Place of living	
urban	64 (73.5)
rural	23 (26.5)
Living arrangement	
single	27 (31)
in a partnership/married	60 (69)
Level of education	
no formal education/elementary school	33 (37.9)
secondary/high school	40 (45.9)
faculty/PHD	14 (16.2)
Employment position	
laborer	48 (55.2)
technical worker (sales, production,	10 (11.5)
maintenance, operation)	5 (5.7)
administrative worker	15 (17.2)
manager (education, health, business)	9 (10.4)
unemployed	
HPV status	
positive	39 (44.8)
negative	48 (55.2)
AJCC Stage	
I	11 (12.7)
II	9 (10.3)
III	26 (29.9)
IV	41 (47.1)
Treatment modality	
OP	9 (10.3)
RT	8 (9.2)
OP + RT	27 (31)
RT + CT,	27 (31)
OP + RT + CT	16 (18.5)

OP – operation; RT – radiotherapy; CT – chemotherapy; HPV – human papilloma virus; AJCC – American Joint Committee on Cancer.

Table 2

EORTC QLQ-C30 and KPS Index scores depending on the investigated parameters (all values are expressed as mean value ± standard deviation)

Characteristics	Global quality of life	Physical functioning	Role functioning	Emotional functioning	Cognitive functioning	Social functioning	Fatigue	Nausea	Pain	Dyspnea	Insomnia	Appetite loss	Constipation	Diarrhea	Financial difficulty	KPS Index
Gender																
male	59.8±22.9	80.4±17.7	75.8±29.9	77±22.7	84.8±21.7	83.6±25.5	22.9±23	5.8±13	26.3±24.9	11.6±21.5	20.8±30.8	14.5±27.1	7.7±20.7	6.2±20	33.8±39.8	80.4±10.5
female	56±31.7	68.1±24	65.7±35.4	52.3±35.2	65.7±27.1	67.6±32.1	45±28.9	12±12.5	27.8±29.1	29.6±34.1	50±36.6	35.2±38.7	22.2±37.9	5.6±17.1	44.4±37.9	79.4±5.4
Place of living																
urban	57.3±25.1	77.8±20.9	73.5±30.8	69.5±29.8	78.8±25.6	78.8±26.9	29.6±27.4	8.7±14.6	28.6±25.8	18.5±25.9	30.7±36.1	21.6±32.3	10.6±25.3	5.8±18.5	37.6±40.8	79.7±10.6
rural	64.4±24.7	76.9±16.6	71.9±33.5	78.8±19.9	85.6±19.4	82.6±28.1	21.7±21	3±6.6	21.9±26.4	6±22.1	16.7±26.7	12.1±26.3	12.1±28.2	7.6±22.8	33.3±37.1	81.4±6.4
Living arrangement																
single	52.5±28.7	74.3±17.4	66.7±38.9	62±32.8	72.2±28.1	75.3±33.1	34.6±28.1	8±12.5	29.6±23.7	14.8±26.7	35.8±40.2	25.9±33.7	13.6±31	8.6±23.7	46.9±38.4	78.9±13.1
in a partnership/married	61.9±22.6	79.4±20.5	76.9±26.8	76.4±23.7	84.7±21.1	82.5±24.6	24.2±24.2	6.7±13.4	25.3±26.7	15.6±24.9	22.8±30.4	15.6±29.1	9.4±23	5±17.2	31.1±39.2	80.8±7.6
Level of education (E)																
no formal E/elementary school	52.5±26.4	75.3±22.7	66.2±29.6	66.2±29.2	73.7±27	77.8±26.9	34±25	10.1±15.6	30.3±28.1	23.2±28.2	31.3±34.3	27.3±35.8	12.1±27.4	6.1±19.5	42.4±41.9	79.1±6.3
secondary/high school	59.6±24.1	77.2±18.3	75.8±34.4	73.9±27.2	83.3±21.7	81.2±30	24.7±27.66	6.7±12.4	26.2±24.1	13.3±24.8	26.7±35.6	15.8±29.2	13.3±28	6.7±21.6	37.5±40.1	79.5±11.7
faculty/PhD	72.6±18.3	85.7±13.8	85.7±20.5	79.8±23	90.5±19.3	83.3±22.6	19.8±19.6	1.2±4.4	19±24.3	2.4±8.9	16.7±28.5	7.1±14.2	0±0	4.8±12.1	16.7±25.31	85±8.5
Employment position																
laborer	53.6±25.5	77.2±18.9	70.1±31.7	72.4±26.7	79.9±23.8	81.9±28.3	27.3±27.5	9±15.3	26.7±25.7	17.4±26.6	25.7±35.2	20.1±32	14.6±27.4	7.6±20.9	36.8±40.8	79.6±10.9
technical worker	69.2±24.9	87.3±14.9	96.7±10.5	85.8±21.5	88.3±19.3	88.3±22.1	14.4±18.2	3.3±10.5	15±19.9	6.7±21.1	16.7±28.3	6.7±21.1	0±0	0±0	33.3±41.6	81±5.7
administrative worker	43.7±15.8	65± 16.7	79.2±31.5	45.8±25	79.2±15.9	70.8±28.4	47.2±16.7	8.3±9.6	45 8±21	16.7±19.2	41.7±31.9	41.7±31.9	25±50	0±0	50±43	75± 10
manager	72.2±17.7	84±14.8	82.2±23.9	80±22.2	91.1±18.7	82.2±22.2	20.7±19.2	1.1±4.3	22.2±26.5	2.2±8.6	15.5±27.8	6.7±13.8	0±0	11.1±27.2	20±27.6	84±9.1
unemployed	63.9±25.3	66.7±30	55.5±39.9	50±32	68.5±28.2	64.8±35.8	44.4±27.8	11.1±11.8	35.2±29.4	33.3±33.3	59.2±27.8	37±42.3	11.1±33.3	0±0	51.8±44.4	78.9±6
HPV status																
positive	68.5±29.4	79.2±14.6	74.2±27.5	73±25.7	83.8±25.4	83.5±27.9	39.9±28	14.8±14	37.8±25.1	29.6±21.5	20.7±32	33.6±25.1	18.7±27	5.3±20	38.3±35.8	80.6±15.5
negative	59±26,6	73.1±21	68.8±31.3	68.2±33.1	78.7±27.2	77.3±30.2	32±26.4	6±12.2	20.3±28.8	10.6±34.1	22±33.7	25.2±27.7	20.2±33.9	6.8±16.4	41.1±36	80±6.7
AJCC Stage																
I	55.8±23.6	82.5±16.4	73.7±33.7	80±22.2	82.5±24.1	85.4±24.2	45.6±24.2	15±18.3	40±21.1	30±29.2	46.7±32.2	30±36.7	13.3±28.1	3.3±10.5	20±32.2	78±9.2
II	68±21.2	81.7±15.4	78.5±28.4	72.2±28.2	84.7±22.5	80.5±27.2	31.7±37.6	11.9±12.6	35.7±32.5	33.3±38.5	38.1±40.5	38.1±48.8	14.3±37.8	4.8±12.6	52.4±37.8	80±5.8
III	66.7±30.4	71.4±36.8	71.4±30	54.8±41.9	73.3±26.3	66.7±33.3	21.7±22.8	4.9±9.2	22.9±25.9	8.3±20.2	25±37.1	6.9±13.8	6.9±24	8.3±24.6	43.1±38.7	80.8±6.5
IV	46.7±27.8	59.3±14.5	65±30.9	57.5±23	73.8±30.2	65±33.7	25±24.6	6.2±13.9	25.4±25.6	11.7±22.1	21.7±30.7	20±31.8	13.3±25.9	6.7±20.2	31.7±39.9	79.7±11.9
Treatment modality																
OP	65.6±20.4	82.1±15	81.2±20.1	84.9±17.3	87.5±15.5	90.6±14.9	42±29.3	14.8±13	35.2±22.7	33.3±23.6	40.7±27.8	22.2±28.9	11.1±33.3	3.7±11.1	22.2±37.3	81.1±9.3
RT	63.5±36.7	71.7±33.7	72.2±31.2	71.3±30.7	82.1±24.9	87±23.3	33.3±33.6	10.4±15.3	31.2±32.6	29.2±33	45.8±43.4	29.1±45.2	12.5±24.8	8.3±23.6	66.7±35.6	78.7±8.3
OP + RT	63.6±24.5	79.7±17.9	68.7±31.4	75±21.3	82.1±22.1	79±29.4	21±26.6	8±15.6	21±20.4	11.1±22.6	25.9±35	14.8±31.1	14.8±29.7	3.7±10.7	28.4±38.9	80.7±14.6
RT + CT,	52.8±20.1	78.3±18.3	78.4±32.3	57.4±27.8	70.4±25	66.7±35.6	30.9±23.7	3.7±8.4	35.2±28.6	13.6±26.6	25.9±33.7	23.4±28.9	9.9±24.1	12.3±29.4	35.8±41.2	79.2±6.1
OP + RT + CT	48.2±31.4	68.9±20.8	66.7±35.5	54.2±39.1	70.8±36.5	57.4±30.2	21.5±18.8	5.2±13.2	14.6±21.8	8.3±19.2	12.5±26.9	10.4±26.4	4.2±16.7	0±0	41.7±35.5	81.2±3.4

EORTC QLQ-C30 – European Organization for Research and Treatment of Cancer Quality -of Life-Questionnaire-C30; KPS – Karnofsky Performance Scale; OP – operation; RT – radiotherapy; CT – chemotherapy; HPV – human papilloma virus; AJCC – American Joint Committee on Cancer.

Table 3

EORTC QLQ - H&N35 scores depending on the investigated parameters (all values are expressed as mean value ± standard deviation)

Characteristics	Pain	Swallowing	Senses	Speech	Eating	Contact	Sexuality	Teeth	Opening mouth	Dry mouth	Saliva	Coughing	Feel ill	Pain killers	Supplements	Feeding tube	Weight loss	Weight gain
Gender																		
male	30.5±27.6	29.1±26.9	9.7±19.7	17.2±21.3	26.2±26.1	8.2±22.1	30.2±30.8	14±28.8	23.7±34.8	44.9±37.8	52.4±36.6	29.5±26.5	21.2±26.8	44.9±50.1	5.8±23.5	1.4±12	29±45.7	10.3±30.6
female	26.1±28	35.2±26	25±29.3	27.8±22.9	39.3±31.1	23.6±24.1	64.8±40.4	9.2±19.5	29.6±37.7	59.2±4.6	52.9±42.6	33.3±30.2	37±30	44.4±51.1	16.7±38.3	0±0	50±51.4	16.7±38.3
Place of living																		
urban	27.1±26.4	33.2±26.5	15.6±25.2	20.4±22.8	30.7±27.6	12.9±25.1	39.7±37.3	14.8±29.8	26.4±36	56.1±37.8	55.2±36.5	31.2±27.3	27±28.6	47.6±50.3	7.9±27.2	1.6±12.6	28.6±45.5	12.9±33.8
rural	35.6±31.3	24.6±26.8	6.1±12.1	18.2±19.5	25.7±28.3	7.9±18.1	32.6±40.4	9.1±18.3	18.2±30.4	27.3±36.6	47±42	28.8±27.8	18.2±26.7	31.8±46.7	9.1±29.4	0±0	45.4±50.9	9.1±29.4
Living arrangement																		
single	33.6±27.4	33.3±29.2	16±23.8	26.3±25.8	34.9±29.9	18.5±27.8	54.3±40.7	7.4±23.3	30.8±35.7	56.8±42.2	58.7±40	35.8±26	33.3±26.1	66.7±48	7.4±26.7	0±0	33.3±48	15.4±36.8
in a partnership/ married	26.8±27.5	29±25.6	11.4±22.2	16.3±19.3	26.2±26.2	8.2±20.4	29.7±30.5	15.6±28.4	22.2±35.1	43.9±37.6	50±36.6	27.8±27.6	20.6±28.1	35±48.1	8.3±27.9	1.7±12.9	33.3±47.5	10±30.2
Level of education (E)																		
no formal																		
E/elementary school	35.8±29	35.3±29.3	15.6±26	22.6±23.3	34.9±29.5	16.4±26.1	42.9±35.1	10.1±22.8	32.3±36.8	43.4±37.7	44.4±37.9	33.3±26.3	31.3±30	60.6±49.6	6.1±24.2	3±17.4	39.4±49.6	9.1±29.2
secondary/high school	25.4±25.8	31.2±25.8	13.7±22.6	18.9±22.7	28.7±28.2	10.6±23.9	37.5±37.1	19.2±32.8	23.3±37.1	60.8±38.4	64.1±37.7	28.3±29.7	23.3±27.4	35±48.3	10± 30.4	0±0	32.5±47.4	15± 36.2
faculty/PhD	22.6±26.8	16.1±17.7	3.6±9.6	13.5±15.2	15.5±14.2	1.8±4.8	23.8±31.2	2.4±8.9	11.9±21.1	21.4±30.9	38.4±26.7	28.6±22.1	11.9±21.1	35.7±49.7	7.1±26.7	0±0	21.4±42.6	7.7±27.7
Employment position																		
laborer	29±25.7	34.7±29.3	17±26.9	20.6±24.5	33.2±30.3	12.8±26.8	37.8±32.6	15.3±29.1	26.4±37	54.2±38	56.2±38.4	30.5±27.4	25±27.9	45.8±50.3	8.3±27.9	2.1±14.4	35.4±48.3	10.4±30.9
technical worker	15± 25.4	14.2±16.2	5±11.2	14.4±20.3	16.7±17.57	14.2±18	33.3±47.16	10±22.5	16.7±36	46.7±39.1	56.7±41.7	33.3±35.1	16.7±17.6	20±42.2	0±0	0±0	30±48.3	30±48.3
administrative worker	22.9±7.9	43.7±14.2	16.7±23.6	19.4±19	35.4±23.97	0±0	41.7±41.97	25±50	33.3±27.2	75±50	22.2±38.5	25±31.9	33.3±27.2	75±50	25±50	0±0	25±50	0±0
manager	27.8±32.7	20±22.9	3.3±9.3	16.3±18.2	16.1±3.9	1.7±4.7	24.4±30.1	2.2±8.6	17.8±30.5	20±30.3	42.8±30.5	26.7±22.5	15.5±24.8	40±50.7	6.7±25.8	0±0	20±41.4	7.1±26.7
unemployed	45.4±30.6	37±23.6	14.8±19.4	24.7±19	37.9±33.9	23.1±27.2	55.5±43.3	18.5±29.4	29.6±38.9	55.6±37.2	59.2±36.4	29.6±26	37±38.9	55.5±52.7	11.1±33.3	0±0	55.5±52.7	11.1±33.3
HPV status																		
positive	38.8±25.4	42.1±26	30.7±25.7	29.2±14.3	26.7±26.4	10.2±25.1	36.8±30.8	13±224.2	25.6±34.6	48.6±30.1	59.2±36.6	33.5±25.2	28.2±28.5	43.5±42.1	5.8±28.4	0.0±0	30.2±35.7	15.6±30.2
negative	22.1±26	35.4±28.2	7±29.6	15.8±20.5	31.3±29.9	13.6±28.5	44.7±30	10.2±21.4	26.6±36.7	52.3±24.9	50.7±35.3	36.7±30.6	33.9±30.7	46.8±40.1	9.7±39.1	0±0	36.5±36.6	18.9±30.6
AJCC Stage																		
I	40±21.8	39.2±21.5	31.7±30.9	30±26.2	35.8±25.5	23.3±26.9	55±31.5	16.7±28.3	36.7±42.9	63.3±33.1	59.2±32.4	43.3±31.6	40±26.3	40±51.6	0±0	0±0	40±51.6	0±0
II	32.1±33.1	28.6±27.6	26.2±38.3	17.5±20.1	26.2±36.8	10.7±19.7	42.8±46	14.3±26.2	14.3±26.2	52.4±42.4	61.9±44.8	33.3±33.3	28.6±40.5	42.8±53.4	14.3±37.8	0±0	14.3±37.8	0±0
III	27.8±32.6	26.7±21.1	6.2±13.7	20.8±22	21.2±16.5	6.6±17	35.4±37.8	16.7±32.6	20.8±35.2	48.6±40.5	61.1±36.3	27.8±28.9	20.8±29.2	37.5±49.4	12.5±33.8	0±0	16.7±38.1	20.8±41.5
IV	25.8±24.7	27.5±29.5	8.3±17.7	15.5±20.7	29.4±29.6	9.8±22.7	32.1±31.9	11.7±25.6	24.2±35.4	40.8±40.3	42.5±38.5	28.3±22.1	24.2±26.1	52.5±50.6	7.5±26.7	2.5±15.8	47.5±50.6	12.5±33.5
Treatment modality																		
OP	28.7±22.9	37.9±24	22.2±32.3	21±18.8	26.8±21.1	19.4±25.7	48.1±37.7	18.5±24.2	22.2±37.3	48.1±41.2	55.3±35.3	25.9±36.4	33.3±23.6	33.3±50	11.1±33.3	0±0	55.6±52.7	11.1±33.3
RT	37.5±35.1	21.9±21.3	16.7±23.7	22.2±24.5	30.2±35.6	15.6±26.5	58.3±42.7	12.5±35.3	16.7±30.9	54.1±43.4	45.8±50.2	31.7±23.6	33.3±39.8	50±53.4	0±0	0±0	12.5±35.3	25±46.3
OP+RT	26.8±25.5	30.2±28.6	14.2±25.6	21.8±27.1	31.2±29.5	13.6±29.4	31.5±37.9	11.1±26.1	23.4±34.4	56.8±41.1	49.4±35	33.3±29.2	23.4±28.9	37±49.2	7.4±26.7	0±0	22.2±42.3	7.7±27.2
RT+CT,	38.6±30	37.3±29.7	8±16.9	18.5±19.7	33±30.1	8±18.8	38.3±30.2	12.3±26.4	29.6±39.6	48.3±36.6	37±35.1	25.9±25	27.1±29.3	62.9±49.2	11.1±32	0±0	44.4±50.6	14.8±36.2
OP+RT+CT	14.6±17.1	18.7±17.9	11.4±20	14.6±17.1	18.7±16.8	6.8±14.3	29.1±34.1	14.6±29.7	25±33.3	45.8±38.2	50±38.5	29.1±24	12.5±16.7	31.2±47.9	6.2±25	6.2±25	31.2±47.9	6.2±25

EORTC QLQ-H&N35 – European Organization for Research and Treatment of Cancer Quality of -Life Questionnaire-Head and Neck 35; OP – operation; RT – radiotherapy; CT – chemotherapy; HPV – human papilloma virus; AJCC – American Joint Committee on Cancer.

Table 4

Statistical differences between the European Organization for Research and Treatment of Cancer Quality-of Life-Questionnaire-C30 (EORTC QLQ-C30) and the European Organization for Research and Treatment of Cancer Quality of- Life Questionnaire-Head and Neck 35 (EORTC QLQ-H&N35) scores depending on the investigated parameters

EORTC QLQ-C30	Parameters (*t*-test; *p*-values)	EORTC QLQ-H&N35	Parameters (ANOVA; *p*-values)
	Gender (male vs. female)		Education (E)
Physical functioning	0.018		no formal E/elementary school vs. faculty/PhD
		Global quality of life	0.039
Emotional functioning	0.01	Dyspnea	0.033
Cognitive functioning	0.002		secondary/high school-faculty/PhD
		Dry mouth	0.004
Social functioning	0.028		HPV status* positive vs. negative
Fatigue	0.001	Pain	0.024
Dyspnea	0.045	Dyspnea	0.043
Insomnia	0.001	Senses	0.26
Appetite loss	0.044		Employment position (manager–unemployed)
Senses	0.48	Insomnia	0.046
Contact	0.12		AJCC stage
	Place of living (urban vs. rural)	Physical functioning	I vs. III: 0.2
Senses	0.022		I vs. IV: 0.008
Dry mouth	0.003	Appetite loss	II vs. III: 0.025
Nausea	0.016	Senses	I vs. III: 0.221
Dyspnea	0.036		I vs. IV: 0.25
	Living arrangement (single vs. in a partnership/married)	Weight loss	II vs. III: 0.2 II vs. IV: 0.23
Emotional functioning	0.048		Treatment modality
Cognitive functioning	0.046	Pain	RT+CT vs. OP+RT+CT: 0.017
Sexuality	0.008	Social functioning	OP vs. RT+CT: 0.033
Feel ill	0.049		
Pain killers	0.006		

*** *t*-test (*p*-value); OP – operation; RT – radiotherapy; CT – chemotherapy; AJCC – American Joint Committee on Cancer; HPV – human papilloma virus.**

The Pearson's correlation test was used to determine the correlation among the EORTC QLQ-C30, EORTC QLQ-H&N35 and KPS Index and other parameters (Table 5). The KPS Index scores did not correlate with any of the variables. Older age of the patients correlated positively with sexuality in the patients, and negatively with occurrence of diarrhea. The level of education correlated positively with the global quality of life and cognitive functioning, and negatively with symptoms of nausea, dyspnea, appetite loss, swallowing, eating and feeling ill. Different employment positions did not correlate with the EORTC QLQ-C30, EORTC QLQ-H&N35 scores. There was a negative correlation among the stages of the disease and physical and emotional functioning scores, also with occurrence of dyspnea, insomnia and swallowing. The more combined therapy modalities patient had, significantly the worse emotional and social functionings were.

Table 5

Significant correlations ($p < 0.05$) between examined parameters and the score values of EORTC QLQ-C30, EORTC QLQ-H&N35 (Pearson's correlation test)

Parameters	Questionnaires	r
	EORTC QLQ-C30	
Age	Diarrhea	-0.228
Level of education	Role functioning	0.221*
	Cognitive functioning	0.253
	Nausea	-0.229
	Dyspnea	-0.288
	Appetite loss	-0.237
AJCC stage of the disease	Emotional functioning	-0.290
	Physical functioning	-0.327
	Social functioning	0.218
	Dyspnea	-0.234
	Insomnia	-0.223
Treatment modality	Emotional functioning	-0.319
	Social functioning	-0.366
	Nausea	-0.236
	Dyspnea	-0.272
	Insomnia	-0.253
	EORTC QLQ-H&N35	
Age	Sexuality	0.215
Level of education	Eating	-0.229
	Feel ill	-0.235
	Swallowing	-0.225
AJCC stage of the disease	Senses	-0.298

EORTC QLQ-C30 – European Organization for Research and Treatment of Cancer Quality-of Life-Questionnaire-C30; EORTC QLQ-H&N35 – European Organization for Research and Treatment of Cancer Quality of-Life Questionnaire-Head and Neck 35; AJCC – American Joint Committee on Cancer; r – Pearson's correlation coefficient.
***statistically significant correlation.**

Discussion

Oropharyngeal cancer has become a growing concern, with rising incidence in the younger male patients [6]. With developing more advanced strategies of head and neck cancer treatment [14, 15], locoregional control of the disease along with the disease-specific survival are significantly better. The expected QOL should be an important factor in choosing an adequate treatment modality, due to its immense influence on the patients' social, physical, psychological and overall functioning [16]. Clinicians are turning to the QOL measures for decision making in daily practice, improving the patient-doctor interaction and monitoring the patient experience with the treatment [17, 18].

Most of the parameters of QOL, are assessed at the lowest 3 months after treatment [17], but in the disease free head and neck patients major improvements in scores happen one year post-treatment [19, 20]. The assessment of QOL parameters in our study was done in that period, which is considered to be a good time for the assessment of QOL, because most of the QLQ-C30 and QLQ-H&N35 scores return to the preoperative values, depending on the treatment [21], and the variations are considered negligible in the absence of recurrent disease [22].

During this study, the demographic and social factors significantly influenced QOL and functional performance in the patients with oropharyngeal cancer, in addition to the stage of the disease and treatment modality. This results were already proven to be significant [23, 24]. Considering the different oropharyngeal sub sites involved, treatment is associated with a wide range of functional and psychosocial deficits. The multiple QOL segments are influenced and the patients are forced to make permanent changes in their eating habits, swallowing, appearance and communication. It is reasonable to expect differences in QOL between the patients treated for oropharyngeal carcinoma depending on their age, marital and educational status and employment. In this study, the women had significantly worse scores in many aspects of functioning, and also regarding fatigue, dyspnea, insomnia, and appetite loss, senses, contacts and sexuality, making gender significant factor which influences the QOL scores in these patients. Marital status influenced limited aspects of QOL, mostly emotional and cognitive functionings, sexual life and felling ill. There were significant differences noted in the patients living in rural areas; they had fewer problems with the senses, dry mouth, felt less nauseous and dyspneic, than those living in urban areas. There are studies that noted the differences in the emotional, functional, and head and neck cancer-specific scores between head and neck cancer survivors living in rural and urban areas, in term of better QOL in rural ones [25].

The level of education significantly influenced some the QOL aspects, like global QOL and cognitive functioning, nausea, dyspnea, appetite loss, swallowing, eating and feeling ill. This was generally noticed in the patients with head and neck carcinoma [23, 26]. Few possible explanations were offered. The patients with the lower education level and lower socioeconomic classes have less accessible health care, which leads to delays in diagnosis and treatment [23]. Some authors suggested that the patients with higher social and cultural level had a better capability of coping with cancer and its consequences. Comparing to the patients with higher education and less physically demanding workplace, the patients with employment that requires physical strength are more likely to be influenced by the disease, and have more trouble in adaptation to other work positions [26]. Considering the

structure of patients in our study, with 83.8% with high-school education and lower and 44% working as laborers, these claims are highly applicable.

The relation between HPV and QOL was explored in a few studies [27, 28]. Sharma et al. [28] found no association between HPV status and QOL one year post-treatment. On the other hand, Maxwell et al. [29] published that the HPV positive patients had significantly better scores considering activity, recreation, swallowing, chewing, speech and overall quality of life a year after the treatment. Production of saliva in the HPV positive patients was poorer comparing to the HPV negative patients in first 12 months, but after that time, the difference was no longer significant. A year after the treatment, the HPV positive patients in our study significantly less complained of pain, dyspnea and on trouble with their senses. Global QOL was better in the HPV positive patients, but differences were not significant. Due to favorable reaction to radiotherapy and better survival rates, we could argue that the HPV positivity surely influences postoperative QOL in the patients with oropharyngeal carcinoma. Recommended modality treatment depending on the HPV status would certainly be a subject for further discussion, with more knowledge accumulated on the subject.

A stage of disease, cancer site, and treatment type are the predictors of post-treatment QOL, particularly disease-specific symptoms [30]. In this study, the patients with more advanced stage of the disease scored worse than those with less advanced stage of the disease in all aspects of QLQ-C30, QLQ-H&N35 and KPS Index scale, which is consistent with previous papers on the subject [30, 31]. Significant differences were noted in physical functioning and with the senses between patients in the stage I of the disease and patients in the stages III and IV of the disease. Oates et al.[30] reported great deterioration of senses, teeth, saliva secretion and coughing in the patients with early-stage cancer and significant deterioration of sexual function and complains of dry mouth in the patients 12 months after the treatment for all four stages of the disease. The findings of statistically significant differences in the QOL scores favoring patients receiving a single therapy compared to the combination therapies are not consistent across studies [19]. In our patients, social functioning was significantly better for those who underwent operative treatment than for those treated operatively with postoperative radiochemotherapy or just with adjuvant radiochemotherapy. Also, the pain was significantly more severe in the patients treated operatively with radiochemotherapy, than in the patients treated only with radiochemotherapy. Some authors published similar findings [27, 31, 32], but in most studies, the results were inconclusive [33, 34]. Good oncological results are the first objective of treatment, but functional preservation could be one of the main challenges after surgical treatment or radiochemotherapy. Comparing to surgery, the patients were primary treated with chemoradiotherapy [35] or with adjuvant therapy [36, 37]. In our study, there were some differences in the functional aspects (eating, swallowing, complaints of dry mouth and saliva production), but they were not significant between the groups of patients considering the treatment modality. Our findings could have been strongly influenced by the time of evaluation. The differences between the QOL scores in the patients treated with different treatment modalities proved to be the greatest 3 months after the treatment, and by 6 and 12 months of follow-up, they were significantly less pronounced [30].

With rising incidence of patients diagnosed with oropharyngeal cancer, there is a great need for better understanding of recovery process, that significantly influences post-treatment QOL and how to educate the patients in terms what to expect after the treatment. After diagnosis and treatment of oropharyngeal cancer, the patients go back to their family and living environment, with distinct personal, social, and economic expectations and duties. These factors are of little variability and are constantly present in the patients' lives pre and post-treatment and it would be crucial to recognize their important influence on overall recovery and survival.

There are some limitations of the study. First, the study assessed QOL and functional performance in the patients with oropharyngeal cancer at a time point, not prospectively, so any changes between the influence of sociodemographic factors and QOL over time was not followed. Second, the number of patients in the study was small and the results of this study should be evaluated cautiously. Last, a number of patients with different subsites of the oropharyngeal carcinoma was also small and it was not analyzed how different oropharyngeal subsites involvement influenced QOL and functional performance.

Conclusion

Clinicians should have in mind the socioeconomic factors and HPV status when planning recovery course after treatment in the patients with oropharyngeal carcinoma. Gender, education level and employment are the variables that form certain risk profiles associated with lower post-treatment QOL. This would ultimately lead to the better functional results, faster recovery and return to everyday life and activities in the patients with oropharyngeal cancer.

Conflict of interest

None.

REFERENCES

1. *Lambert R, Sauvaget C, de Camargo Cancela M, Sankaranarayanan R.* Epidemiology of cancer from the oral cavity and oropharynx. Eur J Gastroenterol Hepatol 2011; 23(8): 633–41.
2. GLOBOCAN. 2012. Available from: http://globocan.iarc.fr/old/summary_table_site_prev.asp?selection=13010&selection=21030&title=Lip%2C+oral+cavity%2C+Other+pharynx&sex=0&africa=1&america=2&asia=3&europe=4&oceania=5&build=6&window=1&so
3. *Warnakulasuriya S.* Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45(4–5): 309–16.
4. *Chandu A, Smith AC, Rogers SN.* Health-related quality of life in oral cancer: A review. J Oral Maxillofac Surg 2006; 64(3): 495–502.

5. *Chaturvedi AK, Engels EA, Pfeiffer RM, Hernandez BY, Xiao W, Kim E*, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J Clin Oncol 2011; 29(32): 4294–301.
6. *Weatherspoon DJ, Chattopadhyay A, Boroumand S, Garcia I*. Oral cavity and oropharyngeal cancer incidence trends and disparities in the United States: 2000-2010. Cancer Epidemiol 2015; 39(4): 497–504.
7. *Cleveland JL, Junger ML, Saraiya M, Markowitz LE, Dunne EF, Epstein JB*. The connection between human papillomavirus and oropharyngeal squamous cell carcinomas in the United States: Implications for dentistry. J Am Dent Assoc 2011; 142(8): 915–24.
8. *Iyer GN, Dogan S, Palmer F, Rahmati R, Nixon IJ, Lee N,* et al. Detailed Analysis of Clinicopathologic Factors Demonstrate Distinct Difference in Outcome and Prognostic Factors Between Surgically Treated HPV-Positive and Negative Oropharyngeal Cancer. Ann Surg Oncol 2015; 22(13): 4411–21.
9. *Morton RP*. Studies in the quality of life of head and neck cancer patients: Results of a two-year longitudinal study and a comparative cross-sectional cross-cultural survey. Laryngoscope 2003; 113(7): 1091–103.
10. *Singhi AD, Westra WH*. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. Cancer 2010; 116(9): 2166–73.
11. *Cai C, Chernock RD, Pittman ME, El-Mofty SK, Thorstad WL, Lewis JS*. Keratinizing-type squamous cell carcinoma of the oropharynx: P16 overexpression is associated with positive high-risk HPV status and improved survival. Am J Surg Pathol 2014; 38(6): 809–15.
12. *Bjordal K, Ahlner-Elmqvist M, Hammerlid E, Boysen M, Evensen JF, Biörklund A,* et al. A prospective study of quality of life in head and neck cancer patients. Part II: Longitudinal data. Laryngoscope 2001; 111(8): 1440–52.
13. *Karnofsky DA, Burchenal JH*. The clinical evaluation of chemotherapeutic agents in cancer. In: *MacLeod CM*, editor. Evaluation of chemotherapeutic agents. New York: Columbia University Press; 1949. p. 196–7.
14. *Röösli C, Tschudi DC, Studer G, Braun J, Stoeckli SJ*. Outcome of patients after treatment for a squamous cell carcinoma of the oropharynx. Laryngoscope 2009; 119(3): 534–40.
15. *Yasumatsu R, Nakashima T, Komune S*. Squamous cell carcinoma of the oropharynx: single-institution outcome analysis of patients treated with concurrent chemoradiotherapy. J Laryngol Otol 2015; 129 Suppl 2: S77–82.
16. *Broglie MA, Soltermann A, Haile SR, Röösli C, Huber GF, Schmid S*, et al. Quality of life of oropharyngeal cancer patients with respect to treatment strategy and p16-positivity. Laryngoscope 2013; 123(1): 164–70.
17. *Binenbaum Y, Amit M, Billan S, Cohen JT, Gil Z*. Minimal clinically important differences in quality of life scores of oral cavity and oropharynx cancer patients. Ann Surg Oncol 2014; 21(8): 2773–81.
18. *Schunemann HJ, Akl EA, Guyatt GH*. Interpreting the results of patient reported outcome measures in clinical trials: The clinician's perspective. Health Qual Life Outcomes 2006; 4: 62.
19. *Infante-Cossio P, Torres-Carranza E, Cayuela A, Hens-Aumente E, Pastor-Gaitan P, Gutierrez-Perez JL*. Impact of treatment on quality of life for oral and oropharyngeal carcinoma. Int J Oral Maxillofac Surg 2009; 38(10): 1052–8.
20. *Bozec A, Peyrade F, Milano G*. Molecular targeted therapies in the management of head and neck squamous cell carcinoma: Recent developments and perspectives. Anticancer Agents Med Chem 2013; 13(3): 389–402.
21. *Al-Mamgani A, Rooij P, Tans L, Verduijn GM, Sewnaik A, Baatenburg JR*. A prospective evaluation of patient-reported quality-of-life after (chemo) radiation for oropharyngeal cancer: Which patients are at risk of significant quality-of-life deterioration. Radiother Oncol 2013; 106(3): 359–63.
22. *Pierre CS, Dassonville O, Chamorey E, Poissonnet G, Ettaiche M, Santini J,* et al. Long-term quality of life and its predictive factors after oncologic surgery and microvascular reconstruction in patients with oral or oropharyngeal cancer. Eur Arch Otorhinolaryngol 2014; 271(4): 801–7.
23. *Vartanian JG, Carvalho AL, Toyota J, Kowalski IGS, Kowalski LP*. Socioeconomic effects of and risk factors for disability in long-term survivors of head and neck cancer. Arch Otolaryngol Head Neck Surg 2006; 132(1): 32–5.
24. *Demiral AN, Sen M, Demiral Y, Kinay M*. The effect of socioeconomic factors on quality of life after treatment in patients with head and neck cancer. Int J Radiat Oncol Biol Phys 2008; 70(1): 23–7.
25. *Thomas AA, Timmons A, Molcho M, Pearce A, Gallagher P, Butow P*, et al. Quality of life in urban and rural settings: A study of head and neck cancer survivors. Oral Oncol 2014; 50(7): 676–82.
26. *Terrell JE, Ronis DL, Fowler KE, Bradford CR, Chepeha DB, Prince ME,* et al. Clinical predictors of quality of life in patients with head and neck cancer. Arch Otolaryngol Head Neck Surg. 2004; 130(4): 401–8.
27. *Allal AS, Nicoucar K, Mach N, Dulguerov P*. Quality of life in patients with oropharynx carcinomas: Assessment after accelerated radiotherapy with or without chemotherapy versus radical surgery and postoperative radiotherapy. Head Neck 2003; 25(10): 833–9; discussion 839-40.
28. *Sharma A, Méndez E, Yueh B, Lohavanichbutr P, Houck J, Doody DR*, et al. Human papillomavirus-positive oral cavity and oropharyngeal cancer patients do not have better quality-of-life trajectories. Otolaryngol Head Neck Surg 2012; 146(5): 739–45.
29. *Maxwell JH, Mehta V, Wang H, Cunningham D, Duvvuri U, Kim S,* et al. Quality of life in head and neck cancer patients: Impact of HPV and primary treatment modality. Laryngoscope 2014; 124(7): 1592–7.
30. *Oates J, Davies S, Roydhouse JK, Fethney J, White K*. The effect of cancer stage and treatment modality on quality of life in oropharyngeal cancer. Laryngoscope 2014; 124(1): 151–8.
31. *Bjordal K, de Graeff A, Fayers PM, Hammerlid E, van Pottelsberghe C, Curran D,* et al. A 12country field study of the EORTC QLQ-C30 (version 3.0) and the head and neck cancer specific module (EORTC QLQ-H&N35) in head and neck patients. EORTC Quality of Life Group. Eur J Cancer 2000; 36(14): 1796–807.
32. *Kim TW, Youm H, Byun H, Son Y, Baek C*. Treatment Outcomes and Quality of Life in Oropharyngeal Cancer after Surgery-based versus Radiation-based Treatment. Clin Exp Otorhinolaryngol 2010; 3(3): 153–60.
33. *Boscolo-Rizzo P, Stellin M, Fuson R, Marchiori C, Gava A, Da Mosto MC*. Long-term quality of life after treatment for locally advanced oropharyngeal carcinoma: Surgery and postoperative radiotherapy versus concurrent chemoradiation. Oral Oncol 2009; 45(11): 953–7.
34. *Mowry SE, Ho A, Lotempio MM, Sadeghi A, Blackwell KE, Wang MB*. Quality of life in advanced oropharyngeal carcinoma after chemoradiation versus surgery and radiation. Laryngoscope 2006; 116(9): 1589–93.
35. *Chen AM, Daly ME, Luu Q, Donald PJ, Farwell GD*. Comparison of functional outcomes and quality of life between transoral surgery and definitive chemoradiotherapy for oropharyngeal cancer. Head Neck 2015; 37(3): 381–5.
36. *Günzel T, Schimmer M*. Quality of life after primary radiation/radiochemotherapy vs. operation therapy in an oropharyngeal cancer. Laryngorhinootologie 2012; 91(7): 451–6; quiz 457–9. (German)
37. *Mendenhall WM, Amdur RJ, Morris CG, Kirwan JM, Li JG*. Intensity modulated radiotherapy for oropharyngeal squamous cell carcinoma. Laryngoscope 2010; 120(2): 218–22.

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Assessment of fluoride intake from drinking water and toothpaste in 3-year-olds: preliminary results in Belgrade, Republic of Serbia

Procena unosa fluorida putem vode za piće i paste za zube kod dece uzrasta od tri godine: preliminarni rezultati u Beogradu, Republika Srbija

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Abstract

Background/Aim. Fluoride has beneficial effect on dental caries prevention and enables high hardness of enamel. However, fluoride intake above optimal levels can have adverse effects on teeth and bones, especially in young children during the period of intense growth and teeth development. The aim of this study was to assess fluoride intake from water and toothpaste among 3-year-old children in Belgrade, Serbia, in the municipalities of Vračar and Novi Beograd. **Methods.** A questionnaire for the parents (n = 40) was used to provide information on the water consumption (tap and/or bottled water) and the brand of toothpaste used by children as well as the frequency of tooth brushing and the amount of toothpaste during brushing. Fluoride concentrations in water and toothpaste samples were determined electrochemically by using fluoride-selective electrode. Fluoride intake was estimated through a mathematical model commonly used by the U.S. Environmental Protection Agency. **Results.** The obtained results indicate no significant difference in daily fluoride intake through drinking water and toothpaste in 3-year-old children in Vračar (n = 19) compared to Novi Beograd (n = 21) ($p > 0.05$). However, all estimated fluoride levels (0.089–0.625 mg/day) are significantly lower than the optimal daily intake level for caries protection (0.7 mg/day for children up to 4 years, FNB-USA National Institute of Medicine) and two to six times lower than tolerable upper fluoride level for the children of same age (1.3 mg/day, FNB-USA National Institute of Medicine). Furthermore, calculated daily fluoride intake per kilogram body weight confirm very low fluoride intake by water and toothpaste in children of investigated municipalities in Belgrade, being significantly below the recommended an adequate intake (0.05 mg/kg/day, EFSA). **Conclusion.** This preliminary study has shown that daily fluoride intake in 3-year-olds is lower than tolerable upper fluoride level, even not sufficient for the prevention of dental caries.

Key words:
flouorides; child, preschool; drinking water; toothpastes; tooth diseases; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Fluoridi imaju pozitivan efekat na prevenciju karijesa zuba kao i povećanje čvrstine zubnog emajla. Međutim, njihov unos iznad optimalnog može imati štetne efekte na zube i kosti, posebno kod male dece tokom intenzivnog rasta i razvoja zuba. Cilj ovog rada bio je da se proceni unos fluorida putem vode za piće i paste za zube kod dece uzrasta od tri godine u Beogradu, na teritoriji dve opštine, Vračar i Novi Beograd. **Metode.** Anketnim upitnicima (n = 40) za roditelje dobijeni su podaci o vrsti vode koju deca piju (česmenska i/ili flaširana), proizvodima koje koriste za čišćenje i negu zuba, učestalosti pranja zuba kao i količini paste za zube koja se koristi po jednom pranju. Sadržaj fluorida u vodi za piće i pastama za zube određen je elektrohemijski sa fluoridnom jonselektivnom elektrodom. Unos fluorida procenjen je korišćenjem matematičkog modela datog od strane Američke agencije za zaštitu životne sredine. **Rezultati.** Dobijeni rezultati pokazuju da nema statistički značajne razlike u dnevnom unosu fluorida putem vode za piće i paste za zube između dece uzrasta od tri godine na Vračaru i Novom Beogradu ($p > 0,05$). Međutim, sve procenjene vrednosti unosa fluorida (0,089–0,625 mg/dan) značajno su niže od optimalnog dnevnog unosa za prevenciju karijesa (0,7 mg/dan za de-

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cu uzrasta do 4 godine, FNB-Nacionalnog instituta medicine, SAD) i dva do šest puta niže od tolerišućeg gornjeg nivoa unosa fluorida za decu istog uzrasta (1,3 mg/dan, FNB-Nacionalnog instituta medicine, SAD). Štaviše, izračunati dnevni unosi fluorida izraženi po kilogramu telesne mase potvrđuju veoma nizak unos fluorida kod dece ispitivanih opština u Beogradu, čak značajno niže od preporučenog optimalnog unosa (0,05 mg/kg/dan, EFSA). **Zaključak.** Ovim preliminarnim istraživanjem pokazano je da je dnevni unos fluorida kod trogodišnjaka u Beogradu značajno niži od tolerišućeg gornjeg nivoa unosa fluorida, čak niži od optimalnog unosa za prevenciju karijesa.

Ključne reči:
fluoridi; deca, predškolska; voda za piće; dentifriciji; zub, bolesti; ankete i upitnici.

Introduction

Fluoride intake in an optimal dose is a safe and efficient way to minimize a risk of dental caries and prevent enamel demineralization [1–3]. However, the range between optimal levels and the concentrations that lead to adverse effects such as dental and skeletal fluorosis is narrow, and indicates the importance of fluoride intake assessment [2, 4, 5]. Furthermore, the opinion of the European Food Safety Authority (EFSA) is that data on fluoride exposure are insufficient [5].

The important sources of fluoride intake for general population are water, drinks and food, particularly some kinds of tea, sea fish, fluoridated foods and fluoride dentifrices [6–8]. Epidemiological studies from the 70's have shown that the most important source of fluoride intake is drinking water which contains 0.5 to 1 mg/L fluoride, depending on climate conditions [4]. On the other hand, fluorosis can be related not only to the regions rich in fluoride but also to the fluoride overuse, particularly from fluoride toothpastes, mouthwashes, gels, etc. [9, 10]. High fluoride intake can be of a special risk in children up to six years of age when tooth growth and development occurs, especially during the phases of enamel formation [10-12]. The period between the 15th and 30th month of life is considered to be of the highest risk, when the fluoride intake should be carefully controlled and balanced between the need for dental caries protection and the risk of fluorosis [13]. A special caution should be taken in two- and three-year-olds since they can swallow high amount of toothpaste as a result of inadequate swallowing reflex [5, 14, 15]. Thus, the Academy of Paediatric Dentistry [American (AAPD) and European (EAPD)] gave detailed recommendations for fluoride content in toothpastes and its amounts for brushing in relation to the age of child [16, 17]. For the majority of European communities, the EAPD recommends the use of appropriate fluoride toothpaste in conjunction with good oral hygiene to be the basic fluoride regimen [17]. The approach of the EAPD was adopted in Serbia, having in mind the importance of adequate fluoride intake through toothpaste for reducing dental caries in children [18].

However, despite a significant reduction in caries in Western and Northern European countries, available data indicate that the prevalence and occurrence of dental caries in Serbian children is high, even the highest in the countries of Eastern and Central Europe (DMFT index 3.4) [19]. According to the DFMT index value (number of decayed, missing due to caries and filled teeth in the primary dentition) relative risk for tooth decay in our country is almost two times higher than the regional average [19]. The prevalence of dental caries in early childhood in Serbia is 8.3% for three-year-olds, while in six-year-olds with permanent teeth the percentage is 0.8% [18]. Data from the latest research in the Autonomous Province of Vojvodina, Republic of Serbia show that the prevalence of early childhood caries is extremely high, approaching 50% [20].

Drinking water with optimal fluoride concentration is the most important source of fluoride intake in childhood, but in the areas with water and food, poor in fluoride, the dominant route of fluoride intake is via toothpaste [7, 8, 21]. The results of de Almeida et al. [21] indicate that toothpaste alone is responsible for about 80% of the daily fluoride intake of 1 to 3-year-old children. Thus, the aim of this study was to assess fluoride intake through drinking water and toothpaste in three-year-olds and obtain preliminary results of fluoride intake in children in Belgrade, Republic of Serbia. To achieve this aim, it was necessary to: conduct a questionnaire for parents to obtain information on the type of water that children consume (tap and/or bottled), the type of toothpaste they use, the frequency of tooth brushing and the amount of toothpaste used per tooth brushing; determine fluoride concentrations in the samples of non-carbonated bottled water, tap water and toothpastes selected on the basis of the questionnaire for parents; estimate the daily fluoride intake in mg/day and mg/kg/day and compare the obtained results with the recommended an adequate intake and tolerable upper level of fluoride intake for children of this age, proposed by the Food and Nutrition Board, U.S. National Academy of Sciences Institute of Medicine and EFSA [5, 22].

Methods

Questionnaire

A previously validated questionnaire for parents was used in order to obtain the general data (sex, age, body weight and dental health of children), information on the type of water that children drink (tap, bottled, type of bottled water), the type of toothpaste their children use, the frequency of tooth brushing, and the amount of toothpaste used per tooth brushing [23]. The questionnaire was anonymous and voluntary. Filling out the questionnaire by the parents of 3-year-olds was carried out in two kindergartens in Belgrade – in one from the municipality of Vračar and one from the municipality of Novi Beograd.

Drinking water and toothpaste

Different non-carbonated bottled waters and toothpastes available on the market in Belgrade, were selected for fluo-

ride determination on the basis of the completed questionnaire for parents. All non-carbonated bottled waters available on the market had no fluoride content, while the content of fluoride was indicated on toothpastes and ranged in a very wide range of 500 to 1450 mg/kg fluoride. Tap water from the territories of Vračar and Novi Beograd was also used for fluoride determination.

Fluoride determination

Fluoride in water was determined directly without previous preparation, while the preparation of toothpastes depended on the chemical form of fluoride present in the toothpaste (NaF, Na-monofluorophosphate, aminofluoride). For the preparation of toothpaste samples the protocol given by Omena et al. [24] was applied. All samples were prepared in duplicate. The fluoride content was determined electrochemically, using fluoride-selective electrode (WTW, ISE type 800 – Consort, Belgium; pH meter Iskra MA 5735). Before measurement, all samples were mixed with the TISAB buffer solution in ratio 1 : 1. All chemicals, obtained from the commercial sources, were of analytical grade purity. The analytical method for fluoride determination was linear in the range of 0.05–5 mg/L ($r = 0.9998$). The obtained limits of determination (LOD) and quantification (LOQ) were 0.003 and 0.009 mg/L, respectively. The recovery values from 98% to 107% indicated an adequate accuracy of the method, while repeatability was confirmed by corresponding coefficient of variation from 2.2% to 4.4%.

Estimation of fluoride intake

Fluoride intake (mean and 95th percentile, P-95) was estimated through a mathematical model employed by the U.S. Environmental Protection Agency (EPA) according to the equations for water and toothpaste [25]:

$$EDI_w = \frac{C \times IR \times CF}{BW}$$

Where: EDI_w– Estimated daily intake by drinking water (mg/day or mg/kg/day)
C – Fluoride concentration in drinking (mg/L)
IR – Intake rate, amount of water intake per day (L) – 0.9 L for 3 years age [26]
CF – Conversion factor
BW – Body weight (kg)

$$EDI_t = \frac{C \times IR \times AF \times EF \times CF}{BW}$$

EDI_t – Estimated daily intake by toothpaste (mg/day or mg/kg/day)
C – Fluoride concentration in toothpaste (mg/kg)
IR – Ingestion or intake rate, amount of toothpaste per one tooth brushing (mg)
EF – Exposure frequency (number per day), or the frequency of tooth brushing
AF – Absorption factor (amount of fluoride swallowed, for the age of 3 years 48%) [14]
CF – Conversion factor
BW– Body weight (kg)

Total daily fluoride intake in mg/day and mg/kg/day by these sources was obtained adding the estimated values for EDI_w and EDI_t.

Data analysis

Statistical analysis was performed using the computer program STATISTICA 7.0 and the MS Excel package 2007. The Student *t*-test was applied to determine statistically significant difference between water and toothpaste intake as well as for the total intake between the two examined groups. Statistical significance was set for $p < 0.05$.

Results

Data obtained from the questionnaire

A questionnaire was filled in by parents from two Belgrade municipalities, Vračar and Novi Beograd. The total number of filled in questionnaires was 40, 19 from Vračar and 21 from Novi Beograd. Twenty questionnaires referred to girls, 19 to boys, and in one questionnaire the gender was not specified. The majority of parents in Vračar considered the dental health of their children to be excellent (47.83%), followed by good (43.48%), moderate (4.38%), while one parent did not provide the answer. The parents in Novi Beograd evaluated the dental health of their children mostly as good (47.62%), followed by moderate (28.57%) and bad (14.29%), while there was only one answer for excellent (4.76%) as well as for very bad (4.76%).

Based on the data obtained from the questionnaire shown in Table 1, about one half of the children drink both tap and bottled water (Vračar 52.6%, Novi Beograd 47.6%). The consumption of tap water only is more common in Novi Beograd (38.1%) than in Vračar, while on the other hand, the consumption of bottled water only, is higher in Vračar (31.6%) than in Novi Beograd (4.8%).

Table 1

Type of water consumed by the 3-year-old children in Vračar and Novi Beograd

Type of water	Vračar n (%)	Novi Beograd n (%)
Tap	3 (15.8)	8 (38.1)
Tap purified	0 (0)	2 (9.5)
Bottled	6 (31.6)	1 (4.8)
Tap and bottled	10 (52.6)	10 (47.6)
Total	19 (100)	21 (100)

n – number of children.

Table 2 presents data obtained from the questionnaire: the type of toothpaste, frequency of tooth brushing and the amount of toothpaste used per tooth brushing. The frequency of tooth brushing in the 3-year-olds in Novi Beograd is once per day, in Vračar it is once or two times per day, while only one child brushes teeth more than two times per day.

The largest percentage of children in both localities (Vračar 52.6%, Novi Beograd 100%) applies a pea-sized amount of toothpaste on the brush. The results of the conducted questionnaire show that the children use toothpastes with a fluoride content that varies to a great extent, from toothpastes which does not contain fluoride, to toothpastes with 500 mg/kg fluorides, to those which contain 1,450 mg/kg fluoride-content equivalent to adult toothpaste. The majority of children in Vračar use the toothpaste *Vademecum junior 2 in 1* – 1,450 mg/kg (26.3%), followed by *Chicco*-non-fluoride (21.1%) and *Colgate Smiles* – 1,000 mg/kg (15.8%), while children in Novi Beograd mostly use *Vademecum junior 2 in 1* (33.3%) – 1450 mg/kg, *Vademecum* (23.8%) – 500 mg/kg and *Aquafresh kids* (23.8%) – 500 mg/kg (Table 2). The obtained results also show that at that time no child took fluoride supplements in the form of tablets, gels or solutions.

Table 2

The frequency of tooth brushing, the amount of toothpaste per brushing and the most commonly used toothpastes by the 3-year-olds in Vračar and Novi Beograd

Questionnaire	Municipality	
	Vračar n (%)	Novi Beograd n (%)
How often do you brush your childs' teeth?		
1x day	9 (47.3)	21 (100)
2x day	9 (47.3)	0 (0)
> 2x day	1 (5.4)	0 (0)
When tooth brushing, do you use a pea-sized amount of toothpaste?		
yes	10 (52.6)	21 (100)
no	9 (47.4)	0 (0)
Which toothpaste does your child most commonly use?		
Aquafresh kids (500 mg/kg)*	1 (5.2)	5 (23.8)
Lacalut (500 mg/kg)*	0 (0)	0 (0)
Vademecum (500 mg/kg)*	2 (10.5)	5 (23.8)
Vademecum junior 2 in 1 (1,450 mg/kg)*	5 (26.3)	7 (33.3)
Colgate Smiles (1,000 mg/kg)*	3 (15.8)	1 (4.8)
Chicco – non fluoride	4 (21.1)	3 (14.3)
Other	4 (21.1)	0 (0)

n = number of children; *fluoride content in tooth paste.

Fluoride concentrations in drinking water and toothpaste

Since the results from the questionnaire showed that the children used only a few brands of non-carbonated bottled drinking water and toothpaste, fluoride content was determined in those brands. All the examined non-carbonated bottled waters (n = 8) contained very low fluoride levels in the range from 0.077 to 0.185 mg/L. Fluoride content in tap water was also low, in Novi Beograd 0.153 ± 0.004 mg/L and in Vračar 0.127 ± 0.003 mg/L.

The results of fluoride content in toothpastes showed that the fluoride concentration in the analyzed toothpastes was in the range of 475–1,475 mg/kg (declared content 500–1,450 mg/kg). The most commonly used toothpastes according to the questionnaire (*Aquafresh kids, Colgate smiles, Vademecum junior 2 u 1*) had fluoride content similar to the declared.

Estimation of the total daily fluoride intake

Based on the mathematical model commonly used by the U.S. EPA, the total daily fluoride intake by drinking water and toothpaste was calculated and the results are presented in Figures 1 and 2 and Table 3.

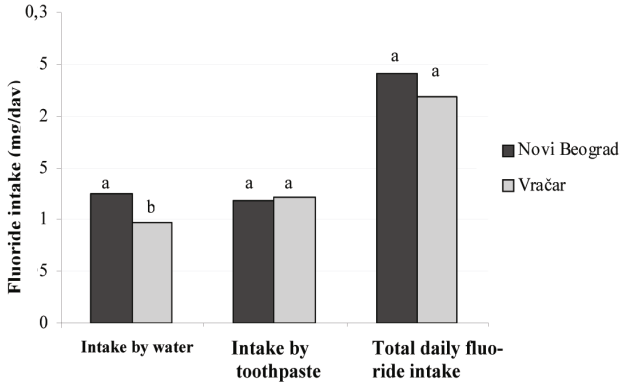

Fig. 1 – Daily fluoride intake by the 3-year-olds in Belgrade (mg/day).

Values are presented as the mean intake of fluoride in Novi Beograd (n = 21) and Vračar (n = 19). Statistical significance of differences was tested by the *t*-test; different letters indicate statistical significance between fluoride intake in 3-year-old children from two investigated municipalities, $p < 0.05$.

Table 3

The lowest and highest calculated daily fluoride intake from drinking water and toothpaste in 3-year-olds in Belgrade

Fluoride intake	Value mg/day	Type of water	Fluoride content in toothpaste (mg/kg)	Amount of toothpastes per brushing	Frequency of tooth brushing
Min – Vračar	0.089	T/B	without fluoride	pea-sized*	once daily
Min – Novi Beograd	0.135	T	without fluoride	pea-sized	once daily
Max – Vračar	0.625	T	500 mg/kg	tooth brush sized**	twice daily
Max – Novi Beograd	0.322	T/B	1450 mg/kg	pea-sized	once daily

T – tap water; T/B – tap and bottled water; *a pea-sized amount of toothpaste is equivalent to the mass of 0.25 g; ** a tooth brush sized amount of toothpaste is equivalent to the mass of 0.75 g [14].

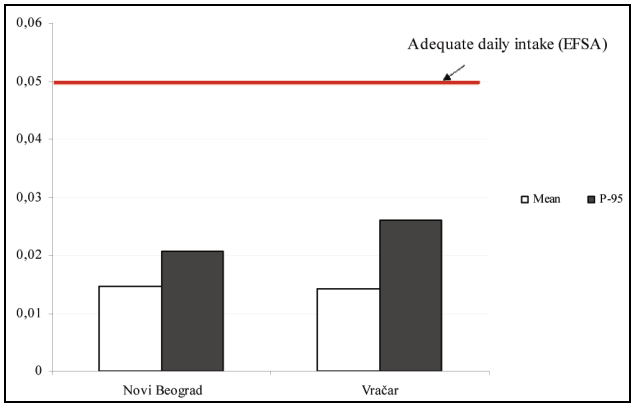

Fig. 2 – Mean and P-95 of the daily fluoride intake from drinking water and toothpaste in the 3-year-olds from two Belgrade municipalities, Novi Beograd and Vračar (mg/kg/day) in comparison with adequate daily intake according to the European Food Safety Authority – EFSA (0.05 mg/kg, based on all fluoride sources).

Figure 1 shows the mean daily fluoride intake by water, toothpaste and the total intake by these sources in Vračar and Novi Beograd, while Figure 2 gives mean and P-95 values for total fluoride daily intake per kilogram body weight in the investigated municipalities in Belgrade. In Novi Beograd, the fluoride intake was somewhat higher through water, in Vračar through toothpaste, although the difference was not statistically significant. A significant difference was obtained only between consumed water in Vračar and Novi Beograd. The total fluoride daily intake was in the range of 0.089 to 0.625 mg/day for children living in Vračar and 0.135 to 0.322 mg/day for children from Novi Beograd, corresponding to 0.0143 mg/kg/day (mean value) and 0.026 mg/kg/day (P-95), and 0.0146 mg/kg/day (mean value) and 0.020 mg/kg/day (P-95), respectively (Figure 2). No significant difference in the total daily fluoride intake among three-year-olds from the two Belgrade municipalities was found (Figure 2).

Table 3 presents the lowest and highest calculated daily fluoride intake from drinking water and toothpaste in 3-year-olds in Belgrade. The lowest calculated daily fluoride intake for both municipalities (0.089 mg/day and 0.135 mg/day in Vračar and Novi Beograd, respectively) was in children who use non-fluoride toothpaste. In Vračar, the highest daily fluoride intake (0.625 mg/day) was calculated for the children who used fluoride toothpaste (500 mg/kg), brushing teeth two times a day and applied a toothbrush-sized amount of the toothpaste per brushing. In other investigated municipalities, the highest daily fluoride intake was two times lower than the values in Vračar (0.322 mg/day) and the result was obtained for the children who used a toothpaste with fluoride (1,450 mg/kg) and applied pea-sized toothpaste per brushing, but brushed the teeth only once day (Table 3).

Discussion

In this study, daily fluoride intake was estimated in 3-years-old children in Belgrade on the basis of the content of fluoride in drinking water and toothpaste. Although fluoride can be ingested by various sources (water, drinks, food, toothpaste, supplements, etc.), drinking water and toothpaste represent the most important sources of fluoride intake in preschool children [27]. When containing the optimal fluoride levels of 0.5–1 mg/L as declared by the World Health Organization (WHO), drinking water is the main contributor to the total daily fluoride intake [4–6]. In many countries, drinking water is fluoridated, although not in our country. Furthermore, the use of bottled water is becoming considerably more common, both in the world and in our country, but nonetheless many of the brands do not declare fluoride content. The results of this study show that the non-carbonated bottled waters consumed by children have levels of fluoride in the range from 0.077 to 0.185 mg/L which are significantly below the recommended ones. The results of the conducted questionnaire show that the majority of children consume both bottled and tap water, tap water in both locations also containing rather low levels of fluoride. These results are in accordance with other recent studies conducted in our country, which indicate low fluoride levels in tap and bottled non-carbonated drinking water, with the exception of a minor area with endemic fluorosis [28–32]. Daily fluoride intake could not be significantly increased by food intake, since food is prepared with poor fluoride level water [5, 17]. Therefore, in areas with a deficit of fluoride in drinking water, toothpaste is an important source of this anion [21]. Besides, the toothpaste has an important role in caries prevention, not only due to fluoride content but also due to its local action on the tooth surface [18, 33]. The local availability of fluoride from the toothpaste was shown to prevent caries by primarily three mechanisms: inhibiting demineralization of tooth enamel; enhancing remineralization of tooth enamel prior to lesion progression; and inhibiting the enzyme activity of cariogenic bacteria [34].

Data obtained from the questionnaire show that the majority of children in Novi Beograd (85.7%) and Vračar (70.8%) use toothpaste with fluorides ranging from 500 mg/kg to 1450 mg/kg while a small number of children brush their teeth with a toothpaste without fluoride. However, it should be emphasised that the obtained levels of fluoride in the toothpastes were under the levels recommended by the EAPD and national protocol in Serbia – 1,000(+) mg/kg for children 2 to 6 years old [17, 18]. Similar results were obtained from toothpastes for children in Chile [35], although in another South American country Brazil fluoride levels > 1,000 mg/kg was determined [21, 36].

Furthermore, fluoride intake depends on the amount of used toothpaste as well as on the frequency of tooth brushing. The results show that most of the children (Vračar 63.6% and Novi Beograd 100%) use the amount of toothpaste corresponding to pea-sized amount of toothpaste, the amount that is in accordance with the national protocol [18]. When considering the frequency of brushing, two-times brushing was reported for the half of investigated children in Vračar, while in Novi Beograd all children brush their teeth once per day.

Estimated total daily fluoride intake through tap and/or non-carbonated bottled water and toothpaste in 3-year-olds in investigated municipalities indicates no significant difference between fluoride intakes in children living in two different municipalities. A significant difference was observed only for fluoride intake via water, and the 3-year-olds in Novi Beograd had a higher intake by water than children in Vračar. Fluoride intake by toothpaste does not differ between the examined groups indicating no influence of the frequency of teeth brushing on the total fluoride intake. That could be explained by higher levels of fluoride in toothpastes used in Novi Beograd. However, the obtained values for total fluoride intake are lower than optimal fluoride intake (0.7 mg/day) for this age, and two to six times lower than tolerable upper intake level 1.3 mg day^{-1} for children up to 4 years of age proposed by the Food and Nutrition Board, U.S. National Academy of Sciences Institute of Medicine [5, 22].

The minimum observed fluoride intake level was 0.089 mg/day in Vračar and 0.135 mg/day in Novi Beograd, while the highest fluoride intake 0.625 mg/day was estimated for Vračar and 0.322 mg/day for Novi Beograd. Low levels of fluoride intake in both municipalities can be explained by low fluoride levels in consumed water and used toothpastes without fluorides. On the other hand, the application of fluoride toothpaste with fluoride content of 1450 mg/kg resulted in the higher total fluoride intake 0.322 mg/day for Novi Beograd. The highest level of total fluoride level (0.625 mg/day) estimated for Vračar can be explained by 2-times brushing with bigger amounts of applied toothpaste (a toothbrush-sized – 0.75 g).

Furthermore, in this study the estimated daily fluoride intake per kg b.w. confirm very low fluoride intake by water and toothpaste, being significantly below the adequate intake level from all sources, including non-dietary sources (0.05 mg/kg/day, EFSA) [5]. Contrary to our results, Omena et al. [24] estimated a daily fluoride intake of 0.128 mg/kg/day in 18 to 36- months-old children consuming water with 0.94 mg/L and toothpastes with > 1,000 mg/kg fluoride. Other literature data also estimated daily fluoride intake through water with optimal and high fluoride concentration, fluoridated food (milk, salts and other dietary sources) and dental products above the recommended values, indicating the opposite risk of fluoride overdosage [37–40].

According to our investigation, the total intake of fluoride in young children, estimated on the basis of consumption water with low fluoride content and toothpaste application is far below the levels known to be beneficial for caries prevention. Moreover, these results indicate that children aged 3 years can safely use the toothpaste with a maximum fluoride concentration of 1450 mg/kg, especially as tooth brushing with fluoride toothpaste is a fundamental measure for the primary prevention of early childhood caries [41]. These findings can serve as a starting point for developing an optimal caries prevention national plan, in addition to other measures as well as patient/parents education, dietary advice, use of nonfluoride caries-preventive agents (xylitol lozenges, sucrose-free chewing gum, chlorhexidine, etc.) and periodic clinical examinations [17, 42].

Conclusion

Based on these preliminary results it can be concluded that the daily fluoride intake by drinking water and toothpaste in children aged 3 years on the territory of two Belgrade municipalities is significantly lower than the adequate intake level thus contributing to the occurrence of dental caries. Further research is needed to cover all children of preschool age from 2 to 7 years of age and other municipalities in Belgrade and elsewhere in Serbia in order to obtain more comprehensive information on the assessment of fluoride intake in children.

Conflicts of interest

The authors declare no conflict of interest.

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R E F E R E N C E S

1. *Petersen PE, Lennon MA.* Effective use of fluorides for the prevention of dental caries in the 21st century: The WHO approach. Community Dent Oral Epidemiol 2004; 32(5): 319–21.
2. *Everett ET.* Fluoride's effects on the formation of teeth and bones, and the influence of genetics. J Dent Res 2011; 90(5): 552–60.
3. *Clark MB, Slayton RL.* Fluoride use in caries prevention in the primary care setting. Pediatrics 2014; 134(3): 626–33.
4. *World Health Organization (WHO).* Guidelines for Drinking-water Quality. 4th ed. Geneva: World Health Organization; 2011.
5. *European Food Safety Agency (EFSA).* Panel on Dietetic Products, Nutrition and Allergies. Scientific opinion on Dietary Reference Values for fluoride. EFSA J 2013; 11(8): 3332.
6. *Fawell J, Bailey K, Chilton J, Dahi E, Fewtrell L, Magara Y.* Fluoride in Drinking-water. U: WHO Water Series. ISBN: 1900222965. London, UK: World Health Organization, IWA Publishing; 2006.
7. *European Food Safety Authority (EFSA).* Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the Tolerable Upper Intake Level of Fluoride. EFSA J 2005; 192: 1–65.
8. *Agency for Toxic Substances and Disease Registry* (ATSDR). Toxicological Profile for Fluorides, Hydrogen Fluoride, and Fluorine. Atlanta, Georgia: U. S. Department of Health and Human Services, Public Health Service; 2013.
9. *Pereira AC, da Cunha FL, Meneghim MC, Werner CW.* Dental caries and fluorosis prevalence study in a nonfluoridated Brazilian community: Trend analysis and toothpaste association. ASDCJ Dent Child 2000; 67(2): 132–5.
10. *Wong MC, Clarkson J, Glenny AM, Lo EC, Marinho VC, Tsang BW, Worthington HV.* Cochrane reviews on the benefits/risks of fluoride toothpastes. J Dent Res 2011; 90(5): 573–9.
11. *Ekambaram M, Itthagarun A, King NM.* Ingestion of fluoride from dentifrices by young children and fluorosis of the teeth: A literature review. J Clin Pediatr Dent 2011; 36(2): 111–21.
12. *Lockner F, Twetman S, Stecksén-Blicks C.* Urinary fluoride excretion after application of fluoride varnish and use of fluoride toothpaste in young children. Int J Paediatr Dent 2017; 27(6): 463–8.
13. *Evans RW, Stamm JW.* An epidemiologic estimate of the critical period during which human maxillary central incisors are most susceptible to fluorosis. J Public Health Dent 1991; 51(4): 251–9.
14. *Ellwood RP, Cury JA.* How much toothpaste should a child under the age of 6 years use?. Eur Arch Pediatric Dent 2009; 10(3): 181–7.
15. *Kobayashi CA, Belini MR, Italiani FM, Pauleto AR, Araújo JJ, Tessarolli V,* et al. Factors influencing fluoride ingestion from dentifrice by children. Community Dent Oral Epidemiol 2011; 39(5): 426–32.
16. *American Academy of Pediatric Dentistry* (AAPD). Guideline on Fluoride Therapy. Clin Pract Guidelines 2014; 37(6): 177–9.
17. European Academy of Paediatric Dentistry (EAPD). Guidelines on the use of fluoride in children: An EAPD policy document. Eur Arch Pead Dent 2009; 10(3): 129–35.
18. Ivanović M, Carević M, Marković D, Vulićević Z, Stevanović R, Petrović V, et al. The protocol for the use of fluoride in caries prevention in children and youth in Serbia. In: Ivanović M, Carević M, Marković D, Vulićević Z, Stevanović R, Petrović V, et al. Protocols in dentistry. Belgrade: University of Belgrade, School of Dentistry. 2009. p. 21–47. (Serbian)
19. *da Silveira Moreira, R.* Epidemiology of dental caries in the world. In: *Virdi M,* editor. Oral Health Care – Pediatric, Research, Epidemiology and Clinical Practices. Rijeka: InTech; 2012. Available from: http://www.intechopen.com/books/oral-health-care-pediatric-research-epidemiology-and-clinical-practices/epidemiology-of-dental-caries-in-the-world [accessed 2016 December 28].
20. *Tušek I, Tušek J, Ukropina S.* Risk factors associated with early childhood caries in autonomous province of Vojvodina, Republic of Serbia. Vojnosanit Pregl 2017; 74(6): 511–9.
21. *de Almeida BS, da Silva Cardoso VE, Buzalaf MA.* Fluoride ingestion from toothpaste and diet in 1- to 3-year-old Brazilian children. Community Dent Oral Epidemiol 2007; 35(1): 53–63.
22. Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington (DC): National Academies Press (US); 1997.
23. *Đukić-Ćosić D, Antonijević E, Vićentijević N, Malešević N, Ćurčić M, Dilber S,* et al. Validation of a questionnaire for assessing fluoride intake in preschool children. MD-Medical Data 2017; 9(2): 95–100. (Serbian)
24. *Omena LM, Silva MF, Pinheiro CC, Cavalcante JC, Sampaio FC.* Fluoride intake from drinking water and dentifrice by children living in a tropical area of Brazil. J Appl Oral Sci 2006; 14(5): 382–7.
25. *U.S. Environmental Protection Agency* (EPA). Guidelines for exposure assessment. Fed Reg 1992; 57: 22887–938.
26. *Food and Nutrition Board* (FNB). Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington DC: Institute of Medicine National Academy Press; 2004.
27. *Erdal S, Buchanan SN.* A quantitative look at fluorosis, fluoride exposure, and intake in children using a health risk assessment approach. Environ. Health Perspect 2005; 113(1): 111–7.
28. *Glavaški M, Ćurčić M, Đukić-Ćosić D, Plamenac-Bulat Z, Matović V.* Fluoride content in bottled mineral waters of Serbia. Arh Farm 2009; 59: 321–30. (Serbian)
29. *Petrović TM, Zlokolica Mandić M, Veljković N, Papić PJ, Poznanović MM, Stojković JS,* et al. Macro and microelements in bottled and tap waters of Serbia. Hem Ind 2012; 66(1): 107–22. (Serbian)
30. *Mandinić Z, Ćurčić M, Antonijević B, Charles P, Lekić CP, Carević M.* Relationship between fluoride intake in Serbian children living in two areas with different natural levels of fluorides and occurrence of dental fluorosis. Food Chem Toxicol 2009; 47(6): 1080–4.
31. *Mandinić Z, Ćurčić M, Antonijević B, Carević M, Mandić J, Djukić-Ćosić D,* et al. Fluoride in drinking water and dental fluorosis. Sci Total Environ 2010; 408(17): 3507–12.
32. *Antonijević E, Mandinic Z, Curcic M, Djukic-Cosic D, Milicevic N, Ivanovic M,* et al. "Borderline" fluorotic region in Serbia: Correlations among fluoride in drinking water, biomarkers of exposure and dental fluorosis in schoolchildren. Environ Geochem Health 2016; 38(3): 885–96.
33. *Twetman S, Axelsson S, Dahlgren H, Holm A, Källestål C, Lagerlöf F,* et al. Caries-preventive effect of fluoride toothpaste: a systematic review. Acta Odontol Scand 2003; 61(6): 347–55.
34. *Weyant RJ, Tracy SL, Anselmo T, Beltrán-Aguilar ED, Donly KJ, Frese WA,* et al. Topical fluoride for caries prevention: executive summary of the updated clinical recommendations and supporting systematic review. J Am Dent Assoc 2013; 144(11): 1279–91.
35. *Giacaman RA, Carrera CA, Muñoz-Sandoval C, Fernandez C, Cury JA.* Fluoride content in toothpastes commercialized for children in Chile and discussion on professional recommendations of use. Int J Paediatr Dent 2013; 23(2): 77–83.

36. *Cury JA, Oliveira MJ, Martins CC, Tenuta LM, Paiva SM.* Available fluoride in toothpastes used by Brazilian children. Braz Dent J 2010; 21(5): 396–400.
37. *Oganessian E, Ivancakova R, Lencova E, Broukal Z.* Alimentary fluoride intake in preschool children. BMC Public Health 2011; 11: 768.
38. *Nascimento HA, Soares FJ, Granville-Garcia AF, Brito CE, Almeida CA, Sampaio FC.* Estimation of toothpaste fluoride intake in preschool children. Braz Dent J 2013; 24(2): 142–6.
39. *Cochran JA, Ketley CE, Duckworth RM, van Loveren C, Holbrook WP, Seppä L,* et al. Development of a standardized method for comparing fluoride ingested from toothpaste by 1. 5-3. 5-year-old children in seven European countries. Part 2: Ingestion results. Community Dent Oral Epidemiol 2004; 32(Suppl 1): 47–53.
40. *Levy SM, Broffitt B, Marshall TA, Eichenberger-Gilmore JM, Warren JJ.* Associations between fluorosis of permanent incisors and fluoride intake from infant formula, other dietary sources and dentifrice during early childhood. J Am Dent Assoc 2010; 141(10): 1190–201.
41. *Kumar S, Tadakamadla J, Johnson NW.* Effect of Toothbrushing Frequency on Incidence and Increment of Dental Caries: A Systematic Review and Meta-Analysis. J Dent Res 2016; 95(11): 1230–6.
42. *Rethman MP, Beltrán-Aguilar ED, Billings RJ, Burne RA, Clark M, Donly KJ,* et al. Nonfluoride caries-preventive agents. Executive summary of evidence-based clinical recommendations J Am Dent Assoc 2011; 142(9): 1065–71.

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Urinary transferrin as an early biomarker of diabetic nephropathy

Urinarni transferin kao rani marker dijabetesne nefropatije

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Abstract

Background/Aim. Diabetic nephropathy is one of the leading cause of chronic kidney disease and end-stage renal disease. It occurs in 20%–40% of patients with diabetes mellitus and microalbuminuria is still considered as the first sign of diabetic nephropathy. Low sensitivity and specificity of microalbuminuria lead to more sensitive biomarkers that may be used to detect diabetic nephropathy at an earlier stage with a higher accuracy. This study was carried out to determine whether urinary transferrin can serve as an indicator of diabetic nephropathy. **Methods.** Our study included 80 type 2 diabetic patients who were classified into two groups: group 1 – normoalbuminuric patients (albumin excretion up to 30 mg/d); group 2 – microalbuminuric patients (albumin excretion from 30–300 mg/d), and 10 healthy controls. All patients were older than 18, having the diabetic disease more than one year, glomerular filtration rate more than 60 mL/min/1.73 m^2. Serum creatinine, glycosylated hemoglobin (HbA1c), and concentration of transferrin in the 24 h urine samples as well as in spot urine were measured using a highly sensitive one-step sandwich enzyme immunoassay kit. **Results.** Urinary transferrin was significantly higher in the microalbuminuric patients than in the normoalbuminuric ones and healthy control subjects. When comparing these goups according to the urinary transferrin concentration, we found a statistically significant positive correlation r = 0.584 ($p < 0.001$). There was no correlation between level of urinary transferrin and glycoregulation, and no correlation was found between transferrin and duration of diabetes. **Conclusions.** The results from this study provide the evidence that the urinary transferrin levels could be used as an early marker of diabetic nephropathy.

Key words:
diabetes mellitus; diabetic nephropathies; albuminuria; biomarkers; transferrin.

Apstrakt

Uvod/Cilj. Dijabetesna nefropatija predstavlja jedan od vodećih uzroka hronične bubrežne bolesti i terminalne bubrežne insuficijencije. Zastupljena je kod 20%–40% bolesnika sa dijabetes melitusom, a kao prvi znak dijabetesne nefropatije još uvek se smatra mikroalbuminurija. Niska senzitivnost i specifičnost mikroalbuminurije su doveli do ispitivanja novih urinarnih biomarkera koji bi mogli biti rani pokazatelji postojanja dijabetesne nefropatije. Ova studija spovedena je da bi se utvrdilo da li urinarni transferin može biti rani marker dijabetesne nefropatije. **Metode.** U našu studiju bilo je uključeno 80 bolesnika sa tipom 2 dijabetesa, podeljenih u dve grupe: grupa 1 – normoalbuminurični bolesnici (ekskrecija albumina do 30 mg/dan); grupa 2 – mikroalbuminurični (ekskrecija albumina od 30–300 mg/dan) i 10 zdravih osoba. Svi bolesnici bili su stariji od 18 godina, imali su dijabetes melitus duže od jedne godine i jačinu glomerulske filtracije veću od 60 mL/min/1,73 m^2. Svim bolesnicima određivan je nivo serumskog kreatinina, glikozilovanog hemoglobina i transferina u urinu. Koncentracija transferina određivana je u 24 časovnom uzorku urina i u prvom jutarnjem urinu primenom visoko senzitivnog ELISA kita. **Rezultati.** Koncentracija urinarnog transferina bila je značajno veća kod bolesnika koji su imali mikroalbuminuriju u poređenju sa bolesnicima koji su bili normoalbuminurični i zdravim osobama, a Pearson-ov koeficijent korelacije bio je r = 0,584 ($p < 0,001$). Nismo dobili povezanost između nivoa urinarnog transferina i glikoregulacije, kao ni nivoa transferina i dužine trajanja dijabetesa. **Zaključak.** Rezultati ove studije pokazuju da bi urinarni transferin mogao biti rani marker dijabetesne nefropatije.

Ključne reči:
dijabetes melitus; dijabetesne nefropatije; albuminurija; biološki pokazatelji; transferin.

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Introduction

Diabetes mellitus (DM) is a chronic disease whose incidence and prevalence show a steady increase. According to the International Diabetes Federation about 415 million people suffer from diabetes around the world, and it is estimated that by 2040 the number of people with diabetes will be around 642 million, with prevalence of 10% [1]. An increasing number of diabetic patients, mostly with the type 2 diabetes (90%) is associated with enhanced rate of diabetic complications, including diabetic kidney disease [2]. Diabetes is considered a leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD). Costs of care for patients with diabetic kidney disease (DKD) are extremely high, especially after they enter ESRD, and it is necessary to establish the diagnosis of diabetic nephropathy as soon as possible [3, 4]. Microalbuminuria (MA) is generally considered to be the earliest non-invasive marker of kidney damage and it was described for the first time in 1960s [5]. Microalbuminuria is defined as persistent elevation of albumin in the urine, of 30–300 mg/day, and it is generally considered the earliest non-invasive marker for the development of diabetic nephropathy (DN), even though the specificity and sensitivity of MA are limited [6, 7]. Some patients with diabetes mellitus progress to DN even if urinary albumin levels are in the normal range, indicating that albuminuria is not the perfect marker for the early detection of DN [8, 9]. Recent studies have shown that some relevant biomarkers associated with DN were found and they potentially could be used to predict DN or progression of the disease [10]. Several different markers of tubular and glomerular damage were investigated to discover DN in its early phase and to start therapy as soon as possible [11].

Urinary transferrin is considered to be an early marker of glomerular injury in diabetic patients. It is a protein, slightly higher molecular weight than albumin (76.5 kDa). Due to its low molecular weight and its ionic load, it filters easily through the glomerular membrane [12]. Some previous studies have shown that increased urinary transferrin excretion can be reported before MA in the normoalbuminuric patients with DM type 2. Because of that, urinary transferrin is considered to be a more sensitive marker of glomerular damage in the diabetic patients [13]. Excretion of transferrin was not associated with glycemic control (hemoglobin A1c), but some studies showed that urinary transferrin concentration was higher in the patients with diabetic retinopathy [14]. The aim of this study was to determine if urinary transferrin can be classified into a group of early biomarkers of DN.

Methods

This cross-sectional study was carried out between September 2015 and December 2016, with the aim to investigate the correlation between MA and urinary transferrin in DN. The study was approved by the Ethics Committee of the Military Medical Academy, Belgrade, Serbia and written informed consent was taken from all patients involved. Eighty patients with type 2 diabetes mellitus (DM2) with duration of the disease for one year or more, estimated glomerular filtration rates more than 60 mL/min/1.73 m^2, and without albuminuria were included in the study. The patients with overt albuminuria (> 300 mg/day), previous renal diseases, urinary tract infection in the last 4 weeks, the use of nephrotoxic drugs, systemic disease, malignant diseases except for basocellular skin carcinoma, were excluded.

The selected patients were studied in detail for the history and physical examination, including ultrasonography of the kidney. Age, gender, duration of diabetes mellitus, weight, height, blood pressure and smoking habits were noted too. The body mass index (BMI) was calculated according to the formula based on the height and weight measurements of the patients. The blood samples were taken after overnight fasting for at least 8 hours and the following parameters were analyzed: serum level of glycaemia, urea, creatinine, glycated hemoglobin (HbA1C). Glomerular filtration rate (GFR) was calculated based on the CKD-EPI formula. [GFR = 141 × min (Scr /κ, 1) α × max (Scr /κ, 1)-1.209 × 0.993 years × 1.018 (for women) = mL/min/1.73 m^2] [15]. Transferrin concentration (ng/mL) and transferrin to creatinine ratio (mg/g of creatinine) were determined in a spot morning urine sample, and albuminuria (30 mg/day or greater) measured in a 24h urine collected on the subsequent day. All samples of urine were immediately processed within 4 hours of collection to ensure optimal protein stability. Urine was centrifuged (1000 × g, 20 min), then divided into 1.5 mL aliquots and frozen at -80°C until the analysis. The levels of urinary transferrin were determined by the commercially available ELISA kits from Elabscience Biotechnology Co., Ltd. Minimum and detectable concentration for urinary transferrin was 1.56 ng/mL.

Statistics

Statistical analyses were performed using the Statistical Package for the Social Science (SPSS) version 19.0. Basic descriptive statistical parameters were presented by the measures of central tendency (mean and median), a measure of variability (standard deviation and variation interval) and were expressed in percentages. To compare continuous variables, the Student's *t*-test was used for independent samples or the Mann-Whitney test, depending on the normality of distribution, which was checked by the Kolmogorov-Smirnov test. For comparison of frequencies for categorical variables, the χ^2-test was used. A statistical hypothesis was tested at 0.05 level of significance, and probability (*p*) value less than 0.05 was regarded statistically significant.

Results

Our study included 80 type 2 diabetic patients, 44 (55%) males and 36 (45%) females, mean age 59.85 ± 8.87 years (range 38–73 years). Prevalence of MA was 41.25% (33 patients) and 58.75% (47 patients) were normoalbuminuric. Among the patients with MA 17 (51.52%) were males and 16 (48.48%) were females. The average duration of diabetes was 13.29 ± 7.69 years, and the average estimated GFR was 86.86 ± 14.18. There were no significant differences in baseline clinical characteristics among examined groups (Table 1).

Table 1

Baseline clinical characteristics of type 2 diabetic patients according to the levels of urinary albumin

Characteristics of the patients	All patients (n = 80)	Normoalbuminuric (n = 47)	Microalbuminuric (n = 33)	Healthy persons (n = 10)	*p*
Gender (M/F), n/n	44/36	27/20	17/16	5/5	0.276
Age (years), mean ± SD	59.85 ± 8.871	60.49 ± 8.73	58.94 ± 9.13	54 ± 10.59	0.014
Duration of DM (Years), mean ± SD	13.29 ± 7.69	13.34 ± 7.74	13.21 ± 7.73	n/a	0.942
BMI (kg/m^2), mean ± SD	27.36 ± 4.42	26.64 ± 3.56	28.38 ± 5.31	25.73 ± 4.77	0.325
Current smoker (%), mean ± SD	39 (48.8)	26 (55.3)	13 (39.4)	3 (30)	
Systolic BP (mmHg), mean ± SD	134.60 ± 14.08	133.47 ± 12.87	136.21 ± 15.71	122 ± 17.02	0.411
Diastolic BP (mmHg), mean ± SD	81.56 ± 7.53	80.96 ± 7.42	82.42 ± 7.72	75.5 ± 10.39	0.389
Serum creatinine (μmol/L), mean ± SD	75.38 ± 15.04	76.64 ± 15.97	73.58 ± 13.64	73.6 ± 7.6	0.360
GFR (mL/min /1.73 m^2), mean ± SD	86.86 ± 14.18	85.78 ± 13.55	88.39 ± 15.12	92.84 ± 9.06	0.430
HbA1c (%), mean ± SD	7.59 ± 1.34	7.25 ± 1.15	8.07 ± 1.45	4.93 ± 0.3	0.074

DM – diabetes mellitus; BMI – body mass index; BP – blood pressure; HbA1c – hemoglobin A1c; GFR – glomerular filtration rate; SD – standard deviation.

Table 2

Correlation of transferrinuria and microalbuminuria

Transferrin concentration (μg/gC$_r$)	Microalbuminuric patients (n = 33) (≥ 30 mg/24h)	Normoalbuminuric patients (n = 47) (< 30 mg/24h)	Perason's *r*	*p*
	mean ± SD	mean ± SD		
Transferrin concentration 24h urine	91.76 ± 68.45	22.56 ± 31.46	0.489	< 0.001
Transferrin concentration spot urine	85.07 ± 56.54	25.63 ± 29.85	0.354	< 0.001

C$_r$ – creatinine; Pearson's test, *r* – correlation coefficient; SD – standard deviation.

Urinary transferrin concentration in spot samples and urinary transferrin concentrations in 24h urine samples showed significant linear correlation, therefore, we used results from spot urine samples for further analyses.

The mean concentration of urinary transferrin in the MA patients was 85.07 ± 56.54 μg/gC$_r$, and for the normoalbuminuric patients it was 25.63 ± 29.85 μg/gC$_r$. We found a statisticaly significant correlation in the transferrin concentration between these two groups (Table 2).

Table 3

Correlation of transferinuria with independent variables

Parameters	All patients (n = 80)	Pearson's *r*
	mean ± SD	
Age (years)	59.85 ± 8.87	0.003
BMI (kg/m^2)	27.3 ± 4.42	0.053
HbA1c (%)	7.59 ± 1.34	0.132
Duration of DM (years)	13.29 ± 7.69	0.127
Microalbuminuria	40.42 ± 40.89	0.584

Pearson's test, *r* – correlation coefficient; SD – standard deviation; BMI – body mass index; HbA1c – hemoglobin A1c; DM – diabetes mellitus.

The correlation analysis for the concentration of urinary transferrin with independent variables is shown in Table 3. Among all variables, we found significant correlation only with MA (Table 3).

Diabetic retinopathy was found in 24 (30%) patients. Those patients had significantly higher urinary transferrin levels, albumin excretion and duration of diabetes.

Sensitivity and specificity of urinary transferrin concentrations expressed as an area under the reciever operating characteristics (ROC) curve (AUC), and it was 87.1%, with the sensitivity 81.8%, and specificity 80.9% [95% confidence interval (CI) – from 0.796 up to 0.945; $p < 0.001$] (Figure 1).

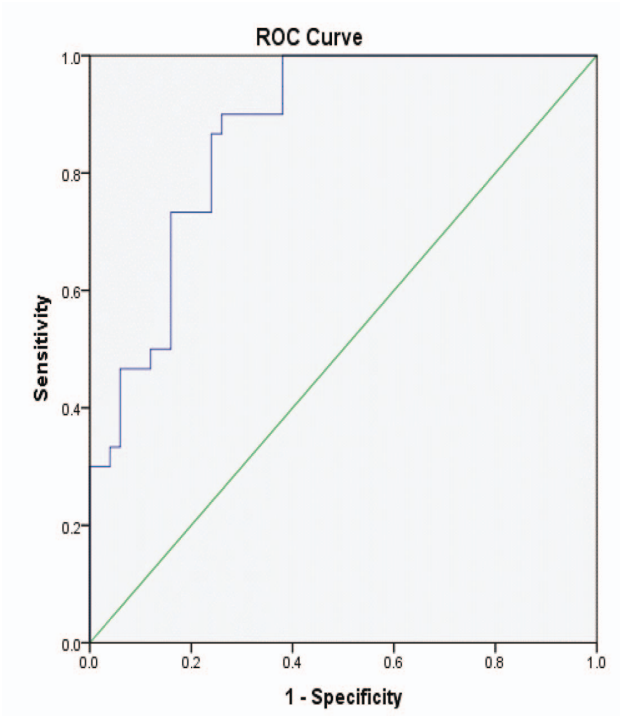

Fig. 1 – Sensitivity and specificity of urinary transferrin concentration.
ROC – receiver operating characteristic.

Discussion

Albuminuria is considered as a marker of kidney (glomerular) damage, and the first clinical indicator of DN presence [16]. Even today it is a clinically useful tool for predicting the outcome and for monitoring a response to the therapy [11]. Discordance between the presence of albuminuria and the decline in renal function is crucial point of clinical significance of albuminuria. The presence of albuminuria is not mandatory in all patients with reduced GFR. Perkins et al. [9] reported the development of advanced CKD (GFR < 60 mL/min 1.73 m^2) without a concomitant progression of albuminuria in the type 1 diabetic patients. Chen et al. [17] compared several studies from 1977 to the present and showed that a portion of diabetic patients with normoalbuminuria had progressive decline in renal function, referred to nonalbuminuric DN. In different studies, the number of nonalbuminuric diabetic kidney disease was from 21.8% reported by Boronat et al. [18] to 56.6% reported in 2014 by Penno et al. [19]. Nonalbuminuric renal impairment was not associated with HbA1c and retinopathy, but some studies found that gender is correlated with nonalbuminuric renal impairment. That is why we need a new biomarker with higher sensitivity and specificity for an earlier detection of DN and more accurate prediction of the progression to ESRD. Therefore, we analyzed urinary transferrin as a biomarker of glomerular injury implicated in the early DN, in nonalbuminuric diabetic patients.

The results of our study showed that increased urinary excretions of transferrin was higher in the patients with MA than in the normoalbuminuric type 2 diabetic patients. We found statistically significant correlation between concentration of urinary transferrin and MA. This is in accordance with the results obtained by Narita et al. [13] who reported that increased urinary transferrin found in the diabetic patients independently of microalbuminuria could also predict the development of MA in the normoalbuminuric DM2 patients. In the 24-month follow-up study with the DM2 patients, Kazumi et al. [20] found that 31% of patients who had transferrinuria at baseline subsequently developed MA, compared with 7% of patients without transferrin excretion. They concluded that in the patients with type 2 diabetes without MA, increased urinary transferrin excretion may predict the development of MA. The same results were found by Kanauchi et al. [21] in the group of 60 DM2 patients. They presented a significant correlation between the urinary excretion of transferrin and albumin. Their findings indicate that urinary transferrin may be useful in detecting DN at an early stage. Al-Rubeaan et al. [22] described similar results in a cross-sectional study in the group of 467 DM2 patients.

Similarly to our results, O'Donnell et al. [23] found no correlation between urinary transferrin levels and glycemic control in the group of 40 DM2 patients at first day of the disease diagnosis and after 6 and 12 weeks of treatment. Urinary excretion rates of transferrin were measured, and they showed that urinary transferrin was not correlated with glycemic control. We found no correlation between the urinary transferrin levels and the duration of diabetes, as well.

Several studies found a relationship between excretion of urinary albumin and diabetic retinopathy, and the famous one was the Japanese study of Moriya et al. [24], which included 2,205 DM2 patients aged 40–70 years. We found similar results. The patients with retinopathy had significantly higher values of urinary transferrin excretion as well as higher levels of MA.

Our sensitivity and specificity analysis of urinary transferrin excretion showed that it could be more sensitive indicator of an early glomerular damage in diabetes mellitus than MA.

Conclusion

Urinary transferrin was significantly increased in DM2 patients with MA. It was independent of diabetes duration and glycemic control. According to our results, the level of urinary transferrin excretion could be used as an early biomarker of diabetic nephropathy.

REFERENCES

1. International Diabetes Federation (IDF). IDF Diabetes Atlas. 7th ed. 2015. Available from: https://www.idf.org/.../diabetes-atlas/13-diabetes-atlas-seventh-...
2. *Tuttle KR, Bakris GL, Bilous RW, Chiang JL, Boer IH, Goldstein-Fuchs J*, et al. Diabetic kidney disease: a report from an ADA Consensus Conference. Diabetes Care 2014; 37(10): 2864–83.
3. *Saran R, Li Y, Robinson B, Abbott KC, Agodoa LY, Ayanian J,* et al. US Renal Data System 2015 Annual Data Report: Epidemiology of Kidney Disease in the United States. Am J Kidney Dis 2016; 67(3 Suppl 1): Svii, S1–305.
4. *Kramer A, Pippias M, Stel VS, Bonthuis M, Abad Diez JM, Afentakis N,* et al. Renal replacement therapy in Europe: A summary of the 2013 ERA-EDTA Registry Annual Report with a focus on diabetes mellitus. Clin Kidney J 2016; 9(3): 457–69.
5. *Loeffler I.* Pathophysiology of Diabetic Nephropathy. In: *Wolf G*, editor. Diabetes and Kidney Disease. Verlag: Wiley-Blackwell; 2013. p. 45–61.
6. *Dragović T.* Microalbuminuria in diabetes: definition, identification techniques, and the significance of early recognition. Vojnosanit Pregl 2006; 63(12): 1027–32. (Serbian)
7. Redon J. Measurement of microalbuminuria: What the nephrologist should know. Nephrol Dial Transplant 2006; 21(3): 573–6.
8. *Dwyer JP, Parving H, Hunsicker LG, Ravid M, Remuzzi G, Lewis JB.* Renal Dysfunction in the Presence of Normoalbuminuria in Type 2 Diabetes: Results from the DEMAND Study. Cardiorenal Med 2012; 2(1): 1–10.
9. *Perkins BA, Ficociello LH, Roshan B, Warram JH, Krolewski AS.* In patients with type 1 diabetes and new-onset microalbuminuria the development of advanced chronic kidney disease may not require progression to proteinuria. Kidney Int 2010; 77(1): 57–64.
10. *Moresco RN, Sangoi MB, de Carvalho JA, Tatsch E, Bochi GV.* Diabetic nephropathy: Traditional to proteomic markers. Clin Chim Acta 2013; 421: 17–30.

11. *Dragović T, Ajdinović B, Hrvacević R, Ilić V, Magić Z, Anđelković Z*, et al. Angiotensin II type 1 receptor gene polymorphism could influence renoprotective response to losartan treatment in type 1 diabetic patients with high urinary albumin excretion rate. Vojnosanit Pregl 2010; 67(4): 273–8.
12. *McCormick CP, Konen JC, Shihabi ZK.* Microtransferrinuria and microalbuminuria. I. In the diabetic human. Clin Physiol Biochem 1990; 8(2): 53–8.
13. *Narita T, Hosoba M, Kakei M, Ito S.* Increased Urinary Excretions of Immunoglobulin G, Ceruloplasmin, and Transferrin Predict Development of Microalbuminuria in Patients With Type 2 Diabetes. Diabetes Care 2005; 29(1): 142–4.
14. *Hellemons ME, Kerschbaum J, Bakker SJ, Neuwirt H, Mayer B, Mayer G,* et al. Validity of biomarkers predicting onset or progression of nephropathy in patients with Type 2 diabetes: A systematic review. Diabet Med 2012; 29(5): 567–77.
15. *Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI,* et al. A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med 2009; 150(9): 604–12.
16. *Bakris GL, Molitch M.* Microalbuminuria as a risk predictor in diabetes: The continuing saga. Diabetes Care 2014; 37(3): 867–75.
17. *Chen C, Wang C, Hu C, Han Y, Zhao L, Zhu X*, et al. Normoalbuminuric diabetic kidney disease. Front Med 2017; 11(3): 310–8.
18. *Boronat M, García-Cantón C, Quevedo V, Lorenzo DL, López-Ríos L, Batista F,* et al. Non-albuminuric renal disease among subjects with advanced stages of chronic kidney failure related to type 2 diabetes mellitus. Ren Fail 2014; 36(2): 166–70.
19. *Penno G, Solini A, Bonora E, Fondelli C, Orsi E, Zerbini G*, et al. Clinical significance of nonalbuminuric renal impairment in type 2 diabetes. J Hypertens 2011; 29(9): 1802–9.
20. *Kazumi T, Hozumi T, Ishida Y, Ikeda Y, Kishi K, Hayakawa M*, et al. Increased urinary transferrin excretion predicts microalbuminuria in patients with type 2 diabetes. Diabetes Care 1999; 22(7): 1176–80.
21. *Kanauchi M, Nishioka H, Hashimoto T, Dohi K.* Diagnostic significance of urinary transferrin in diabetic nephropathy. Nihon Jinzo Gakkai Shi 1995; 37(11): 649–54.
22. *Al-Rubeaan K, Siddiqui K, Al-Ghonaim MA, Youssef AM, Al-Sharqawi AH, AlNaqeb D.* Assessment of the diagnostic value of different biomarkers in relation to various stages of diabetic nephropathy in type 2 diabetic patients. Sci Rep 2017; 7(1): 2684.
23. *O'Donnell MJ, Watson J, Martin P, Chapman C, Barnett AH.* Transferrinuria in Type 2 Diabetes: The Effect of Glycaemic Control. Ann Clin Biochem 1991; 28(Pt 2): 174–8.
24. *Moriya T, Tanaka S, Kawasaki R, Ohashi Y, Akanuma Y, Yamada N*, et al. Diabetic Retinopathy and Microalbuminuria Can Predict Macroalbuminuria and Renal Function Decline in Japanese Type 2 Diabetic Patients: Japan Diabetes Complications Study. Diabetes Care 2013; 36(9): 2803–9.

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Neurocutaneous flaps for soft tissue reconstruction of the knee, lower leg, ankle and foot: clinical experience with 32 patients

Neurokutani režnjevi za rekonstrukciju defekata mekih tkiva kolena, potkolenice, skočnog zgloba i stopala: kliničko iskustvo sa 32 pacijenta

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Abstract

Background/Aim. Neurocutaneous flaps (NF) are the type of fasciocutaneous flaps whose clinical application has increasingly grown over the years. They have become an indispensable step in the reconstructive ladder for the small and medium soft tissue defects of the lower leg and foot. The aim of this study was to analyse the results of the treatment of patients with lower extremity soft tissue defects caused by trauma, infection, tumour removal or unstable scar formation, which were reconstructed with a variety of NF. **Methods.** This retrospective study includes 32 consecutive patients with soft tissue defects of the lower limb, treated in the Clinical Centre of Vojvodina from January 2004 to April 2017. All the operations were performed in regional anaesthesia with pneumatic tourniquet. Design of the flap and length of the pedicle were determined by the size and position of the recipient site after necessary debridement. The flap was harvested, rotated and positioned in the defect region. The patients and flap data were summarized upon their collection. **Results.** The average age of the patients, mostly males (81.2%), was 46.7 years. Distally based sural flaps were used in a majority of patients (56.2%), followed by the distally based saphenous (21.9%), lateral sural (12.5%) and proximally based sural flaps (9.4%). Defects were most often localized on the distal third of the lower leg and on the ankle (53.1%). The most common indication for surgery were trauma (46.9%) and chronic infection (31.2%). A satisfactory coverage of the defect was achieved in all 32 patients with no flap loss. A partial necrosis of the flap due to prolonged venous congestion was noted in 3 (9.4%) patients, which were healed by second intention or with delayed skin grafting. Five (15.6%) patients developed a localised infection. The infection signs withdrew spontaneously in 2 cases and after a surgical revision in 3 cases, where osteitis of the tibia had persisted. One of them required the Ilizarov orthopaedic procedure after bone resection. **Conclusion.** NF proved to be a paramount alternative to free-flap reconstruction of the lower limb. Intensive clinical application can be explained by the fact that it is a less technically demanding and time consuming surgical procedure with no major source vessel sacrifice. The reliability and safety of their utilisation are confirmed by our clinical data.

Key words:
ankle, joint; foot; lower extremity; reconstructive surgical procedures; surgical flaps; treatment outcome.

Apstrakt

Uvod/Cilj. Neurokutani ostrvasti režnjevi su vrsta fasciokutanih režnjeva čija je klinička primena u stalnom porastu poslednjih godina. Oni zauzimaju značajno mesto u rekonstruktivnoj lestvici za pokrivanje malih i srednjih defekata mekih tkiva potkolenice i stopala. Cilj ovog rada bio je da se analiziraju rezultati lečenja pacijenata sa defektima mekih tkiva donjih ekstremiteta usled traume, infekcije, uklanjanja tumora ili nestabilnog ožiljka koji su rekonstruisani različitim vrstama neurokutanih režnjeva. **Metode.** U ovu retrospektivnu studiju bila su uključena 32 pacijenta sa mekotkivnim defektima donjih ekstremiteta, koji su lečeni u Kliničkom Centru Vojvodine od januara 2004. do aprila 2017. Sve operacije su izvedene u regionalnoj anesteziji pod Esmarhovom poveskom. Dizajn režnjeva i dužina peteljki bili su determinisani veličinom i pozicijom recipijentne regije nakon neophodnog debridmana. Režnjevi su podizani,

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rotirani i pozicionirani na mesto defekta. Podaci o režnjevima i pacijentima su neposredno beleženi i sumirani. **Rezultati.** Pacijenti su bili pretežno muškog pola (81,2%), prosečne starosti 46,7 godina. Distalno bazirani suralni režanj je korišćen kod većine pacijenata (56,2%), zatim distalno bazirani safenski (21,9%), lateralni suralni (12,5%) i proksimalno bazirani suralni režanj (9,4%). Lokalizacija defekta je kod većine pacijenata bila na distalnoj trećini potkolenice i skočnom zglobu (53,1%). Najčešće indikacije za operaciju su bile povreda (46,9%) i hronična infekcija (31,2%). Zadovoljavajuće pokrivanje defekta postignuto je kod sva 32 pacijenta, bez ijednog izgubljenog režnja. Delimična nekroza režnja usled produžene venske kongestije je zabeležena kod 3 (9,4%) pacijenta, a zarasla odloženo ili nakon postavljanja slobodnog kožnog transplantata. Lokalna infekcija je zabeležena kod pet (15,6%) pacijenata. Znaci infekcije su se spontano povukli kod dva pacijenta, a posle hirurške revizije kod tri, kao posledica rezidualnog osteitisa tibije. Jedan od njih je nakon resekcije kosti podvrgnut ortopedskoj proceduri po Ilizarovu. **Zaključak.** Neurokutani režnjevi su značajna alternativa slobodnim režnjevima za rekonstrukciju defekata na donjim ekstremitetima. Učestalost kliničke primene se može objasniti činjenicom da ova hirurška procedura nije tehnički i vremenski zahtevna, te da se pri tome ne kompromituje magistralnu arterijsku cirkulaciju. Naša klinička studija potvrđuje pouzdanost i sigurnost korišćenja ovih režnjeva.

Ključne reči:
skočni zglob; stopalo; potkolenica; hirurgija, rekonstruktivna, procedure; režnjevi, hirurški; lečenje, ishod.

Introduction

Reconstruction of soft tissue defects of the lower leg and foot is still a challenge for surgeons. Exposed tendons, bones or joints as a result of trauma, infection or tumour removal require an adequate soft tissue coverage. There are various reconstruction options that can be used such as local, pedicle and free flaps. Since they were introduced into clinical practice, neurocutaneous flap (NF) have become an excellent choice for solving distal lower leg, ankle and foot defects. Previously, these defects could only have been reconstructed with much more demanding and less reliable surgical procedures.

Neurocutaneous island flaps are the type of fasciocutaneous flaps whose clinical application has increasingly grown over the years. They became an indispensable step in the reconstructive ladder for the small and medium soft tissue defects of the lower extremity.

Pontén [1] first described fasciocutaneous flaps in 1981. The anatomical vascular basis of the axillary fasciocutaneous pedicled flap was demonstrated by Cormack and Lamberty [2] in 1984. Vascularization of NF is enabled by longitudinal chain-linked adipofascial plexus as well as the neurovascular axis around the sensitive nerves of the lower leg. In essence, the vessels accompanying the sensitive superficial nerves will allow skin flaps to survive. Nutrition of the distally based adipofascial pedicle flaps is ensured through a retrograde flow from the septocutaneous and musculocutaneous perforators of posterior tibial artery, anterior tibial artery and peroneal artery which were first described by Masquelet et al. [3] in 1992 and Hasegawa et al. [4] in 1994. Dominant vascularization of proximally based flaps is achieved due to direct cutaneous arteries that accompany sensitive nerves on their way through the lower leg. These arteries course subfascially in its proximal third and suprafascially in the distal two thirds of the calf [5].

The aim of this study was to summarize and analyse the treatment results of patients with lower extremity soft tissue defects caused by trauma, infection, tumour removal or unstable scar formation which were reconstructed with NF.

Methods

Data for this retrospective study were collected from the Clinic for Plastic and Reconstructive Surgery and the Clinic for Orthopaedic Surgery and Traumatology, Clinical Centre of Vojvodina from January 2004 to April 2017. This study, approved by the Ethics Board of the same institution, included 32 consecutive patients in whom we used the proximally and distally based sural, lateral sural and distally based saphenous flaps. All the obtained data were statistically processed upon their collection.

The Doppler ultrasound examinations were performed in all patients to evaluate vascularization and to identify the map of perforators. The operation started under regional anaesthesia with a patient in the prone position. Exsanguination was achieved with pneumatic tourniquet to enable better identification and dissection of the vital structures. Design of the flap and length of the pedicle were determined by the size and position of the recipient site after necessary debridement. Adipofascial pedicles were from 3 to 5 cm wide, surrounding the neurovascular bundle equally from each side to ensure the vascularization of the fasciocutaneous island. The raised flaps were rotated from 90^{o} to 180^{o} and placed over the defect through the subcutaneous tunnel in the majority of patients. The donor area was covered either with a split thickness skin graft or with a direct closure if the defect was less than 5–6 cm wide.

Anatomical considerations and surgical approach

Anatomical considerations and surgical technique for the flaps used in this clinical study are given below.

Lateral sural flap

Neurovascular basis of the lateral sural flap consists of the lateral sural nerve, the lateral cutaneous sural artery and two concomitant veins. The lateral sural artery is usually a direct branch of the popliteal artery, originating from the level of the lateral condyle of the tibia. From the popliteal fossa,

the artery courses subfascially alongside the lateral sural nerve, which is a branch of the peroneal nerve, and after 4–6 cm pierces the deep fascia. In its further course, it travels along the surface of the lateral head of the gastrocnemius muscle, forming a subfascial and suprafascial vascular chain along with the extrinsic vascular plexus of the lateral sural nerve. Terminal branches of the lateral sural artery anastomose with musculocutaneous and septocutaneus perforators of the peroneal artery in the middle and distal third of the lower leg [6].

This flap is an excellent solution for covering the soft tissue defects around the knee and proximal part of the lower leg [5]. The flap is elevated along the posterolateral side of upper two-thirds of the lower leg in the subfascial plane. The maximum size ranges from the tibial plateau to the lower margin of the gastrocnemius belly. The lateral sural artery and nerve should be in the mid axis of the flap. A surgical dissection begins from the distal edge of the flap and continues upwards. The pivot point of the fasciosubcutaneous pedicle is usually in the popliteal fossa [7].

Proximally and distally based sural flaps

The neurovascular basis of these flaps consists of the sural nerve and superficial sural artery with two concomitant veins for proximally based and the small saphenous vein for distally based pedicle. In most cases, the superficial (median) sural artery branches directly off from the popliteal artery or from the lateral sural artery. From the popliteal fossa, together with the sural nerve it passes between the two heads of the gastrocnemius muscle, pierces the fascia in the middle third of the lower leg, ending its course along the lateral side of the Achilles tendon and anastomosing with branches of the peroneal artery. The medial sural nerve originates from the tibial nerve and unites with a communicant branch of the lateral sural nerve in the suprafascial plane, forming a common sural nerve in the middle third of the lower leg [4]. The neurovascular axes of both flaps are the same. The proximally based flap circulation mainly depends on the median superficial sural artery together with the extrinsic vascular plexus of the sural nerve. The distally based flap circulation is achieved due to a retrograde flow from septocutaneus perforators of the peroneal artery which anastomoses with the superficial vascular network of the sural artery around the nerve [5].

Proximally based sural flap is a good alternative for the reconstruction of soft tissue defects around the knee and proximal half of the lower leg [8]. The flap is elevated along the posterior side of the upper two-thirds of the calf, from distal to proximal. The subfascial plane of dissection starts with ligation of the median sural artery, the sural nerve and lesser saphenous vein, all of which should be in the middle of the flap axis. The pivot point of the pedicle is usually on the midline of the popliteal skin crease [5].

The distally based sural flap has a widespread clinical use for reconstruction of defects in the distal part of lower leg, ankle and foot [4, 9]. The neurovascular axis extends across the middle of the calf and descends obliquely towards the posterior side of the lateral malleolus. The subfascial dissection begins on the boundary between the proximal and middle third of the calf downwards, with identification and ligation of the median sural artery, sural nerve and lesser saphenous vein. The pivot point of the adipofascial pedicle should be at least five centimetres above the lateral malleolus to preserve anastomosis with an important septocutaneous perforator of the peroneal artery [10].

Distally based saphenous flap

The neurovascular basis of the flap consists of the saphenous nerve, saphenous artery and great saphenous vein. The saphenous nerve is the largest cutaneous branch of the femoral nerve. It runs together with the great saphenous vein, downwards from the medial aspect of the knee to the medial aspect of the foot. The saphenous artery is a constant branch of the descending genicular artery which comes down the lower leg along and in front of the saphenous nerve. In the middle third of the lower leg it forms a vascular network around the nerve and dominantly anastomoses with intermuscular septal branches of the posterior tibial artery. The most distal cluster of perforator vessels around the medial malleolus provides a nutrient basis for the distally based pedicle [6, 11].

The flap is a good solution for soft tissue defect reconstructions around the ankle and heel. The axial line of the flap extends from the anterior margin of the medial malleolus to the medial epicondyle of the femur. Elevation begins at the proximal part of the fasciocutaneous island and includes the saphenous nerve and artery as well as the great saphenous vein. The dissection of the adipofascial pedicle continues into the subfascial plane up to the point of rotation which is usually 5–6 cm above the medial malleolus. The pivot point is determined by the location of the most distal anastomosis with perforator of the posterior tibial artery [12].

Results

The mean follow up period was 12 months (range: 6–18 months). Satisfactory coverage of the defect was achieved in all 32 patients with no flap loss. The average age of the patients, mostly males (81.2%), was 46.7 years. The largest size of the fasciocutaneous island was 17 x 10 cm, and the smallest one was 5 x 3.5 cm. The average time duration for elevation and flap placement was 1 h and 25 min. The patients usually stayed in the hospital for 5.5 days after the surgery.

The clinical summary of patient and flap data is shown in Table 1. The distally based sural flap was most frequently used (56%). Defects were most often localised on the distal third of the lower leg and ankle (53%). The most common indications for surgery were trauma (47%) and chronic infection (31%).

Venous congestion occurred in 4 distally based flaps (2 sural and 2 saphenous), which resulted in partial necrosis in 3 cases. In two of them, which had marginal necrosis, spontaneous wound healing was achieved upon conservative treatment. In the third one, a complete surface necrosis was developed. However, the residual adipofascial flap tissue was well vascularised, allowing a delayed free skin grafting to be performed.

Table 1

Clinical summary of patient and flap data

Characteristics	Values
Number of flaps	32
Age (years), mean (range)	46.7 (20–78)
Sex ratio, (male/female)	4.3
Male, n (%)	26 (81.2)
Female, n (%)	6 (19.8)
Localization, n (%)	
knee	4 (12.5)
proximal third of lower leg	2 (6.2)
medial third of lower leg	2 (6.2)
distal third of lower leg	12 (37.5)
ankle	5 (15.6)
heel	4 (12.5)
foot	3 (9.4)
Etiology, n (%)	
trauma	15 (46.9)
chronic infection	10 (31.2)
tumour	4 (12.5)
unstable scar	3 (9.4)
Type of flaps, n (%)	
distal sural	18 (56.2)
distal saphenous	7 (21.9)
lateral sural	4 (12.5)
proximal sural	3 (9.4)
Time of reconstruction, n (%)	
delayed	24 (75)
immediate	8 (25)
Size of the flap (cm^2), mean (range)	35 cm^2 (9–170)
Tunnelling of the pedicle, n (%)	28 (87.5)
Skin blade harvesting, n (%)	11 (34.4)
Donor site, n (%)	
split thickness skin grafts	25 (78.1)
primary closure	7 (21.9)
Complications, n (%)	
infection	5 (15.6)
distal venous congestion	4 (12.5)
partial necrosis	3 (9.4)
partial dehiscence	2 (6.2)
Follow-up (months), mean (range)	12 (6–18)
Long/wide ratio, mean (range)	5.1 (4–8)

A postoperative flap infection occurred in 5 patients, mostly in cases with a former chronic infection of the soft tissue or osteitis of tibia (4 out of 5). The infection signs withdrew spontaneously in 2 patients, in 2 after a surgical revision and with fistulisation in one patient. The last one required the Ilizarov orthopaedic procedure after bone resection.

Only one quarter of the patients underwent an immediate surgical reconstruction. The reason for the large number of delayed treatments can be explained by the fact that complicated cases with unsolved lower limb conditions were referred to our Clinic from other, regional hospitals.

There was no significant donor site morbidity. All flaps provided a stable defect coverage with a satisfactory colour, texture and contour. A few patients complained of numbness or reduced sensitivity in the skin area which was innervated by a particular sacrificed nerve but it did not influence their daily activities. A vast majority of patients was satisfied with the functional and aesthetic outcome.

Characteristics of four cases are presented below.

Case 1

A 22-year-old male patient was admitted to our Clinic with soft tissue necrosis of the knee and an exposed patella. Three weeks earlier, he was polytraumatized in a severe traffic accident with injuries of the head, chest, liver, spleen and open fracture of the patella (grade 3B). Therefore, among other urgent surgical procedures, a partial patellectomy and reinsertion of the ligament were performed. After stabilisation of the patient's general condition and demarcation of the necrotic tissue, a radical debridement was done with elevation of the lateral sural flap (size 9 x 5.5 cm). The wound healing was completed without any complications. The donor site was primarily closed. Eight months later, he had a normal gait with an excellent function of the knee joint (Figure 1).

Case 2

A 37-year-old male patient referred to our Clinic four weeks after a corn picker injury and traumatic amputation of the lower leg. Reconstruction of the defect was achieved by the distally based direct saphenous flap from the opposite limb as a "cross leg" procedure. The external fixation for both legs was carried out for three weeks. The flap completely survived after separation. The function of the knee joint was fully preserved. Three months later, the patient was able to walk with a lower leg prosthesis (Figure 2).

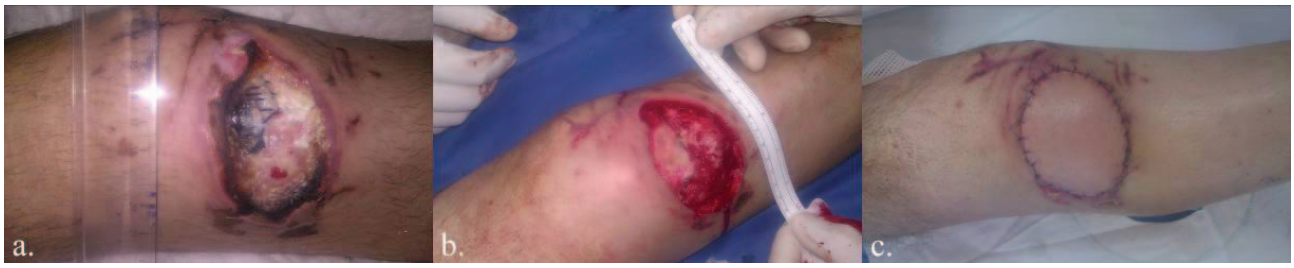

Fig. 1 – a) Necrosis of soft tissue of the knee and exposed patella; b) Wound after radical surgical debridement; c) Reconstruction with lateral sural flap (7 days post-op).

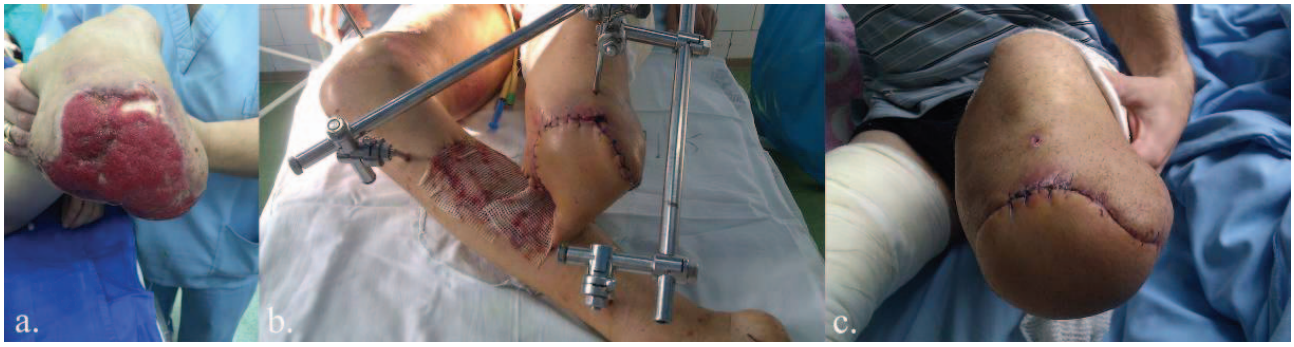

Fig. 2 – a) Amputation stump with granulated tissue and exposed tibia; b) Distally based saphenous flap from the opposite leg as "cross leg" procedure; c) Appearance of the flap 15 days after separation.

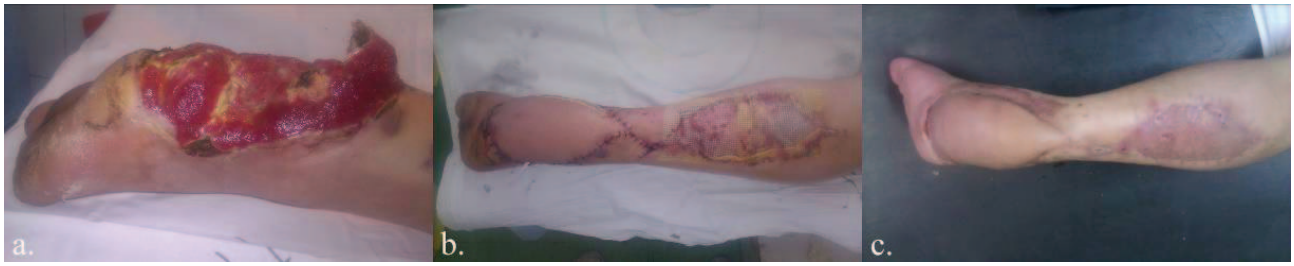

Fig. 3 – a) Defect of soft tissue and calcaneus with avulsion of the Achilles tendon attachment; b) reinsertion of the tendon and reconstruction of the defect with large distally based sural flap; c) appearance of the flap 4 weeks post-op.

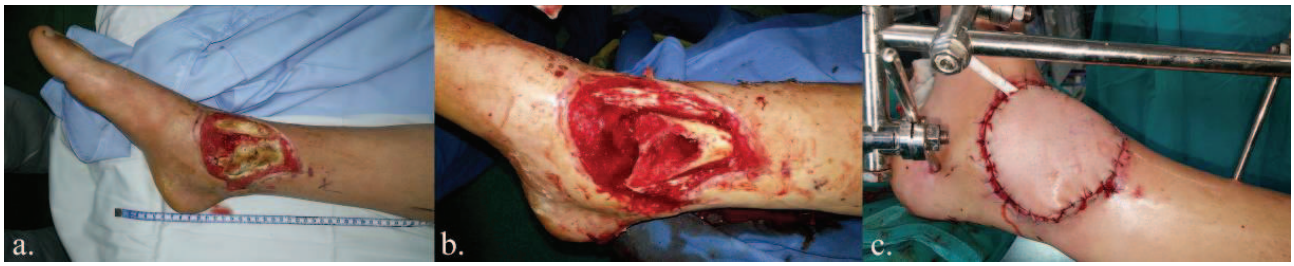

Fig. 4 – a) Necrosis of soft tissue with osteomyelitis of the tibia and exposed tibialis anterior tendon; b) radical surgical debridement with sequestrectomy of the tibia; c) Reconstruction of the defect with distally based sural flap (4 days post-op).

Case 3

A 48-year-old male patient was admitted to our Clinic from a regional hospital one month after a severe lawn-mower injury of the heel and ankle with defects of soft tissue and calcaneus, and avulsion of the Achilles tendon insertion. A radical debridement was performed with the tendon reinsertion and defect reconstruction with the distally based sural flap (size: 17 x 10 cm). A minor dehiscence on the distal part of the flap healed spontaneously five weeks later. Four months after the surgery, the patient had a satisfactory function of the ankle joint and a normal gait with an orthopaedic insert (Figure 3).

Case 4

A 35-year-old male patient referred to our Clinic three weeks after an injury in fight. He sustained an open fracture of the distal part of the tibia. Osteomyelitis and dehiscence of the wound occurred after initial internal osteosynthesis. After the tibia sequestrectomy, the plate was removed and external fixation was placed. Reconstruction of the defect was achieved by the distally based sural flap (size: 8.5 x 8.5 cm). On the seventh postoperative day, an infection developed under the flap. This was followed by debridement of the bone with a focus of the residual infection. After the operation, the infection retreated with a full consolidation of the flap (Figure 4).

Discussion

NF have wide clinical applications, particularly those distally based as they are used for the reconstruction of defects on distal parts of the lower leg, ankle and foot [13]. The reliability and safety of their use are confirmed by our clinical data. Despite the specified complications, there was not a single flap loss. A recent meta-analysis study which have in-

cluded 907 patients, reported a distally based sural flap loss rate of 3.2%. The overall complication and flap failure rate were lower when compared to free flap surgery [14].

The rate of complications is affected by many systemic and local factors. Risk factors may be: patient's age (older than 60 years), smoking, obesity and peripheral vascular disease [14, 15]. Chronic venous insufficiency leads to complications ninefold frequently. For these groups of patients some authors advocate a delayed surgical treatment [16, 17]. It can be a two or three stage procedure that includes elevation of the distal portion of the flap or pedicle, one or two weeks before the final inset of the flap. According to them, this technique provides a better safety for flap survival.

Unlike in the proximally based, a temporary venous congestion is common in the distally based flaps due to the retrograde flow of the venous blood and one-way valvular system. This problem is more pronounced in the distally based saphenous flaps due to more intense blood flow [12]. Small concomitant bypass veins surrounding the small and great saphenous veins enable drainage of the flaps. In our series, when the flap suffered moderate or severe congestion during the operation (22% of cases), it was relieved by ligation of the magistral superficial vein at the proximal base of the pedicle. This procedure is recommended by several authors. Loonen et al. [17] and Wong and Tan [18] suggested anastomosis of the lesser saphenous vein to the surrounding vein at the recipient site. Supercharging the free end of the vein should provide the "flow-through" venous flow and enhance the venous blood return [19]. Salvage of NF with venous congestion can also be achieved using the intravenous cannula inserted into the proximal stump of the cutaneous vein for intermittent bleeding [20]. Despite the proposed solutions, the venous congestion remains a major problem in the elevation of distally based flaps. All partial and superficial necroses of flaps in our series were a consequence of this complication.

Local infection following the neurocutaneous flap surgery is a relatively rare complication. A recent pool-analysis study reported a distally based flap infection rate of 2.5% [14]. In our series, the surgical site infection developed significantly more often (15.6%), which can be explained by a large number of patients with chronic wounds, osteomyelitis or bone non-union. In cases with an underlying osteomyelitis, recurrence of infection occurs in 5% to 20% of flaps. For these patients additional surgical treatment with selected antibiotic therapy is necessary in order to achieve a satisfactory outcome [21].

The length-width ratio of the pedicle as well as the size of the flap may affect flap perfusion. Sound planning of the flap implies that the ratio does not exceed 5 : 1. The accepted opinion is that the localization of the top edge at the lower 7/9 of the calf is safe and reliable for distally based flaps [15, 22]. In our data, the average ratio was 5.1 : 1, which is in accordance with recommendations by other authors. A larger adipofascial pedicle (up to 7 cm) may improve the survival rate, but practical usage is limited due to difficulties with rotation and tunnelisation as well as with bulky appearance [23]. Our pedicles did not exceed a width of over 5 cm.

The size of the flap is also a limiting factor that may significantly affect the number of complications and overall survival. It is recommended that the width of the fasciocutaneous island does not exceed 8 cm [14, 22]. Larger flaps after successful acception could induce prolonged swelling. In the three largest flaps of our series oedema was noted to persist for one to two months.

Liu et al. [24] and Dhamangaonkar et al. [25] showed that a skin blade (1.5–2 cm wide) over the pedicle, from the skin island to the pivot point of rotation, could enhance perfusion of the flap by subdermal vascular network. As a result, the length-width ratio could be increased, which would allow distally based flaps to cover defects of the forefoot. The disadvantage of this procedure is the aesthetic appearance and scarification, which is visible when the pedicle passes on the anterior side of the lower leg and foot. In our clinical practice we used skin extension over the distal part of the pedicle only for large flaps with the arc of rotation exceeding 150°. This procedure surely reduces kinking and protects the pedicle.

Subcutaneous tunnelling of the adipofascial pedicle is a procedure that was most commonly used in our series (88% of cases). We did not use it only in situations where it could jeopardize the perfusion of the flap. The skin condition under the tunnel, thickness and size of the pedicle and flap as well as the rotation arch, should be considered before making a decision on the flap placement method. Many authors believe that subcutaneous tunnelling is a safe procedure unless there is a presence of comorbidities [14, 26, 27]. Additional skin extension of the distal part of the pedicle, which we performed in some cases, can reduce the pressure on the neurovascular bundle. Yildirim et al. [28] and Nuri et al. [29] recommended a back-cut incision up to the defect for long and thick fasciocutaneous pedicles with a high arc of rotation. On the other hand, transverse incisions in the leg could have negative influences on venous and lymphatic drainage. We believe that a wide subcutaneous undermining of the tunnel reduces the risk of complications in a majority of cases and contributes to favourable cosmetic outcomes.

Proximally based flaps possessed normal sensation in the majority of cases. The loss of sensation in distally based flaps could be a problem especially for covering defects on weight bearing areas. Some authors suggested reinnervation for faster sensory nerve recovery 30, 31. Although earlier restoration of sensation after the nerve coaptation was observed, the overall protective sensation after 12 months was similar as compared with the groups of patients without initial reinnervation. Thus, this procedure, which extends the operating time and requires microsurgical skill, is not necessary.

The intrinsic and extrinsic vascular network based on nervous axes is very important for flap perfusion. In order to preserve function, a group of authors spared the sural nerve during the dissection, leaving the perineural vascular network of the flap [32]. Although the results showed that there were no significant consequences on the vitality of the flap, we believe, among many other authors, that this procedure is inadequate and additionally jeopardizes flap circulation [15, 19, 23]. Furthermore, the sensory deficiency caused by the sacrifice of the saphenous or sural nerve has never been emphasized by our patients.

On the base of the vascular communication between the subfascial sural neurovascular axis and musculocutaneous

perforators of the medial and lateral head of the gastrocnemius muscle, musculoneurocutaneous distally based sural flaps were introduced into the clinical practice [33, 34]. At first, the muscle cuff around the intergastrocnemius sural nerve was used in an attempt to improve the vascularisation of the most distal flap portion. However, an increased donor site morbidity does not justify this method except in situations with chronic osteomyelitis or bone defect, when insetting the muscle can solve the problem. Better perfusion of the flap could be achieved by placing the dissection plane just above the epimysium which allows the preservation of the sural neurovascular mesentery structure of the skin flap [15, 22].

The methods of reconstruction of the lower leg and foot with fasciocutaneous locoregional flaps have been constantly improving and evolving over the recent years. Perforator style flaps and their modification such as propeller flaps, are essentially distally based flaps whose perfusion is ensured by a single perforator with sufficient calibre from the peroneal or tibial posterior artery [35–38]. Pedicle circulation is based on the same principles as in NF through the vascular chain surrounding the saphenous or sural nerve. The keystone and V-Y design perforator island flaps have to be also taken into consideration for the reconstruction of the lower limb [39]. All of these flaps have their advantages and limitations which must be taken into account when making decisions about their application.

Conclusion

NF proved to be a paramount alternative to free-flap reconstruction of the lower limb. Their use has been steadily increasing for the last 20 years. They are an excellent choice for solving small and medium soft tissue defects on the lower leg and foot. Utilisation of newly modified techniques to increase the perfusion of distally based flaps can extend the coverage of larger and more distal defects. The reliability and safety of their application are also confirmed through our clinical data. Intensive clinical application can be explained by the fact that it is a less technically demanding and time consuming surgical procedure. Preservation of major source vessels, low donor site morbidity with violation of only the involved limb as well as similarity of surrounding tissue should also be considered as advantages of these flaps. Proper patient selection is very important. Characteristics of the defect and overall patient condition are crucial for the choice of the most appropriate reconstructive surgical procedure.

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REFERENCES

1. *Pontén B.* The fasciocutaneous flap: Its use in soft tissue defects of the lower leg. Br J Plast Surg 1981; 34(2): 215–20.
2. *Cormack GC, Lamberty BG.* A classification of fascio-cutaneous flaps according to their patterns of vascularisation. Br J Plast Surg 1984; 37(1): 80–7.
3. *Masquelet AC, Romana MC, Wolf G.* Skin island flaps supplied by the vascular axis of the sensitive superficial nerves: anatomic study and clinical experience in the leg. Plast Reconstr Surg 1992; 89(6): 1115–21.
4. *Hasegawa M, Torii S, Katoh H, Esaki S.* The distally based superficial sural artery flap. Plast Reconstr Surg 1994; 93(5): 1012–20.
5. *Kojić S.* Significance of neurocutaneous flaps in reconstruction of foot and lower leg defects [dissertation]. Belgrade: University of Belgrade, Faculty of Medicine; 2005. (Serbian)
6. *Nakajima H, Imanishi N, Fukuzumi S, Minabe T, Aiso S, Fujino T.* Accompanying arteries of the cutaneous veins and cutaneous nerves in the extremities: Anatomical study and a concept of the venoadipofascial and/or neuroadipofascial pedicled fasciocutaneous flap. Plast Reconstr Surg 1998; 102(3): 779–91.
7. *Deng C, Wei ZZ, Wang B, Jin W, Zhang W, Tang X,* et al. The proximally based lateral superficial sural artery flap: a convenient and optimal technique for the reconstruction of soft-tissue defects around the knee. Int J Clin Exp Med 2016; 9(8): 15167–76.
8. *Pan H, Zheng Q, Yang S.* Utility of proximally based sural fasciocutaneous flap for knee and proximal lower leg defects. Wounds 2014; 26(5): 132–8.
9. *Ilyas Tahirkheli MU, Ellahi I, Dar MF, Sharif A.* Distal Based Sural Fascio-Cutaneous Flap: A Practical Limb Saviour for Wounds of War and Peace. J Coll Physicians Surg Pak 2016; 26(5): 399–402.
10. *Ciofu RN, Zamfirescu DG, Popescu SA, Lascar I.* Reverse sural flap for ankle and heel soft tissues reconstruction. J Med Life 2017; 10(1): 94–8.
11. *Cavadas PC.* Reversed saphenous neurocutaneous island flap: Clinical experience. Plast Reconstr Surg 1997; 99(7): 1940–6.
12. *Zhong W, Lu S, Chai Y.* Distally based saphenous neurocutaneous perforator flap: A versatile donor site for reconstruction of soft tissue defects of the medial malleolar region. J Foot Ankle Surg. 2016; 55: 391–6.
13. *Zhu Y, Wang Y, He X, Zhu M, Li F, Xu Y.* Foot and ankle reconstruction: An experience on the use of 14 different flaps in 226 cases. Microsurgery 2013; 33(8): 600–4.
14. *de Blacam C, Colakoglu S, Ogunleye AA, Nguyen JT, Ibrahim AM, Lin SJ,* et al. Risk factors associated with complications in lower-extremity reconstruction with the distally based sural flap: A systematic review and pooled analysis. J Plast Reconstr Aesthet Surg 2014; 67(5): 607–16.
15. *Herlin C, Sinna R, Hamoui M, Canovas F, Captier G, Chaput B.* Distal lower extremity coverage by distally based sural flaps: Methods to increase their vascular reliability. Ann Chirur Plastique Esthetique 2017; 62(1): 45–54.
16. *Erdmann D, Gottlieb N, Humphrey SJ, Le TC, Bruno W, Levin SL.* Sural flap delay procedure: A preliminary report. Ann Plast Surg 2005; 54(5): 562–5.
17. *Loonen MP, Kon M, Schuurman AH, Bleys RL.* Venous bypass drainage of the small saphenous vein in the neurovascular pedicle of the sural flap: Anatomical study and clinical implications. Plast Reconstruct Surg 2007; 120(7): 1898–905.
18. *Wong C, Tan B.* Maximizing the reliability and safety of the distally based sural artery flap. J Reconstr Microsurg 2008; 24(8): 589–94.
19. *Fujiwara M, Nagata T, Matsushita Y, Ishikawa K, Yusuke O, Fukamizu H.* Delayed distally based sural flap with temporary venous supercharging. Microsurgery 2013; 33(7): 534–8.
20. *Eker G, Akan I, Aydoğdu E, Aköz T.* Salvage of neurocutaneous flaps with venous congestion using intravenous cannula. Plast Reconstr Surg 2003; 112(4): 1191–2.

21. *Schmitt SK*. Osteomyelitis. Infect Dis Clin North Am 2017; 31(2): 325–38.
22. *Wei J, Dong Z, Ni J, Liu L, Luo S, Luo Z,* et al. Influence of flap factors on partial necrosis of reverse sural artery flap: A study of 179 consecutive flaps. J Trauma Acute Care Surg 2012; 72(3): 744–50.
23. *Tsai J, Liao HT, Wang PF, Chen CT, Lin CH*. Increasing the success of reverse sural flap from proximal part of posterior calf for traumatic foot and ankle reconstruction: Patient selection and surgical refinement. Microsurgery 2013; 33(5): 342–9.
24. *Liu L, Zou L, Li Z, Zhang Q, Cao X, Cai J*. The extended distally based sural neurocutaneous flap for foot and ankle reconstruction: a retrospective review of 10 years of experience. Ann Plast Surg 2014; 72(6): 689–94.
25. *Dhamangaonkar AC, Patankar HS*. Reverse sural fasciocutaneous flap with a cutaneous pedicle to cover distal lower limb soft tissue defects: Experience of 109 clinical cases. J Orthopaed Traumatol 2014; 15(3): 225–9.
26. *Uygur F, Evinç R, Noyan N, Duman H*. Should we hesitate to use subcutaneous tunneling for fear of damaging the sural flap pedicle. Ann Plast Surg 2009; 63(1): 89–93.
27. *Parrett BM, Pribaz JJ, Matros E, Przylecki W, Sampson CE, Orgill DP*. Risk Analysis for the Reverse Sural Fasciocutaneous Flap in Distal Leg Reconstruction. Plast Reconstr Surg 2009; 123(5): 1499–504.
28. *Yildirim S, Akan M, Aköz T*. Soft-tissue reconstruction of the foot with distally based neurocutaneous flaps in diabetic patients. Ann Plast Surg 2002; 48(3): 258–64.
29. *Nuri T, Ueda K, Maeda S, Otsuki Y*. Anatomical study of medial and lateral sural cutaneous nerve: Implications for innervated distally-based superficial sural artery flap. J Plast Surg Hand Surg 2012; 46(1): 8–12.
30. *Santanelli F, Tenna S, Pace A, Scuderi N*. Free flap reconstruction of the sole of the foot with or without sensory nerve coaptation. Plast Reconstr Surg 2002; 109(7): 2314–22.
31. *Orbay H, Ogawa R, Ono S, Aoki S, Hyakusoku H*. Distally based superficial sural artery flap excluding the sural nerve. Plast Reconstruct Surg 2011; 127: 1749–50.
32. *Chen S, Chen T, Wang H*. The distally based sural fascio musculocutaneous flap for foot reconstruction. J Plast Reconstr Aesthet Surg 2006; 59(8): 846–55.
33. *Fodor L, Horesh Z, Lerner A, Ramon Y, Peled IJ, Ullmann Y*. The Distally Based Sural Musculoneurocutaneous Flap for Treatment of Distal Tibial Osteomyelitis. Plast Reconstr Surg 2007; 119(7): 2127–36.
34. *Chen X, Xu Y, Chen J, Ma Z, Guan L, Xu J,* et al. Dominant perforator neurocutaneous flaps for one-staged reconstruction of defects caused by high energy at lower legs, ankles and feet. Zhonghua Zheng Xing Wai Ke Za Zhi 2013; 29(2): 81–7. (Chinese)
35. *Terzić Z, Djordjević B*. Clinical aspects of reconstruction of the lower third of the leg with fasciocutaneous flap based on peroneal artery perforators. Vojnosanit Pregl 2014; 71(1): 39–45.
36. *Toia F, Arpa SD, Pignatti M, Noel W, Cordova A*. Axial propeller flaps: A proposal for update of the "Tokyo consensus on propeller flaps". J Plast Reconstr Aesthet Surg 2017; 70(6): 857–60.
37. *Ozalp B, Aydınol M*. Perforator-based propeller flaps for leg reconstruction in pediatric patients. J Plast Reconstr Aesthet Surg 2016; 69(10): e205–11.
38. *Chaput B, Herlin C, Espié A, Meresse T, Grolleau JL, Garrido I*. The keystone flap alternative in posttraumatic lower-extremity reconstruction. J Plast Reconstr Aesthet Surg 2014; 67(1): 130–2.

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Comparison of pharmacodynamic properties of three different aspirin formualtions in the patients with stable coronary disease

Poređenje farmakodinamskih osobina tri različita preparata aspirina kod bolesnika sa stabilnom koronarnom bolešću

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Abstract

Background/Aim. The platelet aggregation, as a laboratory test for assessment of platelet function, is very efficient for optimal antiplatelet treatment and also to identify individuals who have suboptimal response to antiplatelet drugs, such as aspirin and clopidogrel. The aim of this study was to determine the level of inhibition of platelet aggregation using impedance aggregometry in the patients receiving different preparations of acetylsalicylic acid (ASA) in a dose of 100 mg per day. **Methods.** The examination included 215 patients (110 men and 105 women), treated with one of three different ASA preparations after acute myocardial infarction, as a single therapy or with clopidogrel. Among them, 89 patients were on Aspirin protect® (Bayer, Germany) – Group 1 and 66 patients were on Cardiopirin® (GL Pharma GMBH, Austria) – Group 2, while 60 patients were taking Andol® (Pliva, Croatia) – Group 3. The groups were equal in the presence of factors that can influence platelet aggregation (age, gender, smoking, diabetes, taking other drugs). The platelet function was measured using the impedance aggregometer Multiplate (Multiplate Platelet Function Analyzer, Roche) in the blood samples with heparin for the platelet aggregation activated by the arachidonic acid (ASPI) and by thrombin (TRAP) tests [the area under the aggregation curve (AUC) was used to express the aggregation response over the measured time (AU*min)]. **Results.** Efficacy of ASA preparations showed statistically significant differences among the three investigated groups ($\chi_{KW}^2 = 46.279$; $p < 0.001$), and it was also observed separately in the patients undergoing single therapy ($\chi_{KW}^2 = 26.344$; $p < 0.001$) and dual therapy ($\chi_{KW}^2 = 23.498$; $p < 0.001$). It was found that the patients who were taking Aspirin protect® obtained significantly better antiplatelet efficiency compared to the patients receiving Cardiopirin® ($Z = 5.472$; $p < 0.001$) and Andol® ($Z = 5.387$; $p = 0.022$). There is reduced efficiency of all ASA preparations in smokers, while patients receiving Aspirin protect® were 10.5 times more likely to be responders. **Conclusion.** Different ASA preparations observed in this study showed different efficiency on the platelet function as measured by the method of impedance aggregometry.

Key words:
platelet aggregation; aggregation inhibitors; aspirin; clopidogrel; acute coronary syndrome; treatment outcome.

Apstrakt

Uvod/Cilj. Agregacija trombocita, kao laboratorijski test za procenu funkcije trombocita, je od posebnog značaja za optimalno vođenje antitrombocitne terapije i izdvajanje bolesnika koji pokazuju suboptimalni odgovor na primenu antitrombocitnih lekova, kao što su aspirin i klopidogrel. Cilj rada bio je odrediti stepen inhibicije agregacije trombocita metodom impedantne agregometrije kod bolesnika koji su uzimali različite preparate acetilsalicilne kiseline (ASA) u dozi od 100 mg dnevno. **Metode.** Ispitivanjem je obuhvaćeno 215 bolesnika (110 muškaraca i 105 žena), koji su nakon infarkta miokarda sa naknadnom revaskularizacijom uzimali jedan od tri različita preparata ASA, pojedinačno ili u kombinaciji sa klopidogrelom. Od ukupnog broja, 89 bolesnika je uzimaloAspirin protect® (Bayer, Nemačka) – Grupa 1, Cardiopirin® (GL Pharma GMBH, Austrija) je uzimalo 66 bolesnika– Grupa 2, dok je 60 bolesnika primalo Andol® (Pliva, Hrvatska) – Grupa 3. Grupe su bile jednake u zastupljenosti faktora koji mogu biti od uticaja na agregaciju trombocita (starost, pol, pušenje, diabetes, uzimanje drugih lekova). Funkcija trombocita merena je na impedantnom agregometru Multiplate (Multiplate Platelet Function Analyzer, Roche) iz uzoraka krvi sa heparinom, korišćenjem

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agregacije trombocita aktiviranih arohidonskom kiselinom (ASPI) i trombinom (TRAP) [rezultati su bili izraženi kroz površinu ispod agregacione krivulje u periodu ispitivanja (AU*min)]. **Rezultati.** Ustanovljena je statistički značajna razlika u efikasnosti različitih preparata ASA ($\chi_{KW}^2 = 46{,}279$; $p < 0{,}001$), kako kod bolesnika koji su na pojedinačnoj ($\chi_{KW}^2 = 26{,}344$; $p < 0{,}001$), tako i onih na dvojnoj terapiji ($\chi_{KW}^2 = 23{,}498$; $p < 0{,}001$). Bolesnici koji su uzimali Aspirin protect® su imali značajno bolju antiagregacionu efikasnost leka u poređenju sa bolesnicima koji su uzimali Cardiopirin® ($Z = 5{,}472$; $p < 0{,}001$) i Andol® ($Z = 5{,}387$; $p = 0{,}022$). Postojao je smanjeni efekat svih preparata ASA kod pušača, dok su bolesnici koji su uzimali Aspirin protect® imali 10,5 puta veću verovatnoću da budu responderi. **Zaključak.** Različiti preparati acetilsalicilne kiseline posmatrani u ovom ispitivanju pokazuju laboratorijski značajno različitu efikasnost na funkciju trombocita merenu metodom impedantne agregometrije.

Ključne reči:
trombociti, agregacija; antiagregaciona sredstva; aspirin; klopidogrel; akutni koronarni sindrom; lečenje, ishod.

Introduction

Antiplatelet therapy shows a significant benefit in the treatment of acute coronary syndrome (ACS). For over 100 years acetylsalicylic acid (ASA) has been used as an anti-inflammatory and antipyretic drug, but since the end of 1960s it has been known that ASA also reaches its positive cardiovascular effects in the inhibition of thromboxane A_2 (TxA_2) by acting on the enzymes cyclooxygenase-1 (COX-1) and cydooxygenase-2 (COX-2) [1, 2]. The effect of ASA on COX-1 is irreversible and lasts for the life of the platelets, depending on the production of new platelets which will recover COX-1 activity at a rate of about 10% per day in healthy people. Low doses of ASA are sufficient to suppress more than 95% of TXA_2 synthesis by COX-1, which leads to inhibition of platelet aggregation. However, platelets affected with ASA may still aggregate in the presence of potent platelet agonists such as collagen and thrombin. Higher doses of ASA can inhibit COX-2 mediated synthesis of prostacyclin in endothelial cells, but they retain the ability to regenerate the production of prostacyclin a few hours after ingestion of ASA due to the ability of cells to synthesize the core protein [2, 3].

Despite its significant antiplatelet effect, ASA is not always able to prevent all cardiovascular events. This is far from surprising when considering the complexity of arterial thrombosis and specific platelet physiology [4]. This lack of therapy success was the reason for introduction various diagnostic tests with the intent of guiding and optimizing the clinical treatment of patients. Such tests have resulted in the generation of clinical data that suggest suboptimal response to antiplatelet agents such as ASA or clopidogrel, which is called "resistance" [5–7].

The definition of ASA resistance is quite variable in the literature and has been described as the occurrence of thromboembolic events despite ASA intake, insufficient pharmacological inhibition of COX-1-derived TXA_2 formation with subsequent insufficient inhibition of platelet function, or the inability of the drug to cause prolongation of bleeding time [7–9]. It should be noted that most experts prefer to use the term "variable response" instead of ASA resistance, which indicates that the response to ASA differs among some patients, and may be attributed to various individual-, drug- or disease-related reasons or mechanisms [5, 7, 9]. Several mechanisms were identified to explain the incidence of variable response to ASA: non-compliance, age, sex, smoking, possible drug interactions (nonsteroidal anti-inflammatory drugs – NSAIDs, inhibitors of proton pump), inadequate dosing, alternative pathways of platelet activation, altered platelet response to ASA under some conditions/surgical procedures (e.g., coronary artery bypass grafting – CABG), genetic variations of COX-1 gene or platelet receptors, pre-treatment platelet reactivity and pre-existing clinical conditions (diabetes mellitus, renal failuire, essential thrombocythaemia) [10–15].

The possibility of monitoring the platelet response to the ASA therapy can have a great impact on the management of therapy and significantly reduce the incidence of morbidity and mortality. The most important thing is to distinguish patients who do not receive the necessary protection with ASA, but also to determine possible causes of treatment failure [16, 17]. The most clinically meaningful measure of the platelet-inhibitory effects of ASA is the level of serum thromboxane B_2 (TxB_2) which reflects thromboxane $A_2$$TxA_2$) formation by platelets [18]. Other methods that are efficient in optimal management of antiplatelet therapy and identification of the patients who have a suboptimal response to antiplatelet drugs are light transmission and impedance aggregometry, thromboelastography, bleeding time assay and flow cytometric analysis [19–21].

The aim of this investigation was to test the platelet function by the method of impedance aggregometry in the patients receiving various preparations of ASA in order to determine whether the type of ASA preparation affects the degree of inhibition of platelet aggregation and thus affects the degree of variable response to ASA.

Methods

The study included 215 patients who received a single (ASA) or dual (ASA + clopidogrel) antiplatelet therapy after acute myocardial infarction with revascularization. These patients were not at the same time on NSAIDs, and the patients were not with established thrombocytopenia or thrombocytosis (platelet count was 150–300 x 10^9/L). All patients had normal renal function (creatinine clearance greater than 60 mL /min). The patients were taking 3 types of ASA preparations in the from of tablets in a single dose of 100 mg daily at least for 2 months, but the longest for 6 months: Aspirin protect® (Bayer, Germany) – Group 1, Cardiopirin® (GL Pharma GMBH, Austria) – Group 2 and Andol® (Pliva, Croatia) – Group 3. The groups were equal in the presence of

factors that can influence the platelet aggregation (age, gender, smoking, diabetes, taking other drugs).

The platelet function was measured using impedance aggregometer Multiplate (Multiplate Platelet Function Analyzer, Roche) in the blood samples of 4 mL with lithium heparin as antucoagulant (VenoSafe, Terumo) for the platelet aggregation activated by arachidanic acid (ASPI test) and by thrombin activator peptide (TRAP test). The blood samples were taken 4 hours after taking ASA. The procedure implied adding 300 ml of the heparinized blood and 300 mL of the saline solution into the test cell. After incubation at 37°C for 3 minutes, 20 mL of the selected agonist was added, so the final concentration of arachidonic acid (AA) of 0.5 mM (ASPI test) and TRAP of 3.2 uM (TRAP test) was achieved. A blood sample containing added agonist was automatically stirred (800 U/min) using a magnetic stirrer coated with poly-tetra-fluoro-ethylene (PTFE). The activated platelets adhere to the electrode and increase the electrical impedance between them, which was registered within 6 minutes, and the increase in impedance was converted into arbitrary units aggregation (aggregation arbitary units – AU). The area under the aggregation curve (AUC) was used to express the aggregation response over the measured time (AU*min). According to the manufacturer, the reference values were 923–1509 AU∗ min for TRAP test and 790–1410 AU∗ min for ASPI test. If ASPI was < 400 AU∗ min, the patient was assigned as ASA-responder.

Further, we considered the risk factors that may affect the efficacy of ASA preparations, such as smoking, gender, age, diabetes mellitus or taking other drugs [anticoagulant agents, proton pump inhibitors (PPIs) and β- blockers].

Statistical analysis was performed using the Statistical Package for Social Science (SPSS Software GmbH, Germany), version 20.0. The results were presented in tables and graphs, using the mean values and standard deviations (SD). The efficacy of ASA preparations among the groups was compared using the χ^2-test, ANOVA, Kruskal-Wallis test and Mann Whiney U test. Logistic regression analysis was used to determine the predictive factors in the assessment of drugs efficacy. Statistical significance was determined at the level of $p < 0.05$.

Results

From the total of 215 patients in this study, there were 110 men (110/215 or 51.2%) and 105 women (105/215 or 48.80%). The average age of patients in the study was 55.8 ± 11.2 years; the youngest patient was 24 and the oldest one was 80 years of age. There was no statistically significant difference in the age structure of patients by gender ($t = 1.163$; $p = 0.046$).

All the patients were divided into three groups according to the type of the applied ASA preparation: Group 1 (Aspirin protect®) – 89 (41.4%) patients, Group 2 (Cardiopirin®) – 66 (30.7%) patients, Group 3 (Andol®) – 60 (27.9%) patients. Most of the patients were on a single therapy – 121 (56.3%) patients, 55 (45.4%) of them were in the Group 1, 33 (27,3%) patients in the Group 2 and 33 (27.3%) patients were in the Group 3. On the other hand, 94 patients were at dual therapy, 34 (36.2%) of them in the Group 1, 33 (35.1%) patients were in the Group 2 and 27 (28.7%) patients were in the Group 3.

Table 1 shows the general characteristics of the patients in relation to the type of ASA preparation.

Table 1

General characteristics of the patients

Patients characteristics	Group 1	Group 2	Group 3	χ^2/F*	*p*
	n (%)	n (%)	n (%)		
Sex					
m	45 (50.6)	34 (51.1)	31 (51.7)		
f	44 (49.4)	32 (48.5)	29 (48.3)	0.022	0.989
Age (years), mean ± SD	56.09 ± 11.31	54.29 ± 11.96	57.10 ± 10.48	1.046*	0.353
Diabetes mellitus					
yes	21 (23.6)	16 (24.2)	18 (30.0)		
no	68 (76.4)	50 (75.8)	42 (60.0)	0.862	0.650
Smoking					
yes	33 (37.1)	29 (43.9)	34 (56.7)		
no	56 (62.9)	37 (56.1)	26 (43.3)	5.584	0.061
Anticoagulants					
yes	35 (39.3)	16 (24.2)	19 (31.7)		
no	54 (60.7)	50 (75.8)	41 (68.3)	3.957	0.138
Beta blockers					
yes	41 (46.1)	34 (51.5)	29 (48.3)		
no	48 (53.9)	32 (48.5)	31 (51.7)	0.450	0.798
Proton pump inhibitors					
yes	26 (29.2)	19 (28.8)	18 (30.0)		
no	63 (70.8)	47 (71.2)	42 (70.0)	0.023	0.989

χ^2-Chi sqare test; F-ANOVA – analysis of variance; SD – standard deviation.
Group 1 – Aspirin® protect (Bayer, Germany) á 100 mg; Group 2 – Cardiopirin® (GL Pharma GMBH, Austria) á 100 mg; Group 3 – Andol® (Pliva, Croatia) á 100 mg.

Table 2

Assessment of efficiency of acetylsalicylic acid (ASA) preparations in all groups

Therapy	test	Group 1 (n = 89) mean ± SD	Group 2 (n = 66) mean ± SD	Group 3 (n = 60) mean ± SD	F/$^{*}\chi_{KW}^2$	*p*
Total	TRAP	1166.82 ± 207.23	1186.32 ± 248.78	1125.32 ± 210.63	1.238	0.292
(n = 210)	ASPI	301.93 ± 122.98	521.29 ± 270.71	459.60 ± 185.89	46.279*	< 0.001
Single therapy	TRAP	1184.85 ± 206.26	1152.73 ± 229.19	1131.97 ± 190.14	0.704	0.496
(n = 121)	ASPI	319.84 ± 121.98	576.76 ± 290.15	469.06 ± 200.22	26.344*	< 0.001
Dual therapy	TRAP	1137.65 ± 208.349	1219.91 ± 266.19	1117.18 ± 236.75	1.630	0.202
(n = 94)	ASPI	272.97 ± 120.78	465.81 ± 241.50	448.04 ± 169.79	23.498*	< 0.001

F-ANOVA – analysis of variance; χ_{KW}^2 – Kruskal-Wallis Test; SD – standard deviation.
Group 1 – Aspirin protect® (Bayer, Germany) á 100 mg; Group 2 – Cardiopirin® (GL Pharma GMBH, Austria) á 100 mg; Group 3 – Andol® (Pliva, Croatia) á 100 mg; TRAP test – platelet aggregation activated by thrombin receptor activator peptide; ASPI test – platelet aggregation activated by archidonic acid.

The sex and age distribution did not differ significantly by the group. Also, the groups were homogenous according to the smoking status and the presence of diabetes. The differences did not exist in relation to the use of anticoagulants, beta-blockers and PPIs.

The general assessment of the efficacy of ASA preparations and a comparison between the groups regardless of the type of antiplatelet therapy (single, dual) is shown in Table 2.

The TRAP values did not differ significantly among the groups (F = 1.238; $p = 0.292$), in the patients on single therapy (F = 0.371; $p = 0.069$) as well as on a dual therapy (F = 1.299; $p = 0.280$), which indicated the similar basic function of platelets in all the patients.

The efficacy of ASA preparations showed statistically significant differences among the three investigated groups ($\chi_{KW}^2 = 46.279$; $p < 0.001$), and it was also observed separately in the patients undergoing single therapy ($\chi_{KW}^2 = 26.344$; $p < 0.001$) and dual therapy ($\chi_{KW}^2 = 23.498$; $p < 0.001$). Examining the efficacy of ASA in all patients, it was found that the patients who were taking Aspirin protect® obtained significantly better efficiency compared to the patients receiving Cardiopirin® (Z = 5.472; $p < 0.001$) and Andol® (Z = 5.387; $p = 0.022$). Significantly better efficacy of Aspirin protect® is also determined in both groups of patients on the individual and dual antiaggregation therapy.

Logistic regression model of independent factors for the assessment of effectiveness of ASA preparations for all patients in this study is shown in Table 3, where all patients are divided into responders (ASPI ≤ 400 AU*min) and non-responders (ASPI > 400 AU*min). This model included the following variables: age, diabetes, smoking, anticoagulants, beta blockers, PPIs and the type of applied ASA preparation.

The whole model was highly significant [χ^2 (df = 9, N = 215) = 112.658, $p < 0.001$)] and explained between 40.8% and 54.9% of the variance of efficiency of all ASA preparations according to the ASPI test. The factors that gave statistically significant contribution to the model were smoking [odds ratio (OR) = 0.108; $p < 0.001$) and the use of Aspirin protect® (OR = 10.538; $p < 0.001$)]. In the non-smokers the probability for values of ASPI < 400 AU*min increased for 89.2% compared to the smokers, while the patients receiving Aspirin protect® were 10.5 times more likely to have a value of ASPI < 400 AU*min.

Table 3

Logistic regression model of independent factors for assessing the efficiency of acetylsalicylic acid (ASA) for all the patients according to ASPI test

Factors	OR	95% CI	*p*
Gender	0.658	0.308–1.407	0.281
Age	1.024	0.990–1.059	0.174
Diabetes	0.868	0.315–2.391	0.784
Smoking	0.108	0.040–0.293	< 0.001
Anticoagulants	1.794	0.677–4.753	0.240
Beta blockers	0.812	0.388–1.701	0.581
PPIs	0.398	0.153–1.039	0.060
Aspirin protect®	10.538	3.893–28.526	< 0.001
Cardiopirin®	0.902	0.358–2.271	0.827
Andol®	1.109	0.440–2.792	0.832

OR – odds ratio; PPIs-proton pump inhibitors;
CI – confidence interval; ASPI test – platelet aggregation activated by arachidonic acid.

Table 4 shows the three logistic regression models of independent factors for the assessment of effectiveness of three different ASA preparations, where all patients were also divided into responders (ASPI ≤ 400 AU*min) and non-responders (ASPI > 400 AU*min). The models included the following variables: age, diabetes, smoking, anticoagulants, beta blockers and PPIs.

The first model included the patients from the Group 1. The whole model was highly significant χ^2 (df = 7, N = 89) = 17.299, $p = 0.016$) and explained between 17.7% and 29% of the variance of efficiency of Aspirin protect®. However, none of the variables was marked as statistically significant.

The second model included the patients from the Group 2. The model was highly statistically significant [χ^2 (df = 7, N = 66) = 51.939, $p < 0.001$] and generally explained between 54.5% and 73.2% of the variance of the efficiency of Cardiopirin®. A statistical significant contribution to the model had the following factors: gender (OR = 0.093; $p = 0.020$) and smoking (OR = 0.003; $p < 0.001$).

The third model consisted of patients from the Group 3. The model was highly statistically significant [χ^2 (df = 7, N = 60) = 43.199, $p < 0.001$] and generally explained between 51,3% and 69.4% of the variance of efficiency of Andol®. However, none of the factors was statistically significant.

Table 4

Logistic regression model of independent factors for assessing the efficiency of Aspirin protect®, Cardiopirin® and Andol® according to ASPI test

Factors	OR	95% CI	*p*
Model 1 (Aspirin protect®)			
gender	0.888	0.228–3.458	0.864
age	1.054	0.996–1.115	0.067
diabetes	0.821	0.155–4.343	0.817
smoking	0.498	0.106–2.346	0.378
anticoagulants	7.377	0.834–65.261	0.072
beta blockers	0.709	0.191–2.628	0.607
PPIs	0.622	0.191–2.628	0.592
Model 2 (Cardiopirin®)			
gender	0.093	0.012–0.692	0.020
age	1.048	0.970–1.132	0.238
diabetes	0.157	0.004–6.872	0.337
smoking	0.003	0.000–0.069	<0.001
anticoagulants	0.179	0.024–1.348	0.095
beta blockers	1.476	0.259–8.400	0.661
PPIs	0.203	0.021–1.918	0.164
Model 3 (Andol®)			
gender	0.664	0.104–4.224	0.664
age	0.911	0.822–1.010	0.077
diabetes	0.139	0.006–3.305	0.222

OR – odds ratio; PPIs-proton pump inhibitors; CI – confidence interval; ASPI test – platelet aggregation activated by arachidonic acid.

Discussion

The antiplatelet therapy cannot provide the prevention of all cardiovascular events, but the inhibitory effect on the platelet aggregation significantly decreases the cardiovascular morbidity and mortality. According to data from the American Heart Association (AHA) and the European Society of Cardiology (ESC), the therapy with ASA in a dose of 100 mg daily has significant therapeutic effects in the patients with moderate cardiovascular risk. In the patients with primary coronary intervention (PCI), with or without stenting, it is recommended to use clopidogrel (75 mg per day) in combination with ASA in an initial dose of 300 mg, and subsequently to reduce the dose to 75–100 mg daily [22]. Also, the recommended initial treatment in the patients with acute ischemic attack, who do not have thrombolytic therapy, is ASA in a dose of 150 to 325 mg and in the further therapy, it is recommended to use ASA in a dose of 100 mg daily with clopidogrel, 75 mg daily [23, 24].

The potential relation between the low response to antiplatelet therapy and clinical outcome has not yet been fully explained, mainly due to the fact that there is no universally accepted definition of resistance. The term platelet resistance should not be used lightly, because it can have a bad effect if it is not interpreted correctly. Misidentified, it can produce increased risk of thrombosis if the treatment is discontinued. On the other hand, there is a risk of hemorrhage if a dose of antiplatelet drug is wrongly increased. Some studies showed the inconsistent levels of resistence to ASA. Data range from 1.4%–9.8% [25] to 55% [26], but the majority of studies presented the incidence of 15%–33% of individuals with bad response to ASA [27–31]. The rates are slightly higher in the patients with a stroke. Recent studies have shown that nonresponsiveness to the antiplatelet drugs is a risk factor for thromboembolic events (stroke, myocardial infarction, vascular death). Škorić et al. [32] concluded in their study that initial patency of the infarct-related artery in the patients with the acute ST elevation myocardial infarction is related to the platelet response to aspirin. Also, Gum et al. [33] documented in their investigation a greater than threefold increase in the risk of major adverse events associated with the ASA resistence. A recent meta-analysis of 20 studies included more than 2,900 patients and reported that the patients with the lower response to ASA had a significantly increased risk of having a cardiovascular event [34]. Given these data and conclusions, it is clear that measuring the antiplatelet effect of ASA is of a great relevance.

Nowadays, there is a large number of commercial tests available to monitor the effects of ASA, in order to identify the patients who are at substantial risk for adverse events while they are on therapy. Recent studies have shown that the impedance aggregometry can be reliably used to assess the effect of ASA therapy, because it shows a high degree of sensitivity and good correlation with other testing methods [2, 16]. The variability of response to a given ASA is not a surprise, given that the environment, genetics, and disease can affect the drug's disposition. The most important factors that influence the effectiveness of ASA are the age, gender, the

presence of diabetes, smoking and concomitant therapies, such as the PPIs, antihypertensive drugs and the anticoagulant drugs. It is known that diabetes mellitus is associated with underlying platelet over-reactivity, which may attenuate the response to aspirin [10], tobacco use increases platelet activation and accentuates platelet thrombosis [8], while concomitant administration of PPIs reduces the effect of ASA due to the weaker absorption enhanced by esterases of gastrointestinal mucosa [11]. Our investigation showed that the statistically significant factors for the efficiency of ASA are smoking and the type of ASA preparation, where we can see that the patients receiving Aspirin protect® were 10.5 times more likely to be responders in the ASPI test.

There are not published data yet whether the selection of ASA preparation can affect the therapy itself and whether the kind of ASA preparations taken in the same dosage and in the same way can affect its effectiveness. This is important especially due to the fact that many authors do not recommend increasing the dose of ASA to achieve and maintain an effective level of antiplatelet activity because of the possibility of increased bleeding, especially in the patients with a stroke. Therefore, there are important implications for being able to optimize the efficiacy and safety of ASA preparations.

Our investigation showed that the 3 ASA preparations, which are available in our market, demonstrate their effectiveness comparable to the data in the literature. However, the effectiveness of ASA preparations, which is measured in this investigation by the method of impedance aggregometry, showed a statistically significant difference. In general, regardless whether patients were taking just ASA or ASA with clopidogrel, Aspirin protect® showed significantly higher efficiency compared to Cardiopirin® and Andol® ($\chi_{KW}^2 = 46.279$; $p < 0.001$). The same relation exists in the group of patients on a single therapy ($\chi_{KW}^2 = 26.344$; $p < 0.001$), as well as in the group of patients who had the dual therapy ($\chi_{KW}^2 = 23.498$; $p < 0.001$). Comparing the factors that may influence the efficiency of different ASA preparations, we found that none of the evaluated factors were statistically significant for the effectiveness of Aspirin protect® and Andol®, while gender and smoking were significant for Cardiopirin®. It is known that hormonal changes in women can enhance the platelet activation [8] and ASA bioinactivation by the liver may be slower in older patients than in younger ones, but it is important to point out that the gender was not showed to be an important factor for the efficacy of all ASA preparations, although, thus increasing the bioavailability in this group [35].

The results of our research confirm our assumption that together with all the factors, we know that can influence the effectiveness of ASA, selection of ASA preparation can also impact on the outcome of therapy. Although we can't clinically prove that the type of ASA preparations is of an importance for the effectiveness of antiplatelet therapy, it seems reasonable that the laboratory assessment of the efficiency of ASA preparations is taken into consideration as one of the criteria, and a type of ASA preparation as one of the factors which affect the anti-platelet effect of ASA.

Conclusion

Various preparations of acetylsalicylic acid examined in this investigation showed significantly different laboratory efficiency on the platelet function as measured by the method of impedance aggregometry.

REFERENCES

1. *Reininger AJ*. Primary haemostasis and its assessment by laboratory tests.Hamostaseologie 2006; 26(1): 42–4, 46–7.
2. *Guyer KE*. The present state of aspirin and clopidogrel resistance. Hämostaseologie 2009; 29(3): 285–90.
3. *Gasparyan AY, Watson T, Lip GY*. The role of aspirin in cardiovascular prevention: implications of aspirin resistance. J Am Coll Cardiol 2008; 51(19): 1829–43.
4. *Grove EL*. Antiplatelet effect of aspirin in patients with coronary artery disease. Dan Med J 2012; 59(9): B4506.
5. *Campbell CL, Steinhubl SR*. Variability in response to aspirin: do we understand the clinical relevance. J Thromb Haemost 2005; 3(4): 665–9.
6. *Zimmerman N, Hohlfeld T*. Clinical implications of aspirin resistance. Thromb Haemost 2008; 100(3): 379–90.
7. *Schwartz KA*. Aspirin Resistance: A Clinical Review Focused on the Most Common Cause, Noncompliance. Neurohospitalist 2011; 1(2): 94–103.
8. *Mehta JL, Mohandas B*. Aspirin resistance: Fact or fiction? A point of view. World J Cardiol 2010; 2(9): 280–8.
9. *Patrono C*. The Multifaceted Clinical Readouts of Platelet Inhibition by Low-Dose Aspirin. J Am Coll Cardiol 2015; 66(1): 74–85.
10. *Linden MD, Tran H, Woods R, Tonkin A*. High platelet reactivity and antiplatelet therapy resistance. Semin Thromb Hemost 2012; 38(2): 200–12.
11. *Dawson J, Quinn T, Rafferty M, Higgins P, Ray G, Lees KR*, et al. Aspirin resistance and compliance with therapy. Cardiovasc Ther 2011; 29(5): 301–7.
12. *Cotter G, Shemesh E, Zehavi M, Dinur I, Rudnick A, Milo O*, et al. Lack of aspirin effect: aspirin resistance or resistance to taking aspirin. Am Heart J 2004; 147(2): 293–300.
13. *Kurth T, Glynn RJ, Walker AM, Chan KA, Buring JE, Hennekens CH*, et al. Inhibition of Clinical Benefits of Aspirin on First Myocardial Infarction by Nonsteroidal Antiinflammatory Drugs. Circulation 2003; 108(10): 1191–5.
14. *Grosser T, Fries S, Lawson JA, Kapoor SC, Grant GR, FitzGerald GA*. Drug resistance and pseudoresistance: an unintended consequence of enteric coating aspirin. Circulation 2013; 127(3): 377–85.
15. *Floyd CN, Ferro A*. Mechanisms of aspirin resistance. Pharmacol Ther 2014; 141(1): 69–78.
16. *Harrison P, Frelinger AL, Furman MI, Michelson AD*. Measuring antiplatelet drug effects in the laboratory. Thromb Res 2007; 120(3): 323–36.
17. *Alberts MJ*. Platelet function testing for aspirin resistance is reasonable to do: yes. Stroke 2010; 41(10): 2400–1.
18. *Santilli F, Rocca B, De Cristofaro R, Lattanzio S, Pietrangelo L, Habib A*, et al. Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays:

implications for aspirin "resistance.". J Am Coll Cardiol 2009; 53(8): 667–77.
19. *Paniccia R, Antonucci E, Maggini N, Romano E, Gori AM, Marcucci R,* et al. Assessment of platelet function on whole blood by multiple electrode aggregometry in high-risk patients with coronary artery disease receiving antiplatelet therapy. Am J Clin Pathol 2009; 131(6): 834–42.
20. *Schror K.* What is aspirin resistance? Br J Cardiol 2010 17(Suppl 1): S5–S7.
21. *Adamzik M, Görlinger K, Peters J, Hartmann M.* Whole blood impedance aggregometry as a biomarker for the diagnosis and prognosis of severe sepsis. Critical Care 2012; 16(5): R204.
22. *Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H*, et al. ESC Scientific Document Group . 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2018; 39(2): 119–77.
23. *Goodman SG, Menon V, Cannon CP, Steg G, Ohman E, Harrington RA.* American College of Chest Physicians. Acute ST-segment elevation myocardial infarction: American College of Chest Physicians evidence-based clinical practice guidelines (8th edition). Chest 2008; 133(6): 708S–75S.
24. *Weitz JI, Eikelboom JW, Samama MM.* New antithrombotic drugs: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141(2 Suppl): e120S–e151S.
25. *Hurlen M, Abdelnoor M, Smith P, Erikssen J, Arnesen H.* Warfarin, Aspirin, or Both after Myocardial Infarction. N Engl J Med 2002; 347(13): 969–74.
26. *Buchanan MR, Verma S.* Biological basis and clinical implications of acetylsalicylic acid resistance. Can J Cardiol 2006; 22(2): 149–51.
27. *Cheng X, Xie N, Xu H, Chen C, Lian Y.* Biochemical aspirin resistance is associated with increased stroke severity and infarct volumes in ischemic stroke patients. Oncotarget 2017; 8(44): 77086–95.
28. *Liu L, Cao J, Fan L, Hu G, Hu Y, Zhu B,* et al. Prevalence and Risk Factors for Aspirin Resistance in Elderly Patients With Type 2 Diabetes. Int J Gerontol 2011; 5(2): 112–6.
29. *Alahmari S, Alayed K, Malik A, Abdel GA, Albanyan A, Al-Shaikh Y.* Measurement of platelet function to determine the prevalence of aspirin non-responsiveness among Saudi type II diabetic patients. J Health Spec 2016; 4(1): 31–6.
30. *Mortensen SB, Larsen SB, Grove EL, Kristensen SD, Hvas A.* Reduced platelet response to aspirin in patients with coronary artery disease and type 2 diabetes mellitus. Thromb Res 2010; 126(4): e318–2.
31. *Gluckman TJ, McLean RC, Schulman SP, Kickler TS, Shapiro EP, Conte JV*, et al. Effects of aspirin responsiveness and platelet reactivity on early vein graft thrombosis after coronary artery bypass graft surgery. J Am Coll Cardiol 2011; 57(9): 1069–77.
32. *Škorić B, Miličić D, Lovrić D, Gornik I, Škorić-Narančić K, Sertić J.* Initial patency of the infarct-related artery in patients with acute ST elevation myocardial infarction is related to platelet response to aspirin. Int J Cardiol 2010; 140(3): 356–8.
33. *Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ.* A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. J Am Coll Cardiol 2003; 41(6): 961–5.
34. *Krasopoulos G, Brister SJ, Beattie WS, Buchanan MR.* Aspirin "resistance" and risk of cardiovascular morbidity: systematic review and meta-analysis. BMJ 2008; 336(7637): 195–8.
35. *Rocca B, Petrucci G.* Variability in the responsiveness to low-dose aspirin: pharmacological and disease-related mechanisms. Thrombosis 2012; 2012: 376721.

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GENERAL REVIEW

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Dental management of patients taking antiplatelet, oral anticoagulant and novel anticoagulant medications

Stomatološko zbrinjavanje pacijenata na terapiji antitrombocitnim, oralnim antikoagulantnim i novim antikoagulantnim lekovima

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Key words:
tooth diseases; fibrinolitic agents; anticoagulants; oral surgical procedures; treatment outcome.

Ključne reči:
zub, bolesti; fibrinolitici; antikoagulansi; hirurgija, oralna, procedure; lečenje, ishod.

Introduction

Antiplatelet and anticoagulant drugs are used widely in the long-term prevention and treatment of arterial and venous thrombosis. Oral surgery in the patients taking these drugs is always challenging and the risk of bleeding needs to be balanced against the risk of thromboembolic complication in case of treatment cessation.

Recently, a group of drugs, referred to as new oral anticoagulants (NOACs), including direct thrombin inhibitors and factor Xa (FXa) inhibitors, have started to be in clinical use. These drugs are mostly indicated to prevent a stroke and systemic embolisms in patients with atrial fibrillation, and for prevention of thrombosis after the elective hip and knee surgery [1, 2]. It is likely that NOACs will be increasingly used in the coming years. Therefore, it is very important for dentists to be familiar with the mechanisms of action of the drugs, their interaction with the drugs commonly prescribed in dentistry, possible bleeding complications and their prevention and treatment.

The aim of this article is to show basic characteristics of antiplatelet, oral anticoagulants and NOACs, and based on the literature review, to present current recommendations regarding the dental treatment of patients taking these medications.

Dental treatment of patients taking antiplatelet drugs

Low doses of aspirin, clopidogrel, ticlopidine and dipyridamole are the most frequently administered antiplatelet drugs. The most common indications for a long-term antiplatelet therapy are ischemic cardiovascular and cerebrovascular diseases and peripheral arterial disease [3, 4]. The mechanisms of action of these drugs are different. Aspirin irreversibly inactivates cyclo-oxygenase, the enzyme necessary for synthesis of thromboxane A_2, which is important for platelet aggregation. Thienopyridines (clopidogrel, ticlopidine and prasugrel) are inhibitors of adenosine-diphosphate receptors. Like aspirin, these drugs affect the activity of platelets during their lifetime (7–10 days). Dipiridamole inhibits the reuptake of adenosine and increases cAMP [5].

The patients taking antithrombotic agents may have prolonged bleeding time, but this test is not reliable enough to predict the bleeding risk after oral surgical procedures. Moreover, despite using antithrombotic agents, the bleeding time may be within the normal range [6]. The platelet aggregation test is more sensitive, but it is not in use in everyday practice [6, 7]. That is the reason why any platelet function test is not commonly recommended to the patients taking antiplatelet drugs before the dental surgical procedure.

For fear of prolonged and excessive bleeding, discontinuation of antiplatelet drugs several days before a dental surgery was often recommended in the past. This therapeutic approach can expose patients to the risk of thromboembolism [8–11]. According to the current recommendations based on numerous researches, there is no need to stop antiplatelet medications before most dental surgical procedures, including tooth extraction [6, 7, 12–15].

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A recently published review of the literature have showed that of at least 1,283 patients receiving single or dual antiplatelet medications who underwent at least 2,343 dental surgical procedures, including at least 2,308 simple and surgical tooth extractions in at least 1,334 visits, no more than 35 patients (2.7% of patients and 2.6% of visits) had bleeding complications requiring local measures for hemostasis and only 2 patients (0.2%) needed more than local measures to control hemorrhage. On the other hand, there were several reports of thrombotic complications when antiplatelet drugs were stopped due to dental procedures. The author concluded that bleeding is a rare complication after tooth extractions in the patients taking antiplatelet medications and, therefore, there is no need to discontinue these drugs for a dental surgery [16].

Due to the different mechanisms of action, a combined use of antiplatelet drugs may have a synergistic effect. The combination of low-dose aspirin and clopidogrel is increasingly used. The most common indication for a dual antiplatelet treatment is prevention of thrombotic complications after percutaneous insertion of a coronary stent [5, 17]. As recommended by the American College of Chest Physicians, a dual antiplatelet therapy should not be interrupted perioperatively within 6 weeks of placement of a metal stent or within 6 months of placement of a drug-eluting stent [18]. Premature discontinuation of a dual antiplatelet therapy is well recognized as a risk factor for stent thrombosis [17].

Despite its benefits, a dual antiplatelet therapy increases the risk of spontaneous and postoperative bleeding [16]. A small number of studies with a limited number of patients were conducted in order to estimate the risk of bleeding after oral surgical procedures in the patients taking dual antiplatelet drugs. However, the results of all these studies suggested that dental extractions can be safely done without interrupting a dual antiplatelet therapy applying only local hemostatic measures [12, 13, 15, 16, 19, 20].

Dental treatment of patients taking oral anticoagulant drugs

Oral anticoagulants are coumarin derivatives and vitamin K antagonists. These drugs inhibit vitamin K epoxide reductase, an enzyme responsible for the cyclic interconversion of vitamin K. The lack of the active form of vitamin K which is necessary for carboxylation of the glutamic acid residue on coagulation factors II, VII, IX and X results in the production of biologically inactive coagulation factors [2].

The most common indications for oral anticoagulant therapy (OAT) are atrial fibrillation, the mechanical prosthetic heart valves, deep vein thrombosis and pulmonary embolism [21]. The International Normalized Ratio (INR) is the test used to monitor the effect of OAT. The therapeutic range of the INR values is 2.0 to 3.0 in most cases. For the patients with the highest risk of thromboembolism, for example those with the mechanical prosthetic heart valves, higher INR values, up to 3.5 or even 4.0, are recommended [21–23].

Acenocoumarol, warfarin and phenprocoumon are the most commonly used oral anticoagulants. All these drugs are used orally and they are rapidly absorbed from the gastrointestinal (GI) tract. They have high bioavailability and circulate mostly bounded to plasma proteins, mainly albumin. They are metabolized by the liver via cytochrome P-450 enzyme system, mostly by hydroxylation, and excreted through urine. Half-lives of these drugs are different (acenocoumarol 8–11 h, warfarin 36–42h, phenprocoumon 5–6 days). Coumarin derivatives have a slow onset of action because their antithrombotic effect requires reduction of vitamin K-depending coagulation factors in the plasma and depends on half-lives of these factors. Half-lives of vitamin K-depending factors are different (4–6 hours for FVII, 18–30 hours for FIX, 48 hours for FX and 60–72 hours for FII). Following the administration of OAT, it takes usually 2–3 days for the initial effect on the INR value. On the other hand, the effect of these drugs lasts for several days after cessation of OAT. This time it is necessary to have a complete excretion of the drug from the body for synthesis of new vitamin K-dependent factors [2, 24, 25].

The most serious complication of OAT is bleeding. Depending on the INR levels and severity of the bleeding complication, several therapeutic approaches are recommended: to reduce or temporarily interrupt OAT, or to introduce vitamin K orally or by slow IV infusion. Life-threatening bleeding could be treated by fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), or recombinant factor VIIa (rVIIa) [2, 24, 25].

Many drugs can increase or decrease the anticoagulant effect of oral anticoagulants. The most commonly used drugs in dentistry which can interact with OAT are: carbamazepine, metronidazole, erythromycin, sulphonamides, tetracycline and miconazole (oral gel). Except carbamazepine, all mentioned drugs increase the effect of OAT [25–27]. Due to the risk of bleeding, aspirin and other nonsteroidal anti-inflamatory drugs (NSAIDs) should be avoided. Paracetamol is considered to be the drug of choice for pain relief in the patients taking OAT.

The oral surgical procedures in the patients taking OAT have been studied a lot. The results of most studies show that dental extractions can be performed safely without discontinuing OAT if INR is within the therapeutic range (INR $\leq$ 4.0) and if appropriate local hemostatic measures are provided [14, 28–38]. The most commonly used local hemostatic agents and measures are: oxidized regenerated cellulose, absorbable gelatin or collagen sponges, fibrin glue, antifibrinolytics applied directly into the wound or in the form of a solution as a mouthwash and wound suturing [39–41].

A recently published review of the literature showed that over 99% of anticoagulated patients who continued OAT had no postoperative bleeding requiring more than local hemostatic measures. In more than 5,431 patients who underwent over 11,381 surgical procedures, bleeding that required more than local hemostasis occured only in 31 (~0.6%) of patients. Many of these patients had higher INR therapeutic levels than currently recomended. On the other hand, among at least 2,673 patients whose OAT was reduced or withdrawn for at least 2,775 visits for the dental procedures, there were 22 embolic complications (0.8% of cessations), including 6 fatal events (0.2% of cessations). The authors concluded that

the thromboembolic risk in the patients whose OAT was interrupted for a dental surgery exceeded the risk of significant bleeding in the patients whose anticoagulation is continued [38].

A certain number of patients, mostly those with the highest risk for thrombosis, take OAT and aspirin combined. A strict recommendation for the combined OAT-aspirin therapy was given only to the patients with the prosthetic heart valves [42–44]. However, many patients with atrial fibrillation are receiving the combined OAT-antiplatelet therapy as well [45, 46]. The addition of aspirin to OAT seems a rational therapeutic approach for the patients receiving OAT in whom cardiovascular prophylaxis is indicated. Despite the advantages of this combined therapy, there is a higher risk of experiencing a spontaneous and prolonged, excessive bleeding during and after surgical procedures [34]. There is a lack of data regarding this group of patients who required a dental surgery. The results of the published studies, which comprised a limited number of patients, show that dental extractions can be safely done without interrupting either OAT or antiplatelet therapy if INR is within the therapeutic range, and if proper local hemostatic measures are applied [14, 34].

Dental treatment of patients taking new oral anticoagulants

In the last few years, new oral anticoagulant drugs, direct thrombin inhibitors and FXa inhibitors, are available for clinical use. Compared to vitamin K antagonists, NOACs have certain advantages: rapid onset and direct mode of action, predictable anticoagulant response, wide therapeutic index, limited drug and food interactions and no need for routine monitoring of their effect [47, 48].

Direct thrombin inhibitors

Dabigatranetexilate is a reversible thrombin inhibitor that binds on the thrombin, thus preventing fibrinogen conversion into fibrin. It reversibly inhibits free and clot-bound thrombin. When taken orally, it is rapidly absorbed from the GI tract. After hydrolysis in plasma, it is converted into an active form with a rapid onset of action and reaches the peak plasma concentration after 0.5–4 hours [49]. Its terminal half-life is 12–17 hours and up to 27 hours in the patients with severe renal dysfunction [47–50]. 80%–85% of the drug is eliminated by the kidneys and the rest via the bile. Administered in the common doses of 150 mg, or 110 mg twice daily, dabigatran reaches a stabile concentration in plasma 2–3 days after the initiation of therapy. The duration of its effect is about 22 hours.

The RELY-ABLE multicentre study conducted in 2009 assessed the efficacy and bleeding complications of dabigatran compared to warfarin in the patients with atrial fibrillation. The results of the study showed that patients taking dabigatran in the dose of 150 mg twice daily had lower rates of stroke and systemic embolism, but similar rates of major bleedings. Dabigatran in the dose of 110 mg twice daily showed a similar efficacy in preventing systemic embolism and stroke, but lower rates of major bleedings compared to warfarin [51]. The drug was approved by the European Medicines Agency (EMA), and the Food and Drug Administration (FDA) for the prevention of stroke and systemic embolism in the patients with nonvalvular atrial fibrillation as well as for thromboprophylaxis after the prosthetic hip and knee joint replacement.

A routine coagulation monitoring for the patients taking dabigatran is not required. Thrombin clotting time (TT) and ecarin clotting time are the most sensitive test for monitoring the effect of dabigatran. The activated partial thromboplastin time (aPTT) is less sensitive, but widely available and it can be used to check coagulation in case of emergency. Checking INR is not recommended, because this test is insensitive [47–50].

In case of bleeding in the patients taking dabigatran, a treatment option depends on the severity of a bleeding complication. For minor bleedings, it is recommended to postpone the next dose or discontinue the drug. Any discontinuation of the drug should be carefully considered due to the risk of thromboembolism. In case of a moderate and severe bleeding, treatment options include fluid replacement and hemodynamic support, the use of rVIIa factor prothrombin complex concentrates and hemodialysis [47, 49, 50]. Recently, FDA approved the use of idarucizumab, monoclonal antibody fragment, for reversal of the effect of dabigatran in urgent surgical procedures or in life threatening bleeding situations [49, 52].

There is a risk of prolonged bleeding during and after invasive dental procedures, including dental extractions, in the patients taking dabigatran. It is estimated that there is a similar risk of peri-procedural bleeding in the patients taking dabigatran and warfarin [53, 54]. There is also the opinion that the patients taking dabigatran should be treated similarly to those receiving low-molecular-weight heparins [48]. Up to now, there has been a lack of well-designed clinical trials that would include a number of patients taking dabigatran and require a dental surgery [49, 55, 56].

Some authors suggested skiping the dose on the morning of the procedure [57]. However, most authors suggested that dabigatran should be continued in case of a minor dental surgery, including simple dental extractions [47, 48, 50, 52, 58–60]. To minimize the risk of postoperative bleeding, the procedure should be performed as long after the last dose of dabigatran as possible, trying to avoid the surgery when the drug has a maximal anticoagulant effect. The procedure should be carried out as atraumatic as possible, applying proper local hemostatic measures. Discontinuation of dabigatran, usually 24 hours before the surgery, should be discussed with a patient´s physician and considered only in case of a high risk procedure. Discontinuation of the therapy depends on the risk of postoperative bleeding, the risk of thromboembolism and renal function. Interruption of dabigatran increases the risk of stroke or systemic embolism and if necessary, the drug should be resumed as soon as possible, usually 24–48 hours after the procedure. Paracetamol should be prescribed for pain relief in the postoperative period. Aspirin and other NSAIDs should be avoided. Drugs that decrease the effect of dabigatran such as rifampicin, dexamethasone and car-

bamazepine, or increase its effect such as ketoconazole, itraconazole, erythromycin and clarithromycin, should be avoided or prescribed carefully [47–49, 55].

Factor Xa inhibitors

Rivaroxaban is a reversible direct inhibitor of FXa that catalyzes activation of prothrombin into thrombin. Rivaroxaban is administered orally, once daily. It has a rapid onset of action and reaches the peak plasma concentration after 2.5–4 hours. Its terminal half-life is 5.7–9.2 hours that can be prolonged up to 12–13 hours in the patients > 75 years old. About 66% of the drug is excreted in the urine and the rest in the feces [47–50]. Indications for the use of rivaroxaban include thromboprophylaxis after the prosthetic hip and knee joint replacement. ROCKET-AF, clinical trial showed that rivaroxaban was noninferior to warfarin for the prevention of stroke and systemic embolism in patients with atrial fibrillation [61, 62].

The patients taking rivaroxaban slightly prolonged prothrombin time (PT) and aPTT. The anti-factor Xa assay is considered to be the most accurate test for monitoring the effect of dabigatran, but a routine monitoring of coagulation, similarly to other NOACs, is not required. There is no specific reversal agent for rivaroxaban. In case of a minor bleeding, discontinuation of the drug could be sufficient because duration of the drug effect is short. In case of more serious bleeding complications, rVIIa or prothrombin complex concentrate can be used [47–49].

Drugs that are cytochrome P450 inhibitors such as erythromycin, ketoconazole, itraconazole, voriconazol and posaconazole may increase the risk of bleeding by increasing the concentration of rivaroxaban. Opposite to this, drugs that are cytochrome P450 inducers such as rifampicin may decrease the effect of rivaroxaban [47–49].

Apixaban is recently introduced reversible direct inhibitor of FXa with the same therapeutic indications. The results of the apixaban for Reduction in Stroke and Other Thromboembolic Events (ARISTOTLE) trial showed that apixaban was as effective as warfarin in the prevention of stroke and embolism in the patients with atrial fibrilation with fewer bleeding complications [63]. The drug is administered orally, twice a day. It reaches the maximum plasma concentrations in 3 hours. The half-life of the drug is about 12 hours. About 75% of the drug is eliminated in the feces and 25% in the urine. As for rivaroxaban, the anti-factor Xa assay is considered to be the most accurate test for monitoring its effect and there is no specific antidote. In mild cases of bleeding, discontinuation of the drug could be sufficient, while in more serious cases, the rVIIa or prothrombin complex concentrate can be used [49].

There is insufficient data in the literature about the safety of dental surgical procedures in patients taking FXa inhibitors [49, 54, 56, 59, 64]. However, similar recommendations given for dental treatment of the patients taking dabigatran are applicable to the patients taking FXa inhibitors [47, 48, 50, 52, 58].

Conclusion

Based on the results of the studies, there are clear recommendations in the literature that minor oral surgical procedures, including tooth extractions, can be safely performed in the patients taking antiplatelet and oral anticoagulant drugs without therapy interruption if the proper local hemostatic measures are applied. Similar recommendations were given for the dental treatment of patients taking NOACs. However, these recommendations are mainly based on the experts' opinion, rather than on the results of clinical studies. Therefore, further researches of the safety of dental extractions in the patients taking NOACs are necessary.

REFERENCES

1. *Siegal DM, Crowther MA.* Acute management of bleeding in patients on novel oral anticoagulants. Eur Heart J 2013; 34(7): 489–498b.
2. *Ageno W, Gallus AS, Wittkowsky A, Crowther M, Hylek EM, Palareti G.* Oral anticoagulant therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141(2 Suppl): e44S–e88S.
3. *Antithrombotic Trialists' Collaboration.* Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002; 324(7329): 71–86.
4. *Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J*, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet 2009; 373(9678): 1849–60.
5. *Eikelboom JW, Hirsh J, Spencer FA, Baglin TP, Weitz JI.* Antiplatelet drugs: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141(2 Suppl): e89S–e119S.
6. *Brennan MT, Wynn RL, Miller CS.* Aspirin and bleeding in dentistry: An update and recommendations. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104(3): 316–23.
7. *Nagao Y, Masuda R, Ando A, Nonaka M, Nishimura A, Goto K*, et al. Whole blood platelet aggregation test and prediction of hemostatic difficulty after tooth extraction in patients receiving antiplatelet therapy. Clin Appl Thromb Hemost 2018; 24(1): 151–6.
8. *Biondi-Zoccai GG, Lotrionte M, Agostoni P, Abbate A, Fusaro M, Burzotta F,* et al. A systematic review and meta-analysis on the hazards of discontinuing or not adhering to aspirin among 50,279 patients at risk for coronary artery disease. Eur Heart J 2006; 27(22): 2667–74.
9. *Burger W, Chemnitius JM, Kneissl GD, Rücker G.* Low-dose aspirin for secondary cardiovascular prevention - cardiovascular risks after its perioperative withdrawal versus bleeding risks with its continuation - review and meta-analysis. J Intern Med 2005; 257(5): 399–414.
10. *Maulaz AB, Bezerra DC, Michel P, Bogousslavsky J.* Effect of discontinuing aspirin therapy on the risk of brain ischemic stroke. Arch Neurol 2005; 62(8): 1217–20.

11. *Ferrari E, Benhamou M, Cerboni P, Marcel B.* Coronary syndromes following aspirin withdrawal: a special risk for late stent thrombosis. J Am Coll Cardiol 2005; 45(3): 456–9.
12. *Bajkin BV, Urosevic IM, Stankov KM, Petrovic BB, Bajkin IA.* Dental extractions and risk of bleeding in patients taking single and dual antiplatelet treatment. Br J Oral Maxillofac Surg 2015; 53(1): 39–43.
13. *Napeñas JJ, Oost FC, DeGroot A, Loven B, Hong CH, Brennan MT,* et al. Review of postoperative bleeding risk in dental patients on antiplatelet therapy. Oral Surg Oral Med Oral Pathol Oral Radiol 2013; 115(4): 491–9.
14. *Morimoto Y, Niwa H, Minematsu K.* Risk factors affecting postoperative hemorrhage after tooth extraction in patients receiving oral antithrombotic therapy. J Oral Maxillofac Surg 2011; 69(6): 1550–6.
15. *Lillis T, Ziakas A, Koskinas K, Tsirlis A, Giannoglou G.* Safety of dentalextractions during uninterrupted single or dual antiplatelet treatment. Am J Cardiol 2011; 108(7): 964–7.
16. *Wahl MJ.* Dental Surgery and Antiplatelet Agents: Bleed or Die. Am J Med 2014; 127(4): 260–7.
17. *Grines CL, Bonow RO, Casey DE Jr, Gardner TJ, Lockhart PB, Moliterno DJ,* et al. Prevention of premature discontinuation of dual antiplatelet therapy in patients with coronary artery stents: a science advisory from the American Heart Association, American College of Cardiology, Society for Cardiovascular Angiography and Interventions, American College of Surgeons, and American Dental Association, with representation from the American College of Physicians. Circulation 2007; 115(6): 813–8.
18. *Douketis JD, Spyropoulos AC, Spencer FA, Mayr M, Jaffer AK, Eckman MH,* et al. Perioperative management of antithrombotic therapy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141(2 Suppl): e326S–e350S.
19. *Park M, Her S, Kwon JB, Lee JB, Choi M, Cho JS,* et al. Safety of dental extractions in coronary drug-eluting stenting patients without stopping multiple antiplatelet agents. Clin Cardiol 2012; 35(4): 225–30.
20. *Sadeghi-Ghahrody M, Yousefi-Malekshah SH, Karimi-Sari H, Yazdanpanah H, Rezaee-Zavareh MS, Yavarahmadi M.* Bleeding after tooth extraction in patients taking aspirin andclopidogrel (Plavix®) compared with healthy controls. Br J Oral Maxillofac Surg 2016; 54(5): 568–72.
21. *Guyatt GH, Akl EA, Crowther M, Gutterman DD, Schuünemann HJ.* American College of Chest Physicians Antithrombotic Therapy and Prevention of Thrombosis Panel. Executive summary: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guideline. Chest 2012; 141(Suppl 2): 7S–47S.
22. *Keeling K, Baglin T, Tait C, Watson H, Perry D, Baglin C,* et al. British Committee for Standards in Haematology: Guidelines on oral anticoagulation with warfarin-fourth edition. Br J Haematol 2011; 154(3): 311–24.
23. *Vahanian A, Baumgartner H, Bax J, Butchart E, Dion R, Filippatos G,* et al. Guidelines on the management of valvular heart disease: The Task Force on the Management of Valvular Heart Disease of the European Society of Cardiology. Eur Heart J 2007; 28(2): 230–68.
24. *Hirsh J, Fuster V, Ansell J, Halperin JL.* American Heart Association/American College of Cardiology Foundation. American Heart Association/American College of Cardiology Foundation guide to warfarin therapy. J Am Coll Cardiol 2003; 41(9): 1633–52.
25. *Ansell J, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G.* American College of Chest Physicians. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008; 133(Suppl 6): 160S–98S.
26. *Carter G, Goss AN, Lloyd J, Tocchetti R.* Current concepts of the management of dental extractions for patients taking warfarin. Aust Dent J 2003; 48(2): 89–96; quiz 138.
27. *du Breuil AL, Umland EM.* Outpatient management of anticoagulation therapy. Am Fam Physician 2007; 75(7): 1031–42.
28. *Jeske AH, Suchko GD.* ADA Council on Scientific Affairs and Division of Science; Journal of the American Dental Association. Lack of a scientific basis for routine discontinuation of oral anticoagulation therapy before dental treatment. J Am Dent Assoc 2003; 134(11): 1492–7.
29. *Aframian DJ, Lalla RV, Peterson DE.* Management of dental patients taking common hemostasis-altering medications. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 103 Suppl: S45.e1–11.
30. *Bajkin BV, Popovic SL, Selakovic SD.* Randomized, prospective trial comparing bridging therapy using low-molecular-weight heparin with maintenance of oral anticoagulation during extraction of teeth. J Oral Maxillofac Surg 2009; 67(5): 990–5.
31. *Aldridge E, Cunningham LL Jr.* Current thoughts on treatment of patients receiving anticoagulation therapy. J Oral Maxillofac Surg 2010; 68(11): 2879–87.
32. *Bacci C, Maglione M, Favero L, Perini A, Di Lenarda R, Berengo M,* et al. Management of dental extraction in patients undergoing anticoagulant treatment. Results from a large, multicentre, prospective, case-control study. Thromb Haemost 2010; 104(5): 972–5.
33. *Bajkin BV, Todorovic LM.* Safety of local anaesthesia in dental patients taking oral anticoagulants: Is it still controversial. Br J Oral Maxillofac Surg 2012; 50(1): 65–8.
34. *Bajkin BV, Bajkin IA, Petrovic BB.* The effects of combined oral anticoagulant-aspirin therapy in patients undergoing tooth extractions: A prospective study. J Am Dent Assoc 2012; 143(7): 771–6.
35. *Eichhorn W, Burkert J, Vorwig O, Blessmann M, Cachovan G, Zeuch J,* et al. Bleeding incidence after oral surgery with continued oral anticoagulation. Clin Oral Investig 2012; 16(5): 1371–6.
36. *Hong C, Napenas JJ, Brennan M, Furney S, Lockhart P.* Risk of postoperative bleeding after dental procedures in patients on warfarin: A retrospective study. Oral Surg Oral Med Oral Pathol Oral Radiol 2012; 114(4): 464–8.
37. *Broekema FI, Minnen B, Jansma J, Bos RR.* Risk of bleeding after dentoalveolar surgery in patients taking anticoagulants. Br J Oral Maxillofac Surg 2014; 52(3): e15–9.
38. *Wahl MJ, Pinto A, Kilham J, Lalla RV.* Dental surgery in anticoagulated patients-stop the interruption. Oral Surg Oral Med Oral Pathol Oral Radiol 2015; 119(2): 136–57.
39. *Marjanović M.* Use of thrombin powder after tooth extraction in patients receiving anticoagulant therapy. Vojnosanit Pregl 2002; 59(4): 389–92. (Serbian)
40. *Bajkin BV, Rajic NV, Vujkov SB.* Dental extraction in a hemophilia patient without factor replacement therapy: a case report. J Oral Maxillofac Surg 2012; 70(10): 2276–7.
41. *Bajkin BV, Selaković SD, Mirković SM, Šarčev IN, Tadič AJ, Milekič BR.* Comparison of efficacy of local hemostatic modalities in anticoagulated patients undergoing tooth extractions. Vojnosanit Pregl 2014; 71(12): 1097–101.
42. *Salem DN, O'Gara PT, Madias C, Pauker SG.* Valvular and structural heart disease: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest. 2008; 133(6 Suppl): 593S–629S.
43. *Bonow RO, Carabello BA, Chatterjee K, de Leon AC Jr, Faxon DP, Freed MD,* et al. 2006 Writing Committee Members; American College of Cardiology/American Heart Association Task Force. 2008 Focused update incorporated into the ACC/AHA 2006 guidelines for the management of patients with valvular

heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Revise the 1998 Guidelines for the Management of Patients With Valvular Heart Disease): endorsed by the Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. Circulation 2008; 118(15): e523–661.

44. *Dentali F, Douketis JD, Lim W, Crowther M*. Combined aspirinoral anticoagulant therapy compared with oral anticoagulant therapy alone among patients at risk for cardiovascular disease: A metaanalysis of randomized trials. Arch Intern Med 2007; 167(2): 117–24.
45. *Akins PT, Feldman HA, Zoble RG, Newman D, Spitzer SG, Diener HC,* et al. Secondary stroke prevention with ximelagatran versus warfarin in patients with atrial fibrillation: pooled analysis of SPORTIF III and V clinical trials. Stroke 2007; 38(3): 874–80.
46. *Douketis JD, Arneklev K, Goldhaber SZ, Spandorfer J, Halperin F, Horrow J*. Comparison of bleeding in patients with nonvalvular atrial fibrillation treated with ximelagatran or warfarin: Assessment of incidence, case-fatality rate, time course and sites of bleeding, and risk factors for bleeding. Arch Intern Med 2006; 166(8): 853–9.
47. *Little JW*. New oral anticoagulants: Will they replace warfarin? Oral Surg Oral Med Oral Pathol Oral Radiol 2012; 113(5): 575–80.
48. *Firriolo JF, Hupp WS*. Beyond warfarin: the new generation of oral anticoagulants and their implications for the management of dental patients. Oral Surg Oral Med Oral Pathol Oral Radiol 2012; 113(4): 431–41.
49. *Costantinides F, Rizzo R, Pascazio L, Maglione M*. Managing patients taking novel oral anticoagulants (NOAs) in dentistry: a discussion paper on clinical implications. BMC Oral Health 2016; 16: 5.
50. *Breik O, Cheng A, Sambrook P, Goss A*. Protocol in managing oral surgical patients taking dabigatran. Aust Dent J 2014; 59(3): 296–301; quiz 401.
51. *Connolly S, Ezekowitz M, Yusuf S, Eikelboom J, Oldgren J, Parekh A,* et al. Dabigatran versus warfarin in patients with atrial fibrillation. N Engl J Med 2009; 361(12): 1139–51.
52. *Mauprivez C, Khonsari RH, Razouk O, Goudot P, Lesclous P, Descroix V*. Management of dental extraction in patients undergoing anticoagulant oral direct treatment: A pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol 2016; 122(5): e146–e155.
53. *Healey JS, Eikelboom J, Douketis J, Wallentin L, Oldgren J, Yang S,* et al. Periprocedural bleeding and thromboembolic events with dabigatran compared with warfarin: results from the Randomized Evaluation of Long-Term Anticoagulation Therapy (RELY) randomized trial. Circulation 2012; 126(3): 343–8.
54. *Johnston S*. An evidence summary of the management of patients taking direct oral anticoagulants (DOACs) undergoing dental surgery. Int J Oral Maxillofac Surg 2016; 45(5): 618–30.
55. *Muñoz-Corcuera M, Ramírez-Martínez-Acitores L, López-Pintor RM, Casañas-Gil E, Hernández-Vallejo G*. Dabigatran: A new oral anticoagulant. Guidelines to follow in oral surgery procedures. A systematic review of the literature. Med Oral Patol Oral Cir Bucal 2016; 21(6): e679–e688.
56. *Lusk KA, Snoga JL, Benitez RM, Sarbacker GB*. Management of Direct-Acting Oral Anticoagulants Surrounding Dental Procedures With Low-to-Moderate Risk of Bleeding. J Pharm Pract 2017; 1: 897190017707126.
57. *Miclotte I, Vanhaverbeke M, Agbaje JO, Legrand P, Vanassche T, Verhamme P*, et al. Pragmatic approach to manage new oral anticoagulants in patients undergoing dental extractions: A prospective case-control study. Clin Oral Investig 2017; 21(7): 2183–8.
58. *van Diermen DE, van der Waal I, Hoogstraten J*. Management recommendations for invasive dental treatment in patients using oral antithrombotic medication, including novel oral *anticoagulants. Oral Surg Oral Med Oral Pathol Oral Radiol 2013; 116(6): 709–16*.
59. *Thean D, Alberghini M*. Anticoagulant therapy and its impact on dental patients: A review. Aust Dent J 2016; 61(2): 149–56.
60. *Sivolella S, De Biagi M, Brunello G, Berengo M, Pengo V*. Managing dentoalveolar surgical procedures in patients taking new oral anticoagulants. Odontology 2015; 103(3): 258–63.
61. ROCKET AF Study Investigators. Rivaroxaban-once daily, oral, direct factor Xa inhibition compared with vitamin K antagonism for prevention of stroke and Embolism Trial in Atrial Fibrillation: rationale and design of the ROCKET AF study. Am Heart J 2010; 159(3): 340–347.e1.
62. *Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W*, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. N Engl J Med 2011; 365(10): 883–91.
63. *Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M,* et al. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med 2011; 365(11): 981–92.
64. *Morimoto Y, Yokoe C, Imai Y, Sugihara M, Futatsuki T*. Tooth extraction in patients taking nonvitamin K antagonist oral anticoagulants. J Dent Sci 2016; 11(1): 59–64.

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Unilateral agenesis of the right ovary and Fallopian tube in an infertile patient with a normal uterus

Jednostrana agenezija desnog jajnika i jajovoda kod infertilne pacijentkinje sa normalnom matericom

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Abstract

Introduction. Unilateral ovarian and Fallopian tube agenesis is an extremely rare anomaly that is usually associated with uterine and renal anomalies. This anomaly is usually incidentally detected during laparoscopy or laparotomy performed for other indications. We have reported a rare case of unilateral ovarian and Fallopian tube agenesis in an infertile patient with a normal uterus. **Case report.** A 34-year-old infertile patient was admitted to our clinic for laparoscopy and hysteroscopy, indicated for the right Fallopian tube occlusion and an endometrial polyp. The patient underwent laparoscopy and hysteroscopy. Unilateral right ovarian and Fallopian tube agenesis was diagnosed during laparoscopy. Upon exploration of peritoneal surfaces, omentum and intestinal serosa, neither ectopic nor remnant tissues of the ovary or Fallopian tube were found. Renal anomalies were not identified on the ultrasound examination. The hormone panel and karyotype were normal. Her partner's semen analysis was normal. The patient conceived spontaneously one year later. Serial ultrasound examinations showed normal fetal intrauterine growth and development. The patient vaginally delivered a live, female newborn at 40 weeks' gestation, weighing 3,350 grams, 53 cm long, with 9/10 Apgar scores at birth. The patient and her newborn were discharged home on the third postpartum day. **Conclusion.** Unilateral ovarian and Fallopian tube agenesis is rarely associated with a normal uterus. Laparoscopy is the gold standard in the diagnosis of ovarian and Fallopian tube agenesis. Unless other obstetric indications are present, this anomaly is not specific and it is not an indication for a Caesarean section, and vaginal delivery is a possibility at term pregnancy.

Key words:
congenital abnormalities; diagnosis; fallopian tubes; infertility; laparoscopy; ovary.

Apstrakt

Uvod. Jednostrana agenezija jajnika i jajovoda je ekstremno retko stanje i obično je udružena sa anomalijama materice i bubrega. Ova anomalija se obično otkriva slučajno na laparoskopiji i laparotomiji, koje se izvode zbog nekih drugih ginekoloških i opstetričkih razloga. Prikazali smo redak slučaj jednostrane agenezije jajnika i jajovoda kod infertilne pacijentkinje sa normalnom matericom. **Prikaz bolesnika**. Pacijentkinja stara 34 godine, sa primarnim infertilitetom, primljena je na našu kliniku za laparoskopiju i histeroskopiju zbog okluzije levog jajovoda i endometrijalnog polipa u šupljini materice. Urađena je laparoskopija i histeroskopija. Na laparoskopiji je dijagnostikovana unilateralna agenezija desnog jajnika i desnog jajovoda, sa normalnom matericom. Pri eksploraciji peritonealnih površina, omentuma i serozecreva nije nađeno ektopično tkivo ni za ostalo tkivo jajnika i jajovoda. Ultrazvučnim pregledom urinarnog trakta isključene su anomalije bubrega. Hormonske analize i kariotip kod pacijentkinje su bili normalni. Spermogram partnera je bio uredan. Godinu dana kasnije pacijentkinja je spontano ostala trudna. Serijskim ultrazvučnim pregledima u toku trudnoće dijagnostikovan je normalani intrauterusni razvoj ploda. Pacijentkinja se porodila u 40. nedelji trudnoće, spontano, vaginalnim imputem. Rodila je žensko, živo novorođenče, telesne mase 3,350 gr, dužine 53 cm, Apgar score 9/10 na rođenju. Pacijentkinja je zajedno sa novorođenčetom treći dan posle porođaja otpuštena kući. **Zaključak.** Jednostrana agenezija jajnika i jajovoda je retko udružena sa normalnom matericom. Laparoskopija predstavlja zlatni standard za dijagnozu agenezije jajnika i jajovoda. Ukoliko ne postoje druge akušerske indikacije za carski rez, ova anomalija nije indikacija *per se* za carski rez i moguć je vaginalni porođaj u terminskoj trudnoći.

Ključne reči:
anomalije; dijagnoza; jajovodi; neplodnost; laparoskopija; jajnik.

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Introduction

Unilateral ovarian and Fallopian tube agenesis is a very rare anomaly with an unknown incidence. In the article printed in 1986, Sivanesaratnom [1] reported the incidence of 1 in 11,240 women. The research is limited and knowledge pertaining to this anomaly is based mostly on case reports [2]. Three hypotheses are postulated in the etiology of ovarian and Fallopian tube agenesis. Firstly, it is hypothesized that adnexal torsion during fetal or early neonatal period may be the cause of this anomaly. Secondly, anomalous embryologic development of the gonadal ridge and upper ends of Mullerian ducts may also be involved in etiology of this anomaly. The third etiologic factor may be a vascular accident with ischemic injuries to the upper ends of Mullerian ducts during embryologic development [3, 4]. Since it is asymptomatic, this anomaly is most commonly an incidental finding during laparoscopy or laparotomy indicated for other obstetric/gynecologic conditions. It is thought that this anomaly can decrease a woman's fertility. However, it is not considered the cause of primary infertility if the contralateral Fallopian tube is patent.

Case report

We reported a case of a 34-year-old patient with primary infertility, who was admitted to our clinic for laparoscopic and hysteroscopic surgery, indicated for the right Fallopian tube occlusion and an endometrial polyp. Her menstrual cycles were regular, occurring every 26 to 28 days; menarche occurred at 14 years of age. The patient was trying to conceive for the past year. There was no history of previous pelvic or abdominal surgeries and no history of acute pelvic pain. The uterine cavity was morphologically normal and the left Fallopian tube patent on hysterosalpingography, but the right Fallopian tube was not seen. An endometrial polyp was suspected after a transvaginal ultrasound was performed. Hormone panel was within normal reference ranges. The partner's semen analysis was normal. Upon admission to our clinic, pelvic examination and ultrasound were performed. The pelvic exam revealed normal appearance of the external genitalia, cervix and vagina. On bimanual examination, a normal and mobile uterus was palpated; the left ovary was palpable, while the right ovary could not be palpated. Transvaginal ultrasound demonstrated an anteverted and anteflexed uterus, measuring 50 x 33 x 27 mm, with a focal 5 x 5 mm endometrial thickening resembling an endometrial polyp; the left ovary had a normal appearance, measuring 28 x 20 mm, while the right ovary was not visualized. The patient underwent diagnostic laparoscopy and hysteroscopy. Upon the hysteroscopic exploration, a normal endometrial cavity, without a polyp, was seen, and the endometrium was biopsied; the left tubal ostium was visible, while the right tubal ostium was not visible. Pathohistology of the endometrial biopsy demonstrated a proliferative phase endometrium. Laparoscopy demonstrated the uterus of normal size and shape as well as normal appearance of the left ovary and Fallopian tube (Figure 1). However, the right ovary and Fallopian tube were absent (Figure 2). The infundibulopelvic, broad, round and cardinal ligaments were normal. The vesicouterine and the pouch of Douglas peritoneum had a normal appearance. The omentum had a normal appearance and adhesions were not seen in the pelvic cavity. Neither ectopic nor remnant ovarian and Fallopian tube tissues were seen on the omentum, peritoneal and serosal surfaces. Transcervical chromopertubation with methylene blue revealed a patent left Fallopian tube. On the first postoperative day, abdominal and urinary tract ultrasounds were performed, and did not reveal any abnormalities. Both kidneys were of normal size and contours in their usual anatomic location. The patient was discharged home. Her karyotype was normal. One year postoperatively, the patient conceived spontaneously. Serial first, second and third trimester ultrasounds confirmed a normal pregnancy with normal fetal growth and development, normal insertion of the placenta and amniotic fluid volume. Doppler flow ultrasound of the uteroplacental, umbilical and cerebral circulation was normal. At 40 weeks of pregnancy, the patient delivered vaginally a live healthy female newborn, weighing 3,350 grams, 53 cm long, with 9/10 Apgar score at birth. The mother and newborn were discharged home on the third postpartum day.

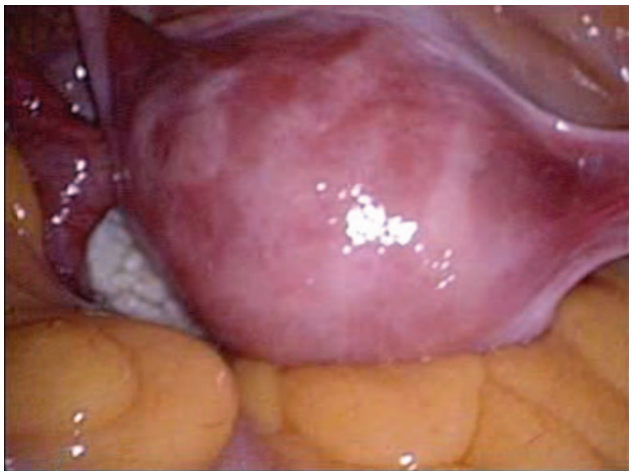

Fig. 1 – Laparoscopic appearance of a normal uterus with the left ovary and Fallopian tube.

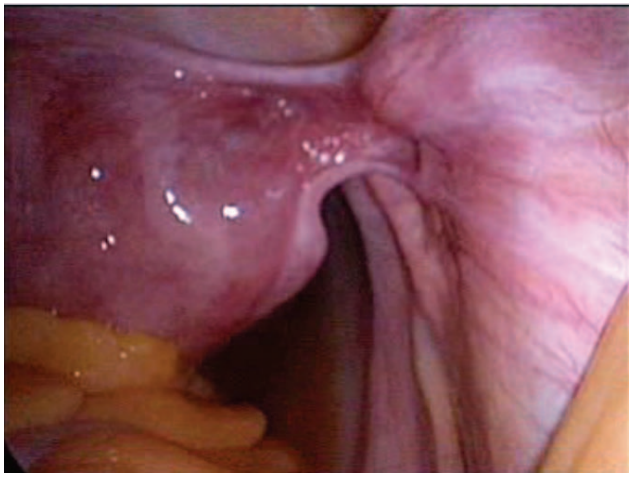

Fig. 2 – Laparoscopis appearance of a normal uterus, the right ovary and Fallopian tube are absent.

Discussion

In terms of anatomy and function, the ovary and Fallopian tube are closely associated, but their embryologic origins differ. The upper ends of Mullerian ducts produce Fallopian tubes, while the ovaries arise from the gonadal ridge [5]. Except for streak ovaries, occurring in cases of gonadal dysgenesis, other ovarian anomalies are very rare. The complete absence of an ovary is very rare, and is usually associated with ipsilateral Fallopian tube and renal ageneses. Fallopian tube anomalies are usually asymptomatic, and the absence of one Fallopian tube is usually associated with uterine anomalies, most commonly unicornuate uterus. Mullerian duct anomalies can coexist with gonadal anomalies. We reported a case of a patient with primary infertility who was laparoscopically diagnosed with unilateral agenesis of the right ovary and Fallopian tube, while her uterine and renal anatomy was normal. After attempting for one year, the patient was unable to conceive despite regular sexual intercourse and normal semen analysis. Since the research is solely based on case reports, the exact incidence of unilateral ovarian and Fallopian tube agenesis is unknown. Only a few cases have been reported in the literature, and the majority of authors cite the incidence as reported by Sivanesartnam [1], who found the incidence of unilateral ovarian and Fallopian agenesis to be 1 in 11,240 women based on two cases at one centre. This anomaly is usually asymptomatic and incidentally detected during laparoscopy or laparotomy performed for other obstetric and gynecologic indications. Unilateral adnexal agenesis is commonly associated with uterine malformations, such as unicornuate uterus and unilateral renal agenesis. A case of women with unicornuate uterus and unilateral ovarian agenesis was reported by Stuti et al. [6]. Unilateral agenesis of the ovary and Fallopian tube can occur in the presence of a normal uterus and kidneys. Our patient had a normal uterus and kidneys. Chen et al. [7] reported a case of an infertile patient with a normal uterus and unilateral agenesis of the left ovary and Fallopian tube. At the time of Caesarean section of a multiparous patient, Maurya and Gupta [8] found unilateral right ovarian and Fallopian tube agenesis without uterine and renal anomalies. A case of an infertile patient with unilateral left ovarian, Fallopian tube and round ligament agenesis in the presence of normal uterus and kidneys was reported by Rastogi et al [9].

The etiology of unilateral ovarian and Fallopian [3] tube agenesis is unknown. Three hypotheses regarding the etiology are postulated in the literature. Adnexal torsion, leading to ischemia, necrosis and organ resorption, during fetal or the early neonatal period is considered as a potential etiologic factor. However, adnexal torsion is also possible during childhood. The symptoms of adnexal torsion may be minimal, and may not present typically with acute pain in the lower abdomen. Remnant ovarian tissue residing on the omentum was found in a patient with unilateral left ovarian and distal Fallopian tube agenesis, which would support the previously described hypothesis [3]. Furthermore, this hypothesis is also supported by a case reported by Yerebasmaz et. al. [10] who described left ovarian and Fallopian tube agenesis with remnant ovarian and tube tissues on the intestinal serosa. Anomalous embryologic development of the gonadal ridge and upper ends of Mullerian ducts may also play a role in the etiology of unilateral adnexal agenesis. The next hypothesis postulates a vascular accident as an etiologic factor of unilateral adnexal agenesis. Namely, ischemia of the upper ends of mullerian ducts during embryologic development can lead to autocrine and paracrine dysregulation, which may lead to gonadal agenesis and tubal malformation [4]. Disturbance in embryologic development is the least likely in our case since we did not find congenital anomalies of other organs and the patient did not have a history of acute abdomen. For this reason, we are considering a vascular accident as a potential etiologic factor of unilateral ovarian and Fallopian tube agenesis in our patient. Despite the lack of history of acute abdominal pain, we cannot rule out with certainty adnexal torsion during the fetal period. Our patient had a normal karyotype, which rules out aberrant chromosomes that would be potentially associated with this anomaly. Unilateral adnexal agenesis is rarely associated with primary infertility. Nevertheless, this anomaly was discovered in numerous patients who underwent infertility investigations. Unilateral agenesis of the right ovary and Fallopian tube were discovered in a patient with primary infertility by Sukhadiya and Grover [11, 10]. Unilateral agenesis of the left ovary and tube in an infertile patient with a normal uterus was reported by Chen et. al. [7]. Fruzzetti et. al.[12] reported the case of a nulliparous patient, who was undergoing laparoscopic surgery for an ovarian teratoma, and at the time of this surgery, unilateral andexal agenesis was discovered. Barsky et al. [2] reported two cases of unilateral ovarian and Fallopian tube agenesis in nulliparous patients, who were undergoing surgery for the contralateral ovary. The first case involved agenesis of the left adnexa, while the second case involved the agenesis of the right adnexa; the existing ovaries were removed in both cases leading to premature menopause. A study by Yerebasmaz et. al. [10] reported four cases of nulliparous patients with the ovarian and Fallopian tube agenesis. The first case involved a patient with unilateral right adnexal agenesis. The second case was that of the left ovarian and Fallopian tube agenesis, with remnants of the fimbrial and ovarian tissue, measuring 5 x 5 mm on the intestinal serosa. The third case was a patient with the left ovarian and Fallopian tube agenesis. The fourth case was patient with the right unicornuate uterus and right ovarian agenesis. Whether unilateral adnexal agenesis could contribute to infertility is a conundrum. There is a lot of controversy among researchers and clinicians. On one hand, researchers believe that unilateral adnexal agenesis cannot contribute to infertility unless it is associated with uterine malformations. On the other hand, researchers believe that unilateral adnexal agenesis can contribute to infertility, but that conception is possible if the contralateral adnexa are functional. This statement is reinforced by case reports, which describe patients with unilateral adnexal agenesis, but who achieved normal pregnancies ending in deliveries. Erkilinc et. al. [13] reported an incidentally discovered case of unilateral Fallopian agenesis in a patient undergoing Caesarean section. A case of multigravida with unilateral

right adnexal agenesis, normal uterus and kidneys discovered during a Caesarean section was reported by Maurya and Gupta [8].

Conclusion

Unilateral agenesis of the ovary and Fallopian tube with a normal uterus and kidneys is a very rare anomaly. More commonly, this anomaly is associated with a unicornuate uterus and ipsilateral renal agenesis. Unilateral adnexal agenesis is usually asymptomatic and incidentally discovered in fertile and infertile women during laparoscopy or laparotomy performed for other obstetric and gynecologic indications. This anomaly does not cause infertility, but it is contemplated that it can lead to decreased fertility. Laparoscopy is the gold standard in the diagnosis of ovarian and Fallopian tube agenesis. Unless other obstetric indications are present, this anomaly is not specific and it is not an indication for a Caesarean section, and vaginal delivery is a possibility at term pregnancy.

R E F E R E N C E S

1. *Sivanesaratnom V*. Unexplained unilateral absence of ovary and fallopian tube. Eur J Obstet Gynecol Reprod Biol 1986; 22(1–2): 103–5.
2. *Barsky M, Beaulieu AM, Sites CK*. Congenital ovarian-fallopian tube agenesis predisposes to premature surgical menopause: A report of two cases. J Androl Gynaecol 2015; 3(1): 3.
3. *Uckuyu A, Ozcimen EE, Sevincciftci FC*. Unilateral congenital ovarian and partial tubal absence: Report of four cases with review of the literature. Fertil Steril 2009; 91(3): 936.e5–8.
4. *Pabuccu E, Kahraman K, Taskın S, Atabekoglu C*. Unilateral absence of fallopian tube and ovary in an infertile patient. Fertil Steril 2011; 96(1): e55–7.
5. *Sadler TW*. Langman's medical embryology. 13th ed. Philadelphia, Pennsylvania: Wolters Kluwer Health; 2015.
6. *Stuti T, Suchitra J, Surinder S, Pragya Y*. An incidental finding of unicornuate uterus with unilateral ovarian agenesis and ipsilateral twining of fallopian tubes during cesarean. JARMS 2015; 7(1): 11–3.
7. *Chen B, Yang C, Sahebally Z, Jin H*. Unilateral ovarian and Fallopian tube agensis in an infertile patient with a normal uterus. Exp Ther Med 2014; 8(3): 831–5.
8. *Maurya NR, Gupta AS*. A case of unilateral complete absence of fallopian tube and ovary. JPGO 2015; 3: 1.
9. *Rastogi R, Gupta Y, Gupta B, Sinha P, Chaudhary M, Parashar S,* et al. Unilateral agenesis of adnexa: A rare clinico-radiological condition. J Med Diagn Meth 2016; 5: 228.
10. *Yerebasmaz N, Dilbaz B, Şengül Ö, Altınbaş S, Çakır L*. Four cases with congenital unilateral absence of ovary and fallopian tube: Review of the literature. J Clin Anal Med 2016; 7(Suppl 3): 275–8.
11. *Sukhadiya M, Grover SV*. Unexplained unilateral absence of fallopian tube and ovary: A rare occurrence. J South Asian Feder Menopause Soc 2014; 2(1): 46–7.
12. *Fruzzetti F, Bucci F, Perini D, Gadducci A*. Unilateral adnexal agenesis and dermoid cyst: Fertility implications. Gynecol Endocrinol 2015; 31(6): 438–40.
13. *Erkilinc S, Güzel AI, Doganay M, Özer I, Ümit C*. Incidental unilateral tubal absence detected during cesarean section: Report of a case. Gynecol Obstet Reprod Med 2014; 20: 60–1.

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CASE REPORT

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Unusual case of Marchiafava-Bignami disease presenting as axial hypotonia

Neobičan slučaj Marchiafava-Bignami bolesti koja se manifestovala kao aksijalna hipotonija

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Abstract

Introduction. Marchiafava-Bignami disease is a rare disorder mostly associated with chronic heavy alcohol consumption that results in progressive demyelination and necrosis of the corpus callosum. **Case report.** We reported a 35-year-old woman with a history of alcohol consumption and malnutrition. Neurological examination revealed axial hypotonia, dysarthric speech and lack of motor coordination. The brain multislice computed tomography imaging demonstrated hypodense lesion of the corpus callosum. On the basis of her history, clinical features and imaging studies, the diagnosis of an acute form of Marchiava-Bignami disease was made. Definite diagnosis was confirmed at autopsy. **Conclusion.** Marchiafava-Bignami disease is of a medical emergency and early recognition and early aggressive treatment are critical for a good clinical outcome. To our knowledge, this is the first case of Marchiafava-Bignami disease presented with axial hypotonia.

Key words:
alcoholism; corpus callosum; diagnosis; marchiafava-bignami disease; tomography, x-ray computed.

Apstrakt

Uvod: Marchiafava-Bignami bolest je veoma retka i najčešće je udružena sa hroničnom konzumacijom alkohola koja dovodi do progresive demijelinizacije i nekroze korpusa kalozuma. **Prikaz bolesnika.** Prikazana je žena stara 35 godina sa istorijom konzumiranja alkohola i pothranjenosti. Neurološkim pregledom nađena je aksijalna hipotonija, dizartričan govor i gubitak motorne koordinacije. Primenom multislajsne kompjuterizovane tomografije mozga otkrivena je hipodenzna lezija korpus kalozuma. Na osnovu anamneze, kliničkog nalaza i neuroradiološke eksploracije postavljena je dijagnoza akutne forme Marchiava-Bignami bolesti. Definitivna dijagnoza je potvrđena autopsijom. **Zaključak.** Marchiafava-Bignami bolesti je urgentno medicinsko stanje i njeno rano prepoznavanje i rano agresivno lečenje su ključni za povoljan ishod. Prema našem znanju, ovo je prvi slučaj Marchiava-Bignami oboljenja koje se kod bolesnika prezentovalo kao aksijalna hipotonija.

Ključne reči:
alkoholizam; corpus callosum; dijagnoza; marchiafava-bignami bolest; tomografija, kompjuterizovana, rendgenska.

Introduction

Marchiafava-Bignami disease (MBD) is a rare disorder mostly associated with chronic heavy alcohol consumption. MBD is characterized by the primary demyelination and necrosis of the corpus callosum [1]. The computerized tomography (CT) and magnetic resonance imaging (MRI) are helpful in diagnosis in the early stages of the disease [2].

Case report

We reported a 35-year-old woman with a history of drinking red wine and very poor nutritional status. One month before the admission, the patient suddenly developed gait ataxia and slurred speech. The patient was hospitalized because she experienced acute onset vomiting and mental confusion. Physical examination showed asthenia. Neurological examination revealed dysarthric speech, lack of motor coordination and pronounced

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axial hypotonia. Hypotonia was presented primarily of neck musculature with the impossibility of keeping the head and achieving a vertical position with her head kept falling off the back. The results of routine blood tests and cerebrospinal fluid examinations were all within the normal limits. Electroencephalography (EEG) showed diffuse slow waves of 6–8 Hz without epileptiform discharge. The brain CT, which was performed only in transverse plane immediately at admission, showed no significant abnormalities.

Three days later, her level of consciousness rapidly deteriorated and she became comatose [Glasgow Coma Scale (GCS) score was 3] with respiratory failure that required mechanical ventilation. The follow-up brain multislice CT (MSCT) imaging performed one month after the onset of symptoms demonstrated hypodense lesion of the corpus callosum involving genu, body and splenium, in sagittal plane (Figure 1). The diagnosis of an acute form of MBD was made. The patient was treated with a high-dose of thiamine (vitamin B_1), 100 mg per day. A high dose of intravenous corticosteroids was also administered. Three days after the onset of the therapy the patient showed improvement in her consciousness and become sopor, but 11 days after the admission to hospital, the patient got pneumonia and died. A clinical diagnosis was confirmed by postmortem pathologic findings. The main pathologic change was the degeneration of the corpus callosum with demyelination and fragmentation of some axons. Demyelination was accompanied by the focal collections of macrophages and present proliferation astrocytes (Figure 2). General autopsy observation included fibrinopurulent pneumonia and lung abscess on the left side.

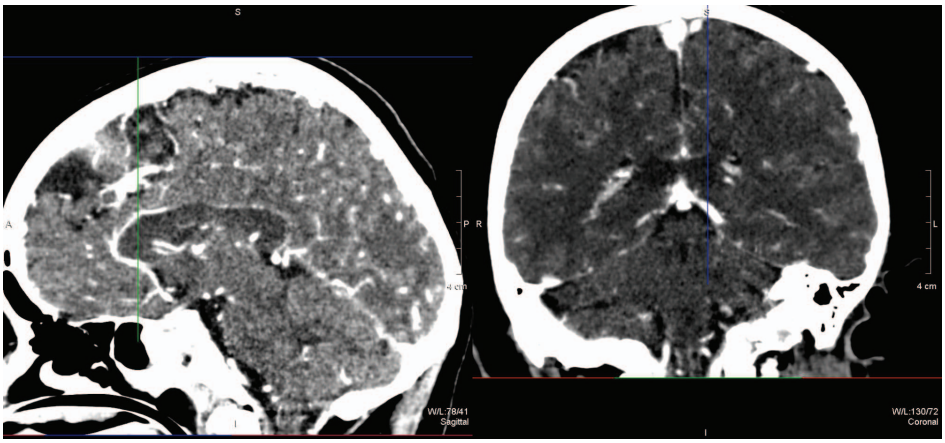

Fig. 1 – Hypodense of the corpus callosum involving genu, body and splenum.

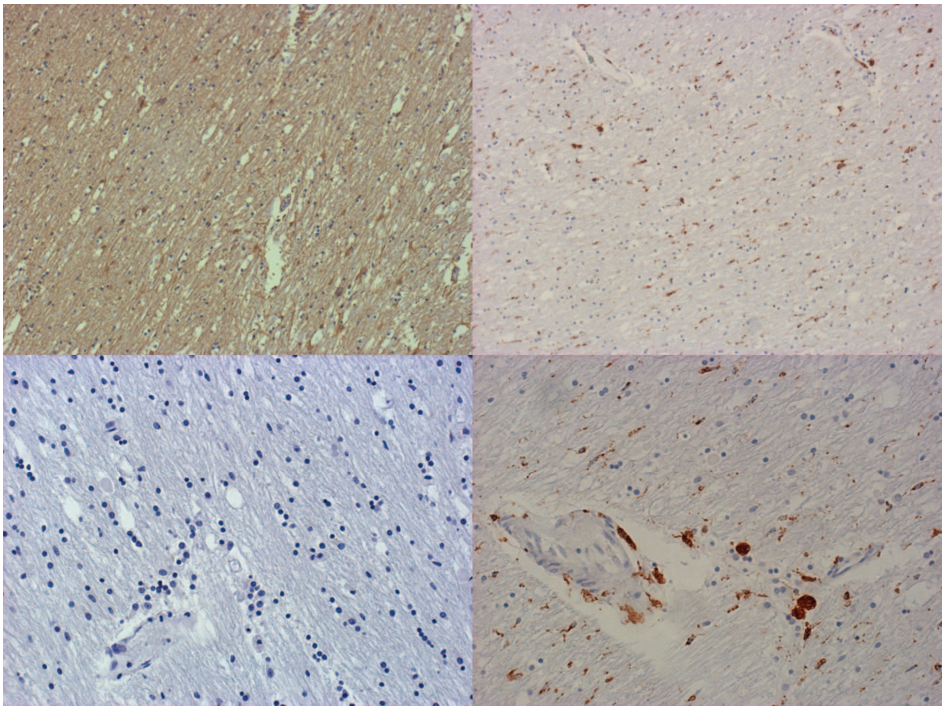

Fig. 2 – Immunohistochemical application for antibodies to neurofilament (NF), macrophage (CD 68) and astrocytes (EFP): A) Demyelination with relative sparing of the axons (100×); B) Humerous lipid-laden macrophages (100×); C) Amyloid corpuscles with macrophage mobilization (100×); D) Blood vessels proliferation and hyalinization of their walls (200×).

Discussion

MBD is a rare disorder mostly associated with chronic alcoholism. Although several cases of MBD were associated with non-alcoholic patients, most instances of MBD have occurred as a result of malnutrition. Our patient had a history of malnutrition and consummation of red wine for an unknown period. It is generally accepted that the disease is due to the deficiency of the vitamin B_1, thiamine [3, 4].

The syndrome is in most instances seen in middle-aged to elderly men drinkers [5]. Our patient was a young women with the acute form of MBD.

In the acute stage, the patient often has non-specific neurologic changes such as dysarthria, seizures, confusion, coma, generalized muscular hypertonia and clinic diagnosis of MBD can be difficult [6, 7]. All authors discussing MBD emphasized the difficulty of making the clinical diagnosis during life because the disease is rare and its manifestations are non-specific [8]. The course of the disease in our patient was acute and presented with unusual finding such as axial hypotonia.

MRI and CT are more useful for early diagnosis and detailed analysis of the distribution of lesions [2]. Our patient presented the characteristic follow-up brain MSCT imaging, performed one month after the onset of the symptoms, and then the disease was recognized.

The most important is the early recognition and detection of MBD. In the era before CT scanning and MRI, MBD was confirmed almost exclusively at autopsy. Our patient had an acute form of MBD that had a rapid course resulting in the fatal outcome. No standardized treatment protocols were established in MBD. The early aggressive treatment is often associated with marked clinical improvement. Clinical improvement was documented when using a high dose of corticosteroids and thiamine [9].

Conclusion

MBD is of a medical emergency and the early recognition and early aggressive treatment are critical for a good clinical outcome. To our knowledge, this is the first case of MBD presented with axial hypotonia.

R E F E R E N C E S

1. *Carrilho PE, Santos MB, Piasecki L, Jorge AC.* Marchiafava-Bignami disease: A rare entity with a poor outcome. Rev Bras Ter Intensiva 2013; 25(1): 68–72. (English, Portuguese)
2. *Hillbom M, Saloheimo P, Fujioka S, Wszolek ZK, Juvela S, Leone MA.* Diagnosis and management of Marchiafava-Bignami disease: a review of CT/MRI confirmed cases. J Neurol Neurosurg Psychiatr 2014; 85(2): 168–73.
3. *Garcia-Santibanez R.* Marchiafava-Bignami disease presenting as acute dysarthria and ataxia. Alcohol Alcohol 2015; 50(2): 256–7.
4. *Kumar KS, Challam RJ, Singh WJ.* Marchiafava - Bignami Disease: A Case Report. JCDR 2014; 8(8): RD01–RD02.
5. *Murthy KP.* Magnetic resonance imaging in Marchiafava-Bignami syndrome: a cornerstone in diagnosis and prognosis. Case Rep Radiol. 2014; 2014: 609708.
6. *Wagh SJ, Dabhi AS, Thorat PB, Vasava JF, Modia JP, Shah MJ.* Marchiafava-Bignami disease: a rare presentation of chronic alcoholism. J Indian Acad Clin Med 2012; 13(4): 59–61.
7. *Tung CS, Wu SL, Tsou JC, Hsu SP, Kuo HC, Tsui HW.* Marchiafava-Bignami disease with widespread lesions and complete recovery. AJNR Am J Neuroradiol 2010; 31(8): 1506–7.
8. *Koeppen AH, Barron KD.* Marchiafava-Bignami disease. Neurology 1978; 28(3): 290–4.
9. *Dujmovic I, Nikolic I, Gavric-Kezic M, Dackovic J, Mesaros S, Drulovic J.* Teaching NeuroImages: reversible widespread brain MRI lesions in Marchiafava-Bignami disease. Neurology 2015; 84(11): e81–2.

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Prophylactic use of the Angel® catheter in a patient with paraneoplastic syndrome scheduled for surgical tumor resection. A case report and literature review

Profilaktička primena Angel® katetera kod bolesnika sa paraneoplastičnim sindromom planiranim za hiruršku resekciju tumora – prikaz bolesnika i pregled literature

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Abstract

Introductio. The Angel® catheter (BiO2 Medical Inc, San Antonio, Texas, USA) is a novel device that combines a triple lumen central venous catheter with an inferior vena cava filter for prevention of pulmonary embolism (IVC filter-catheter). **Case report.** We present the case of a 53- year-old male patient with renal carcinoma and a history of recent deep venous thrombosis (DVT) on oral anticoagulation who was scheduled to undergo open radical nephrectomy. Because of concerns about the risks from documented pre-existing DVT, we decided to insert the Angel® catheter preoperatively in order to have central venous access during surgery and also to reduce the risk of perioperative pulmonary embolism. On the first postoperative day, active gastric bleeding was detected and nadroparine was stopped. Before removal of the Angel® catheter, a pre-removal cavagram revealed large thrombus mass in the catheter filter. Because of the presence of the thrombus mass, the catheter was removed surgically, after a permanent vena cava filter was inserted. **Conclusion.** This case suggests that the use of the Angel® IVC filter/3-lumen central catheter combination could be a reasonable option for pulmonary embolism prophylaxis in the patients at a high risk for DVT, such as the patients with malignant disease, paraneoplastic syndrome and chemotherapy who need to undergo surgery.

Key words:
neoplasms; para neoplastic syndromes; venous thrombosis; pulmonary embolism, vena cava filters; central venous catheters; anticoagulants.

Apstrakt

Uvod. Angel® kateter (BiO2 Medical Inc, San Antonio, Texas, USA) predstavlja kombinaciju trolumenskog centralnog venskog katetera i vena kava filtera koji se koristi u prevenciji plućnog embolizma (IVC filter-kateter). **Prikaz bolesnika.** U radu je prikazan bolesnik, starosti 53 godine, kod koga je planirana radikalna nefrektomija zbog dijagnostikovanog renalnog karcinoma. Duboka venska tromboza (DVT) je prethodila dijagnozi tumora, zbog čega je bolesnik bio na terapiji oralnim antikoagulantima. Uzevši u obzir rizike vezane za ranije tretiranu DVT, odlučili smo se za preoperativno plasiranje Angel® katetera u cilju obezbeđivanja centralnog venskog pristupa i sniženja rizika od nastanka plućne embolije. Prvog postoperativnog dana došlo je do pojave aktivnog gastričnog krvarenja, što je dovelo do ukidanja primene nadroparina. Kavagram urađen po protokolu, pre uklanjanja Angel® katetera, prikazao je veliku trombotičnu masu u filteru. Nakon plasiranja trajnog vena kava filtera, pristupilo se hirurškom uklanjanju Angel® katetera. **Zaključak.** Primena kombinovanog Angel® IVC filter/3-lumenskog centralnog venskog katetera, može predstavljati spasonosnu meru profilakse plućne embolije ukoliko je planiran hirurški zahvat kod bolesnika sa visokim rizikom nastanka DVT, kao što su bolesnici sa malignom bolešću, paraneoplastičnim sindromom i hemioterapijom.

Ključne reči:
neoplazme; paraneoplastički sindromi; tromboza, venska; pluća, embolija; v. cava filteri; kateteri, centralni venski; antikoagulansi.

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Introduction

The Angel® catheter (BiO2 Medical Inc, San Antonio, Texas, USA) is a novel device that combines the inferior vena cava (IVC) filter with triple lumen central venous catheter (IVC filter-catheter). It is used as a temporary IVC filter for pulmonary embolism (PE) prevention and also provides central venous access in the critically ill patients where the routine PE prophylaxis methods, such as anticoagulation, or mechanical compression devices, are contraindicated [1, 2]. The Angel® catheter received United States Food and Drug Administration (FDA) approval for clinical investigation use in the United States in 2013, followed by FDA 510(k) clearance in 2016 as a medical device for protection of critically ill patients at a high PE risk when anticoagulation is contraindicated [3].

Current data support the use of a IVC filter in the patients with documented thromboembolism where anticoagulation is contraindicated, caused complications, or has failed, while a prophylactic IVC filter use is controversial [4–13].

The Angel® catheter advantages include less invasive placement and the convenience of bedside placement, thereby eliminating the need to transport patients to radiology, which can cause delays and increase deep venous thrombosis (DVT) and a PE risk [1]. Published data suggest that the Angel® catheter is safe and effective for short-term PE prophylaxis in the high risk patients with contraindications to anticoagulation [14]. The Angel® catheter placement may also bring benefit to the patients with major trauma, intracerebral hemorrhage, stroke, venous thromboembolic events or active bleeding [1, 14].

In this report we present a patient with renal carcinoma and documented recent DVT under oral anticoagulation who needed open radical nephrectomy. This case is published with the patient's approval.

Case report

A 53-year-old man with hypertension and chronic gastritis was admitted for open radical nephrectomy. Multislice computerized tomography (MSCT) revealed a 39 x 42 x 46 mm tumor in the right kidney with central vascularization and necrosis, a subdiaphragmatic 11 mm mass in the liver and a 32 x 27 mm left suprarenal gland enlargement. Although metastasis of renal cell carcinoma in the suprarenal gland are rare [15], with estimated incidence of 0.5% on the contralateral side based on the data from the European Association of Urology (EAU) [16], preoperative assessment in this case included hormonal measurements (metanephrin, normetanephrin, chromogranin A, cortisol level at 8 a.m.), and results were all within normal range. Because of the documented right superficial femoral and popliteal vein DVT, the patient started oral warfarin 5 mg daily. After two months of warfarin treatment and seven days before surgery, ultrasound with Doppler showed partial (20%) thrombus re-canalization in the superficial femoral and popliteal veins. Because the patient had renal cancer, a surgical treatment was indicated and it was not advisable to delay surgery until complete thrombus recanalization, since the process of thrombus recanalization is long and unpredictable, and "almost complete recanalization" can take up to 12 months [17]. Therefore, in preparation for tumor resection, the patient started low molecular weight heparin (LMWH) nadroparin 0.6 mL subcutanously and discontinued warfarin. One month before surgery, the patient had right renal vein embolization (AZUR®Peripheral Embolization System; Terumo Corporation, Tokyo, Japan). Preoperative laboratory evaluation showed elevated laktate dehydrogenase (LDH) (243 IJ/L), C-reactive protein (CRP) (29.7 mg/L), close to normal creatinine (126 umol/L = 1.43 mg/dL) and mild leukocytosis (11.35×10^9/L).

Because of concern about the risk from the documented DVT and calculated Caprini score 12 [18], we decided to use the Angel® catheter in order to reduce the risk of PE and have the central venous access. The patient was informed that the Angel® catheter was a new promising but not extensively evaluated device and gave written informed consent. The Angel® catheter was inserted through the left femoral vein. After appropriate catheter placement was confirmed with ultrasound and abdominal radiography in accordance with manufacturer instructions [19], the patient underwent uneventful right trans-peritoneal nephrectomy.

On postoperative day 1, after the patient reported malaise, vomited hemorrhagic content and became pale and hypotensive, nadroparin was discontinued and hypotension was treated with volume. Gastroscopy revealed anterior gastric wall ulceration with bleeding, which was stopped by the adrenalin injection. On postoperative day 2, the patient started to walk. On day 6, the patient was mobile and ready for discharge and we decided to remove the Angel® catheter. However, the pre-removal cavagram done based on the manufacturer recommended removal protocol [19, 20] revealed large vena cava filter thrombus (Figure 1). Therefore, because of the Angel® catheter filter thrombus, a permanent vena cava filter (ALN filter, Ghisonaccia, France) was placed via the right jugular vein and only then the Angel® catheter was removed surgically. After control of the proximal and distal femoral vein, the Angel® catheter was removed, a Prolene 4-0 suture was placed and the femoral vein was reconstructed. During the catheter removal, large thrombi located in the vein and the vena cava filter were also removed.

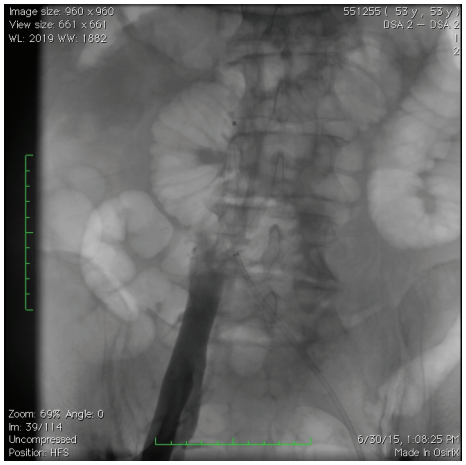

Fig. 1 – Phlebography showing thrombus in the vena cava filter.

Discussion

We report the use of the Angel® IVC filter-central line catheter in a patient with renal cancer and paraneoplastic syndrome with the documented DVT. We decided to place the Angel® catheter due to known DVT despite LMWH prophylaxis, in an attempt to reduce the risk of perioperative PE.

After the Angel® catheter placement and confirmation of appropriate position with ultrasound and abdominal radiography, we proceeded with planned nephrectomy. Although, based on the current literature, indications for placement of the Angel® catheter are debatable, the use of the Angel® catheter in this case allowed us to discontinue perioperative prophylactic LMWH while providing protection against PE.

Based on literature data, pancreas, lung and stomach cancers are associated with DVT, whereas renal cell carcinoma is not [21–23]. However, other studies show that incidence of DVT in renal cancer and paraneoplastic syndrome patients is 10%–40% [16, 24].

In our patient, the femoral vein thrombus formation occurred despite the preoperative anticoagulation and LMWH prophylaxis. The incidence of venous thromboembolism (VTE) is 117/100 000 in the general population, but the risk is markedly higher in cancer, with postmortem studies demonstrating VTE in 50% of cancer patients [25, 26]. Compared to the general population, the cancer patients undergoing chemotherapy have 6.5-fold higher VTE risk, so that 1 in 200 malignancy patients develop VTE [27]. Although prophylactic LMWH reduces the risk by 50%–60% [27–29], thrombosis can still occur [30, 31].

Although anti–factor Xa assay was used for monitoring anticoagulant therapy, we did not measure the anti-factor Xa levels because the routine anti-factor Xa level monitoring is not recommended in stable cancer patients with normal renal function. The anti-factor Xa level monitoring is recommended in the patients with renal dysfunction, but our patient had almost normal renal function [32].

Table 1 shows published data on the Angel® catheter use. There are no data on the IVC filter placement in the patients with paraneoplastic syndrome, history of DVT and a risk for gastric bleeding. In our patient, there were no indications for a permanent vena cava filter placement . However, we were concerned that, based on the history of deep venous thrombosis and calculated Caprini score = 12, this patient was at a high risk for postoperative thromboembolic complications [33]. Therefore, we believed that prophylactic placement of the Angel® catheter was appropriate in this case because the catheter can be easily placed preoperatively by the anesthesiologist and there is no need for surgical removal after surgery. Furthermore, the placement of the Angel® catheter was not associated with the serious complications reported regarding the standard IVC filters.

It is worth pointing out that when planning for this particular case, the anesthesiologist and the surgeon agreed that the risk of significant bleeding during surgery was high. Because this patient had a poor peripheral IV access and the risk of intraoperative bleeding was high, the placement of central venous catheter was indicated for IV access, intraoperative monitoring and administration of intravenous vasoactive infusions [34] regardless of whether or not the Angel® catheter would be used. Furthermore, the published data suggest that when the Angel® catheter is placed in accordance with current recommendations for the central venous catheterization, the risk of infectious complications is very low even in cases where the Angel® catheter remained in place for prolonged periods in the intensive care unit [2, 14].

Because the Angel® catheter could help avoid the traditional IVC filter complications, such as the vena cava perforation, filter tilting, filter migration, and irretrievability, the Angel® catheter use seems reasonable and deserves further investigation. The Angel® catheter use could also be reasonable in the patients who need postoperative LMWH prophylaxis discontinued because of bleeding or other concerns [1]. In this report, we used the IVC filter-catheter because the patient was at a risk for postoperative bleeding complications, including gastrointestinal bleeding and postoperative bleeding and was also at risk for PE due to known DVT.

Table 1

Published data on the clinical use of the Angel® catheter.

Author /Year	Patient number	Comorbidities	Duration (days)	Comments
Cadavid CA et al. 2013 [2]	8	Critical illness: multiple trauma, intracranial hemorrhage or PE	3.8 ± 1.6	Ultrasound guidance in 5 cases, no guidance in 3 cases. Large clot trapped in filter in one case, no complications.
Serednicki W et al. 2015 [1]	1	Critical illness: trauma after a fall	3	Thrombus lodged in the tip of the filter, uneventful catheter removal.
Taccone FS et al. 2015 [14]	60	Critical illness: major trauma, intracerebral hemorrhage, stroke or PE	6 (4–8)	Insertion without fluoroscopy in 90% of cases Reported problems: Guidewire kinked (1 case), filter migration > 2 cm (2 cases), inadvertent removal (4 cases), inability to visualize vena cava (1 case), death (12 cases).

PE – pulmonary embolism.

Conclusion

The Angel® catheter is a novel, less invasive device that combines the IVC filter and three-lumen central venous catheter for a temporary use in the patients at risk for DVT and PE with contraindications to anticoagulation. The preliminary data suggest the Angel® catheter is safe and easy to place and could broaden indications for the IVC filter placement. However, published clinical data are limited, and the true risks and benefits of this promising device are unknown. Large prospective multi-center studies are necessary to better define the role of the Angel® catheter for the PE prophylaxis in different patient populations, including the patients with malignancy, risk of postoperative bleeding and paraneoplastic syndromes.

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Before the procedure, the patient was fully informed that theAngel® catheter is a new device that had not been extensively evaluated, and gave written informed consent. This work was supported solely by the Department funds.

R E F E R E N C E S

1. *Serednicki W, Dobrowolska E, Kałuża K, Kopacz M, Wordliczek J.* Angel(®) Catheter use for pulmonary embolism prophylaxis in a polytrauma patient. Injury 2015; 46(6): 1167–70.
2. *Cadavid CA, Gil B, Restrepo A, Alvarez S, Echeverry S, Angel LF,* et al. Pilot study evaluating the safety of a combined central venous catheter and inferior vena cava filter in critically ill patients at high risk of pulmonary embolism. J Vasc Interv Radiol 2013; 24(4): 581–5.
3. FDA. 510(k) Premarket Notification. Silver Spring, Maryland: U.S. Food & Drug Administration; 2016.
4. *Siskin GP, Bartholomew K.* Inferior vena cava filters. Available from: http://emedicine.com/radio/topic762.htm. [updated 2005 December]
5. *Montgomery JP, Kaufman JA.* Inferior Vena Cava Filters: Indications, Outcomes, and Evidence. Curr Treat Options Cardiovasc Med 2015; 17(9): 401.
6. *Stein PD, Matta F.* Vena cava filters in unstable elderly patients with acute pulmonary embolism. Am J Med 2014; 127(3): 222–5.
7. *Ozturk C, Ganiyusufoglu K, Alanay A, Aydogan M, Onat L, Hamzaoglu A.* Efficacy of prophylactic placement of inferior vena cava filter in patients undergoing spinal surgery. Spine (Phila Pa 1976) 2010; 35(20): 1893–6.
8. *Knudson MM, Ikossi DG, Khaw L, Morabito D, Speetzen LS.* Thromboembolism after trauma: an analysis of 1602 episodes from the American College of Surgeons National Trauma Data Bank. Ann Surg 2004; 240(3): 490–6; discussion 496–8.
9. *Spencer FA, Bates SM, Goldberg RJ, Lessard D, Emery C, Glushchenko A,* et al. A population-based study of inferior vena cava filters in patients with acute venous thromboembolism. Arch Intern Med 2010; 170(16): 1456–62.
10. *Moore PS, Andrews JS, Craven TE, Davis RP, Corriere MA, Godshall CJ,* et al. Trends in vena caval interruption. J Vasc Surg 2010; 52(1): 118–25.e3; discussion 125–6.
11. *Duszak R Jr, Parker L, Levin DC, Rao VM.* Placement and removal of inferior vena cava filters: national trends in the medicare population. J Am Coll Radiol 2011; 8(7): 483–9.
12. *Stein PD, Matta F, Hull RD.* Increasing use of vena cava filters for prevention of pulmonary embolism. Am J Med 2011; 124(7): 655–61.
13. *Haut ER, Garcia LJ, Shihab HM, Brotman DJ, Stevens KA, Sharma R,* et al. The effectiveness of prophylactic inferior vena cava filters in trauma patients: a systematic review and meta-analysis. JAMA Surg 2014; 149(2): 194–202.
14. *Taccone FS, Bunker N, Waldmann C, De Backer D, Brohi K, Jones RG,* et al. A new device for the prevention of pulmonary embolism in critically ill patients: Results of the European Angel Catheter Registry. J Trauma Acute Care Surg 2015; 79(3): 456–62.
15. *Paul R, Mordhorst J, Leyh H, Hartung R.* Incidence and outcome of patients with adrenal metastases of renal cell cancer. Urology 2001; 57(5): 878–82.
16. *Ljungberg B, Bensalah K, Canfield S, Dabestani S, Hofmann F, Hora M,* et al. EAU guidelines on renal cell carcinoma: 2014 update. Eur Urol 2015; 67(5): 913–24.
17. *Puskás A, Balogh Z, Hadadi L, Imre M, Orbán E, Kósa K,* et al. Spontaneous recanalization in deep venous thrombosis: a prospective duplex ultrasound study. Int Angiol 2007; 26(1): 53–63.
18. *Caprini JA, Arcelus JI, Hasty JH, Tamhane AC, Fabrega F.* Clinical assessment of venous thromboembolic risk in surgical patients. Semin Thromb Hemost 1991; 17 Suppl 3: 304–12.
19. BiO2 Medical I. Angel® Catheter & Angel® Catheter Accessory Kit Instructions for Use. 2016. Available from: http://www.bio2medical.com/angel-catheter. 2016.
20. BiO2 Medical I. Angel® Catheter Care & Maintenance Guidelines. 2016. Available from: www.aquilantinterventional.com/.../AD-023_Rev_A_Nursing
21. *Lee AY, Levine MN.* Venous thromboembolism and cancer: risks and outcomes. Circulation 2003; 107(23 Suppl 1): I17–21.
22. *Yokom DW, Ihaddadene R, Moretto P, Canil CM, Reaume N, Le Gal G,* et al. Increased risk of preoperative venous thromboembolism in patients with renal cell carcinoma and tumor thrombus. J Thromb Haemost 2014; 12(2): 169–71.
23. *González J, Ciancio G.* Increased risk of preoperative venous thromboembolism in patients with renal cell carcinoma and tumor thrombus: comment. J Thromb Haemost 2014; 12(4): 577–8.
24. *Palapattu GS, Kristo B, Rajfer J.* Paraneoplastic syndromes in urologic malignancy: the many faces of renal cell carcinoma. Rev Urol 2002; 4(4): 163–70.
25. *Heit JA, O'Fallon WM, Petterson TM, Lohse CM, Silverstein MD, Mohr DN,* et al. Relative impact of risk factors for deep vein thrombosis and pulmonary embolism: a population-based study. Arch Intern Med 2002; 162(11): 1245–8.
26. *Donnellan E, Kevane B, Bird BR, Ainle FN.* Cancer and venous thromboembolic disease: from molecular mechanisms to clinical management. Curr Oncol 2014; 21(3): 134–43.
27. Hull RD, Pineo GF, Brant RF, Mah AF, Burke N, Dear R, Wong T, Cook R, Solymoss S, Poon MC, Raskob G; LITE Trial Investigators. Long-term low-molecular-weight heparin versus usual care in proximal-vein thrombosis patients with cancer. Am J Med 2006; 119(12): 1062–72.
28. *Leizorovicz A, Cohen AT, Turpie AG, Olsson CG, Vaitkus PT, Goldhaber SZ.* PREVENT Medical Thromboprophylaxis Study Group. Randomized, placebo-controlled trial of dalteparin for the prevention of venous thromboembolism in acutely ill medical patients. Circulation 2004; 110(7): 874–9.
29. *Cohen AT, Zaw HM, Alikhan R.* Benefits of deep-vein thrombosis prophylaxis in the nonsurgical patient: The MEDENOX trial. Semin Hematol 2001; 38(2 Suppl 5): 31–8.
30. Kontny F, Dale J, Abildgaard U, Pedersen TR. Randomized trial of low molecular weight heparin (dalteparin) in prevention

of left ventricular thrombus formation and arterial embolism after acute anterior myocardial infarction: the Fragmin in Acute Myocardial Infarction (FRAMI) Study. J Am Coll Cardiol 1997; 30(4): 962–9.

31. *Simonneau G, Laporte S, Mismetti P, Derlon A, Samii K, Samama CM,* et al. FX140 Study Investigators. A randomized study comparing the efficacy and safety of nadroparin 2850 IU (0.3 mL) vs. enoxaparin 4000 IU (40 mg) in the prevention of venous thromboembolism after colorectal surgery for cancer. J Thromb Haemost 2006; 4(8): 1693–700.
32. *Easaw JC, Shea-Budgell MA, Wu CM, Czaykowski PM, Kassis J, Kuehl B,* et al. Canadian consensus recommendations on the management of venous thromboembolism in patients with cancer. Part 1: prophylaxis. Curr Oncol 2015; 22(2): 133–43.
33. *Laryea J, Champagne B.* Venous thromboembolism prophylaxis. Clin Colon Rectal Surg 2013; 26(3): 153–9.
34. *Spyropoulos AC, Douketis JD.* How I treat anticoagulated patients undergoing an elective procedure or surgery. Blood 2012; 120(15): 2954–62.

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One hundred and thirty years from the birth of a medical lieutenant colonel and academician Kosta Todorović: warrior, physician, scientist... humanist

Stotrideset godina od rođenja sanitetskog potpukovnika i akademika Koste Todorovića: ratnik, lekar naučnik... humanista

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Key words:
history of medicine; serbia; military medicine; famous persons; communicable diseases; history, 20th century; sociology.

Ključne reči:
istorija medicine; srbija; medicina, ratna; osobe, poznate; zarazne bolesti; istorija medicine, xx vek; sociologija.

"I admit that I am very sorry and will regret the departure of Dr. Kosta Todorović from the Army, but I will bewail as the soldier and his former chief; but as a Serbian and physician, I may not be sorry just because I am convinced that Dr. Kosta at this Serbian pet child – our young Faculty of Medicine, will be the honor and pride of our complete medical branch, that he will from that position even more be useful for the general cause and his own war fellows, among whom he has been such nicely nurtured" [1].

Introduction

At the beginning of July 2017, one hundred and thirty years passed from the birth of a founder of the Yugoslav infectious disease, a participant in the Balkans War and the First World War, a medical lieutenant colonel and an academician, but above all a patriot and humanist, Prof. Dr. Kosta P. Todorović. The subject of the paper is an attempt to indicate his scientific contribution, especially as a member of military medical corps, in the field of infectious illness, epidemiology and bacteriology, but also regarding his pioneer contribution to the foundation of the sociology of health and diseases, not only with us. The scientific goal is the more comprehensive perception of the personality and work of this great scientist, physician, humanist and war medical officer, contributing to studies in the field of history of medicine and history of sociology of health and illness.

The historical approach was implemented primarily in this research. Regarding the operational method, a quantitative content analysis was used both for the archive material and original works of professor Todorović, but also for the works which others wrote about him.

Growing up, education, idealism formed at the period

The academician Kosta Todorović was born in Belgrade, on 5 July 1887, from the father Pavle, a veterinarian and the mother Brigitte de Piliany, a Viennese by birth, as the third child in the family with thirteen children born, of which nine survived. Due to his father's profession, who was transferred forom the place to place according to the needs of the service, he finished the primary school in Vranje and he attended the first two grades of the grammar school in Knjaževac (there was no grammar school with all grades at the time in this city), then in Zaječar, where he graduated in 1906. He was excused from the final examinations since he was an excellent pupil during his schooling. He had to live alone in Zaječar for some time, separated from the family, subsisting by teaching German [2]. He had a rather clear picture of the ideals he stood up for already as a secondary school leaver, which is obvious from his speech at the St. Sava School Leaving Ball held in the Guildhall in Zaječar on 27 January 1906: "It is an undeniable truth that the future of a nation is reflected in its youth and if the youth is more prepared for the life, if it is animated with a national feeling, then it will bet-

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ter understand the hard task awaiting them in future life...". The Ball also had a humanitarian character, since assets for the school leaving trip should be gathered, and its goal could be seen in the words of the secondary school leaver Todorović: "Even the smallest sacrifice from your side is given for the noblest deed: for meeting our landscapes, saturated with blood and seeded with bones of our forefathers. How big a fire will make the damped spark of patriotism when they see: Mostar, Deligrad, Varvarin, Ravnje, Ljubić, Dublje, sad Kosovo, proud Bosnia, rugged Herzegovina, oppressed Macedonia and old Serbia? All that will surely influence the gentle soul of a young man, feeding it with self-confidence and enthusiasm, so much needed in these gloomy days for our Serbian cause, because Serbia may not expect a smile of a ruddy dawn and a clear sky over the agonized Serbs until it started to grow such sons" [3]. In the self-analysis of his speech, 63 years later, academician Todorović indicated to the agony of the Serbian people under the Ottoman authority, as well as the fact that under such hard circumstances the people had preserved a freedom-loving spirit and belligerence, concluding that these were "the basic elements for establishing a life philosophy of our men of that region and time" [3]. He simultaneously indicated to the ignorance ruling the society, to the lack of contacts of broad masses with developed countries of the world, as well as that "literacy during the slavery had been kept only in churches and monasteries, were not only the preserved monuments of the previous power and greatness of the Serbian state, but also the strongholds of the national consciousness and pride" [3]. In this work, he pointed out that his generation had suffered great temptations and had achieved their ideals through the foundation of Yugoslavia, the common state in which all South Slavs were united. We can notice in this autobiographic contribution of the academician Todorović several of his virtues manifested already in this period of being a secondary school leaver, faithful to them to the end of his life. These were: feeling of national ties in the moments when the Serbian people was oppressed (not only in the sense of political oppression but also socially oppressed: kept illiterate, ignorant, in general misery and poverty) and patriotic feelings for his country[1], Yugoslavianism as an orientation and a great confidence in the youth as bearers of its future and progress, visionary encouraging and supporting them to specialize in various fields of infectious disease, especially those that were risky, because these were absent for a long time and had not enough attention in education. Due to this characteristic of the academician Todorović, Yugoslavia was able to face readily the epidemic of smallpox in 1972. [4–8].

Ready to serve to his people and the Homeland, young Kosta Todorović became a scholarship holder of the Ministry of Military in 1906 and graduated from the studies of medicine in Graz, where he was promoted to the physician of general practice on 23 March 1912. Just in time to become involved in the wars ensued for Serbia and to help his people in the best possible manner – by curing them. During the studies in Graz, he participated in the foundation of the Association of Yugoslav Medical Students in 1909. According to the Todoric's testimony, the association was founded on one hand as a reaction to discrimination of German students concerning the others, and on the other hand, as the need that students – Slovenians, Croats and Serbs - felt for mutual rapprochement in the professional field, but with clear presence of the idea of Yugoslavianism [9]. Between the two World Wars, Dr. Kosta Todorović also took part in establishing a joint Yugoslav Physician Society, the president of which was Dr. Milan Jovanović Batut. He considered that it presented accomplishment of the "a long standing wish of Serbian physicians, members of the Serbian Physician Society, to unite in their noble work with the members of existing physician societies in Croatia, Slovenia, Bosnia and Herzegovina, Montenegro, Macedonia and Dalmatia", but also indicating that "this idea existed before, even before the Balkan Wars, obtaining its form even in 1909 when the Association of Yugoslav Physicians was established from the physicians' progeny – the Yugoslav medical students in Graz" [7].

Participation in wars, suppression of great national plagues, scientific works conceived at front

In the First Balkan War, he was allocated as the physician of the Third Field Hospital of the Timok Division of the first requisition. Since then, he participated in all the wars that Serbia fought for liberation and unification in various medical posts – the regimental physician of the Timok horse regiment of the first requisition, the physician of the Timok permanent military hospital, the regimental physician of the XVIII infantry regiment, the Danube horse regiment of the first requisition, XIII infantry regiment "Hajduk Veljko", the physician in the Central Military bacteriological laboratory in Thessaloniki, founded and managed by the famous Polish scientist Dr. Ludwik Hirszfeld (1884–1954), the chief of the Bacteriological Laboratory of the II Army, at service in the I and the II bandage place of Sumadija regiment and the II bandage place of the Timok regiment, the director of Dubrovnik temporary military hospital, the acting Chief of the Timok permanent military hospital and the Chief of the Internal Department of the same hospital. In 1924, on his personal request, he was transferred from his last post with a rank of medical lieutenant colonel to civilian medical service [10].

A year before the end of his life, summarizing the influences shaping him as the person, the academician Todorović pointed out: "The Army had an unusual positive influence on me... the Army thought me a disciplined work, appropriate fulfillment of obligations, complete and conscious surrender to a task. The Army relies on a healthy and useful principle: it can only help a smart person. I should thank a great deal to this self-discipline gained in the Army for the later results in my work, as a clinical physician, as a University professor and as a scientist-researcher" [11]. This statement of Dr. Todorović is certainly sincere and truthful. If his early works published in the "Serbian Archive" are viewed, they are mainly the result of his work as a physician and simultaneously scientific-research work during the First World War. In that sense, the statement is correct but

[1]According to the judgement of the author, three categories of individual's feelings for the community should be distinguished, one of them being very harmful – chauvinism, and the other two are not harmful, but may be very beneficial [4]. At that, nationalism is the love for the nation and patriotism is the love for the country [5] and here congruency may or may not exist, depending if the issue is the identity state-nation or multinational state.

incomplete in that that Kosta Todorović "designed his works standing by sick-bed" [12]. It should be added – at the front also. He gained his first war experience in the First Balkan War. The Timok Division, where he was the physician in the third field hospital as a medical captain of the 2nd class, was facing different infectous diseases during the siege of Adrianople, mostly with epidemics of dysentery, typhoid fever and cholera that reigned in Bulgarian and Turkish armies "which were very soon transferred to Serbian divisions. Objectively, poor dwelling conditions facilitated the appearance of the disesases (weather conditions and accomodation), but a part of the problem was in the unpreparedness of medical corps to fight the challenges in such a complex situation in which it was. Negligence regarding the development of military prophylaxis that lasted for decades, burdened the Serbian Army even in the peacetime, emerged for the first time in war conditions in its full actuality" [13]. Dr. Kosta Todorović pointed out this just after the end of the Second Balkan War in the questionnaire of the Serbian Red Cross regarding the role of that organization in Balkan Wars: "When dangerous infectious diseases appeared (especially cholera, Typhus exanthematicus), the help of the Red Cross was arriving late. It would be good to have a considerable number of Deker's barracks and the complete hospital accessory (beds, blankets, warm socks) in order to be able to subvene immediately. The fast help – is the double help" [14]. Zealously performing his duty in very complex epidemic conditions, young Dr. Todorović had two roles in one moment – he was a physician and a patient. Healing others, he went down with Typhus exanthematicus. "When I came home for recovery, even my own mother did not recognize me, it was such an illness" [11]. This experience directed him to study infectious diseases.

In his first work published in the referred magazine just after the end of the First World War in 1919, Todorović presented conclusions that he reached regarding the epidemic of cholera among the Serbian army in the Rrashbull Camp at the beginning of 1916, where his unit was located. "Rrashbull Camp provided a picture of a filthy and a polluted place. Apart from the wasted food, clothes and footwear, the whole camp was polluted with human feces. The troops were located there for a short period, ready to start for Durres as soon as a transport vessel arrives. The smallest attention was not paid to the cleanliness of the camp. Several fresh barrows and crosses marked graves of soldiers that had died in the camp. The troops of a new echelon arrived to such polluted camp. The soldiers of the 9th, 18th and horse regiment occupied all the places and tented, like the troops before them. There was no place for chambers of this echelon in the meadow. They had to be located in a field, where no one before had camped..." [15].

Todorović in his text then indicated that after three days the soldiers started to go down with Asian type of cholera. However, he noticed that only the soldiers located in the meadow went down, the same space where the earlier troops were located, while no-one went down among the soldiers located in the field that had not been used for camping before. Considering what might be the source of the plague, he excluded both the food and drinking water, although he considered that drinking water was "much more suspicious". "Muddy, due to intensive pumping, polluted with mud and surface water with alot of scraps and distasteful – water was rightfully considered to be dangerous. Was it polluted in this case, was it the cause of going down with cholera? The answer to this question with a great probability would be yes. However, if the drinking water was the source of the plague, would not there appear many cases of cholera among the soldiers? If not all, but the majority of soldiers should go down since they all had drank water from the same well". Considering the referred facts, he concluded that the illness was transferred "indirectly, from the polluted soil", warning on the differences regarding the (not) going down of the soldiers that were located in the previously used space and in the space that was not used before for camping, "although that part of the echelon had the same food and water, lived under the same circumstances, endured the same efforts as the others. Pollution of the Rrashbull Camp by human feces was an obvious danger to the health of soldiers and fresh barrows indicated that there were soldiers dying in the camp in the previous echelons. If we connect these two and add a sporadic appearance of cholera – we shall get a clear picture of the infection from the polluted terrain". This was a decisive conclusion about the polluted terrain and location of troops "that had to camp in the same place where other troops had camped before them, in spite of the known principles of military sanitation" [15]. He admitted that he could not make a bacteriological test for this diagnosis, but the clinical picture was completely clear. Since Todorović had graduated at one of the most prestigious medical faculties of the period, he was familiar with the work of Louis Pasteur (1822–1895) regarding anthrax and his famous neologism *les champs maudits* regarding the grazing land previously infected with anthrax bacillus, where infected cattle had dunged, or infected animals were dying and were left to rotten, so that the disease was transferred to healthy cattle and from them to people. Todorović considered that Pasteur was a "genius", holding that "infectious diseases became the subject of thorough discussions only after Pasteur" [8]. It is no wonder that he reasoned the conclusion about cholera in the Rrashbull Camp by the implementation of analogy concerning the Pasteur's argumentation regarding the spreading of anthrax.

Kosta Todorović was a regiment physician who was able to treat a huge number of Serbian soldiers diseased from malaria during 1916–1917. He applied the quinine therapy, not only in treatment but also in prophylaxis, testing how much it would be successful. He published the results of his researches after the end of the war [16], establishing the prophylactic use of quinine as beneficial, "when it is performed properly". In the already mentioned letter to Prof. Dr. Dj. Nešić about the success of Kosta Todorović in the treatment of diseased from tropical malaria, Dr. S. Popović wrote among other things: "the vast number of our agonized healed after injections of Dr. Kosta and were again ready to fight. You also, Mr. Nešić, together with me, as well as many of our colleagues, were coming to Novoselce, to the troop physician of the XIII infantry regiment, to the captain Dr. Kosta Todorović, to get informed about the famous training work of Kosta, regarding suppression and treatment of tropical malaria, which choked us in those swamps of Moglen... All these people, almost without exemption, were successfully treated only in the front in our famous bandage places according to the instructions of Dr. Kosta Todorović, which he was giving in silence from

Novoselce" [1]. Certainly, Todorović's credit reflects also on the fact that he created or systematically was taking a series of preventive and anti-epidemic measures regarding the improvement of soldiers' hygiene, control of water that soldiers were drinking, suppression of lousiness, etc.. Everything was done in almost impossible conditions in which the Serbian army was on the Thessaloniki Front. In his explanation of malaria epidemic in the Serbian Army located near Topcin, he provided a "good description of Moglen valley" [17], considering geographical, hydrological and vegetational conditions at that place to be extremely favorable for the development of the epidemic. This epidemic, in the suppression of which he directly participated, left a deep trace in his life and research work, so that he remembered it for decades. Not only he was gathering medically relevant knowledge on the basis of the acquired empirical insights, but also he left valuable evidence on the heroism of a small, brave nation, outcast from the country, fighting to return to it and liberate it from the conquerors. "Let us mention only the number of diseased from malaria. It was so great during summer and in autumn that in companies, batteries and other units only a dozen of healthy soldiers remained, while diseased – expressing this as a personal experience – did not leave their units and their arms would be engaged in their combat functions as soon as fever stopped" [11, 18]. He did not exclude his evidence on the fallen was comrades and their ideals, "the scene experienced at Kajmakčalan after the end of the battle" [19], while the description of the tenacity in motives and character and the resolution in accomplishing the goal of regaining the freedom for their own country and liberation of enslaved Serbs, the modesty of Serbian soldier, the gusle-instrument transferring a "distant message", he left for Raoul Labry, the administrative officer of the French army, who retreated with the Serbian army across Montenegro and Albania, keeping a diary starting from March 1915. Todorović translated and published an abstract from that diary [20].

Todorović pointed out in both his works [15, 16] to the social factors contributing to the outburst of infectious disease epidemics. In the first case, they were military rules for choosing a camping site, in the second case these are, among others, zoning reasons: insanitary dwellings (e.g., absence of sewerage), their proximity, both mutual and with the Serbian army troops; low level of health organization reflected in the fact that the inhabitants of those places at Moglen field were chronically untreated malaria sufferers ("Locals are the main source of the infection, mosquitoes are transmitters" [16]; the professions of the people requiring increased physical efforts – inhabitants were predominantly farmers, while soldiers were exposed to great efforts in combats, especially during the summer months and in autumn, when the number of diseased was increasing; lack of medical and culture in general. The inclusion of social factors into the consideration of the problem of epidemics of the infectious diseases was not by accident for Dr. Todorović. He pointed out their importance also in his inaugural when taking over the Department for infectious diseases at the newly founded Belgrade Medical Faculty and when he was appointed the director of the Infectious clinic in 1926. "Transfer of infectious diseases was supported by a close contact of certain communities, especially by movement of greater masses, like migrations or wars. War luck was often determined by losses due to war infections and until recently war infections were causing much greater losses than combat weapons" [21, 22]. He considered them to be so important that in his famous textbook he explicitly stated that a contemporary physician may not lose sight of the "influence of social factors on the emergence of acute infectious diseases". He rated among those factors "various customs, superstitions, habits", "profession of the diseased" that "often gives the reason for infection and for the disease" [8, 15], "social position" that had an "undeniable influence to the outbreak of certain infectious diseases. Some of them occured more often among the poor, other ones among the wealthy. Certain diseases were rare among village people, more often among citizens. Homeless people and people without a profession, without the most required conditions for a sanitary life, more often went down due to acute infectious diseases than other people. People that did not pay enough attention to body cleanliness, changing clothes and bedding, and people that came in contact with such people, were more exposed to the danger of getting infected and going down with various infections, transmitted by lice, fleas, bedbugs and other insects (Typhus exanthematicus, Typhus recurrencsand others)" [8, 15]. With such attitudes, Dr. Kosta Todorović is certainly the founder of medical sociology (sociology of health and disease) with us, significantly before this discipline is considered to be established in the world. He was among the first who noticed and clearly showed the influence of social factors on the health of and illness in people [23].

From the infantry regiment "Hajduk Veljko", Dr. Kosta Todorović was transferred to the Central Military Laboratory in Thessaloniki, managed by Dr. Hirszfeld. Namely, the decision was to make a central laboratory at the level of the complete military medical corps, then one within each army. However, it was necessary to provide staff for their functioning. Therefore, Dr. Todorović was transferred to the Central Military Laboratory. Dr. Hirszfeld evidenced that. "We decided, together, military medical chief Colonel Stajic and me, to educate Serbian bacteriological staff and add a laboratory to each army... Colonel Stajić told me that he had met a young physician who was studying tropical medicine while sitting in a trench during bombarding. – 'Colonel, that is our man, send him to me, please' – It was Doctor Kosta Todorović" [24]. In the foreword of the Serbian edition of Hirszfeld's book "The Story of One Life", writing about his war medical teacher, Dr. Todorović summarized the results that this laboratory, in extremely improvised conditions, owing to Hirszfeld's "talent for improvisations" (Todorović), acquired in the production of anti-typhoid-paratyphoid vaccine, which Dr. Hirszfeld tested firstly on himself, then on his associates and finally on soldiers. "This vaccine was used on 100,000 Serbian soldiers at Thessaloniki front and successfully protected them from enteric typhoid and paratyphoid. The result was that Serbian army returned to the homeland after the end of the First World War without single typhoid and paratyphoid diseased among the vaccinated soldiers" [10, 25]. There is no need to point out how big success it was, especially considering that in Serbia, according to Todorović, during the winter 1914 and spring 1915 between 500 and 600 thousand people went down with Typhus exanthematicus. "Serbia met the World War completely military and medically unprepared... Typhus exanthematicus soon joined the war misery. The more we were approaching Valjevo, once

progressive and festive small town, the more often we could see the great misery... There was no house without a diseased in the town (Lazarevac, author's remark). Apart from measles, whooping cough, scarlatina, diphtheria, Typhus recurrens and enteric typhoid, the Typhus exanthematicus had the greatest number of diseased. Indescribable fear spreaded around, especially when frantic diseased in delirium started to run away and attack. A poor man from Podrinje, in his delirium, jumped into a well in open sight of haggard refugees... One artillery sergeant, lying with complete armament in the coffee house 'Plow', swept out to the railway station, started to throw bombs around, believing that he was in a combat with the enemy, until his power failed and one activated bomb exploded in his hand, pacifying this sergeant-fighter for good... The diseased were lying densely in a room of the Bank of Požarevac, almost one across another. While one of them was easing himself at the door and could not find his bed like he was lost, there were dying people in the room. In a dark corner, a candle was fuming in the hand of a diseased dressed in new farmer's clothes, with a new belt, his hands folded on his chest. He and his parents were expecting the last moment. Death was salvation" [26]. Todorović indicated that in the Danube horse regiment of the I call, with the aim to suppress the epidemic of the Typhus exanthematicus, "it seemed" that "Serbian barrel" for disinfection had been designed, "even before Hunter" [26]. In explanation of spreading epidemic of Typhus exanthematicus also, Todorović insisted on social factors. "Progress of enemy army, movement of great military masses and refugees were convenient for spreading epidemic... Huge traffic, inevitable during the war, contributed to spreading of Typhus exanthematicus even to the most outlandish regions and entangling the whole country" [26]. Contemporary physicians add to these factors also the fact that Serbia, after the Balkan wars, extended to regions of Sandzak around Novi Pazar, Kosovo and Metohija and Macedonia, endemic seats of Typhus exanthematicus at the period [27], which also represented a confirmation of findings about the influence of social (political) factors on spreading epidemics, especially. However, regarding the suppression of epidemics, Todorović indicated the importance of creativity, innovation and the ability for improvisation, due to which individuals gifted with these abilities, in almost impossible conditions, managed to save many lives. One of the most skilled people was Todorović's chief at that period, Dr. Hirszfeld, who not only managed to make a successful antityphoid-paratyphoid vaccine in an improvised laboratory, but he also discovered the bacillus of paratyphoid C later named after him, and achieved a great success in the field of transfusion medicine on the basis of the immunobiological research of blood types with different nations, contributing to the successful implementation of blood transfusion in the Serbian army with a control of blood types of donors and recipients. His findings in the field of transfusion medicine regarding the "implementation of serological methods in studying human races" were the most significant [12, 25].

Civil physician, founder of Yugoslav infectious disease, professor, academician, scientist

After the end of the First World War, Dr. Kosta Todorović remained in active military service until 1924, when he resigned and left for specialization in infectious diseases to Paris hospital "Claude Bernard", and to attend a bacteriological-epidemiological course at the Pasteur Institute in Paris.

After finished specialization in infectious diseases, Kosta Todorović was appointed an associate professor of acute infectious diseases at the Faculty of Medicine in Belgrade and the director of the Infectious Diseases Clinic in 1926. Eight years later, he was appointed a full professor. In his paper "Nos recherches et nos expériences dans la question du virus scarlatin" presented at the convention of French physicians at Montpellier in 1929, based on his own research of "blood, blood plasma, pharyngeal mucus and urine of patients with scarlet fever, professor Todorović pointed out that he could never raise a single visible virus from that material nor to generate an experimental scarlet fever with it. On the basis of his research, supported by the experiments on humans, he claimed that there was no visible or invisible germs in blood plasma, but only toxins" [28]. These findings confirmed the etio-pathogenesis of scarlatina based on toxic nature of this disease, with pathogenic hemolytic streptococcus taking "place of the main etiologic factor for scarlatina" [29]. This verified the results of previous researches of the disease, especially of the so-called "American school" guided by the Dick couple. Since then, this issue was considered to be solved, bringing to Dr. Todorović an international scientific affirmation. Professor Todorović, probably due to his war experience, dedicated a great part of his scientific career to the researches of typhoid and paratyphoid diseases, publishing more than 30 scientific works. "The referred matter was elaborated studiously and documentary, during a series of years, from all aspects, making, we may say, a world contribution to the research of typhoid diseases" [30]. During the Second World War, when he was made available both as the professor of the Faculty of Medicine in Belgrade and as the physician at the Infectious Diseases Clinic, defending his ethics and patriotic feelings, he refused to sign the disgraceful "Appeal to Serbian People" organized by the minister of education Velibor Jonić in collaborating government of Nedić, but he accepted to go to Bajina Bašta in May 1942 and face the epidemic of Typhus exanthematicus and enteric typhoid there caused by the surge of refugees from Bosnia, Kosovo and other regions of occupied Yugoslavia to that small place in the Western Serbia [31]. After the end of the Second World War, although he was not a member of the party, the academician Todorović continued to be the director of the Infectious Diseases Clinic until his retirement in 1957. Just after the liberation, he was also the rector of the Great Medical School (1950–1951), then the chief of the Department of Internal Medicine (1951–1957). His professional reputation with all physicians surely contributed to his election, especially internists, electing him also the president of the Internist Association of Yugoslavia (1956) [32]. According to the social-political system of the period, party organizations and their representatives were giving characteristics about everyone, therefore about the academician Todorović. As a historical source, these surely must be taken with a certain reserve, regarding the significant ideological-party position from which these were given, especially for nonmembers as professor Todorović was. However, we consider justified to cite some of the statements, not only for the purpose of understanding the personality and for the purpose of actions of this acade-

mician, who rose the Yugoslav infectious diseases to such significance that his students with good reason declared the whole period of his practice as the "medical epoch of Kosta Todorović in this country. He became a living medical legend, the legend of knowledge and the legend of humanity" [33]. "The most famous physician in Yugoslavia in the 20th century certainly was and remained Dr. Kosta Todorović" [11]. We mention abstracts from these characteristics in order to enable a reader in the present period to make conclusions about the social context after the end of the Second World War in the socialist Yugoslavia, where the scientist and physician of such a format successfully performed his job. In one of them, it was stated, among other things: "He is an expert. The best clinician with huge experience and knowledge. He is the professor and the physician with the greatest reputation of all the physicians of the PR Serbia" [34]. In another: "He is the best expert on infectious diseases in our country" [35]. Although the characteristics were very positive, in the later it animadverted that "one of his very serious oversight was that he did not gather around him a suitable staff, nor did he create his school at the clinic. At the clinic, there was no unity of method and work, both in practice and in teaching... he often takes care not to antagonize, acts opportunistically, often in the Department and in the Faculty Council" [35]. The "serious oversight" from this characteristic was denied by the time, since after him and just because of him, which we mentioned at the beginning of this paper, a constellation of experts remained, who had the most important place in the Yugoslav infectious diseases and successfully struggled with the most complex problems, like caring for diseased from highly contagious diseases, e.g., smallpox. Great number of them remained devoted personnally, proudly considering him to be their teacher. His patriotism was determined in both notes, it was established that he had never been politically active and only one political attitude was mentioned, which we may understand to be literal since it was mentioned in the direct speech. Regarding the Resolution of Cominform, the academician stated that it was "misfortune of our nations" [34].

Streptomycin was discovered in these years and the academician Todorović ventured to its therapeutic implementation, publishing a series of works on that subject, "making solid foundations for the therapy of tuberculous meningitis with us" [30], but obliged also the world medical science. The World Health Organization (WHO) helped him with that, "placing at his disposal great quantities of streptomycin, the medication that was lacking at that time, for the research and scientific experiments. The trust shown to a Serbian scientist by the highest medical forum was a great recognition for our young medical science" [2]. Besides, he was dealing also with other forms of infectious meningitis, and the results published in the works about meningoencephalitis had a consequence that in 1960, the Ciba Foundation from London organized in his honor a scientific gathering, where the works about this disease were presented from various countries of the world [36].

According to the evidence of his students, "working day of professor Todorović lasted at least 12 to 14 hours. The job was very extensive, starting from every day the examinations of seriously diseased, always very patiently and very precisely. On the way, he never refused to examine someone and to give advice, regardless the social status; a minister had no privileges, a beggar was never rejected. Only a more serious disease had an advantage in the effort and exertion to get help" [12]. However, those efforts must not be assigned to the "notorious enthusiasm and partly fanaticism". He was "accomplishing even the impossible because he was an excellent organizer, who subordinated everything to the treatment of diseased" [12], with good mental and physical health even in the old age. As an influential factor to origination and maintenance of such a zeal, it surely should be considered that "his obligations of a military physician in war implied superhuman efforts" [12].

Humanist

He died in his 88th year, in 1975. At that time, he was actively working on the organization of celebrating the half of a century from the foundation of the Infectious Diseases Clinic, which bears his famous name since then.

Academician Kosta Todorović was trying to solve by himself some issues from his life and work that he considered important. One of the facts is his direct report about the influence of the army to his life and work [11]. Secondly, it is the explanation of how he had determined to deal with infectious diseases. "Actually, I opted for infectious diseases in wars" [11], referring to the epidemic of Typhus exanthematicus during the First Balkan War, when he himself went down with this dangerous national plague. Todorović mentioned the epidemic of cholera in Rrashbull camp as "one of the crucial moments" in his interest for infectious diseases, while he was definitely determined to study these diseases. He said: "at the Thessaloniki front. I encountered an unbelievable epidemic of malaria" [11]. Thirdly, it is his definition of a diseased "It is a miserable, helpless man expecting... help" [11]. Fourthly, he discovered the ideal organization of a clinic at the Paris Clinic Claude Bernard. "I was with the famous expert for infectious diseases professor Tessier, I saw what they were doing and what importance they were giving to scientific research. Everything at professor Tessier's clinic was so organized to be for the benefit of a diseased" [11]. Todorović found his personal model at scientific researches in Dr. Ludwik Hirszfeld, who "was accessing the scientific work with such an enthusiasm that was able to inspire others" [11]. On his behalf, Hirszfeld recorded that from all of his Thessaloniki students "for me Kosta was my favorite student, not only because of his extraordinary ability but also because of the enthusiasm and virtues of his character" [24]. Dr. Kosta Todorović insisted very much on the general education of a physician and his general culture that "had to be an inherent part of the personality of a contemporary physician" [11]. Therefore, his companionship with respectable writers like Ivo Andric is no wonder, but also the friendship with his "war buddy", Prince Djordje Karadjordjević, who often visited him at the Infectious Disease Clinic. The seventh is the belief of Todorović regarding the relationship physician-patient: "a physician must get close to a patient so that he is sure that you are his friend and not a clerk that approached him to feel his pulse and ask if he had a temperature. If a patient has no confidence in his physician, everything goes much harder" [11]. "We are here for patients; they are not here for us. Therefore, treat them like everyone of them is your child, mother, father, sister, brother", he was advising – we would say a moral impera-

tive – his students and younger colleagues, were not allowed to awake a sleeping patient during his examinations, "because patient's sleep is more important than any examination, which we can do later" [37].

Conclusion

The love for the country, libertinism and praxis patriotism were among the motives on which the academician Todorović insisted the most. He was a Serbian physician and Yugoslav patriot in the literal sense of the words. Reasons that predestined him for the professional military service were just those that we mentioned in the previous conclusion, while the reasons that predestined him to study medicine might be found in a deep establishment in the social environment from where he emanated and in his wish to be of general social service to his nation. His dealing with infectious diseases was connected with the determination to face the most serious diseases, and in that way, to serve the nation to which he belonged, which he clearly explained in the manner as presented in the paper. Just the sense of understanding the consequences of poverty and ignorance, understanding the greatness of the suffering of the Serbian nation, not only in war but also in the period under occupation, his ability to recognize that as the factor of influence to development of diseases in people, resulted in the scientific contribution, not only in the field of the basic science that he was interested in – the infectious diseases but also in the field of the sociology of health and illness, and finally, in the field of the history of medicine, especially in the history of the Serbian military medical corps, leaving the valuable evidence on various infectious diseases and his medical experience of them during the Balkan wars and during the First World War. The fact that he was the founder and the president of the Association of Recipients of the Albanian Commemorative Medal 1915–1916, whose goals were taking care and improvement of the social position of old warriors-heroes, his fellow-soldiers, and then cherishing the memory of their past and glorious history of the Serbian army, all that is the best evidence about how much he insisted on his fellow soldiers and the memory of those who gave their lives for the freedom of the Homeland as well as on heritage of the historical truth about their unprecedented heroism. The highest ideal of the academician Todorović was to help a man with a desease. In that sense, one may say that an innate humanism was on the pedestal of his values which was leading him throughout his life and work.

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R E F E R E N C E S

1. Letter to Prof. Dr. Djordje Nešić from Dr. Sava Popović. In: *Petrović Z*, editor. Memorial to academician Kosta Todorović. Memorials, book VII. Department for medical sciences, Book 1. Belgrade: SASA; 1988. (Serbian)
2. *Velimirović M.* Dr. Kosta Todorović. Razvitak 1975; XV (4–5) July-October: 58–66. (Serbian)
3. *Todorović KP.* Expectations and accomplishments: How a secondary school leaver of Grammer School in Zajecar in 1906, subsequent physician, imagined his life mission and what has been realized. Belgrade: Naučno delo; 1971. (Serbian)
4. *Tadić, Lj.* Science on politics. Belgrade: BIGZ; 1996. (Serbian)
5. *Primorac I.* Patriotism: ethical and ground. ARHE 2005; 2(4): 247–63. (Serbian)
6. *Baljosević S, Pavlović M.* Memorial to professor Vojislav Šuvaković. Belgrade: Academy of Medical Sciences of Serbian Association of Physicians; 2017. (Serbian)
7. *Todorović K.* Foundation of Association of Yugoslav medical students in Graz 1909. Jugoslovenski istorijski časopis (JIČ) 1976; (1-2): 123–9. (Serbian)
8. *Todorovic, K.* Activity of Serbian Association of Physicians between two World Wars 1918 – 1941. In: *Djuric DS*, editor. Serbian Association of Physicians: Memorial 1872-1972. Belgrade: Serbian Association of Physicians; 1972. p. 147–58. (Serbian)
9. Biographical data and data about professional and scientific activity of Prof. Dr. Kosta P. Todorović, director of Infectious Diseases Clinic of Great Medical School in Belgrade. Files of deceased members of SASA – Prof. Dr. Kosta Todorović. Belgrade: ASASA; 2013. (Serbian)
10. *Adamović D.* Dialogues with contemporaries. Kosta Todorović (1887-1975). Belgrade: Privredna stampa; 1982. p. 364–7. (Serbian)
11. *Šuvaković V.* Kosta Todorović: physician and humanist (1887-1975). Acta Infectologica Yugoslavica 2001; 6(2): 243–9. (Serbian)
12. *Nedok AS.* Balkan Wars 1912-1913. Operations of Serbian military medical corps. Belgrade: Medija centar "Odbrana"; 2012. (Serbian)
13. *Subotić VM.* Serbian physicians and Serbian volonteer nurses in wars with Turks and Bulgarians 1912 and 1913. Belgrade: Serbian Red Cross; 1919. (Serbian)
14. *Todorović K.* Epidemiology of choleraic disease in Rrashbull camp near Durres. SA 1919; XXI(4): 170–2. (Serbian)
15. *Todorović K.* Accute infectious diseases. Belgrade: National printing house; 1933. . (Serbian)
16. *Todorović K.* About malarian preventive at Thessaloniki front. SA 1920; XXII (7–8): 320–38. (Serbian)
17. *Popović B, Mikić D, Zeljković J Čekanac R, Vidanović M.* Malaria in Serbian army at Thessaloniki front with special retrospective to outburst of epidemic in middle of 1916. In: *Beleslin A,* editor. Serbian military medical service in 1916. Monographs of scientific meetings AMN SLD, series B. Belgrade; AMN SLD; 2007; 2(1): 115–33. (Serbian)
18. *Todorović K.* Foreword. In: *Todorović K*, editor. Through Albania 1915-1916. Belgrade: Prosveta; 1968. p. 444–7. (Serbian)
19. *Todorović K.* At Kajmakčalan after battle. In: *Todorović K*, editor. Thorny path of Serbia 1914-1918. Belgrade: BIGZ; 1974. P. 330–2. (Serbian)
20. *Labry R.* From Diary. Translation K. Todorović. In: *Todorović K.* editor. Through Albania 1915-1916. Belgrade: Prosveta; 1968. p. 444–7. (Serbian)

21. *Todorović K.* Major moments from history and present of Infectious Diseases. SA 1927; XXIX (10): 721–37. (Serbian)
22. *Šuvaković U, Baljošević S, Obradović Ž.* Smallpox and globalization or the first achieved plannetary goal. Vojnosanit Pregl 2014; 71(3): 301–6.
23. *Šuvaković U.* Academician Kosta Todorović – Serbian military physician in First World War and his understanding of influence of social factors to diseases and health of people (sociological-medical contribution). In: *Šuvaković U*, editor. Century of Serbian Golgotha (1915-2015), vol. 3 Social sciences. Kosovska Mitrovica: Faculty of Philosophy of University of Priština; 2016. p. 527–40. (Serbian)
24. *Hirszfeld L.* The Story of One Life. Belgrade: SKZ; 1962. (Serbian)
25. *Todorović K.* Ludwik Hirszfeld. In: *Hirszfeld L*, editor. The Story of One Life. Belgrade: SKZ; 1962. p. 5–16. (Serbian)
26. *Todorović K.* Memories on epidemic of Typhus exanthematicus from 1914/15. In: *Stanojević V,* editor. Memoir of unpublished works presented in Section, vol. II In commemoration of fifty years from Albanian Golgotha. Belgrade: Serbian Medical Society, Section for History of Medicine and Pharmacy; 1965. p. 60–3. (Serbian)
27. *Mikić D, Nedok A, Popović B.* Infectious diseases in Serbian army and nation in 1914 and 1915. In: *Nedok A, Popović B*, editor. Serbian Military Medical Corps 1914-1915. Belgrade: Ministry of Defence, Directorate for Military Health Care, Academy of Medical Sciences of Serbian Medical Society; 2010. p. 181–204. (Serbian)
28. *Nesic DJ, Radosavljavic A.* To Medical Faculty Council. Belgrade: AS, G-200 UB Rectorate (University professors) f. VIII – 22. (Serbian)
29. *Todorovic K.* About Etiology of Scarlatina. SA 1929; XXXI (3): 177–206. (Serbian)
30. *Nikolić M.* Academician Professor Dr. Kosta P. Todorović (1887-1975). In: *Pavlović J*, editor. Fifty years of work of Clinic for Infectious Diseases of Faculty of Medicin in Belgrade 1926-1976. Belgrade: Galenika; 1976. p. 259–64. (Serbian)
31. *Ignjić S.* Bajina Bašta and surroundings: until 1941, vol. 1. Bajina Bašta: National Master Library, Workers' University "Miloš Trebinjac"; 1985. (Serbian)
32. SASA. Kosta P. Todorović, full member. Yearbook 1959; LXV for 1958: 229–38. (Serbian)
33. *Perišić Ž.* Academician Prof. Dr. Kosta Todorović (5 July 1887 – 22 September 1975). SA 1975; 103(10): 911–4. (Serbian)
34. *Vučenov D.* Characteristic of Todorović (Pavle) Kosta. 1949 September 28. Belgrade: AS, G-216, Ministry of Public Health. Files of physicians, f. 169–28. (Serbian)
35. S.N. Characteristic of Todorović (Pavle) Kosta. 1950 August 21. Belgrade: AS, G-216, Ministry of Public Health. Files of physicians, f. 169–28. (Serbian)
36. *Nikolić S.* One hundred years from birth of professor Kosta Todorović. Belgrade: Razvitak 1987. (Serbian)
37. *Petrović M.* Reminiscence: "We are here for diseased." In: *Savićević M.* editor. Professors of Faculty of Medicine in Belgrade: from foundation to fifties of 20th century. 2nd ed.Belgrade: Faculty of Medicine of University in Belgrade; 2003. p. 270–1. (Serbian)

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

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

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

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

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

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