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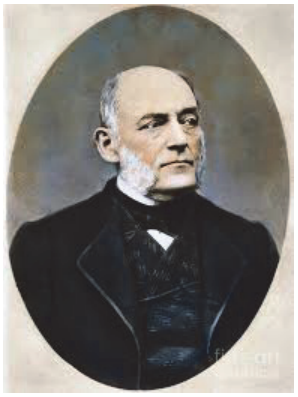

Carl von Rokitansky (19 February 1804– 23 July 1878), famous Check physician, pathologist, humanist and liberal politician. His name is associated with several diseases/morphologic features of diseases including Mayer-Rokitansky-Küster-Hauser syndrome, Rokitansky's diverticulum, Rokitansky's triad (pulmonary stenosis), Rokitansky-Aschoff sinuses (in the gallbladder), Rokitansky-Cushing ulcer, Rokitansky-Maude Abbott syndrome, etc. Rokitansky also developed a method of autopsy which consisted mainly of *in situ* dissection. He said that he "supervised 70,000 autopsies and personally performed over 30,000, averaging two a day, seven days a week, for 45 years". This year, July 23, it reaches 140 years since his death.

Karl fon Rokitanski (19. februar 1804 – 23. jul 1878), čuveni češki lekar, patolog, humanista i liberalni političar. Po njemu je nazvano više bolesti/morfoloških karakteristika bolesti, uključujući Mayer-Rokitansky-Küster-Hauser sindrom, Rokitansky diverticulum, trijada Rokitansky (plućna stenoza), Rokitansky-Aschoff-ovi sinusi (u žučnoj kesi), Rokitansky-Cushing-ov ulkus, Rokitansky-Maude Abbott sindrom, itd. On je, takođe, razvio metodu atopsije baziranu na disekciji *in situ*. Govorio je da je „nadgledao 70 000 autopsija, lično izvršio njih 30 000, prosečno dve na dan, sedam dana u nedelji, tokom 45 godina". Ove godine, 23. jula, navršava se 140 godina od njegove smrti.

E D I T O R I A L

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Inclusion of the Vojnosanitetski Pregled into Science Citation Index Expanded (SCIe) database – ten years later

Ulazak „Vojnosanitetskog pregleda" u *Science Citation Index Expanded* (SCIe) bazu – deset godina kasnije

Silva Dobrić

University of Defence, Institute for Scientific Information, Belgrade, Serbia

Last month it was exactly 10 years after the *Vojnosanitetski Pregled* (VSP) entered indexing system of the famous citation database Science Citation Index Expanded, originally produced by the Scientific Information Institute from Philadelphia and now maintained by Clarivate Analytics (previously the Intellectual Property and Science Business of Thomson Reuters). This meant an inclusion into the group of the most influential journals in the world in the field of General and internal medicine (in 2008, 153 journals were included in that group, and in 2017, 154), and obtaining the impact factor on the basis of which journals are ranked according to their influence in the field. In accordance with the known methodology of calculating an impact factor, the first impact factor of a journal was obtained only two years after the beginning of indexing in the citation bases of the aforementioned Institute. Just to remind, an impact factor for a given year is a quotient between the total number of citations in that year for articles published in the previous two years and the total number of articles published in those two years). In view of this, the first impact factor of our journal was obtained for 2010 and it was published in the Journal Citation Reports in June 2011. Its value was 0.199. Since then, the value of IF of the VSP has steadily increased, with the exception of value for 2011, when it was somewhat lower than the previous one. The last IF, released on June 26 this year, is 0.405 (Table 1). Since we have been trying for years to keep the number of published articles in one year of the Journal roughly the same, this increase in the value of IF reflects, above all, an increase in the number of citations, which is particular satisfaction.

The entry among the journals covered by the SCIe meant greater visibility of the Journal on the international scene, a greater citation of articles published in it and an increasing influx of new manuscripts not only from Serbia and the region, but also from many European countries as well as from the USA and Asia. According to the EBSCO database through which articles from the VSP can be downloaded in full text, the number of accesses and downloads of the VSP articles has progressively increased since 2008 (e.g. in 2008 there were 2,340 downloads, a year later, 5 times more – 11,562, and in the last two years, 2016 and 2017, there were 80,664 and 60,887 downloads, respectively, or, on average, about 200 per day).

We hope that the authors' interest to publish their papers in the VSP as well as the readers to use and cite them, would continue to grow in the following period. This obliges us to continue with the practice of publishing only high-quality papers, primarily original research, critical reviews and rare and interesting case reports. In this, so far, we expect a great support, first of all, from the authors as well as members of the Editorial Board and, of course, the reviewers who by their expertise greatly influence the improvement of the quality of manuscripts submitted to our Journal.

I use this opportunity to thank all of them for their contribution in raising the quality and impact of the Journal because the new, increased impact factor is our mutual success!

Table 1

Values of impact factor of the *Vojnosanitetski pregled*

Year	Impact factor
2010	0.199
2011	0.179
2012	0.210
2013	0.269
2014	0.292
2015	0.355
2016	0.367
2017	0.405

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ORIGINAL ARTICLES

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Ovarian reserve in patients who have undergone endometriosis surgery

Ovarijalna rezerva kod bolesnica operisanih od endometrioze

Olivera Džatić Smiljković, Mladenko Vasiljević, Ivana Rudić, Jelena Vugdelić, Aleksandar Ristić, Rada Vugdelić

University of Belgrade, Faculty of Medicine, University Clinic of Gynecology and Obstetrics "Narodni Front", Belgrade, Serbia

Abstract

Background/Aim. Endometriosis is a gynaecological disorder characterized by the presence of endometrial tissue outside the uterine cavity. The aim of this paper was to determine the effect of laparoscopic cystotomy and cystectomy on ovarian function, as well as to compare these two methods in terms of qualitative and quantitative damage to the ovaries, achieved pregnancies and recurrence. **Methods.** The prospective study, conducted in "Narodni Front" Obstetrics and Gynaecology Clinic in Belgrade at the Endoscopic Infertility Treatment Ward, included a total of 150 patients. The study group was represented by 100 patients who underwent a surgical treatment of endometrial ovarian cysts. The patients in the study group were divided into 2 subgroups: Subgroup I consisted of 50 patients who underwent a laparoscopic cystotomy (incision of the cyst and thermal coagulation) and subgroup II which included 50 women who underwent a laparoscopic cystectomy. The control group consised of patients who underwent a surgery due to tubal factor infertility. The following parameters of the ovarian function were tested: the ovarian volume, the antral follicle count, the presence of the preovulatory follicle on the operated ovary, the serum levels of anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH), ovarian tumor marker (Ca 125), inhibin B, as well as the rate of achieved pregnancies one year after the surgery. **Results**. The ovarian volume and the antral follicle count as well as the FSH values were significantly higher in the control group in comparison with the patients in the study group. There were no significant differences in the ovarian volume, the antral follicle count, the AMH values and inhibin B values in the study group between the patients with cystectomy and those with the incision and coagulation of the cyst. **Conclusion.** Both surgical techniques diminished the ovarian reserve: cystectomy was more aggressive method in terms of the damage inflicted on the ovarian tissue, and incision with coagulation carried a higher risk of recurrence.

Key words:
endometriosis; infertility; laparoscopy; cystectomy; cystotomy; treatment outcome.

Apstrakt

Uvod/Cilj. Endometrioza je ginekološko oboljenje koje karakteriše prisustvo endometrijalnog tkiva van materične šupljine. Cilj ovog rada bio je da se utvrdi uticaj laparoskopske cistotomije i cistektomije na funkciju jajnika, kao i da se uporede ove dve metode, po pitanju kvalitativnog i kvantitativnog oštećenja jajnika, ostvarenih trudnoća i recidiva. **Metode**. Sprovedena je prospektivna studija u Ginekološko-akušerskoj klinici (GAK) „Narodni Front" u Beogradu, u Odeljenju za endoskopsko lečenje infertiliteta. Studijom je bilo obuhvaćeno ukupno 150 bolesnica. Ispitivanu grupu je sačinjavalo 100 bolesnica koje su operisane zbog postojanja endometriotičnih cista na jajniku. Bolesnice ispitivane grupe su podeljene u dve podgrupe: podgrupu I sačinjavalo je 50 operisanih bolesnica kod kojih je urađena laparoskopska incizija ciste sa termokoagulacijom (cistotomija), dok je podgrupa II obuhvatila 50 operisanih bolesnica kod kojih je urađena laparoskopska cistektomija. Kontrolnu grupu su činile bolesnice operisane zbog tubarnog faktora infertiliteta. Ispitivani su parametri ovarijalne funkcije: volumen jajnika, broj antralnih folikula, prisustvo preovulatornog folikula na operisanom jajniku, serumski nivoi anti-milerovog hormona (AMH), folikulostimulirajućeg hormona (FSH), inhibina B, tumorskog markera jajnika (CA 125), kao i stopa ostvarenih trudnoća nakon operacije. **Rezultati.** Volumen jajnika i broj antralnih folikula kao i vrednosti FSH su bili značajno veći u kontrolnoj grupi u odnosu na ispitivanu grupu bolesnica. Nije nađena značajna razlika u volumenu jajnika, broju antralnih folikula, vrednostima AMH i Inhibina B u ispitivanoj grupi bolesnica između onih sa

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cistektomijom i onih sa incizijom i koagulacijom ciste. **Zaključak.** Obe operativne tehnike smanjuju ovarijalnu rezervu: cistektomija je agresivnija metoda u smislu oštećenja tkiva jajnika, a incizija nosi veći rizik od recidiva.

Ključne reči:
endometrioza; neplodnost; laparoskopija; cistektomija; cistotomija; lečenje, ishod.

Introduction

Endometriosis is a benign estrogen-dependent gynaecological disorder characterized by the presence of endometrial tissue outside the uterine cavity. It is associated with pelvic pain, dysmenorrhoea and infertility [1]. Pelvic organs and peritoneum are the most commonly affected areas. It ranges from minimal lesions to massive ovarian cysts and adhesions which disturb the tubo-ovarian anatomy. The prevalence in the female population is around 5%–10%, although in particular groups of women with severe gynaecological disorders as well as in women who underwent a laparoscopic sterilisation, the frequency of around 20% was recorded [2]. A very high prevalence, ranging from 20% to 50%, is present in women with pelvic pain and decreased fertility [3]. The etiology of endometriosis has still not been fully explained. It is evident that the process of etiopathogenesis of endometriosis includes, besides retrograde menstruation, numerous mechanisms such as an altered immune response, genetic predisposition as well as environmental factors. There are two types of endometrial cysts, depending on the origin, the clinical course, the histopathological findings and the response to treatment: TYPE I or primary endometriomas and TYPE II, the so-called secondary endometriomas [4]. The mechanisms that are related to decreased fertility are numerous: ovular dysfunction, luteal phase defect, luteinised unruptured follicle syndrome, multiple abortions, altered immune response, intraperitoneal inflammation, implantation dysfunction [5]. Laparoscopy is a 'golden standard' in the treatment of endometriosis. A complete excision of the capsule is an acceptable method in terms of complete removal of endometriosis and prevention of the recurrence of the disease. Various parameters can be used to estimate the ovarian reserve. The use of a colour Doppler ultrasound makes it possible to estimate the ovarian volume, the antral follicle count, to analyse the stromal blood flows and to assess the vascular network in the ovary. The determination of the basal serum concentrations of the follicle stimulating hormone (FSH), luteinsing hormone (LH), estradiol, inhibin B and anti-Müllerian hormone (AMH) is of great significance in the estimation of the ovarian reserve [6].

The aims of this paper were the following: to estimate the ovarian reserve in patients who have undergone a surgical treatment due to ovarian endometriomas (endometriotic ovarian cysts); to estimate which of the two applied laparoscopic techniques, the laparoscopic cystotomy or the laparoscopic incision (cystectomy), causes greater damage to the ovarian function, i.e. diminishes the ovarian reserve; to estimate the percentage of recurrence of endometriosis during the first year after the surgery, depending on the surgical technique used, i.e. cystotomy and cystectomy; to determine the percentage of achieved pregnancies in the first year after the surgery and to estimate whether that rate depends on the type of the laparoscopic surgery that was performed.

Methods

A prospective study was conducted in Obstetrics and Gynaecology Clinic "Narodni Front" in Belgrade at the Endoscopic Infertility Treatment Ward. A total of 150 patients were included in the study. The study group was represented by 100 patients who underwent a surgical treatment of endometrial ovarian cysts. The patients in the study group were divided into 2 subgroups: Subgroup I consisted of 50 patients who underwent a laparoscopic cystotomy and subgroup II which included 50 women who underwent a laparoscopic cystectomy. The decision to perform a surgical procedure was made when endometrial cyst was suspected based on the case history, clinical findings, ultrasound findings and the findings of the colour Doppler as well as the serum concentrations of ovarian tumor marker (CA-125). A definitive diagnosis was made using diagnostic laparoscopy and as soon as the diagnosis was made, the laparoscopic surgical procedure was continued. The ultrasound exams were performed using a 5–7 MHz transvaginal probe. The control group consisted of 50 patients who underwent a surgical treatment due to tubal factor infertility. Patients up to 35 years of age who previously had not had an ovarian surgery participated in the study. Patients who were given medical treatment prior to surgery, that is, gonodotropin-releasing hormone (GnRh) analogues as well as those who had a history of polycystic ovaries were excluded from the study. All the patients were selected randomly. A type of the surgical technique, cystectomy or cystotomy, was selected at the discretion of the surgeon and based on the intraoperative findings. The surgical procedures performed on all the patients were laparoscopic, monitored on a TV screen. Cystotomy was performed by opening the capsule of the cyst on the antimesenteric side of the ovary and emptying the contents of the cyst using an aspirator. The inside of the cyst was flushed with saline solution and then laparoscopic camera was inserted into the cyst and the interior wall of the cyst was inspected. If pockets of endometriosis were found on the capsule, they were cut out with scissors and sent to the histopathological (HP) analysis. After that, the place where the pocket was found was coagulated using the bipolar. Cystectomy was performed by making an incision on the wall of the cyst on the antimesenteric side of the ovary using the large general cutting scissors (LCS) (ultrasonic laparoscopic coagulation shears), after which the contents of the cyst was aspirated. The inside of the cyst was flushed. The capsule of the cyst was, in part sharply and in part bluntly, separated from the ovary, making sure that ovarian tissue was preserved to the maximum extent. Haemostasis using a bipolar was performed where there was bleeding. The removed

tissue was sent to a HP analysis. Based on the stage of the disease according to the Revised American Fertility Society (r-AFS), the patients in the study group were divided into those who were in stage II (mild), III (moderate) and IV (severe). The patients with minimal endometriosis (stage I) were not included in the study group. Ovarian reserve was estimated before and after the surgery in all patients in both the study and control groups. The estimation of the ovarian reserve was based on the following: a) ovarian volume and the antral follicle count, b) hormonal status was determined radioimmunoassay (RIA) by on the second or the third day of a menstrual cycle: FSH, LH, estradiol, inhibin B, AMH. The analyses were performed before the surgery and 1 month after the surgical treatment if no postoperative therapy was introduced (GnRh analogues, oral contraceptives, progestogens). If postoperative therapy was applied, the analyses were performed after the patient got her first regular menstruation. The RIA of the hormonal status was performed on the second or the third day of a menstrual cycle. The ultrasound examinations were performed as follows: for the ovarian volume in the early follicular phase of the cycle and the volume of the operated ovary was compared to the volume of the ovary on the same side in the control group, while the ultrasound exam of the preovulatory follicle was performed between the 10th and the 14th day of the menstrual cycle. In order to determine the antral follicle count, the measurement was performed in one cross-section. The serum concentration of the CA-125 tumour marker was determined before and after the surgical procedure. The percentage of the recurrence of the endometrioma as well as the percentage of spontaneous pregnancies during the first year after surgery were analysed in all patients in the study group. The results obtained during this study were compared within the study group between the patients who had undergone laparoscopic cystotomy and those who had undergone laparoscopic cystectomy. Each of these two subgroups of the patients from the study group was compared with the patients from the control group. All the obtained data was statistically processed using the χ^2-test, Fisher's test, the Kruskal-Wallis single-factor analysis of variance, the Kolmogorov-Smirnov test and the obtained results were compared with the results of the domestic and foreign researchers. Certain conclusions were made based on the obtained results.

Results

On the basis of the statistical data analysis shown in Figure 1, the study group and the control group showed extremely high statistically significant difference in the ovarian volume after the surgical procedure ($p < 0.001$). No statistically significant difference was determined between the two subgroups of the patients who had undergone surgical treatment ($p > 0.05$). Statistically highly significant difference was recorded between the control group and the cystotomy subgroup ($p < 0.001$) as well as between the cystectomy subgroup and the control group ($p < 0.001$).

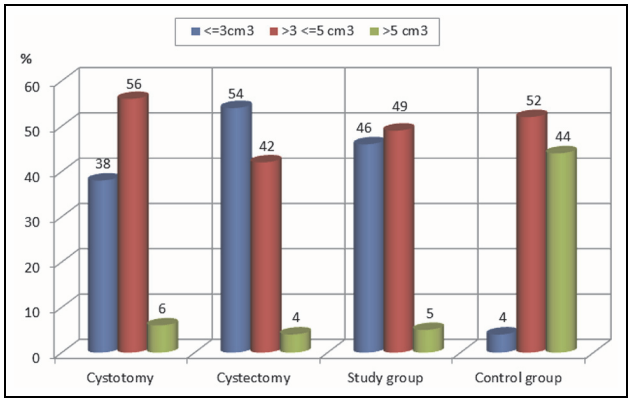

Fig. 1. – Ovarian volume after surgical procedures.

Table 1 shows the presence of a preovulatory follicle on the operated ovary in the patients from the study group in comparison with the ovary on the same side in the patients from the control group. In the study group, 72% of the patients did not have a preovulatory follicle on the operated ovary. There was no preovulatory follicle in 68% of the patients in the cystotomy subgroup nor in 76% of the patients in the cystectomy subgroup. A preovulatory follicle was not present on the ovary on the same side in 56% of the patients in the control group. The study group and the control group were statistically significantly different ($p < 0.05$), as well as the control group and the subgroup of the cystectomy patients ($p < 0.05$).

As for the antral follicles, the majority of patients in the control group (62%) had 3–5 on the operated ovary while the majority in the study group, 66%, had 2 or fewer follicles: 62% of the cystotomy patients and 70% of the cystectomy patients. No statistically significant difference was determined between the two subgroups of the patients who had undergone surgical treatment, cystotomy or cystectomy ($p > 0.05$). Statistically highly significant difference was recorded between the control group and the cystotomy subgroup ($p < 0.001$) as well as between the cystectomy subgroup and the control group ($p < 0.001$).

Table 1

Presence of a preovulatory follicle

Preovulatory follicle	Study group			Control group n (%)
	Cystotomy n (%)	Cystectomy n (%)	Total n (%)	
Yes	16 (32)	12 (24)	28 (28)	22 (44)
No	34 (68)	38 (76)	72 (72)	28 (56)
Total	50 (100)	50 (100)	50 (100)	50 (100)

A Figure 2 shows AMH values in the study group before and after the surgery, depending on the stage of the endometriosis as well as in the control group. Before the surgery, the highest AMH values were recorded in the patients with stage II endometriosis (5.7 ± 1.9 ng/mL), while the lowest value was recorded in patients with stage IV endometriosis (3.53 ± 1.6 ng/mL). In the cystotomy subgroup, the highest AMH value after the surgery was recorded in the patients with stage II endometriosis (5.2 ± 2.4 ng/mL); the highest AMH value was also recorded in the same stage of endometriosis in the cystectomy subgroup (5.0 ± 2.2 ng/mL). The highest AMH value was recorded in the control group (6.9 ± 2.8 ng/mL). The lowest AMH value after the surgery was recorded in the patients with stage IV endometriosis who had undergone cystectomy and cystotomy. Although the mean AMH value in the control group was higher than the values in both subgroups of the study group, no statistically significant difference was determined between the study and the control groups ($p > 0.05$).

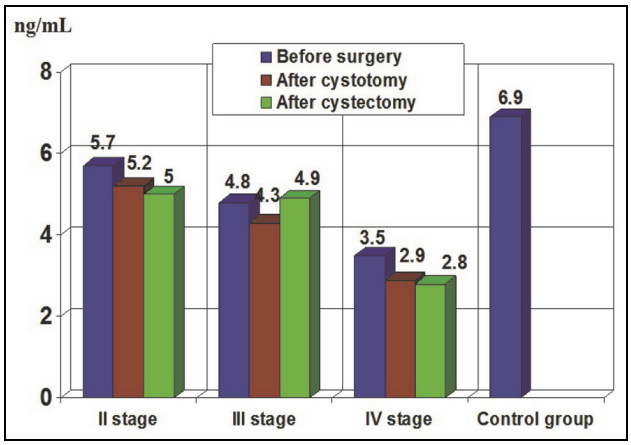

Fig. 2 – Anti-Müllerian hormone (AMH) average values in presenting groups.

The highest FSH value after the surgery in the cystotomy subgroup was recorded in patients with stage IV endometriosis (8.5 ± 1.9 mIU/L). This was also the case in the cystectomy subgroup where the highest FSH value was recorded in stage IV endometriosis (8.7 ± 1.5 mIU/L) (Figure 3).

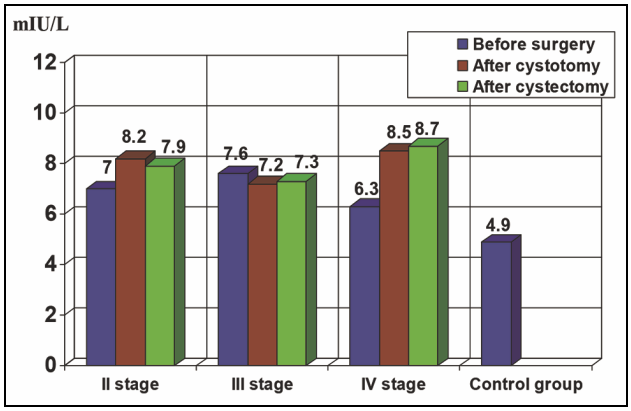

Fig. 3 – Follicle-stimulating hormone (FSH) average values in presenting groups.

The FSH values after the surgery showed statistically highly significant difference ($p < 0.001$) in relation to the control group, regardless of the type of the surgical technique and the stage of the disease.

The highest preoperative inhibin B values were recorded in the patients with stage II endometriosis (97.4 ± 25.1 pg/mL) while the lowest value was recorded in patients with stage IV endometriosis (42.6 ± 18.4 pg/mL). After the surgery, the highest inhibin B values were recorded in the patients in stage II of the disease. The lowest mean inhibin B values were recorded in patients with stage IV endometriosis, both in the cystectomy and the cystotomy subgroups. The mean inhibin B value in the patients from the control group was higher than the value in the patients in both subgroups of the study group (Figure 4). No statistically significant difference ($p > 0.05$) was determined between the two subgroups of the study group nor in relation to the control group.

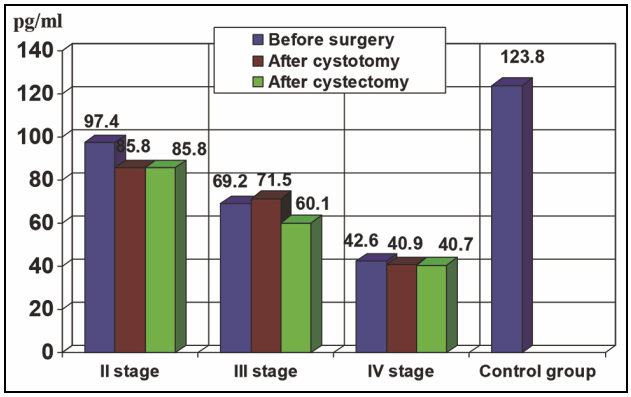

Fig. 4 – Inhibin B average values in presenting groups.

The mean values of the CA-125 tumour marker before the surgical procedure showed statistically highly significant difference ($p < 0.001$) in relation to the values of the CA-125 tumour marker after the surgical procedure in all stages of endometriosis, both in the subgroup of the patients who had undergone cystotomy and the patients who had undergone cystectomy. The mean CA-125 values between the subgroups of cystotomy and cystectomy patients were statistically significantly different only in the patients with stage II endometriosis (Figure 5).

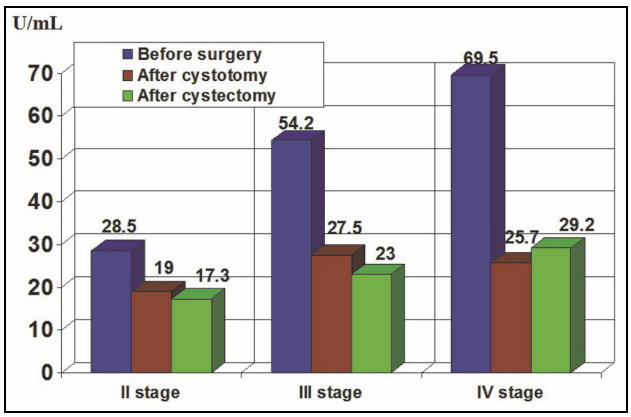

Fig. 5 – Ovarian tumor marker (CA-125) average values in presenting groups.

The mean values of the CA-125 tumour marker before the surgical procedure showed extremely high statistically significant difference ($p < 0.001$) in relation to the values of the CA-125 tumour marker after the surgical procedure in all stages of endometriosis both in the subgroup of the patients who had undergone cystotomy and the patients who had undergone cystectomy.

Figure 6 shows the distribution of achieved pregnancies one year after the surgery in the patients in both the study group and the control group. Pregnancy was achieved in 19% of the patients from the study group, more precisely, in 16% of cystotomy patients and 22% of cystectomy patients. There was no statistically significant difference between the study group and the control group ($p > 0.05$).

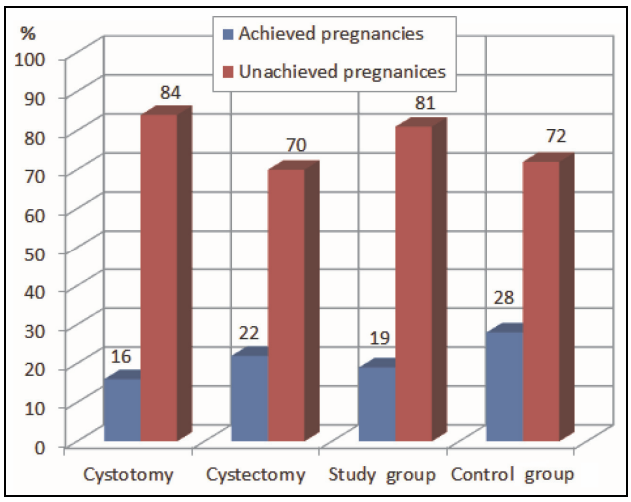

Fig. 6 – Achieved pregnancies.

The recurrence of endometriosis was diagnosed in 46% of the patients who had undergone cystotomy, whereas in patients who had undergone cystectomy the percentage of recurrence was 36%. The cystotomy and cystectomy subgroups did not show statistically significant difference in the rate of recurrence of endometriosis after the surgical procedure ($p > 0.05$).

Discussion

Various parameters can be used to estimate the ovarian reserve. The use of an ultrasound makes it possible to estimate the ovarian volume and the antral follicle count. The change in the ovarian volume and the direct correlation with the hormonal status was first noticed by Goswamy et al. [6]. Many studies conducted later indicated that a decrease in the ovarian volume together with a decrease in the antral follicle count represented a possible indicator of a weaker response to a controlled ovarian stimulation [7]. Diminished ovarian reserve was directly confirmed by the decreased ovarian volume and lower antral follicle count and the ultrasound parameters were in direct correlation with the decrease in estradiol values, increase in FSH values, decreased number of aspirated oocytes and lower pregnancy rate [8]. There was data in literature that showed that the antral follicle count was better indicator of a poor ovarian response than the basal level of FSH [9]. Serum levels of FSH, estradiol and inhibin B in the early follicular stage of the menstrual cycle are used for the estimation of the ovarian reserve. All these hormones are a part of the pituitary-ovarian feedback loop, hence their values are mutually dependent and the levels show significant variability during the cycle. AMH is the only serum marker not controlled by gonadotropin and it is very useful in determining ovarian aging. The excision of the fibrous capsule of the endometrium as well as thermal cauterisation for the purpose of achieving haemostasis, reduces the healthy ovarian tissue and in cystotomy, after the incision and drainage of the cyst, the endometrial pockets, which can take up large areas of the cyst capsule, are thermally cauterised. This surgical technique may cause thermal damage to the ovary [10].The retraction of the scar tissue after the surgery can also lead to the decreased ovarian volume. Electrosurgical instruments achieve high-frequency electric current and thus cause the tissue to heat up, so that haemostasis of smaller blood vessels is achieved in addition to the dissection. Cutting, coagulation or cauterisation can be performed by adjusting the power. When using these instruments, the depth of the thermal action must be taken into consideration. Candiani et al. [11] determined by ultrasound examinations a significant decrease in ovarian volume after the excision of the endometrial cyst in comparison with the contralateral ovary. The mean recorded decrease in the volume of the operated ovary was 33%, which was not linked to the damaged vascularisation of the ovary because the stromal blood flows did not change significantly. Other papers also indicated that there was a significant decrease in the basal volume of the operated ovary as well as in the number of the preovulatory follicles in the process of the controlled ovarian stimulation [12]. Based on the ultrasound measurements of the antral follicle (AF) count on the operated ovary, it was concluded that the largest percent (2/3) of the operated patients in the study group had up to 2 antral follicles (measured in one cross-section) in comparison with the control group in which the largest percentage of women (62%) had 3–5 AFs. There were differences between the study subgroups (the cystotomy patients had a larger number of antral follicles), but they were not statistically significant. In a paper written by Chinese authors, a significant reduction in the antral follicle count was recorded both in the ovarian suture and in the haemostasis by electrocoagulation, with the decrease in antral follicle count being more progressive in the second group [13]. There was a difference in the reduction of the ovulation rate of the operated ovary in a non-stimulated cycle in relation to the control group. The largest number of the patients in the study group did not have a preovulatory follicle on the operated ovary, 68% in the cystotomy patients and 76% in cystectomy patients, to be precise. In the control group, the monitoring of the ovulation on the ovary on the same side showed that 56% of the patients did not have a preovulatory follicle. There was a statistically significant difference between the subgroup of patients who underwent cystectomy in relation to the control group ($p < 0.001$), while the same did not apply to the cystotomy subgroup in relation to the control group (p

> 0.05). That suggests that cystotomy is a less invasive procedure than cystectomy. In their study, Beretta et al. [14] stated a statistically significant difference ($p < 0.05$) in the 24-month cumulative pregnancy rate in the cystectomy group (66.7%) in comparison with the cystotomy group (23.5%). Ragni et al. [15] evaluated the reduced ovarian response to hyperstimulation in women who underwent unilateral endometriosis surgery. Comparing the operated ovary with the contralateral one in the same patient, the rate of dominant follicles, the number of oocytes, embryos and high quality embryos, they reached a conclusion that a laparoscopic excision of the endometrium was associated with quantitative (but not qualitative) damage to the ovarian reserve. The comparison of the results in the patient groups depending on whether they underwent excision of the cyst capsule or cystotomy showed similar hormonal levels, and in relation to the control group the FSH level is significantly higher. Different results of studies can be found in literature: from those that corresponded with the results obtained in this study where significantly higher FSH levels after the surgery spoke in favour of diminished ovarian reserve [16] to those in which no significant differences were recorded in the FSH levels, indicating that the endometrial cyst surgery did not compromise ovarian function [17]. A significant increase in FSH levels after endometrial surgery was also recorded by other authors. A larger extent in electrocoagulation than in ovarian suture was applied for the purpose of achieving haemostasis [17]. The values of serum AMH levels in all patients who underwent endometriosis surgery showed the inverse correlation with the stage of the disease and with a mild decrease in the values after the surgery. Furthermore, these values were lower than those in the control group. However, due to the numerical factor, these differences were not statistically significant. The highest values before the surgery were present in the patients with stage II endometriosis (5.7 ± 1.9 ng/mL), and the lowest in the patients with stage IV of the disease (3.53 ± 1.6 ng/mL). The highest value among the operated patients in the cystotomy subgroup was in the patients in stage II (5.2 ± 2.4 ng/mL) and in the cystectomy subgroup the highest value was also in stage II (5.0 ± 2.2 ng/mL). The lowest values were recorded in stage IV (cystotomy: 2.9 ± 1.2 ng/mL; cystectomy: 2.9 ± 1.9 ng/mL). The obtained results corresponded to those of other studies [18]. They suggested that the damage to the ovarian tissue was similar in both surgical techniques: the cystotomy with a coagulation of endometrial pockets and the excision of the endometrioma capsule. Normal inhibin pg/mL values were > than 45 pg/mL. The highest values before the surgery were recorded in the patients with stage II endometriosis (97.4 ± 25.1 pg/mL), and the lowest in the patients with stage IV of the disease (42.6 ± 18.4. pg/mL). After the surgery, the highest mean inhibin B value was in the patients in stage II of the disease who underwent cystotomy (85.8 ± 32.5 pg/mL) and the lowest in the patients with stage IV endometriosis who underwent cystectomy (40.7 ± 19.4 pg/mL). The mean inhibin B value in the patients belonging to the control group was 123.8 ± 36.8 pg/mL. It can be noted that inhibin B level in the patients with endometriosis was lower even before the surgical procedure and that it depended on the extensiveness of the disease, which means that the very presence of the endometrial process had a negative impact on the ovarian reserve. The mean inhibin B values in the control group were higher than the values in the patients from both subgroups of the study group, which means that they had better ovarian fertility potential. A mild decrease in the level of this hormone after the surgery, regardless of the surgical technique applied, indicated that the surgery made further damage to the healthy ovarian tissue, whether by coagulation or by excision of the endometrioma capsule. The clinical significance of the determination of inhibin B level in the serum was reflected in the estimation of ovarian reserve, as well as the number and quality of the follicles. The serum values of this hormone can be used as the basis for estimating the ability of the ovary to activate a larger number of follicles during stimulation of ovulation by medication. No statistically significant difference was determined between the inhibin B values in both subgroups of the study group ($p > 0.05$) and the same applies to AMH values, due to the small amount of available data. The highest CA-125 values before the surgery were recorded in the stage IV patients in the study group, 69.5 ± 9.3, and the lowest in stage II of the disease, 28.5 ± 8.2 IV/L. After the surgery, the mean CA-125 values in all stages of the disease in both subgroups of the study group were statistically significantly lower in comparison with the preoperative values. The CA-125 values were in direct correlation with the stage of the disease, which can be seen from the results of the study. Cheng et al. [19] stated that the values higher than 65 IU/L were associated with later stages of endometriosis, the pouch of Douglas obliteration and endometriosis that spreaded into the bladder. The serum levels were significantly higher in women with ovarian endometriosis as well as in cases of deep endometriosis, but not in mild forms of the disease. It was assumed that endometrial lesions contained higher amounts of CA-125 than normal endometrium. When these lesions were damaged due to constantly present inflammation, CA-125 was released [20].

No significant difference was found in the number of achieved pregnancies between the subgroups of the study group one year after the surgery. The percentage of achieved pregnancies in the study group was 19%, with 22% in cystotomy patients and, 16% in cystectomy subgroup, but this difference was not statistically significant. The reconstruction of normal pelvic anatomy is achieved surgically. The success rate in decreased fertility cases depends on the severity of endometriosis. The treatment of moderately severe disease resulted in approximately 60% pregnancy rate, while in severe endometriosis the rate was 35% [21, 22]. Recurrence was diagnosed in 41% of the patients in the study group, with the cystotomy subgroup having a higher recurrence rate (46%) while in the cystectomy subgroup that rate was lower (36%). However, this difference was not statistically significant. There are studies in which similar conclusions were reached. Hemmings et al. [23] published in a paper that the recurrence rate 36 months after the laparoscopic surgery was similar, regardless of whether the laparoscopic technique applied was cystectomy or cystotomy. The reop-

eration rate after 18 months was 6.1% in the cystectomy group, in comparison with the 21.9% in the cystotomy group. The reoperation rate after 48 months was 23.6% after cystectomy and 57.8% after cystotomy. Alborzi et al. [24] stated that the reoperation rate was statistically significantly lower after cystectomy than after cystotomy.

Conclusion

Laparoscopic surgery represents a golden standard in the treatment of ovarian endometriosis. The excision technique gives better results than the drainage of endometrial cysts in terms of recurrence, pain and spontaneous pregnancies in women who suffer from decreased fertility. However, the excision and the ablation of the capsule may cause damage to the normal cortex of the ovary. When performing the excision of the capsule, healthy ovarian tissue is often also removed, resulting in follicular loss. The ablation of the capsule may result in thermal damage to the ovarian tissue under the cyst capsule. Considering the fact that endometriosis is a disorder affecting young women, surgical treatment should be performed with utmost caution in order to preserve the reproductive function. Due to the proximity of the fibrous pseudocapsule of the endometrial cyst and the ovarian tissue, it must be taken into account that any surgical technique may cause damage of the ovaries and consequently decrease the ovarian reserve.

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Characteristics of empathy and psychopathy among pathological Internet users and opiate-dependent persons

Karakteristike empatije i psihopatije kod osoba koje patološki upotrebljavaju internet i kod zavisnika od opijata

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Abstract

Background/Aim. The debate about diagnostic criteria for differences and similirities as psychosocial characteristics of pathological Internet users and Internet and substance addicts still continued. The aim of the study was to investigate the differences between empathy and psychopathy among subjects with pathological Internet use (PIU) and opiate dependence. **Methods.** The cross sectional study was performed at the Methadone Center of the Clinical Centre of Vojvodina, Novi Sad, Serbia, with 40 consecutively recruited patients with opiate dependence (OD group) and compared to age matched (25 to 35 years) random online recruited subjects (n = 80). They were divided into two groups: 40 subjects with PIU and 40 controls according to cut-off score of 39 or less obtained at Questionnaire regarding the Internet use assessment. The questionnaires for sociodemographic data, Emotion Specific Empathy (EMI) questionnaire and Psychopathy Assessment Questionnaire (PAQ) were applied. Descriptive statistics, χ^2-tests, ANOVA and MANOVA analysis were used for data assessment which was performed by the SPSS software (version 20.0) and $p < 0.05$ was applied. **Results.** There were more differences among psychopathy dimensions than empathy dimensions among PIU subjects, controls and heroin dependent subjects. The PIU group demonstrated the lowest psychopathy dimensions except for the lifestyle dimension which was higher than in the control group. On the contrary, the PIU group showed the highest empathy, with two dimensions as the most prominent ones: empathy with negative emotions and emotional reactions provoked by empathy. **Conclusion.** The PIU subjects are characterized by high empathy and low psychopathy. These findings suggest that it would be useful to consider seriously the dimensions of empathy and psychopathy when developing strategies to prevent PIU and planning educational programs for persons with problems related to the Internet overuse disorder.

Key words:
empathy; antisocial personality disorder, internet; opioid related disorders.

Apstrakt

Uvod/Cilj. Debata oko dijagnostičkih kriterijuma i sličnosti između problematične upotrebe interneta i zavisnosti od supstanci, kao i razlika između njihovih psiholoških karakteristika još traje. Cilj istraživanja je bio da se ispitaju razlike između empatije i psihopatije kod zavisnika od opijata i osoba koje prekomerno upotrebljavaju internet. **Metode.** U kliničku studiju preseka konsekutivno je bilo regrutovano 40 opijatskih zavisnika lečenih u Metadonskom centru Kliničkog centra Vojvodine (OD grupa), koji su upoređeni sa 80 ispitanika nasumično odabranih i uparenih po starosti, koji su popunili upitnike preko internet mreže i koji su razvrstani u dve grupe: 40 ispitanika koji patološki upotrebljavaju internet (PIU) grupa i 40 ispitanika koji ne ispunjavaju kriterijume za PIU (kontrolna grupa). Svi ispitanici su popunili saglasnost, Sociodemografski upitnik, Upitnik za procenu upotrebe interneta, Upitnik za procenu psihopatije i Upitnik za procenu empatije. U obradi rezultata korišćena je deskriptivna statistika, χ^2-test, analize varijanse (ANOVA i MANOVA) u SPSS programu (verzija 20.0). statistički značajan rezultat smatran je za $p < 0.05$. **Rezultati.** Između ispitanih grupa utvrđene su veće razlike u dimenzijama psihopatije nego empatije. Grupa PIU je imala niže skorove za sve dimenzije psi-

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hopatije, osim dimenzije životnog stila. Međutim, PIU grupa je imala najviši skor za empatiju uz najizraženije dve dimenzije u odnosu na ostale dve grupe: empatija sa negativnim emocijama i emocionalne reakcije provocirane empatijom. **Zaključak.** Osobe sa PIU se karakterišu visoko izraženom empatijom i niskom psihopatijom. Rezultati ove studije ukazuju na to da bi bilo korisno da se ozbiljno razmotre i procene dimenzije empatije i psihopatije u razvoju strategija u prevenciji PIU, kao i u planiranju edukativnih programa za osobe sa PIU.

Ključne reči:
empatija; ličnost, antisocijalni poremećaji; internet; poremećaji izazvani opioidima.

Introduction

In the last century, all around the world the Internet use spread to all aspects of life [1]. The Internet as a medium has an important role in making certain behaviors addictive, but the popular concept of the Internet addiction (IA) needs to be distinguished from patterns of excessive or abnormal Internet use [2]. Currently, there is no strong enough evidence base to provide support for the Internet addiction disorder [3]. The models of the Internet addiction and substance addiction share some criteria, such as feeling a loss of control over its use, ensuing psychological, social professional conflicts or problems and preoccupation with Internet content when not using it.

A lack of formalized diagnostic criteria and valid and reliable assessment instruments for pathological Internet use (PIU), make assessment and treatment paradigms difficult [4]. Nonetheless, there are a growing number of researches in this field.

Given a lack of consensus on the subject of the Internet addiction, a focus on problematic behaviors appears to be guaranteed [5]. There is raising interest in empathy assessment for better understanding of the Internet overuse. Empathy is not a single but a multidimensional ability to perceive, feel and understand the emotional states of others. The core components of empathy are emotion recognition, perspective taking and affective responsiveness, however the most studies are focused on single component such as cognitive or affective ones [6].

The behavioral-addiction perspective suggests that the IA could share similar characteristics with substance dependence.

Successful social behavior and empathic abilities are fundamental for many disorders of social cognition, including psychopathy [7].

Low empathy is associated with aggressive behavior and externalizing psychopathology [8]. There are some neurobiological evidence for a dialectic between empathy and predatory violence with suggestions for an early introduction of empathy training in treatment-resistant psychopathy [9].

The present study was designed to examine the characteristics of empathy and psychopathy among persons with problematic Internet use and opiate dependence.

Methods

Setting and sample

The cross-sectional study was performed at the Methadone Center of the Clinical Centre of Vojvodina, Novi Sad, with 40 consecutive recruited patients with opiate dependence (OD group). They were both gender patients who met International Classification of Diseaes – 10th Revision (ICD-10) criteria for opiate dependence and had been taking methadone therapy at least for six months.

They were compared to 80 subjects who were randomly recruited online and divided into two groups: 40 subjects with PIU (PIU group) and 40 controls without PIU (control group) according to cut-off score of 49 or less on Questionnaire for the Internet use assessment [10].

The inclusion criteria for all participants (N = 120) was the age from 20 to 35 years.

The exclusion criteria for all participants were: presence of any co-morbid mental disorder and severe co-morbid medical disorder. After receiving information about the study, all subjects provided their written informed consent prior entering the study procedures.

Ethics committee permission and institutional approval were obtained for this study.

Instruments

Sociodemografic data were collected by means of a questionnaire constructed for this study: age, gender, marital status, employment, residence, socio-economic status and parents' marital status. The subjects were investigated about motives for beginning the Internet or heroin use.

The Internet use was assessed by the Internet problematic use (IPU) questionnaire [10]. The IPU consisted of three factors. According to the total score there was the following scoring scale: the normal Internet use scored 0–49, the mild problematic Internet use 50–79 and severe in Internet use scored 80–100. The scale had good inernal consistency (α = 0.944, $\alpha 1$ = 0.912, $\alpha 2$ = 0.878 and $\alpha 3$ = 0.771) and good discriminative ability, so, 89.6% of the subjects were properly classified into categories.

Emotion Specific Empathy (EMI) questionnaire consisted of 42 items with five-point answers on Lickert scale [11]. The EMI had four subscales: Empathy with negative emotions (EN), Empathy with positive emotions (EP), Empathy as a social role (SR) and Emotional reactions (ER) provoked by empathy. Permission to use the scale was obtained from the author.

Psychopathy Assessment Questionnaire (PAQ), consisted of 40 items with yes-no answers [12]. Factor analysis identified four factors: Antisocial behaviour, Lyfestyle, Psychopatic affect and Interpersonal relations.

Data analysis

Descriptive statistics, χ^2-tests, ANOVA and MANOVA analysis were applied for data assessment using SPSS software version 20.0.

Results

Sociodemografic data were explored. Each group consisted of 40 subjects. The mean age [± standard deviation (SD)] (M ± SD) in the OD group was 24.7 ± 5.4 years, in the PIU group 24.4 ± 5.2 years and 24.6 ± 5.8 years in the control group, without a significant difference among the groups ($p = 0.979$).

More male than female subjects were in the total sample (male vs. female was 62.5% vs. 37.5%, respectively), without gender differences among the groups ($\chi^2 = 4.48$, $p = 0.106$). The most subjects lived in the urban enviroment (80%), without a significant difference among the groups ($\chi^2 = 4.06$, $p = 0.131$). A significant difference was not recorded for employment status ($\chi^2 = 4.48$, $p = 0 .106$), but little more unemployed subjects among OD than PIU and fewer among the subjects in the control group. There was a significant difference in a level of education among the groups. In the PIU group most frequent answer was 12 years of education (45%) so as in the controls (75%), but a majority of the subjects in the OD group had only 8–11 years of education (55%). The financial status analysis showed the low level among almost a half of the OD group (45%), but a majority of the controls and the PIU group subjects declared middle financial level (95% and 92.5%, respectively). The controls were mostly married (77.5%), but both the PIU and OD group had only 32.5% married subjects. The majority of the subjects had no children (71.7%), without group differences.

The parents' marital status demonstrated that the majority of controls (77.5%) and only 32.5% of subjects in both the OD and PIU groups had married parents. The divorced parents situation was the most frequently present in the PIU group (55%), then in the OD group (27.5%) and the control group (10%).

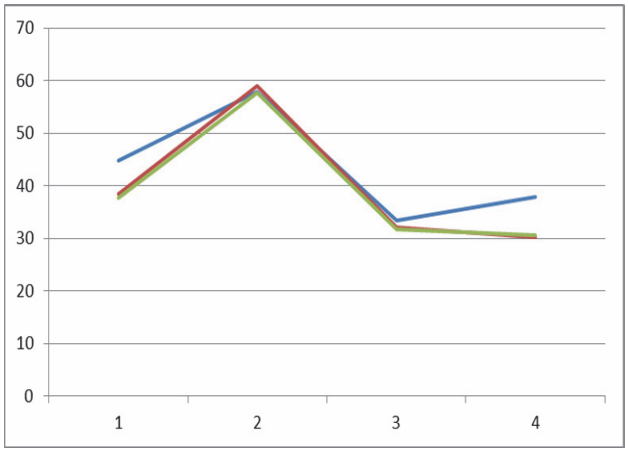

Fig. 1 – The group differences for empathy dimensions on Emotion Specific Empathy questionnaire (EMI). 1. Empathy with negative emotions (EN); 2. Empathy with positive emotions (EP); 3. Empathy as a social role (SR); Emotional reactions provoked by empathy (ER); blue – pathological internet use (PIU) group; red – opiate dependence (OD) group; green – control group.

Analysis of motives at the beginning of the Internet use were peer influence, school problems and psychological problems among the subjects in the PIU group, but in the OD group, the most frequent motives were curiosity, fun and boredom.

The empathy was investigated in all three groups (Figure 1).

Assessment of empathy performed by EMI scale and MANOVA (Figure 1) showed that the PIU group demonstrated the highest score for three dimensions of empathy: EN, SR and ER provoked by empathy. The only two dimensions showed significant differences among the groups: EN ($p = 0.003$) and ER ($p = 0.009$). The OD group had the highest score only for EP but without significant difference, in relation to other two groups.

Psychopathy assessment

Related to the total PAQ score, analysis of variance showed a significant difference among groups ($F = 84.295$, $df = 2$, $p = 0.000$). The OD group had the highest total score. The differences were significant among all three groups, (Wilks $\lambda = 0.118$, $F= 54.327$, $p = 0.000$). MANOVA was applied and the statistically significant group differences for each psychopathy dimension was rated. The OD group had the highest average score for all four psychopathy dimensions. The highest score showed the lifestyle dimension (score 16.88) but less prominent were antisocial behaviour vs. interpersonal relations vs. psychopatic affect dimensions (13.08, vs. 14.23 and 12.08, respectively). The control group had a significantly higher average score vs. the PIU group for the following three dimensions: interpersonal relations (11.90 vs. 10.50, respectively), psychopathic affect (10.83 vs. 9.88, respectively) and antisocial behavior (10.75 vs. 8.18, respectively). Only the lifestyle dimension showed a significantly higher score between the PIU and control group (11.88 vs. 10.70, respectively) and lower than the OD group score (16.83).

Discussion

In this study, the persons aged from 25 to 35 years were explored. This age was chosen as a sample-level moderator because of the literature suggestions that there may be important differences in psychosocial factors related to age, since younger individuals may show higher vulnerability to the negative consequences of the IA than adults under similar levels of duration and intensity of the Internet use [13]. The different social roles and lyfestyle between youth and adults was usually observed, and this is related to emotional, behaviour and social aspects of their functioning. Also, exploring the psychopathy dimensions is more suitable to young adults than to adolescents.

In this paper more male than female subjects were in the groups, but without any significant differences. The higher prevalence of male subjects was reported for PIU in adolescents, which was associated with strong emotional-motivational states and different underlying motivations that may require different prevention and treatment program [4].

The PIU group and the control group showed most frequently a level of 12 years of education, but a majority in the

OD group achieved a level of 8–11 years of education. The largest number of unemployed persons were found among opiate dependent persons, the fewer of them in the PIU group and the fewest among the controls with a significant difference. This is consistent with reports of other authors for the PIU subjects [14]. The majority of all subjects had average financial status, but almost a half of opiate addicts had the low level financial status. The groups did not differ in residence; the majority lived in urban environment. A majority of subjects from all groups were without children. These could be explained by matching age from 25 to 35 years and similar social and cultural background of all subjects. A majority of the controls were married, but there were fewer married subjects in the PUI group while the fewest of them were in the OD group. The parents' marital status demonstrated that the majority of the controls had married parents. The largest number of divorced parents was found in the PIU group and single parents in the OD group. The literature data revealed that a young person suffering from the Internet addiction and substance use shared similar family factors and both were considered to be a behavioral problem syndromes and family–based preventive approach was suggested [15].

Analysis of motives to start using the Internet were peer influence, school problems and psychological problems among the PIU group but among the opiate dependents the most frequent motives were curiosity, fun and boredom. The peer influence for the majority of subjects (82.4%) who started using heroin, was reported to be, on average, at age of 20 years [16].

The IA might be explained from a perspective of interpersonal difficulties since the online space provides a rewarding sense of warmth, belonging and well-being. In other words, the virtual space functions as a substitute for a lack of actual relationships. Some researchers reported that interpersonal problems such as social anxiety and poor social competence positively correlated with the IA [13].

Empathy is not a single, but a multidimensional ability to perceive, feel and understand the emotional states of others. However, most studies were focused on single aspects of empathy, such as emotion perception [6]. In this study the PIU group showed the highest mean score on three dimensions subscale (Figure 1). The highest EN dimension in the PIU group indicated that this group showed the most prominent ability for emotional engagement in unpleasant situations. By contrast, reports from Germany and China presented some data with evidence that across both cultures more PIU was associated with low empathy among students while self-report measured problematic use of the Internet [17]. There are many screening and diagnostic instruments for PIU and the IA, which led to very different estimation of the PIU or IA, ranging from < 1% to 27% [18]. The heterogeneous instruments influence the concept of PIU and empathy, but also the sample selection could be taken into account when explaining such long intervals of their assessment [19].

In this paper the psychopathy was explored as a multidimensional feature, too. Related to the total PAQ score analysis of variance, a significant differences among all three groups was shown (Figure 2). The opiate dependence group showed the highest total score. Also, a significant group differences for each psychopathy dimension were analyzed (Figure 2). The OD group had the highest average score for all four psychopathy dimensions (Figure 2). The highest score showed lifestyle dimension, but less prominent were antisocial behavior vs. interpersonal relations vs. psychopatic affect dimensions, respectively.

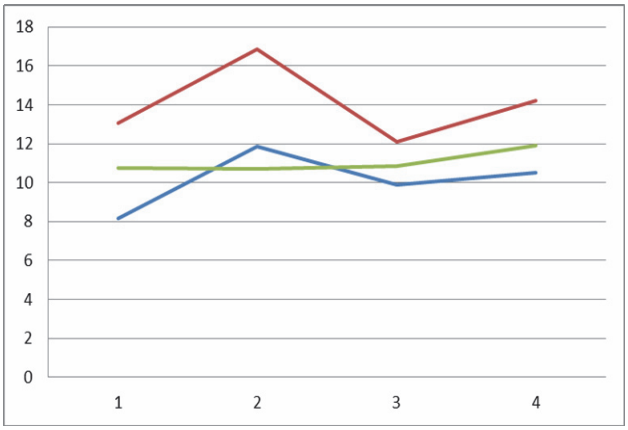

Fig. 2 – Psychopathy dimensions score on the Psychopathy Assessment Questionnaire (PAQ).
1 – Antisocial behaviour; 2 – Lyfestyle; 3 – Psychopatic affect; 4. Interpersonal relations; blue – pathological internet use (PIU) group; red – opiate dependence (OD) group; green – control group.

The control group had a significantly higher average score for three dimensions comparing to the PIU group: interpersonal relations, psychopatic affect and antisocial behavior.

The PAQ assessment showed the lowest mean scores in the PIU group for three psychopaty dimensions, but only the lifestyle dimension showed a significantly higher score in the PIU group comparing to the control group and lower than the OD group score. The lifestyle dimension is related to an outstanding need for stimulation, unresponsibilities and substance abuse. These could be factors that motivate subjects with PIU to use the Internet excessively. If these factors are seriously considered, clinical interventions may be designed to improve resiliency with an aim that individuals susceptible to PIU may better cope with adversity [4]. Also, some researchers suggested that PIU demonstrated significant psychosocial and functional impairments and shared many features with impulse control disorders and substance dependence [20, 21].

Regarding negative consequences of PIU, there are some reports on disrupted interpersonal relationships, increased delinquent and criminal behavior, poor work and school performance.

Persons with prominent psychopathy were characterized to have a tendency for substance abuse which was confirmed by neurobiological findings [22]. The hemodynamic neuron activity was tested and Hare Psychopathy Checklist-Revised Cope (HPC-R) was applied to over two hundred adult heroin dependent subjects. The results of this neuroradiological investigation confirmed a correlation between of psychopathy with dysfunction of limbic and paralimbic system, which is related to substance craving, making moral decisions and fear of adjustment and emotional recall [23].

The presented study has some limitations due to the cross-sectional design and sample size. Further prospective investigations with large samples and within longer period of time are needed. It is necessary to take into account that psychosocial variables of the problamatic Internet use reflected cultural differences from various parts of the world. However, the findings suggested that more vulnerable individuals could be identified by assessing psychopathy and empathy dimensions so that further methods of protection from the IA could be developed.

Conclusion

There were more differences in psychopathy dimensions than in empathy dimensions among the PIU, controls and OD subjects. The PIU group demonstrated the low prominent psychopathy dimensions except the lifestyle dimension which was significantly higher than in the control group. On the contrary, the PIU group showed the highest empathy level, but the most prominent were the following two dimensions: EN and ER provoked by empathy.

These findings suggest that it would be useful to consider the dimensions of empathy and psychopathy seriously when developing strategies to prevent PIU and planning educational programs for persons with the PIU.

Conflict of interest

The authors declare no conflict of interest.

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Surgical treatment of hiatal hernia: a ten-year experience

Desetogodišnje iskustvo u hirurškom lečenju bolesnika sa hijatus hernijom

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Abstract

Background/Aim. Today, hiatal hernia and the accompanying gastroesophageal reflux disease (GERD) are the most common benign disorders of the upper gastrointestinal tract. The aim of this study was to present the results of the hiatal hernia and GERD laparoscopic surgery in finding out for best type of fundoplication in each individual case. **Methods.** The study included 132 patients with the diagnosis of hiatal hernia and GERD, operated in the period from May 2004 to December 2014 at the Clinic for Abdominal, Endocrine and Transplantation Surgery of the Clinical Center Vojvodina, Serbia. The patients were selected for surgery on the basis of the findings of esophago-gastroscopy, barium contrast upper gastrointestinal series, 24-hour pH monitoring and esophageal manometric studies. **Results.** All the patients in this series underwent a posterior hiatoplasty with direct sutures. An additional mesh reinforcement was performed in 21 (16%) patients with a large hiatal hernia. There were 68 Nissen, 59 Toupet, and 5 Door fundoplications. Recently, the short-floppy Nissen fundoplication has predominantly been performed due to good postoperative outcomes. Intraoperative complications were: the parietal pleura lesion (3 patients), the spleen capsule laceration (4 patients), a minor injury of the adventitia of the distal esophagus (1 patient) and a thermal injury of the gastric fundus (1 patient). The postoperative complications were as folows: one fistula of the gastric fundus, transitory subcutaneous emphysema in the neck (5 patients), minor left-sided pleural effusions (6 patients), a transitory dysphagia (23 patients). The overall recurrence rate was 18.2% (24 patients). There was one fatal outcome. **Conclusion.** Laparoscopic surgery is considered a safe and effective surgical procedure for the treatment of hiatal hernia. The hiatal repair with a mesh reinforcement is recommended in selected cases. Today all consider the "short floppy" Nissen fundoplication as procedure of choice for the adequate hiatal repair.

Key words:
hernia, hiatal; surgical procedures, operative; surgical mesh; treatment outcome.

Apstrakt

Uvod/Cilj. Najčešći benigni poremećaji gornjeg dela gastrointestinalnog trakta u današnje vreme su hijatus hernija i pridružena gastroezofagusna refluksna bolest (GERB). Cilj rada bio je da se prikažu rezultati laparoskopskog hirurškog lečenja hijatus hernije i GERB u traženju najboljeg tipa fundoplikacije za svakog pojedinačnog bolesnika. **Metode.** Studijom su obuhvaćena 132 bolesnika sa dijagnozom hijatus hernije operisana u periodu od maja 2004. do decembra 2014. godine na Klinici za abdominalnu, endokrinu i transplantacionu hirurgiju Kliničkog centra Vojvodine. Selekcija za hirurško lečenje izvršena je na osnovu nalaza ezofagogastroskopije, rendgena gastroduodenuma sa kontrastom, 24-časovnog pH monitoringa i ezofagealne manometrije. **Rezultati.** Kod svih bolesnika izvršena je posteriorna hijatoplastika direktnim suturama, a kod 21 (16%) bolesnika sa velikom hijatus hernijom, uz direktnu suturu dodato je ojačanje mrežicom. Ukupno je izvedeno 68 Nissen-ovih fundoplikacija, 59 Toupet-ovih i samo 5 Door-ovih fundoplikacija. Zadnjih godina se uglavnom primenjuje „*short-floppy*" Nissen-ova fundoplikacija zbog dobrih postoperativnih rezultata. Od intraoperativnih komplikacija bile su prisutne: lezija parijetalne pleure (kod tri bolesnika), laceracija kapsule slezine (četiri bolesnika), minimalna povreda adventicije distalnog ezofagusa (kod jednog bolesnika) i termalna povreda

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fundusa želuca (jedan bolesnik). Od postoperativnih komplikacija zabeležena je jedna fistulizacija fundusa želuca, tranzitorni emfizem u predelu vrata (kod pet bolesnika), manji pleuralni izliv sa leve strane (kod šest bolesnika) i kod 23 (17,4%) bolesnika bila je prisutna tranzitorna disfagija. Stopa recidiva bila je 18,2% (kod 24 bolesnika). Desio se jedan fatalni ishod. **Zaključak.** Laparoskopska operacija se smatra bezbednom i uspešnom hirurškom procedurom u lečenju hijatus hernije. Hijatoplastika ojačana mrežicom preporučljiva je procedura u odabranim slučajevima. Posle adekvatne hijatoplastike preporučuje se primena dobro mobilisane „*short flopy*" Nissenove fundoplikacije.

Ključne reči:
hernija, dijafragmalna; hirurgija, operativne procedure; hirurška mrežica; lečenje, ishod.

Introduction

Hiatal hernia (HH) and the accompanying gastroesophageal reflux disease (GERD) are the most common benign disorders of the upper gastrointestinal tract all over the world nowadays [1]. Due to a constantly increasing prevalence of these diseases, there is a growing number of studies and papers in literature. Earlier, these disorders were predominantly treated conservatively, applying diverse diets, antacid drugs, specific H2-receptor blockers, proton pump inhibitors etc. and a surgery was considered only in case the former measures were ineffective. However, larger HH with a severe reflux and the assumption that GERD and subsequent Barett's esophagitis may induce cancer significantly contributed to a better understanding and more efficient treatment of this disorder [2, 3]. Furthermore, all these facts contributed to extend the scope of indications significantly and more readily decide to perform a surgery.

The treatment strategy both for the patients with GERD and those with HH has been significantly modified in a few recent decades due to anti-reflux surgery which is performed laparoscopically. Laparoscopical approach has considerable advantages as compared with the open, conventional surgery and it enables Nissen fundoplication with a minimally invasive trauma and significantly reduced postoperative sequels [4, 5]. Due to a reduced morbidity, good results and well-known advantages of laparoscopic approach, an increasing number of GERD patients are selected for surgical treatment. Nissen (complete fundoplication) or Toupet (partial fundoplication) have soon become the procedures of choice in the surgical treatment of the patients with GERD-accompanied by HH. The standard Nissen fundoplication was accompanied with a higher morbidity rate as well as with dysphagia and postprandial bloating. Partial Toupet fundoplication was mainly reserved for the patients with slow pulsating motions on the manometric analysis and this fundoplication type was associated with a lower morbidity rate. Since 2009, the choice of fundoplication has been modified, favoring the Nissen *short-floppy* fundoplication.

The aim of this study was to present performed fundoplication types in the patients operated on for HH and accompanying GERD at the Clinic for Abdominal, Endocrine and Transplantation Surgery of the Clinical Center of Vojvodina, Serbia, in order to find out the best type of fundoplication in each individual case.

Methods

Data source and clinical trial design

A retrospective, single-arm, observational trial was conducted from May 2004 to December 2014. The Institutional Review Board approved this study in compliance with all applicable federal regulations in February 2015 (No 33/15). Study participants were recruited from the patients who underwent surgeries in the hiatal area. Informed consent for this study was not obtained from each study subject because of a retrospective nature of the study.

Study population

Adult male patients and nonpregnant female patients (18 years of age or older) admitted and operated on for any elective surgical procedure in the hiatal area were eligible for this trial. Those having the diagnosis other than HH, other than GERD with or without HH (especially achalasia) were excluded.

Patients having access to the peritoneal cavity other than laparoscopy, those with incomplete medical data, and those who did not fullfilled the required 12-month telephone follow-up (including those who died during that period) were excluded.

Study interventions

Study participants were scheduled for laparoscopy before study enrollment. Demographic, medical history, laboratory, perioperative, operative, and surgical outcomes data were collected retrospectively from medical records.

Demographic variables included age, gender and body mass index (BMI). Medical history data included preoperative risk assessement using American Society of Anaesthesiologists (ASA) score, preoperative GERD symptoms evaluation (heartburn, regurgitation, postprandial dysphagia, anaemia, chest pain, vomiting, cough and dyspnea), preoperative assessing the type of HH (obtained from diagnostic studies), hystory of diabetes, history of smoking, preoperative functional and morphologic studies and surgical indications. Laboratory parameters recorded at the time of operation included hemoglobin, white blood cell count, blood urea nitrogen, bilirubin, and total protein level. Perioperative and operative data collected included antibiotics and/or antico-

agulants administered within 1 hour of operation, operative time, intraoperative confirming of preoperative assesing of HH size, need for conversion in laparotomy, intraoperative transfusion (mL), type of the used procedure in historical phases, use of prosthetic material, use of peritoneal drains and intraoperative complications. Surgical technique was given in brief. Surgical outcomes and follow-up data included length of stay, postoperative in-hospital and outpatient complications and postoperative telephone follow-up time.

Follow-up

All study subjects were clinically and by telephone followed up for a minimum of 12 months for recurrence and adverse events including dysphagia, distention, epigastric pain, heartburn and belching by gastroenterologist and surgeon. The follow-up data, including the final telephone questionnaire, was collected by personnel blinded to the surgical procedure performed. Patients were followed up 6 month and 12 month and symptom questionnaires were completed at these phone call visits for all contactable patients. The information obtained with this questionnaire included symptoms, recurrence and Visick questionnaire. Out of the 218 patients who were screened for this study, 86 were excluded. There were 132 evaluable patients who represented the study population for this analysis. Telephone follow-up was continued later only for reccurence.

Surgical treatment

Indications for the surgical treatment in the patients with HH (paraesophageal or mixed type) were established on the basis of the upper endoscopy findings (esophagogastroscopy), upper contrast (barium) gastrointestinal series in the Trendelenburg position and 24-hour pH manometric studies in the GERD patients. Esophageal manometric studies provided useful information about the motor activity of the esophageal body, assessing the quality of the esophageal peristalsis. The values of the esophageal manometric studies over 12–16 mmHg allowed a safe Nissen fundoplication, while lower manometric studies values, due to a failure of the esophageal propulsion, suggested that the Toupet fundoplication should be performed.

All the patients had an adequate preoperative preparation for the elective surgical operations and were admitted to hospital a day prior to the scheduled surgery. All operations were performed in the elective surgery program in general anesthesia. Prophylactic doses of antibiotics (second generation cephalosporines) were administered to all patients one hour before the surgery. All patients were placed in the reversed Trendelenburg position on the table inclined at 20°–25°, with separated legs. The surgery started by induction of pneumoperitoneum, placing a supraumbilical port for the optics and another four working ports (two 10 mm and two 5 mm *in situ* respectively) to enable the most flexible manipulation of atraumatic forceps. A surgical exploration was always performed first, followed by a careful dissection. Having the total dissection performed, a posterior crural repair was routinely performed in all cases, with three or four non-absorbable interrupted sutures Ethibond® 00. In the large paraesophageal HH, with the crural defect of 4–6 cm or more, the posterior crural repair with interrupted sutures was reinforced with a mesh in the assymetrically "U" or a key-hole shape. We applied a dual, two-component Proceed® mesh. It was placed behind the esophagus and fixed with a few extracorporaly sutures Ethibond® 00 (Figure 1). The selected fundoplication type followed. In the first several years (2004–2005), predominantly performed standard procedure was the ordinary Nissen fundoplication. Due to threatening postoperative complications, such as dysphagia and bloating with the symptoms of stenosis which usually required a re-operation, this fundoplication type was soon (after the 3 years, in 2006) replaced by the partial Toupet fundoplication. In the last few years (2010–2014), due to a high relapse rate, the choice of fundoplication was predominantly based on the "short-floppy" Nissen fundoplication preceded by a complete mobilization of the gastric fundus and a part of its corpus with cutting of short gastric vessels, reducing the wrap length to 2 cm. The Toupet fundoplication was performed only in patients with significant esophageal motility disorders registered at manometric studies.

The primary outcomes variable was the noted surgical outcome one year after the operation.

Secondary outcomes variable was only recurrence noted by telephone follow-up later than one year.

Sample size was not calculatated because of single-arm retrospective observational study that included all eligible subjects.

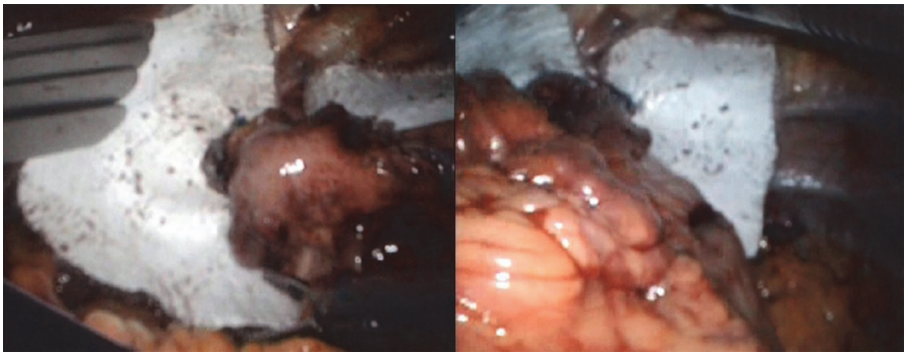

Fig. 1 – Display of the hiatal repair with a mesh reinforcement.

Statistical analysis

Results are expressed in descriptive manner only with basic statistical parameters.

Results

Patient characteristics

The study population was operated by a surgery team, consisting of 4 surgeons, at the Clinic for Abdominal, Endocrine and Transplantation Surgery from May 2004 till December 2014. Medical records of all patients having an operation in hiatal region were screened and enrolled in the study in May 2015. A total of 218 patients were enrolled in the trial and 86 were excluded. They were excluded because of an access to peritoneal cavity via laparotomy (n = 48), diagnosis of achalasia (n = 22), carcinoma of cardia preoperatively undiagnosed (n = 1), mortality (n = 1), incomplete data (n = 6), incomplete six-months follow-up (n = 6) and because of incomplete twelve-months follow-up (n = 2). Then, 132 patients were followed up for adverse events minimum 12 months. Statistical analysis included these 132 patients. The flow of participants through each stage of the trial is demonstrated in Figure 2.

Over the mentioned period there were 132 patients who underwent a laparoscopic surgery at the Clinic due to a diagnosed HH without GERD (solely HH) in 10 (7.6%) patients, due to diagnosed GERD without HH (solely GERD) in 12 (9.1%) and diagnosed HH accompanied with GERD in 110 (83.3%) patients. The study population was composed of twice more women than men (88 or 66.7% *vs* 44 or 33.3%, respectively) with a mean age at presentation of 56.04 ± 13.47 years (ranging from 26 to 78 years). Mean body mass index (BMI) for the study population was 25.6 ± 13.5 kg/m^2. Nearly half of the patients in this study 58/132 (43.9%) were overweight BMI (> 25 kg/m^2).

Types of hiatal hernia

All patients had clear documentation regarding the type of hernia. On the basis of the obtained findings, X-ray screening visualized a larger hiatal hernia in 23 (17.4%) patients. HH types were assessed preoperatively with endoscopy and upper gastrointestinal contrast series and finally confirmed at the operation.

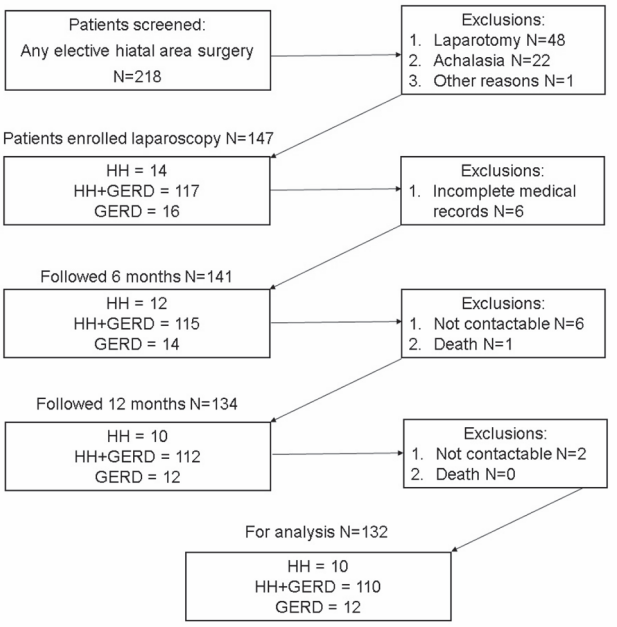

Fig. 2 – The flow of participants through each stage of the trial. HH – hiatal hernia; GERD – gastroesophageal reflux disease.

Twenty-three (17.4%) patients had the so-called large HH, which was intraoperatively in the diameter of ≥ 5 cm between the crura, or, over one half of the stomach was localized in the hernial sac, intrathoracally. Type I (paraesophageal), type II (sliding) or type III (mixed) HH was registered in 39 (29.55%), 57 (43.18%) and 36 (27.27%) patients, respectively. Only one female patient had an extremely large HH involving the whole of the stomach, a part of the colon, a few small intestine loops and most of the omentum in thorax. Some authors consider this to be type IV HH, occurring rarely [6].

Diagnosis

The major symptoms registered in our patients included: heartburn, regurgitation, postprandial dysphagia, anemia, chest pain, vomiting, cough and dyspnea. HH symptoms registered in our examined patients are reviewed according to the HH type in Table 1. The most common symptoms correlated to those were characteristical for GERD.

Table 1

Clinical preoperative symptoms*

Major clinical symptoms	Type I	Type II	Type III	Total
Heartburn	25	43	29	97
Regurgitation	21	41	25	87
Postprandial dysphagia	15	24	14	53
Anemia	4	3	2	9
Chest pain	10	12	5	27
Vomiting	7	15	3	25
Cough	5	10	4	19
Dyspnea	2	5	2	9

***All data are expressed as number of patients.**

Esophageal manometric studies established a reduced function of the lower esophageal sphincter (LES) and the esophageal body in 72 (54.5%) patients, and pH monitoring revealed a pathologic exposition of the esophageal acid into the distal esophagus in 39 (29.5%) patients and 12 (9%) patients had a clearly established "Barrett`s esophagus" due to HH-induced GERD.

The preoperative risk was assessed using the American Society of Anesthesiology (ASA) Score System. The ASA I score was registered in 18 (13.7%) patients. Most patients – 85 (64.4%) had the ASA II score and the remaining 29 (21.9%) patients had the ASA III score. The incidence of diabetes mellitus was recorded in 21 (15.9%) patients. Active tobacco use was reported in nearly half of the study patients – 64 (48.5%).

Mean serum leukocyte, hematocrit, blood urea nitrogen, total protein, and bilirubin levels were 12.5 ± 7.6 x 10^3/L (range 3.6 to 10.0 x 10^3/L), 38.7% ± 6.3% (range 38% to 49%), 6.4 ± 4.8 mmol/L (range 1 to 7.5 mmol/L), 6.44 ± 0.76 g/dL (range 6.0 to 8.0 g/dL), and 0.708 ± 0.766 mg/dL (range 0.2 to 1.23 mg/dL), respectively.

Surgery

Preoperative prophylactic antibiotics and preoperative venous thromboembolism prophylaxis with heparin were administered to all patients in this study. The average length of surgery was 103.4 ± 28.7 (ranging 60 to 160) minutes when performing the hiatal repair only by direct suturing or 20 minutes longer when applying a mesh. Intraoperative confirming of preoperative assesing of hiatal hernia size are mentioned in the section of patient characterisctic.

Eleven (8.3%) patients underwent a conversion into the upper laparotomy; in 9 patients it was predominantly due to adhesions of former surgeries and in 2 patients due to an excessive hemorrhage induced by the injuries of the spleen capsule in one case and by inadequately cutting of branch of the short gastric arterial vessels in the other, respectively. The mean blood loss was 60 mL (range, 0–800 mL). Two patients had 500 to 800 mL of blood loss. In the selected cases with a large HH [21 (15.9%) patients] a mesh reinforcement was performed. Use of peritoneal drains were noted in all patients.

Intraoperative complications

The parietal pleura was injured in the course of the circular peeling and esophageal preparation in 3 patients with larger hernias. The complications occurred in all 3 patients resolved by laparoscopical suturing and aspirating the pleural space with the assistance of an anesthesiologist who inflated the lungs with no need for thoracic drains. Minor laceration of the spleen at the greater omentum adherence site to the spleen capsule occurred in 4 patients. In 3 of them, hemostasis was achieved laparoscopically by ultrasound scissors, inserting the Surgicel® patch, while the fourth patient had a conversion to laparotomy due to excessive bleeding and since hemostasis was impossible to achieve, splenectomy was performed. One patient had the adventitia of the distal esophagus injured and the complication was resolved by laparoscopical suturing with two intracorporeal sutures. During the gastric mobilization, one patient had a thermal injury of the gastric fundus when cutting the short gastric vessels with ultrasound scissors. In the postoperative course, on the 9th postoperative day, this patient developed a fistula with signs of the intraabdominal abscess and excessive pleural effusions, confirmed on abdominal CT scans. The patient underwent a reoperation with midline upper laparotomy, abscessotomy and excising the damaged gastric fundus by a linear stapling device.

Surgical outcomes and follow-up data

The average length of stay was 3.8 ± 2.7 (ranging from 2 to 19) days. Three patients stayed 10 to 19 days for various reasons including chronic obstructive pulmonary disease-related symptoms, postoperative pain, and postoperative pleural effusion. Average time of recurrence presentation was 9.2 ± 12.8 months. The follow-up period ranged from 6 months to 6 years and 4 months.

Postoperative complications

Regarding the postoperative complications, one fistulization occurred at the site of the thermal damage of the gastric fundus (which has been already described), 5 patients developed the signs of a transitory mild subcutaneous emphysema in the neck area, 6 patients had minor left-sided pleural effusions, 4 patients had pulmonary atelectases, and 2 patients reported transitory retrostemal pains. There were no fatal outcomes.

Twenty-three (17.4%) patients had a transitory dysphagia in the first postoperative month, which persisted in the next three months in only 9 of them. There was one fatal outcome on the 6 month follow-up, and no fatal outcome on the 12 month follow-up. The "short" esophagus was registered in 2 patients with a recurrence of the disease, who were initially converted to open surgery. The Collis-Nissen fundoplication was not performed in these cases. The recurrence rate was 18.2% (24 patients) noted at the 12 month follow-up. 16 patients had a resurgery (11 laparoscopically and 5 open).

Postoperative satisfaction

The total Patients' postoperative satisfaction with any HH surgery type was evaluated 6 and 12 months after the surgery, using the Visick scale, as shown in Table 2 as well. No symptoms (Visick 1) were noted by 83 and 102 patients after the 6 months and after the 12 months, respectively. Reoperation required (Visick 4) were noted by 10 and 6 patients after the 6 months and after the 12 months, respectively.

Table 2

Patients' postoperative satisfaction with hiatal hernia (HH) surgery (Visick scale)*

Visick scale (grades)	After six months	After a year
I – no symptoms	83 (61.9%)	102 (77.3%)
II – mild symptoms	26 (19.4%)	15 (11.4%)
dysphagia	19/26	11/15
distention	8/26	5/15
epigastric pain	5/26	5/15
III – medicamentous treatment	15 (11.2%)	9 (6.8%)
dysphagia	11/15	7/9
heartburn	7/15	5/9
belching	5/15	4/9
IV– reoperation required	10 (7.5%)	6 (4.5%)
Total	134	132

***Data are given as number (percentage) of patients meeting appropriate Visick grade.**

Discussion

In a few recent decades, laparoscopic antireflux surgery has become a golden standard for GERD treatment. Numerous studies report its high efficacy (over 90%) with exceptionally good functional results and improvement of patients' quality of life [7–13]. Many studies have also confirmed that the laparoscopic treatment of large HH is safe and easily performed [6, 13, 14]. This minimally invasive procedure enables much better visualization of the hiatal region than laparotomy facilitated by a pneumoperitoneum [6].

The laparoscopic approach also enables the adequate crural repair in HH, with good results tested by numerous studies in various periods. Some studies reported the relapse rate of primary laparoscopic procedures of 2%–7% in smaller HH, while in large paraesophageal HH (≥ 5 cm diameter between the crura), the relapse rate may approach to even 45% [14–17]. This high relapse rate aroused a great debate regarding the laparoscopic repair of HH. Due to this unacceptably high relapse rate, some technical detailes were proposed (the necessity of antireflux procedure, gastropexy, hiatal repair using a prosthesis, etc.), inducing a constant professional debate [18–20]. Due to a high relapse rate of larger HH with direct suturing of the hiatal defect, it was recommended to apply a prosthesis, especially in cases with an excessively large hiatal defects in order to reduce tension at the crural suture site and produce stronger scar tissue by an incorporated mesh [18, 21]. Following the first insertion of a mesh for a hiatal closure reported in 1993, numerous techniques of prosthesis insertion were applied with the aim to reduce the relapse rate [22]. The selective application applied to the patients in whom a sufficient closure of the hiatal defect could not be achieved, with the hiatal defect site as the major criterion [18, 23, 24]. In our patients, we applied a dual, two-component Proceed® mesh, thin and flexible, with polypropylene on one side fitting on the diaphragm, and oxidized regenerated cellulose on the other, providing the bio-resorbable layer towards the viscera. The application of prosthetic materials could induce unpleasant postoperative complications, such as esophageal strictures, mesh migrations, and visceral erosions. To prevent prosthetic migrations, crural approximation was recommended whenever possible, followed by an onlay mesh installation to reinforce the hiatal defect and prevent intrathoracic fundoplication migrations [21, 23].

Most HH relapses and the resulting symptoms usually occurred on the repaired crura, no matter whether it is a stenosis of the hiatal aperture and the resulting obstruction at the gastroesophageal connection site, or migration of the fundoplication cuff into the thoracic cavity through the hiatal aperture [7, 23]. In some studies authors measured the diameter of hiatal crura and its relationship to the relapse of the disease. They found a smaller relapse rate (0.9%) when the diameter was < 4.5 cm, and the relapse rate of 11% when the diameter was > 5 cm ($p < 0.001$), making a statistical significance [25]. The initial diameter in the antero-posterior aspect could therefore be regarded as a selective indication for a mesh reinforcement of the crura.

The crucial moment contributing to a dramatic change in the adoption of anti-reflux surgery was the short, free, excessively mobilized Nissen fundoplication which dramatically reduced the postoperative complications of antireflux surgery and improved the control of the gastric content reflux into the esophagus [4, 7]. In a few recent years (2010–2014), we applied this fundoplication type in all patients with normal esophageal motility, even when the esophageal manometric findings were at the limit of the normal values. The procedure was more demanding as a more excessive mobilization of the fundus was needed, with obligatory cutting of short gastric vessels. Postoperative results of these fundoplications were much better than the standard Nissen ones with a minor mobilization of the fundus and no cutting of short gastric vessels.

Despite the good surgical results, some patients sometimes redeveloped the symptoms of gastroesophageal reflux with regurgitation, or even dysphagia, predominantly transitory in character [26, 27]. A problem appeared when dysphagia persisted over a longer period of time, which obligatory requires additional diagnostic studies. Numerous studies were carried out to select the adequate surgical procedure and principles in order to avoid failures and complications, such as dysphagia and a relapse of the disease. The "tailored approach" was mostly used to select the type of fundoplication, based on esophageal motility [7]. According to this approach, the patients with normal esophageal motility findings were

selected for the Nissen 360º-fundoplication, unlike the patients with motility disorders who were predominantly submitted to the Toupet 220º procedure [7]. This concept was based on reducing the undesirable postoperative side effects. Due to the severity of dysphagia and bloating, the Toupet fundoplication was routinely performed by some authors, regardless the esophageal manometry findings [1, 28]. The effects of these treatments were reviewed in prospective studies which evaluated the results of a five-year surgical practice in 100 patients submitted to laparoscopic Toupet fundoplication regardless esophageal manometry findings [1]. On the basis of this study results and the analysis of the previously obtained results and the patients' satisfaction, a relatively low relapse and morbidity rates were recorded. When the safety and efficacy of the procedure were already confirmed, it was recommended as the method of choice for the primary surgical treatment of patients with GERD and minor HH [1]. Nevertheless, many authors consider dysphagia is morphologically primarily due to ineffective hiatal closure, rather than to an inadequate fundoplication [7, 27]. Analyzing the major causes of postoperative dysphagia, one study reported that the morphologic-anatomic causes of dysphagia might be attributed to the difficulties with hiatal closing in 90% of the cases and in only 10% to an inadequate fundoplication (too long or too tight), or the intrathoracic fundoplication migration was involved, possibly resulting in a partial or total disruption and obstruction [7]. The cases of recurrent or persistent dysphagia due to the so-called "slipped Nissen", induced by a partial or total disruption of the primary posterior hiatal repair were also reported [7, 28]. In addition, postoperative dysphagia may largely be due to an excessively tight hiatal repair, i.e. to an artificially created hiatal stenosis because of a great number of sutures. To avoid this, many authors recommended calibrated probes for esophageal hernias. The prosthetic material for crural reinforcement can also produce postoperative dysphagia, which may induce adhesions, particularly as the result of the scar tissue developing at the crura.

Another important component is recognition of the so-called short esophagus, considered to be the major cause of the relapse. In these cases some authors recommend the "wedge-Collis" gastric repair which produces less tension on the hiatal repair and reduces a relapse of the disease [29]. In our study we did not note so-called short esophagus, nor performed "wedge-Collis" gastric repair.

The operative approach was individualized. Depending on the type of a surgery performed at our clinic, the patients were subclassified in three historical phases according to the type of fundoplication performed after the hiatal repair. The posterior hiatal repair was carried out in all patients, applying interrupted Ethibond® 00 sutures.

At the beginning, in the first historical phase (2004–2005), when we introduced laparoscopic procedures, we initially performed the Door fundoplication (5 patients). Soon afterwards, in the second historical phase (2005–2010), we started to perform the standard Nissen fundoplication. In the next 3–4-year period, we performed it in 33 (25%) patients and the Toupet fundoplication in 8 patients. Due to dysphagia and postprandial bloating as common postoperative complications of the Nissen fundoplication, the Toupet fundoplication was introduced and routinely performed in the next 44 (33.3%) patients, while the standard Nissen fundoplication was performed in 9 patients in the following period. In the third historical phase (2010–2014), the "short-floppy" Nissen fundoplication was predominantly performed – in 26 (19.7%) patients. It was usually performed in the patients without serious motility disorders registered on esophageal manometric series. However, the Toupet procedure was performed in only 7 patients with abnormal esophageal motility. In the analyzed period, the total of 68 (51.5%) Nissen, 59 (44.7%) Toupet, and 5 (3.8%) Door fundoplications were performed.

Limitation of this study are its retrospective nature and only one group of the patients suitable for analysis because we reviewed only historical phases. This can be improved by new prospective studies on at least two groups of patients.

Conclusion

Based on our experience, laparoscopic surgery of the HH with the hiatal repair and fundoplication is considered a safe and effective treatment modality. Posterior hiatal repair with a mesh is recommended in selected cases (in large HH ≥ 5cm in diameter, and in recurrent HH). Whenever inserting the prosthetic material, two-component meshes should be applied to reduce the mesh-related complications. We tried to formulate an acceptable solution to perform the HH surgery in a safe and effective way. We proposed a well mobilized "short floppy" Nissen fundoplication if no significant pressure reduction was registered by the preoperative manometric studies. Acceptable solution is and Toupet fundoplication in selected cases.

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Self-rated health among older adults in two fast ageing European countries: evidence from Italy and Serbia

Samoprocena zdravlja starih u dve evropske zemlje brzog starenja: dokazi iz Italije i Srbije

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Abstract

Background/Aim. Self-rated health (SRH) is a widely adopted tool to compare health across countries. Relationships of socio-demographics with SRH in later life have been extensively cross-nationally observed. However, cross-comparisons of the effects of health behaviors (i.e., eating habits, smoking, and alcohol consumption) and health status (i.e., chronic diseases) on SRH are less frequent. Our aim was to examine SRH differences between older adults in Italy and Serbia and to observe the role of predictors of SRH particularly referring to health behaviors in both countries. **Methods.** Two samples of 4,406 Italians and 3,539 Serbs aged 65 and older were extracted from national health surveys conducted in 2013. For this secondary analysis, SRH, socio-demographics, health status variables, and health behavior factors were selected. In the multivariate logistic regression models, SRH was the dependent variable while the selected independent predictors were socio-demographics, characteristics related to health status and to health behavior. **Results.** Both Italians (30.3%) and Serbs (22.3%) reported lower values of good- or very good-SRH than the European average (36.9%). The logistic regressions showed that Serbs reported poor–SRH significantly more often than Italians. Moreover, gender, education level, chronic diseases, and daily life limitations resulted as significant predictors of SRH in both national samples. In addition, vegetables intake was positively associate to SRH among Italians, while among Serbs an adequate fruits intake was positively associated to SRH. **Conclusion.** Health behavior and health status factors are associated with better SRH in the population aged 65 and older. The effects differ between countries. It is essential that decision-makers of the implementation of international preventive strategies take into account the specific characteristics of countries in the organization of interventions for the aged population.

Key words:
aged; aged, 80 and over; health; self-assessment; surveys and questionnaires; italy; serbia; risk factors.

Apstrakt

Uvod/Cilj. Samoprocena zdravlja (*Self-rated health* – SPH) je široko prihvaćen postupak za potrebe komparacije zdravstvenog stanja različitih zemalja. Povezanost socijalno-demografskih osobina sa SPH starijih osoba uočena je u nacionalnim istraživanjima. Međutim, ova istraživanja retko obuhvataju poređenja povezanosti zdravstvenog ponašanja (tj. navika u ishrani, pušenju i konzumiranju alkohola) sa zdravstvenim stanjem (tj. prisustvom hronične bolesti). Cilj je bio da se ispita razlika u SPH starih osoba u Italiji i Srbiji, kao i i da se odredi uloga prediktora SPH, posebno zdravstvenog ponašanja u obe zemlje. **Metode.** U okviru nacionalnih istraživanja zdravlja u Italiji i Srbiji, sprovedenih na reprezentativnim uzorcima domaćinstava u 2013. godini, posmatrane su osobe starije od 65 godina: 4 406 Italijana i 3 539 Srba. U modelima multivarijantnih logističkih regresionih analiza SRH je predstavljala zavisnu varijablu, a nezavisne prediktore činile su socijalno-demografske osobine ispitanika, obeležja u vezi sa zdravstvenim stanjem i ona koja predstavljaju faktore zdravstvenog ponašanja. **Rezultati.** U obe zemlje manji broj ispitanika starijih od 65 godina procenio je svoje zdravlje kao dobro i veoma dobro (Italijani: 30,3% i Srbi 22,3%) u odnosu na evropski prosek (36,9%). U logističkim regresi-

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jama uočeo je da je samoprocenjeno zdravlje češće lošije u Srbiji nego u Italiji. Ipak u oba nacionalna uzorka značajni prediktori samoprocenjenog zdravlja bili su isti: pol, obrazovanje, hronične bolesti i ograničenje dnevnih aktivnosti. Posmatrajući zdravstveno ponašanje, u Italiji je unos povrća bio značajno povezan sa osećanjem boljeg zdravlja, dok je u Srbiji istu povezanost imao unos voća u svakodnevnoj ishrani. **Zaključak.** Istraživanje pokazuje da postoje faktori zdravstvenog ponašanja koji su udruženi sa boljom SPH u populaciji starijoj od 65 godina, ali i da se oni razlikuju među zemljama. Neophodno je da donosioci odluka o implementaciji međunarodnih preventivnih strategija uvaže posebne specifičnosti zemalja pri organizaciji intervencija za populaciju starih.

Ključne reči:
stare osobe; stare osobe, 80 i više godina; zdravlje; samoprocena; ankete i upitnici; italija; srbija; faktori rizika.

Introduction

The factors associated with older people's self-rated health (SRH) have been studied extensively. Health-related factors, such as chronic diseases and daily life limitations were identified among the principal determinants of SRH for this age group [1]. In addition, health behavior factors were also reported to affect older people's SRH [2]. Rarely these factors were analyzed together in a multi-national study. The present study aimed at comparing elderly population in Italy and Serbia, two Southern European countries that are currently facing specific challenges in the public health domains concerning older people.

Italy is a country that for a long time has enjoyed high fertility and birth rates. However, since the 1970s, these figures started to decline rapidly, leading to a dramatic population aging that reached the level of one in five citizens over 65 years old [3]. Currently, Italy ranks second in the world regarding population aged 65 and older (22%), right behind Japan [4]. This picture could even get worse since Italy is about to experience one of the largest worldwide growths in over 65 years old population [5]. In fact, a cohort born between 1945 and 1964, so-called "baby boomers", is progressively aging and this trend will continue up to the 2030s, leading to a top-heavy age structure [6]. An older population carries major challenges to health and social care since the number of "super-utilizers" grows inevitably. They are a particular part of the elderly population composed of patients with multiple ambulatory care-sensitive chronic conditions consuming a rich portion of the medical resources [7]. The optimization of the resource allocation needs to be guided by strong indicators and SRH has been found to be the most efficient measure of the general state of health [8].

Serbia is expected to face an increase in the portion of people aged 65 and above, ranging from 17% to 33% by 2061 [9]. This demographic picture is also affected by migration problems: in 2009, 44% of young Serbs (age between 18 and 24) indicated an intention to migrate. This could lead in turn to top-heavy age structure in the country [10]. The risk of a mass migration is real since 80% of migration seekers declared to have connections abroad, such as relatives or friends who could ease the transition dynamics [10]. This running aging process is most visible in the city of Belgrade: the capital has seen a constant rise in the proportion of 65+ age groups, paralleled with a decrease of youngsters [11]. Moreover, a major risk factor for the elderly population of Serbia is poverty: a recent survey on the living standard of the population showed that the poverty rate among older adults (aged 65 and above) is significantly higher than the overall population and, also, the poverty risk among the older people is higher than the Serbian average [12].

Overall, comparison of different countries in Southern Europe is recommended in order to evidence comparability of results to support intervention aimed at improving health among portions of the population at risk such as the elderly [13]. Accordingly, the present study was designed to investigate and compare levels of SRH in the 65+ population of Italy and Serbia and to compare associations with socio-demographics, health status, and health behaviors characteristics.

Methods

Study population and data

We used cross-sectional population-based data from two national health surveys in Italy and Serbia. Both countries dispose of nationally representative data that include measures of SRH and health-related habits as a result of the alignment to international standards in health surveys. More specifically, for this secondary analysis we used data from the 2013 Italian National Health Survey, section Multi-scope Survey on the Family Aspects of Daily Life [14] and from the 2013 National Health Survey for the population of Serbia [15]. The current national data were harmonized with those of the European Health Interview Survey wave 2, with the aim of reaching comparability with results of other European Union countries [16]. Both surveys targeted members of private households and adopted paper and pencil technique to submit questionnaires. For Italy, in order to obtain a nationally representative sample, households were selected through a complex stratified multistage design, while a stratified two-stage cluster probability sampling was adopted in Serbia. The inhabitants of the Autonomous Province of Kosovo and Metohija were not included in the Serbian survey. A total of 30,914 respondents (20,275 Italians and 16,623 Serbs) completed the examination, with a response rate of 78.9% and 88.9% in Italy and Serbia respectively. According to the definition of older adults given by the World Health Organization (WHO) [17] we restricted this study to adult respondents aged 65 years and older. This left a total of 4,406 Italians (1,854 males and 2,552 females) and 3,539 Serbs (1,527 males and 2,012 females).

Study variables

As dependent variable, SRH was measured using a single-item question, namely "How is your health in general?",

with five possible answers: very good, good, fair, poor, and very poor. The first two answers were then grouped as "good" and the last two were grouped as "poor", like in other studies [18, 19].

The independent variables were selected after reviewing the research literature on factors influencing SRH. The selected items were divided into three groups: socio-demographics, health status, and health behaviors.

Socio-demographics included: age, gender, education level, geographical distribution, and living alone. Age was categorized as follows: 65–74, 75–84, and 85+. Gender was classified as males and females. Education level was reclassified according to the International Standard Classification of Education (ISCED) [20]: low (ISCED 0–2), medium (ISCED 3–4), and high (ISCED 5–8). The geographical distribution was summarized in North, Center, and South because of the similar demographic macro-regions. For Italy, North West and North East were grouped in North, Center was left as such, and South incorporated Islands too. For Serbia, Vojvodina and Belgrade represent the North, Šumadija and West fall into Center, while South integrates also East. Living alone was calculated with the following item: "Total number of persons in a household". The dichotomization was then carried out separating the people living alone from the people not living alone.

Health status was summarized into two variables: the Age-adjusted Charlson Comorbidity Index (AaCCI) [21] for the chronic diseases and daily life limitations. Longstanding diseases or health problems were extracted from a long list of conditions occurred in the past year. The following were available in both surveys and, consequently, included: diabetes (scoring 1), hypertension (1), infarction (1), angina pectoris (1), chronic obstructive pulmonary disease (1), asthma (1), allergies (1), neoplasm (2), liver calculus (2), cirrhosis (1), osteoarthritis (1), and mental diseases (1). The age adjustment was then implemented by weighting 0 the 65–74 group, 1 the 75–84 group, and 2 the 85+ group. By summing up the eventual presence of chronic diseases and the age weight, an overall score was obtained and therefore classified as 1, 2, 3, 4, and 5+. General activity limitations were investigated through one question, namely "For at least the past 6 months, to what extent have you been limited because of a health problem in activities people usually do?" Three options were given: severely limited, limited but not severely, and not limited at all.

Items regarding health behaviors included: intake of fruits, intake of vegetables, binge drinking, smoking, and body mass index (BMI). BMI was measured using the formula: weight divided by height squared (kg/m^2), and then categorized into three groups, according to the WHO classification [22]: under/normal weight (< 25 kg/m^2), overweight (25 to 29.99 kg/m^2), and obese (≥ 30 kg/m^2). The frequencies of vegetables and fruits intake were measured through two similar questions: "How often do you eat vegetables or salad (excluding juice and potatoes)?", and "How often do you eat fruits (excluding juice)?" Answers were then rearranged into two levels, following the WHO report recommending a minimum of 400 g of fruits and vegetables per day for the prevention of several diseases [23]: inadequate ("never", "less than once a week", "1 to 3 times a week", and "4 to 6 times a week"), and adequate ("once or more a day"). Binge drinking, or risky single occasion drinking, was estimated using the following single-item: "During the past 12 months, how often have you had six or more drinks on one occasion?" The answers were dichotomized into non-binge drinkers ("never" and "not during the past 12 months"), and binge drinkers ("less than once monthly", "once a week", and "every day"). Smoking habit was measured with a question: "Do you smoke at all nowadays?" The three possible responses were dichotomized into non-smokers ("no") and smokers ("yes, daily" and "yes, occasionally").

Statistical analysis

Data were analyzed using SPSS version 21.0 software (SPSS Inc., Chicago, IL, USA). Analyses consisted of descriptive statistics and univariate and multivariate multinomial logistic regression methods.

Table 1 reports frequencies for independent and dependent variables and results of χ^2 tests assessing significant differences between Italy and Serbia. Table 2 reports the results of univariate and multivariate (adjusted for age and gender and all study variables) logistic multinomial regression analysis testing for SRH differences across Italy and Serbia. Tables 3 and 4 report the results of univariate and multivariate multinomial logistic regression analyses using good-SRH vs. poor-SRH and good-SRH vs. fair-SRH as dependent variables in the Italian and Serbian sample respectively. The use of multivariate modeling enabled us to distinguish among different determinants: socio-demographics, health status, and health behaviors. Odds ratios (ORs) in univariate analysis were calculated and then the adjustment for age and gender was introduced in the second model. Only the variables that were significant in the model adjusted for age and gender were entered into the final multivariate multinomial logistic regression model to identify the predictors of SRH using good SRH as the reference category. AaCCI and daily life limitations were excluded from these last analyses because of multicollinearity problems. At last, the descriptions of the predictor coefficients was based on the previous models, while their comparison was established on the new multinomial model with SRH as dependent variable and country* predictor as independent variable. Specifically, we analyzed the interaction term between two countries (Italy/Serbia) and each predictor wherever it was generated in the previous models for either Italy or Serbia. In all the analyses, a p-value of < 0.05 was considered statistically significant.

Results

As it can be seen from Table 1, the two countries significantly differed on all analyzed variables, except for gender. SRH as a discriminant was always significant for the Serbian sample and the entire sample while for the Italian sample it was not significant for binge drinking and fruit consumption. Overall, Serbs were younger, higher educated, and consumed more vegetables, while Italians reported better health, less chronic diseases, less general activity limitations, lower BMI, declared to eat fruits more frequently and to drink and smoke less than Serbs (Table 1).

Table 1

Self-rated health, socio-demographics, health status, and health behavior characteristics of Italians, Serbs, and in both countries

Variables	Italy (n = 4,406) n (%)	Serbia (n = 3,539) n (%)	Both countries (n = 7,945) n (%)	*p**
		Self-rated health		
Very good	112 (2.5)	102 (2.9)	214 (2.7)	
Good	1221 (27.7)	687 (19.4)	1908 (24.0)	< 0.001
Fair	2202 (50.0)	1334 (37.7)	3536 (44.5)	
Poor	719 (16.3)	1076 (30.4)	1795 (22.6)	
Very poor	152 (3.4)	340 (9.6)	492 (6.2)	
		Socio-demographics		
Age (years)				
65–74	2181 (49.5)	1954 (55.2)	4135 (52.0)	< 0.001
75–84	1642 (37.3)	1385 (39.1)	3027 (38.1)	
85+	583 (13.2)	200 (5.6)	783 (9.9)	
Gender				
male	1854 (42.1)	1527 (43.1)	3381 (42.6)	0.331
female	2552 (57.9)	2012 (56.9)	4564 (57.4)	
Education level				
high	199 (4.5)	529 (14.9)	728 (9.2)	< 0.001
middle	619 (14.0)	934 (26.4)	1553 (19.5)	
low	3588 (81.4)	2076 (58.6)	5664 (71.3)	
Geographical distribution				
north	1895 (43.0)	1608 (45.4)	3503 (44.1)	< 0.001
center	810 (18.4)	1012 (28.6)	1822 (22.9)	
south	1701 (38.6)	919 (26.0)	2620 (33.0)	
Living alone				
yes	3053 (69.3)	2771 (78.3)	5824 (73.3)	< 0.001
no	1353 (30.7)	768 (21.7)	2121 (26.7)	
		Health status		
AaCCI				
0	713 (16.2)	368 (10.4)	1081 (13.6)	
1	1053 (23.9)	719 (20.3)	1772 (22.3)	
2	977 (22.2)	836 (23.6)	1813 (22.8)	< 0.001
3	661 (15.0)	690 (19.5)	1351 (17.0)	
4	448 (10.2)	439 (12.4)	887 (11.2)	
5+	554 (12.6)	487 (13.8)	1041 (13.1)	
Daily life limitations				
not limited	1866 (45.5)	1192 (33.7)	3058 (40.0)	< 0.001
limited	1524 (37.1)	1453 (41.1)	2977 (39.0)	
seriously limited	715 (17.4)	887 (25.1)	1602 (21.0)	
		Health behavior		
BMI (kg/m^2)				
less than 25	1752 (39.8)	1002 (30.5)	2754 (35.8)	< 0.001
25–29	1980 (44.9)	1330 (40.5)	3310 (43.1)	
30+	674 (15.3)	950 (28.9)	1624 (21.1)	
Fruits				
adequate	3698 (85.5)	1751 (49.5)	5449 (69.3)	< 0.001
inadequate	627 (14.5)	1788 (50.5)	2415 (30.7)	
Vegetables				
adequate	2155 (49.7)	2002 (56.6)	4157 (52.8)	< 0.001
inadequate	2180 (50.3)	1537 (43.4)	3717 (47.2)	
Binge drinking				
no	4144 (97.7)	887 (74.5)	5031 (92.6)	< 0.001
yes	98 (2.3)	304 (25.5)	402 (7.4)	
Smoking				
no	4045 (91.8)	3155 (89.1)	7200 (90.6)	< 0.001
yes	361 (8.2)	384 (10.9)	745 (9.4)	

AACCI – Age-adjusted Charlson Comorbidity Index; BMI – body mass index;

***Differences between Italy and Serbia according to χ^2 test.**

Levels of SRH as potential discriminators between the two countries of interest are reported in Table 2.

Levels of poor-SRH compared to levels of good-SRH were significantly higher in the Serbian sample than in the Italian sample. This hold true in the univariate model (OR 2.75), as well as in multivariate models after adjusting for age and gender (OR 3.19), and all variables from Table 1 (OR 2.25). Levels of fair-SRH compared to good-SRH did not differ between national samples (Table 2).

Table 2

Differences of self-rated health levels between Italy and Serbia – results of univariate and multivariate logistic regression models

Self-rated health	Serbia (0) vs. Italy (1)		
	OR	95% CI	*p*
Univariate			
Poor	2.75	2.43–3.10	< 0.001
Fair	1.02	0.92–1.14	0.682
Good (ref cat)	1.00		
Adjusted on age and gender			
Poor	3.19	2.81–3.63	< 0.001
Fair	1.10	0.98–1.23	0.117
Good (ref cat)	1.00		
Adjusted on all variables			
Poor	2.25	1.53–3.31	< 0.001
Fair	0.98	0.79–1.21	0.815
Good (ref cat)	1.00		

Ref cat = reference category; OR – odds ratio; CI – confidence interval.

The results of the multinomial logistic regression analyses for Italy are presented in Tables 3a and 3b. Predictors of SRH in the groups of Italians respondents that reported significantly better health than their respective referent categories in both good-SRH vs. Poor-SRH and fair-SRH vs. Poor-SRH were: 65–74 and 75–84 years of age, males, high education, living in north and center, not living alone, AaCCI scores lower than 5, no daily life limitations, non-serious daily life limitations, BMI lower than 25, and adequate vegetables consumption. Binge drinking and smoking were not significant in the adjusted models, therefore, they were not included the final model (Tables 3a, 3b).

The results of the multinomial logistic regression analyses for Serbia are presented in Tables 4a and 4b. Similar results were recorded among Serbs: being a male, better educated, not living in the South, not living alone, scoring less than 5 at the AaCCI and not being seriously limited were factors leading to better health than their respective referent categories in both the analyses. The only age category that significantly correlated with SRH in all analyses was the 75-84 group, for what concerning specifically the good-SRH vs. poor-SRH model [OR, 1.44; 95% confidence interval (CI), 1.17–1.77]. BMI resulted to be a significant predictor only in the good–SRH vs. poor–SRH model: only the group reporting a value lower than 25 kg/m^2 was significantly different in the multivariate analysis (OR, 1.49; 95% CI, 1.15–1.93) from the obese group. A healthy diet was always a discriminant in the univariate and in the adjusted models, but only an adequate intake of fruits positively influenced health when taking into account all the other variables in the good-SRH vs. poor-SRH analysis (OR, 1.34; 95% CI, 1.06–1.29). Binge drinking and smoking did not enter the multivariate models (Tables 4a, 4b).

Discussion

One of the goals of the present study was to investigate and compare levels of SRH in the 65+ population of Italy and Serbia. Serbia reported poor-SRH significantly more often than Italians (40.0% vs. 19.8% respectively). The effect of nationality on SRH hold true also after controlling for gender and age and for all the variables in the final model. Our results confirmed the evidence that Eastern Europeans report worse self-perceived health than Westerners [24]. Serbia is still facing the consequences of the war period during the 1990s and health implications are enormous especially for older generation [25]. Based on the present data and analyses, a possible explanation could be found in the high prevalence of chronic diseases and daily life limitations among Serbian elderly, since these two variables resulted as the most determinant in explaining SRH in both national samples while their prevalence was higher among the Serbian population (21.4% vs. 34.4% with no chronic diseases and 33.8% vs. 45.5% with no daily limitations in favor of Italy). The effect of health status on SRH was also proved in other researches [1].

Comparing the poor-SRH results for the population of interest with those of other European countries [26], we see that Italy would rank 15th and Serbia 26th out of 31. On the other hand, for the good-SRH results, Italy would occupy the 21st spot and Serbia the 23rd. The better results achieved by Italy could be explained by longevity in the country: thanks mainly to the Mediterranean diet and the role played by olive oil [27], Italy ranks second in overall life expectancy, only behind Japan, with 83 years, while Serbia is 67th, with 75 years [4].

Another goal of the present research was to investigate the association between socio-demographics, health status, and health behavior factors with SRH. Within the socio-demographics, the only factor to remain significant in all the models across the two countries was education. This factor was found to be crucial also in another recent study on older adults in Europe [28]. Education after World War II was considered an exclusive for richer part of the population so that among today's older generation a higher educational level implies a privileged family background [29]. In our sample, education mirrors financial resources that in turn are associated with subjective health in before and after retirement population [12].

Although older Italians declared to eat more fruits than vegetables, it is the latter that had a positive influence on health when all the variables were taken into account. Something similar could be seen in the Serbian sample where participant declared to eat more vegetables than fruits, but it was only the latter to be significant in the good-SRH vs. poor-SRH multivariate model.

Table 3a

Predictors of self-rated health for Italians in multinomial logistic regression models (Socio-demographics)

Variables	Good (1) vs. poor (0) self-rated health [OR (95% CI); *p*]			Good (1) vs. fair (0) self-rated health [OR (95% CI); *p*]		
	Univariate	Adjusted on age and gender	Adjusted on all variables	Univariate	Adjusted on age and gender	Adjusted on all variables
Age* (years)						
65-74	7.22(5.41-9.65); 0.000	6.78(5.07-9.07); 0.000	5.92(4.31-8.13); 0.000	2.80(2.17-3.61); 0.000	2.68(2.07-3.45); 0.000	2.60(2.00-3.39); 0.000
75-84	3.59(2.95-4.36); 0.000	3.51(2.88-4.27); 0.000	3.48(2.80-4.33); 0.000	1.65(1.42-1.91); 0.000	1.62(1.40-1.89); 0.000	1.60(1.37-1.88); 0.000
85+ (ref cat)	1.00			1.00		
Gender*						
male	1.83(1.53-2.18); 0.000	1.63(1.36-1.96); 0.000	1.58(1.28-1.93); 0.001	1.47(1.28-1.69); 0.000	1.40(1.22-1.61); 0.000	1.30(1.12-1.51); 0.000
female (ref cat)	1.00			1.00		
Education level						
high	3.69(2.25-6.03); 0.000	2.90(1.75-4.81); 0.000	2.76(1.59-4.81); 0.000	1.96(1.45-2.66); 0.000	1.75(1.29-2.38); 0.000	1.62(1.18-2.23); 0.003
middle	1.11(0.64-1.93); 0.705	1.16(0.66-2.05); 0.756	1.27(0.69-2.35); 0.444	1.05(0.75-1.47); 0.780	1.07(0.76-1.50); 0.696	1.06(0.74-1.50); 0.761
low (ref cat)	1.00			1.00		
Geographical distribution*						
north	3.07(2.52-3.73); 0.000	3.01(2.46-3.69); 0.000	2.82(2.25-3.54); 0.000	2.07(1.77-2.42); 0.001	2.05(1.75-2.40); 0.000	1.84(1.56-2.17); 0.000
center	1.46(1.14-1.87); 0.003	1.44(1.11-1.85); 0.005	1.40(1.06-1.86); 0.018	1.36(1.13-1.64); 0.001	1.35(1.12-1.63); 0.002	1.38(1.14-1.67); 0.001
south (ref cat)	1.00			1.00		
Living alone*						
no	3.30(2.72-4.01); 0.000	3.27(2.65-4.03); 0.000	3.33(2.67-4.16); 0.000	2.38(2.02-2.81); 0.000	2.36(1.99-2.79); 0.000	2.22(1.87-2.63); 0.000
yes (ref cat)	1.00			1.00		

***Variables weighting more for Italy than for Serbia; ref cat – reference category; OR – odds ratio; CI – confidence interval.**

Table 3b

Predictors of self-rated health for Italians in multinomial logistic regression models (Health status and Health behavior)

Variables	Good (1) vs. poor (0) self-rated health [OR (95% CI); *p*]			Good (1) vs. fair (0) self-rated health [OR (95% CI); *p*]		
	Univariate	Adjusted on age and gender	Adjusted on all variables	Univariate	Adjusted on age and gender	Adjusted on all variables
			Health status			
AaCCI†						
0	276.19(151.27-504.24); 0.000	384.89(201.09-736.67); 0.000		15.56(9.77-24.79); 0.000	20.41(12.32-33.84); 0.000	
1	77.66(44.98-134.07); 0.000	99.73(55.67-178.64); 0.000		10.47(7.23-15.18); 0.000	13.25(8.83-19.88); 0.000	
2	24.45(15.07-39.65); 0.000	29.34(17.52-49.15); 0.000		5.53(4.27-7.15); 0.000	6.84(5.10-9.17); 0.000	
3	9.25(5.80-14.75); 0.000	10.57(6.47-17.27); 0.000		3.36(2.72-4.15); 0.000	4.04(3.17-5.14); 0.000	
4	2.96(1.82-4.83); 0.000	3.11(1.90-5.11); 0.000		1.97(1.62-2.40); 0.000	2.17(1.76-2.67); 0.000	
5+ (ref cat)	1.00			1.00		
Daily life limitations†						
not limited	848.57(459.96-1565.512); 0.000	723.32(389.48-1343.31); 0.000		15.70(9.05-27.25); 0.000	13.46(7.72-23.47); 0.000	
limited	26.69(18.75-38.00); 0.000	23.90(16.74-34.12); 0.000		5.45(4.59-6.46); 0.000	5.06(4.25-6.02); 0.000	
			Health behavior			
BMI*						
less than 25.00	1.66(1.29-2.15); 0.000	2.13(1.63-2.79); 0.000	1.48(1.09-2.01); 0.012	1.42(1.15-1.76); 0.001	1.61(1.30-2.01); 0.000	1.43(1.14-1.80); 0.002
25.00-29.99	1.01(0.84-1.22); 0.910	1.16(0.95-1.41); 0.135	0.95(0.76-1.18); 0.624	1.11(0.96-1.29); 0.162	1.21(1.04-1.40); 0.014	1.09(0.93-1.28); 0.285
30.00 and more (ref cat)	1.00			1.00		
Fruits*						
adequate	1.30(1.02-1.65); 0.033	1.32(1.03-1.69); 0.030	1.14(0.85-1.53); 0.366	1.04(0.85-1.27); 0.704	1.05(0.86-1.29); 0.624	
inadequate (ref cat)	1.00			1.00		
Vegetables*						
adequate	1.52(1.28-1.80); 0.000	1.56(1.31-1.87); 0.000	1.26(1.02-1.55); 0.034	1.26(1.10-1.45); 0.001	1.29(1.12-1.49); 0.000	1.24(1.07-1.43); 0.004
inadequate (ref cat)	1.00			1.00		
Drinking*						
no	0.51(0.26-1.01); 0.053	0.87(0.43-1.78); 0.709		0.99(0.64-1.54); 0.977	1.34(0.86-2.10); 0.200	
yes (ref cat)	1.00			1.00		
Smoking*						
no	0.55(0.40-0.76); 0.000	0.88(0.62-1.24); 0.457		0.69(0.55-0.88); 0.002	0.86(0.67-1.09); 0.200	
yes (ref cat)	1.00			1.00		

***Variables weighting more for Italy than for Serbia; † Variables not included in the final model because of multicollinearity;**
AaCCI – Age-adjusted Charlson Comorbidity Index; BMI – body mass index; ref cat – reference category; OR – odds ratio; CI – confidence interval.

Table 4a

Predictors of self-rated health for Serbs in multinomial logistic regression models (Socio-demographics)

Variables	Good (1) vs. poor (0) self-rated health [OR (95% CI); *p*]			Good (1) vs. fair (0) self-rated health [OR (95% CI); *p*]		
	Univariate	Adjusted on age and gender	Adjusted on all variables	Univariate	Adjusted on age and gender	Adjusted on all variables
Age (years)						
65-74	1.75(1.19-2.57); 0.005	1.57(1.06-2.32); 0.023	1.34(0.85-2.11); 0.213	0.94(0.62-1.42); 0.759	0.88(0.58-1.33); 0.540	
75-84	1.68(1.39-2.02); 0.000	1.67(1.38-2.01); 0.000	1.44(1.17-1.77); 0.001	1.13(0.93-1.36); 0.218	1.12(0.93-1.35); 0.233	
85+ (ref cat)	1.00			1.00		
Gender						
male	2.53(2.12-3.03); 0.000	2.51(2.10-3.00); 0.000	1.97(1.61-2.42); 0.000	1.74(1.46-2.08); 0.000	1.75(1.46-2.09); 0.000	1.61(1.33-1.94); 0.000
female (ref cat)	1.00			1.00		
Education level						
high	5.01(3.87-6.48); 0.000	3.94(3.02-5.14); 0.000	3.16(2.36-4.22); 0.000	2.06(1.64-2.61); 0.000	1.79(1.41-2.28); 0.000	1.57(1.23-2.01); 0.000
middle	1.88(1.42-2.49); 0.000	1.80(1.35-2.39); 0.000	1.67(1.23-2.27); 0.001	1.51(1.18-1.94); 0.001	1.44(1.12-1.86); 0.004	1.36(1.06-1.76); 0.017
low (ref cat)	1.00			1.00		
Geographical distribution						
north	1.89(1.52-2.36); 0.000	1.97(1.58-2.47); 0.000	1.75(1.36-2.25); 0.000	1.61(1.29-2.02); 0.000	1.66(1.32-2.07); 0.000	1.61(1.28-2.03); 0.000
center	1.80(1.46-2.22); 0.000	1.88(1.51-2.33); 0.000	1.60(1.26-2.04); 0.000	1.47(1.19-1.82); 0.000	1.51(1.22-1.87); 0.000	1.45(1.17-1.81); 0.001
south (ref cat)	1.00			1.00		
Living alone						
no	2.33(1.84-2.94); 0.000	2.23(1.75-2.83); 0.000	2.00(1.55-2.59); 0.000	1.61(1.27-2.05); 0.000	1.49(1.17-1.91); 0.001	1.47(1.14-1.88); 0.002
yes (ref cat)	1.00			1.00		

OR – odds ratio; CI – confidence interval.

Table 4b

Predictors of self-rated health for Serbs in multinomial logistic regression models (Health status and Health behavior)

Variables	Good (1) vs. poor (0) self-rated health [OR (95% CI); *p*]			Good (1) vs. fair (0) self-rated health [OR (95% CI); *p*]		
	Univariate	Adjusted on age and gender	Adjusted on all variables	Univariate	Adjusted on age and gender	Adjusted on all variables
			Health status			
AaCCI*†						
0	140.78(75.29-263.24); 0.000	210.79(109.33-406.40); 0.000		12.62(7.04-22.63); 0.000	18.90(10.24-34.89); 0.000	
1	42.21(25.86-68.91); 0.000	63.18(37.40-106.73); 0.000		5.71(3.73-8.74); 0.000	8.48(5.35-13.45); 0.000	
2	18.50(12.20-28.05); 0.000	26.19(16.72-41.04); 0.000		4.13(3.00-5.68); 0.000	5.85(4.10-8.37); 0.000	
3	9.77(6.53-14.61); 0.000	12.63(8.25-19.33); 0.000		4.06(3.05-5.42); 0.000	5.29(3.85-7.27); 0.000	
4	3.02(2.00-4.56); 0.000	3.28(2.16-4.99); 0.000		2.24(1.70-2.95); 0.000	2.48(1.86-3.31); 0.000	
5+ (ref cat)	1.00			1.00		
Daily life limitations*†						
not limited	146.15(95.36-224.01); 0.000	153.12(99.14-236.50); 0.000		3.84(2.50-5.90); 0.000	4.04(2.62-6.22); 0.000	
limited	16.23(12.42-21.21); 0.000	15.41(11.75-20.22); 0.000		3.94(3.23-4.80); 0.000	3.87(3.16-4.74); 0.000	
seriously limited (ref cat)	1.00			1.00		
			Health behavior			
BMI (kg/m^2)						
less than 25.00	1.57(1.24-1.99); 0.000	1.50(1.17-1.92); 0.001	1.49(1.15-1.93); 0.003	1.22(0.96-1.55); 0.097	1.14(0.89-1.45); 0.303	
25.00-29.99	0.98(0.79-1.22); 0.872	1.00(0.81-1.25); 0.969	1.02(0.81-1.29); 0.864	1.04(0.85-1.29); 0.694	1.03(0.83-1.27); 0.779	
30.00 and more (ref cat)	1.00			1.00		
Fruits						
adequate	1.70(1.42-2.02); 0.000	1.64(1.37-1.96); 0.000	1.34(1.06-1.29); 0.016	1.28(1.07-1.53); 0.006	1.28(1.07-1.53); 0.007	1.09(0.87-1.36); 0.442
inadequate (ref cat)	1.00			1.00		
Vegetables						
adequate	1.41(1.18-1.69); 0.000	1.38(1.15-1.65); 0.001	1.08(0.85-1.38); 0.537	1.27(1.06-1.52); 0.009	1.27(1.06-1.52); 0.010	1.19(0.95-1.49); 0.121
inadequate (ref cat)	1.00			1.00		
Drinking						
no	0.59(0.41-0.85); 0.004	0.70(0.49-1.02); 0.062		1.06(0.79-1.43); 0.686	1.13(0.83-1.53); 0.436	
yes (ref cat)	1.00			1.00		
Smoking						
no	0.67(0.51-0.87); 0.003	0.83(0.63-1.10); 0.187		0.77(0.59-1.00); 0.50	0.82(0.62-1.08); 0.151	
yes (ref cat)	1.00			1.00		

***Variables weighting more for Italy than for Serbia; † Variables not included in the final model because of multicollinearity;**
AaCCI – Age-adjusted Charlson Comorbidity Index; BMI – body mass index; ref cat – reference category; OR – odds ratio; CI – confidence interval.

Similar positive results were found in a research that measured the associations between fruits and vegetables intake and SRH among older adults [30]. Those findings together with our results show that even a relatively cheap food, such as seasonal fruit and vegetable, may play a substantial role in improving health in both Italy and Serbia.

BMI resulted to be significantly correlated to SRH: in the Italian sample, both under/normal weight and overweight respondents rated their health better than the obese ones, while among Serbs under/normal weight individuals did not significantly differ from the reference category. The significant interaction between BMI and SRH at later stages of life is already known in literature: a cross-sectional research in India reports that subjects with good/fair SRH tended not to have problematic BMI, while in the poor-SRH category 55% of males and 47% of females were below 19 units of BMI [31]. Regarding the average levels of BMI, it is noteworthy to underline that, being a normal BMI between 18.5 kg/m^2 and 24.9 kg/m^2, both countries have to be recognized as overweight on average since Italy scored 25.1 kg/m^2 (25.7 kg/m^2 for males and 24.4 kg/m^2 for females) and Serbia 25.9 kg/m^2 (26.3 and 25.4 kg/m^2, respectively) [21].

Both binge drinking and smoking did not represent a major threat to SRH for the current population of interest: none of them was significant in the adjusted models so that they were not included in the multivariate analysis. However, the descriptive analysis showed a quite different reality between the two countries: Serbs declared to smoke and drink more than what Italians did. As shown in a longitudinal Serbian study, heavy drinkers, who were usually smokers too, exhibited significantly higher relative risks for all-cause mortality and myocardial infarction [32]. Accordingly, even though after the age of 65, levels of SRH also depend on other variables, these factors may still be taken into account in future studies based on Serbian samples.

Several limitations to the present study should be noted. First, given the cross-sectional nature of the data analyzed here, it is not possible to establish causal relationships between levels of SRH and studied factors. Second, chronic diseases and daily life limitations were measured trough self-reported items, so that information bias could not be prevented. Finally, some of the variables that have been found significant predictors of SRH among older adults in previous studies, such as levels of physical activity or access to health care [33], could not be included in the current study since they were omitted or differently measured in the two national databases analyzed here.

Conclusion

In sum, the present study confirmed that socio-demographics, health status, and health behavior factors are major factors affecting SRH in late life, as previously reported by other authors. Implications for the health and social care of non-institutionalized seniors in Italy and Serbia can be also drawn thanks to the representative samples included here. In both countries, the strategies of prevention should focus mainly on relieving the burden of chronic diseases and daily life limitations, which appeared to be the main determinants of poorSRH. Improving self-perception of health status among elderly could also drive the promotion of well-being. Our findings could be also informative for other countries in the Southern European region. In the future, it would be valuable to carry out similar studies including more countries from this geographical area.

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Conflicts of interest

None declared.

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Oral health in children with special needs

Stanje oralnog zdravlja dece sa posebnim potrebama

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Abstract

Background/Aim. Due to their primary medical condition, children with special needs often display lower levels of oral hygiene, larger prevalence of caries and other oral diseases. The aim of this study was to estimate the prevalence of dental caries, oral cleanliness and presence of malocclusion in children with disabilities, as well as to evaluate eruption time of the permanent molars. **Methods.** Case–control study was carried out on a group of 107 children with disabilities at the Faculty of Dental Medicine, University of Belgrade, Serbia. The control group comprised of 104 healthy school children. **Results.** Children with disabilities had statistically higher mean [decayed missing and filled teeth – dmft for primary DMF for permanent dentition (dmft DMFT)] values in both dentitions than children from the control group ($p < 0.05$). Oral cleanliness level was much lower in children with disabilities. A significantly higher percentage of Class II malocclusions and a higher tendency to have a delayed time of eruption of permanent molars were observed in the test group in permanent dentition. **Conclusion.** Considering poor oral health status and higher tendency for development of malloclusions and delayed eruption, it is necessary to develop preventive dental programmes for children with special needs, as well as improve public awareness about these issues.

Key words:
disabled persons; child; adolescent; oral health; risk assessment; tooth eruption; malocclusion.

Apstrakt

Uvod/Cilj. Deca sa posebnim potrebama često zbog svoje primarne bolesti, imaju niži nivo oralne higijene i veću prevalencu karijesa i drugih oralnih oboljenja. Cilj rada bio je da se proceni prevalenca karijesa, nivoa oralne higijene i prisustvo malokluzija kod dece sa posebnim potrebama, kao i vreme erupcije stalnih molara. **Metode.** Ispitivanjem oralnog zdravlja obuhvaćeno je 107 dece sa posebnim potrebama na Klinici za dečju i preventivnu stomatologiju Stomatološkog fakulteta u Beogradu. Kontrolnu grupu je činilo 104 zdrave školske dece. **Rezultati.** Deca sa posebnim potrebama imala su statistički značajno viši nivo indeksa karijesnih, ekstrahovanih i plombiranih zuba (KEP) u obe denticije, u odnosu na kontrolnu grupu ($p < 0.05$). Nivo oralne higijene bio je lošiji kod dece sa posebnim potrebama. Takođe, primećeno je statistički značajno povećanje malokluzija klase II, kao i kasnije vreme erupcije stalnih molara kod dece sa posebnim potrebama u odnosu na kontrolnu grupu. **Zaključak.** Zbog lošijeg stanja oralnog zdravlja i povećane verovatnoće razvoja malokluzija i odloženog nicanja zuba, neophodno je formirati preventivne stomatološke programe za decu sa posebnim potrebama, kao i poboljšati informisanost javnosti o ovom problemu.

Ključne reči:
invalidi; deca; adolesenti; usta, zdravlje; rizik, procena; zub, nicanje; malokluzija.

Introduction

Over the past decade, children with disabilities have emerged as a major public health concern in many countries, but nationwide surveys conducted in the Western Balkan region on the oral condition of these children are lacking. According to the Health Statistical Year Book of the Republic of Serbia published in 2014, there are 22,000 children with disabilities under the age of 7. There are approximately 100,000 children with disabilities in Serbia, counting for 6% of total children population in Serbia. Out of those, it is estimated that there are around 2,400 children with cerebral palsy [1]. Children with disabilities need special or intensive medical care, requiring the interest of clinicians not only in

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the prevention of the primary medical condition, but also in the prevention of the problems related to it, such as dental caries, periodontal disease and malocclusions [2, 3]. Caries experience in these children has been attributed to disability-related factors, medications, diet, inadequate oral hygiene and unavailability of dental treatment [4].

Inadequate dental care or poor dental public health measures may have negative influences on oral health status of children with disabilities. There are no recent data on the dental health status of such patients in Serbia, and most of the epidemiological data gathered on the subject in the area of Western Balkan are scarce and in need updating.

The aim of this study was to determine the prevalence of oral disorders including dental caries, oral cleanliness, eruption time of first and second permanent molar teeth and malocclusions in children with disabilities in Serbia.

Methods

The study was in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki [5]. Approval for the study was obtained from the Ethics Committee of the Faculty of Dental Medicine, University of Belgrade and prior to data collection, written informed consent was obtained from all parents of the children that participated in the study.

The study group comprised 107 children with disabilities aged betweeen 6–16 years who were referred to the Clinic of Paediatric and Preventive Dentistry in the period of one year. They were examined for dental caries, oral cleanliness, time of molar eruption and presence of malocclusions. Information about the medical conditions of the children was obtained from the referring paediatricians. Treatment included both preventive and prophylactic measures (dental and oral hygiene examinations, mechanical removal of plaque and calculus, pit and fissure sealants, topical fluoride applications, parental motivation and oral health education), and dental treatment (treatment planning, restorations and extractions).

Inclusion factors for determining the study group were: children with disabilities that have demonstrated sufficient cooperation level to be examined in a dentist chair.

Exclusion factors for determining study group were: institutionalised patients; patients whose primary medical condition also includes: blood dyscrasia, congenital heart disease, diabetes, autoimmune conditions, kidney diseases, and patients undergoing chemo- or radiation therapy; patients that previously had undergone dental treatment under general anaesthesia; patients originating from the areas where endemic fluorosis was present.

A group of 104 non-medically compromised children who attended regular schools in Belgrade and were matched for age, gender, and type of dentition (mixed or permanent) served as the control group. They were examined in the order they appeared at the Clinic for Paediatric and Preventive Dentistry. Control participants did not use any medication that could affect oral health.

A single, trained and calibrated examiner carried out all procedures and intraexaminer reliability was calculated by reexamination of 10% of children from the control group at two different visits.

Caries diagnosis at the cavity level was performed according to standard World Health Organisation (WHO) methodology, and decayed, missing and filled teeth (dmft for primary dentition; DMFT for permanent dentition) were recorded [6].

Oral cleanliness was assessed by visually evaluating the presence of plaque on the buccal and lingual surfaces of upper and lower incisors and canines using the oral hygiene index proposed by James et al. [7]: score 0 = no evidence of plaque (good oral cleanliness), score 1 = some plaque at retention sites and/ or food accumulation (fair oral cleanliness) and score 2 = marked presence of plaque and/or food accumulation on most examined surfaces (poor oral cleanliness). The children teeth were not brushed nor professionally cleaned prior to the examination.

For the presence of permanent teeth the following criteria were applied: noting the presence of first permanent molars in 5–7 years old children and noting the presence of second permanent molars in 12–13 years old children. Code 0 was used for non-erupted teeth and code 1 was used if any part of the molar crown was visible in the oral cavity [8]. Occlusion was recorded according to the Angle Classification system, and if patients had only primary dentition, it was recorded according to the terminal plane of the primary molars.

Statistical significance levels was set at $p < 0.05$. Statistical calculations were performed by SPSS, version 14.0 for Windows (SPSS inc., Chicago, IL, USA). To establish the statistical distribution of data, the following tests were used: Mann Whitney test, Fisher's exact test, and Chi-square test.

Results

Intra-examiner reliability calculated by Cohen Kappa score was 0.91.

Distribution of age and gender of the study and the control groups is presented in Table 1.

The study group consisted of 107 children of whom 55 had mixed dentition (ages 6–11 years), and 52 children with permanent dentition (ages 12–16 years). Mean age of the study group was 11.19 ± 3.36 years.

The control group consisted of 104 non-medically compromised children out of whom 51 had mixed dentition (age 6–11 years), and 53 had permanent dentition (age 12–16 years). Mean age of the control group was 10.83 ± 3.30 years.

The medically compromised children had statistically higher mean dmft/DMFTvalues in both dentitions than children from the control group ($p < 0.001$) [Table 2 (Mann Whitney test)].

When comparing dmft/DMFT among the study subgroups [autism, cerebral palsy (CP) and mental retardation], no statistical significance was observed in the dmft. In the DMFT range, statistical significance was only observed in decayed teeth (DT) between autism and mental retardation study subgroups ($p < 0.006$), and in filled teeth (FT) between autism and cerebral palsy study subgroups ($p < 0.005$), and autism and mental retardation study subgroups ($p < 0.003$).

Table 1

Distribution of age and gender in children with special needs

Age (years)	Autism		Cerebral palsy		Mental retardation		Control	
	male	female	male	female	male	female	male	female
6–11	10	9	7	4	14	10	23	28
12–16	9	4	4	6	7	11	28	25

***All values are expressed as number of subjects.**

Table 2

Comparison of decayed, missing and filled surfaces in primary and permanent dentitions in the 6-11-year and 12-16-year age range in the study and control group of patients

Index	n (6–11 years)	n (12–16 years)	mean ± SD	*p*
dt	55		8.1 ± 4.5	0.000*
S	51		3.7 ± 4.4	
C				
DT				
S	55		4.0 ± 3.0	0.000*
C	51		0.6 ± 1.1	
S		52	13.7 ± 6.3	0.000*
C		53	4.9 ± 3.6	
Mt				
S	55		4.0 ± 3.7	0.000*
C	51		1.6 ± 2.4	
MT				
S	55		1.3 ± 1.5	0.000*
C	51		0.1 ± 0.3	
S		52	5.9 ± 4.2	0.000*
C		53	1.2 ± 1.5	
ft				
S	55		0.3 ± 0.8	0.024*
C	51		0.7 ± 1.3	
FT				
S	55		0.1 ± 0.3	0.073
C	51		0.4 ± 0.9	
S		52	1.2 ± 2.0	0.082
C		53	1.5 ± 1.7	
sound				
S	55		2.9 ± 2.7	0.000*
C	51		10.0 ± 5.6	
SOUND				
S	55		5.9 ± 3.0	0.025*
C	51		8.5 ± 5.5	
S		52	11.0 ± 5.6	0.000*
C		53	19.3 ± 5.0	
dmft				
S	55		12.4 ± 7.7	0.000*
C	51		5.7 ± 6.9	
DMFT				
S	55		5.4 ± 4.4	0.000*
C	51		1.0 ± 1.7	
S		52	20.9 ± 9.6	0.000*
C		53	7.7 ± 5.3	

***statistically significant (Mann Whitney test), SD – standard deviation; n – number of subjects; S – study group; C – control group; dt – decayed teeth; mt – missing teeth; ft – filled teeth (for primary dentition); DT – decayed teeth; MT – missing teeth; FT– filled teeth (for permanent dentition); sound – sound for primary dentition; SOUND – sound for permanent dentition.**

†decayed, missing and filled teeth (dmft for primary dentition, DMFT for permanent dentition).

There was a statistically significant difference between the study and control groups regarding plaque accumulations on the buccal and lingual surfaces of the upper and lower anterior teeth ($p < 0.01$), while there was no difference in oral cleanliness among medically compromised children with different handicaps in the study group [Figure 1 (Mann Whitney test)].

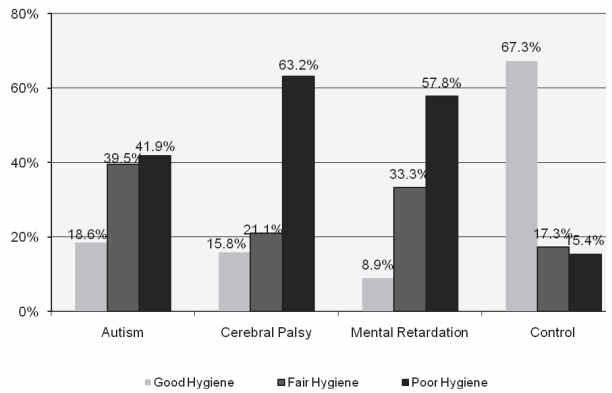

Fig. 1 – Oral cleanliness in the study and control group of subjects.

A tendency to have a delayed time of eruption of permanent molars was observed for all study subgroups in relation to the control group [Table 3 (Fisher's test)].

Class II malocclusions were more frequent in the study group while for both groups incorrect molar occlusions were observed ($p = 0.000$) [Table 4 (Chi-square test)].

Significantly higher percentage of Class II malocclusions for males in both mixed and permanent dentitions and for females in the mixed dentition of the study group ($p < 0.01$) was observed [Table 5 (Chi-square test)].

A higher percentage of class II malocclusions was observed in patients with cerebral palsy in relation to patients with autism and mental retardation, but without statistical significance [Table 6 (Chi square-test)].

Table 3

Comparison of the presence of the first permanent molar in the mixed dentition and of the second permanent molar in the permanent dentition in the study and control group

Age (years)	Autism		Cer. palsy		Ment. retardation		Control		*p*
	erupted	non erupted	erupted	non erupted	erupted	non erupted	erupted	non erupted	
5–7	0	10	3	1	5	5	19	12	
12–13	2	7	2	3	2	5	22	4	
Total	2	17	5	4	7	10	41	16	0.000*

***statistically significant (Fisher's test).**

Table 4

Comparison of the presence of the first permanent molar in the mixed dentition and of the second permanent molar in the permanent dentition in the study and control groups

Molar occlusion	Control group	Study group	Total
	n (%)	n (%)	n (%)
Class I	55 (53)	20 (18)	75 (35)
Class II	43 (41)	83 (78)*	126 (60)
Class III	6 (6)	4 (4)	10 (5)
Total	104 (100)	107 (100)	211 (100)

***$p = 0.000$ (χ^2-test).**

Table 5

Comparison of the presence of malocclusions (Class II Division 1) in mixed and permanent dentition in the study (S) and control (C) group

Age (years)	Gender	Group	n	Malocclusion (%)	*p*
6–11	male	S	28	60.71	0.005*
6–11	male	C	23	21.73	
6–11	female	S	27	70.37	0.001*
6–11	female	C	28	28.57	
12–16	male	S	24	79.17	0.008*
12–16	male	C	28	42.86	
12–16	female	S	28	64.29	0.077
12–16	female	C	25	40.00	

***statistically significant (χ^2-test).**

Table 6

Types of molar occlusion in relation to the patients condition

Molar Occlusion	Autism	Cerebral Palsy	Mental retardition	Total
	n (%)	n (%)	n (%)	n (%)
Class I	9 (45)	2 (10)	9 (45)	20 (100)
Class II	35 (42)	18 (22)	30 (36)	83 (100)
Class III	1 (25)	0 (0)	3 (75)	4 (100)

χ2-test – no significance.

Discussion

Children with disabilities included in this study exhibited a higher prevalence of oral disease in comparison to the healthy children. Other studies reported that the prevalence of dental caries was higher in medically compromised patients when compared with healthy children. Oral hygiene, diet, living conditions, water fluoridation, social factors and institutionalisation were recognised as important contributing factors to the prevalence of oral diseases in medically compromised patients [9–12]. The results of the present study support the findings of reports that demonstrated a high caries prevalence, alongside with a higher proportion of untreated lesions [10, 11, 13] as well as higher prevalence of malocclusion in children with disabilities when compared to non-medically compromised patients [14, 15]. However, there are other studies that reported comparable or no appreciable difference, or even lower oral disease levels in children with disabilities [16]. There are also studies that show that children with severe disabilities can demonstrate lower levels of DMFT in comparison to the children with mild or moderate disabilities [17, 18], indicating that further research is needed in this field. The preventive and restorative treatment needs a large number of children in the present study, where unmet and high priority in public dental funding should be given to the prevention and treatment needs of these patients. There is an opinion that because of their complex treatment needs, children with disabilities require specialist care and general anaesthesia which could improve quality of their dental treatment [19].

In this study the dmfs and DMFS indices of the study group, related to age, have significantly higher values than those of the control group. Our results can be compared with the study of Shmarak and Bernstein [20] who summed dmfs and DMF calculated per tooth surface (DMFS) in the mixed dentition and found higher caries levels in children with cerebral palsy. Nielsen [21] found that motor alterations in handicapped persons were the best caries predictors and that the presence of residual food was the result of the inability of the tongue, cheeks and lips to perform normal deglutition [21]. Contrarily, Swallow [22] demonstrated a trend of a lower caries incidence in the primary teeth of children with a wide range of physical and medical handicaps. When comparing dmfs and DMFS in the children with mixed dentitions Rodrigues dos Santos et al. [2] did not find any difference between the children with CP and healthy children. However, in the permanent dentition the children with CP had significantly higher values of DMFS compared to the healthy controls [2, 11, 21].

Choi and Yang [14] reported that the dft, dfs and DMFT indices of the medically compromised subjects were significantly lower than those for healthy individuals and that DMF, DMFS and DMFT indices increased with age in both of the examined groups. The results in the present study showed significantly higher dmft and DMFT indices in children with disabilities. It was observed that the decay component (dt; DT) of the mean dmft and DMFT index was the largest component of the index for both groups. Children with disabilities have had low levels of restorative care as demonstrated through the low number of filled teeth. The restorative component was lower in children with disabilities, which is attributed to the lack of conservative approach to the treatment of dental caries and is in agreement with other studies [10, 16]. The explanation for this might be found in the greater difficulty of treating children with disabilities. The majority of children with special care needs spend most of their time at home, and only a few hours daily at specialist daycare centres and other support institutions. Therefore these children receive their daily dental oral care from their parents with little emphasis placed on prevention and therefore they have poor dental attendance record. The severity of the handicap should also be taken into account since it is a determining factor, not only for oral hygiene status, but also for dental therapy which can be further hampered by the inability of those children to fully communicate and cooperate during dental treatment.

Clinical experience in Serbia shows that medically compromised children are taken to the dentist usually when they experience symptoms of acute pain, and that the higher incidence of caries could be due to the lack of awareness about the importance of regular dental visits and preventive and prophylactic care.

It is shown that individuals with autistic disorder, mental disorders and other pervasive developmental disorders may have lower learning abilities than healthy individuals [23]. Consequently, this may affect their oral hygiene [24]. In our study oral hygiene level was shown to be rather poor in the study group compared to their healthy counterparts, and these findings are in agreement with other similar studies [14]. Most of the studies in the literature reported unsatisfactory oral hygiene in patients with disabilities [10, 14]. Difficulties in maintaining satisfactory oral hygiene and effective brushing were obvious in the children with disabilities group. The presence of mental disorders, motor alterations and dyskinetic movements coupled together with pathological oral reflexes, such as biting and vomiting, may also be considered important factors for the difficulties in mechanical removal of plaque, and may hamper dental hygiene. Dental cleanliness values observed in the test group of our study were significantly lower in the children in the permanent dentition. Increasing discrepancy of oral hygiene related with age between medically compromised and non-medically compromised children was also noticed and it was in agreement with other studies [25].

Delay in the time of permanent molar eruption observed in this study is in accordance with the previously reported results of other authors [2, 11, 14, 21].

Brown and Schodel [25] reported that orthopaedic handicapped groups could have an increased incidence of malocclusion, which was attributed to a lack of muscular co-ordination with dyskinetic movement, tendency to develop increased overjet due to buccal breathing and tongue thrusting. This is in accordance with our results where children with disabilities had malocclusions in 82% of the cases, of which 95% were Class II malocclusions. Swallow [22] did not show significant differences from the norm for malocclusion in a group of physically and medically compromised children.

The male subjects in the study group with permanent dentitions exhibited a significantly higher percentage of malocclusions than in the control group. The presence of malocclusion can further complicate the child's disability, as seen in children with cerebral palsy or epilepsy who are predisposed to trauma because of large overjets. As reported previously, periodontal disease, functional problems, speech impairment and temporomandibular joint dysfunction can develop [11]. Oral health status of patients with disabilities can be further complicated and worsened if they exhibit signs of psychiatric disorders or are on antidepressant therapy [26].

In spite of longstanding efforts of clinicians to modify parental behaviour and approach to maintaining oral hygene in children with disabilities and other efforts to reduce caries-risk in this population, little has been changed so far in the caries rates for medically compromised children.

Eastern European countries are facing problems in the treatment of this group of patients due to their low socioeconomic status. It is considered that the best approach for treating children with disabilities is conventional dental approach [27], but, limited cooperation with these patients as well as a large number of untreated caries lesions and other oral pathology may be exhibited, which often leads us to use general anaesthesia in their treatment [19]. Future efforts must be directed at finding effective national preventive strategies for the children with disabilities who continue to be afflicted with extensive caries. Until these strategies become available, clinicians need to improve their efforts to protect the dentition of handicapped children through appropriate preventative and restorative care.

Conclusion

Significantly high levels of caries prevalence and low levels of oral hygiene status for permanent teeth were observed in the children with disabilities group. The major component of the dental caries index was attributable to the decayed component and lack of conservative approach to the treatment was confirmed in the study group of children with disabilities. In children with disabilities there was a higher tendency for a delayed time of eruption of permanent molars comparing to healthy children. Class II division 1 malocclusions were significantly higher in a group of medically compromised children with permanent dentitions.

Conflict of interests

The authors declare that there is no conflict of interest.

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Bone tunnel enlargement after the reconstruction of anterior cruciate ligament using bone-tendon-bone graft

Uvećanje koštanog kanala nakon rekonstrukcije prednje ukrštene veze kolena upotrebom kost-tetiva-kost grafta

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Abstract

Background/Aim. Bone-tendon-bone (BTB) graft is one of the strongest biological grafts and it provides a strong initial fixation with the application of interference screws making possible the primary bone healing and bone integration of the graft on the place of fixation during arthroscopic reconstruction of the arterior cruciate ligament of the knee. The aim of our research was to determine if BTB graft from which, throughout the surgical treatment, soft tissue and periosteum are removed, leads to the reduction of the enlargement in the femoral and tibial bone after the arthroscopic reconstruction of the anterior cruciate ligament. **Methods.** The first phase consisted of bio-mechanical study on 12 pairs of cadaveric BTB grafts. The testing was performed on the mechanical testing machine. The second phase involved clinical testing. The first group consisted of 40 patients treated with the classical BTB autograft. The second group consisted of 56 patients who had two thirds of the bony parts of the bone-tendon-bone autografts of the soft tissue and periosteum removed. We measured the distance between the edge of the sclerotic tunnel on the tibial and femoral bone in three different points: proximal (F1;T1), middle (F2;T2) and the lower part (F3;T3). **Results.** The experimental part of the study showed no statistically significant difference in graft breakout force expressed in N/mm2 between classically treated BTB graft and graft with a partially removed soft tissue and periosteum. By comparing the expansion of tunnels in all segments in both bone tunnels between study groups, statistically significantly lower enlargement was measured in the group with BTB grafts with partially removed soft tissues and periosteum ($p < 0.05$). **Conclusion.** The use of BTB grafts with partially removed soft tissues and periosteum provides less bone tunnel expansion as compared to classically treated grafts of the anterior cruciate tendon.

Key words:
arthroscopy; knee; anterior cruciate ligament; transplants; rehabilitation.

Apstrakt

Uvod/Cilj. Kost-tetiva-kost (KTK) graft jedan je od najčvršćih bioloških graftova i obezbeđuje jaku inicijalnu fiksaciju i primenu interferencijalnih zavrtanja, čime se postiže primarno zaceljenje kosti i integracija grafta za kost na mestu fiksacije tokom artroskopske rekonstrukcije prednje ukršene veze kolena. Cilj našeg istraživanja bio je da utvrdi da li KTK autokalem kod koga se tokom hirurške obrade uklanjaju meka tkiva i periost dovodi do smanjenja uvećanja kanala u butnoj kosti i golenjači nakon artroskopske rekonstrukcije prednje ukrštene veze kolena. **Metode.** Prva faza sastojala se od biomehaničkog ispitivanja na 12 parova kadaveričnih KTK kalemova. Testiranje smo vršili na mehaničkoj kidalici. Druga faza je podrazumevala kliničko ispitivanje. Prvu grupu činilo je 40 ispitanika sa klasično obrađenim KTK autokalemom. Drugu grupu činilo je 56 ispitanika kod kojih su sa dve trećine koštanih delova KTK autokalema odstranjena meka tkiva i periost. Merili smo rastojanje između sklerotičnih ivica tunela na golenjači i butnoj kosti u tri različite tačke: proksimalno (F1;T1), u sredini (F2;T2) i u donjem delu (F3;T3). **Rezultati.** Eksperimentalni deo studije pokazao je da ne postoji statistički značajna razlika u sili kidanja kalema izražene u N/mm^2 između klasično obrađenog KTK kalema i KTK grafta sa delimično uklonjenim mekim tkivima i periostom. Uporedivanjem proširenja kanala u svim segmentima u oba koštana tunela između ispitivanih grupa, statistički značajno manje uvećanje kanala izmereno je u

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grupi sa KTK kalemom sa delimično uklonjenim mekim tkivima i periostom ($p < 0.05$). **Zaključak.** Upotreba KTK autokalema sa delimično uklonjenim mekim tkivima i periostom daje manje proširenje koštanih kanala u odnosu na klasično obradivane kaleme prednje ukrštene veze.

Ključne reči:
artroskopija; koleno; ligament, prednji ukršteni; graftovi; rehabilitacija.

Introduction

Nowadays, the primary arthroscopic reconstruction of the anterior cruciate ligament (*ligamentum cruciatum anterius* – LCA) has reached a significant level of precision and is routinely performed. In the last two decades, in literature, one can find the problem of enlargement of bone tunnel in the femoral and tibial bone after the primary reconstruction of the anterior cruciate knee ligament [1–3]. In 1994 Fahey and Indelicato [4] were the first who published the study pointing out the significance of this problem. The widening of the tunnel has no effect on the clinical results of the reconstruction of the cruciate ligament [5, 6]. However, this phenomenon can significantly complicate the revision surgery of the LCA.

All the agents responsible for the development of bone tunnel enlargement in the femoral and tibial bones are divided into two large groups. The first group consists of biological factors such as: the immune response of the body when using allografts [7], toxic effect (sterilization with ethylene oxide) [8], non-specific inflammatory response (the effect of cytokines on synovial fluid) [9], bone-cell necrosis caused by high temperature during the drilling of the bone tunnel [10] and avascular necrosis of bone cells of the graft itself [3, 9]. The second group consists of the so called mechanical reasons and these are : the movement of graft in the bone tunnel [9, 11] and its effect on the walls of the tunnel, aggressive rehabilitation [12, 13], and the effect of strong forces on the badly positioned graft [14, 15]. The extension of the tunnel is larger and it occurs more often when using hamstring tendon and allografts than in the application of bone-tendon-bone (BTB) autograft [16].

BTB autograft is used daily by orthopedic surgeons in the reconstruction of the anterior cruciate ligament because it gives good clinical results. Franke [17] says that BTB autograft is one of the most popular grafts equally represented both in the USA and in Europe thanks to the studies of Eriksson [18] and Clancy [19].

The application of BTB autograft in 80–90% of the cases has excellent clinical results and some of the most common complications are related to the lesion of extensor appliance of the given region [20, 21]. BTB graft is one of the strongest biological grafts and it provides a strong initial fixation with the application of interference screws making possible the primary bone healing and bone integration of the graft on the place of fixation during arthroscopic reconstruction [20, 21].

Over the years of using this graft the manner of its treatment has not significantly been changed. Pujol et al. [22] describe the arthroscopic double bundle LCA reconstructive technique making two BTB grafts from one donor place. Imitating the anatomical LCA reconstruction Shino et al. [23] and Herbort et al. [24] tried to improve the BTB technique, creating a rectangular tunnel and bony part of the graft, neglecting the importance of the cross-sectional area of the classic BTB graft, with a width of 10 mm and a thickness of 3–5 mm which ultimately depends on the individual patient's characteristics [25–28].

The aim of this study was to reduce the level of the enlargement of bone tunnel in the femoral and tibial bones by using different BTB grafts.

Methods

The experimental part of the research

Biomechanical testing was performed on 12 pairs of cadaveric BTB grafts. The age of cadaveric donors ranged from 19–38 years (average age was 25), 10 men and 2 women. The average time from taking the graft to testing on mechanical test machine was 6 weeks (3–12 weeks).The pairs consisted of BTB grafts of the same size (25 mm long and 10 mm thick) taken from the same cadaver where soft tissues and periosteum (up to bone part cortex of BTB graft) were partially removed. We wrapped thus obtained pairs into aluminum foils after washing them in the physiological solution and placed them into plastic bags which were kept at the temperature of -20 ºC. After that, before testing, we kept them at the temperature of 4 ºC for 12 hours and then at room temperature on the test day.

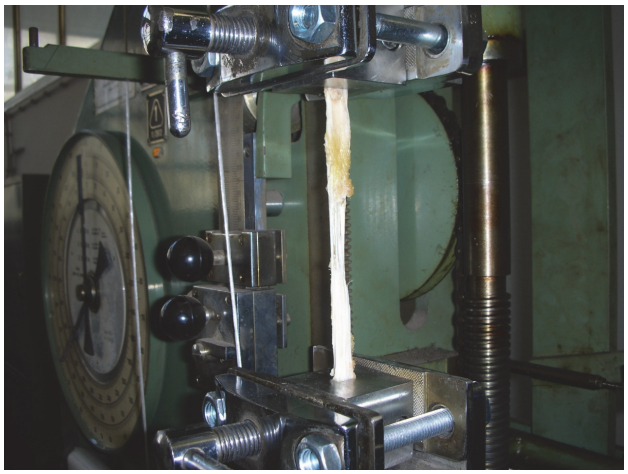

Fig. 1 – Mechanical testing machine (model WPM-Rauenstein, Universal Testing Machine, Leipzig, Germany).

The testing was performed on the mechanical testing machine (Figure 1) (»WPM- Rauenstein, Universal Testing Machine, Leipzig, Germany) with the maximum load of 5 kN to check whether the treated BTB grafts (with no perio-

steum and soft tissue) would retain the same force expressed in N/mm^2 in comparison to classically treated and prepared BTB grafts. The force expressed in N/mm^2 at which the graft cracks was recorded for each sample. During the testing, we always used the same graft holder. Each cadaver BTB graft was placed in the same way – so that the bone part is in the graft holder precisely to the joint of BTB [29]. Having realized that there was no statistically significant difference in the force required for tearing the graft (expressed in N/mm^2, between the classically treated graft and BTB graft with partially removed soft tissue and periosteum, we could start the clinical trial.

Clinical part of the research

The study was randomized and prospective. The tested group consisted of 96 patients of both sexes, aged from 18 to 48 years with unilateral complete rupture of the anterior cruciate ligament. The patients involved in the study were not previously treated surgically for these injuries and were divided into 2 groups. The groups were comparable according to all demographic characteristics and there was no statistically significant difference when it comes to gender, laterality, age, level of sports activity, the time passed from injury to surgery, combined lesions of meniscus and lesions of cartilage. The only difference between the groups was in the way the BTB graft was treated. With all the patients the uniform program of rehabilitation was carried out according to Shelborn's modified protocol [12]. The study did not include patients with multiligament injuries, degenerative changes and deformities of the knee joint.

There were 40 patients in the first group with the classically treated BTB autograft, the average age being 27 ± 5.514 years and the average body mass index (BMI) 21.94 kg/m^2. In the second group, there were 56 patients who had BTB autografts the soft tissue and periosteum partially removed and their average age was 26 ± 4.982 years and the average BMI 21.37. In the first group, the average duration of the injury was 8.2 ± 5.036 months and in the second 9.4 ± 4.131 months. There was no statistically significant difference in the duration of injury between the groups ($t = -1.242$; $p > 0.05$) nor when it comes to the age of the patients and BMI.

From the patients in the first group, during the surgery with the normal vertical incision above the patella ligament, a BTB graft was taken, on which, after the treatment, bone blocks were cylindrical with the diameter of 10 mm and length 25 mm. During the preparation of the graft itself soft tissue and periosteum were not removed from the bone parts of the future ligament. In the second group, very same technique of taking the graft was applied as in the first group, but the processing method was different. During the preparation of bone blocks, from two thirds of the bone part of the graft soft tissue and periosteum were completely removed (to the cortex of bone part of the BTB graft) with a scalpel. During the processing the cylinders obtained also had the diameter of 10 mm.

After a detailed introduction to the planned procedure, the voluntarily registered candidates who agreed to participate in the study were selected. On those patients the parameters obtained with the analysis of radiographic images seven days after the surgery in the period from 3 to 12 months after the surgery was analyzed and compared. We determined preoperatively and postoperatively the Lachman test [30] (positive and negative), Pivot shift test [31] (subluxation of the tibia relative to the femur), Lysholm [32], Tegner [33] and International Knee Documentation Committee (IKDC) score [34] for the knee. Arthrometric measurements implied the determination of front motion of the tibia relative to the femur (Lachman test) in mm using the arthrometer. The front mobility was measured by the arthrometer on both the operated and the opposite knee and the differences were registered.

Surgical treatment and postoperative protocol

The patients were in the dorsal position with a leg on the arthroscopic support in general or epidural anesthesia. The surgeries were carried out in ischemia. All the patients were operated on with the same arthroscopic single-bundle anatomic technique. A 10 mm diameter drill was used for both bone tunnels (on the tibia and the femur). The graft was fixed with cannulated titanium screws (Grujić & Гpyjић, Novi Sad, Serbia), 8 x 25 mm. After the surgery, all patients had an elastic bandage for 14 days. In the rehabilitation process, we used a modified Shelbourne protocol [12]. From the first day after the surgery, all the patients were subjected to continuous passive motion of the operated knee with the help of kinetec. A partial support was allowed after two weeks and the full support six weeks after the surgery. All the patients followed the same rehabilitation program with the chain of kinetic exercises for the reinforcement and restoring the muscle strength of the anterior and posterior thigh. The patients were checked a week after the completion of hospital treatment, and then 6 weeks after the surgery when they get the full support on the operated leg, and then 3 and 6 months after the surgery when they are practically in the full competitive form. We did the clinical check-ups again in the interval from 1 to 4 years (the average of 2 years). All the patients within our study were operated on by the same orthopedic surgeon, using the same graft fixation method and the uniform program of the rehabilitation was carried out by the same doctor.

The monitoring of radiological parameters

The lateral and anterior-posterior (AP) radiograph of the operated knee was done 7 days after the surgery and 6 and 24 months after the surgery. The AP images were made with the knee in full extension and the lateral images with the knee in a passive extension (with the heel set on the base around 10 cm high. The source of radiographic rays was located 100 cm from the cassette and positioned vertically to it. The diameter of the tunnel on the AP and lateral images were determined according to the modified Peyrache et al. [35] method. We measured the distance between sclerotic edges of the tunnel on the tibia and femur in three different points: proximal (F1; T1), medial (F2; T2) and the lower part

(F3; T3), i.e., at the level of the joint cavity and 1 cm and 2 cm distal to the tibia and proximal to the femur (Figure 2). The results were compared to the width of the drill, with which the tunnel was drilled during the surgery. The obtained results could be viewed as the percentage of the diameter change which was calculated in the following way:

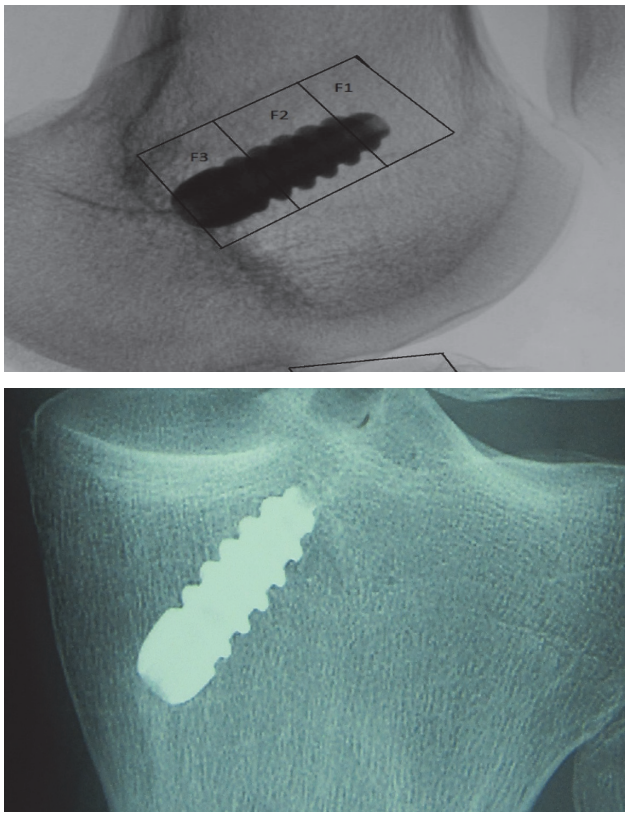

Fig. 2 – Distance between sclerotic edges of the fibia and femur in three different points (F1, F2, F3).

Measured diameter of the tunnel-intraoperative diameter of the tunnel

Enlargement of the tunnel was defined as every enlargement of the tunnel compared to intraoperative diameter of the tunnel (which was determined by the size of the drill). The shape of the tunnel was classified as a cone, cavity and a line type of bone tunnel enlargement [36]. The measurements were performed on the scanned radiograms using the high precision calibration. The reproducibility was checked on the measures obtained by 3 different doctors. The precision of the measurement of the angles was 0.5° and linear measurements were 0.5 mm. For each of the parameters that were considered, the average, min. and max. values were calculated as well as standard deviation.

The study was approved by the Ethics Committee of the Medical Faculty of the University of Novi Sad and Ethics Committee of the Clinical Centre of Vojvodina in Novi Sad.

Statistical data analysis

In the statistical analysis the Kolmogrov-Smirnov test was used and depending on the nature of the data parametric tests were used (Student's *t* test, *t*-test for pair samples and ANOVA) or an equivalent non-parametric statistical tests (Mann-Whitney test, Wilcoxon test for pair samples and Kruskal-Wallis test). The values $p < 0.05$ were considered statistically significant. In the data analysis software MS Office Excel 2007 was used, with the statistical software Analyze – lt with the appropriate text comment. The statistical analysis of the results obtained with arthrometric evaluation was performed with the help of the Student's *t*-test. The values $p < 0.05$ were considered statistically significant.

Results

There was no statistically significant difference in the postoperative values of IKDC score between the observed groups ($\chi^2 = 4.265$; $p > 0.05$). With the statistical analysis of the data using the non-parametric Wilcoxon test, it was found that there was a statistically significant difference between the Tegner score of the activities before and after the surgery for each group individually [(I group – Z = -5.561; $p < 0.01$); (II group – Z = -6.534; $p < 0.01$)]. There was also a statistically significant difference between Lysholm score of the activity before and after the surgery for each group individually [(I group – Z = -5.515; $p < 0.01$); (II group Z = -6.511; $p < 0.01$)]. Comparing the difference between the healthy and the diseased knee before and after completing the surgical treatment we did not find a statistical significance between the observed groups (Table 1).

With the statistical data analysis using the Student's *t*-test, we did not determine the existence of statistically significant differences in the tearing force expressed in N/mm^2 between 12 pairs of cadaveric BTB grafts (t = 0.058; $p > 0.05$). The force in N/mm^2 , at which the graft rupture occured, was recorded for each sample. The average value of the tearing force in N/mm^2 of the classically treated BTB graft amounted to 1155.42 ± 157.94 and the average value of tearing force in N/mm^2 of the BTB with partially removed soft tissues and periosteum was 1150.25 ± 157.69 (Figure 3).

Table 1

Functional scores and arthrometric measurements

Type of BTB	Tegner score before / after	Lysholm score before / after	Lachman test (mm) before / after
Classic BTB	1.73 / 8.15	46.73 / 95.93	14.428 / 3.90
BTB without periosteum and soft tissue	1.63 / 8.34	48.43 / 97.14	14.025 / 4.26

BTB – bone-tendon-bone graft.

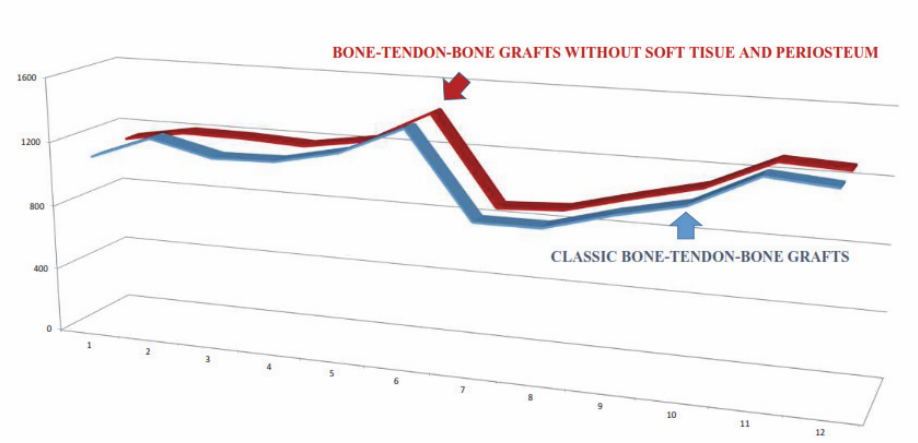

Fig. 3 – Measurement of the tearing force in N/mm^2.

Comparing the radiographic results according to segments (T1, T2, T3 segment in AP projection and profile and F1, F2 and F3 segment in AP and profile) between the examined groups, we found out that the best results were achieved by using the BTB graft with partially removed soft tissues and periosteum. In this group, the level of bone tunnel enlargement in the femoral and tibia bones was significantly lower compared to the classically treated BTB grafts (Figures 4–6) and (Tables 2 and 3)

Table 2

Results of the measurements of bone tunnel percentage of enlargement in tibia by segments among the observed groups

Tibial segment	BTB without periosteum and soft tissue	Classic BTB	*p*
	mean	mean	
Antero-posterior			
T1 proximal third	0.0214	0.5100	< 0.01
T2 middle third	0.0179	0.7175	< 0.01
T3 distal third	0.0179	0.5300	< 0.01
Lateral			
T1 proximal third	0.0161	0.5375	< 0.01
T2 middle third	0.0125	0.7175	< 0.01
T3 distal third	0.0125	0.5300	< 0.01

BTB – bone-tendon-bone graft.

In our sample of 96 patients the conical shape of the bone tunnel enlargement was determined in 28 (29.1%) patients, cavity shapes in 14 (14.58%) patients, and line shapes in 7 (7.29%) patients. Bone tunnel enlargement was not found in 47 (48.95%) patients.

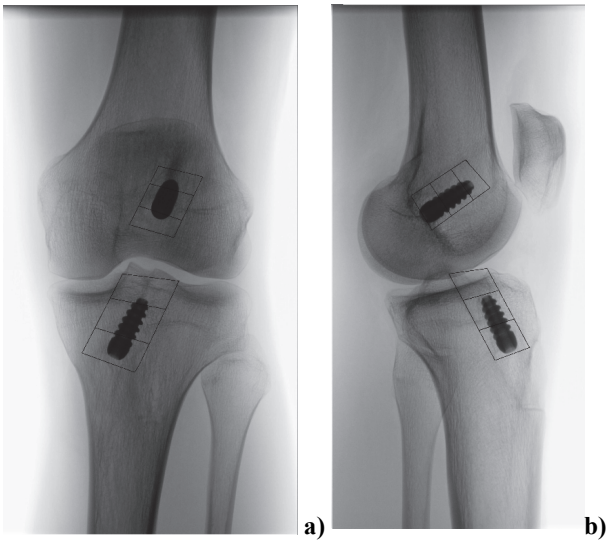

Fig. 4 – Radiographs of the knee.
a) Anteroposterior (AP) position; b) Profile.

Table 3

Results of the measurements of bone tunnel percentage of enlargement in femur by segments among the observed groups

Femoral segment	BTB without periosteum and soft tissue	Classic BTB	*p*
	mean	mean	
Antero-posterior			
F1 proximal third	0.0161	0.3675	< 0.01
F2 middle third	0.0089	0.3675	< 0.01
F3 distal third	0.0018	0.3775	< 0.01
Lateral			
F1 proximal third	0.0125	0.4325	< 0.01
F2 middle third	0.0107	0.4325	< 0.01
F3 distal third	0.0018	0.3350	< 0.01

BTB – bone-tendon-bone graft.

Discussion

The reason why bone tunnel enlargement occurs after the anterior cruciate ligament reconstruction is still unexplained, but it is certainly multifactorial and conditioned by biological and mechanical reasons [37, 38]. Although, any correlation between clinical results and bone tunnel enlargement has not been found in the published studies until now, this phenomenon plays an important role in revision surgery of LCA which is required in 8% of the patients after the primary reconstruction [1–3].

The experimental part of our study which related to biomechanical research showed that there was no statistically significant difference regarding the tearing force of the graft expressed in N⁄mm^2 between the classically treated BTB graft (with soft tissue and periosteum) and BTB graft with partially removed soft tissue and periosteum (soft tissue and periosteum were completely removed from the two thirds of the bone part of the graft). In an experimental study Handl et al. [29] showed on 21 pairs of cadaveric BTB grafts that the tearing force expressed in N/mm^2 is 1482 ± 211. As their sample showed, the force required to tear the natural anterior cruciate ligament ranged from 1246 ± 243 N⁄mm^2. The results of the experimental part of our study showed that the average value of the tearing force in N⁄mm^2 of the classically treated BTB grafts was 1155.42 ± 157.94 and the average value of the tearing force in N⁄mm^2 of the BTB graft with partially removed soft tissues and periosteum was 1150.25 ± 157.69. The results of the experimental part of our study were similar to the results of numerous experimental studies [39–41] which investigated the tearing force of the BTB graft in N⁄mm^2 regardless of the new way of treating the graft and the partial removal of soft tissues and periosteum from bone parts.

Peyrache et al. [35] and Jaureguito and Taulos [39] thought that bone tunnel enlargement in the femoral and tibia was an important complication and that it may be the first, early sign of graft failure. This phenomenon did not affect the clinical stability of the knee but it significantly complicated revision surgeries [1, 40]. One of the few exceptions was the perspective randomized study published in 2008 by Järvelä et al. [41], which showed that with the application of the so-called anatomical "double bundle" technique for a lower level of a bone tunnel enlargement on the tibial insertion (for both tunnels) was obtained comparing to the "single bundle" enlargement technique with a better postoperative knee stability. The author himself admitted that the study was limited by a small number of respondents and mixed measurement methodology. According to literature data [5, 6], despite the significant differences in the size of bone tunnel in the femur and tibia, there was no statistically significant difference among the observed groups regarding the obtained postoperative knee stability.

Comparing the obtained radiographic results between the groups of classically treated BTB graft and BTB graft with partially removed soft tissue and periosteum, in both radiographic projections (AP and profile) we found a statistically significant reduction of bone tunnels enlargement in the femoral and tibial bones in the group with the grafts with partially removed soft tissue and periosteum. Measuring precisely and comparing the changes in the diameter of bone tunnels in this group of respondents, we determined that the best results were obtained in F3 segment (which was one third of the tunnel in the femoral which was closest to the joint cavity), because, it was the part of the tunnel where the bone part of the graft is usually positioned. The graft treated this way without soft tissue and periosteum has a full biological potential of healing.

Although there was a statistically significant reduction in the bone tunnel enlargement on the tibia compared to the classically treated BTB graft, the percentage of the reduction was lower than in the femur. Published data [42–44] confirm that the enlargement is bigger on the tibia than on the femur due to the fact that it is more difficult to position the graft on the tibia in the vicinity of joint cavity so that the movements of both bone and ligament part of the neoligaments are still higher than on the femur. Drogset et al. [45] and Cameron et al. [9] believed that such a radiological result was also a consequence of the fact that the entire femoral tunnel was in cancellous bone unlike the tibial that was localized in the cancellous bone only juxta-articularly. Radiographic measurements showed that the bone tunnel enlargement both in the group with classical BTB graft and in the group with BTB graft without the soft tissue and periosteum was the biggest in T1 and T2 segments (medial and proximal third of the tibial tunnel), i.e., in the segments which were more often without the bone part of the graft. Such a result is probably due to the so-called "windshield wiper effect" or micro movement of the ligament part of the graft in the transverse plane of the tibial tunnel [11]. However, regardless of the numerous impacts of mechanical and biological factors [3, 7–16] on the tibial tunnel and tibial part of the graft, in the group with BTB graft with partially removed soft tissue and periosteum, we got a significantly smaller enlargement of the bone tunnel of the tibia in all three segments (T1,T2 and T3) in both radiographic projections.

If the graft fixation point is more distant from the initial part of a bone tunnel, then its movement in the tunnel itself is higher and, consequently, a degree of osteolysis as well. Such movement of the graft in the bone tunnel in the longitudinal direction is marked as "bungee effect" (i.e., the effect of graft stretching or the effect of longitudinal displacement of the graft) [46]. This effect is stronger when using hamstring tendons in reconstruction of the LCA, but it is not exclusively a characteristic of anterior cruciate ligament reconstruction with these tendons. Contrary to this opinion Kobayashi et al. [47] believed that the way of graft fixation did not affect the degree of osteolysis of bone tunnels. They also believed that the movement of the graft inside the tunnel was not the only reason for the occurrence of this phenomenon. Within our study in both groups of patients, the fixation in the bone tunnels and femur and tibia was done with interferential titanium screws in the third of the tunnel which was nearest to the joint cavity and thus the effect of longitudinal displacement of the graft was reduced to minimum. The published data also showed that this method of fixation minimized the movement of the graft within the bone tunnels [48, 49]. Regarding the application of metal or titanium screws, it should be mentioned that although Maloney et al. [50] in *in vi-*

tro conditions demonstrated decreased cell proliferation with the increasing concentration of particles of titanium and aluminium alloys and chromium particles, no clinical study proved that the interference screws after the LCA reconstruction caused the enlargement of bone tunnel.

All the studies that were carried out, and so did ours, indicated that in the application of BTB graft the "windshield wiper effect" dominates with a significant enlargement of the tunnel [11]. Within our study, with the altered way of treatment of the BTB graft with the partially removed periosteum and soft tissue and its adequate positioning with the use of anteromedial portal we got statistically significantly smaller enlargement of the tunnel, both on the tibia and the femur, in comparison to the classical, uniform treatment of this most commonly used graft.

The most common form of bone tunnel enlargement on our sample was on both bones in both radiographic projections of conical type which was in accordance with the published data [51].

The accelerated rehabilitation protocol may result in the functioning of strong mechanical forces on the graft before its complete biological incorporation finishes [13]. In both groups of patients a uniform program of rehabilitation was conducted according to the so-called modified Schelbourne protocol [12] in order to prevent the possible influence of different physical procedures on bone tunnel enlargement in the femoral and tibial bones.

Non-anatomical position of the neoligament increases the intensity of the forces acting on it, and the cadaveric studies [14, 15] have shown that those forces are 3–4 times stronger than the forces that the natural LCA endures. Such mechanical forces increase the level of osteolysis especially at the beginning of bone tunnel where these forces are the strongest [10]. With all the patients included in this study the femoral tunnel on the profile images was located in the so-called green field [52], and on the tibia it was about 1 cm medially from tuberosity, drilled at the angle of 50–60 degrees with intraarticular position of about 7 mm in front of the rear cruciate ligament. With such a position of the graft we tried to maximally prevent the influence of its inappropriate position on bone tunnel enlargement. The size and the localization of the enlargement of bone tunnel in the femur and tibia depends, substantially, on the surgical techniques used in the reconstruction of the LCA. The variations in surgical techniques [53] can be the reason for different results when researching the effect of bone tunnel enlargement in the femur and tibia. Therefore, it is important to correlate and compare the size of osteolysis and the type of surgical techniques in order to obtain the best results in treatment of anterior cruciate ligament injuries. This was one of the aims of this study.

The study that we conducted has some potential flaws. The fact is that most authors believe that the computed tomography (CT) evaluation of this phenomenon is more precise than the radiographic. However, according to a study conducted by Webster et al. [54] in which they compared these two methods, the digital radiography was considered quite satisfactory and it was emphasized that its convenience was significantly lower price, especially in the research with a greater number of respondents (in our study 96 patients were monitored), and a lower dose of ionizing radiation to which the patients were exposed. Digital radiographic evaluation of bone tunnel enlargement in the femoral and tibial bone was absolutely comparable with the results in CT research with a better economic effect and shorter time needed to carry out the research on which Jo et al. [55] agreed in their study.

The most significant result of this prospective randomized experimental-clinical study was that we have improved the characteristics of the most common replacement for LCA and approached it more to the so-called "ideal graft" making revision surgery easier by reducing the bone tunnel in the femoral and tibial bone.

Conclusion

An application of BTB autograft with partially removed periosteum and soft tissue provides significantly better results in the reduction of the degree of osteolysis of bone tunnel in the femoral and tibial bones than using the classically treated graft of the anterior cruciate ligament. The use of such treated grafts significantly facilitates possible revision procedures whose number increases from year to year because of the increasing number of primary reconstructions of this ligament.

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Evaluation of choledochoduodenal anastomosis function in benign biliary obstruction

Procena funkcije holedohoduodenalne anastomoze u hirurškom lečenju benignih opstruktivnih oboljenja žučnih puteva

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Abstract

Background/Aim. Choledochoduodenostomy has been reported as an effective treatment of benign biliary obstructions, but associated with a certain percentage of complications, (primarily cholangitis and the "sump" syndrome), as the consequence of duodenobiliary reflux which may occur. The aim of our study was to evaluate the safety, effectiveness and technical feasibility of choledochoduodenostomy for the treatment of distal benign biliary obstruction and to present its minimal postoperative complications. **Methods.** This propective study included 50 operated patients who had choledochoduodenal anastomosis created for benign biliary obstructions. The symptoms, biochemical and echosonographic parameters of cholestasis, operative technique, recovery features and complications were analayzed and compared. Based on the analysis of obtained data, safety, efficacy and competence of choledochoduodenal anastomosis were determined. **Results.** Specific early anastomosis-related complications were observed in 12.0% of patients (mostly minor surgical complications). During the immediate postoperative course, aerobilia as an indirect sign of duodenobiliary reflux, occurred in 91.7% of patients, but it was reduced to 16.7% after 30 days (and was not always associated with symptomatology). Choledochoduodenostomy was associated with a low incidence of cholangitis (2%) and anastomosis dehiscence (2%). Transitory duodenogastric reflux was identified in 6% of patients. The rate of intrahospital mortality was very low, considering patients' very complex conditions (4%). During early postoperative period, the "sump" syndrome was not identified. **Conclusion.** Choledochoduodenostomy is a simple and effective method in the management of certain types of biliary obstruction. Serious complications can be avoided by proper selection of patients and careful surgical technique. This type od anastomosis has to be included in basic skills of every general surgeon.

Key words:
anastomosis, surgical; biliary tract surgical procedures; choledochostomy; duodenum; gallstones.

Apstrakt

Uvod/Cilj. Holedohoduodenalna anastomoza se uspešno primenjuje u hirurškom lečenju benignih bilijarnih opstrukcija. Medutim, smatra se da je povezana sa izvesnim procentom komplikacija, (holangitis i "sump" sindrom), kao posledice duodeno-bilijarnog refluksa, koji može postojati nakon njenog kreiranja. Cilj rada bio je procena efikasnosti, sigurnosti i tehnike izvođenja holedohoduodenalne anastomoze u terapiji distalnih benignih bilijarnih opstrukcija, u cilju utvrđivanja uticaja izbora indikacija i operativne tehnike na pojavu postoperativnih komplikacija. **Metode.** Prospektivnom studijom je obuhvaćeno 50 bolesnika operisanih zbog benignih bilijarnih opstrukcija, primenom holedohoduodenalne anastomoze. Analizom i komparacijom kliničke slike, biohemijskih parametara holestaze, ultrazvuka i parametara operativne tehnike, kao i analizom ranog postoperativnog perioda i detekcijom komplikacija, utvrđivani su efikasnost, sigurnost i kompetentnost holedohoduodenoanastomoze. **Rezultati.** Neposredne specifične postoperativne komplikacije, vezane za anastomozu, zabeležene su kod 12% bolesnika (uglavnom minorne hirurške komplikacije). Neposredno nakon kreiranja anastomoze, aerobilija kao indirektni znak duodenobilijarnog refluksa, registrovana je kod 91,7% bolesnika, ali se taj procenat do tridesetog dana smanjio na 16,70% i nije bio uvek praćen patološkim posledicama.

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Holangitis je potvrđen kod jednog (2%) bolesnika, kao i dehiscencija anastomoze (2%), dok "sump" sindrom nije detektovan u posmatranom periodu. Tranzitorni duodenogastrični refluks je identifikovan kod 6% bolesnika. Stopa intrahospitalnog mortaliteta bila je niska (4%), s obzirom na kompleksno stanje bolesnika podvrgnutih operaciji. **Zaključak.** Holedohoduodenalna anastomoza je jednostavna i efikasna metoda u lečenju benignih bilijarnih opstrukcija. Ozbiljne komplikacije moguće je izbeći pravilnim izborom indikacije i pažljivom operativnom tehnikom, zbog čega njihovo poznavanje mora biti deo osnovne edukacije svakog opšteg hirurga.

Ključne reči:
anastomoza, hirurška; hirurgija žučnih puteva, procedure; holedohostomija; duodenum; žučni kamenci.

Introduction

The number of surgical interventions on bile ducts is constantly increasing and in many surgical units these are the most frequent abdominal operations. Benign biliary obstructions (BBOs) are among the most common biliary diseases, following gall bladder calculosis. Most common causes of BBOs are hepatico-choledocholithiasis (HCHL) (present in 10%–20% of patients with gall bladder calculosis), stenosis of the sphincter of Oddi, stenosis of the papilla (SP), rarely fibrous chronic pancreatitis (CP) and then inflammatory and iatrogenic strictures of the hepaticocholedochus (HCH). Parasitoses (especially echinococcosis), juxtapapillary duodenal diverticulum, congenital HCH cysts, congenital biliary atresia and sclerosing cholangitis are less frequent causes of BBOs [1–3].

BBOs are treated endoscopically or surgically (using laparoscopic or classical "open" approach). One of the methods of treatment is choledochoduodenal anastomosis (CDA) which has been debated about since its introduction in 1888. It is described as a "reflux" procedure due to the risk of reflux of duodenal content into the bile duct after the creation of anastomosis as well as alkaline duodenal content back into the stomach, with possible complications such as recurrent cholangitis (cholangiocarcinoma as well, in the long run), "sump" syndrome (creation of choledochal stump as a remnant of created anastomosis) and alkaline gastritis. In the era of endoscopy, interventional radiology and laparoscopy and with an increasing use of hepaticojejunal anastomosis (HJA), it was almost completely abolished back in the 1980s. However, numerous studies have shown that CDA is an anastomosis that can be easily created, with good long-term results, while the complications can be kept at a minumum rate by a careful selection of indications and meticulous surgical technique. In everyday clinical practice, it is most commonly used in the elderly, those at higher surgical risks with distal (retropancreatic or supraduodenal) stenoses (CP, injuries, inflammations), those with calculi stuck in the papilla as well as in patients with multiple, residual or recurrent calculosis. In addition to the above indications, CDAs can be applied as well after unsuccessful endoscopic procedures [endoscopic papillotomy (EPT), stenting] and in the cases when these are not available [4–9].

The aim of this study was to evaluate the safety, effectiveness and technical feasibility of choledochoduodenostomy for the treatment of distal benign biliary obstruction and to demonstrate that, in properly selected patients with careful surgical technique, postoperative complications of this method may be kept to a minimum.

Methods

The prospective study included 50 patients treated at the General Surgery Clinic, Clinical Center Niš, Serbia, in the period from 2010 to 2014. All the patients underwent surgery and CDA for BBOs.

In all 50 patients preoperative clinical symptoms (icterus, pain, fever) and biochemical parameter of cholestasis [bilirubin, aspartat aminotransferase (AST), alanine aminotransferase (ALT), gama glutamyl transferase (GT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH)] were analyzed and HCH diameters were sonographically measured preoperatively in all 50 patients.

The parameters of the surgical technique were directly monitored (length of surgery, creation and duration of CDA itself, blood losses). Distal longitudinal choledochotomy (25–30 mm), duodenal mobilization and duodenotomy closest to choledochal incision, were a condition of creating anastomosis without any tension. Latero-lateral (L-L) CDA was created using interrupted suture technique with Vicryl 3.0 or 4.0 resorbable sutures in 46 patients. In 4 patients, due to Mirrizi's syndrome type II, III and IV, a part of the HCH was resected and terminolateral (T-L) CDA was created. The length of the whole surgical intervention was measured as well as the duration of a CDA creation from the moment of completion of all the preparations. Also, the total intraoperative blood loss was measured. After the creation of anastomosis, its circumference was measured in order to establish possible interdependence of the bile duct diameter and anastomosis itself.

Biochemical cholestatic parameters were observed on postoperative days 1, 3, 7 and 30. HCH diameters were sonographically measured and possible complications were evaluated on days 7 and 30 as well as the indirect and direct signs of duodenobiliary reflux, assessing thus the efficacy and safety of CDA in resolving the cholestasis. Nineteen patients underwent hepatobiliary Tc^{99m}-hepatobiliary iminodiacetic acid (HIDA) scintigraphy and 4 had esophagogastroduodenoscopy (with biopsies of antral mucosa), if alkaline reflux was suspected according to clinical, labaratory and ultrasonographic (US) findings.

Functional assessment of the liver and bile ducts using hepatobiliary scintigraphy was done in total of 19 patients at the Nuclear Medicine Center, Clinical Center Niš, using

^{99m}Tc-ethyl-hexa-imino diacetic acid (^{99m}Tc-EHIDA). Liver morphology, changes in the bile ducts and the onset of bowel activity were qualitatively analyzed as well as the onset and duration of duodenogastric reflux (DGR). As the semiquantitative indicators of liver function, the following factors were determined: time to maximal activity - T_{max} (in minutes), semielimination time – T/2 (in minutes), and residual activity – R (in %) of a radiopharmaceutical, time to the onset of DGR, detection of biliary-to-bowel transit (BBT), i.e. time of the activity onset in the bowels were established visually and semiquantitatively (the values up to 30 minutes were considered normal).

In all patients, early postoperative complications were recorded and analyzed (those occuring in the first 30 postoperative days), both specific and non-specific, i.e. the complications caused by comorbidities or operation itself, without any direct association with CDA.

Complications in this study were graded according to Clavien-Dindo classification scale [10].

Statistical analysis

SPSS 16.0 statistical software package was used for statistical data processing. We used *t*-test (or Mann-Whitney test), χ^2 test and Fisher's test of exact probability, variance analysis (ANOVA), and as a *post hoc* analysis Tukey's and afterwards Kruskal-Wallis test, Wilcoxon and Friedman test. The statistical hypothesis was tested at the level of significance $\alpha = 0.05$, i.e. the difference between the samples was considered significant if $p < 0.05$.

Results

The mean age of examined patients was 63.08 ± 8.82 years (ranging from 42 to 84, years). There were 52% men and 48% women. All the enrolled patients were divided into three groups based on indications: 56% of the patients with HCHL (3.58% with recurrent; 10.71% with residual; and 85.71% with secondary calculosis); 28% of the patients, with benign stenosis of the HCH or the ampulla of Vater (53.57% with stenosing papillitis; 35.71% with inflammatory stenosis of the HCH; 10.72% with distal bile duct lesions); 14% of patients with chronic fibrous pancreatitis (62.50% with alcoholic origin). There was not any statistically significant difference in age related to the indications ($p = 0.947$), nor a statistically significant difference in gender distribution related to the indications ($p = 0.242$). All patients had preoperative biochemical cholestatic syndrome, with or without clinically evident jaundice. Icterus was observed in 92% of patients and was usually accompanied by upper abdominal pain (67%), nausea and vomiting (63%) and fever (32%). The classical Charcot's cholangitis triad was present in a smaller proportion of cases (21%). All the patients in the prospective group (100%) before the surgery had sonographically confirmed mild or larger dilation of major bile ducts (exceeding 8 mm), with clinically manifested icterus or without it. Average HCH width was 17.57 ± 6.03 mm (range 10.5 to 32). Most patients had values ranging from 10 to 12.5 mm, and from 17.5 to 20 mm.

Surgical technique

In 92% of patients, L-L anastomosis was created, while in 8% of cases T-L CDA was done. Average lenght of operation was 89.30 ± 17.53 minutes (min. 60 minutes, max. 130 minutes). The creation of anastomosis took 14.98 ± 2.54 minutes (min. 11.00, max. 22.00), while the average blood loss during the surgery was 126.28 ± 44.86 mL (min. 78, max. 255 mL).

The average width of HCH, measured preoperatively, was 17.57 ± 6.03 mm (min. 10.50, max. 32 mm). The average width of anastomosis was 19.78 ± 4.29 mm (min. 13, max. 29 mm). A strong statistical correlation was observed between these two parameters (r = 0.949, $p < 0.001$) (Figure 1).

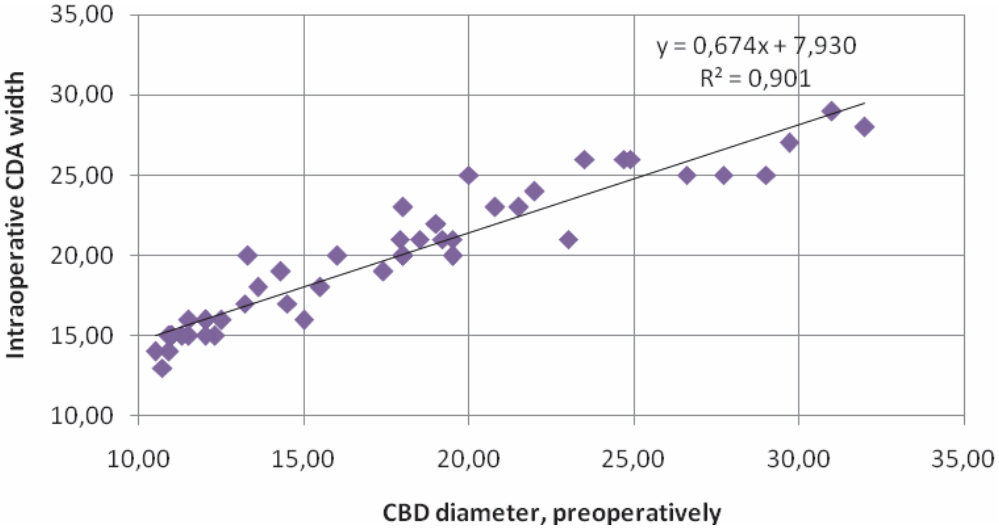

Fig. 1 – Association of hepaticocholedochus (HCH) diameter with intraoperative choledochoduodenal anastomosis (CDA) width.
CBD – common bile duct.

After measuring the biochemical parameters of cholestasis on postoperative days 1, 3, 5 and 7 and comparing them values, it was demonstrated that their regression occurred on the first postoperative day, immediately after the creation of anastomosis and continued to decrease on day 3 and on day 7. Further surveillance of bilirubin, ALT, AST, ALP and LDH revealed that, up to the termination of early postoperative period (the first 30 days), their values were significantly lower than preoperative ones ($p < 0.001$) in most of the cases (94%).

On postoperative day 7, the average HCH diameter decreased to 14.59 ± 4.13 mm (min. 10, max. 25 mm), while on day 30 it was 7.73 ± 1.40 mm, (min. 6, max. 11 mm). HCH diameter statistically diminished during the 30 days compared to the preoperative values ($p < 0.001$) (Figure 2).

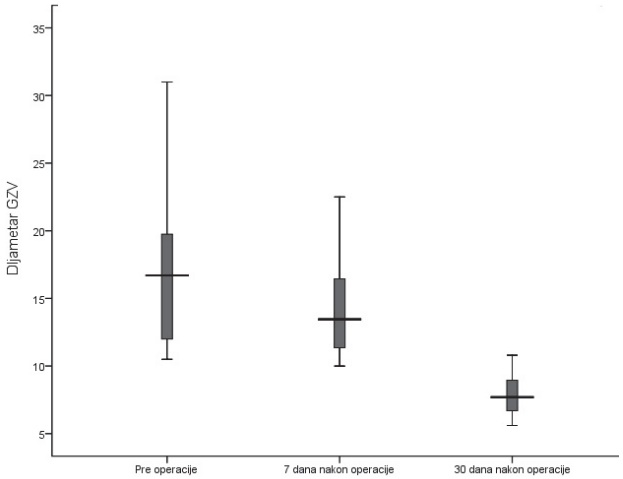

Fig. 2 – Common bile duct (CBD) diameter before surgery, 7 and 30 days after surgery (S).

Aerobilia was present in most of the examined patients (91.7%) 7 days after surgery. The number of patients with aerobilia significantly fell up to postoperative day 30 - 8 (16.7%) patients; ($p < 0.001$).

In 19 patients HIDA scintigraphy was done during the first 30 postoperative days. In 9 of them, abnormal liver morphology was encountered, but the values of semielimination and retention of the radiopharmaceutical were within the physiological ranges (30 minutes and 50%, respectively). In addition to morphological abnormalities of the biliary tree present in almost all examined patients, the mean values of semielimination and retention of the radiopharmaceutical were elevated compared to control values (30 minutes and 50%, respectively) in 15 patients. In all patients, biliary-to-bowel transit (BCT) was visualized within one hour, which indicated the competence of the created CDA and fast transit into the duodenum. In all patients DGR was detected, but only 3 had symptoms of alcaline gastritis.

The overall hospital mortality was 2% and overall morbidity was 28%. Indication for CDA and cause of death were icterus and HCHL and acute myocardial infarction on postoperative day 3 in the first patient and icterus and postcholecystectomy syndrome and cholagitis and hepatorenal syndrome on postoperative day 9 in the second patient, respectively. The total time of postoperative hospital stay for all the enrolled patients was 10.22 ± 44.60 days.

Specific early complications (related to CDA) were present in 12% of patients (Table 1). Non-specific complication were present in 16% of patients (2 wound infection and 6 with cardiovascular, pulmonary and renal complications).

Table 1

Specific early choledochoduodenal anastomosis (CDA)-related complications in operated patients

Complications	Choledocholithiasis	Stenosis	Fibrous pancreatitis	Total
	n (%)	n (%)	n (%)	n (%)
Fistula	1 (3.57)	0 (-)	0 (0)	1 (2.00)
Cholangitis	0 (-)	1 (7.14)	0 (0)	1 (2.00)
Nausea/vomiting	1 (3.57)	1 (7.14)	1 (12.50)	3 (6.00)
Intraabdominal bleeding	0	0 (-)	1 (12.50)	1 (2.00)
Total	2 (7.14)	2 (14.28)	2 (25.00)	6 (12.00)

n (%) – number (%) of operated patients.

Most patients with complications in our study (11/50) had grade I and II complications (minor complications which did not require any serious therapy, without the need for endoscopic, surgical or radiological interventions). One patient (1/50) was classified as grade IIIb (intraabdominal bleeding due to portal hypertension, requiring surgical reintervention). Two deceased patients (2/50) belonged to grade V by this classification (69-years-old patient with chronic coronary disease and 84-years-old patient with prolonged icterus and hepatorenal syndrome).

Discussion

Benign biliary obstructive diseases constitute the group of the most common biliary diseases after gall bladder calculosis. CDA still has its place in the management of certain forms of BBO as a simple, fast, effective and safe alternative, in spite of the development of endoscopic procedures and the trends of minimally invasive approaches to this pathology. Indications for CDA remained almost the same as they were in 1974, when they were established for the first time by Degenshein (except for the malignant ones) [9] and can be termed all together as distal biliary obstructions, comprising the most common bile duct lithiasis, stenosing papillitis and CP. In our series there were 58% of HCHL, 28% of papillary stenosis and 14% of CP.

By surveilling the intraoperative parameters, we were able to confirm that it was a fast to perform and technically relatively simple procedure, taking into account the complexity of the procedure itself and a patient's characteristics (aged mostly 60 to 70 years, usually with comorbid conditions, icterus and coagulation abnormalities). The complete

surgery lasted 89.30 ± 17.53 minutes, creation of an anastomosis took on the average 14.98 ± 2.54 minutes, while the average blood loss during the operation was 126.28 ± 44.86 mL.

In 92% of cases it was a L-L anastomosis, although some authors [11] recommended a T-L type, especially for younger patients, in order to avoid the "sump" syndrome and cholangitis. However, the creation of such an anastomosis prolongs the surgery, it can compromise blood supply to the proximal end of CBD, while the "sump" syndrome is a rare complication; therefore, we believe that T-L CDA should be created only when intraoperative findings mandate it [12].

The decision about the type of biliary drainage was made based on pre- and intraoperative findings. One of the common alternatives in the management of BBO is hepaticojejunal anastomosis (HJA), which, together with endoscopy, almost completely superseded CDA during the 1980s. One of the recent studies [13], comparing these two anastomoses in 121 patients operated for BBOs, demonstrated a higher risk of postoperative cholangitis and "sump" syndrome in CDA (10.41% and 4.17% respectively), compared to a complete absence of these complications with HJA, while HJA was associated with a higher risk of dehiscence and postoperative stenosis (6.67% and 4.44%, respectively) with a complete absence of these complications with CDA. Another group of authors demonstrated with their series of 314 patients surgically treated for lithiasis of intrahepatic bile ducts using HJA or T-draining that this type of anastomosis was not free from risk of postoperative cholangitis (present in 24% in this series) due to disturbed motility of the Roux loop, which, without physiological stimulation by food and with reduced peristalsis, favours bacterial colonization and enterobiliary reflux [14, 15]. HJA is more burdensome in view of a more complicated technique involved, greater losses of blood, longer duration, and its use is reserved for younger patients with lower surgical risks, and primarily those with proximal biliary obstructions. Perhaps its most important use (most commonly as the only one solution) is in biliary tract reconstruction in iatrogenic lesions of the extrahepatic bile ducts [16–18].

Early postoperative complications after CDA creation occur in 9.8% to 28% of patients [16, 19, 20]. Specific complications were present in 12.0% of the patients in our study.

Careful abiding by the surgical technique, valid for all biliodigestive anastomoses, is the sole most important factor of prevention of postoperative complications. CDA is defined as a reflux anastomosis and its characteristic complications can be minimized by the creation of a sufficiently wide, tension-free anastomosis on a dilated, well vascularized bile duct. Some authors suggested that a CBD diameter had to measure >16 mm [5] or > 15 mm [6], but De Almeida et al. [18] thought that a dilation exceeding normal values (i.e. over 10 mm) is sufficient for a wide CDA to be created. According to the literature data, the percentage of cholangitis after a CDA creation ranges from 0% [21], 3% [6], 4.2% [5], to 7% [22]. Almost all of the authors have described cholangitides as the consequence of inappropriate indications and inadequate techniques. During our study, as a CDA complication, cholangitis occurred in the early postoperative period in 1 patient who was conservatively treated until the disease was completely cured, without any recurrent cholangitis episodes in the first 30 days. In the literature, different results were presented regarding cholangitis as a long-term complication of CDA [12, 23]. The most serious complication of this anastomosis is certainly cholangiocarcinoma, which may appear after many years of chronic, recurrent cholagitis. Tocchi et al. [23] found that all biliodigestive dainage operations (HDA, HJA, transduodenal sphincterotomy) were also the risk factors leading to carcinoma. Recurrent cholangitis was identified as the sole independent factor of influence on the incidence of cholangiocarcinoma. Therefore, in order to prevent malignant alteration, regular follow-up of patients with reflux complaints is necessary [utilizing endoscopic retrograde cholangiopancreatography (ERCP) biopsies].

In the early postoperative period, we did not detect any case of "sump" syndrome in the studied patients. In addition to enteral reflux, stenosis of the papilla of Vater, wide distal choledochus, residual calculosis, an insufficently wide anastomosis is also one of the major factors in the onset of the syndrome (adequate draining and irrigation of the blind segment of choledochus are thus hampered). Nevertheless, the syndrome remains a rare complication occurring after CDA in 0%–9.6% of cases [5, 7, 10, 22, 23]. It is generally recommended that in patients with confirmed residual calculosis or stenosis of the papilla of Vater, after the creation of a wide anastomosis, endoscopic papillotomy or calculus extraction should be performed in order to prevent this complication [1].

Aerobilia or the presence of gas in bile ducts is an ultrasound finding that should be expected immediately after the creation of anastomosis, and it differs from the gas present as the consequence of anaerobic infection of the hepatobiliary system (diffusely present throughout the liver parenchyma or within an abscess collection) or the presence of gas in the portal venous system as the consequence of intestinal ischemia or inflammation (predominantly peripheral gas distribution). Aerobilia occurring after the creation of CDA is situated in central portions of the liver, towards the *porta hepatis*, as the result of "hepatofugal" bile flow, which via constant excretion prevents gas and other reflux, enteral contents, to pass into more peripheral parts of the liver. In later postoperative course, it represents an indirect proof of duodenobiliary reflux. In our study, immediately after surgery, aerobilia was present in most patients of the prospective group (91.70%), but up to postoperative day 30 it was confirmed in only 8 (16.70%) patients. Except for the 2 patients of the prospective group with complications (cholangitis and biliary fistula), in whom, in addition to the air CBD dilation was detected, in 6 patients aerobilia was also detected by ultrasonography (US), but with the CBD diameter normalization and without other (clinical and laboratory) signs of reflux. Detected in competent CDAs, with normalized bila duct diameter, it shows that biliary reflux does not always have pathological consequences and can also be a temporary phenomenon. After the CDA creation and switching off the sphincter of Oddi, reflux of duodenal contents is a normal phenomenon, transitory and mostly without any consequences thanks to continued production and excretion of

bile, which, due to a difference in pressures between the choledochus and duodenum, constantly irrigates the bile ducts through a sufficiently wide anastomosis [24].

CDA is associated with a low incidence of dehiscence (from 0% to 3.5%) [20–22, 25]. In one patient in our study, a minimal anastomotic dehiscence with the appearance of a controlled biliary fistula was reported, which was conservatively treated and controlled till it resolved (without any reintervention). It was considered that an error in the surgical technique caused the anastomotic dehiscence (insufficient mobilization of the duodenum, inadequate suture placement, absence of adaptation of bile duct epithelium to duodenal mucosa) [18, 26].

During the early postoperative period, nausea and vomiting of biliary contents were observed in 3 patients in our study after CDA creation. All of them were included in scintigraphic examination and gastroduodenoscopy with biopsy (an acute non-specific inflammation and edema of the gastric mucosa were found). The patients were treated with the appropriate therapies after which their complaints subsided. DGR is a sporadic, physiological phenomenon occurring after meals or during sleep and its consequences have not been elucidated nor studied sufficiently. It is thought that it results from a disturbed antro-pyloro-duodenal antireflux barrier and an inadequate, non-physiological biliary drainage which occur in biliary tract surgery. This pathological reflux was most frequently attributed to sphincterotomy and CDA. However, numerous studies [23, 27 29] have not been able to demonstrate that any of the operations carries a higher risk of the onset of reflux and its consequnces, nor the similar for cholecystectomy, where DGR is also present. Moreover, its presence is not a direct evidence of altered gastric mucosa. Kuran et al. [24] found that there was a significant association between the patient age and onset of reflux, with reflux occurring mostly in the elderly.

By monitoring the dynamics of regression of cholestasis and reduction of the US bile duct diameters after surgery as well as by morpho-functional studies using HIDA scintigraphy, it was established that this type of anastomosis, in addition to its efficacy, was characterized by a rapid resolution of the cholestatic syndrome. CDA is recommended, above all, for patients with evident icterus, altered general status, with abnormal coagulation factors and hepatic and renal function, not only as the final solution for their cholestasis, but also as a lifesaving procedure.

Conclusion

Benign obstructive diseases of the bile ducts are the most common biliary diseases after gallbladder calculosis. Despite the development of endoscopic procedures and trends of minimally invasive approaches to pathology, CDA is still important in the management of certain forms of BBOs. Based on our results (technical fesibility, low incidence of complications, fast postoperative recovery) CDA was show as safe and effective metod in the menagement of certain type of biliary obstruction. It is recommended not only as a definitive, but also as a rapid solution for cholestasis which qualifies the procedure as a "lifesaving" one. Serious complications occur in small percentages and can be avoided by proper selection of patients and meticulous surgical technique each general surgeon should have the thorough knowledge of.

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Complete histopathological regression in rectal cancer after neoadjuvant chemoradiotherapy and sphincter preserving surgical treatment

Kompletna patohistološka regresija karcinoma rektuma nakon neoadjuvantne radiohemioterapije i sfinkter prezervirajućih operacija

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Abstract

Background/Aim. Multimodal approach to locally advanced rectal cancer treatment results in better disease outcome. Preoperative chemoradiotherapy improves disease local control, reduces risk of local recurrence and in the majority of patients with complete or substantial regression of the tumors significantly improves survival rates. According to the literature data, approximately 20% of patients had achieved complete histopathological response (pCR) after neoadjuvant chemoradiation therapy. The aim of this study was to evaluate overall survival in rectal cancer patients treated with preoperative chemoradiotherapy and sphincter preserving surgery. **Methods.** This retrospective study included 191 patients. Patients received preoperative radiation therapy and chemotherapy-chemoradiation therapy (CRT) followed by operation that favorized sphincter preservation with total mesorectal excision (TME) from June 2000 until December 2010. Diagnosis was established according to the following algorithm: patient history, digital rectal examiantion, colonoscopy with biopsy and histopathology verification, and preoperative clinical staging. Patients with tumors located below promontorium were included in the study and patients with metastatic disease and local recurrence were excluded from the study. For tumors located below the promontorium preoperative radiotherapy was used with total dose of 50.4 Gy, divided into daily doses of 1.8 Gy, during 28 days. Chemotherapy followed radiotherapy with 5-fluorouracil and folic acid (Leucovorin®) on days 1, 2, 10, 11, 20 and 21. Six to ten weeks after neoadjuvant therapy, magnetic resonance imaging (MRI) to restage tumors and operation were performed. **Results.** Of all patients that received preopertive chemoradiation, 163 had radical sphincter preservig surgery and 28 patients had paliative operations. Histopathological examination of the specimens showed that the complete histopathological regression was achieved in 21.4% of the patients, downstaged was found in 63.2% of them and unchanged stage was found in 15.3% of the patients. The five-year survival rate was 63.3% and 50.5 % in the patients with pCR and patients with incomplete histopathological regression, respectively. Survival rates between two groups were not statistically significant ($p > 0.05$). **Conclusion.** The preoperative chemoradiotherapy is very important in achieving optimal clinical care for patients with locally advanced rectal cancer.

Key words:
rectal neoplasms; carcinoma; digestive surgical procedures; chemoradiotherapy; neoadjuvant therapy; prognosis.

Apstrakt

Uvod/Cilj. Multimodalni pristup u lečenju lokalno uznapredovalog karcinoma rektuma rezultira boljom prognozom bolesti. Preoperativnom primenom kombinovane radioterapije i hemioterapije-radiohemioterapije (RHT) poboljšava se lokalna kontrola bolesti, smanjuje rizik od pojave lokalnih recidiva i vodi boljem preživljavanju kod većine bolesnika kod kojih je postignuta kompletna ili nekompletna histološka regresija tumora.

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Oko 20% bolesnika nakon neoadjuvantne terapije ima potpunu patohistološku regresiju tumora. Cilj ovog istraživanja je bio evaluacija preživljavanja bolesnika nakon neoadjuvantne terapije i sfinkter prezervirajućih operacija. **Metode.** Retrospektivnom studijom obuhvaćen je 191 bolesnik. Bolesnici su primili neoadjuvantnu radiohemioterapiju a nakon toga su operisani u periodu od juna 2000. do decembra 2010. godine. Dijagnostika je izvođena prema sledećem algoritmu: anamneza, digito-rektalni tuše, kolonoskopija sa biopsijom i patohistološkom verifikacijom. U studiju su uključeni samo bolesnici sa tumorima lokalizovanim ispod promontorijuma. Iz studije su isključeni bolesnici sa udaljenim metastazama i sa reicidivima. Ukupna doza zračenja od 50.4 Gy je bila podeljena u dnevne doze od 1.8 Gy. Hemioterapija [5-fluorouracil i folna kiselina (Leucovorin®)] je pratila radioterapiju 1. 2. 10. 11. 20. i 21. dana. Šest do osam nedelja nakon ove terapije urađen je pregled male karlice magnetnom rezonancom (MR) radi novog stejdžinga. Bolesnici su operisani u periodu od 8 do 12 nedelja po završetku hemioiradijacije. **Rezultati.** Od svih bolesnika koji su primili neoadjuvantnu terapiju, kod 163 je urađena sfinkter prezervirajuća operacija sa totalnom mezorektalnom ekscizijom. Kod 28 bolesnika izvedena je palijativna operacija. Patohistološkim nalazom je utvrđeno da 21,4% bolesnika ima kompletnu histološku regresiju tumora, 63,2% nekompletnu, dok je 15,3% bolesnika bilo nepromenjenog statusa. Petogodišnje preživljavanje kod bolesnika sa kompletnom histološkom regresijom bilo je 63,3%, kod onih sa nekompletnom histološkom regresijom 50,5%. Razlika u preživljavanju između ove dve grupe bolesnika nije bila statistički značajna ($p > 0,05$). **Zaključak.** Preoperativna radiohemioterapija obezbeđuje optimalno kliničko zbrinjavanje bolesnika sa lokalno uznapredovalim karcinomom rektuma.

Ključne reči:
rektum, neoplazme; karcinomi; hirurgija digestivnog sistema, procedure; radiohemioterapija; lečenje, neoadjuvantno; prognoza.

Introduction

The managment of rectal cancer has changed over the last twenty years, due to improvement of surgical techinques and introduction of neoadjuvant radiation therapy and chemotherapy-chemoradiation therapy (CRT).

Multimodal approach to locally advanced rectal cancer treatment results in better disease outcome. Preoperative CRT improves local disease control and reduces risk of local recurrence. Some patients respond to neoadjuvant CRT with pathological complete response (pCR) in approximately 20% of casses [1]. Multimodal treatment for rectal cancer includes: introducing effective surgery (total mesorectal excision), neoadjuvant radiotherapy, and modern cytotoxic chemotherapy [2].

Preoperative chemotherapy and/or radiotherapy followed by surgery currently represents the standard approach for locally advanced rectal cancer, providing survival benefit for the patients compared to surgery alone. The most of the patients with complete or substantial regression of the tumors showed improved survival rates [3–8].

After preoperative CRT significant tumor downstaging and downsizing, greater rates of sphincter preservation surgery and better functional results have been reported [9].

Tumor response to neoadjuvant CRT is not consistent. It is connected with many factors such as specific treatment regimens, timing of surgery after CRT completion, tumor/patient characteristics and tumor biology [10].

Two most frequent regimens used in the preoperative treatment of patients with resectable clinical T3-T4 rectal cancers are fractionated long-course CRT followed by surgery after 6–8 weeks or pelvic short-course irradiation with 25 Gy in five fractions followed by immediate surgery [10].

The study of Maas et al. [11] showed that patients who achieved the pCR after neoadjuvant CRT had outcomes similar to those who underwent surgery. This fact was used for introduction of a concept based on "wait and see policy" instead of surgical treatment.

The aim of this study was to evaluate overall survival in patients after preoperative CRT and sphincter preserving surgical treatment.

Methods

This was a retrospective study which included 191 patients: 134 males and 57 females, (mean age 65.10 years; range 32–81 years). Patients received preoperative chemoradiation followed by operation that favorized sphincter preservation with a total mesorectal excision from June 2000 until December 2010. Diagnosis was established according to the following algorithm: patient history, digital rectal examiantion, colonoscopy with biopsy and histopathology verification, and nuclear magnetic resonance (NMR) of pelvis. Only the patients with tumors below the promontorium were included in evaluation, patients with metastatic disease and local recurrence were exluded from the study.

The radiation therapy regimen: total dose of 50.4 Gy was divided into daily doses of 1.8 Gy, during 28 days. Chemotherapy followed radiotherapy with 5-fluorouracil and folic acid (Leucovorin®) on days 1, 2, 10, 11, 20 and 21. Six to ten weeks after neoadjuvant therapy, magnetic resonance imaging (MRI) was performed in order to restage the tumor. Prior to surgery, complete blood count and biochemical analysis were performed as well as liver ultrasonograpahy.

Histopathological analysis of surgical specimen was used to determine the patients with pCR and incomplete pathological regression. The patients with metastatic disease and local recurrence were excluded from the study. The resected specimens were fixed in 4% formaldehyde overnight. After a specimen had been opened, the tumourous or fibrotic area was identified and described macroscopically. For an obvious residual primary tumor, a minimum of four paraffin blocks were processed. If no tumor was visible, the whole area suggestive of disease was sliced and embedded. Hematoxylin-eosin-stained sections were reviewed, and proximal, distal, and circumferential resection

margins were evaluated. All lymph node that was found was sampled and microscopically examined.

Histopathological assessment was performed according to the Rectal Cancer Regression Grade (RCRG), which classifies tumor regression into three levels: RCRG 1 – the tumour is either sterilised or only microscopic foci of adenocarcinoma remain; RCRG 2 – marked fibrosis with macroscopic tumour still present; RCRG 3 – little or no fibrosis in the presence of abundant macroscopic tumour. RCRG 1 and 2 were considered to represent significant tumour regression [12]. In cases where only acellular pools of residual mucin were noted, the response was considered to be complete.

For statistical analysis and survival rates both the patients with complete and incomplete histopathological regression were included. Software SPSS v17 was used for statistical analysis.

Results

Among 191 patients that had were examined, male predominated, and male:female ratio was 1.42 : 1. All patients received preoperative CRT, and 163 of them underwent sphincter preserving operations with total mesorectal exscision (TME). In 28 patients only palliative operation was possible (Table 1).

Table 1

Clinical characteristics of patients

Patient characteristics	Value
Mean age range (years)	65.1 (32–81)
Gender M/F, ratio	134/57 (1.42:1)
Type of surgery, n (%)	
sphincter preserving	163(85.4)
paliative	28 (14.6)
Preoperative CRT, n (%)	191(100)
HP finding, n (%)	
complete HP regression	35 (21.4)
incomplete HP regression	103 (63.2)
without change	25 (15.3)
Clinical T stage, n (%)	
cT3	155 (81)
cT4	36 (19)
Clinical N stage, n (%)	
cN(-)	123 (64.2)
cN(+)	68 (35.8)
Histology, n (%)	
well/moderately diferentiated	122 (63.8)
poorly diferentiated	69 (36.2)
Lymphovascular invasion, n (%)	43 (22.4)

M/F – male/female; CRT – chemoradiation therapy; HP – histopathological.

Figure 1 shows preoperative chemoradiated patients and types of operations (radical or palliative) practiced in our department over the years in the patients with locally advanced rectal cancers.

Histopathological examination of the operative specimen showed that 35 out of 163 (21.4%) patients treated with neoadjuvant treatment and sphincter preserving surgery with TME, achieved pCR (Figure 2); 103 (63.2%) were downstaged and only in 25 (15.3%) patients no change in tumor stage could be detected (Figure 3). The patients with pCR were younger compared to the patients with incomplete tumor regression (Spearman`s rho test; $p = 0.03$). There were no correlation between pCR or incomplete tumor regression with other clinical parameters such as gender, histology, lymphovascular invasion, initial (before preop-CRT) clinical T and N stages (Spearman`s rho test, $p > 0.05$).

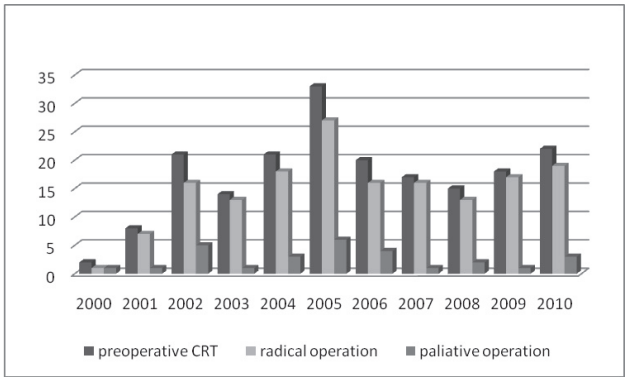
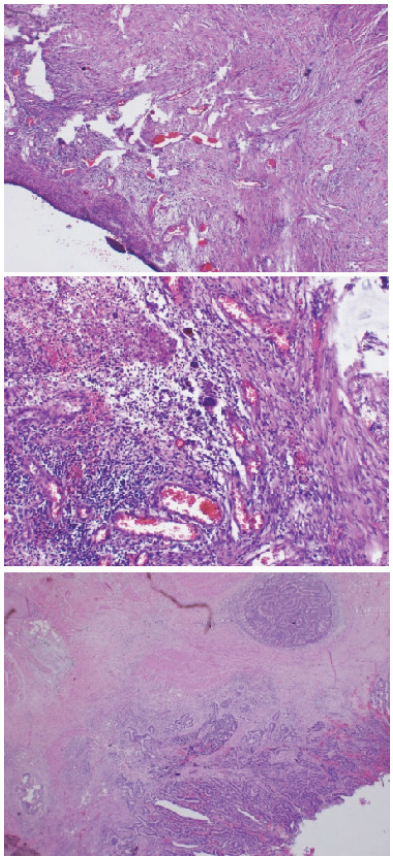


Fig. 1 – Preoperative chemoradiated patients and types of operations (radical or palliative) practiced in our department by year in the patients with locally advanced rectal cancers.



A)

B)

C)

Fig. 2 – Therapy response grade: A) complete therapy response with marked fibrosis without cancer tissue; B) near complete therapy response with few tumour cells outgrown by fibrosis and inflammation; C) poor therapy response showed residual cancer outgrowing fibrosis characterized by a scant presence of regressive changes (H&E, ×40).

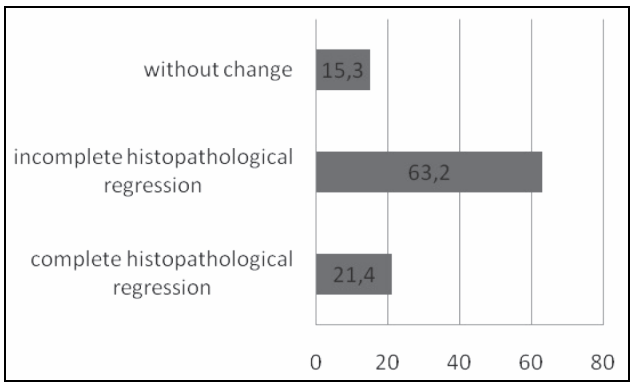


Fig. 3 – Histopathological examination of the operative specimen.

The five-year survival of patients with pCR was 63.3% while survival rate of the patients with incomplete histopathological regression was 50.5% (Figure 4). There were no statistical significance between survival rates of the pCR patients and patients with incomplete histopathological regression ($p > 0.05$).

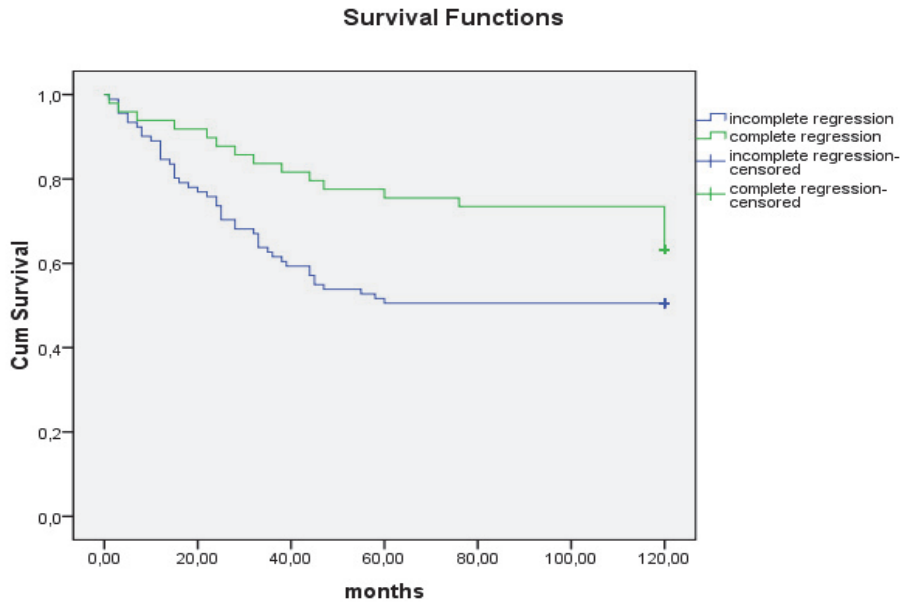


Fig. 4 – Patient survival in relation to histopathological tumor regression ($p > 0.05$).

Discussion

Modern concepts of treatment for locally advanced rectal cancer include preoperative chemoradiation followed by surgery. A large number of studies have demonstrated that preopearative chemoradiation for locally advanced rectal cancer can lead to tumor regression [13]. It is also shown that such treatment could reduce local recurrence rates and increase number of sphincter preserving operations, and, also increase patients' survival and thier quality of life [8, 14].

Complete pathological response appears in 10%–30% of patients who were treated with chemoradiotherapy.

The patients with pCR have better survival compared to patients with incomplete histopathological regression [9, 15].

In our study, among 163 patients who underwent sphincter preserving operations after preoperative chemoradiotherapy, pCR was found in 21.4% of the patients while incomplete histopathological regression in 63.2% of them. Cases with complete regression or RCRG 1 showed an absence of histologically identifiable residual cancer with predominant fibrosis extending through the different layers of the rectal wall or there were only microscopic foci of small cluster or individual tumor cells. Cases with RCRG2 or partial regression were characterized by presence of residual cancer tissue which was still outgrown by fibrosis. In grade 3 (poor regression) there were huge areas of residual cancer outgrowing fibrosis characterized by a scant presence or the complete absence of regressive changes.

Shivnani et al. [13] reported similar results. Their study included 100 patients, 25% with pCR and 60% without pCR. Our results are similar to data presented by Onaitis et al. [14] who analyzed 146 patients with locally advanced rectal cancer after preoperative chemoradiotherapy and surgery. They found 20% of patients with pCR and 57 % without pCR. In the study of Garcia-Aguilar et al. [15], among 168 patients who received preoperative chemoradiation therapy, only 13% were with pCR and 55.4% of them with incomplete histopathological regression.

Our study showed that five-year survival rate (63.3%) was better in patients with pCR compared to those with in-

complete histopathological regression (50.5%). However, the survival rate of our patients, regadless the HP outcome, were smaller compared to the reported data [14, 16, 17]. Stipa et al. [17] showed that five-year survival in patients with complete and incomplete histopathological regression was 90% and 68%, respectively. Similar results reported Garcia-Aguilar et al. [15], i.e., five-year survival in patients with pCR was 95.2%, while in patients with incomplete histopathological regression it was 55.4%. Shivnani et al. [15] showed that five-year survival in patients with pCR was 89% and 75% of the ones with incomplete regression.

Although tumor regression grade basically scores the ratio of residual cancer cell to radiation-induced fibrosis, a standard method for scoring tumor regression grade still does not exist. This is important because documentation of tumor regression grade (TRG) can be different depending on the methods used to prepare slides, the number of slides reviewed per tumor, staining and the experience of the reviewers. There are several grading systems for TRG. Some of them such as Mandard et al. [18] Dworak et al. [19], and Ruo et al. [20] proposed fivepoint grading systems while Rodel et al. [9] and Wheeler et al. [12] advocated three-point grading systems. Comparative studies showed that although prognostic impact might be the same, the three-point TRG was better with respect to intra- and inter-observer agreement [21, 22]. The three-point grade has the advantage of better reproducibility, with similar prognostic significance. Thus, we used the three-point grading system according to RCRG [12]. TRG was uniformly found by univariate analysis to get a prognostic value for survival and recurrence in rectal cancer after irradiation [14, 18]. Most series, however, failed to establish TRG as an independent prognostic factor stronger than ypT or ypN [23, 24].

New studies with “wait and see policy” were initiated. This concept is based on omitting surgery in patients with pCR after chemoradiation therapy. After a mean follow-up of 60 months, the results for the ”wait and see group” were impressive, with a five-year survival of 93% [25]. However complete response of the primary tumor cannot predict response in regional lymph nodes which were involved in 7%–17% of patients who have pCR of the primary tumor [26, 27]. So this concept has to be proved in randomized trials.

Conclusion

Complete histopathological response is now accepted as an independent predictor of long-term outcomes following neoadjuvant chemoradiotherapy for locally advanced rectal cancer. Further work is needed to determine the clinical importance of lesser degree of pathological regression. A robust and internationally accepted system for the grading of tumour regression in rectal cancer following neoadjuvant chemoradiotherapy is currently required. Such consistency will help with clinical decision-making and will influence surgical strategies, postoperative adjuvant therapy and surveillance intensity.

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SHORT
COMMUNICATIONS

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Severe clinical forms of Mediterranean spotted fever: a case series from an endemic area in Bulgaria

Teške kliničke forme mediteranske pegave groznice: serija slučajeva iz endemskog područja Bugarske

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Abstract

Background/Aim. Mediterranean spotted fever (MSF) belongs to Rickettsioses, the Spotted fever group (SFG). The causal agent is *Rickettsia conorii conorii* and the transmission to humans occurs through dog tick *Rhipicephalus sanguineus* bites. The aim of this study was to describe clinical and laboratory characteristics in patients with severe form of Mediterranean spotted fever admitted to Bulgarian university hospital in endemic region. **Methods.** A retrospective study was conducted at Stara Zagora University Hospital (Southeastern Bulgaria) between April 2015 and August 2016. During the analyzed period, 58 cases had clinical and laboratory data for MSF. Serological tests were applied for the etiological diagnosis. MSF-specific immunoglobulin (IgM) and IgG antibodies were detected in serum by indirect immunoenzyme assay (ELISA IgG/IgM, Vircell, Spain) – *R. conorii* ELISA IgG sensitivity 85%, specificity 100% and *R. conorii* ELISA IgM sensitivity 94%, specificity 95%. Statistical analysis was made by MS Excel 2007 and SPSS Statistics, version 19.0. **Results.** Eighteen patients presented as severe forms. The predominant gender of them were males (78%) and 22% were females. The median age of the analyzed group was 55 years (range: 14–78 years). Ten patients developed hepatic disorder while 4 had neurological signs. Laboratory data showed thrombocytopenia in 15 patients, mean value of platelet (PLT) count for the whole group was 108.6 ± 53.8 × 10^9/L. Liver enzymes were elevated with mean value of aspartate aminotransferase (AST) 161.4 ± 90.1 IU/L and alanine aminotransferase (ALT) 163.9 ± 81.5 IU/L. Acute phase reactant as C-reactive protein (CRP) had mean value of 140.3 mg/L (range: 9–230 mg/L). Kidney function was impaired in some cases; the mean value of creatinine for the studied group was 134.7 μmol/L (range: 78–313 μmol/L) and mean value of urea was 9.6 mmol/L (range: 4.2–27.4 mmol/L). **Conclusion.** Bulgaria is an endemic area for tick-borne diseases. Cases of MSF are reported annually. Severe forms of MSF are not rare. Typical clinical and laboratory markers for severity should be actively searched for. Early diagnosis and proper treatment is the key to avoid complications and enable patient recovery.

Key words:
rickettsia; boutonneuse fever; ticks; bulgaria; serology; blood chemical analyses; diagnosis.

Apstrakt

Uvod/Cilj. Mediteranska pegava groznica (MPG) pripada rikeciozama (grupi pegave groznice) – izaziva je *Rickettsia (R.) conorii conorii*, a prenosi se na ljude ujedom krpelja pasa *Rhipicephalus sanguineus*. Cilj ove studije bio je da se opišu kliničke i laboratorijske karakteristike bolesnika sa teškom formom MPG koji su bili hospitalizovani u univerzitetskoj bolnici u endemskom području Bugarske. **Metode.** Retrospektivna studija sprovedena je u Univerzitetskoj bolnici Stara Zagora (južnoistočna Bugarska) u periodu od aprila 2015. godine do avgusta 2016. godine. Tokom analiziranog perioda 58 bolesnika imalo je kliničke i laboratorijske znake MPG. Za potvrdu etiološke dijagnoze primenjeni su serološki testovi. Za MPG specifična imunoglobulinska (IG) antitela za IgM i IgG pronađena su u serumu imunoenzimskim indirektnim esejom (ELISA IgG/IgM, Vircell, Španija). *R. conorii* ELISA IgG senzitivnost bila je 85%, specifičnost 100%, a *R. conorii* ELISA IgM senzitivnost bila je 94%, specifičnost 95%. Statistička analiza urađena je u programu MS Excel 2007 i SPSS verzija 19.0. **Rezultati.** Osamnaest bolesnika imalo je težak oblik MPG. Muškarci (78%) su bili brojniji u odnosu na

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žene (22%). Srednja vrednost starosti kod 18 analiziranih bolesnika bila je 55 godina (14–78 godina). Kod 10 bolesnika pojavili su se poremećaji funkcije jetre, a kod četiri, neurološki simptomi. U laboratorijskim analizama trombocitipenija se javila kod 15 bolesnika, a srednja vrednost trombocita u celoj grupi iznosila je 108,6 ± 53,8 × 10 9/L. Jetreni enzimi bili su povišeni, a srednja vrednost aspartat aminotransferaze (AST) iznosila je 161,4 ± 90,1 IU/L, a alanin aminotransferaze (ALT) 163,9 ± 81,5 IU/L. Srednja vrednost reaktanata akutne faze kao sto je C-reaktivni protein (CRP) iznosila je 140,3 mg/L (9–230 mg/L). Kod nekih bolenika javio se poremećaj bubrežne funkcije. Srednja vrednost kreatinina u posmatranoj grupi iznosila je 134,7 μmol/L (78–313 μmol/L), a ureje iznosila je 9,6 mmol/L (4,2–27,4). **Zaključak.** Bugarska je endemsko područje za bolesti nastale zbog ujeda krpelja. Slučajevi MPG se analiziraju na godišnjem nivou. Teški oblici MPG nisu retki. Tipične kliničke i laboratorijske markere za procenu težine bolesti treba aktivno pratiti. Rana dijagnoza i odgovarajuće lečenje ključni su za izbegavanje komplikacija i oporavak bolesnika.

Ključne reči:
rickettsia; groznica, boutonneuse; krpelji; bugarska; serologija; krv, hemijske analize; dijagnoza.

Introduction

Mediterranean spotted fever (MSF) belongs to Rickettsioses, the Spotted fever group (SFG). The agent is *Rickettsia conorii conorii* and transmission to humans occurs through dog tick *Rhipicephalus sanguineus* bites. The disease is also called "Boutonneuse fever", "Marseilles fever", etc. and depends on the geographic region of its appearance [1]. Historically, the illness was described for the first time in Tunisia in 1909 by Conor and Bruch [2]. In 1923, the typical cutaneous lesion at the place of tick bite (tache noire) was drawn by Pieri in Marseilles [3]. The disease is endemic for Mediterranean region and northern Africa, although cases are reported in the central and eastern Europe, India, and southern Africa [1, 4]. Most of the Balkan countries (Bulgaria, Croatia, Greece, Romania, Serbia, Turkey) have reported the infection or serological data for the circulation [4–10]. In Bulgaria, the infection was identified for the first time in 1948 by Vapzarov [11]. For the period from 1948 to 1959 human cases are reported annually [11]. After 1960, the incidence of cases dramatically decline because of the good veterinarian control of homeless dogs, rabies prophylaxis and improved agricultural measures [12]. From 1993 to 2003, a new phase of re-emerging of the disease started, following a phase of declines for the period of 2004 to 2011 [11]. Since then (2011), some cases have been annually reported in the endemic area of Bulgaria [5, 13–16]. These territories are located around the Maritsa riverside settlements and Black Sea. Few Bulgarian researchers reported their results for MSF.

The aim of this study was to describe clinical, epidemiological and laboratory characteristics in patients with severe forms of MSF who were admitted to Bulgarian University Hospital in the endemic region.

Methods

A retrospective study was conducted between April 2015 and August 2016 at the Department of Infectious Diseases, Stara Zagora University Hospital (Southeastern Bulgaria). Patients over 14 years with clinical, epidemiological and laboratory data for MSF were enrolled. Patients' records, historical and physical features, laboratory parameters, imaging investigations [radiography, abdominal ultrasound, computed tomography (CT)] and invasive procedure (lumbar puncture) were collected and analyzed. Diagnostic criteria by Raoult et al. [17], were applied and patients with diagnostic score more than 25 were analyzed further.

For severity, a complex criteria were enclosed. Severe forms are defined as presence of at least two laboratory criteria and at least one clinical criterion. Clinical symptoms were fever above 39ºC, headache, nausea/vomiting, muscle/joint pain, abundant hemorrhagic rash (acute onset of intense skin rash with hemorrhagic characteristics with reddish to bluish-purple spots), clinical manifestation of one organ damage, elderly patients. Laboratory parameters were: white blood cells more than 12×10^9/L or less than 5×10^9/L; platelet count < 120×10^9/L; hemoglobin level bellow 110g/L; elevation of liver enzymes; creatinine and urea above the normal range.

The patients whose clinical and laboratory features fulfilled the criteria for severe forms continue the evaluation. Their symptoms, clinical findings, epidemiological data and laboratory features were analyzed.

Etiological diagnosis was made by serology tests. MSF-specific IgM and IgG antibodies were detected in serum by indirect immunoenzyme assay (ELISA IgG/IgM, Vircell, Spain) – *Rickettsia conorii* ELISA IgG sensitivity 85%, specificity 100% and *Rickettsia conorii* ELISA IgM sensitivity 94%, specificity 95%. Serum samples were collected at the day of admission and fourteen days later. The presence of antibodies of class IgM and following appearance of antibodies of class IgG were accepted for serological confirmation of clinical, epidemiological and laboratory data.

The medical procedures of this study were approved by the Local Ethics Committee of Stara Zagora University Hospital, Bulgaria (2 Stoletov Str., 6000 Stara Zagora)

Statistical analysis was made by MS Excel 2007 and SPSS Statistics, version 19.0. Average values (mean ± SD) and a range were calculated for the laboratory parameters.

Results

For the period of one and a half year, 58 pearsons with MSF were hospitalized at Stara Zagora University Hospital (Southeastern Bulgaria, endemic area for MSF). Thirty-one percentages fulfilled the criteria of severe course and were

further analyzed. Males (77.8%) prevailed. The mean age was 54.9 ± 16.4 years. Underlying diseases were marked in 7 patients – all had hypertension except one who had diabetes mellitus type 2. The median of hospital stay was 7.5 days. Sixteen patients developed the illness during the typical season for the occurrence (May-October). All analyzed cases were residents from the endemic area of *R. conorii conorii*. Contacts with dogs (direct or dog care) were mentioned in 61.1% of the cases, but 9 noted tick bites. The patients developed typical clinical presentation (fever, rash, flu-like symptoms) and they mentioned epidemiological risk factors, but the severity of the illness was an indispensable reason for the hospital admission (Figures 1 and 2). Main clinical signs are presented in Table 1. Mean value of fever height was 39.2ºC ± 0.5ºC and headache was mentioned in 50% of the cases. Craniopharyngeal syndrome (a presentation of red face, redness of conjunctiva, hyperemia of neck and upper thorax to clavicle) was marked in 7 cases. Gastrointestinal signs were presented in 33.3%, central neural system disorders were noted in 22.2%, pulmonary involvement was found in 16.7% and kidney failure in 27.8% of cases. In contrast to these manifestations, hepatic injury was estimated in 77.8% of the cases.

Fig. 1 – Rash in a 47-year-old man with Mediterranean spotted fever (MSF).

Fig. 2 – Rash in a 45-year-old patient infected by *Rickettsia conorii conorii.*

Laboratory findings are presented in Table 2. The median value of hemoglobin was 130.5g/L (range: 99 g/L–165 g/L), white blood cells had the median of 7.4×10^9/L (range: 1.4×10^9/L–16×10^9/L), the median value of platelet count was 106×10^9/L (range: 37×10^9/L–226×10^9/L). The mean value of fibrinogen was 3.7 ± 0.8 g/L, erythrocyte sedimentation rate (ESR) had mean value of 27.2 ± 20.8 mm/h and the mean value of CRP was 140.3 ± 69.8 mg/L. Liver enzymes were elevated in almost all cases with the median value of AST 154.4 U/L (range: 36 U/L–356.7 U/L) and 174.0 U/L of ALT (range: 36 U/L–299 U/L). Kidney parameters demonstrated deviations: the mean values of creatinine was 134.7 ± 73.1 μmol/L and 9.6 ± 6.7 mmol/L of urea. Cerebrospinal fluid (CSF) abnormalities were estimated in 3 examined cases.

Serological tests confirmed the disease. Specific antibodies of class IgM were detected from the first serum samples and two weeks later, antibodies of class IgG were found.

A complex therapy was applied. Fluoroquinolones were administered as a first choice; it was recommended to take 400 mg of Ciprofloxacin intravenously twice a day over 7–10 days in all patients. Seven patients were treated with a combination therapy doxycycline (100 mg twice a day) and ciprofloxacin (400 mg twice a day) for 10 days. Ceftriaxone (2.0 g once a day intravenously) was added empirically to the therapy in cases with pneumonia. Supportive therapy as

infusions, hepatoprotective drugs, corticosteroids and antipyretic medications were used. All patients recovered after a complex treatment. There were no complications and consequences in the follow-up period.

Table 1

Epidemiological and clinical data of patients with severe Mediterranean spotted fever (MSF)

Patient (N)	Gender/Age	Month of admission	Tick bites	Eschar	Hemorrhagic rash	Muscle/Joint pain	Hepatomegaly/ Splenomegaly
1	M/60	August	Yes	No	No	Yes	Yes/No
2	M/59	June	No	Yes	Yes	Yes	Yes/No
3	F/45	May	Yes	No	No	No	Yes/No
4	M/64	July	Yes	Yes	Yes	Yes	Yes/Yes
5	M/32	June	No	Yes	Yes	Yes	Yes/No
6	M/51	May	No	No	No	Yes	No/No
7	M/60	August	Yes	No	No	Yes	No/Yes
8	M/14	August	No	No	No	Yes	Yes/No
9	M/78	August	Yes	No	No	Yes	No/No
10	M/51	April	Yes	Yes	No	No	Yes/No
11	F/75	July	No	No	No	Yes	No/No
12	F/70	July	No	No	No	Yes	Yes/No
13	M/47	July	No	No	No	Yes	Yes/No
14	M/77	May	No	No	Yes	Yes	Yes/Yes
15	M/47	April	Yes	Yes	Yes	Yes	Yes/Yes
16	M/45	October	No	Yes	No	Yes	Yes/No
17	M/67	May	Yes	Yes	No	Yes	Yes/No
18	F/47	July	Yes	Yes	No	Yes	Yes/No

M – male; F – female.

Table 2

Laboratory data of 18 patients with severe Mediterranean spotted fever (MSF)

Patient	WBC (3.5–10.5 $\times 10^9$/L)	PLT (150–400 $\times 10^9$/L)	PT (70–110%)	CRP (0.0–5.0 mg/L)	AST/ALT (5–40 IU/L)	Creatinine (58–110 μmol/L)	Urea (2.8–8.3 mmol/L)	Diagnosis
1	10.6	96	46	217	162/240	85	6.4	MSF & Hepatic Involvement
2	10.5	226	76	ND	103/97	99	7.6	MSF & Hepatic Involvement
3	6.7	138	69	210	253.9/220.6	78	9.3	MSF & Hepatic Involvement
4	11.7	119	98	123	140/165	141	21.36	MSF & Meningoencephalitis
5	3.6	64	97	138.5	165/231	99	5.4	MSF & Hepatic Involvement
6	5.2	168	89	43.2	50/44	91	4.5	MSF & Pneumonia
7	16	168		ND	36/51	111	7.6	MSF & Pneumonia
8	2.8	56	84.3	230	50/36	90	6	MSF
9	13.9	62	104.2	ND	167.8/89.3	105	7.9	MSF & Neurological Disorder
10	4.4	51	85.1	ND	222.5/155.3	303	13.5	MSF & Hepatic Involvement
11	8.1	135	83.3	187	147/183	313	27.4	MSF & Hepatic Involvement
12	9	82	89	135.6	98.9/86.5	211	20.1	MSF & Encephalitis
13	6.2	93	96.1	ND	193.2/209.5	103	5.2	MSF & Hepatic Involvement
14	1.4	42	113.2	9	290/247.5	105	4.3	MSF & Hepatic Involvement
15	10.8	37	124	136.4	356.7/299	203.6	8.5	MSF & Hepatic Involvement
16	6.4	116	92.4	ND	74/134	101	7.1	MSF & Pneumonia
17	6.4	184	80	ND	123.3/265	85	6.4	MSF & Hepatic Involvement
18	11.6	117	85	113.4	272/196	101	4.2	MSF & Meningoencephalitis

Note: WBC – White blood cells; PLT – Platelet count; PT – Prothrombin time; CRP – C-reactive protein; AST – aspartate transaminase; ALT – alanine transaminase; ND – no data available.

Discussion

The present study described clinical, epidemiological and laboratory data in 18 cases with MSF presented as severe clinical forms. The aim of the analysis was to exclude mild and moderate forms and to characterize the specific features in cases with severe disease course. The estimate of 31% of severe forms for the presented period was a high percentage when compared to other publications [8, 13, 18, 19]. One of the explanations is the limitation of the study. Patients with signs of complications or other disorders were directly hospitalized. Probably many cases with mild and moderate forms were treated as outpatients and they were not included in our analysis.

The mean age of 54.9 years is similar to other reports [4, 8, 20]. Males were the predominant gender in our study. Reports from France [18], Greece [20] and Romania [4] also estimated high proportion of male gender. In contrast, Kuloglu et al. [8] found slight prevalence of females. The sex ratio is influenced by local characteristics regarding agricultural work, livestock farming and housekeeping. The seasonal occurrence for MSF in our study is the typical for the rickettsioses [10, 20–22]. Most of our patients had contacts with dogs and 50% of them mentioned tick bites which were predispositions for developing a tick-borne disease. Similar epidemiological data were identified by Pitigoi et al. [4], Romdhane et al. [23] and Kuloglu et al. [24]. The clinical symptoms and signs were similar to ones presented in other studies [4, 8, 13, 15, 20, 23–26]. The leading clinical features in severe forms were high fever with severe headache, hepatomegaly/splenomegaly and the combination of older age and underlying diseases. Similar symptoms are reported by Raoult et al. [19] and Kuloglu et al. [8]. The accompanying diseases according to our data were hypertension and *diabetes mellitus* which did not differ from others studies [8, 21, 24]. Neurological disturbances and pulmonary disorders were described by other authors as leading clinical findings in severe and malignant forms [8, 20, 27, 28]. Laboratory data from the presented study established liver impairment, kidney failure, marked elevation of CRP, alteration in CSF and thrombopenia. Those laboratory findings were not distinguished by other studies in Europe [4, 8, 19, 20, 25, 27, 28]. Severe case with neurological deterioration, renal impairement, thrombocytopenia and enlarged liver enzymes was reported by Tzavella et al. [28]. Aliaga et al. [27] presented an old diabetic man with encephalitis, thrombocytopenia and acute renal failure. Our neurological cases characterized with similar symptoms and disorders. In our study, no neurological sequences were detected as in Greek [28] and Turkish reports [8]. In contrast, Spanish report described developing of severe sequelae [27]. Pulmonary infiltrates were demonstrated in 27% of a Greek study [20], one patient had pneumonia in other report [8], 43% of severe cases of pneumonitis was displayed in a French investigation [19]. In comparison, 3 of our patients developed pneumonia. Gastrointestinal signs were not rare in the presented study. Yilmaz et al. [25] observed gastrointestinal complaints in 25% and Raoult et al. [19] noted diarrhea in 14% of severe cases. Acute renal failure was mentioned as a risk factor for fatal outcome [16]. Kidney disorders at different levels were found in 5 of our patients, but with no lethal outcome. Renal dysfunction was revealed in the case of severe MSF with encephalitis. The man survived with neurological complications [27]. In our study, timely and appropriate treatment was applied. Therefore, no deaths or subsequent complications occurred in the follow-up period.

The region of Stara Zagora (Southeastern Bulgaria) is an endemic area for tick-borne diseases and close attention to rickettsioses was paid. In summer time, every suspected case with fever and rash is carefully examined and those suspected of severe forms of infectious diseases are directly guided to an infectious diseases specialist. Because of that procedure, there is a high percentage of hospitalized cases with severe forms of MSF and with favorable outcome. The clinical signs does not differ from those in other reports. Severe cases are characterized by typical laboratory disturbances and predominant liver and kidney involvement, CSF manifestation and pulmonary and gastrointestinal symptoms. The discrete differences between the reports are due to the local characteristics, national habits, investigations features, time and place of study.

Conclusion

The MSF is still endemic disease in Bulgaria and a trend towards an increase of severe cases is real. The surveillance of this disease should be continuous with an emphasis on severe cases and risk factors. In any case with fever, rash and epidemiological data for vector-borne disease, MSF should be suspected. An appropriate treatment should be provided for positive outcome.

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Conflicts of interest

None of the authors have any associations that might be deemed a conflict of interest to the publication of this manuscript. Parts of this manuscript are coming to be presented at 6th International Meeting on Emerging Diseases and Surveillance (IMED 2016), 04–07 November 2016, Vienna, Austria (Abstract #0610 – Poster session).

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Effect of live ammunition shooting from an automatic rifle on sense of hearing in proffesional military personnel

Uticaj bojevog gađanja iz automatske puške na čulo sluha kod profesionalnih vojnih lica

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Abstract

Background/Aim. A short-term high-intensity noise from a gunshot impulse that occurs when using infantry weapon, can result in onset of auditory symptoms such as tinnitus, impaired hearing or feeling of pressure in one or both ears. The aim of this study was to examine the effect of live ammunition shooting from an automatic rifle on the hearing sense in professional military personnel. The examination was done in correlation with the conditions of the common shooting practice procedure. **Methods.** The research has been conducted on 22 professional soldiers serving in the Serbian Military, during their regular peacetime training shooting from the automatic rifle AP 7.62 mm M70. The training was conducted on the formational shooting field "Bubanj Potok". The written consent from all subjects was acquired. All subjects were submitted to medical examination prior and after the shooting session. The medical examination consisted of anamnesis, clinical otorhinolaryngological examination, audiometry and impedancemetry. All subjects filled out the following questionnaire forms: "The Questionnaire prior to shooting" and "The questionnaire after shooting." Subjects who were incapable to undergo shooting practice, whether from psychological or physical reasons were excluded from this study. **Results.** After the assessment of received data from 22 subjects, the results were as followed: in 2 (9%) subjects hearing impairment was diagnosed. Tinnitus was registered in 5 (22.7%) patients. One (4.5%) patient reported the feeling of pressure in one ear. One of two cases of one ear hearing impairment was on the rifle holding side and second case was on opposite lateral side. In both cases, hearing loss was of sensorineural type of milder degree, with a scotoma at 4,000 Hz in one case up to 50 dB and the second up to 55 dB. **Conclusion.** Auditory effects of impulse noise that occurs when shooting from automatic rifles cause hearing impairment, tinnitus and a feeling of pressure in the ears. The practical significance of this study lies in prevention which includes the mandatory use of personal protective equipment and functional arrangement of the practice shooting field.

Key words:
military medicine; firearms; noise; hearing tests; serbia.

Apstrakt

Uvod/Cilj. Kod pucanja iz pešadijskog naoružanja nastaje impulsna buka zbog koje se pojavljuju auditivni efekti. Ispoljavaju se u vidu zujanja, pritiska ili oslabljenog sluha na jedno ili oba uva. Cilj rada bio je da ispitamo uticaj bojevog gađanja iz automatske puške na čulo sluha kod profesionalnih vojnih lica u korelaciji sa uslovima izvođenja bojevog gađanja. **Metode.** Istraživanje je, uz pisani pristanak, sprovedeno nad 22 profesionalna pripadnika Vojske Srbije, koji su bili na svom redovnom mirnodopskom zadatku obuke gađanja iz automatske puške AP 7,62 mm M70, na formacijskom strelištu „Bubanj Potok". Kod svih je pre i posle gađanja urađena: anamneza, klinički otorinolaringološki pregled, audiometrijski i impedanciometrijski pregled. Popunjen je

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„Upitnik pre gađanja" i „Upitnik posle gađanja". Iz istraživanja su bili isključeni svi koji psihofizički nisu bili sposobni za gađanje. **Rezultati.** Posle kompletne obrade dobijenih rezultata od 22 ispitanika u dva (9%) slučaja je registrovano oštećenje sluha. Tinitus je registrovan kod pet (22,7%) ispitanika. Kod jednog (4,5%) ispitanika registrovan je osećaj pritiska u uvu. Registrovano je jedno oštećenje uva sa iste i jedno oštećenje uva sa suprotne strane u odnosu na rame o koje su oslanjali pušku. U oba slučaja oštećenje sluha je bilo senzorineuralnog tipa, lakšeg stepena, sa skotomom na 4 000 Hz, u jednom slučaju do 50 dB, a u drugom do 55 dB. **Zaključak.** Impulsna buka prilikom gađanja iz automatske puške dovodi do pojave auditivnih efekata. To su oštećenje sluha, zujanje i osećaj pritiska u ušima. Praktični značaj ove studije ogleda se u prevenciji, koja podrazumeva obaveznu upotrebu ličnih zaštitnih sredstava i uređenje strelišta.

Ključne reči:
medicina, vojna; puška, automatska; buka; sluh, ispitivanje; srbija.

Introduction

Sound is a physical phenomenon, which refers to creation of sound waves that stimulate the sense of hearing. Sound is produced by all objects and bodies that vibrate. The sound is created if the object oscillates and forms a tunable, harmonic vibration. Production of complex and inconsistent vibration is defined as noise [1]. Effects of noise can be non auditive, psychogenic, but primarily, the noise affects the hearing sense organ. Acute acoustic trauma is a consequence of short-term high-intensity noise, for example the gunshot. Due to the nature of their work and exposure to impulse noise from the gunshot, professional military personnel are at greater risk of developing hearing impairment. Symptoms that appear during the use of infantry weapons are: cloged ears, feeling of pressure in ears, tinnitus, impaired hearing (hearing impairment), ear pain (otalgia).

Acoustic trauma afects the inner ear in two ways: by mechanical energy, so-called blast waves, and by acoustic energy wich is absorbed by the sensory cells in cochlea [2]. The acoustic pulse waves formed by a gunshot from infantry weapons has a mechanical effect. Mechanical impulse creates vortices in fluid of the inner ear. The vortices are spread along the basilar membrane and cause direct damage to the ciliated cells, especially external ciliated cells. This may cause a rupture of Reisner membrane, folowed by mixing of endolymph and perilymph, and damage to the sensory cells.

Impulse noise leads to functional overload of sensory structure, caused by metabolic effects. The catabolic processes in cells prevail over the anabolic, which, followed by reduced blood perfusion, leads to vasospasm and hypoxia, causing the functional disorders in reaction of sensory cells [3].

The consequences of these changes are shorter or longer hearing impairment, followed by a „stage of adaptation", later by the "stage of fatigue" which is characterized by exhaustion of sensory cells and ultimately definite degenerative changes and loss of the cell.

Commonly, it is well known that short time after the exposure to the noise, a person has weaker sense of hearing. This is temporary hearing loss, which recovers after a certain period of time spent in silence. By audiometric parameters, it is described as temporary hearing impairment [Temporary Threshold Shift (TTS)]. If exposure to noise is prolonged, permanent hearing loss occurs [Peomanent Treshold Shift (PTS)] [4].

The aim of this study was to examine the effect of live ammunition shooting from an automatic rifle on the hearing sense in professional military personnel. The examination was done in correlation with the conditions of the common shooting practice procedure.

Methods

This prospective observational study was conducted as a pilot project. Approval to conduct the survey was obtained from the Ethics Committee of the Military Medical Academy (MMA), Belgrade, Serbia. The study was conducted on 22 subjects, professional soldiers serving in the Serbian Military, during their regular peacetime training shooting from the automatic rifle AP 7.62 mm M70. The shooting was done on the shooting range "Bubanj Potok". The written consent from all the subjects was acquired. The medical examination prior and after the shooting was done. It consisted of anamnesis, clinical otorhinolaryngological examination, audiometry and impedancemetry. Tonal liminar audiometry was performed by using Madsen Xeta aparatus and tympanometry was done by using Madsen Zodiac 901 aparatus. All subjects filled out the following questionnaire forms: "The questionnainre prior to shooting" and "The questionnaire after the shooting".

"The questionnaire prior to the shooting" had questions that explore previous exposure to impulse noise during active military service, data on how often were subjects exposed to shooting noise during one year, use or non-use of personal protective equipment. It also concidered information on the presence of symptoms after the shooting and whether the treatment was conducted. "The questionnaire after the shooting" contained questions that indicated use or non-use of personal protective equipment and presence of symptoms after the shooting. Subjects who were incapable of undergoing shooting practice, whether from psychological or physical reasons, were excluded from this study.

Noise measurements were done at the shooting range "Bubanj Potok", in the working environment, by professional MMA authority which issued an expert report. The measurement of sound pressure level was done on instrument for noise measurement and analysis, model 2250 D-DOO, with condenser microphone model 4941 manufactured by Brüel & Kjaer, Denmark.

Statistical methods used for the analysis of primary data were descriptive statistical methods and method for statisti-

cal hypotheses testing. Descriptive statistical methods that were used were measurement of central tendency (mean, median), rate variability (standard deviation) and the relative numbers (structure indicators). The methods for testing statistical hypotheses were Wilcoxon test and the McNemar test. The statistical hypotheses were tested at the level of statistical significance (alpha level).

Results

The measured level of impulse noise on the site for shooting supervisor, site number 2, during live ammunition single shooting from AP 7.62 mm M70, on the line of fire in the right sector was 133.1 dB. The measured level of impulse noise on the site for shooting supervisor, site number 2, during burst shooting from AP 7.62 mm M70, on the line of fire in the right sector was 138.2 dB.

Table 1 shows some of variables which were followed during this research and were examined in the questionnaire prior to shooting.

An average age of the subjects was 35.5 ± 6.3 years, the youngest subject was 27 and the oldest one was 51 years old.

The median of active military service of the subjects was 13 years (range 4–26).

The median frequency of shooting with an automatic rifle in one year was 4 times a year (range 2– 11 times a year).

The median frequency of shooting from smaller caliber arms in one year was 2 (range 0–11).

The median frequency of shooting from weapons of greater caliber in one year was 0 (range 0–3).

Forteen % of the subjects relied a rifle on the left shoulder, while 86% of the subjects relied a rifle on the right shoulder.

Personal protective equipment in the previous training shooting sessions was never used by 32% of the subjects, was occasionally used by 41% of the subjects while it was continuously used by 27% of the subjects.

For regular medical assessment 18% of the subjects reported once per year, 73% of the subjects reported once in every two years, while 9% of the subjects reported less frequently than specified. Nine percent of the subjects reported acute hearing problem. Nine percent of the subjects reported different health problems. All the subjects (100%) were physically and psychologically fit for the shooting.

Seven (32%) subjects used personal protective equipment during shooting. Two (9%) subjects used ear plugs, 5 (23%) subjects used cotton plugs. Fifteen (68%) subjects did not use any kind of protection.

Table 2 shows objective examination findings before and after the shooting (otoscopy, regular othorinolaringology examination, audiogram, tympanogram).

All the subjects (100%) had normal otoscopic examination findings before and after the shooting both on left and right ear.

Normal findings had 59% of the respondents, 36% had a diagnosis of *Deviatio septi nasi*, and 5% had a diagnosis *Polyposis nasi*.

Ninety-one percent of the subjects had normal tympanogram on the left ear prior and after the shooting which was calculated as not statistically significant ($p = 1.000$).

The tympanogram of the left ear prior and after the shooting was: Type A had 91%, Type B had 4.5% and Type C had 4.5% of the subjects.

Eighty-six percent of the subjects had regular tympanogram on the right ear prior and after the shooting which was calculated as not statistically significant ($p = 1.000$).

Tympanogram on the right ear prior and after the shooting was: Type A had 86%, Type B had 5% and Type C had 9% of the subjects.

Regular audiogram after the shooting was found in 91% of the subjects. One subject had a damage on the same side as the gun relied shoulder, and one had a damage on the opposite side from the side on which the gun was relied.

In relation to the degree of hearing impairment, in both cases sensorineural hearing loss of the lower level was registered in the form of scotoma at 4,000 Hz. In one case up to 50 dB, while in the other one up to 55 dB.

Table 1

Variables examined in the questionnaire prior to shooting

Variables	Values
Number of subjects	22
Age (years), x ± SD	35.5 ± 6.3
Years of service, median (range)	13.0 (4.0–26.0)
Fequency of shooting from AR/year, median (range)	4.0 (2.0–11.0)
Fequency of shooting from arms of smaller calliber than that of that of AR/year, median (range)	2.0 (0.0–11.0)
Fequency of shooting from arms of greater calliber than AR/year, median (range)	0 (0–3.0)
Rifle lining shoulder, n (%)	
left	3 (14)
right	19 (86)
Use of protective equipement in previous shooting, n (%)	
never	7 (32)
periodically	9 (41)
permanently	6 (27)

***Variables according to "Questionnaire prior to shooting".**
AR – automatic rifle

Table 2

Objective examination findings before and after the shooting

Variables	Values
Otoscopy finding left prior to shooting – normal, n (%)	22 (100)
Otoscopy finding right prior to shooting – normal, n (%)	22 (100)
ORL finding prior to shooting, n (%)	
normal	13 (59)
DSN	8 (36)
polyposis nasi	1 (5)
Audiogram prior to shooting, n (%)	
normal	15 (68)
right impairment	0 (0)
left impairment	3 (14)
both sides impairment	4 (18)
Tympanogram left prior to shooting - normal, n (%)	20 (91)
Tympanogram right prior to shooting - normal, n (%)	19 (86)
Otoscopy finding left after shooting- normal, n (%)	22 (100)
Otoscopy finding right after shooting- normal, n (%)	22 (100)
ORL finding after shooting, n (%)	
normal	13 (59)
DSN	8 (36)
polyposis nasi	1 (5)
Audiogram after shooting, n (%)	
normal	13 (59)
right impairment	1 (5)
left impairment	4 (18)
both sides impairment	4 (18)
Tympanogram left after shooting – normal, n (%)	20 (91)
Tympanogram right after shooting – normal, n (%)	19 (86)

ORL – otorhinolaryngology; DSN – *deviatio septi nasi.*

Table 3 shows summary audiogram for the left ear prior and after shooting at frequences from 250 Hz to 8,000 Hz.

Table 4 shows sumary audiogram for the right ear prior and after shooting at frequences from 250 Hz to 8000 Hz.

The median value of audiograms prior and after shooting for the left and right ear are given in Tables 4 and 5. A statistically significant difference in median values of audiogram prior and after the shooting, for the left ear was present at 2 KHz (p = 0.046). The difference was close to significance for the the left ear at 6 and 8 KHz and at 2 KHz for the right ear to, but did not reach significance threshold.

Table 3

Summary audiogram for the left ear before and after shooting

Audiogram (left ear) at various frequences	Prior to shooting	After shooting	p
	median value (range)	median value (range)	
250 Hz	10 (10–20)	10 (10–25)	0.250
500 Hz	10 (10–20)	10 (10–25)	0.518
1 KHz	10 (10–20)	10 (10–20)	0.480
2 KHz	10 (10–15)	10 (10–20)	0.046
4 KHz	10 (10–40)	15 (10–50)	0.337
6 KHz	15 (10–45)	15 (10–50)	0.062
8 KHz	15 (10–55)	15 (10–65)	0.088

Table 4

Summary audiogram for the right ear before and after shooting

Audiogram (right ear) at various frequences	Prior to shooting	After shooting	p
	median (range)	median (range)	
250 Hz	10 (10–40)	10 (10–35)	1.000
500 Hz	10 (10–35)	10 (10–35)	0.180
1 KHz	10 (10–35)	10 (10–40)	0.317
2 KHz	10 (10–35)	10 (10v35)	0.083
4 KHz	10 (10–50)	10 (10–65)	0.389
6 KHz	15 (10–60)	15 (10–65)	0.439
8 KHz	17.5 (10–65)	20 (10–65)	0.378

Table 5 shows the auditory effects (hearing loss, tinnitus, pressure in the ear) and their total sum.

Table 5

Auditory effects after shooting (total sum)

Variables	n (%)
Number of subjects, n (%)	22 (100)
Number of auditive effects, n (%)	8 (36)
Number of hearing impairments, n (%)	2 (9)
Number of reported – tinitus, n (%)	5 (23)
Number of feeling of ear pressure, n (%)	1 (5)

Tinnitus was registered in 5 (23%) patients, hearing loss in 2 (9%) and in one (5%) case, feeling of pressure in the ears. In total, some of the auditory effects of shooting from an automatic rifle were registered in 8 (37%) subjects. In 14 (63%) subjects non-auditory effect was detected.

Discussion

Aleksić [3] was examining the frequency and degree of hearing impairment as a result of the use of small arms among members of the Serbia and Montenegro Military and came to the following conclusions. When shooting with an automatic rifle 7.62 mm M70, maximum sound pressure levels ranged between 133 dB and 161 dB (threshold limit values were 140 dB by civil and military standards). Audiological tests showed significantly higher incidence and degree of hearing loss in the group of exposed subjects, as compared to the control group [3].

Weckl et al. [5], were examining the impact of use of small arms on hearing in the Army of Brazil and found that 20.79% of the subjects had a hearing loss, with a significant difference in incidence compared to civilians.

Heupa et al. [6] were examining soldiers who were exposed to noise from infantry weapons and reported that in 23% of the cases the shooting was folowed by onset of tinnitus, and in 7.6% of the cases of hearing loss.

Dhammadejsakdi et al. [7], examined the use of protective headphones at subjects from Thailand military and concluded that the incidence of acute acoustic trauma was reduced 15 times [7].

Honet et al. [8], confirmed that hearing damage could occur following the first exposure to shooting without protection at a shooting range, which emphasizes the importance of preventive use of personal protective equipment.

Guida et al. [9], recorded the occurrence of noise with a peak to 146 dB, by measuring the level of noise at shooting ranges and found that it was was significantly above the recommended 120 dB.

Berg et al. [10], reported more frequent incidence of damage to the left compared to the right ear due to the shadow effect of screening.

Meinke et al. [11] concluded that a risk of hearing damage was reduced if shooting from a standing position, and when using personal protective equipment.

Saedi et al. [12] in the results of their research confirmed that exposure to impulse noise when shooting from automatic small arms, without adequate protection can pose a major threat to hearing, particularly at high frequencies.

Our research confirmed the influence of impulse noise which occurs following gunshot from an automatic rifle on the sense of hearing in professional military personnel. In our case, the observed effects on 22 subjects were: the loss of hearing, tinnitus and feeling of pressure in the ear. Hearing impairment was recorded in 2 (9%) of the cases. It was of the sensorineural type, milder degree, in a form of scotoma at 4,000 Hz, in one case, up to 50 dB, and in another up to 55 dB. It is understandable that the damage occurs at peak value of 4,000 Hz. This is because the short duration high-intensity noise (bang) that occurs following a gunshot from small arms affects mainly the cells in the cochlear basal curve of Chorty body, corresponding to the area C5 (4000 Hz). The effect is mechanical damage to the cell organ of Chorty. Sudden strong impulse is followed by movement of endolimfe and creation of vortices in the basal membrane. This leads to direct damage to the ciliated cells, primarily external (followed by anatomical order) and then, according to sound intensity, to the internal cells which are partially anatomically protected [1–4].

Tinnitus, immediately after the shooting, was registered in 7 cases, 2 of which had spontaneous recovery and in 5 cases tinnitus was present in the examination done few hours after the shooting. Tinnitus occurs as a result of stimulation of the cochlea and it has higher incidence than hearing impairment. It usually heal spontaneously in a period of few hours after exposure to the noise, but it can remain as permanent impairment [1–4].

In one (5%) patient, the feeling of pressure in the ear was registered. This is a subjective feeling which is hard to distinguish from the feeling of fullness or humming in the ear.

It is noted that in both patients with registered hearing impairment there was no subjective symptoms (hearing loss, tinnitus or any other symptom). It was also noted that none of 2 subjects, in which hearing impairment was registered, did not use any protective agent.

Further researchs on a larger sample are maded for more detailed analysis of an influence of live ammunition shooting on sense of hearing in proffesional military personel.

Conclusion

The effect of pulse noise following live ammunition shooting from automatic rifle can be divided into extraauditory and auditory. Primarily the effect is audible and is expressed as: tinnitus, loss of hearing, feeling of pressure in the ears. This study confirmed the occurrence of those auditory effects. Further research on a larger sample are needed to confirm the statistical significance of these effects. The practical significance of this study lies in prevention where the mandatory use of personal protective equipment and organization of the shooting range, can have a direct impact on reduction in incidence of hearing loss, as the most important, tinnitus and other auditory effects. All this can influence the decrease of treatment cost regarding these diseases.

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Cilioretinal arteries and collateral vessels after occlusion of central retinal artery

Cilioretinalne arterije i kolateralni krvni sudovi posle okluzije centralne retinalne arterije

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Abstract

Background/Aim. Central retinal artery occlusion (CRAO) is a disease of the eye where the flow of blood through the central retinal artery is blocked. It causes sudden, painless, unilateral and usually severe vision loss. The aim of our study was to examine significance of cilioretinal artery on collateral and neovasculatization development after occlusion of the central retinal artery. **Methods.** This study retrospectively reviewed all fluorescein angiography (FA) cases with confirmed CRAO and presenting, one or more, cilioretinal arteries on initial examination. The study included patients referred to the Clinic of Ophthalmology, Clinical Center Kragujevac for the examination in the period from January 2010 to January 2015. Ten eyes of 10 patients with confirmed CRAO and existing cilioretinal artery on initial examination were found and analyzed in this study. **Results.** This study included 10 (6 males and 4 females) patients from 50 to 76 years old (mean 66.3 ± 10.078 years). Visual acuity on initial examination presented on the decimal scale was from 0.01 to 0.2 (mean 0.087 ± 0.066). Intraocular pressure measured by applanation tonometry was in the range from 14 to 20 mmHg (mean 16.7 ± 2.540 mmHg). Cilioretinal artery was revealed on the first FA examination of all eyes. On control FA, in three eyes *de novo* collaterals were discovered. In the first eye, collaterals were discovered after two months, in the second eye after four months, and in the third eye after seven months of the performing the initial angiogram. Visual acuity was checked after one year. It was from light perception to 0.03 (mean 0.016 ± 0.009). **Conclusion.** The presence of cilioretinal arteries with preexisting or *de novo* developed collaterals was not enough to preserve visual acuity and prevent neovascularisation over a longer period after CRAO.

Key words:
retina; retinal artery; arterial occlusive diseases; collateral circulation; retinal neovascularization; diagnostic techniques and procedures.

Apstrakt

Uvod/Cilj. Okluzija centralne retinalne arterije (OCRA) je bolest oka gde je prekinut protok krvi kroz centralnu retinalnu arteriju. Izaziva iznenadni, bezbolni, jednostrani i najčešće težak gubitak vida. Cilj ove studije bio je da ispita značaj cilioretinalne arterije za razvoj kolaterala i neovaskularizacije posle okluzije centralne retinalne arterije. **Metode.** Ovom retrospektivnom studijom pregledani su svi angiografski potvrđeni slučajevi OCRA sa prisutnom jednom ili više cilioretinalnih arterija na prvom pregledu. Studija je uključila bolesnike koji su pregledani u Klinici za oftalmologiju Kliničkog Centra Kragujevac u periodu od januara 2010. do januara 2015. godine. Deset očiju od 10 bolesnika sa potvrđenom OCRA i prisutnim cilioretinalnim arterijama pregledano je i dalje analizirano. **Rezultati.** Studija je uključila 10 bolesnika (šest muškaraca i četiri žene) starosti od 50 do 76 godina (srednja vrednost 66,3 ± 10,078). Vidna oštrina na prvom pregledu izražena na decimalnoj skali kretala se između 0,01 i 0,2 (srednja vrednost 0,087 ± 0,066). Intraokularni pritisak meren aplanacionim tonometrom kretao se između 14 i 20 mmHg (srednja vrednost 16,7 ± 2,540 mmHg). Na prvom pregledu fluoresceinskom angiografijom kod svih očiju uočeno je prisustvo cilioretinalne arterije. Na kontrolnim pregledima fluorescenskom angiografijom kod tri oka uočeno je *de novo* stvaranje kolaterala. Kod prvog oka kolaterale su otkrivene posle dva meseca, kod drugog oka posle četiri meseca, a kod trećeg oka posle sedam meseci od prvog angiografskog pregleda.

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Vidna oštrina je proverena posle godinu dana i iznosila je od osećaja svetlosti do 0,03 (srednja vrednost 0,016 ± 0,009). **Zaključak.** Prisustvo prepostojećih cilioretinalnih arterija ili *de novo* stvaranje kolaterala nije dovoljno da očuva vidnu oštrinu i spreči neovaskularizaciju tokom dužeg perioda posle okluzije centralne retinalne arterije.

Ključne reči:
mrežnjača; a. centralis retine; arterije, okluzione bolesti; krv, kolateralna cirkulacija; mrežnjača, neovaskularizacija; dijagnostičke tehnike i procedure.

Introduction

Central retinal artery occlusion (CRAO) is a disease of the eye where the flow of blood through the central retinal artery is blocked, accompanied by sudden, painless, unilateral and usually severe vision loss [1, 2]. The majority of CRAOs are secondary to intraluminal thrombosis or embolism. It is associated with significant systemic pathologies, such as hypertension, diabetes, and carotid atherosclerotic disease [3]. Cilioretinal arteries are reported to be present from 10% to 50% of eyes and are considered to be the commonest retinal vascular anomaly [2, 4, 5]. Cilioretinal collateral vessels are a connection between cilioretinal arteries and retinal vascular network [4]. Neovascularization of retina or optic disk after OCRA is rare, with the prevalence of neovascularization varying from 3% to 18%, which can occur about 8 weeks after the accident (range 2–16 weeks) [6]. We reported a series of cases of CRAO with cilioretinal arteries present and with the development of collaterals and neovascularization.

Methods

This study retrospectively reviewed all fluorescein angiography (FA) cases with confirmed CRAO and existing, one or more, cilioretinal artery on initial examination. The study included patients referred to the Clinic of Ophthalmology, Clinical Center Kragujevac, Serbia for the examination in the period from January 2010 to January 2015, with sudden painless unilateral sever vision loss for detailed clinical examination. Ten eyes of 10 patients with confirmed CRAO and presented cilioretinal artery on initial examination were found and analyzed in this study. Initially, the standard ophthalmic examination was performed in all the patients: the best corrected visual acuity, applanation tonometry, slit lamp examination, and posterior segment examination by indirect ophthalmoscopy, photofundus and FA (Visucam Lite Fundus Camera, Carl Zeiss Meditec AG, Jena, Germany). Photofundus check-up, color, and green mode, as well as FA, were performed for all the patients, in mydriasis, under the same conditions, by the same digital fundus camera after seven days, two months and then every three months until one year. All patients received the same non-invasive therapy which included intraocular pressure lowering maneuvers, oral administration of acetazolamide and anticoagulant therapy.

Results

Patients characteristics

This study included 10 patients (6 males and 4 females), 50 to 76 years old (mean 66.3 ± 10.078 years). Visual acuity on initial examination presented on the decimal scale was from 0.01 to 0.2 (mean 0.087 ± 0.066). Intraocular pressure measured by applanation tonometry was in the range from 14 mmHg to 20 mmHg (mean 16.7 ± 2.540 mmHg).

Fundoscopic examination

Early fundoscopic findings performed within seven days of CRAO showed the following results: retinal opacity in the posterior pole (5 eyes), cherry-red spot (10 eyes), cattle trucking (2 eyes), retinal arterial attenuation (5 eyes), optic disk oedema (4 eyes) and pallor (5 eyes) (Table 1, and Figure 1).

Table 1

Inital fundoscopic findings within 7 days of central retinal artery occlusion

	Photofundus examination						Flurescein angiography	
Patient	Retinal opacity in the posterior pole	Cherry-red spot	Cattle trucking	Retinal arterial attenuation	Optic disk oedema	Pallor	Ciliortetinal artery (n)	Collateral present
1	+	+	-	-	+	+	1	-
2	+	+	-	+	-	-	1	-
3	+	+	-	+	-	-	1	-
4	-	+	+	+	-	+	1	-
5	-	+	-	-	+	-	1	-
6	-	+	-	-	-	+	2	-
7	+	+	+	-	+	-	1	-
8	-	+	-	+	+	+	1	-
9	-	+	-	+	-	-	1	-
10	+	+	-	-	-	+	4	+

+ – present; - – absent.

Fig. 1 – Early fundoscopic findings (7 days from central retinal artery occlusion).

Fluorescein angiography examination

First fluorescein examination was performed four days after the initial exam. On the first FA examination, the presence of cilioretinal artery was revealed in all eyes. Cilioretinal arteries were filled during the choroidal phase of FA. In 8 eyes cilioretinal artery was acknowledged in the middle part of intrapapilary region. Two eyes had a specific presentation of cilioretinal arteries: in the first eye two cilioretinal arteries and in the second eye four cilioretinal arteries were found. Angiogram of the eye with four cilioretinal arteries reveled pre-existing collaterals between cilioretinal arteries and retinal vascular network (Figure 2). This preexisting collaterals enabled fulfillment of the drained retinal network in both directions: retrograde was from perifoveal capillary arcade and anterograde was from collateral between a cilioretinal artery and inferior temporal artery on optic disk border (Figure 3). Control examination of these patients was performed after seven days, two months and then every three months until one year. At later control examinations, fundoscopic findings showed optic atrophy (7 eyes), retinal arterial attenuation (6 eyes), cilioretinal collaterals (4 eyes), and macular retinal pigment epithelial changes (1 eye) (Table 2).

The control FA was carried out two months after the first angiogram. On the control FA in 3 eyes, *de novo* collaterals were discovered. In the first eye, collaterals were discovered after two months, in the second eye after four months and in the third eye seven months after the initial angiogram.

Visual acuity was checked after one year. It was from light perception to 0.03 (mean 0.016 ± 0.009). Patients included in this study had no prior ocular history. Cardiovascular risk factors included: hypertension (7 patients), diabetes mellitus (4 patients), hyperlipidemia (6 patients), and smoking (6 patients) (Table 3). Significant (> 50% occlusion) carotid stenosis was present in 14% of the CRAO patients, and stenosis with mild to moderate plaque in 30%. Cardiac valve disease was present in 23% of the patients in our study.

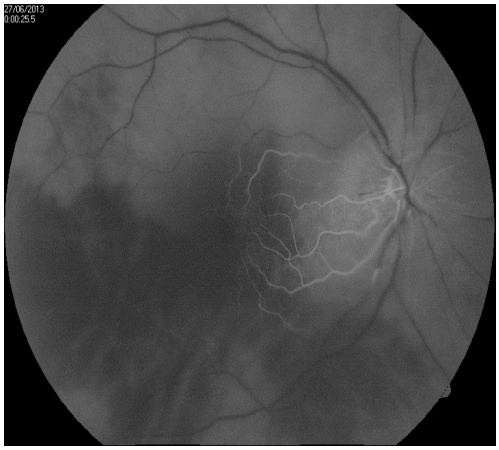

Fig. 2 – Angiogram of the eye with four cilioretinal arteries.

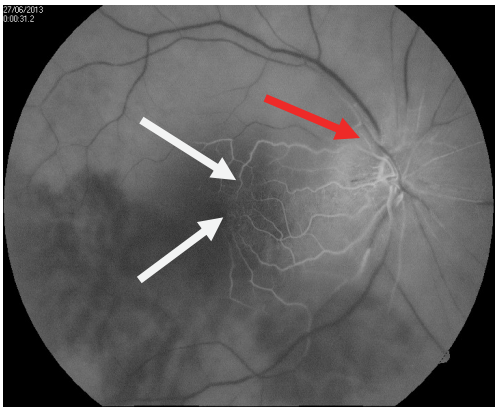

Fig. 3 – Retrograde fulfillment of drained retinal network (red arrow) and anterograde fulfillment of drained retinal network (white arrows).

Table 2

Later fundoscopic findings

	Photofundus examination			Fluorescein angiography		
	Optic atrophy	Retinal arterial attenuation	Macular retinal pigment epithelial changes	Neovacularization present/absent (±)	Ciliortetinal artery (n)	Collateral present/atsent (±)
1	+	-	+	+	1	-
2	+	+	-	-	1	+
3	+	+	-	-	1	-
4	+	+	-	-	1	+
5	+	-	+	-	1	-
6	-	-	-	-	2	+
7	+	+	+	-	1	-
8	-	+	+	+	1	-
9	+	+	-	-	1	-
10	-	-	-	+	4	+

+ – present; - – absent.

Table 3

Cardiovascular risk factors

Patient	Hypertension	Diabetes mellitus	Hyperlipidemia	Smoking
1	+	+	-	+
2	+	-	+	+
3	-	-	-	+
4	+	+	+	-
5	-	+	+	+
6	+	+	+	+
7	+	-	-	+
8	-	-	-	-
9	+	-	+	-
10	+	-	+	-

+ – present; - – absent.

Discussion

The inner retina is supplied primarily or exclusively by the central retinal branch of the ophthalmic artery. Several studies suggested that CRAO lasting for about 240 min results in massive, irreversible retinal damage [7]. Occasionally the central retinal artery is assisted by one or more cilioretinal arteries. When present, cilioretinal arteries vary in size, number, distribution, and point of origin from the optic disc [8]. In a healthy eye, the presence or absence of a cilioretinal artery is clinically insignificant. If a retinal vascular occlusion occurs, the presence of a cilioretinal artery can be a significant factor influencing visual morbidity. Cilioretinal arteries are reported to be present from 10% to 50% of eyes [9]. It is of clinical relevance that a temporal cilioretinal artery supplying the fovea, may spare the fovea in the case of central retinal artery occlusion [10]. Collaterals can be native (pre-existing) or can be formed after CRAO. They are arteriole-to-arteriole anastomoses that cross-connect a small fraction of the outer branches of adjacent arterial trees and are present in most tissues [4, 10]. When the trunk of one of the trees becomes obstructed, collateral-dependent anterograde and retrograde perfusion significantly decreases tissue injury caused by hypoxia. The amount of protection depends primarily on the extent of collaterals present, plus the perfusion pressure across the collateral network and vascular resistance above and below it [10, 11]. In our study we acknowledged that the presence of pre-existing collaterals are rare, only in one case. The presence of collaterals maintains the circulation in the papillomacular and macular regions of the retina, sparing central vision in that way. These collaterals can fill emptied retinal arterial network and this fulfillment can be in a normal anterograde or retrograde direction. We noticed that retrograde direction started from perifoveal capillary arcade. Hence, collaterals enable retrograde fulfillment of emptied retinal arterial vascular network after CRAO. This retrograde fulfillment is common in branch retinal vein occlusion, but it is rarely seen in central retinal artery occlusion [6]. Earlier data reported that *de novo* development of arterial collaterals can be observed in around 30% eyes with CRAO. Time from occlusion to the first detection of collateral formation was three to twelve months [4]. We found the presence of collaterals in 4 eyes. In our study, collaterals formed two to seven months after CRAO. Pre-existing collaterals were found only in 1 eye. Collaterals formation can be explained in few various ways. If a relatively focal blockage exists in the retinal arterial tree and if sufficient retinal and choroidal flow exist to maintain at least minimal retinal and vascular viability, collaterals will be able to improve the hypoxic condition. Alternatively, gradual closure of the retinal vessel by sclerosis or thrombus formation, or by recurrent tiny emboli, might encourage the widening of collateral channels. That can be the reason why collaterals are not present in every eye with the cilioretinal artery [12, 13]. In the long-term period, visual acuity was not preserved. We found statistically lower visual acuity between visual acuity on initial examination and one year after CRAO. In our patients with multiple cilioretinal arteries and *de novo* formation of collaterals, visual acuity was not retained. These facts can indicate that atrophy of retinal tissue and optical nerve atrophy could not be prevented even with the existence of cilioretinal arteries and collaterals. Previous studies reported that development of neovascularization after CRAO occurred in 3% to 18% [6, 8, 13] of CRAO eyes. We reported in our study that only 3 eyes developed neovascularization. This can be explained by the presence of cilioretinal arteries and collateral vessels. The presence of these vessels allows sparing of retinal tissue in the papilomacular region, but at the same time leads retina to "semi-ischemic" stadium. This condition can be stimulus for the production of angiogenic growth factors. Most neovascularization vessels were formed in the eye with multiple cilioretinal arteries and pre-existing collaterals.

Conclusion

The presence of cilioretinal arteries with pre-existing or *de novo* developed collaterals was not enough to preserve visual acuity and prevent neovascularization over a longer period after CRAO.

Declaration of interest

The authors declare no conflict of interest for this study.

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GENERAL REVIEW

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Vaccination and autoimmunity: influenza vaccination and association with multiple sclerosis

Vakcinacija i autoimunost: vakcina protiv gripa i povezanost sa multiplom sklerozom

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Ključne reči:
vakcinacija; grip; autoimunske bolesti; multipla skleroza; imunski faktori.

Introduction

Influenza, generally known as the flu, is one of the most common viral respiratory infections with the capacity to disseminate around the world, one could say at lightning speed, in seasonal epidemics, reaching its summit over the course of winter. Influenza is a serious infectious disease caused by RNA viruses which belong to the Orthomyxoviridae family. The first influenza pandemic was documented in 1580 and it has remained a viral disease of global dimension ever since [1]. The four researchers analyzed medical literature reported during the Spanish Flu pandemic from 1918 to 1920. The meta-analysis of these data showed that treatment of new victims of the virus in 1918 with convalescent whole blood, plasma or serum collected from patients who had recovered from Spanish Influenza resulted in reduced case-fatality rate of severely ill patients by 50% [2]. In 1931, Goodpasture was the first who discovered a viral growth in embryonated hen's egg, and in the 1940s, the US military developed the first approved inactivated vaccines for influenza, used during World War II [1]. Unlike other viral vaccines, annual influenza vaccination is recommended due to fast evolution of influenza viruses, evolve one million times faster than mammals, which results in high mutation rates and antigenic variations known as antigenic drift, a minor change such as amino acid substitution in virus surface proteins hemagglutinin (HA) and/or neuraminidase (NA), and antigenic shift, a new combination of different flu genes emerged to infect people [3–5].

Traditional influenza vaccine composition

There are three distinct types of influenza viruses, designated A, B, and C, with types A and B of influenza viruses being the major pathogens in humans. Influenza A can infect humans, birds, pigs and other species [6]. Influenza B and C are primarily human pathogens. Unlike influenza A and B viruses, influenza C virus is of little clinical importance [7]. The core of the A and B viruses is surrounded by a lipid in nature membrane, or 'envelope' derived in part from modified host cell membranes, from which may protrude spikes, glycoprotein complexes, corresponding to the hemoglutinin (HA) trimer ligand and the neuraminidase (NA) tetramer ligand [8]. HA surface protein enables the virus to get attached to sialic acid-containing receptors and viral entry by membrane fusion. NA surface protein is a receptor-destroying enzyme responsible for viral release and cell-to-cell spreading [9]. There are 17 HA subtypes of influenza virus whereas 9 subtypes of NA are known to be present [10]. Nowdays traditional flu vaccines (called "trivalent" vaccines), composed of two types of inactivated influenza viruses: an influenza A (H1N1) subtype virus, an influenza A (H3N2) subtype virus, and an influenza B virus, are used worldwide to protect from influenza and its serious complications [7].

Influenza vaccination: Pros and cons

A vaccine is a preparation of killed or inactivated microbes (parasites, viruses, bacteria), or purified products deri-

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ved from them, used to elicit the immune system to a particular disease [11]. Human vaccines are regarded as one of the safest medical products available, and the most effective method of prophylaxis we have against infectious diseases for the general population. Medical community considers current human vaccines also safe and effective for patients with autoimmune diseases [12, 13], but like any other medical product, there may be risks. In general, all inactivated virus vaccines are considered safe and effective[11].

Even though influenza infection *per se* has seldom been associated with various organ-specific and systemic autoimmune diseases, systemic and neurological autoimmune phenomena have been reported following influenza vaccine that consists of inactivated purified surface fragments and no viral genetic material [14]. In June 2009 the World Health Organization declared the new influenza of swine origin, A (H1N1), which originated in Mexico around March 18, 2009, was pandemic [15]. A concern about pandemic influenza A (H1N1) 2009 vaccine was the possible occurrence of neuroimmunological adverse events, including Guillain-Barré syndrome (GBS). A causal relationship between the vaccine and this autoimmune neurological disease was suggested by the original Centers for Disease Control study during an outbreak of GBS in 1976 that was caused by the swine flu vaccine [16]. A more than seven-fold increment in risk of GBS was observed when the influenza A (H1N1) subtype A/New Jersey/76 (A/NJ/76) vaccine had been applied in the United States in 1976. As a consequence, the vaccination campaign had to be suspended brusquely [17]. In contrast to the A/NJ vaccine, in 1978-1979 introduction of a new HA type of influenza vaccine have failed to show a statistically significant excess risk of acquiring vaccine-related GBS [18]. A retrospective epidemiological study done by Lasky et al. [19] on seasonal influenza vaccines used in 1992–1993 and 1993–1994 showed modest increases in risk of GBS. For the two seasons combined the risk of occurrence of GBS showed 95% confidence interval (1.0, 2.8). The adjusted (age, sex, and vaccine season) relative risk of 1.7 ($p = 0.04$) suggested slightly more than one additional case of GBS within six weeks after vaccination in one million people.

A population-based cohort study in Stockholm, Sweden, investigated over a period of 8–10 months, the risk of neurological and autoimmune disorders of special interest in people vaccinated against pandemic A (H1N1) with monovalent Pandemrix® (GlaxoSmithKline, Middlesex, UK) 2009 vaccine compared with those who remained unvaccinated. The study population comprised 1.98 million people registered in Stockholm county with more than one million people vaccinated and 900,000 unvaccinated. This retrospective cohort study was devised for the purpose of linking individualized data on pandemic vaccinations to an inpatient and specialist database on healthcare utilization in Stockholm county for follow-up during and after the pandemic period. The overall relative risk for GBS, multiple sclerosis (MS), type 1 diabetes, or rheumatoid arthritis among vaccinated compared with unvaccinated people remained unchanged [20].

Three major neurological manifestations of an autoimmune nature have been viewed in conjunction with vaccination: the GBS, MS and autism [21]. Among autoimmune adverse events, GBS remains the most frequently reported influenza vaccine adverse neurological autoimmune event. For that reason, and the issue around GBS in 1976 another prospective multinational case-control study in Europe [22] with an objective to evaluate any association between GBS and adjuvanted pandemic influenza A (H1N1) 2009 vaccine was performed. The main outcome measure was a relative risk estimate for GBS after influenza vaccine. The point estimate showed no association between pandemic influenza vaccination and GBS, although the upper confidence limit was 2.7 meaning a potential increase in risk up to 2.7-fold or three excess cases per one million vaccinated people.

GBS is a transient, often preceded by a respiratory or gastrointestinal illness, acute polyneuropathy, in Europe mostly presents as acute inflammatory demyelinating polyradiculoneuropathy, characterized by areflexic symmetrical motor paresis with mild sensory disturbances [23]. The infections *per se* are usually not enough for triggering autoimmune diseases. There are clearly other factors involved: genetic, immunological and hormonal [24]. This is probably why the role of influenza vaccination as a trigger of GBS remains controversial [22].

Hence, the GBS-vaccine link controversy continuous. Two recent studies from the United Kingdom identified influenza-like illness/upper respiratory tract infection as a strong risk factor. An increased risk of GBS was seen shortly thereafter, consistent with observations that GBS is often preceded by a respiratory illness. It is however difficult to associate GBS with influenza virus infection solely since other respiratory pathogens, that can present as influenza-like illness, are also at their height in the winter. This study found no causal association of GBS with influenza vaccine; instead, it pointed out to increased risk of GBS after influenza-like illness. Furthermore, this study suggests that influenza vaccine should protect against GBS and also finds equally valuable to make an overall risk-benefit assessment - the risk of such events due to pandemic influenza *vs* the degree of vaccine protection [25, 26].

While the substance of epidemiologic evidence, evaluated over the last 30 years, does not support the association between influenza vaccination and GBS, the Committee of the Institute of Medicine (2011) found that an association cannot be ruled out with confidence, particularly because the annual antigenic reformulation of the influenza vaccine varies from year to year and the potential for risk of GBS also varies [27].

Pathophysiological concepts of multiple sclerosis with respect to antigen presenting cells

In general, MS is heterogeneous disorder of the central nervous system. Although the factors that contribute to its heterogeneity are still confounding, at the same time we are quite assured that we talk about a complex genetic trait that is influenced by environmental variables such as exposure to infections, climate etc. There is no doubt that cellular and humoral immune mechanisms are implicated in the pathoge-

nesis of demyelinating autoimmune diseases in the CNS in humans and animal models [28, 29]. Furthermore, pathophysiological studies suggest that MS results from an immunological attack on white matter in the CNS and consequent breakdown of the myelin around axons and the possibility of secondary axonal damage as well. Plaques, the pathological signature of MS, are small round areas of demyelination that may occur anywhere within the white matter. Depending on their stage of development the varying proportions of immune cells and immunoreactive substances can be detected [30].

Antigen presenting cells (APCs) are necessary for the pathogenesis of murine models of MS [31,32]. APCs are involved in multiple stages during MS pathology, thenceforth the growing interest in studying these cells. MS seems primarily to be a disease that involves an immune response to antigens presented by major histocompatibility complex (MHC) class II molecules. Perivascular macrophages are an abundant cell type in the CNS. This location enables them to encounter pathogens and assist in controlling innate and adaptive immune responses in the CNS. They are especially plentiful in actively demyelinating lesions and are characterized by higher levels of MHC class II and CD45 in both rodents and humans compared to microglia [33]. Although microglia are thought to exert a detrimental role on the brain, their precise contribution to brain inflammatory demyelination is largely unknown [34]. In humans MHC class II is expressed in MS lesions [35]. Additionally, human astrocytes express MHC class II *in vitro* upon IFN-γ stimulation [36]. Further, the accumulation of dendritic cells (DCs) within the CNS [37–39] is evident, yet the mechanisms of recruitment of DCs to the CNS continue to be an area of ongoing research. These infiltrating DCs may seem to some of us excessive, given the fact that the CNS has resident APCs (microglia and astrocytes). DCs are of hematopoietic origin, they evolve from lymphoid and myeloid precursors, respectively. There are two main subsets of DCs: conventional DCs (cDCs) and plasmacytoid DCs (pDCs) [40]. DCs, upon activation through either Toll-like receptor (TLR) signaling or encountering with antigen (Ag), travel from resident tissues or sites of inflammation to the lymph nodes (LN). Classically, T cell activation occurs in the LN where DCs migrate after Ag uptake in peripheral tissues. In experimental autoimmune encephalomyelitis (EAE) model, naive T cells are first introduced to myelin antigens in the periphery [41]. Irla et al. [42] proposed that MHCII expression by pDCs confers natural protection against EAE by stimulating the selective expansion of myelin-Ag-specific natural regulatory T cells in secondary lymphoid tissues. In our previous study we suggested that pDCs might be the one to make a connection between mild clinical signs expressed in the myelin oligodendrocyte glycoprotein (MOG) variant of EAE-induced mice and the expression of MHC class II molecules in the secondary lymphoid organs [43]. Activation of DCs leads to their maturation, that is, upgrades the expression of MHC class II as well as co-stimulatory molecules (CD80, CD86, CD40). This way DCs become more efficient at presenting cognate Ag to naive as well as memory T cells, which is essential in the coordination of both the innate and adaptive immune responses [44].

Overly simplified view of the functionally balanced division of CD4(+) T cells into Th1 and Th2 lymphocytes remains useful and remains a model of MS pathogenesis [45, 46]. However, exceptions to Th1-driven MS model may be much more prominent, often plentiful, clonal expansion of CD8+/MHC class I-restricted T lymphocytes, especially in active lesions, whereas the CD4+ phenotype predominates in the perivascular space [47]. Current data suggest that MS is driven by Th1 and Th17 subsets, although they are mechanistically different from each other [38, 48]. Various pathological, experimental, clinical and immunological findings also collectively indicate a pathogenic role of antibodies in MS. Serum autoantibodies that targeted extracellular MOG, the outermost lamellae of the myelin sheath and hence easily accessible to antibody attack, in its native structure were shown to be lytic *in vitro*, supporting a potential pathogenic role of these antibodies in MS [49]. EAE is the most extensively studied mouse model of MS [50] and MOG induced EAE more closely resembles MS than other EAE variants in which inflammation greatly predominates over demyelination [51]. More work is however required to learn the pathogenic details of the demyelinating events observed in the CNS caused by antibodies reactive with myelin constituents in EAE and to determine whether these mechanisms are indicative in MS.

Influenza infection or vaccination as probable trigger of MS

MS is the most common chronic neurological disease in young adults, affecting about 2.5 million people worldwide [52]. It is now generally acknowledged that the etiology of autoimmune diseases, even though still not clear, includes the genetic, immunological, hormonal and environmental factors skewing the immune response towards autoreactivity [53]. Environmental factors, especially infections, are considered to be probable, although usually not sufficient, triggers of autoimmune response and can elicit or exacerbate autoimmune diseases [54, 24]. More recent studies divulge that peripheral B-cell responses are closely involved in the immune pathology of MS through proinflammatory mechanisms, bystander activation, or through regulatory functions [55]. It is proposed that aberrant proinflammatory cytokine responses exhibited by episodically triggered B cells of MS patients mediate bystander activation of disease-related proinflammatory T cells resulting in relapsing disease activity [56]. Since abnormal cell-mediated and humoral immunity play a role in the pathogenesis of disease, a higher susceptibility to infections in MS patients is therefore expected. Although the unbalanced immune system is evident in patients with MS, immune defense against common viral and bacterial infections appears to be preserved. The infection rate of commonly occurring infectious diseases is not increased among MS patients [57, 58]. Additionally, several reports suggested that virus infections could trigger a relapse typically observed in relapse-remitting (RR) form of the MS patients [59]. A possible explanation for disease exacerbation after influenza infection could be loss of down-regulation

within DC clusters and consequent increase of activated cells [60]. Therefore, immunization is considered important for the MS patients not only to prevent an infectious ailment, but also to potentially prevent the MS relapses. The debate about vaccine safety in patients with MS is still wide open [61]. Clinical onset and the MS disease activity after vaccination have been reported [57, 62, 63].

Hepatitis-B vaccine has been of particular concern and most extensively studied in connection with the MS onset. The focus on this vaccine followed case reports, in France and the US, of the CNS demyelization that have been documented days to weeks post-vaccination [64]. A number of epidemiological studies and medical records-based investigations of hepatitis B vaccine and MS followed these case reports and most of these have shown neither increased risk of experiencing a demyelinating episode nor occurrence of MS for vaccinated *vs* unvaccinated individuals [65–67]. Studies that did show the relationship between hepatitis B vaccine and increased risk of MS noted that this vaccine does not represent a widespread risk factor for the disease [68–71]. In 2002, the National Academy of Sciences' Institute of Medicine, after having examined the published, peer-reviewed scientific and medical literature, concluded the lack of association between hepatitis B vaccination and the CNS demyelinating disease [72]. Additionally, studies that have examined the potential effect of hepatitis B vaccination on relapses in people diagnosed with the MS disease could not demonstrate an increased risk of relapse [73, 74].

Other vaccines with potential implications for the MS patients that were studied include influenza, because of its widespread use, tetanus and measles vaccines. There is no definite increase of either occurrence of MS or the risk of triggering the MS bouts following the influenza vaccination that has been reported.

Vaccination has proved effective in neutralizing infectious agents by inducing strain-specific antibodies [14]. Back in 1962 Sibley and Foley [57] observed 24 patients with MS following the administration of influenza vaccine. The quadrivalent inactivated-virus vaccine contained three type A strains and one type B strain. Antibodies to all four viral strains were determined in the MS patients and control patients with other neurological disease. The vaccine was well tolerated in the group of patients with MS with no convincing evidence of adverse reactions to vaccine. Antibody response to influenza vaccination in both groups showed that immunologic responsiveness was similar, with usual rise in titer by an average of twofold to fourfold after vaccination. In a clinical study done by Moriabadi et al. [75] "Influenza vaccination in MS" mean antibody response against influenza A virus was increased in both MS patients and healthy controls after 2 weeks post immunization. He also argues against a general immune stimulation by influenza vaccination since no increase of myelin protein–reactive T-cells was observed after immunization. The overall data presented in this study support the effectiveness and safety of influenza vaccination in the patients with MS. Our group was reported that anti-influenza antibody titers in healthy vaccinated mice and in MOG induced EAE-vaccinated mice, 4 weeks after vaccination with inactivated influenza vaccine (split virion), was significantly higher compared to the unvaccinated control groups, indicating long-lasting antibody response as well as preserved immune response in MOG induced EAE mice [76]. A possible role of several viruses, including influenza A virus, was investigated in a case-control study in 152 children with MS and a significantly higher concentration of antibodies was found in the MS patients comparing with controls. This study pointed out more to a complex infectious background of MS rather than, 'a specific virus causes a specific disease' [77].

Analyzed medical records, which included influenza vaccine among other vaccines found no association between influenza vaccine and increased risk of MS [67, 71]. The studies that analyzed whether influenza vaccination affected the risk of the MS exacerbation or disability progression demonstrated no relation between influenza vaccine and subsequent flare of the disease [78].

Apart from a risk of MS or the MS relapse from vaccination in general, a few studies investigated the issue of vaccine efficacy in the MS patients due to immune system dysfunction in genetically predisposed persons, and found no sufficient evidence to make a determination [79, 80]. The antibody levels following various virus vaccination were present at similar levels in both MS patients and healthy subjects [81]. This suggests that vaccination is likely as effective in the MS patients as in the healthy subjects.

There is a long-lasting concern that a mechanism by which immunization may trigger the MS activity may be shared among other autoimmune diseases such as GBS. In theory, intensified immune response against live attenuated viruses (e.g. measles and varicella), inactive viruses (e.g. seasonal influenza and hepatitis A), or portions of viruses or bacteria [e.g., hepatitis B, human papiloma virus (HPV), and pneumococcus] vaccines, that have been included among the environmental factors, might also induce an aberrant immune response against self-antigens [82]. Many common infections are known to induce a transient rise in autoantibody production. A similar rise in autoantibody production has been observed after various vaccinations. Recently, our group has detected a significant increase of anti-MOG antibodies in sera of MOG induced EAE mice and MOG induced EAE-influenza vaccine vaccinated mice, compared to influenza vaccine vaccinated and intact groups. No difference was found between influenza vaccine vaccinated *vs* intact groups and a positive correlation was found between anti-MOG antibody titer and the development of the EAE clinical signs. The overall data presented in this study indicate that influenza vaccine has no effect on production of autoantibodies and development of clinical signs [76]. A number of cohort studies demonstrated a transient change in autoantibody production, with presumably no clinical significance, after influenza vaccination in apparently healthy participants [83, 84]. These autoantibodies usually resolve within a period of 2 months [85] but can persist in rare cases. Based on several reported studies, the post-vaccination stimulation of autoantibody production became one of the criteria of establishing vaccine safety. Although autoantibodies have the potential of pathogenicity

in several diseases it is often not clear whether they mirror events of certain antigens important in the development of the illness or represent the causal factor [21]. As of now, however, the pathological relevance of antibody-mediated autoimmune encephalomyelitis in MS remains unclear regardless their significant deposits in some demyelinating MS lesions [86]. A recent case report done by Amano et al. [87] supported the view that anti-MOG antibodies were linked to longitudinally extensive transverse myelitis (LETM) established after influenza infection. LETM, defined as a spinal cord lesion that extends over three or more vertebrae, is classically associated with neuromyelitis optica. Spinal cord lesions may, however, arise from a number of autoimmune and inflammatory diseases that involve the CNS such as MS, sarcoidosis or Sjögren syndrome or infectious diseases with the CNS involvement [88]. The clinical significance of anti-MOG antibodies for diagnosis, treatment, and prognosis has yet to be ascertained. Although autoantibodies against MOG are found in the clinical spectrum of MOG associated diseases in humans as well as in different experimental models, the role of anti-MOG antibodies in pathogenesis is still unclear [88].

The mechanism of host response applies equally to an infectious invasion and to vaccination [89]. However, the pathogenic mechanism underlying the association between viruses and MS is still uncertain. Since vaccinations prime the immune system and immune factors are viewed as major players in MS, it is plausible that vaccines might work as the infections do and molecular mimicry, which is immunologic similarity between antigenic determinants of the infectious agent or an antigen in a vaccine and an autoantigen, such as a myelin peptide in MS, remains the most appealing hypothetic mechanism by which infections/vaccines may trigger autoimmune tissue damage [90, 91]. The T cell cross-reactivity is a general property of T cell recognition probably needed to balance the requirement to recognize non-self antigens and to reduce the possibility of loss of self-tolerance. In many cases of autoimmunity, it leaves an open question whether cross-reactivity represents an epiphenomenon or a breakdown in the ability of T-cells to distinguish self from non-self antigens through the mechanism of epitope spreading [92, 93]. It is to be, however, remembered that molecular mimicry usually requires several weeks following first exposure to an antigen, while a second exposure to the same antigen might elicit a response within a shorter period of time [94]. Other possible mechanisms, including pathways of innate immunity that have been offered for explaining vaccine induced autoimmunity are: latent viral infection that could possibly persist in the target tissue and potentially result in demyelination; the potential of induction of autoantibodies production and abnormal cytokine production triggered by vaccination [95].

Influenza vaccine impact on spatial learning and memory in MS

MS affects motor, sensory as well as behavioral and cognitive functions. In the end, it is worthwhile to consider the fact that clinically evident cognitive disorders start early in the MS disease [96]. Cognitive deficits experience approximately up to 65% of the MS patients [97]. The most common cognitive symptoms include deficits in the ability to concentrate, mental processing speed, impairment of short-term and working memory. Among these cognitive deficits, memory dysfunction is especially common [98]. What causes memory dysfunction in MS is currently unknown, but neuroimaging studies show demyelination in the hippocampal (a small region of the brain that forms part of the limbic system) structures suggesting that hippocampal pathology is involved regardless of cognitive status [99]. The number of demyelinating plaques in the *corona radiata* (a pair of white matter tracts adjacent to the body of the lateral ventricle), insula (highly important island of cerebral cortex), and hippocampus is especially correlated with cognitive impairment [100]. A study performed by Sacco et al. [101] indicated that gray matter and hippocampal atrophy occured in the MS patients with and without cognitive deficits [101]. Studies in humans and animal models have provided evidence that hippocampus plays a compelling role in spatial memory; the part of memory with an ability to regulate and encode information about the surroundings and to navigate in space [102, 103]. Nonetheless, the hippocampal involvement in cognitive functions has been poorly examined in the MS patients and the underlying pathophysiology of cognitive symptoms has yet to be unraveled. Further, increasing evidence from EAE model suggests that deficits in hippocampal-dependent learning and memory are in correlation with early microglial activation, synaptic alterations and neurodegeneration. The studies of cognitive deficit in animal model EAE can help understand the early molecular and physiologic events in the MS pathogenesis and they represent a diagnostic tool for an early diagnosis of the disease in individuals with familial susceptibility to MS [104].

Neurological and cognitive effects associated with influenza infection have been reported throughout history but the mechanisms underlying these symptoms remain unclear [105–107]. Most influenza strains, including those responsible for pandemics, are considered non-neurotropic, suggesting that neurological symptoms possibly following an influenza infection are not a result of direct inoculation of virus into the CNS areas relevant to cognitive behavior, instead it may be due to neuroinflammation induced by peripheral viral infection [108–110]. Recent experiments have been designed to investigate whether peripheral infection with influenza virus can impact the brain and behavior and to yield insight for preventing inflammation and neuronal damage associated with peripheral influenza infection. Altered cognitive behaviors accompanied by increased microglial reactivity in the hippocampus of influenza infected mice as well as influenza induced alterations in hippocampal neuron morphology provide the first evidence that neuroinflammation and architectural changes to hippocampal neurons may underlie functional deficits in hippocampal-dependent learning and memory during influenza infection as shown in the doctoral thesis done by Jurgens [111].

There is as yet insufficient data concerning the effects of influenza vaccination on cognitive function. At one point,

the goal of our team was to use a MOG induced EAE model to ascertain the effects of influenza vaccine on memory loss and locomotor dysfunctions in mice using Morris Water Maze (MWM) test. MWM is a test of spatial learning for rodents. The basic procedure for the MWM is that the rodent is supposed to find a platform, either visible (non-spatial version) or invisible (spatial version of the test), to escape the water by using various cues [112, 113]. Cognitive dysfunction in infected mice is related to their inability to efficiently navigate to the relocated platform, which is shown by an increase in time and path length to find a new platform [111]. Our findings mainly confirm that there is no effect of influenza split vaccination on memory impairment in MOG induced EAE mice and no significant influence on hippocampal-dependent spatial learning. Additionally, although clinical signs of EAE are very subtle, the lesions that have been found in the hippocampal region of the brain could be associated with memory dysfunction observed during the course of MWM testing [114]. Even though the initial studies have demonstrated that influenza infection induces deficits in spatial learning and memory loss in adult mice at day 7 post infection, conflicting data exist regarding the connection between MS and other autoimmune illnesses and vaccination [95, 111].

Conclusion

The debate as to whether vaccination brings more risk or benefit continues to be a speculative one and to date neither the advantage of vaccination has been refuted nor the vaccination related autoimmune diseases have been irrevocably proved. Nonetheless, the medical community contemplates current human vaccines, including influenza vaccine, safe and effective for patients with autoimmune diseases, and immunization is considered important for these patients not only to prevent an infectious ailment, but also to potentially prevent relapses. It has become evident that immunization *per se* is not enough for triggering autoimmune diseases, and that genetic, immunological and hormonal factors are equally involved.

A wide work is still ahead of us due to a lack of a well-established pathophysiology of the central demyelinating events and pathogenetic mechanisms underlying the adverse neurological events possibly caused by influenza vaccination.

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Conflict of interest

The authors report no conflicts of interest.

R E F E R E N C E S

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CASE REPORTS

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Successful surgical closure of an aortopulmonary window associated with Holt-Oram syndrome in adulthood

Uspešno hirurško zatvaranje aortopulmonalnog prozora udruženog sa Holt-Oramovim sindromom u odraslom dobu

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Abstract

Introduction. Aortopulmonary window (APW) is a rare congenital anomaly caused by incomplete division of the embryonic common arterial trunk which allows direct and usually unobstructed communication between the ascending aorta and pulmonary artery trunk. Holt-Oram syndrome (HOS) is an autosomal dominant disorder caused by the mutation in the TBX5 gene and it is characterized by bones abnormalities in at least one limb while the association with APW is extremely rare. **Case report.** We report a case of a female patient in her thirties with an extremely rare combination of the HOS and APW window that reached the adulthood without surgical correction. The adult patient came to our clinic with signs of severe heart failure and pulmonary hypertension. Although previously diagnosed as inoperable, after the decongestive medical treatment and detailed diagnostic procedures we proved reactive pulmonary vascular resistance and the patient was successfully surgically treated. **Conclusion.** This case confirms the absolute necessity of cautious and comprehensive examinations of each patient with congenital heart disease and pulmonary hypertension irrespective of age.

Key words:
aortopulmonary septal defect; holt-oram syndrome; adult; cardiovascular surgical procedures; treatment outcome.

Apstrakt

Uvod. Aortopulmonalni prozor (*aortopulmonary window* – APW) je retka kongenitalna anomalija uzrokovana nepotpunim razdvajanjem zajedničkog embrionalnog arterijskog stable koja se manifestuje direktnom i neuobičajenom komunikacijom između aorte i plućne arterije. Holt-Oramov sindrom je antozomno-dominantni poremećaj uzrokovan mutacijom TBX5 gena koga odlikuju anomalije kostiju bar na jednom od gornjih ekstremiteta. Njegova udruženost sa APW je veoma retka. **Prikaz bolesnika.** U radu je prikazana bolesnica u četvrtoj deceniji života sa vrlo retkom kombinacijom Holt-Oramovog sindroma i APW koji nije bio zatvoren do odraslog doba. Bolesnica je hospitalizovana sa znacima teške srčane insuficijencije i plućne hipertenzije. Iako prethodno dijagnostikovana kao inoperabilna, posle odgovarajućeg medicinskog tretmana i detaljnih dijagnostičkih procedura dokazana je još uvek značajno reaktivna plućna vaskularna rezistencija nakon čega je bolesnica uspešno operisana. **Zaključak.** Ovaj slučaj potvrđuje neophodnost pažljive i sveobuhvatne dijagnostike bolesnika sa hemodinamski značajnim urođenim srčanim manama i sekundarnom plućnom hipertenzijom bez obzira na njihovo životno doba.

Ključne reči:
aortopulmonalni septalni defect; holt-oram sindrom; odrasle osobe; hirurgija, kardiovaskularna, procedure; lečenje, ishod.

Introduction

Aortopulmonary window (APW) is a rare congenital anomaly caused by incomplete division of the embryonic common arterial trunk which allows direct and usually unobstructed communication between the ascending aorta and pulmonary artery trunk [1]. Usual morphological classification according to the nomenclature reported by Jacobs et al. [2] is based according to APW localization. Closure of APW, either surgical or percutaneous, is necessary and the optimal time to be performed is in early childhood, before irreversible pulmonary hypertension (PHT) and Eisenmenger syn-

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drome are developed [1]. If the pulmonary vascular disease is formed, there is often a "gray zone" between its reversibility or irreversibility [3, 4], which represents a diagnostic challenge and requires serious diagnostic procedures before pronouncing an adult with APW and PHT as inoperable.

Holt-Oram syndrome (HOS) is an autosomal dominant disorder caused by the mutation in the TBX5 gene [5–7] and it is characterized by bones abnormalities in at least one limb. Also, about 75% of individuals with Holt-Oram syndrome have heart abnormalities, most commonly atrial and ventricular septal defect and cardiac conduction disease while the association with APW is extremely rare [8].

Case report

We present a 31-year-old female patient admitted because of symptoms and signs of heart failure [New York Heart Assotiation Functional Classification (NYHA) class III]. According to her past medical history, aortopulmonary window was diagnosed in childhood as part of Holt-Oram syndrome (complete absence of the right hand). Lack of symptoms was the reason for her rejecting the recommended surgical closure. In August 2013, she experienced attacks of severe dyspnea and palpitations followed by leg edema and ascites. On admission, she was dyspneic, acyanotic (SpO_2 99%) and afebrile. The auscultation revealed bilateral basal inspiratory crackles, sinus tachycardia with a heart rate of 110 beats per minute (bpm), a 3/6 systolic and short diastolic murmur over the aorta and systolic regurgitated murmur at the apex. Her blood pressure was 120/50 mmHg on both arms. The abdomen was distended due to hepatomegaly and ascites. ECG showed atrial fibrillation and left ventricular strain. Chest X-ray confirmed cardiomegaly (cardiothoracic index – CTI) 0.75 with increased hilar vascularity and pulmonary congestion. Echocardiography revealed significant enlargemen and moderate reduced contractility of the left ventricle [end-systolic diameter (ESD) 7.9 cm, end-by diastolic diameter (EDD) 6.7 cm, ejection fraction (EF) 37%] with mitral regurgitation grade +3/4 (Table 1). The pulmonary artery was dilated (diameter 3.9 cm) with continuous left–to-right flow (maximal velocity 0.6 m/s by Doppler echocardiography) through a 10 mm wide aorto-pulmonary fenestration best seen on the parasternal short axis view. Moderate pulmonary regurgitation was registered in the normal sized right ventricle with +3/4 tricuspid regurgitation and estimated right ventricular systolic pressure about 74 mm Hg. There were no signs of pericardial effusion.

The patient was treated with diuretics, angiotensin-converting enzyme (ACE) inhibitors, beta blockers, digoxin, amiodarone and anticoagulant therapy. After a few weeks under the therapy her condition was significantly better. After disappearance of ascites and regression of congestive heart failure, cardiac multislice computed tomography (MSCT) was performed for better visualization of the aorto-pulmonary window. Type I of APW diameter 10 mm was diagnosed, localized 39 mm above the sinotubular aortic junction. Cardiac catheterization was performed with testing the pulmonary vascular reactivity by breathing 100% oxygen and nitric oxide, and the results revealed that her mean pulmonary artery pressure decreased from 69 mmHg to 57 mmHg (>10%) and pulmonary vascular resistance decreased from 8.9 WU/m^2 to 0.72 WU/m^2, followed by significant increase of left-to-right shunting (Qp/Qs from 2.2 to 15). Aortography in caudal position revealed left-to-right aortopulmonary shunt through APW (Figure 1), but on pulmonary angiography there were no signs of right-to-left shunt. Since the attempt of percutaneous closure with an Amplatzer device had failed, surgery was indicated. In May 2014, she was successfully operated in Geneva, closing the APW by Gore Tex patch. Although, postoperative treatment with sildenafil was advised, yet she did not include it to her therapy consisting of digoxin, oral anticoagulant therapy and small doses of ACE inhibitors, beta blockers and amiodarone. One month after the operation, our patient was free of symptoms and with good exertion tolerance (NYHA class I). Atrial fibrillation persisted with well-controlled heart rate and blood pressure 130/80 mmHg. Echocardiographic examination revealed a decrease of left ventricle diameters (EDD 6.9 cm, ESD 5.4 cm) and significantly improved global systolic function (EF 55%) by reduction of mitral regurgitation grade to + 2/4. Systolic right ventricle pressure also decreased to about 43 mmHg estimated according to mild tricuspid regurgitation (Table 1).

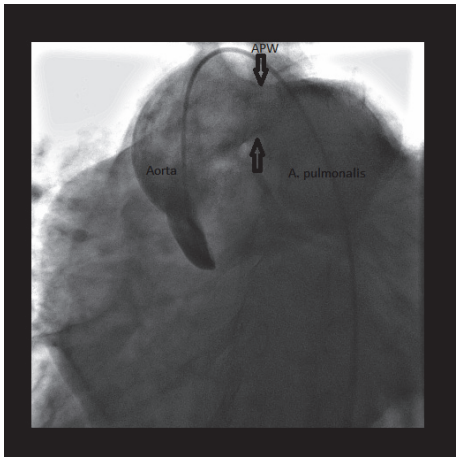

Fig. 1 – Aortography in caudal position revealed left-to-right aortopulmonary shunt through aortopulmonary window.

Table 1

Clinical and echocardiographic parameters before and after the operation of aorto-pulmonary window

Parameters	Preop.	Postop
LVD (mm)	79	69
LVS (mm)	67	54
EF (%)	37	55
MR (grade)	+ 3/4	+ 2/4
TR (mmHg)	74	43
NYHA class	III	I

LVD – left ventricular diastolic dimension; LVS – left ventricular systolic dimension; EF – ejection fraction; MR – mitral regurgitation; TR – pressure gradient of tricuspid regurgitation.

NYHA class – New York Heart Assotiation Functional Classification; Preop – preoperative; Postop – postoperative.

Table 2

Catheterization data before and after operation of aortopulmonary window

Parameters	Before testing	After testing with oxygen and nitric oxide	Postop.
Right atrium pressure (mmHg)	12	12	4
Pulmonary capillary wedge pressure (mmHg)	11	11	8
Mean PA pressure (mmHg)	69	58	18
Mean PA pressure / mean arterial pressure (mmHg)	0.97	0.77	0.22
Qp/Qs	2.2	15	0
Pulmonary vascular resistance (WU/m^2)	8.9	0.72	
Rp/Rs	0.44	0.05	

PA – pulmonary artery; Qp/Qs – ratio of pulmonary and systemic arterial flow; Rp/Rs – ratio between pulmonary and systemic arterial vascular resistance.

On the anterolateral wall of the aorta towards the pulmonary artery, a 12 mm large hiperechogenic zone was visualized, corresponding to the postoperative patch without signs of residual aortopulmonary shunt. Postoperative cardiac catheterization confirmed normalization of pressure in the pulmonary circulation (Table 2) and the absence of aortopulmonary shunt on aortography (Figure 2).

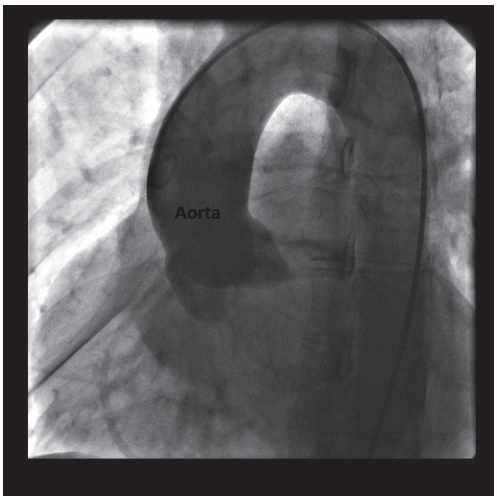

Fig. 2 – The absence of aortopulmonary shunt on aortography.

Discussion

Aortopulmonary window is a very rare cardiac anomaly with incidence from 0.1% to 0.2% of all congenital heart defects [1], described for the first time by Elliotson in 1830. It can be a simple, isolated defect presenting as the only cardiac malformation or associated with other cardiovascular malformations (in 47%–77% of the cases [1]). When diagnosed, APW should be promptly closed, whether surgically or with transcatheter intervention [9]. Diagnosis are usually made early at birth or in childhood and there are extremely rare reports of adults with this diagnosis and especially rare with successful treatment in adulthood. Su-Mei and Ju-Le [10] reported an adult female patient with Eisenmenger syndrome due to untreated APW that survived into adulthood, gave birth to three children in her early thirties, and died in her sixties.

Holt-Oram syndrome, also known as heart-hand syndrome, was first described by Holt M. and Oram S. in 1960 [7]. It is characterized by congenital heart abnormalities and skeletal malformations of the upper limb, ranging from subtle changes such as hypoplasia or absence of the thumb to complete absence of the hand with the left side usually being more often affected [8]. HOS must be differentiated from Okihiro syndrome [11], as those two syndromes have certain clinical features overlap, such as various forms of forearm malformations and ventricular and atrial septal defects that are sometimes presented in Okihiro syndrome. Unlike HOS, Okihiro syndrome is associated with Duane anomaly (limitation of eye abduction associated with retraction of the eye globe and narrowing of the palpebral fissure on adduction). Anal stenosis, renal abnormalities, pigmentary disturbance, hearing impairment, external ear malformations and facial asymmetry can also appear as part of Okihiro syndrome. Finally, Okihiro syndrome is caused by the mutation of SALL4 gene, and HOS by the TBX5 gene mutation.

Epidemiological and clinical aspects of HOS patients were presented using data from European Surveillance of Congenital Anomalies (EUROCAT) registries [12]. The mean prevalence of HOS diagnosed in European registries was 0.7 per 100,000 births or 1:135,615 births. EUROCAT studies collected data from 1990 to 2011 from 34 registries and identified a total of 73 cases of HOS. Congenital heart defects (CHD) were revealed in 78.7% (48/61) of patients. Isolated septal defects were present in 54.2% (26/48) while 25% (12/48) of the patients had complex/severe CHD. In EUROCAT registries there were not any patients with HOS in association with APW.

There are only two cases of APW associated with HOS in the literature (according to Medline database search); one was described by Ulrich et al. [8] in 2004, as the first such case. They presented a female patient with Mayer-von Rokitansky-Kuster-Hauser syndrome in association with a HOS and aortopulmonary window treated surgically when she was 6 months old. The other one is from 2014. Srinivas et al. [13]

presented a 4-month-old male infant who had absence of radius and the first metacarpal and phalangeal bones on the right side together with aortopulmonary window and small secundum atrial septal defect. APW was successfully treated by device closure. The main contraindication for the APW treatment in adulthood is already established pulmonary hypertension. In our case, the patient was hospitalized with signs of acute cardiac failure and atrial fibrillation for which she was initially treated with decongestive, antiarrhythmic, anticoagulant and pulmonary vasodilatation therapy. After the stabilization of the cardiac function and control of atrial fibrillation the next step was to define whether the pulmonary arterial hypertension was reversible or irreversible by testing pulmonary vasoreactivity during cardiac catheterization. Testing is usually conducted using short-acting vasodilators such as inhaled nitric oxide, intravenous epoprostenol, adenosine and inhaled 100% oxygen [14]. We performed the test with 100% oxygen and nitric oxide and got a reduction of pulmonary pressure for > 10%, significant increasing of left-to-right shunt and fall of pulmonary vascular resistance (Table 2). Those results, together with improved cardiac function and general health condition of the patient after intensive decongestive and antiarrhythmic therapy suggested that the closure of APW is possible with a relatively low operative risk.

After detailed examinations, APW was successfully surgically closed without complications in the postoperative period. Postoperatively, we registered significant clinical, echocardiographic and hemodynamic improvement in the patient with complete normalization of pressure in pulmonary circulation (Tables 1 and 2). Although the quality and length of life of the patient was significantly improved, it should be kept in mind that specific long-term consequences of left-right shunt in the form of moderate mitral regurgitation and left heart dilatation with atrial fibrillation still remain.

Conclusion

The outcome of hemodynamically significant congenital heart disease in adult patients can sometimes be surprisingly favorable, but making good decisions about further treatment requires detailed diagnosis and careful cross-analysis of numerous medical histories, clinical and hemodynamic parameters.

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CASE REPORT

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Successful implementation of rituximab in patients with severe refractory forms of granulomatosis with polyangiitis

Uspešna primena rituksimaba kod bolesnika sa teškom refraktarnom formom granulomatoze sa poliangiitisom

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Abstract

Introduction. Wegeners's granulomatosis is a disease characterized by granulomatous inflammation of the upper and/or lower respiratory tract, glomerulonephritis with varying degrees of small vessel vasculitis and classic anti-neutrophil cytoplasmic antibodies (c-ANCA) findings. The treatment uses different modalities of immunosuppressive therapy which does not always lead to remission. We presented the efficacy of biological therapy in a patient with refractory form of the Wegeners's granulomatosis. **Case report**. A 23-years-old patient, was treated in August, 2011 at the Clinic of Otorhinolaryngology and Maxillofacial Surgery of the Clinical Center of Serbia because of suppurative otitis media, resulting twice in mastoidectomy. On the day 7 after the surgery, hemoptysis and fever occurred. Considering lung x-ray that showed presence of the round soft-tissue changes on both sides, nonspecific inflammatory syndrome in laboratory analysis and positive c-ANCA (1 : 160) with high titers the antibodies to the proteinase 3 (anti-PR 3), Wegener's granulomatosis was diagnosed. Due to the fact that administration of glucocorticoids, cyclophosphamide and immunomodulatory dose of immunoglobulin did not lead to clinical remission, it was decided to apply rituximab. After its application clinical remission occurred and it lasted fifteen months. **Conclusion**. Application of biologic therapy might be successful in the treatment of patients with severe form of refractory granulomatosis with polyangiitis.

Key words:
granulomatosis with polyangiitis; therapeutics; biological therapy; rituximab; treatment outcome.

Apstrakt

Uvod. Wegener-ova granulomatoza je oboljenje koje karakterišu granulomatozna inflamacija gornjih i/ili donjih disajnih puteva, glomerulonefritis, uz različit stepen vaskulitisa malih krvnih sudova i nalaz c antineutrofilnih citoplazmatskih antitela (ANCA). U lečenju se koriste različiti modaliteti imunosupresivne terapije koji ne dovode uvek do remisije. Prikazana je efikasnost biološke terapije kod bolesnika sa refraktarnom formom Wegener-ove granulomatoze. **Prikaz bolesnika**. Bolesnik, star 23 godine, lečen je avgusta 2011. godine na Klinici za otorinolaringologiju i maksilofacijalnu hirurgiju Kliničkog Centra Srbije zbog gnojne upale srednjeg uva. U dva navrata je rađena masteidektomija, a sedmog dana nakon operacije došlo do pojave hemoptizije i febrilnosti. S ozirom na rendgentski snimak pluća na kome su viđene okrugle mekotkivne promene obostrano, laboratorijske analize koje su ukazale na nespecifični zapaljenski sindrom i pozitivne c ANCA (1 : 160) uz povišen titar antitela protiv proteinaze 3 (anti-PR3 antitela), postavljena je dijagnoza Wegener-ova granulomatoze. S obzirom na to da primena glikokortikoida, ciklofosfamida, imunomodulatorne doze imunoglobulina nisu doveli do kliničke remisije, odlučeno je da se primeni rituksimab. Nakon njegove primene uspostavljena je klinička remisija koja je trajala petnaest meseci. **Zaključak**. Primena biološke terapije može biti uspešna u lečenju bolesnika sa teškom refraktarnom formom granulomatoze sa poliangiitisom.

Ključne reči:
vegenerova granulomatoza; lečenje; biološka terapija; rituksimab; lečenje, ishod.

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Introduction

Granulomatosis with polyangiitis is a systemic autoimmune disease which affects upper airways, lungs, kidneys, but any organ could be affected [1]. Diagnosis is based on clinical manifestation of systemic vasculitis and histologically proven necrotizing vasculitis of the blood vessels or granulomatous inflammation [2]. A specific laboratory parameter is the emergence of classic anti-neutrophil cytoplasmic antibodies (c-ANCAs) in almost 90% of patients. These are the antibodies to the proteinase 3 (anti-PR 3) which is found in the granules of neutrophils. Height of the c-ANCAs titer can be an indicator of disease activity [1]. The clinical picture is severe, remissions are followed by higher percentage of relapses and it is a potentially life-threatening systemic autoimmune disease. The use of aggressive initial immunosuppressive therapy [glucocorticoids and cyclophosphamide (CYC)] is justified because of the high rate of the mortality. Half of the untreated patients died within 6 months while 90% of patients died during 2 years, mainly because of respiratory or renal insufficiency [3].

We presented a patient with refractory form of granulomatosis with polyangiitis (Wegener's granulamatosis) successfully treated with biological therapy.

Case report

Male patient, born in 1988, from Belgrade, was treated in August, 2011 at the Clinic of Otorhinolaryngology and Maxillofacial Surgery Clinical Centre of Serbia, due to purulent otitis on the right ear. Mastoidectomy with radical trepanation in the process at the top of the pyramid to the right was done. Since inflammatory syndrome maintained after intensive use of antibiotics, reintervention was done. Seven days after reintervention, due to the poor clinical course, despite the antibiotic therapy (clindamycin, ciprofloxacin) – maintenance of febrile status and elevated inflammatory factors were registered. Bloody sputum occurred. Radiographic findings, and, then, the thoracic computed tomography (CT) showed presence of the round soft-tissue changes on both sides in the lung parenchyma with suspected white shadows. The largest one was localized left, paracardially and was later confirmed by multi-slice (MSCT). Therefore, in September 2011, the patient was transferred to the Clinic of Pulmonology, Clinical Centre of Serbia. Laboratory results showed inflammatory syndrome while all other findings were within normal values including the urine sediment. Immediately on admission, combined antibiotic therapy was administered: clindamycin, cefuroxime, metronidazole, in the further course vancomycin 2 × 1 g, imipenem 3 × 1 g, sulfamethoxazole and trimethoprim, 2 × 2 amp, and at all times patients was febrile. During this hospitalization, pathogenic bacterial flora was not isolated on the bacteriological examination of sputum and by direct microscopy of sputum acid resistant bacilli (ARBs) were not observed (3 ×). Also, there were negative Löw culture, and blood negative on ARBs (3 ×). Tumor markers had normal values.

Skin tests and sputum for aspergillus bronchoalveolar lavage (BAL) as antibodies to the antigen aspergillus were negative.

Preserved renal function involved proteinuria and creatinine clearance normal within 24 hours. Bronchial endoscopy indicated signs of inflammation, fiber aspirate was negative on ARBs, and PH bronchoscopy findings revealled chronic bronchitis. After the results of immunoassays: cANCA 1 : 160, anti-PR3 42.3 relative units (RU)/mL, polyangiitis with granulomatosis was diagnosed and patient was transferred to the Clinic of Allergology and Immunology at the Clinical Center of Serbia. Methylprednisolone 3 × 80 mg, was introduced into the therapy. One day after the introduction of the corticosteroid, the patient become afebrile.

During hospitalization, patient received high doses of glucocorticoids (1 mg/kg) followed by gastroprotective therapy and also sulfamethoxazole and trimethoprim. Pulse therapy with CYC at the dose of 1,000 mg, was started and the patient continued to receive it at monthly intervals for the next 5 months, with good clinical and laboratory effects: reduction of the sedimentation (SE) from 90 g/L to 6 g/L, fibrinogen from 6.2 g/L to 2.2 g/L (normal range 2–4 g/L) C-reactive protein (CRP) from 75 mg/dL (normal range 0–10 mg/dL) to 0.2 mg/dL c ANC 1:160 to 1:20, anti-PR3 from 42.3 to 29.9.

In March 2012 the patient was admitted with clinical deterioration: febrile, body temperature up to 39°C, with epistaxis, followed by cough and expectoration of bloody and mucus sputum. Physical findings showed both-sided late-inspirium breaks, more on the left side. Laboratory results showed inflammatory syndrome: SE 90 g/L, fibrinogen 9.9 g/L, CRP 28 mg/dL, leukocites (Le) 22 × 10^9/L (normal range 4.5–11 × 10^9/L), trombocytes (Tr) 609 × 10^9/L (normal range 150–400 × 10^9/L), c-ANCA 1 : 80 and anti-PR3 96.8 RU/mL. Lung and heart X-ray showed one nodose shadow in the mid-lung in midclavicular line on the right and a large nodose shadow in the mediastinum. In the mid-lung on the left, closer to the mid-shadow, a rounded shadow was visible as well as a nodose shadow, closer to the thoracic wall, which corresponded to the primary disease confirmed by the MSCT (Figure 1). The patient was treated with antibiotics, ceftriaksone and ciprofloxacin, with higher doses of glucocorticoids, including pulse doses of methylprednisolone 1,000 mg daily and 6 pulses of CYC at a dose of 1,000 mg, but with incomplete therapeutic effect of the applied therapy. He was discharged with a dose of 40 mg of prednisolone and from May, a therapy with CYC orally at a dose of 150 mg per day was applied. The clinical course of the disease did not improve. Besides activities and primary disease complications, especially colliquation of nodular and necrotic changes, clinical picture was complicated by superimposed respiratory infections and adverse effects of applied glucocorticoid therapy (signs of Cushing's syndrome). In October 2012, patient was again admitted in deterioration, febrile 38.5°C, with cough and expectoration of yellow-green sputum, chest pain, more pronounced in the inspiratory phase, with abundant physical findings in the lungs with low pitch wheezes and rales present in inspirium and expirium. Labo-

ratory results showed inflammatory syndrome, cANCA 1 : 160, anti-PR 3 131 RU/mL. Sputum on bacillus Koch (BK), [mycobacteria growth indicator tube (MGIT) test] was negative. In sputum, *pseudomonas aueriginosa* was isolated. In the lungs, parenchyma round shadows on both sides and nodular shadows in the upper lung field were found as well as abscess mass paracardially on the left with pulmonary condensation zone, indicating poliangiitis with granulomatosis. The patient was treated with a long-term combined antibiotic therapy (piperacillin, imipenem, amikacin) according to the sputum antibiogram in which repeatedly *Pseudomonas aueriginosa* was isolated and with intravenous (*iv*) application of immunoglobulin at a dose of 400 mg/kg for 5 days.

Fig. 1 – Multislice computed tomography (MSCT) before application of rituximab reveals rounded shadow in mid-lung and large nodose shadow in the mediastinum.

All modalities of standard immunosuppressive and immunomodulatory therapy (CYC, glucocorticoids, immunoglobulins) were used, but the disease still had a progressive course which was further complicated by the formation of numerous cavities with colliquative necrosis and superimposed infection. For all these reasons, a consultative decision on the application of rituximab (RTX) was made. It was implemented as an induction dose of 375 mg/m^2, once a week, for 4 weeks, with appropriate premedication, which was approved by the Ethics Committee of the Clinical Center of Serbia. During January and February 2013, an induction of RTX was conducted, at a dose of 375 mg/m^2, once a week, during 4 weeks with premedication (paracetamol at a dose of 1,000 mg *iv*, methylprednisolone 80 mg *iv*, desloratadin 1 × 1 tbl, ranitidin amp in a small infusion). The patient was in good health condition during the treatment. During that hospitalization, the patient was firstly treated with imipenem at a dose of 1.5 g (according to the sputum antibiogram done due to isolated *Pseudomonas auriginosa*), after which the results were normal in repeated samples. Afterwards, the patient had ambulatory follow-ups and was in remission for 15 months. Lung and heart X-ray showed a stationary findings in terms of morphological changes in the lungs. Immunological findings were as follows: cANCA 0 and anti-PR3 19 RU/mL. During periods of remission, B-cell depletion in the sample was verified and it was detected a scarce population of B Ly CD19.8/μL (the reference range 80–490/μL) with elevated absolute lymphocytes count, elevated absolute T-lymphocytes count, elevated absolute CD4+ T cells and, CD8+ T cells (CD3, 3,386/μL; CD3/CD4 1,759/μL and CD3/CD8/1,693 μL). Bacterial smear of specimens from throat, nose, ear and sputum were negative.

Considering that RTX in our country is not registered for use in Wegener's granulamatosis, we were not able to apply any protocol except the "watch and wait" principle of treating. The following relapse occurred in May 2015. Clinical picture included weight loss, cough and hemoptysis with inflammatory syndrome, reactive thrombocytosis, reconstitution of B-cells [immunophenotyping results showed that B lymphocytes population was in relative and absolute values within the reference range, while the absolute values of the total T lymphocytes, subpopulation CD4+ T lymphocytes and natural killer (NK) lymphocytes were significantly reduced], and positivisation of c-ANCA 1:80 and anti-PR3 150 RU/mL. The patient was hospitalized and reapproval of the Ethics Committee for the application of RTX was requested. In the meantime, the patient received high doses of immunoglobulins (human immunoglobulin during 5 days at a dose of 30 g) with combined antibiotic and antimycotic therapy. In June 2014, RTX was repeated during 4 weeks at a dose 4 × 375 mg/kg. During that therapy, the patient received imipenem and fluconazole with probiotics and was re-introduced in a stable clinical remission with complete calming of the inflammatory syndrome and ANCAs negativisation. Lung X-ray finding after the application of rituximab is shown in Figure 2. Since then, the patient has been on regular therapy with prednisone at a dose of 20 mg and methotrexate at a dose of 15 mg per week along with other symptomatic therapy.

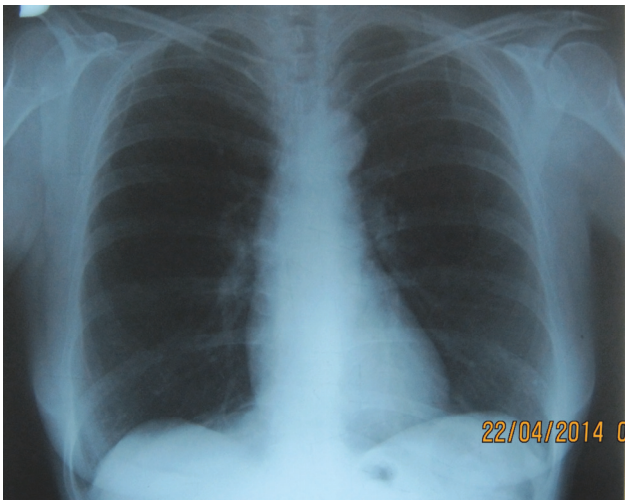

Fig. 2 – Lung X-ray after application of rituximab.

Discussion

European League Against Rheumatism (EULAR) published valid recommendations for the treatment of Wegener's granulamatosis in Europe. For induction of remission in generalized forms of the disease it is recommended to apply CYC orally during 3–6 months, or, 6–9 cycles *iv* with application of corticosteroids or, application of RTX at a dose of 375 mg/m^2 in 4 cycles. As therapeutic option for life-threatening vasculitis, especially in rapidly progressive glomerulonephritis and pulmonary hemorrhage plasmapheresis is occommended [4, 5]. Maintenance regimen with azathioprine has been replaced by metothrexate, leflunomide and mycophenolate mofetil [4, 6, 7].

Refractory disease is defined as a disease in which the maximum tolerated dose of CYC did not have a therapeutic effect, or, CYC could not be applied because of some adverse toxic effects (leukopenia, hemorrhagic cystitis) or contraindications such as the existence of malignancy [8]. For refractory disease or relapse, intravenous application of immunoglobulins for 5 days is recommended, or, application of RTX at a dose of 375 mg/m^2 in 4 cycles. As an option, infliximab or mycophenolate mofetil are proposed [1].

The presence of B-lymphocytes is essential for the regulation of the immune response and production of antibodies. Also, they have multiple roles such as expression of costimulating molecules, production of cytokines, the role of antigen presenting cells, regulation of the activation and differentiation of T-lymphocytes and dendritic cells [9]. They are also responsible for the production of ANCAs that have multiple proinflammatory effects leading to damage of various tissues, organs, and vasculitis [9–11].

RTX is a chimeric monoclonal antibody against the CD20 antigen, which is superficial cell antigen located on pre-B and mature B lymphocytes, but not on the pro B- cells or plasma cells and blasts. RTX allows cell-mediated cytotoxicity, complement-mediated cytotoxicity and inhibits the early B-cell activation and differentiation resulting in rapid and prolonged depletion of B lymphocytes [12].

Two randomized controlled trials, RAVE (RTX in ANCA associated vasculitis) and RITUXIVAS (RTX *vs* CYC in ANCA asociated vasculitis), have shown that RTX is effective as CYC for induction of remission in newly diagnosed patients with severe ANCA associated vasculitis and even superior in severe relapses, resistant cases and also, in such cases when it would be desirable to avoid the side-effects of CYC [10, 13, 14]. Most published reports speak in favor of the extraordinary therapeutic response to the application of RTX with a minimum of side effects and rare relapses. Despite the fact that the B-cells level reduction after RTX administration was not observed, increased incidence rate of serious infections in these patients was noticed [13].

The standard protocols of RTX application have not yet been determined. Studies should demonstrate whether it is more efficient principle of RTX administration cycles at certain fixed intervals, or „watch and wait“ approach when a decision about re-application of RTX, in case of relapse, is made based on monitoring of biomarkers and/or the clinical picture. Advised protocols of maintenance therapy are repeated dose of RTX, at a dose of 1 g, in 4-month intervals or 2 per year [4].

Specks et al. [15] showed that neither the number of B-cells ur ANCA titer were predictors of relapse, but as long as both the B-cells and ANCA antibodies were not detectable, a risk of relapse was very low [15]. Expected reconstitution of peripheral B-cells was up to 18 months [2]. Cartin-Ceba R et al. [14] study followed a group of 53 patients with a diagnosis of granulomatous polyangiitis who received at least 2 cycles of RTX on average. They all had depletion of B lymphocytes, all relapses were followed by the B-cells reconstitution and an increase of the c-ANCA level, except in the case of one negative ANCA patient. The average time of reconstitution was 8.5 months. In the case of our patients, remission followed by depletion of B lymphocytes lasted 15 months after the first administration of RTX. Monitoring of the ANCA titer i.e. their positivity, and CD19+ B lymphocytes number, i.e. the reconstitution of their number, were reliable markers of relapse, together with inflammatory syndrome and the clinical picture. In periods of remission, there was a negativation of ANCA titer antibodies and depletion of B19+ B lymphocytes number.

Conclusion

The application of standard immunosuppressive therapy does not always result in remission of the Wegener's granulamatosis. Therefore, it is necessary to think about new treatment modalities. Previous worldwide experience in the treatment of this disease, as well as our case reported in which RTX was applied for the first time in Serbia, confirms the importance and effectiveness of biologic therapy. RTX is a promising alternative therapy to the previous immunosuppressive therapy. Also, repeated RTX treatment can be individualized by monitoring laboratory parameters such as CD19+ B lymphocyte numbers and c-ANCA level based on a "watch and wait" principle.

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LETTER TO THE EDITOR

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An equation for tacrolimus daily dose calculation in renal transplant patients – simple and cost saving?

Formula za izračunavanje dnevne doze takrolimusa kod bolesnika sa transplantiranim bubregom – jednostavna ušteda novca?

Dear Editor,

One of the most important services given by clinical pharmacologists in the Serbian health care system is therapeutic drug monitoring (TDM) [1]. In the Military Medical Academy, tertiary health care hospital in Belgrade, TDM for tacrolimus, one of the most important immunosuppressant used for prevention of allograft rejection in kidney transplantation, is used in routine clinical settings [2]. This is due to the fact that inappropriate tacrolimus blood concentrations are often met as a result of considerable between-and within-subject pharmacokinetics variability. Numerous factors identified as contributors are gender, body mass index, hematocrit, albumin concentration, liver dysfunction, corticosteroids and other comedication, food, gene polymorphism, etc. [2–7]. Since tacrolimus through concentrations (TTC) are routinely monitored and the dose is adjusted, based mainly on measurements, the influence of aforementioned multiple factors are not supervised in the consistent manner by various transplantation centers. In an attempt to obtain a novel, better approach, tacrolimus concentration/dose (C/D) ratio, a relatively simply obtained TDM tool, has been suggested to better define tacrolimus exposure profile [3, 8]. Gender, comedication of renal transplant recipients with proton pump inhibitors, diuretics, calcium channel blockers, various doses of corticosteroids as well as genetic polymorphisms of CYP3A4 and CYP3A5 enzymes and P-glycoprotein drug transporter, were shown as the most important factors affecting both TTC and tacrolimus C/D ratio [5, 6, 8]. However, even more practical approach was needed until validation of a target C/D ratio is performed, i.e., a correlation with clinical endpoints in the real clinical settings. Therefore, relatively simple equation for tacrolimus dosing was constructed depending on variables analyzed. It was obtained by a multiple regression analysis and related total daily dose of tacrolimus per body weight, corticosteroid dose, gender as well as proton pump inhibitors dose, since they were shown as the most prominent factors in our analysis [8]. Former equations concerning improved tacrolimus dosing are based on population pharmacokinetics principles [9, 10], while ours result from parameters used in everyday practice related to TTC. Equation 1 goes as following:

Tacrolimus daily dose per body weight (mg/kg) = 0.032628 + 0.00312*TTC + 0.005220*gender – 0.013887*proton pump inhibitors + 0.012488*corticosteroid

where TTC is desired value previously set, gender value is 1 for females and 0 for males; proton pump inhibitors has value 1 if they are used, while 0 is correct if they are not used; corticosteroid value should be 1 for its doses < 0.15 mg/kg, 2 for 0.15–0.25 mg/kg dose and 3 if it exceeds values of 0.25 mg/kg. According to this equation table 1 presents recommendations for tacrolimus dosing.

Table 1
The recommended total daily dose of tacrolimus per body weight (mg/kg), according to equation 1

Gender	Corticosteroid dose (mg/kg)	Proton pump inhibitors mean [95% confidence interval (lower-upper values)]	
		Applied	Not applied
male	< 0.15	0.056 (0.050–0.062)	0.070 (0.066–0.074)
female		0.061 (0.055–0.068)	0.075 (0.070–0.081)
male	0.15–0.25	0.069 (0.064–0.074)	0.083 (0.079–0.086)
female		0.074 (0.069–0.079)	0.088 (0.084–0.092)
male	> 0.25	0.081 (0.075–0.087)	0.095 (0.091–0.099)
female		0.086 (0.081–0.092)	0.100 (0.096–0.105)

This relatively simply constructed calculations based on the gender of the transplant recipients, proton pump inhibitors use, corticosteroid dose applied and preset value of tacrolimus through concentrations may help in optimization of tacrolimus daily dosing in patients subjected to numerous control examinations in our transplantation center after surgery [2]. We do hope that its introduction and validation would

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also make cost savings possible both for patients and our health care system as a whole.

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I N M E M O R I A M

Svetlana Kovačević (1950–2017)

Krajem prošle godine (2. decembra 2017), posle duge i teške bolesti, napustila nas je Svetlana Ceca Kovačević, dugogodišnji član kolektiva Instituta za naučne informacije Vojnomedicinske akademije u Beogradu (sada Univerziteta odbrane u Beogradu), i lektor za srpski jezik časopisa „Vojnosanitetski pregled" u periodu 1992-2008. godina.

Rođena je 7.6.1950. u Osijeku. Od rođenja do završetka školovanja živela je u Beogradu. Posle završene gimnazije, 1969. godine, upisuje se na Filološki fakultet Univerziteta u Beogradu, Odsek za srpskohrvatski jezik i jugoslovensku književnost, na kome diplomira 1975. godine. Još od srednjoškolskih dana bila je društveno aktivna. Učestvovala je na dve radne akcije (1968. i 1969) na kojima je proglašena za udarnika, na fakultetu je bila sekretar osnovne organizacije Saveza komunista Jugoslavije Odseka za srpskohrvatski jezik i jugoslovensku književnost, a kasnije i član Komisije za kulturu Skupštine opštine Palilula.

Odmah posle diplomiranja zaposlila se kao profesor srpskohrvatskog jezika u Osnovnoj školi Vajska, gde je radila do 1976. godine. Godinu dana kasnije (1977) prelazi na službu u Ministarstvo odbrane i tu ostaje da radi do kraja svoje profesionalne karijere 2011. godine, kada odlazi u zasluženu penziju. Na početku svoje „vojne" karijere bila je referent za kulturu i domaćin Doma Jugoslovenske narodne armije u Pivki, Slovenija (od 1977. do 1980). Od 1980. do 1982. godine radila je kao rukovodilac arhiva Vojne bolnice u Ljubljani u kojoj, posle položenog državnog ispita za bibliotekara 1982. godine, prelazi na dužnost bibliotekara. U tom periodu bila je aktivni član sindikata u toj ustanovi.

Posle raspada SFRJ 1991. godine, dolazi u Vojnomedicinsku akademiju (VMA) u Beogradu, u Odsek za informisanje Uprave VMA kao pomoćnik načelnika te službe. Sa tog radnog mesta prelazi u stručnu biblioteku Instituta za naučne informacije, u kome je od 2006. godine do penzionisanja bila načelnik bibliotečke službe.

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Prof. dr Silva Dobrić

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Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

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Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

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2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure **– Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi – **Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

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Tabele

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

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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