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SADRŽAJ

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VALUE OF PROTECTIVE STOMA IN RECTAL CANCER SURGERY

ZNAČAJ PROTEKTIVNE STOME U HIRURGIJI KARCINOMA REKTUMA

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Summary

Introduction. Anastomotic leakage is the most serious surgical complication in rectal surgery. The aim of this study was to find out whether a protective stoma was capable of lowering the rate of clinical anastomotic leakage and to evaluate the rate of anastomotic leakages requiring re-surgery. **Material and Methods.** A retrospective study included a sample of 149 consecutive patients with rectal cancer who had undergone elective rectal resection with primary anastomosis. After total mesorectal excision, the anastomosis was created using either the single stapling or double stapling anastomotic technique. Anastomotic integrity was verified by transanal air insufflations with the pelvis filled with saline. A protective covering colostomy was added in selected cases and according to the surgeon's preference. **Results.** A protective stoma was created in 31% of patients. Clinical anastomotic leakage occurred in 6.7% of patients (10/149). Anastomotic leakage occurred in 8.5% of the patients with a protective stoma (4/47) and in 5.9% of those without a protective stoma (6/102), which was not statistically significant. Surgery lasted significantly longer when a stoma had to be created than in case when it was not needed (p=0.024). The overall rate of re-surgery due to postoperative surgical complications was 5.3% and in three cases this happened because of anastomotic leakage. All patients with a protective stoma and clinical anastomotic leakage were treated conservatively, compared to 50% of patients without a protective stoma who suffered anastomotic leakage and had to be operated. **Conclusion.** A stoma cannot prevent but it can surely minimize surgical complications related to anastomotic leakage and it does reduce the rate of re-surgery.

Key words: Rectal surgery; Anastomotic leakage; Protective stoma.

Sažetak

Uvod. Popuštanje anastomoze predstavlja najozbiljniju hiruršku komplikaciju u hirurgiji rektuma. Cilj naše studije bio je da utvrdimo da li protektivna stoma smanjuje učestalost klinički manifestnog popuštanja anastomoze i učestalost reoperacija usled popuštanja anastomoze. **Materijal i metode.** Sprovedena je retrospektivna studija sa 149 bolesnika sa karcinomom rektuma kod kojih je načinjena elektivna resekcija rektuma sa primarnom anastomozom. Nakon totalne mezorektalne ekscizije, kreirana je anastomoza korišćenjem jednostaplerske i dvostruke staplerske tehnike. Integritet anastomoze je proveravan pomoću transanalne insuflacije vazduha sa karlicom ispunjenom fiziološkim rastvorom. Protektivna kolostoma je kreirana u odabranim slučajevima u skladu sa mišljenjem hirurga. **Rezultati.** Protektivna stoma je kreirana kod 31% bolesnika. Klinički manifestno popuštanje anastomoze potvrđeno je kod 6,7% (10/149) bolesnika. Kod bolesnika sa protektivnom stomom popuštanje anastomoze utvrđeno je kod 8,5% (4/47) u poređenju sa 5,9% (6/102) kod bolesnika kod kojih nije kreirana protektivna stoma, te nije utvrđena statistički značajna razlika. Vreme operacije je bilo statistički značajno duže kod bolesnika kod kojih je kreirana protektivna stoma u poređenju sa operacijama kod kojih stoma nije kreirana (p = 0,024). Ukupna stopa reoperacija zbog postoperativnih komplikacija bila je 5,3% i u tri slučaja uzrok je bilo popuštanje anastomoze. Svi bolesnici sa protektivnom stomom i kliničkim popuštanjem anastomoze tretirani su konzervativno u poređenju sa 50% bolesnika bez protektivne stome kod kojih je utvrđeno popuštanje anastomoze i koji su morali biti operisani. **Zaključak.** Stoma ne može da prevenira, ali sigurno može da smanji rizik od hirurških komplikacija koje su u vezi sa popuštanjem anastomoze i može da smanji učestalost reoperacija.

Ključne reči: Hirurgija rektuma; Popuštanje anastomoze; Protektivna stoma

Introduction

During the last two decades there was an increased proportion of sphincter-saving procedures in rectal cancer surgery due to better staging, surgical technique, introduction of staplers and preoperative irradiation [1–4]. However, this has resulted in an increased number of patients exposed to the risk of anastomotic leakage (AL). AL is the most serious surgical complication in rectal surgery with incidence ranging from

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Abbreviations
AL – anastomotic leakage
CSA – circular single stapled anastomosis
DSA – double stapled anastomosis
ASA – American Society of Anesthesiologists
CRT – chemoradiotherapy

1.5 to 23%. Most studies give leakage rates in the range of 9% to 12% [5–8] and the associated risk of postoperative mortality is between 6% and 22% [5]. Since morbidity and mortality resulting from AL are considerable, the routine use of defunctioning stomas has been suggested for high-risk anastomosis. The aim of our study was to find out whether a protective stoma was capable of lowering the rate of clinical AL and to evaluate the rate of AL requiring re-surgery.

Material and Methods

All the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 7th Revision of the Declaration of Helsinki from 2008.

This retrospective study included 149 consecutive patients with rectal cancer (within 15 cm from anal verge), who had undergone elective rectal resection with primary anastomosis between 2006 and 2010 at the Institute for Oncology of Vojvodina, Department of Surgical Oncology. Data on age, gender, stage of tumor, distance from the anal verge, histological grade, neoadjuvant treatment, type of anastomosis technique (single stapling vs. double stapling), complications, anastomotic leakage rate, duration of operation and hospital stay were collected.

Rectal cancer was defined as a tumor located within 15 cm of the anal verge. The distal resection margin was defined as the distance from the lowest border of the tumor to the distal mucosal end of the fixed specimen.

The patients with locally advanced rectal cancer were referred for neoadjuvant chemoradiation con-

Table 1. Patients' characteristics
Tabela 1. *Karakteristike bolesnika*

	Total *Ukupno*	With protective stoma *Sa protektivnom stomom*	No stoma *Bez stome*
Number of patients operated on/*Broj operisanih bolesnika*	149	47	102
Mean age (range)/*Srednja vrednost godina (rang)*	65 (39-88)	65 (40-82)	64 (39-88)
Gender/*Pol*			
male/*muški*	86 (57.7%)	30 (64%)	56 (55%)
female/*ženski*	63 (42.3%)	17 (36%)	46 (45%)
ASA/*Američko udruženje anesteziologa*			
1	8 (5.4%)	2 (4%)	6 (6%)
2	81 (54.4%)	28 (60%)	53 (52%)
3	60 (40.3%)	17 (36%)	43 (42%)
Number of patients with preoperative CRT *Broj bolesnika sa preoperativnom hemioradioterapijom*	54 (36%)	26 (55.4%)	28 (27.5%)
Number of patients with complete pathological response after CRT/*Broj bolesnika sa kompletnom histološkom regresijom nakon hemioradioterapije*	4 (2.7%)	1 (2.1%)	3 (2.9%)
Stage/*Stadijum*			
0 (Tis)	2 (1.3%)	1 (2.1%)	1 (2.1%)
I	45 (30.2%)	16 (34%)	29 (28.4%)
IIa	25 (16.8%)	8 (17%)	17 (16.7%)
IIb	2 (1.3%)	1 (2.1%)	1 (1.0%)
IIIa	9 (6%)	4 (8.5%)	5 (4.9%)
IIIb	30 (20.1%)	7 (14.9%)	23 (22.5%)
IIIc	20 (13.4%)	8 (17%)	12 (11.8%)
IV	12 (8.1%)	1 (2.1%)	11 (10.8%)
Length of procedure – mean, range (min) *Dužina trajanja procedure – srednja vrednost, rang (min)*	132 (60-250 min)	140 (90-250 min)	128 (60-185 min)
Tumor distance from anal verge, mean/range) *Udaljenost tumora od analne linije, srednja vrednost, rang*	8.2 (2-14 cm)	6.6 (2-12)	8.9 (2-14 cm)
Clinical AL/*Klinički znaci popuštanja anastomoze*	10 (6.7%)	4 (8.5%)	6 (5.9%)
Re-surgeries due to AL/*Reoperacije usled popuštanja anastomoze*	3/10 (30%)	0/4 (0%)	3/6 (50%)
Re-surgeries (in hospital or <30 days) (due to AL and other complications)/*Reoperacije (unutar 30 dana) usled popuštanja anastomoze ili drugih komplikacija*	8/149 (5.3%)	2/47 (4.25%)	6/102 (5.9%)
Mortality in hospital/*Mortalitet tokom bolničkog lečenja*	1 (0.7%)	0	1 (1%)

Table 2. Relationship between type of anastomosis and complications in anastomotic integrity
Tabela 2*. Povezanost tipa anastomoze i komplikacija integriteta anastomoze*

		Complications in anastomotic integrity *Komplikacija integriteta anastomoze*			
		No complications *Bez komplikacija*	Incomplete rings *Nepotpuni prstenovi*	Air test positive *Pozitivan test insuflacije vazduha*	Total *Ukupno*
Type of anastomosis *Tip anastomoze*	CSA/*JSA* *(jednostaplerska anastomoza)*	47	4	4	55
	DSA/*DSA* *(dvostaplerska anastomoza)*	89	1	4	94
	Total/*Ukupno*	136	5	8	149

sisting of three field external beam radiation therapy in the total dose of 50.4 Gy in 25 fractions along with bolus infusion sensitizing chemotherapy 5-Fluorouracil (25 mg/m^2/day) with leucovorin (20 mg/m^2/day) administered on the 1st, 2nd, 10th, 11th, 20th and 21st day of irradiation. Surgery was conducted 8 to 12 weeks after completion of chemoradiation.

All rectal resections were performed by three trained colorectal surgeons who applied an open procedure and according to the principles of rectal cancer surgery as described in details elsewhere [8, 9]. The circular staplers used for the circular single stapled anastomosis (CSA) were Premium Plus CEEA™ Stapler 29, 31 and 34 mm (Covidien). In the double stapled anastomosis (DSA), the rectal stump was closed with Contour™ Curved Cutter Stapler (Ethicon Endo-Surgery) and the anastomosis was created with the same circular staplers as in the CSA group. The doughnuts were always inspected for completeness. Anastomotic integrity was verified by transanal air insufflations with the pelvis filled with saline. Additional suturing of the "dog ear" or line of anastomosis was done only in cases with verified anastomotic defect. A pelvic drain was always used.

A protective covering colostomy was added in selected cases and according to the surgeon's preference. No differentiation of the stoma (e.g., ileostomy or colostomy) was undertaken. The usefulness of a protective stoma, in terms of the rate and severity of anastomotic leakage, was examined by comparing the early postoperative results of surgery with and without covering the stoma.

The definition of anastomotic leakage adopted in this study included the following: 1) lower abdominal pain with fever, leucocytosis, tenderness, tachycardia, increased white cell count and/or prolonged ileus after operation with pelvic collection adjacent to the anastomosis with demonstration of anastomotic leakage - by rectal examination, rectoscopy or imaging study; 2) gas, pus or fecal discharge from the drainage site or discharge of pus from the rectum. Anastomotic leakage requiring surgery was defined as a clinical leakage needing an unplanned re-surgery for anastomotic dehiscence.

The association of leakage and the protective stoma as an independent variable was studied by univariate analysis. The target criteria were anastomotic leakage requiring surgery and leakage overall. The following parameters were examined: continuous parameters (distance of the tumor from the anal verge, age, duration of operation), dichotomous parameters (gender, type of anastomosis (CSA vs. DSA) and provision of protective stoma) and ordinal parameters (American Society of Anesthesiologists (ASA), tumor stage). For the selection of the parameters, a stepwise approach was applied. P values less than 0.05 were considered statistically significant.

The SPSS for Windows version 16.0 software program was used for the statistical analyses.

Results

A protective stoma was created in 47 (31%) patients out of 149 who were enrolled in this study. A stoma was created more frequently in males, i.e. 35% of the males (30/86) and 27% of the females (17/63) received a stoma (p=0,356). The patients' characteristics are summarized in **Table 1**.

After total mesorectal excision, the anastomosis was created using CSA or DSA technique. The preferred method for creation of rectal anastomosis was DSA, which was performed in 89 patients (60%). The incidence of incomplete anastomotic rings and/or positive air test and their relation to anastomotic technique are presented in **Table 2.**

Clinical AL occurred in 6.7% of patients (10/149). AL occurred in 8.5% of the patients with a protective stoma (4/47) and in 5.9% of those without a protective stoma (6/102), which was not statistically significant **(Table 3)**.

The median tumor level above the anal verge for the patients having AL was 7 cm (ranging from 5 to 10 cm). ASA score was 2 for five out of ten patients with AL (50%) and 3 for the remaining 50% of patients. Three patients did not have comorbidity and were otherwise healthy, while six had cardiovascular pathology and one had diabetes and cardiovascular pathology. Preoperative radiotherapy was performed in 70% of patients who later suffered from AL (7/10).

Nine patients who developed AL did not have any complications related to creation of anastomosis, whi-

Table 3. The rate of AL and the need for re-surgery in those patients
Tabela 3. *Učestalost popuštanja anastomoze i neophodnost reoperacije*

		Protective stoma/*Protektivna stoma*		
		No/*Ne*	Yes/*Da*	Total/*Ukupno*
Anastomotic leakage *Popuštanje anastomoze*	No/*Ne*	96	43	139
	Yes – without re-surgery *Da – bez reoperacije*	3	4	7
	Yes – with re-surgery/*Da – sa reoperacijom*	3	0	3
	Total/*Ukupno*	102	47	149

le air test was positive in one patient. Surgery lasted significantly longer when a stoma had to be created than in case when it was not needed (p=0.024).

The overall rate of re-surgery due to postoperative surgical complications was 5.3% (8/149) and in three cases this happened because of AL. All patients with a protective stoma and clinical anastomotic leakage were treated conservatively (0/4), compared to 50% of patients without a protective stoma who suffered anastomotic leakage and had to be reoperated (3/6).

The mean hospital stay in patients without AL was 9 days, while those who suffered an anastomotic leakage were discharged from hospital on the median day 14.5 (p=0.056).

The patients without a stoma stayed in hospital for 10 days (range 7-43) while the mean hospital stay for the patients with a stoma was 12 days (range 7-39), p=0.14.

The 30-day mortality rate was 0.7% (1/149).

Discussion

The routine use of protective stoma in rectal cancer surgery is still under debate. First of all, there are no clear indications for creating a stoma after rectal resections. Secondly, what is better: colostomy or ileostomy?

There is no risk-free anastomosis and according to literature there are some factors that may cause a higher rate of AL. These are preoperative irradiation, male sex, obesity and level of anastomosis [10–12]. In these cases, the surgeon should always consider creating a protective stoma.

Other risk factors may be a low preoperative serum albumin level (lower than 3.5 g/dL), steroid use, intraoperative blood loss of 200 mL or more, comorbidity, increased duration of surgery (operative time of 200 minutes or more) and/or intraoperative transfusion requirement [13]. No significant difference in the leakage rate between anastomosis created by single or double stapling technique was found by Radovanović et al. in a randomized study [14].

A stoma does not prevent leakage, but it is capable of minimizing anastomotic complications and the rate of re-surgery [15, 16]. It is associated with better clinical outcome or even the absence of clinical signs of dehiscence. In our study, there were 4 patients with a protective stoma who developed clinical AL and all were treated conservatively, while 50% of patients (3/6) with AL without a stoma had to be reoperated. Karanjia et al. found in their retrospective study that the percentage of anastomotic leakage was 8.3% if protective colostomy was created and 17.7% in patients without a stoma [16]. Poon et al. described an anastomotic leakage of 3.3% in the patients with a protective stoma and 12.6% in the patents without a protective stoma. In this study, 148 colorectal anastomosis were performed by using stapling technique [17]. One of the most important randomized multicenter studies in this field is Norwegian RECTODES which has shown that a protective stoma significantly decreases the incidence of clinically significant anastomotic leakage [18]. Unlike other studies, in this randomized multicenter study the surgeon was not the one who decided whether a protective stoma should be performed or not. Our study is observational, not a randomized trial, and there were no significant differences in the leakage rate between the groups of patients with and without a stoma but the rate of re-surgeries was largely different.

Creating a protective stoma itself may carry possible complications. In addition, it prolongs operative time as we have shown in our study. After the surgery, the patient needs to adjust to the new quality of life. Another possible outcome is stenosis of the anastomosis.

Branagan et al. included 1839 patients who had radical resection of colorectal cancer in their large retrospective study. There were 633 resections of rectum with 6.3% of AL in this group, the early postoperative mortality being 10% in the group with AL and 2% in the group without AL (p=0.014). They also showed a statistically significant difference in the local recurrences and 5-year overall survival rates between the patients with and without AL [19]. This effect of creating a protective stoma and its influence on the local recurrence and 5-year overall survival was reported in several studies published during the last decades [20–22].

However, creating a protective stoma requires another surgery which carries significant risks of morbidity and mortality and it may involve the use of considerable medical and economic resources [23, 24]. The stoma closure-related mortality rate is around 0.5% [25, 26]. The stoma carries morbidity risk from its creation until the moment of its closure [27].

When creating ileostomy, the surgeon should be well aware of its complications, such as increased

loss of electrolytes and liquid, irritation of the skin, and prolapse. In their meta-analysis with 1204 patients from 7 studies, Tilney et al. compared protective ileo and transversostomy [28]. A statistically increased level of electrolyte loss was found when creating ileostomy, while the wound infection and postoperative hernia were detected in closing colostomy more often than ileostomy. Some differences were observed in other compared parameters, such as AL, operative time, duration of hospital stay, prolapse, quality of life, etc. but they were not statistically significant. Edwards et al. also showed in their study that there were no statistically significant differences in creating and closing ileo and colostomy; however, according to the surgeon's opinion, ileostomy closure carries a greater risk [29, 30]. To summarize, there are no big differences between protective ileo and colostomy, so the hospital protocol and the preference of the surgeon are crucial when deciding which stoma should be created.

Conclusion

Although it is the surgeon's preference whether to create a protective stoma after rectal resection, there are obvious high risk factors for anastomotic leakage in which a stoma should be placed. This includes preoperative irradiation, low anastomosis (below 7 cm from the anal verge), in the patients with serious comorbidities and in cases of incomplete stapler's rings or positive air test. A stoma cannot prevent but it can surely minimize surgical complications related to anastomotic leakage and it does reduce the rate of re-surgeries.

References

1. Miucin-Vukadinovic I, Kozic D, Adjic O, Radovanovic Z, Breberina M, Borkov B. Rectal cancer: possibilities of MRI in detection of local recurrence. Med Pregl. 2008;59(3-4):157-63.

2. Radovanovic Z, Radovanovic D, Breberina M, Petrovic T, Golubovic A, Borkov B. The value of endorectal ultrasonography in rectal cancer staging. Med Pregl. 2008;59(11-12):557-61.

3. Lukic D, Radovanovic Z, Petrovic T, Breberina M, Golubovic A, Skoric-Jokic S. Oncologic superiority of extralevator abdominoperineal excision for low rectal cancer. Arch Oncol. 2013;21(1):11-3.

4. Radovanovic Z, Petrovic T, Radovanovic D, Breberina M, Golubovic A, Lukic D. Single versus double stapling anastomotic technique in rectal cancer surgery. Surg Today. 2014;44(6):1026-31.

5. Karanjia ND, Corder AP, Bearn P, Heald RJ. Leakage from stapled low anastomosis after total mesorectal excision for carcinoma of the rectum. Br J Surg. 1994;81(8):1224-6.

6. Law WI, Chu KW, Ho JW, Chan CW. Risk factors for anastomotic leakage after low anterior resection with total mesorectal excision. Am J Surg. 2000;179(2):92-6.

7. Averbach AM, Chang D, Koslowe P, Sugarbaker PH. Anastomotic leak after double-stapled low colorectal resection. Dis Colon Rectum. 1996;39(7):780-7.

8. Heald RJ. Rectal cancer: the surgical options. Eur J Cancer. 1995;31A(7-8):1189-92.

9. Murty M, Enker WE, Martz J. Current status of total mesorectal excision and autonomic nerve preservation in rectal cancer. Semin Surg Oncol. 2000;19(4):321-8.

10. Telem DA, Chin EH, Nguyen SQ, Divino CM. Risk factors for anastomotic leak following colorectal surgery: a case-control study. Arch Surg. 2010;145(4):371-6.

11. Vignali A, Fazio VW, Lavery IC, et al. Factors associated with the occurrence of leaks in stapled rectal anastomoses: a review of 1,014 patients. J Am Coll Surg. 1997;185:105-13.

12. Pakkastie TE, Luukkonen PE, Jarvinen HJ. Anastomotic leakage after anterior resection of the rectum. Eur J Surg. 1994;160:293-7.

13. Wong NY, Eu KW. A defunctioning ileostomy does not prevent clinical anastomotic leak after a low anterior resection: a prospective, comparative study. Dis Colon Rectum. 2005; 48:2076-9.

14. Radovanovic Z, Petrovic T, Radovanovic D, et al. Single versus double stapling anastomotic technique in rectal cancer surgery. Surg Today. DOI 10.1007/s00595-013-0646-x,2013. (Epub ahead of print)

15. Mealy K, Burke P, Hyland J. Anterior resection without a defunctioning colostomy: questions of safety. Br J Surg. 1992;79:305-7.

16. Karanjia ND, Corder AP, Bearn P, Heald RJ. Leakage from stapled low anastomosis after total mesorectal excision for carcinoma of the rectum. Br J Surg. 1994;81:1224.

17. Poon RT, Chu KW, Ho JW, et al. Prospective evaluation of selective defunctioning stoma for low anterior resection with total mesorectal excision. World J Surg. 1999;23:463-7.

18. Matthiessen P, Hallböök O, Rutegård J, Simer G, Sjödahl R. Defunctioning stoma reduces symptomatic anastomotic leakage after low anterior resection of the rectum for cancer. A randomized multicenter trial. Ann Surg. 2007;246:207-14.

19. Branagan G, Finnis D. Wessex Colorectal Cancer Audit Working Group. Prognosis after anastomotic leakage in colorectal surgery. Dis Colon Rectum. 2005;48(5):1021-6.

20. Walker KG, Bell SW, Rickard MJ, et al. Anastomotic leakage is predictive of diminished survival after potentially curative resection for colorectal cancer. Ann Surg. 2004;240:255-9.

21. Bell SW, Walker KG, Rickard MJ, et al. Anastomotic leakage after curative anterior resection results in a higher prevalence of local recurrence. Br J Surg. 2003;90:1261-6.

22. Fujita S, Teramoto T, Watanabe M, Kodaira S, Kitajima M. Anastomotic leakage after colorectal cancer surgery: a risk factor for recurrence and poor prognosis. Jpn J Clin Oncol. 1993;23:299-302.

23. Camilleri-Brennan J, Steele RJ. Prospective analysis of quality of life after reversal of a defunctioning loop ileostomy. Colorectal Dis. 2002;4:167-71.

24. Remzi FH, Fazio VW, Gorgun E. The outcome after restorative proctocolectomy with or without defunctioning ileostomy. Dis Colon Rectum. 2006;49:470-7.

25. Hallbook O, Matthiessen P, Leinskold T. Safety of the temporary loop ileostomy. Colorectal Dis. 2002;4:361-4.

26. Gastinger I, Marusch F, Steinert R. Protective defunctioning stoma in low anterior resection for rectal carcinoma. Br J Surg. 2005;92:1137-42.

27. Chen F, Stuart M. The morbidity of defunctioning stomata. ANZ J Surg. 1996;66:218-21.

28. Tilney HS, Sains PS, Lovegrove RE, et al. Comparison of outcomes following ileostomy versus colostomy for defunctioning colorectal anastomoses. World J Surg. 2007;31:1142-51.

29. Edwards DP, Leppington-Clarke A, Sexton R. Stoma-related complications are more frequent after transverse colostomy than loop ileostomy: a prospective randomized clinical trial. Br J Surg. 2001;88:360-3.

30. Huh JW, Park YA, Sohn SKA. Diverting stoma is not necessary when performing a handsewn coloanal anastomosis for lower rectal cancer. Dis Colon Rectum. 2007;50:1040-6.

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POSTOPERATIVE VISUAL RECOVERY FOLLOWING SURGICAL TREATMENT OF CRANIOPHARYGIOMAS

VIDNA OŠTRINA NAKON OPERATIVNOG LEČENJA KRANIOFARINGEOMA

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Summary

Introduction. Craniopharyngiomas are rare tumors which are typically focused in the sellar and suprasellar region. Secondary to mass effect, these tumors commonly mediate neurologic, endocrinologic or visual functions. The purpose of this study was to investigate the pre and postoperative visual acuity in patients with a craniopharyngioma in the area of the optic chiasm. **Material and Methods**. This retrospective study included 42 patients with a craniopharyngioma demonstrated by computerized tomography or magnetic resonance imaging. The visual status was analyzed both before and after surgery (10 days, one month and six months after surgery). **Results**. Progressive loss of visual acuity was a typical initial eye symptom. Postoperatively, improvement in visual acuity was seen in 47.2% of eyes. Normal vision was attained in the majority of eyes (from 27% preoperatively to 40% six months after surgery). The percentage of eyes with heavily reduced visual acuity decreased as well (from 38% preoperatively to 11% six months after surgery). Visual acuity improved at least in one eye in 36.58% of patients, and 28% of patients achieved normal visual acuity in both eyes, six months after surgery. The improvement of 0.5 and better at least in one eye was observed in 33% of patients. The majority of eyes showed immediate improvement after surgical decompression, during first ten postoperative days. **Conclusions.** The majority of patients with craniopharyngioma show a significant improvement of visual function, particularly in the first ten postoperative days.

Key words: Craniopharyngioma; Recovery of Function; Visual Acuity; Vision Disorders; Treatment Outcome; Signs and Symptoms; Magnetic Resonance Imaging; Tomography, X-Ray Computed

Sažetak

Uvod. Kraniofaringeomi su retki tumori prvenstveno lokalizovani u selarnom i supraselarnom prostoru. Zbog svog mas efekta ovi tumori dovode do neuroloških i endokrinoloških poremećaja, kao i oštećenja vidne funkcije. Cilj ovog rada je da ispita stanje vidne oštrine pre i posle operacije kraniofaringeoma lokalizovanih u zoni optičke hijazme. **Materijal i metode.** Retrospektivno je analizirano 42 pacijenta sa kraniofaringeomom koji je utvrđen pregledom kompjuterizovanom tomografijom ili magnetnom rezonancijom. Pacijenti su podvrgnuti detaljnom oftalmološkom pregledu preoperativno i postoperativno (pri otpustu – 10 dana posle opeacije, posle mesec dana i posle šest meseci). **Rezultati.** Progresivni gubitak vidne oštrine bio je tipični inicijalni simptom. Poboljšanje vidne oštrine uočeno je u nešto manje od polovine ispitivanih očiju – 47,2%, sa najizraženijim promenama u smislu povećanja broja očiju sa normalizovanom vidnom oštrinom – od 27% preoperativno do 40% posle 6 meseci i smanjenjem broja očiju sa teško redukovanom vidnom oštrinom od 38% očiju preoperativno do 11% 6 meseci postoperativno. U odnosu na broj pacijenata – 36,58% pacijenata pokazuje poboljšanje vidne oštrine (bar na jednom oku), 28% postiže za 6 meseci normalnu vidnu oštrinu na oba oka, dok 33% pacijenata postiže bar na jednom oku vidnu oštrinu od 0,5 i veću. Većina očiju pokazuje brz oporavak vidne oštrine, nakon hirurške dekompresije tokom prvih deset dana posle operacije. **Zaključak.** Većina pacijenata sa krniofaringeomom pokazuje signifikantno poboljšanje vidne oštrine, naročito u prvih 10 dana nakona operacije.

Ključne reči: Kraniofaringeom; Oporavak funkcije; Vidna oštrina; Poremećaji vida; Ishod lečenja; Znaci i simptomi; MRI; CT

Introduction

Craniopharyngiomas are histologically benign, dysembryogenetic tumors, originating from embryonic epithelial remnants of invagination of primary oral cavity – craniopharyngeal canal and Rathke's pouch. They represent 2.5 – 4% of all intracranial tumors, accounting for one third of all supratentorial and one half of all suprasellar tumors in children [1, 2].

Up to a half of these tumors occur in childhood and during adolescence by the age of 20. However, in cases with slow growth, their discovery is often postponed up to the fourth and fifth decade of life, making craniopharyngiomas part of adult pathology.

Craniopharyngiomas are often characterized by extensive growth, reaching gigantic proportions, and predisposition to recur. Their localization can be intrasellar, suprasellar or parasellar and basically anywhere alongside the craniopharyngeal canal [1, 2]. Macroscopically, they are usually presented by big solitary mass composed of solid and cystic parts. Walls of cysts commonly contain calcifications.

The first detailed description of cystic suprasellar craniopharyngioma was made in 1857 by Zenker. More than 40 years later, in 1899, Mott and Barett hypothesized that tumor originated from the cells of embryonic epithelial remnants of craniopharyngeal

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Abbreviations

CT	– computed tomography
MRI	– magnetic resonance imaging
VA	– visual acuivity
NLP	– no light perception
CF	– counting fingers
LP	– light perception
HM	– hand movement

canal, while Lewis in 1910 published the first attempt of its surgical removal.

Hypopituitarism, visual function impairment, vegetative and psychological disturbances (thermoregulation, hunger, sleep, affect and memory disorders) and increase of intracranial pressure occur as the consequence of the compression of pituitary gland and its stalk, optical pathways, hypothalamus and third ventricle.

Clinical presentation, tendency to grow, predisposition to recur, possibility of its complete removal, and the prognosis differ significantly in various age groups. In childhood, craniopharyngiomas are more prone to show expansive, aggressive growth, recidivate more often, and cause more severe endocrine disorders [1–4]. In adults, as a consequence of a long term irritation due to the leakage from cystic parts of tumor, numerous fibrous adhesions with surrounding structures are formed, making radical surgical removal much harder [1, 2, 4–8].

The decrease of mortality rate itself is not the only measure of the successful treatment. Today, the aim is to preserve and recover any neurological, psychoemotional and hormonal, as well as visual functions, leading to longer lifetime expectancy and improved quality of life of patients.

The goal of this research was to determine the degree of recovery of visual acuity after surgical treatment of craniopharyngioma by decompression of optochiasmal region.

Material and Methods

Forty two patients with optic chiasmal craniopharyngioma, confirmed by computerized tomography (CT) and/or magnetic resonance imaging (MRI) scan, were included in the study. All of them were operated between 1999 and 2004 at the Institute for Neurosurgery, Clinical Centre of Serbia, Belgrade and the Department of Neurosurgery, Clinical Centre of Vojvodina, Novi Sad. Based on patient's history and neuro-ophthalmological examination, 37 patients (88%) with positive ophthalmological findings and with no prior disease of the eye or optic nerve were selected. Of those, 31 patients (83.7%) were operated by transsphenoidal surgical approach, and 6 (16.2%) by transcranialsurgical approach. Complete tumor resection was achieved in 29 patients (78.4%), while surgical therapy was incomplete in 8 (21.6%).

Detailed ophthalmologic examination included visual acuity, color vision, visual field, pupillary reaction to light, oculomotor nerves functions, as well as measurement of eye bulbs protrusion and fundus inspection. The examination was done preoperatively and postoperatively, on the day of patient's discharge from hospital (10^{th} day postoperatively), after one month and after six months.

Visual acuity (VA) was determined using Snellen optotype, from the distance of 6 meters, in the light of constant brightness and with best refractive correction. Based on VA, the patients were classified into five groups:

1. with no visual acuity decrease BCVA=1.0;
2. with mild decrease BCVA=0.9 – 0.5;
3. with moderate decrease BCVA=0.4 – 0.1;
4. with severe decrease BCVA= counting fingers (CF), hand movement (HM), light perception (LP);
5. blind patients BCVA = no light perception (NLP).

Null hypothesis that there is no change in visual acuity before and after surgery was tested using Student t-test.

Results

Out of 42 patients with diagnosed craniopharyngioma, 37 had positive ophthalmologic findings. The study sample consisted of 18 males (48.64%) and 19 females (51.35%) according to demographic data, whose average age was 30.4 years (12 – 67 yrs.). There were three children up to the age of 14 (8.1%),

Table 1. Proportion of neuroophthalmological disturbances
Tabela 1. *Zastupljenost neurooftalmoloških poremećaja*

Disturbance/*Poremećaj*	No of patients/*Broj pacijenata*	%
Decreased visual acuity/*Pad vidne oštrine*	28	75.6%
Impaired color vision/*Pad kolornog vida*	28	75.6%
Visual field defects/*Ispadi u vidnom polju*	31	83.7%
PNO atrophy/*Atrofija PNO*	25	67.5%
RAPD	8	21%
Papiloedema/*Papiledem*	6	16%
Oculomotor palsies/*Okulomotorne pareze*	4	10.8%
Proptosis/*Proptoza*	0	0

PNO - papilla nervi optici, RAPD - relativni aferentni pupilarni defekt

Table 2. Visual acuity at different time intervals - eyes
Tabela 2. *Vidna oštrina u različitim vremenskim intervalima - oči*

	10 days after surgery *10 dana posle operacije*		1 month after surgery *1 mesec posle operacije*		6 months after surgery *6 meseci posle operacije*	
Unchanged/*Nepromenjena*	43	59.7%	34	47.2%	28	38.9%
Improved up to 2 lines *Poboljšana do 2 linije*	15	20.8%	13	18%	11	15.3%
Improved more than 2 lines *Poboljšana preko 2*	5	6.9%	15	20.8%	23	31.9%
Decreased/*Pogoršana*	9	12.5%	10	13.9%	10	13.9%
Total/*Ukupno*	74	100%	72	100%	72	100%

26 patients were between 15 and 40 years of age (70.27%), and 8 patients were over 40 years of age (21.62%). More than half of the patients (56.7%) were in their first three decades of life. Three patients (8.1%) were over 60 years of age.

Out of all signs and symptoms in the clinical presentation of craniopharyngioma, a decrease in visual acuity (31 patients/83.7%) and endocrine disorders (13 patients/35.1%) were the most common ones. Headache was present in 12 patients (32.4%) as an isolated finding and in combination with nausea and vomiting, as a symptom of raised intracranial pressure, it was observed in 6 patients (16.2%). Neurological disorders leading to motor and mental deficit (muscular weakness, memory loss, disorientation, depression), as well as paresis of external eye muscles were observed in 4 patients (10.8%).

There was an interval from 6 weeks to 9 years (24.4 months on average) from the onset of symptoms to the confirmation of diagnosis. In 16 cases (43.2%) the diagnose was established within 6 months from the beginning of symptoms, in 8 cases (21.6%) during the interval from 7 months to 2 years, and in 13 cases (35.1%) it took more than 2 years to confirm the true diagnosis.

All the patients with pronounced neuro-ophthalmologic manifestations had tumor of suprasellar localization. Intrasellar localization with suprasellar propagation was found in 12 patients (32.4%). Suprasellar tumors of extarventricular localization were present in 19 patients (51.3%), four of which with parasellar and five with retrosellar spreading. Intraventricular location of tumor was observed in 6 patients (16.2%).

The size of tumor, determined by its greatest diameter, was measured using MRI or CT scanning techniques.

Twenty-six patients had complete data on the size of tumor. Most of them were tumors of medium size, between 20 and 40 mm, found in 15 patients (57.6%), followed by small-sized tumors up to the 20 mm in diameter, found in 7 patients (26.9%), while large tumors, bigger than 40 mm were present in 4 patients (15.3%).

The most common neuro-ophthalmological disorders were visual field scotoma, decrease in VA, optic nerve head atrophy, color vision disturbances and relative afferent pupillary defect (RAPD), while papilledema and oculomotor paresis were seen much less frequently **(Table 1)**.

Out of the total number of 74 eyes involved, the normal preoperative VA was found in 20 eyes (27.02%); mild and moderate reduction was found in 24 eyes (32.4%), while 28 eyes (37.8%) had severely decreased VA, including CF, HM and LP. Two eyes (2.7%) were totally blind, with NLP.

Normal binocular VA was found in 8 patients (21.6%). Monocular decrease of VA was observed in only 4 patients (10.8%) and binocular in the remaining 25 patients (67.5%). Severe reduction of VA, at least in one eye, was found in more than half of the patients (23 patients, 62.1%).

Thirty-six patients have completed the study, since one patient, aged 52, with tumor size of 45 mm passed away in the first few days postoperatively. He had had symptoms for 6 years prior to surgery, as well as the total loss of monocular visual function.

The check-ups after 10 days, one month and 6 months revealed postoperative normal VA in 22 (30.5%), 23 (31.94%) and 29 eyes (40.28%); mild and moderate loss in 28 (38.8%), 30 (41.66%) and 24 eyes (33.33%); severe reduction in 18 (25%), 8 (11.11%) and 8 eyes (11.11%); while NLP in 4 (5.5%), 4 (5.5%) and 11 eyes (15.28%), respectively.

Visual acuity remained the same in 43 eyes (59%) 10 days after surgery, including 20 eyes with normal preoperative visual acuity (VA=1.0). It remained unchanged in 34 (47%) and 28 (39%) one month and 6 months after surgery, respectively. Improvement in VA was found in 20 eyes (28%) after 10 days with the tendency of rising at 1 month and 6 month check-ups (28 eyes, i.e. 39% and 34 eyes, i.e.47.22%, respectively). More than 2 lines of Snellen were gained in 5 (7%), 15 (21%) and 23 eyes (32%), 10 days, 1 month and 6 months after surgery. Vision aggravated in 9 eyes (13%) 10 days postoperatively and in 10 eyes (14%) one and six months after surgery **(Table 2, Graph 1)**.

At first, binocular normal VA was present in 9 patients (25%) 10 days after surgery and then 10 patients (27.78%) were found to have it at the last check-up 6 months later. Initially, VA in the better eye was normal in 4 patients (11%) on the 10th day postoperatively and then in 5 patients (13.89%) and finally in 9 patients (25%) at two following check-

Table 3. Visual acuity of both eyes in relation to patients
Tabela 3. *Vidna oštrina oba oka u odnosu na pacijente*

	Preoperative *Pre operacije*		10 days after surgery *10 dana posle operacije*		1 month after surgery *1 mesec posle operacije*		6 months after surgery *6 meseci posle operacije*	
Bilateral normal/*Oba normalna*	8	21.6%	9	25%	9	25%	10	27.8%
Unilateral normal/*Jedno normalno*	4	10.8%	4	11.1%	5	13.9%	9	25%
Unilateral 0,5-0,9/*Jedno 0,5-0,9*	7	18.92	9	25%	13	36.1%	12	33.3%
Bilateral under 0,5/*Oba ispod 0,5*	18	48.6%	14	38.9%	9	25%	5	13.9%
Total/*Ukupno*	37	100%	36	100%	36	100%	36	100%

ups. Visual acuity lower than 0.5 in the better eye was found in 14 patients (39%) at first, but that number decreased with time, ending up with 5 patients (13.89%) 6 months after surgery **(Table 3)**.

Null hypothesis that postoperative VA does not change with time after surgery was established and tested with Student t-test. The following values of t and p were calculated: t= -3.23881 (p=0.0012) 10 days after surgery, t = -4.53335 (p=0.0002315) one month after surgery and t= -5.79627 ($p = 3.8 \times 10^{-7}$) 6 months after surgery. The negative values of t and $p<0.01$ indicate that null hypothesis was wrong, showing that there was a statistically significant increase in postoperative VA over the time, with the level of confidence of 99%.

Improvement of VA was observed in 47.22% of eyes, with the most prominent change in the eyes with normal VA from 27% preoperatively to 40% 6 months after surgery. There was also a significant decrease in the number of eyes with severely impaired VA: from 38% preoperatively to 11.6% after 6 months. Visual acuity remained unchanged in 39% and deterioration of vision was noted in 14% of eyes.

Regarding the number of patients, 36.58% of them had improvement of VA (at least in one eye), normal binocular VA was reached in 28% of them 6 months postoperatively, while 33% of patients achieved monocular VA of 0.5 and higher. There was no change in 28% of patients and a decrease in vision was noted in 14% of patients.

Discussion

Craniopharyngiomas have always been controversial and "mysterious problem" for neurosurgeons, according to one of the pioneers of modern neurosurgery Harvey Cushing. This view is supported by their close relationship and adherence to vital structures at the base of the brain, their size and composition, as well as high mortality and postoperative morbidity in the form of motor and VA impairment, psycho-emotional and vegetative disturbances as the direct consequence of intraoperative injury of hypothalamus.

A number of new microsurgical techniques were developed in the last couple of decades which allowed for better visualization of tumor capsule, its relationship with surrounding tissues and better surgical removal of tumor itself. These advancements led to a significant decrease in mortality and postoperative morbidity of patients and to an increase in survival rates with suitable quality of life.

The issue of quality of life is of great importance for patients operated on for benign tumors such as craniopharyngioma. Successful treatment involves normal motor, cognitive and intellectual development, and emotional stability of the patients, their ability to integrate into social and professional life and to provide for themselves. One of the most important factors for this is the reduction of neuropsychological sequels, one of them being visual impairment.

Craniopharyngiomas, tumors of dysembryogenic type, are diagnosed in young people, mostly in the second decade of life, and in middle age in the fourth and fifth decade. The patients included in our study were between 13 and 68 years of age, the average age being 30.5 years, which corresponds well with the scope of 27 to 42 years found in available literature [4, 9–11]. There were 92% of people over 16 years of age. The peak incidence of craniopharyngioma was in the second (37.83%) and fourth and fifth decade of life (32.43%), that being similar with findings of other authors [9–11]. Gender distribution was basically equal, 19:18 in favor of women.

It is well known that the patient's age is an important factor for clinical presentation, tendency for growth, relapse affinity, as well as for the treatment prognosis of this tumor.

Our study showed no statistically significant difference in VA improvement among various age groups. However, several authors have correlated a poorer postoperative recovery of vision with young age, speculating that the reason for that is delayed tumour diagnosis in this age group, leading to more advanced visual impairment preoperatively [4, 10–12].

Two of the most common features in clinical picture of craniopharyngioma in our patients were a decrease in VA (83.7%) and endocrine disorders (35.1%), mostly in the form of gonadal insufficiency and diabetes insipidus. Headache, as an isolated symptom, was present in 12 patients (32.4%), and combined with nausea and vomiting, it was as a part of raised intracranial pressure in 6 patients (16.2%). Neurological disorders, with predominant motor or mental deficit (motor weakness, obliviousness, disorientation or depression) and paresis of oculomotor nerves, were observed in 4 patients (10.8%).

The majority of authors agree that monocular or binocular loss of vision is a prevalent finding in young

and adult craniopharyngioma patients [4, 7, 9, 11, 13–16]. Van Effenterre and Boch [4] have found it in 80% of cases, while other studies have shown it to be in the range from 42% to 72% [7, 11, 13, 15]. In our patient series there were 75.6% of patients with a decrease in VA, most of them binocular (67.5%). Severely reduced vision, at least in one eye was found in 65.7% of patients, and 83.7% had scotomata in visual field, regardless of their effect on the vision itself.

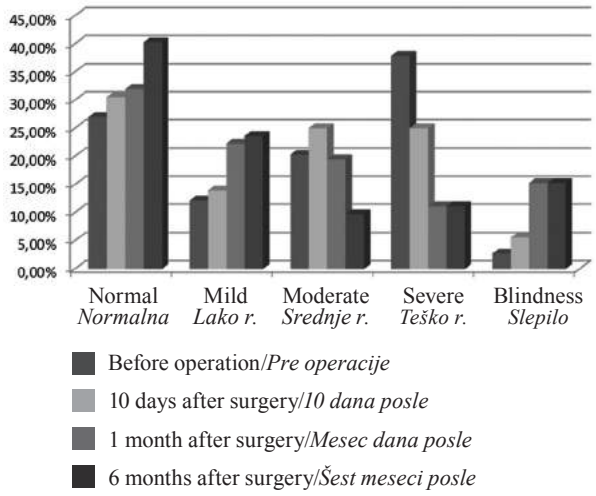
Such a high proportion of craniopharyngioma patients with affected vision can be explained with its retrochiasmal location, which primary leads to the destruction of crossed fibres of the optic nerve serving central parts of visual field.

Six months after surgery, 58.33% of our patients showed an improvement in VA, at least in one eye. In comparison with the situation before surgery when 21.62% of patients had normal VA in both eyes, it was evident that 6 months after surgical treatment there were 27.78% of patients with normal values of binocular VA. Furthermore, there were additional 25% of patients with normal monocular vision. Monocular visual acuity of 0.5 and higher was observed in 33.33% of patients, in contrast to 13.89% of patients who had binocular VA lower than 0.5. No change in vision after 6 months was recorded in 27.77% of our patients, most of them preserving normal preoperative values of VA (96.2%). As for the number of eyes involved, 39% of them maintained preoperative VA, 47% showed improvement, and only 14% suffered from a drop in vision 6 months after surgery.

Van Effenterre and Boch [4] reported an improvement in VA in 70% of eyes at the 2-month follow-up, half of them having achieved normal VA, and 15% of them showing no change, whereas VA deteriorated in 15% of eyes. Yamada et al. [14] studied a group of 61 patients and found that an improvement was observed in 90.2% of cases after surgery. Numerous recent papers report various degrees of postoperative improvement of VA, ranging from 52% to 93% [9, 14, 16–21].

Examples of significant improvement of postsurgical VA in the presence of severe or total preoperative loss of vision have also been known. Stark et al. [22] have described the case of a nine-year old child with NLP who had experienced a full visual recovery within a year after surgery and remained stable throughout the five year follow-up. The pathophysiological explanation for such an astonishing recovery includes gradual remyelination of nerve fibers and reorganization of neural connections in the lateral geniculate nucleus, leading to the improvement in synaptic transmission.

Usual intraoperative findings in patients with severely reduced preoperative VA include compression of large portions of optic nerve and optic chiasm with tumor tissue, leading to ischemia, stretching of the nerve or penetration of tumor cells into the nerve itself, making any surgical manipulation potentially hazardous. Compression on optic nerve fibers itself causes venous stasis and disruption of fast and slow phase of axoplasmatic transport. Edema and anoxia



Graph 1. Eyes (%) and visual acuity in different time intervals

Grafikon 1. *Vidna oštrina u odnosu na broj očiju u različitim vremenskim intervalima*

of nerve fibers ensue and later degeneration and demyelination of nerves rendering nerve impulse transmission defective or even impossible.

It was perceived that after surgical treatment of craniopharyngioma there was a quick improvement of VA in the first 10 to 14 days, followed by a slower recovery during the period of several months or even years [4, 14–16]. Our data show that there was a statistically significant improvement of VA in 36% of patients and 28% of eyes, in the first 10 days after surgery.

Elimination of this so called physical block of impulse transmission is at the core of early, quick recovery of visual function. Sometimes, prompt desorption of local edema of reversibly damaged nerve fibers happens. A later phase of the recovery is believed to be caused by remyelination and axoplasmc transport restoration along nerves [23, 24].

Conclusion

It has been concluded that for the period of 6 months there is a statistically significant improvement of visual acuity regarding both the number of eyes and the number of patients reaching normal vision, at least in a better eye, which enables the patients to perform everyday vision related tasks.

Visual acuity in most of craniopharyngioma patients improves significantly in postoperative period, thus improving the quality of their life.

Craniopharyngioma and its treatment pose several challenges for patients suffering from it. One of the most common among them is an impairment of visual function. Our research shows that there is a constant and statistically significant improvement of visual acuity during the postoperative period of 6 months. Further studies are needed in order to make a proper assessment of the scope of its impact on the patients' quality of life.

References

1. Thapar K, Kovach K, Scheitauer BW. Classification and pathology of sellar and parasellar tumors. In: Tindall GT, Cooper PR, Barrow DL. The practice of neurosurgery. Baltimore: William Wilkins; 1996. p. 1021-65.

2. Ivanović SP i saradnici. Tumori hipofize i karniofaringeomi. Beograd: Nova Evropa; 1995.

3. Suharwardy J, Elston J. The clinical presentation of children with tumours affecting the anterior visual pathways. Eye. 1997;11:838-44.

4. Van Effenterre R, Boch AL. Craniopharyngioma in adult and children: a study of 122 surgical cases. J Neurosurg. 2002; 97:3-11.

5. Fahlbusch R, Honegger J, Paulus W. Surgical treatment of craniopharyngiomas: experience with 168 patients. J Neurosurg. 1999;90:237-50.

6. Duff JM, Meyer FB, Ilstrup DM. Long-term outcome for surgically resected craniopharyngiomas. Neurosurgery. 2000; 46:291-305.

7. Karavitaki N, Brufani C, Warner JT. Craniopharyngioma in children and adults: systematic analysis of 121 cases with long-term follow up. Clin Endocrinol (Oxf). 2005;62:397-409.

8. Stamm AC, Vellutini E, Harvey RJ, Nogeira JF, Herman DR. Endoscopic transnasal craniotomy and the resection of craniopharyngioma. Laryngoscope. 2008;118:1142-8.

9. Campbell PG, McGettigan B, Lungibuhl A, Yadla S, Rosen M, Evans JJ. Endocrinological and ophthalmological consequences of an initial endonasal endoscopic approach for resection of craniopharyngiomas. Neurosurg Focus. 2010;28:1-10.

10. Maira G, Anile C, Albanese A, Cabezas D, Pardi F, Vignati A. The role of transsphenoidal surgery in the treatment of craniopharyngiomas. J Neurosurg. 2004;100:445-51.

11. Repka MX, Miller NR, Miller M. Visual outcome after surgical removal of craniopharyngiomas. Ophthalmology. 1989; 96:195-9.

12. Eliott RE, Sands SA, Strom RG, Wisoff JH. Craniopharyngioma clinical status scale: a standardized metric of preoperative function and posttreatment outcome. Neurosurg Focus. 2010;28:(4)E2.

13. Chen C, Okera S, Davies PE, Selva D, Crompton JL. Craniopharyngioma: a review of long term visual outcome. Clin Experiment Ophthalmol. 2003;31:220-8.

14. Yamada S, Fukuhara N, Oyama R, Takeshita A, Ito J, Inoshita N. Surgical outcome in 90 patients with craniopharyngioma: an evaluation of transsphenoidal surgery. World Neurosurg. 2010;74:320-30.

15. Gucev ZS, Danilovski D, Tasic V. Childhood craniopharyngioma in Macedonia: incidence and outcome after subtotal resection and cranial irradiation. Word J Pediatr. 2011;7:74-8.

16. Gardner PA, Kassam AB, Snyderman CH, et al. Outcomes following endoscopic, expanded endonasal resection of suprasellar craniopharyngiomas: a case series. J Neurosurg. 2008;109:6-16.

17. Laufer I, Anand VK, Schwartz TH. Endoscopic, endonasal extended transsphenoidal, transplanum transtuberculum approach for resection of suprasellar lesions. J Neurosurg. 2007; 106:400-6.

18. Michael ES, Yang I, Kane AJ. Endocrinologic, neurologic, and visual morbidity after treatment for craniopharyngioma. J Neurooncol. 2011;101:463-76.

19. Jane JA, Prevedello DM, Alde TD, Laws ER. The transsphenoidal resection of paediatric craniopharyngiomas: a case series. J Neurosurg Pediatr. 2010;1:49-60.

20. Jane JA, Kiehna E, Payne SC, Early SV, Laws ER. Early outcome of endoscopic transsphenoidal surgery for adult craniopharyngiomas. Neurosurg Focus. 2010;28(4):E9.

21. Kim EH, Ahn JY, Kim Sh. Technicue and outcome of endoscopy-assisted microscopic extended transsphenoidal surgery for suprasellar craniopharyngiomas. J Neurosurg. 2011; 114:1338-49.

22. Stark KL, Kaufman B, Lee BC. Visual recovery after a year of craniopharyngeoma-related amaurosis: report of nine year old child and a review of patophysiologic mechanism. J AAPOS. 1999;3:366-71.

23. Hanke J. Anatomical correlations of intrinsic axon repair after partial nerve crush in rats. Ann Anat. 2002;184:113-23.

24. Grković D, Bedov T, Čanadanović V, Babić N, Barišić S. Postoperative visual recovery following surgical treatment of suprasellar meningiomas. Med Pregl. 2012;65(7-8):309-14.

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ANTIVIRAL TREATMENT OF HEPATITIS C IN SERBIAN PRISON SETTING: MEDICAL TREATMENT OUTCOMES AND PATIENTS' ADHERENCE

ANTIVIRUSNO LEČENJE HEPATITISA C U ZATVORSKIM USLOVIMA U SRBJI: ISHOD LEČENJA I ADHERENCIJA PACIJENATA

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Summary

Introduction. Seroprevlence of chronic hepatitis C viral infection in correctional facilities ranges from 16% to 49%. However, there are only very limited data available on the course of hepatitis C viral infection and outcomes of treatment with pegylated interferon plus ribavirin in correctional settings. The aim of this study was to assess the feasibility and effectiveness of use of pegylated interferon plus ribavirin treatment in the Serbian correctional setting. **Material and Methods.** The study sample consisted of the patients with hepatitis C hospitalized in the Special Hospital for Prisoners in Belgrade (Serbia) during 2007-2013. Health authorities approved treatment for 32 patients out of 76 treatment-naive patients referred to this institution. The patients (N=32) received 180 mcg pegylated interferon alfa -2a once a week plus oral ribavirin in dosage of 800mg or 1000/1200 mg/day for 24 or 48-week treatment. All patients who completed therapy were assessed at the end of an additional 24-week treatment-free period for a sustained virological response. **Results.** Sustained virological response was achieved in 53.8% of hepatitis C viral infection genotype 1 patients and in 73.3% and 66.6% of patients with hepatitis C viral infection genotype 3 and 4, respectively. One patient with mixed genotype (1, 2) did not achieve sustained virological response. The overall safety profile of the treatment regimen was very good. The incidence of influenza-like symptoms and depression were low. A serious adverse event was recorded only in 6.4% of patients. **Conclusion.** The results showed that pegylated interferon alfa-2a plus ribavirin given once a week was well tolerated among prisoners and the regimen had the same adherence and effectiveness as in general population.

Key words: Hepatitis C; Antiviral Agents; Prisons; Prisoners; Serbia; Treatment Outcome; Patient Compliance; Seroepidemiologic Studies; Interferon-alpha; Ribavirin

Sažetak

Uvod. Seroprevalencija hepatitis C virusne infekcije u zatvorima varira između 16–49%. Međutim, veoma je mali broj podataka o toku HCV infekcije i ishodu lečenja pegilovanim interferonom i ribavirinom u zatvorskim uslovima. Cilj istraživanja je ispitivanje izvodljivosti i uspešnosti primene pegilovanog interferona i ribavirina u ustanovama zatvorskog tipa u Srbiji. **Materijal i metode.** Tokom period 2007–2013. godine analizirali smo pacijente sa infekcijom virusom hepattisa C u Specijalnoj zatvorskoj bolnici u Beogradu (Srbija). Zdravstvene vlasti odobrile su lečenje 32 pacijenata od ukupno 76 naivnih pacijenata predloženih za lečenje. Pacijenti (N = 32) su primali peiglovani interferon alfa-2a u dozi od 180 mcg jedanput sedmično plus ribavirin *per os* u dozi od 800 mg ili 1000/1200 mg/dan tokom 24 ili 48 nedelja lečenja. Pacijenti koji su primili kompletnu terapiju kontrolisani su 24 nedelje nakon završetka terapije u cilju provere stabilnosti virusološkog odgovora. **Rezultati.** Stabilan virusološki odgovor je postignut kod 53,8% pacijenata sa HCV genotipom 1. Među pacijentima sa hepatitis C virus genotipom 3 stabilan virusološki odgovor je postignut kod 73,3% a sa hepatitis C virus genotipom 4 kod 66,6%. Jedan pacijent sa mešovitim genotipom (1,2) nije postigao stabilan virusološki odgovor. Ukupan bezbednosni profil terapijskog protokola je bio veoma dobar. Incidencija *influenza-like* simptoma i depresije bila je niska. Ozbiljni neželjeni događaji su zabeleženi kod svega 6,4% pacijenata. **Zaključak.** Rezultati primene pegilovanog interferona alfa-2a jedanput nedeljno, plus ribavirin, u zatvorskoj populaciji, pokazali su isti stepen adherencije, podnošljivosti i uspešnosti kao u opštoj populaciji.

Ključne reči: Hepatitis C; Antivirusni lekovi; Zatvori; Zatvorenici; Srbija; Ishod lečenja; Saglasnost pacijenta; Seroepidemiološke studije; Alfa Interferon; Ribavirin

Introduction

Chronic hepatitis C virus (HCV) infection is a global public health problem [1].

This infection affects up to 170 million people worldwide especially some risk groups such as intravenous drug users (IVDUs) and prisoners. It is estimated that seroprevalence of HCV infection in a prison setting is much higher than in general population and ranges from 16% to 49% [1–3]. Obtaining treatment in a prison setting is a huge problem not only in Serbia but in developed countries as well. The main reasons are limited financial resources and the fact that most of the prisoners belong to the group of IVDUs and some of them have psychiatric comorbidities as well. Since the introduction of dual therapy with pegylated interferon (PE-

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Abbreviations

HCV	– hepatitis C virus
ALT	– alanin aminotransferase
SVR	– sustained virological response
PEGIFN	– pegylated interferon
RBV	– ribavirin
IFN	– interferon
IVDUs	– intravenous drug users
HCV RNA	– hepatitis C virus ribonucleic acid
AE	– adverse event

GIFN) and ribavirin (RBV), this therapeutic protocol has been the treatment of choice for most of the patients [4–6]. The current standard for a successful therapy is to achieve a sustained virological response (SVR) defined as an undetectable serum hepatitis C virus ribonucleic acid (HCV RNA) level, 24 weeks after cessation of treatment. This treatment is associated with an SVR rate of between 40% and 50% of patients. Easier access to diagnosis and treatment among socially marginalized groups such as prisoners should reduce disease transmission and medical costs [3–6]. Antiviral treatment in a prison setting leads to lower recidivism among IVDUs and avoidance of other risk behavior [7]. Awareness that the prevalence of HCV infection is much higher among prisoners than in general population should give rise to community efforts for early diagnosis and treatment for transmission prevention purpose [8]. Concerns relating to adverse events (AEs), adherence to treatment and lack of experienced physicians have limited the number of treated patients [1, 9–13]. This problem exists in community setting as well because patients with alcohol or other drug dependence rarely receive treatment [1, 14–17].

Successful treatment of prisoners in the HCV infection with standard interferon (IFN) has been demonstrated [14]. However, there are limited data on the treatment outcomes and adherence among prisoners who receive PEGIFN.

The aim of this study was to analyze the efficiency, adherence and tolerability of PEGIFN-RBV treatment in HCV infected people in a Serbian prison setting.

Material and Methods

The sample consisted of adult interferon-naive patients (between the ages of 18 and 65 years) with proven HCV infection. The mandatory criteria for inclusion were HCV RNA level higher than 100000 copies/ml, elevated alanin aminotransferase (ALT) level on at least two visits during the preceding six months and a liver biopsy performed within three years before treatment. The exclusion criteria were the presence of severe cardiac disease, seizure disorders, cancer, psychoses, coinfection with human immunodeficiency virus (HIV) and hepatitis B virus, active alcohol and drug dependence within one year before treatment, significant comorbidity, unstable thyroid dysfunction, current pregnancy or breast feeding of infants.

Data from the medical history were analyzed based on both primary outcomes (response at the end of therapy - EOTR) and secondary outcomes (response at week 24 after completion of therapy - SVR). The patients who had met the entry criteria received PEG(40kd)IFN alfa-2a once a week subcutaneously and orally RBV in dosage of 800 mg or 1000/1200 mg/day. The patients were administered therapy in a prison setting for 24 or 48 weeks depending on HCV genotype. After the cessation of treatment they were followed up for the next 24 weeks. All laboratory tests, which include assessment of plasma HCV RNA levels, viral genotyping and serum ALT concentration were performed in central laboratories in the Clinical Centre of Serbia. Pretreatment biopsy specimens were evaluated by a hepatopathologist. During therapy, HCVRNA level was first measured after 12 weeks of treatment of patients with HCV genotype 1 and 4 to assess early virological response.

Monitoring of AEs was mandatory due to safety reasons. All laboratory changes were documented after 1, 2 and 4 weeks of the study and then every month during the treatment. In the follow-up period, the visits to the Department were scheduled every 12 weeks. A dose reduction was necessary for the patients with AEs or significant abnormalities in laboratory analyses. In these patients, the reduction in the assigned dose was 25%, 50%, or 75%.

The goal of the therapy was SVR, defined as undetectable levels of HCVRNA (<100 copies/mL or <50 iu/mL) as measured by a polymerase chain reaction (PCR) at the end of the follow-up period. Biochemical response defined as a normal ALT level

Table 1. Reasons for deferral of hepatitis C therapy
Tabela 1. *Razlozi za odlaganje lečenja hepatitisa C*

Reasons/*Razlozi*	n=44 No. (%) of patients/*br.(%) pacijenata*
Normal ALT level/*Normalan nivo ALT**	10 (22.7)
Normal biopsy finding/*Normalan nalaz biopsije*	11 (24.9)
Patient was discharged too early/*Prerani otpust pacijenta*	3 (6.8)
Patient refused therapy/*Pacijent odbio terapiju*	2 (4.4)
Non compliant patient/*Nekooperativni pacijent*	1 (2.2)
Uncontrolled psychiatric disease/*Nekontrolisana psihijatrijska bolest*	1 (2.2)
Frequent transfer/*Čest premeštaj*	12 (27.2)
Other/*Ostalo*	4 (9.0)

**ALT - alanine aminotransferaza*

was monitored as well at the end of the follow-up period, but it was not considered a primary end point.

HCV RNA levels were measured at baseline visit, after 12 weeks of treatment (only for the HCV genotype 1 and 4 patients), at the end of the treatment and at the end of the follow-up period.

Safety analysis included all patients who received at least one dose of study medication and if they had at least one post base-line safety assessment. Efficacy and baseline predictors were analyzed by using the Cochran-Mantel-Haenszel test and intention to-treat analysis. Fischer's exact test was used to compare virological responses between different genotype groups.

Results

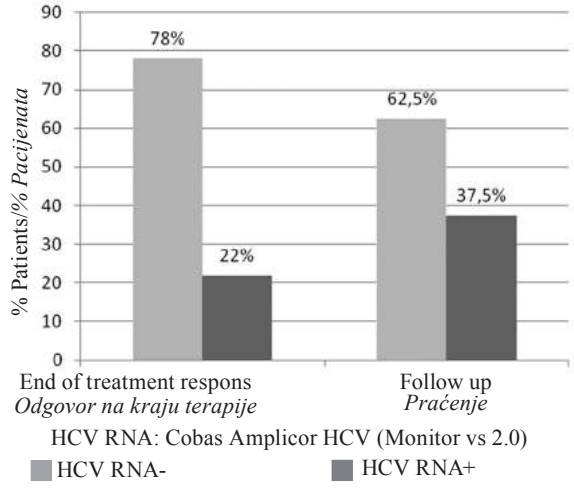
Of the 76 patients screened, 32 met the criteria for the antiviral treatment.

The main reasons for deferral of hepatitis C treatment are presented in **Table 1** and include uncontrolled psychiatric disease, normal ALT concentration and the absence of fibrosis. Four patients were excluded for other reasons. Base-line characteristics of the patients are presented in **Table 2.**

All patients received 180 mcg PEGIFN alfa-2a because PEGIFN alfa-2b was not available. Because of an underlying disease most of our patients used psychiatric medications during hepatitis C treatment: benzodiazepine (89.5%), Methadone (9.3%), and antidepressant (12.5%). None of the patients used antipsychotic medication. In general, therapy was going well **(Table 3)**. The treatment and follow-up was completed in 28 (87.5%) and 27 (84.3%) patients, respectively. The overall virological response at the end of therapy and at the end of the follow-up period was estimated in 78% and 62.5% of patients, respectively **(Graph 1).** A few patients discontinued the antiviral therapy. The main reasons for discontinuation were poor viral responses or comorbidities (9.4%). The treatment was interrupted in one patient because of relapse into drug dependence. All of the patients who achieved SVR had an early virological response after 12 weeks or > 2 - log fall in viral load. The patients who achieved SVR had a normal ALT level 24 weeks after therapy completion as well.

The correlation of some baseline predictors such as genotype and fibrosis has shown that SVR is much better in the subgroup of genotype 3 patients and subgroup of patients with lower fibrosis. Genotype 3 is the most prominent in our patients. Virological response by genotype is presented in **Graph 2.** The rates of SVR by histopathological finding are presented in **Graph 3.**

Adverse events were monitored carefully in all treated patients. Fatigue, headache, myalgia, rigors, and pyrexia were among the most commonly reported AEs (84.3%). Dose modification was necessary only in 6.4% of patients due to AEs. Dose modification was defined as a reduction or omission of one or more doses of study medication. Careful monitoring of blood count during treatment showed mild thrombocytopenia in most of the patients. The treatment completed prematurely in three of the patients but only in one patient the reason for withdrawal was relapse into drug dependence. One death was reported four months after the follow up period because of drug overdose. None of the patients died either during the treatment or in the follow up period.



HCV RNA - Hepatitis C virus ribonucleic acid/*Hepatitis C virusna ribonukleinska kiselina*

Graph 1. HCV RNA at the end of treatment
Grafikon 1. *HCV RNK na kraju terapije*

Discussion

The primary objective of this study was to analyze the efficiency, tolerability and adherence to antiviral treatment in HCV infected people in a Serbian prison setting. Most prisoners in Serbian prisons are IVDUs. The prevalence of HCV infection in this subgroup of prisoners is very high, going up to 80%. However, treatment for HCV infection is limited for all prisoners especially for IVDUs. That is the main reason for limited data available on the outcomes of treatment of HCV infection in prisoners [1, 15, 16].

This study sample included mostly IVDUs (93.8%). Despite this proportion of IVDUs the overall SVR rate was 62.5%. These results are comparable with the results from community setting. In this study sample, 37.5% of patients were infected with HCV genotype 1 [15, 16]. The overall SVR rate was much higher than the results reported by Duncan Smith-Rohrberg Maru [1] and better than SVR rate among prisoners in Canada [14]. There are several reasons which could describe the difference. The first one may be attributed to the fact that our subjects were infected mainly with genotype non-1. The Duncan study included difficult-to-treat patients (28% of patients were co-infected with HIV and 75% were infected with genotype 1 HCV). The second reason for the difference between our results and the

Table 2. Characteristics of the patients included in the study
Tabela 2. *Karakteristike pacijenata uključenih u studiju*

Characteristics/*Karakteristike*	N=32/No of patients/*Broj pacijenata*
Age (years)/*Uzrast (godine)*	33.2+/-6.8
HCVRNA/*HCVRNK* (k/ml)	1832625+/-2628731
Gender (No/%)/*Pol (br./%)*	
Male/*Muškarci*	28 (87.5%)
Female/*Žene*	4 (12.5)
Route of transmission (No/%)/*Put prenosa (br./%)*	
Drug use/*Upotreba droge*	30 (93.8%)
Transfusion/*Transfuzija*	1 (3.1%)
Other/*Ostalo*	1 (3.1%)
Metavir fibrosis score (No/%)/*Metavir fibroza skor (br./%)*	
Mild/*Laka*	17 (53.1%)
Moderate/*Umerena*	4 (12.5%)
Severe/*Teška*	6 (18.7%)
Cirrhosis/*Ciroza*	5 (15.6%)
Genotype (No/%)/*Genotip (br./%)*	
1	12 (37.5%)
Non-1	20 (62.3%)

HCVRNK - hepatitis C virusna ribonukleinska kiselina

results from Canadian prisoners is probably because our patients did not receive standard but pegylated IFN. The third one could be the fact that our patients were very motivated to be treated. They were aware that otherwise they would not be treated easily because of drug dependence. In addition, Serbian hospitals for prisoners are affiliated to the Ministry of Justice not to the Ministry of Health, which also affects the availability of the treatment in a prison setting. Budget for chronic liver diseases is small and there is no policy to treat prisoners for HCV. The Ministry of Justice is not interested in giving a small budget for the long-standing treatment of patients who will go back to the community setting in a year or less. The average duration of incarceration in Serbian prisons is about twelve months. This is one of the main barriers to expanding treatment. The facts such as short duration of incarceration and long duration of treatment sometimes serve as excuses not to treat infected prisoners.

The completion rate is 87.5%, which is also better than the rates from other studies conducted in a prison setting [1, 14, 17]. Therapy was discontinued in 9.3% of our study population (one patient experienced relapse of pulmonary tuberculosis, one took an overdose of drug and one gave up on therapy). Only one patient did not achieve an early virologic response which was the reason to discontinue therapy. These results are comparable with the Duncan study and are better than in other North American prisoners' studies [1, 14].

Some physicians argue that the treatment of HCV in a prison setting is not only an ethical duty but also cost benefit. The reasons for this point of view lie in reducing new infections as well as future medical costs due to complications of advanced liver disease. On the other side are the opponents. Their opinion is that the treatment is expensive not only for the prisoners but for the people without health insurance as well. These expenses are paid by the taxpayers and they should be asked if they want to pay for it or not. This problem was investigated by Sammy Saab et al. [18–20]. The starting point was that treatment is not cost-effective because of relatively high re-infection rates and comorbidity rates in the prison population. In spite of the expectations they found that therapy was cost saving both in strategies with and without biopsy, and concluded that therapy should not be denied to the prisoners with hepatitis C.

Our patients underwent liver biopsy because it was the only way to get treatment at that time according to the Ministry of Health policy concerning treating HCV infected patients. All except one showed very compliant behavior. SVR leads to reducing the risk of progression of liver disease and complications such as decompensated cirrhosis and hepatocellular carcinoma.

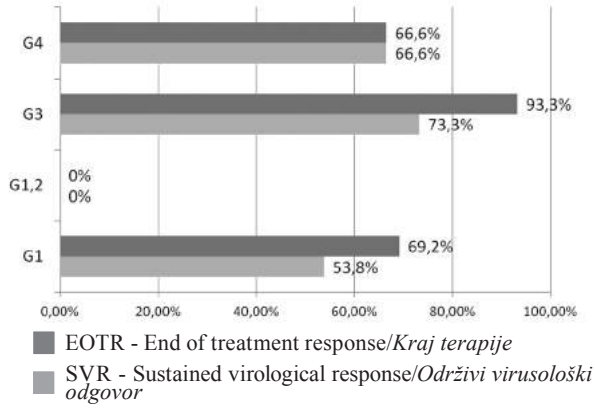
Antiviral treatment is time consuming and requires experienced physicians not only hepatologists but other multidisciplinary experts. Sometimes PEGIFN-RBV treatment is accompanied with serious AEs which should be managed promptly.

A high cure rate especially in the patients with genotype 3 is not enough to persuade authorities that treatment of prisoners is not only ethical but cost saving as well. Still, only a very small number of prisoners with chronic HCV infection receive treatment. Unfor-

tunately the number of prisoners who have access to antiviral treatment in Serbia has rapidly declined since 2013, when the last patient was included in the treatment. Since 2013 very few of the prisoners have received antiviral therapy for HCV infection because of the financial crisis and lack of policy to treat prisoners for hepatitis C. Difficulties related to treatment administration in prisoners are most prominent, but for some reason they are not completely understood. Rather high cure rates were demonstrated in our study. The overall rates of SVR are 62.5% and 53.8% for HCV genotype 1 infection. The study results show that 93.8% of patients belong to IVDUs. That means that they could transmit HCV to other prisoners if they involved themselves in risky behaviors. It is a major risk for the other prisoners. In such circumstances many prisoners require easier access to antiviral treatment and advice how to avoid risky behavior in order to manage their HCV. A risk of contracting HCV due to a high prevalence of this infection amongst prisoners is significantly increased in those prisoners not currently infected because of close contact with HCV infected IVDUs [1, 2].

Incarcerated IVDUs affect society at a number of levels. Some effects are positive, such as putting a stop to criminal behavior. On the other hand, there are negative effects because incarcerating of IVDUs increases the risk of transmission of HCV infection and other blood-born infections to other prisoners. Not only prisoners but also the community is exposed to the increased risk of transmission of HCV infection. The main reason for this is relatively short incarceration time and the return of the prisoners to the community with active untreated HCV infection.

Those who are against the treatment of prisoners should face the fact that avoiding the treatment leads to a higher risk of transmission of HCV infection in the general population and therefore higher long-term medical costs. From the clinicians' point of view all people are entitled to have appropriate health care. This is one of the basic human rights in all democratic societies. Whether a prisoner is treated in prison or after they return to the community is irrelevant. Some expenses cannot be avoided. It is actually far more beneficial to treat the patients earlier rather than later in order to avoid transmission, the progression of the disease and further complications.



Graph 2. Virologic response by genotype
Grafikon 2. *Virusološki odgovor prema genotipu*

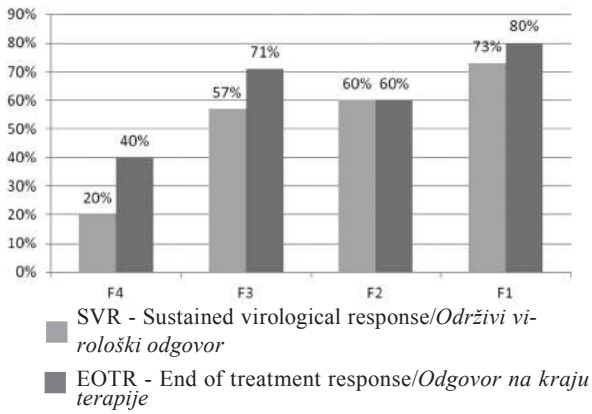
Preventing HCV transmission in prison setting is a complex issue [20, 21]. One of the crucial problems is the risky behavior of active IVDUs. Drug use by injection is even less safe in prison because clean needles and syringes are not available. Another problem is lack of medication. This leads to an increased risk of transmission of HCV infection not only amongst IVDUs but other prisoners as well. A prison setting alters the behavior in most cases and even prisoners who are not HCV positive display risky behavior (tattooing, piercing etc).

Incarceration is not always a limiting factor for treatment. It can be an opportunity to teach prisoners about their disease through workshops or other forms of education. Physicians from prison hospitals would take part in the education of prisoners along with experts in order to find the best solution how to reduce risky behavior, make early diagnosis and administer the necessary treatment [1, 14].

This practice was running very well in Serbia in the period between 2007 and 2013 as a part of the National strategy for HCV treatment. Furthermore, for some prisoners incarcerated at that time it was a unique chance for treatment. However, the program stopped due to economic reasons (lack of financial support). Nowadays we are faced with high prevalence of HCV infection in prisons on the one hand, and lack of appro-

Table 3. Treatment course by HCV genotype
Tabela 3. *Tok lečenja prema genotipu virusa hepatitisa C*

Outcome/*Ishod*	HCV genotype/*HCV genotip*		
	G1	G non-1	All/*Svi*
Completed therapy/*Završena terapija*	11	17	28
Disrupted treatment/*Prevremeni završetak terapije*	1	2	3
Median duration of therapy (weeks)/*Medijana trajanja terapije (nedelje)*	9	12	21
Non-response to treatment/*Odsustvo odgovora na terapiju*	5	7	12
Psychiatric issues/*Psihijatrijski problemi*	2	3	5
Adverse effects/*Neželjeni efekti*	12	15	27
Other (missing data)/*Ostalo (nema podataka)*		1	1



Graph 3. SVR by histopathological finding
Grafikon 3. *Održivi virusološki odgovor prema histopatološkom nalazu*

priate treatment and educational programs on the other hand. This creates a high amount of pressure on public health systems since the majority of prisoners will reenter the community after a short period of time. A relatively short time of incarceration means that they will put other people at risk of getting infected. Therefore, the problem of prevention and treatment of HCV infected people in prison represents a challenge for the entire community. Long term follow-up of HCV infected patients has shown that the "wait and see" approach is not a good choice. Treatment of HCV infection in the early stage of fibrosis is directly related to higher rates of SVR which reduces complications in the final stages of liver disease. Treatment of HCV infection in the prison population is complex and time consuming. Early assessment gives the opportunity to treat only those patients who seek treatment. Our study showed that only 42% of HCV infected prisoners are eligible for treatment. The others are not suitable for a number of reasons, among which are family reasons (pregnancy etc), drug dependence, and some of them are just not committed and dependable enough to go through with the PEGIFN-RBV treatment. In addition, there are a few in a group of well educated prisoners who want to wait for better options (IFN-free regime). Nevertheless, the results of our study showed very good compliance and high SVR rate which is comparable with general population [21].

Treatment of HCV infection should be a multidisciplinary action since most of the patients require psychiatric support to prepare for the treatment. One of the inclusion criteria is a stable abstinence from drug use for at least 12 months before starting the treatment. Therefore, it is necessary to provide careful monitoring of the patient's behavior and regular drug testing. It is also necessary to provide a physician specialized in HCV treatment. Even our study with a small number of patients has shown that prisoners manage their HCV infection fairly well when they have support [22–25].

According to our opinion and the principals of good medical practice HCV infected prisoners should not be treated any differently than others. They have very good adherence and are very motivated to control drug dependence when they are on the treatment. Our data can be very useful for planning a strategy of putting HCV infection in prison settings under control.

Conclusion

Our findings have shown that the prisoners are more compliant and motivated than what is generally thought, and that the treatment of hepatitis C virus infected prisoners is manageable, efficient and safe in a Serbian prison setting.

References

1. Maru DS, Bruce RD, Basu S, Altice FL. Clinical outcomes of hepatitis c treatment in a prison setting: feasibility and effectiveness for challenging treatment populations. Clin Infect Dis. 2008;47(7):952-61.

2. Altice FL, Bruce RD. Hepatitis C virus infection in United States correctional institutions. Curr Hep Rep. 2004;3:112-8.

3. Spaulding AC, Weinbaum CM, Lau DT, et al. A framework for management of hepatitis C in prisons. Ann Intern Med. 2006;144:762-9.

4. Younossi Z, Kallman J, Kincaid J. The effects of HCV infection and management on health-related quality of life. Hepatology. 2007;45:806-16.

5. Spiegel BM, Younossi ZM, Hays RD, et al. Impact of hepatitis C on health related quality of life: a systematic review and quantitative assessment. Hepatology. 2005;41:790-800.

6. Bruno S, Stroffolini T, Colombo M, et al. Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. Hepatology. 2007;45:579-87.

7. Mulhall BP, Younossi Z. Impact of adherence on the outcome of antiviral therapy for chronic hepatitis C. J Clin Gastroenterol. 2005;39(Suppl 1):S23-7.

8. Grebely J, Genoway K, Khara M, et al. Treatment uptake and outcomes among current and former injection drug users receiving directly observed therapy within a multidisciplinary group model for the treatment of hepatitis C virus infection. Int J Drug Policy. 2007;18:437-43.

9. Rifai MA, Moles JK, Short DD. Hepatitis C treatment eligibility and outcomes among patients with psychiatric illness. Psychiatr Serv. 2006;57:570-2.

10. Rifai MA, Moles JK, Lehman LP, et al. Hepatitis C screening and treatment outcomes in patients with substance use/dependence disorders. Psychosomatics. 2006;47:112-21.

11. Sylvestre DL, Zweben JE. Integrating HCV services for drug users: a model to improve engagement and outcomes. Int J Drug Policy. 2007;18:406-10.

12. Sylvestre DL, Litwin AH, Clements BJ, et al. The impact of barriers to hepatitis C virus treatment in recovering heroin users maintained on methadone. J Subst Abuse Treat. 2005;29:159-65.

13. Sylvestre DL. Treating hepatitis C virus infection in active substance users. Clin Infect Dis. 2005;40(Suppl 5):S321-4.

14. Farley J, Vasdev S, Fischer B, et al. Feasibility and outcome of HCV treatment in a Canadian federal prison population. Am J Public Health. 2005;95:1737-9.

15. Pawlotsky J-M. Treating hepatitis C in "difficult-to-treat" patients. N Engl J Med. 2004;351:422-3.

16. Schaefer M, Hinzpeter A, Mohmand A, et al. Hepatitis C treatment in "difficult-to-treat" psychiatric patients with pegylated interferon-alpha and ribavirin: response and psychiatric side effects. Hepatology. 2007;46:991-8.

17. Seal KH, Currie SL, Shen H, et al. Hepatitis C treatment candidacy and outcomes among 4318 US veterans with chronic hepatitis C virus infection: does a history of injection drug use matter? J Clin Gastroenterol. 2007;41:199-205.

18. Tan JA, Joseph TA, Saab S. Treating hepatitis C in the prison population is cost-saving. Hepatology. 2008;48(5):1387-95.

19. McCombs JS, Yuan Y, Shin J, Saab S. Economic burden associated with patients diagnosed with hepatitis C. Clinical Therapeutics. 2011;33(9):1268-80.

20. Hunt DR, Saab S. Viral hepatitis in incarcerated adults: a medical and public health concern. Am J Gastroenterol. 2009;104(4):1024-31.

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21. Bojovic K, Simonovic J, Katanic N, Miloševic I, Pešic I, Delic D, et al. The comparison of chronic hepatitis C treatment outcome between Intravenous Drug Users and Non-intravenous Drug Users. Biomedicine & Pharmacotherapy. 2013;67(6):517-20.

22. Matthews G, Kronborg IJ, Dore GJ. Treatment for hepatitis C virus infection among current injection drug users in Australia. Clin Infect Dis. 2005;40(Suppl 5):S325-9.

23. Hallinan R, Byrne A, Amin J, et al. Hepatitis C virus prevalence and outcomes among injecting drug users on opioid replacement therapy. J Gastroenterol Hepatol. 2005;20:1082-6.

24. Klein SJ, Wright LN, Birkhead GS, et al. Promoting HCV treatment completion for prison inmates: New York State's hepatitis C continuity program. Public Health Rep. 2007;122(Suppl 2): S83-8.

25. Larrey D, Ripault MP, Pageaux GP. Patient adherence issues in the treatment of hepatitis C. Patient Prefer Adherence. 2014;8:763-73.

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Ključne reči koje su adekvatne:

Key words: Electrosurgery; Equipment Safety; Surgical Instruments; Lasers; Argon PlasmaCoagulation; Electrocoagulation; Hemostasis, Surgical; Patient Safety; Patient Care Team; Treatment Outcome

Ključne reči: Elektrohirurgija; Sigurnost opreme; Hirurški instrumenti; Laseri; Argon plazma koagulacija; Elektrokoagulacija; Hirurška hemostaza; Sigurnost pacijenta; Timsko osoblje; Ishod lečenja

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TICK-BORNE ENCEPHALITIS VIRUS INFECTION IN HUMANS

INFEKCIJA VIRUSOM KRPELJSKOG MENINGOENCEFALITISA KOD LJUDI

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Summary

Introduction. Tick-borne meningoencephalitis virus is a flavivirus that causes the most important vector-borne central nervous system infection in many countries of Europe and Asia. There are three subtypes of tick-borne encephalitis virus: European, Siberian and the Far-Eastern subtype. **Transmission.** In endemic areas, the virus remains in transmissive cycles between Ixodes ticks and small rodents. **Clinical picture.** In most cases (70−98%) infection goes asymptomatically. In about one-third of meningitis cases, meningoencephalitis or meningomyelitis is developed. Postencephalytic syndrome may be the complication of the infection, presenting with neurological symptoms. **Diagnosis.** Etiologic diagnosis of tick-borne meningoencephalitis is only made on basis of laboratory analyses. Reverse transcription-polymerase chain reaction is used for determining the presence of virus in the blood and cerebrospinal fluid. Antibodies in blood and cerebrospinal fluid can be detected by serological tests. **Prevention.** The most efficient way to control this potentially severe disease with possible serious long-term consequences is vaccination. It should be recommended to persons who live or travel to endemic areas. **Conclusion.** In Serbia, tick-borne encephalitis virus infection belongs to the list of reportable diseases; however, there are no reported cases because the diagnostics is not performed routinely. We believe that the significance of this zoonosis must be examined in our country and some of its parts because of preliminary positive serological findings found out in Vojvodina as well as because of reported cases in neighboring countries such as Hungary and Croatia and its worldwide distribution.

Key words: Encephalitis, Tick-Borne; Tick-Borne Diseases; Encephalitis Viruses, Tick-Borne; Diagnosis; Epidemiology; Disease Transmission, Infectious; Neurologic Manifestations; Vaccination; Endemic Diseases; Zoonoses

Sažetak

Uvod. Virus krpeljskog meningoencefalitisa je flavivirus, uzročnik najznačajnije vektorske infekcije centralnog nervnog sistema u mnogim zemljama Evrope i Azije. Postoje tri podtipa virusa krpeljskog meningoencefalitisa: evropski, sibirski i dalekoistočni. **Prenošenje.** U endemskim područjima virus se održava u transmisivnom ciklusu između krpelja roda *Ixodes* i malih glodara. **Klinička slika.** U većini slučajeva (70−98%) infekcija je asimptomatska. Kod oko jedne trećine slučajeva meningitisa razvijaju se meningoencefalitis ili meningomijelitis. Kao komplikacija infekcije može se razviti postencefalitisni sindrom, ispoljen neurološkim manifestacijama. **Dijagnoza.** Etiološka dijagnoza krpeljskog meningoencefalitisa zasniva se na laboratorijskim analizama. Reakcija lančane polimerizacije sa reverznom transkripcijom primenjuje se za dokazivanje virusne nukleinske kiseline u serumu i likvoru. Antitela protiv virusa krpeljskog meningoencefalitisa mogu biti dokazana serološkim testovima. **Prevencija.** Najefikasniji način kontrole ove potencijalno opasne bolesti sa mogućim dugotrajnim posledicama je vakcinacija. Vakcina se preporučuje osobama koje žive ili putuju u endemske krajeve. **Zaključak.** U Srbiji je ova infekcija na listi bolesti koje podležu obaveznom prijavljivanju, no prijavljenih slučajeva nema jer se dijagnostika ne sprovodi rutinski. Smatramo da bi trebalo ispitati značaj koji ova zoonoza ima u našoj zemlji i pojedinim njenim područjima zbog nalaza seropozitivnih u prvim preliminarnim serološkim ispitivanjima sprovedenim u Vojvodini, zbog slučajeva koji se registruju u susednoj Mađarskoj i Hrvatskoj kao i rasprostranjenosti ove infekcije u svetu.

Ključne reči: Krpeljni encefalitis; Bolesti izazvane krpeljima; Virus krpeljnog encefalitisa; Dijagnoza; Epidemiologija; Prenos zaraznih bolesti; Neurološke manifestacije; Vakcinacija; Endemske bolesti; Zoonoze

Introduction

During the last thirty years, tick-borne encephalitis virus (TBEV) infection has been dwelling in natural foci located in wide geographic areas of Japan, China, Russia, south Europe, central Europe and north Europe. The growing number of registered cases of TBEV is the consequence of improving di-

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Abbreviations

TBE – tick-borne encephalitis
TBEV – tick-borne encephalitis virus
ORF – open reading frame
PCR – polymerase chain reaction techniques
EU – European Union
WNVI – West-Nile virus infection
TLR – Toll-like receptor
RNA – ribonucleic acid

agnostic possibilities and changes of social, environmental and climatic factors. New foci of infection are constantly registered in European countries [1]. TBEV infection (TBEVI) appears to be an increasing local as well as global public health problem with significant economic consequences for human society [2]. TBEVI is endemic in 27 European countries [3]. It is not known whether TBEVI is present in Serbia and Vojvodina because of the lack of routine use of suitable diagnostic tests, so both the prevalence and the incidence of tick-borne encephalitis (TBE) in Serbia and Vojvodina remain unknown.

Preliminary serologic investigations suggest the presence of TBEV activity in south Backa district population (7,9% examined healthy persons were IgG – positive to TBEV), whereas there were no seropositive subjects in Nisava District (south Serbia) (4). There is a possibility of scenario similar to that related to West-Nile virus infection (WNVI). Namely, WNVI had been diagnosed in Serbia and Vojvodina long before its presence was confirmed in humans [5].

Tick-borne encephalitis virus is a flavivirus that causes TBE. Viral particle is spherical in shape, about 50nm in diameter, has an icosahedral capsid surrounded by a lipid layer. Viral genome is a single-stranded positive RNA 11kb in length which is limited with 5′ - non-coded region (100 nucleotides) and 3′ - non-coded region with variable length of 100 to 700 nucleotides. The open reading frame encodes three structural proteins: envelope glycoprotein E, core protein C and membrane protein M. At the envelope surface there are 180 copies of glycoprotein E positioned parallel to viral surface in form of dymer. Glucoprotein E has 3 domains: I, II and III, whereby the domain III is responsible for virus adsorption to the cell receptor, while domain II is responsible for endosomal fusion. The bearers of humoral immunity against TBEV are neutralization antibodies against antigen E. The genome encodes 7 non-structural proteins significant for virus replication in infected cell: NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. NS5 antigen of TBEV plays the role of interferon antagonist [6]. In cell cytoplasm, NS5 binds to prolidase enzyme, which is necessary for maturation of receptors for interferon IFNAR-1. This leads to the loss of IFNAR-1 receptors at the cell surface by which the virus suppress antiviral effect of interferon [7].

There are three subtypes of TBEV: European, Siberian and the Far-Eastern subtype. European subtype is widespread in Northern, Western, Central and Eastern Europe. Siberian subtype circulates in all endemic areas of Russia, whereas far eastern subtype covers China, Japan and Eastern Russia [8]. The co-circulation between various subtypes in the same geographic area is possible [9]. The presence of European and Siberian subtype of virus has been confirmed in rodents *Microtus agrestis* and *Myodes glareolus voles* in Finland [10]. The co-circulation between European and Siberian subtype in ticks *Ixodes ricinus* was verified on the Crimean peninsula in the period from 1980 to 1990 [11]. European subtype of TBEV was verified in ticks *Ixodes persulcatus* collected from the taigas of the Eastern Siberia. In Siberia, there is the predominance of Siberian subtype, whereas the European subtype is sporadic [12].

The presence of fourth and fifth genotype of TBEV has been found out by using deoxynucleotide probe set. The fourth genotype is represented by the 178-79 isolate from the region of Irkutsk (Russia), whereas the fifth genotype is represented by 10 isolates [9]. Phylogenetic analyses are mostly based on the analyses of E, NS3 and NS5 genes and show the best congruence between the Siberian and Far-Eastern subtype [6].

Transmission

In European woods as well as in Euroasian taiga and in the Far East, the virus is maintained by the transmission between ticks and small rodents. The main vectors are ticks [1]. The absence of enzymes in tick digestive tract makes a tick a suitable species for the transmission of various microorganisms [13]. A well as TBEV, ticks may transmit bacteria, for example, causative agents of Lyme disease and ehrlichiosis/anaplasmosis [14]. *Ixodes ricinus* is the vector in European countries, *Ixodes persulcatus* plays the same role in eastern European countries, Russia and the Far East, while *Ixodes ovatus* serves as a vector in Japan [15]. Ticks become infected by feeding on blood of infected animals during viremic stage. The tick must feed on a vertebrate at least once in each of its developmental stages before its transformation into the next stage. The infection of the tick remains for the whole life [8, 13]. The tick excretes the viruses by saliva and transfers them during the blood meal. All developmental stages, nymph, larva and adult form, may be infected with the virus and are capable of transmitting infection to the humans and animals. Ticks become active at the temperature of 8ºC and humidity of 70-80%. European subtype (adult tick) is the most active in the period of May – June and September - October [13].

About one hundred animals (mammals, birds, reptiles) may be infected with TBEV. The primary hosts for the virus in nature are small rodents, in which viremia lasts for 2 – 8 days with high viral load. Larger animals like foxes, rabbits, deer, wild boars, sheep, cattle and dogs do not enable tick infection because viral load following infection of these animals is not sufficiently high for tick to be infected.

Once infected, a tick can infect a human by bite. Tick saliva contains substances which act anti-in-

flammatory, anticoagulant and have an analgesic effect, so a tick bite may go unnoticed [13]. In about one third of confirmed cases, the patients do not remember the tick bite [16]. In endemic areas, humans may be infected by consuming dairy products from non-pasteurized milk of infected animals, especially goats. Outbreaks caused by consuming infected goat milk have been described [17,18]. The human is an accidental host and has no significant role in the virus maintenance in nature. Human-to-human transmission of infection has not been verified.

Pathogenesis

The primary replication occurs at the site of tick bite in Langerhans cells and granulocytes. The virus is spread by the lymph into the regional lymph nodes where it replicates. The virus then reaches various tissues including reticuloendothelial system (liver, spleen, bone marrow) where it replicates intensely. It is neurotropic and affects large neurons of the anterior horns of the spinal cord, medulla oblongata, pons, dentate nucleus, Purkinje cells and the striatum. Spreading to the central nervous system is carried out through the blood-brain barrier by means of incompletely explained mechanism. It is believed that olfactory endothelium and transcytosis play the role in virus transmission to the central nervous system through brain capillary endothelium [15]. The virus spreads through the brain from a cell to a cell per continuitatem.

The infection drives mechanisms of nonspecific and specific immune response. Specific IgM antibodies are detected shortly after infection in serum and cerebrospinal fluid and dwell for at least 6 weeks, while IgG antibodies dwell for lifetime as markers of past infection. Mononuclear cells, mostly CD4+ lymphocytes, CD8+ lymphocytes and, to a lesser degree, natural killer cells and B lymphocytes appear in the cerebrospinal fluid [6]. CD8+ lymphocytes are the most important in the development of cellular immunity.

According to the research results, inflammatory reaction and CD8+ lymphocyte activity lead to neuron impairment with severe consequences. In the research done by Růzek and al., mice with severe immunodeficiency or CD8-/- had prolonged survival after being inoculated with neuroinvasive strain of TBEV, that being indicative of immunopathologic mechanisms in the brain damage [19].

Genetic factors in the host and the virus virulence contribute to the development of TBE as well. It is possible that symptomatic forms of TBE are in connection with the deletion of human gene for chemokine receptor CCR5. Namely, it has been found out that a 32-base pair deletion is significantly more common in TBE patients than in the patients with aseptic meninoencephalitis of other etiology [20]. The research performed by Kindberg et al. has shown that functional Toll-like receptor (TLR) is related to TBE [21]. TLR 3 recognizes double-stranded ribonucleic acid (RNA) and it is related to the production of type 1 interferon and inflammatory cytokines like tumor necrosis factor α (TNFα). It has also been shown in the same research that rs3775291 mutation on gene for TLR3 represents a risk factor for encephalitis in humans. It was confirmed earlier that the virus virulence is connected with the gene for E protein of viral envelope. It is now clear that other genes might be connected with the virus virulence as well. By analyzing TBEV isolated from the persons with asymptomatic form of infection, the following three mutations have been discovered: deletion of aminoacid 111 in C protein of capside; substitution of Ser1534→Phe in NS3 which leads to the mistakes in the assembly of viral particle without RNA and substitution Ser917→Gly which results in substitution of hydrophilic aminoacid, specific for highly virulent strains by hydrophobic acid [22]. Belikov et al. have found that deletions on structural C protein of capsid and substitutions in nonstructural proteins NS3 and NS5 can reduce virulence of TBEV due to disorders in RNA replication and assembly of viral particle and processing of polyproteins [23].

Clinical Picture

The incubation period lasts for 4 – 28 days, usually 7-10 days. It is shorter (3-4 days) in cases of infection transmitted by unpasteurized milk and dairy products. Even 70 – 98% of cases of TBEV infections are asymptomatic [15]. In clinically ill persons infected by European subtype of virus, during viremia, a ''flu-like'' syndrome without neurological manifestations may develop, lasting for 2 – 7 days. The patient suffers from fever, myalgia, headache and malaise. After the first stage of disease, it may end up with recovery. In about one-third of clinically ill persons, there may be the second stage of disease after afebrile period which lasts for 1 – 20 days [13]. The second stage of disease presents with high-grade fever (>39ºC), signs of meningitis, meningoencephalitis or meningoencephalomyelitis. TBE in European countries manifests most commonly as meningitis (50% cases), then as meningoencephalitis in 40% of cases and as meningoencephalomyelitis in 10% of cases. Having followed 1,500 patients infected by TBEV in the period of 1991 – 2000, Keiser reported disturbance of consciousness in 31%, ataxia in 18%, an extremity paresis in 15% and cranial nerve palsy in 11% of patients [24]. The fatal outcome was registered in 1% of patients. In regard to laboratory findings, leukocytosis was registered in 75%, high sedimentation rate in 91%, high level of C-reactive protein in 82% and pleocytosis in cerebrospinal fluid in 100% of patients. Pathologic electroencephalography and magnetic resonance imaging findings were found in 77% and 18% of patients, respectively [25].

Various neurological sequelae, the so-called "post-encephalitis TBE syndrome" remain after 35-58% of

cases of TBE. A prospective study of 124 persons diagnosed with TBE during the 13- year period confirmed post-encephalitis TBE syndrome in 39.5% of cases presenting with spinal nerve paresis/paralysis, hearing impairment, dysarthria and severe mental disorders [26]. In infections caused by Siberian subtype, chronic progressive course of TBE is possible. For endemic area of West Siberia, chronic progressive form of disease was recorded in 1 – 1.7% of TBE cases [27]. The progressive course of disease is considered to be associated with mutations on NS1 gene and inadequate T lymphocyte response.

The mortality and severity of disease depend on the patient's age and the virus subtype. Older persons are less often asymptomatic and get severely ill more often than children [28]. Infection caused by the Far-Eastern subtype is responsible for monophasic course and the most severe clinical forms of disease. The onset is gradual with fever, headache, loss of appetite, malaise, nausea, vomiting and photophobia, followed by neurological symptoms like extremity paralyses and vision impairment [13]. The Far-Eastern subtype causes severe forms of encephalitis with involvement of the brainstem and the spinal cord. While infections caused by European and Siberian subtype lead to death in about 1%, the mortality in infection caused by the Far-Eastern subtype has reached even 30 – 40% by 1990s. The mortality in TBE caused by the Far-Eastern subtype has been reduced to 13% over the last two decades.

Diagnosis

Etiologic diagnosis of TBE is only made on basis of laboratory analyses. polymerase chain reaction (PCR) test with reverse transcription (RT – PCR) is used for determining the presence of virus in the blood and cerebrospinal fluid. This test has no significance in routine diagnostics because the majority of patients seek medical help when neurological symptoms appear, when viremia is no longer present. This is also the reason why the diagnosis is rarely made by virus isolation. The viremia lasts for about 6 days [29]. For virus isolation biosafety level (BSL) 4 is required.

In addition to neurological symptoms, antibodies in blood and cerebrospinal fluid appear. They can be detected by serological tests, most commonly by enzyme-linked immunosorbent ssay (ELISA) test. IgM antibodies are detectable at the onset of disease (during the first 6 days after the symptoms of encephalitis appeared) and can last for more than 6 weeks of disease. IgM antibodies may appear in the cerebrospinal fluid earlier than in the blood. The highest titer of IgG antibodies can be recorded in the sixth week of disease. Intrathecal IgM antibodies have been detected up to the 6th day of disease in 41% patients, as intrathecal IgG antibodies are detectable between the 21st and 61st day of disease in 98% of TBE patients [30].

The long-term presence of IgM antibodies can be a real problem in result interpretation and evaluation of time of infection. In serological diagnostics, problems arise from cross reactions within the *Flaviviridae* family [31]. These cross reactions are found in the persons infected with Dengue virus (serotypes 1–4) or those who have been vaccinated against yellow fever or Japanese encephalitis [32]. Consequently, the persons initially or repeatedly vaccinated against TBEV produce IgM and IgG antibodies against TBEV. In fatal cases, TBEV may be isolated or diagnosed with PCR from the brain tissue [33].

Distribution

During the period of 2000 - 2010, 17,741 cases of TBE were reviewed in 30 countries of European Union (EU) and European Free Trade Association (EFTA). Most of the cases were reviewed in the Czech Republic, Lithuania, Latvia and Slovenia. In Europe, the disease was recorded most frequently in males, predominantly in the period of July - October [3]. The European Network for Diagnostics of "Imported" Viral Diseases carried out a study on the distribution of TBE in Europe in the period of 2007 – 2009 and established by interviewing that TBE was on the rise in Austria, the Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Latvia, Lithuania, Russia, Slovenia, Sweden and Switzerland [34].

Since September 5th, 2012 TBE has been on the list of reportable diseases in EU countries [35]. TBE cases have been recorded in 20 out of 30 members of EU. Four countries have not recorded a single TBE case (Greece, Ireland, Spain and France). In total, 2.560 cases have been recorded. Incidence for EU is 0.52/100,000 residents. The highest incidence (13.35/100,000 inhabitants) in EU was in Estonia in 2012. The incidence in Lithuania and Slovenia was 11.69 and 7.98/100,000 respectively, whereas the incidence in the Czech Republic, Latvia and Sweden ranged from 3 – 5.5/100 000 inhabitants. A low incidence has been recorded in Austria (0.45), Finland (0.72) and Poland (0.31). The lowest incidence was found in Belgium (0.02) and Germany (0.24) [36].

In the period of 1990 – 2009, several thousands of TBE cases were recorded in Russia per year [37]. The decrease in mortality from TBE may be the consequence of the significant improvement in treatment options as well as the appearance of less virulent viral strains.

Treatment

Etiologic treatment does not exist. Therefore, the patients are treated symptomatically, including bed rest in intensive care units at the departments of infectious diseases. Bed rest until a decrease in body temperature and a significant reduction in neurological symptoms are important for better recovery.

Prevention

The best protection against TBEV infection is achieved by vaccination. Vaccines have enabled the

reduction of morbidity in areas with high incidence of TBE [38]. In Sverdlovsk region, Russia, the vaccination has been carried out since 1996 with the resulting decline in the incidence of TBE from 42.1/100,000 in 1996 to 5.1/100,000 in 2006 [16]. In pre-vaccine era, 200-700 TBE cases were reported in Austria per year contrary to 50–100 cases per year after vaccination was introduced within vaccination coverage of 85% [3]. According to Franz et al., the introduction of massive vaccination in Austria has resulted in reduction of TBE incidence by approximately 16% in relation to pre-vaccine era. The incidence remains high for unvaccinated population in Austria [39]. Vaccination is recommended for those living in endemic areas and persons who stay in affected areas for professional, tourism or recreational purposes. Complete vaccination is carried out with three doses followed by booster doses, if needed. According to available literature in English, there are four vaccines applicable in human medicine; two of them are made in EU, and the other two are produced in Russia [16]. The first generation of vaccine was developed in Russia in 1937 by cultivating vaccine strains on the mouse brain. Adverse reactions were a significant disadvantage of the first generation – vaccines for TBE. Contemporary vaccines are produced from the whole virion by cultivating on the primary cultures of chick embryo fibroblast cell which are inactivated by formaldehyde. These vaccines are highly refined and contain aluminium hydroxide as adjuvant. A study performed by Leonov on 290 subjects completely vaccinated by commercially available Russian or European vaccines has shown that all vaccines stimulate good humoral immune response and major production of high avidity neutralizing antibodies so that all available vaccines are suitable for mass vaccination against TBEV infection [5, 41].

Conclusion

In Serbia, tick-borne encephalitis virus infection belongs to the list of reportable diseases; however, there are no reported cases because the diagnostics is not performed routinely. We believe that the significance of this zoonosis must be examined in our country and some of its parts because of preliminary positive serological findings found out in Vojvodina as well as because of reported cases in neighboring countries such as Hungary and Croatia and its worldwide distribution.

References

1. Fischer M, Rabe IB, Rollin PE. Traveler's Health. In: Infectious Diseases Related to Travel - Tickborne Encephalitis. CDC. Chapter 3, 2016. Available from: http://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/tickborne-encephalitis

2. Šmit R, Postma MJ. Review of tick-borne encephalitis and vaccines: clinical and economical aspects. Expert Rev Vaccines. 2015;14(5):737-47.

3. Stokholm European Centre for Disease Prevention and Control, ECDC. Epidemiological situation of tick-borne encephalitis in the European Union and European Free Trade Association countries. 2012. [Internet]. Available from: http://www.ecdc.europa.eu/

4. Hrnjakovic Cvjetkovic I, Patic A, Nikolic N, Radovanov J, Kovačevic G, Galovic JA, et al. Seroprevalence of West Nile Virus and Tick-borne encephalitis virus in South Backa District and Nisava District. Abstract book of 48th Days of Preventive Medicine. Nis, Serbia. 2014: 35-48 P. 35. Available from: http://www.izjz-nis.org.rs/daniprevmed/14/Zbornik%2048.%20Dana%20preventivne%20medicine.pdf

5. Hrnjaković-Cvjetković I, Cvjetković D, Petrić D, Milošević V, Jerant Patić V, Zgomba M. Savremena saznanja o infekciji virusom Zapadnog Nila. Med Pregl. 2009;62(5-6):231-5.

6. Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T. Tick-borne encephalitis virus: a review of an emerging zoonosis. J Gen Virol. 2009;90(Pt 8):1781-94.

7. Lubick KJ, Robertson SJ, Mcnally KL, Freedman BA, Rasmussen AL, Taylor RT, et al. Flavivirus antagonism of type I interferon signaling reveals prolidase as a regulator of IFNAR1 surface expression. Cell Host Microbe. 2015;18(1):61-74.

8. Amicizia D, Domnich A, Panatto D, Lai PL, Cristina ML, Avio U, Gasparini R. Epidemiology of tick-borne encephalitis (TBE) in Europe and its prevention by available vaccines. Hum Vaccin Immunother. 2013;9(5):1163-71.

9. Demina TV, Dzhioev YP, Verkhozina MM, Kozlova IV, Tkachev SE, Plyusnin A, et al. Genotyping and characterization of the geographical distribution of tick-borne encephalitis virus variants with a set of molecular probes. J Med Virol. 2010;82(6):965-76.

10. Tonteri E, Jääskeläinen AE, Tikkakoski T, Voutilainen L, Niemimaa J, Henttonen H, et al. Tick-borne encephalitis virus in wild rodents in winter, Finland, 2008-2009. Emerging Infect Dis. 2011;17(1):72-5.

11. Iurchenko OA, Vinograd NA, Dubina DA. Molecular genetic characteristics of tick-borne encephalitis virus in the crimea. Vopr Virusol. 2012;57(3):40-3.

12. Adelshin RV, Melnikova OV, Karan LS, Andaev EI, Balakhonov SV. Complete genome sequences of four european subtype strains of tick-borne encephalitis virus from Eastern Siberia. Russia. Genome Announc. 2015;3(3):e00609-15.

13. Tick-Borne Encephalitis [Internet]. Vienna: Baxter; 2007. Available from: http://www.tbe-info.com/upload/media-library/Monograph_TBE.pdf.

14. Potkonjak A, Čanak G, Lako B, Ružić E, Sabljić V, Dinić U, et al. Vektorski prenosive zoonoze u Vojvodini. Medicina Danas. 2012;11(10-12):287-93.

15. Bogovic P, Strle F. Tick-borne encephalitis: a review of epidemiology, clinical characteristics, and management. World J Clin Cases. 2015;16;3(5):430-41.

16. Vaccines against tick-borne encephalitis:WHO position paper. Wkly Epidemiol Rec. 2011;86(24):241-56.

17. Hudopisk N, Korva M, Janet E, Simetinger M, Grgič-Vitek M, Gubnšek J, et al. Tick-borne encephalitis associated with consumption of raw goat milk, Slovenia, 2012. Emerging Infect Dis. 2013;19(5):806-8.

18. Balogh Z, Ferenczi E, Szeles K, Stefanoff P, Gut W, Szomor KN, et al. Tick-borne encephalitis outbreak in Hungary due to consumption of raw goat milk. J Virol Methods. 2010;163(2):481-5.

19. Růzek D, Salát J, Palus M, Gritsun TS, Gould EA, Dyková I, Grubhoffer L. CD8+ T-cells mediate immunopathology in tick-borne encephalitis. Virology. 2009;384(1):1-6.

20. Kindberg E, Mickiene A, Ax C, Akerlind B, Vene S, Lindquist L, et al. A deletion in the chemokine receptor 5 (CCR5) gene is associated with tickborne encephalitis. J Infect Dis. 2008;197(2):266-9.

21. Kindberg E, Vene S, Mickiene A, Lundkvist A, Lindquist L, Svensson L. A functional toll-like receptor 3 gene (TLR3) may be a risk factor for Tick-borne Encephalitis Virus (TBEV) Infection. J Infect Dis. 2011;203(4):523-8.

22. Belikov SI, Leonova GN, Kondratov IG, Romanova EV, Pavlenko EV. Coding nucleotide sequences of tick-borne encephalitis virus strains isolated from human blood without clinical symptoms of infection. Genetika. 2010;46(3):356-63.

23. Belikov SI, Kondratov IG, Potapova UV, Leonova GN. The Relationship between the structure of the tick-borne Encephalitis Virus Strains and Their Pathogenic Properties. PLoS One. 2014;9(4):e94946.

24. Kaiser R. Tick-borne encephalitis. Infect Dis Clin North Am. 2008;22(3):561-75.

25. Kaiser R. Tick-borne encephalitis (TBE) in Germany and clinical course of the disease. Int J Med Microbiol. 2002; 291(33):58-61.

26. Misić Majerus L, Daković Rode O, Ruzić Sabljić E. Post-encephalitic syndrome in patients with tick-borne encephalitis. Acta Medica Croatica. 2009;63(4):269-78.

27. Poponnikova TV. Specific clinical and epidemiological features of tick-borne encephalitis in Western Siberia. Int J Med Mirobiol. 2006;296(40):59-62.

28. Lindquist L,Vapalahti O. Tick-borne encephalitis. Lancet. 2008;31;371(9627):1861-71.

29. Hrnjakovic Cvjetkovic I, Radovanov J, Kovacevic G, Jovanovic Galovic A, Petric D, Petrovic T, et al. Diagnostics of human West Nile virus infection during outbreak in Vojvodina (Serbia) in 2013 16th ICID Cape Town South Africa April 2-5, 2014. Available from: https://www.xcdsystem.com/icid/authors2.html#P

30. Günther G, Haglund M, Lindquist L, Sköldenberg B, Forsgren M. Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis. Long-term follow-up related to clinical course and outcome. Clin Diagn Virol. 1997;8(1):17-29.

31. Hrnjakovic Cvjetkovic I, Petric D, Petrovic T, Kovacevic G, Radovanov J, Jovanovic Galovic A, et al. Cross-reactions in serological diagnosis of flavivirus infections. Book of Abstracts, "One Health-New Challenges" First International Symposium of Veterinary Medicine (ISSVM 2015), May 21-23,2015, editor in chief Tamas Petrovic, Vrdnik organized by Scientific Veterinary Institute Novi Sad and Institute of Veterinary Medicine of Serbia, p. 229-33.

32. Allwinn R, Doerr HW, Emmerich P, Schmitz H, Preiser W. Cross-reactivity in flavivirus serology: new implications of an old finding. Med Microbiol Immunol. 2002;190(4):199-202.

33. Holzmann H. Diagnosis of tick-borne encephalitis. Vaccine. 2003;21(Suppl 1):S36-40.

34. Donoso MO, Escadafal C, Niedrig M, Pfeffer M. Working group for Tick-borne encephalitis virus. Tick-borne encephalitis in Europe, 2007 to 2009. Euro Surveill 2011;16(39) [Internet]. Sept pii=19976. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19976

35. Amato-Gauci A, Zeller H. Tick-borne encephalitis joins the diseases under surveillance in the European Union. Euro Surveill 2012;17(42) [Internet]pii=20299. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20299

36. European Centre for Disease Prevention and Control. Annual epidemiological report 2014: Emerging and vector-borne diseases. 2014. Nov. Available from: http://ecdc.europa.eu/en/publications/Publications/emerging-vector-borne-diseases_annual-epidemiological-report-2014.pdf

37. Lindquist L. Tick-borne encephalitis. In: Tselis AC, Booss J, editors. Neurovirology: Handbook of Clinical Neurology Series Vol. 123 (3rd series). Philadelphia: Elsevier; 2014. p. 531-51.

38. Heinz FX, Stiasny K. Flaviviruses and flavivirus vaccines. Vaccine. 2012;30(29):4301-6.

39. Heinz FX, Stiasny K, Holzmann H, Grgic-Vitek M, Kriz B, Essl A, et al. Vaccination and tick-borne encephalitis, central Europe. Emerging Infect Dis. 2013;19(1):69-76.

40. Leonova GN, Pavlenko EV. Characterization of neutralizing antibodies to Far Eastern of tick-borne encephalitis virus subtype and the antibody avidity for four tick-borne encephalitis vaccines in human. Vaccine. 2009;27(21):2899-904.

41. Hrnjaković Cvjetković I, Cvjetković D, Stefan Mikić S, Patić A, Nikolić N, Milošević V. Chikungunya: a serious threat for public health. Med Pregl. 2015;68(3-4):122-5.

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THE TIBIAL APERTURE SURFACE ANALYSIS IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION PROCESS

ANALIZA POVRŠINE OTVORA TUNELA NA GOLENJAČI PRILIKOM REKONSTRUKCIJE PREDNJEG UKRŠTENOG LIGAMENTA

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Summary

Introduction. The tibial tunnel aperture in the anterior cruciate ligament reconstruction is usually analyzed as an ellipse, generated as an intersection between a tibial plateau and a tibial bone tunnel. The aim of this study is to show that the tibial tunnel aperture, which utilizes 3D tibial surface bone model, differs significantly from common computations which present the tibial tunnel anterior cruciate ligament aperture surface as an ellipse. **Material and Methods.** An interactive program system was developed for the tibial tunnel aperture analysis which included the real tibia 3D surface bone model generated from a series of computed tomography images of ten male patients, their mean age being 25 years. In aperture calculation, the transverse drill angle of 10º was used, whereas sagittal drill angles of 40º, 50º and 60º were used with the drill-bit diameter set to 10 mm. The real 3D and 2D tibial tunnel aperture surface projection was calculated and compared with an ellipse. **Results.** According to the calculations, generated 3D aperture surfaces were different for every patient even though the same drill parameters were used. For the sagittal drill angles of 40º, 50º and 60º, the mean difference between the projected 3D and 2D area on the tibial plateau was 19.6 ± 5.4%, 21.1 ± 8.0% and 21.3 ± 9.6%, respectively. The difference between the projected 3D area on the tibial plateau and ellipse surface was 54.8 ± 16.3%, 39.6 ± 10.4% and 25.0 ± 8.0% for sagittal drill angles of 40º, 50º and 60º, respectively. **Conclusion.** The tibial tunnel aperture surface area differs significantly from the ellipse surface area, which is commonly used in the anterior cruciate ligament reconstruction analysis. Inclusion of the 3D shape of the tibial attachment site in the preoperative anterior cruciate ligament reconstruction planning process can lead to a more precise individual anatomic anterior cruciate ligament reconstruction on the tibial bone. Both tibial aperture area generated in 3D and its projection on a tibial plateau are larger than the ellipse surface; therefore, individual characteristics of each patient have to be taken into consideration.

Key words: Anterior Cruciate Ligament; Anterior Cruciate Ligament Reconstruction; Arthroscopy; Tibia; Imaging, Three-Dimensional; Tomography, X-Ray Computed; Knee Joint

Sažetak

Uvod. Kod rekonstrukcije prednjeg ukrštenog ligamenta kolena otvor tunela na golenjači prikazuje se i analizira kao elipsa koja nastaje u preseku zglobne površine golenjače i tunela u golenjači. Cilj ovog rada je da površinu otvora na golenjači prikazanu u prostoru (3D) uporedimo sa uobičajenim načinom prikazivanja otvora tunela kao elipse. **Materijal i metode.** Razvijen je interaktivni kompjuterski program za analizu površine otvora tunela na osnovu realne prostorne 3D površine dobijene iz serije snimaka kompjuterzovane tomografije kod deset muškaraca prosečne starosti 25 godina. U izračunavanju je korišćen transferzalni ugao bušenja od 10 stepeni, burgija prečnika 10 milimetara, dok su sagitalni uglovi bili 40, 50 i 60 stepeni. Realne 3D i 2D projekcije površina otvora tunela na golenjači su izračunate i upoređene sa površinama elipsa. **Rezultati.** Izvršena izračunavanja pokazala su da su 3D površine otvora tunela na golenjači različite za svakog pacijenta, sa istim parametrima bušenja. Za sagitalni ugao bušenja 40, 50 i 60 stepeni, prosečne razlike između 3D i 2D površine bile su 19,6 ± 5,4%, 21,1 ± 8% i 21,3 ± 9,6%. Prosečne razlike za iste uglove između 3D projekcije i površine elipse bile su 54,8 ± 16,3%, 39,6 ± 10,4% i 25 ± 8%. **Zaključak.** Površine otvora tunela na golenjači značajno se razlikuju od površine elipse koja se uobičajeno koristi u analizi rekonstrukcije prednjeg ukrštenog ligamenta kolena. Uvođenje prostornog 3D oblika pripoja prednjeg ukrštenog ligamenta kolena u preoperatrivno planiranje dovodi do preciznije i individulano anatomske rekonstrukcije na golenjači. Prostorna 3D površina otvora tunela na golenjači i njegova 2D projekcija na zglobnoj površini golenjače veći su nego površina elipse, te se moraju uzeti u obzir individualne karakteristike svakog pacijenta.

Ključne reči: Prednji ukršteni ligament; Rekonstrukcija prednjeg ukrštenog ligamenta; Artroskopija; Golenjača; 3D Imidžing; CT; Zglob kolena

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Abbreviations
CT – computed tomography
ACL – anterior cruciate ligament
PCL – posterior cruciate ligament
SB – single bundle
DB – double bundle
ACL-R – anterior cruciate ligament reconstruction
BTB-SB – bone to bone single bundle

Introduction

One of the most common surgical interventions on a knee is anterior cruciate ligament (ACL) reconstruction [1]. An optimal anatomical replacement of ACL is essential to achieve the knee stability [2]. The ultimate goal of anatomic reconstruction surgery is to restore the native anatomy, i.e. to create femoral and tibial tunnel apertures that are similar in size and orientation to the native anterior cruciate ligament insertion [3]. Graft failure, such as a graft impingement and graft stretching, may be caused by malpositioned or nonanatomic tunnel placement resulting in the failed restoration of knee kinematics and persistent instability [4].

A great interest in the tibial insertion morphology of the ACL is shown in two critical reviews [5, 6] which have tried to draw a large number of conclusions in order to enable improvements in surgical procedures. It seems that standard tunnels actually reproduce only a fraction of the native ACL. Tibial tunnel aperture varies with the tunnel diameter and angle [7, 8]. The shape, size and position of the intraarticular aperture of the drilled tibial tunnel affects the drill-bit diameter, and sagittal (angle at which the tunnel intersects the tibial plateau) and transverse angle (tibial drill-guide adjustment by rotating the guide around the tibial shaft) [8, 9]. In recent studies, tibial aperture was analyzed as an ellipse, which is generated as an intersection between a tibial plateau and a tibial bone tunnel [7, 10–12]. Since human anatomy has a complex 3D structure with considerable individual differences, additional anatomic research using 3D imaging analysis and its clinical application are necessary in order to improve the ACL reconstruction [13–15].

Since ACL is attached to the bone three-dimensionally, the aim of this study is to show that tibial tunnel aperture area surface calculated by means of the 3D bone surface model differs from common computations which present tibial tunnel aperture area as an ellipse.

Material and Methods

The procedure of the study was approved by the Local Human Research Ethical Committee. The study sample included 10 male patients, their mean age being 24.9 ± 6.2 years, who agreed to participate in the research and had computed tomography (CT) knee scans done.

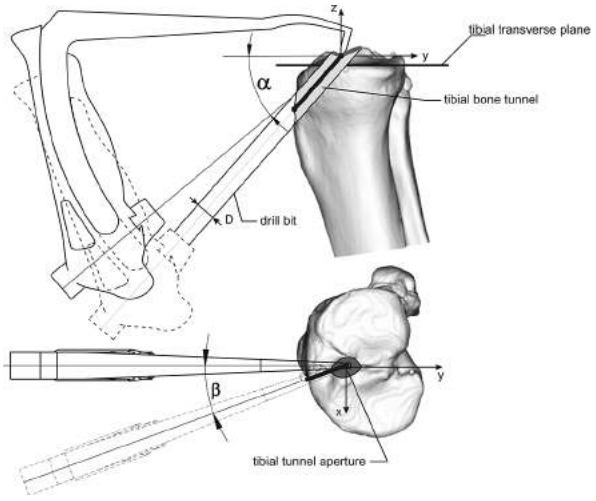

Figure 1. Parameters in the ACL reconstruction process. Transverse drill angle (b), sagittal drill angle (a), drill-bit diameter (D)
Slika 1. *Parametri u procesu rekonstrukcije prednjeg ukrštenog ligamenta. Transferzalni ugao bušenja (*b*), sagitalni ugao bušenja (*a*), prečnik burgije (D)*

The parameters of the ACL reconstruction process are presented in **Figure 1.** All 10 patients had 3D aperture surface and 3D aperture surface projected on tibial plateau calculated for a drill-bit diameter (D) of 10 mm, transverse drill angle (β) of 10° and drill-guide angles (α) of 40°, 50° and 60°.

The centre of the tibial ACL footprint was consistently located at 44% along the length of the tibial plateau, measured from the anterior edge of the tibia [16] in reference to the Amis-Jakob line [17]. Anatomic centre of the ACL tibial footprint is two-fifths of the medial-lateral width of the interspinous distance [18].

A system based on the open-source library Visualization ToolKit (VTK) [19] is developed for tibial 3D surface model generation, resulting in a tibial 3D surface model in STL (STereoLithography) file format (as a collection of many triangle surfaces). The orientation of the generated model depends on the patient's position during a CT scan. For correct analysis results, the bone model is orientated to the appropriate position, which is done interactively using the developed program system. A generated tibial bone of one patient is shown in **Figure 2** (left), produced on the MakerBot Replicator 2X printer. **Figure 2** (right) shows an enlarged complex 3D shape of ACL tibial attachment site.

The main purpose of the developed program system is to calculate the aperture surface on the tibial bone. If the program system creates the aperture surface as a hole in the bone, the geometry information of 3D surface is lost. Because of that, the drill-bit is defined as a cylindrical shape within the developed system and is approximated with vectors **(Figure 3)**. The tibial guide position as well as

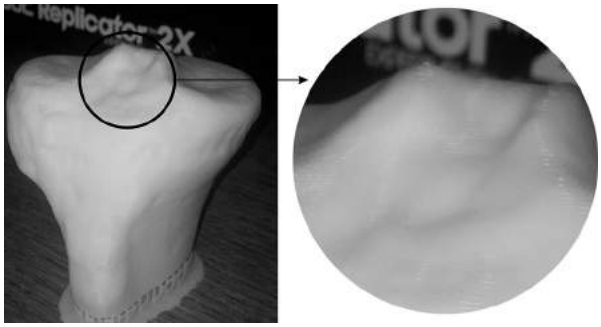

Figure 2. Generated tibial bone model of one patient produced on the MakerBot Replicator 2X printer (left), and enlarged complex 3D shape of the ACL tibial attachment site (right)
Slika 2. *Generisani model golenjače izrađen na MakerBot Replicator 2X štampaču (levo) i uveličani prikaz kompeksnog 3D pripoja prednjeg ukrštenog ligamenta na golenjači (desno)*

transverse (β) and sagittal drill angles (α) are set in the program system too. To calculate the aperture surface, intersections of all drill tool vectors in a bone model should be determined. As shown in **Figure 3** (left), a drill tool vector can intersect the bone model at many points. For aperture surface generation, only the points that intersect the tibial plateau surface are important. After the intersection points with the tibial plateau are determined, a triangulated aperture surface can be generated by connecting adjacent points (**Figure 3**, right). Subsequently, 3D surface area can be calculated as a sum of these triangle areas. For fast intersection calculation, AABB (Axis-Aligned Bounding Box) algorithm from CGAL (Computational Geometry Algorithms Library) is used [20].

Results

The calculated tibial tunnel aperture surfaces for all 10 patients are presented in **Figure 4**. Apertures generated on the left and right knees are shown in numbers 1–5 and numbers 6–10, respectively. The ellipse surfaces are the same for all patients, as already presented in **Figure 4** (left). However, it can be seen that aperture surfaces differ for every patient, the drill parameters being the same, because the tibial attachment site surface is different for every patient and is 3-dimensional.

Table 1 gives the calculated values for 3D tibial aperture area (3D), aperture area projected on tibial plateau (2D), ellipse area (EA), difference between 3D aperture area and 3D aperture area projected on tibial plateau (3D-2D), and difference between 3D aperture area projected on tibial plateau and ellipse surface (2D-EA). The ellipse surface area depends only on a drill-bit diameter and sagittal-drill angle, and is 122.2 mm^2, 102.5 mm^2 and 90.7 mm^2 for sagittal-drill angles of 40^0, 50^o and 60^0, respectively.

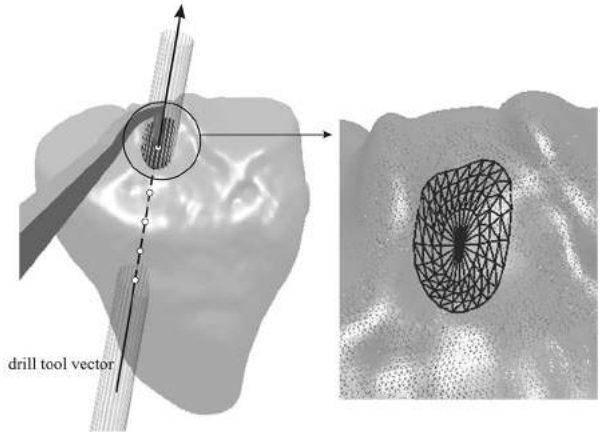

Figure 3. Drill-bit approximated with vectors (left), and generated triangulated 3D aperture surface on the tibial bone (right)
Slika 3. *Burgija aproksimirana vektorima (levo) i generisana 3D površina otvora na golenjači (desno)*

The mean 3D aperture surface areas for 40^0, 50^o and 60^0 sagittal drill angles are 225.6 ± 19.6 mm^2, 173.2 ± 16.0 mm^2 and 137.7 ± 15.5 mm^2, respectively. 3D aperture surfaces projected on tibial plateau (2D) areas for 40^0, 50^o and 60^0 sagittal drill angles are 189.2 ± 19.9 mm^2, 143.1 ± 10.7 mm^2 and 113.3 ± 7.8 mm^2. For sagittal drill angle of 40^o, the mean difference between the 3D surface and the projected 3D surface on tibial plateau are 19.6 ± 5.4%, whilst the difference between the projected 3D surface on tibial plateau and ellipse are 54.8 ± 16.3%. For sagittal drill angle of 50^o, the mean difference between the 3D surface and the projected 3D surface is 21.1 ± 8.0%, and the difference between the projected 3D surface and ellipse is 39.6 ± 10.4%. The calculated results for sagittal drill angle of 60^o show that the mean difference between the 3D surface and the projected 3D surface on tibial plateau is 21.3 ± 9.6% and the difference between the projected 3D surface and ellipse is 25.0 ± 8.0%.

Discussion

The most important finding of this study is that the generated tibial tunnel aperture surface is different for every patient in the ACL reconstruction process and it differs significantly from the ellipse (by which aperture surface is mostly presented) for the same drill parameters. When the sagittal drill angle increases, the difference between the projected 3D aperture surface on tibial plateau and the ellipse surface decreases. But even for the sagittal drill angle of 60^o, this difference is 25.0 ± 8.0. **Figure 5** shows the tibial bone profile analysis for the patient No. 4, for the used sagittal-drill angle of 50^o and transverse angle of 10^o. The generated tibial bone profile line on 3D tibial bone is shown in **Figure 5** (left). A drill bit intersection with the real bone profile is presented in **Figure 5** (middle). It may be seen that the attachment profile line is not flat, as it is assumed if

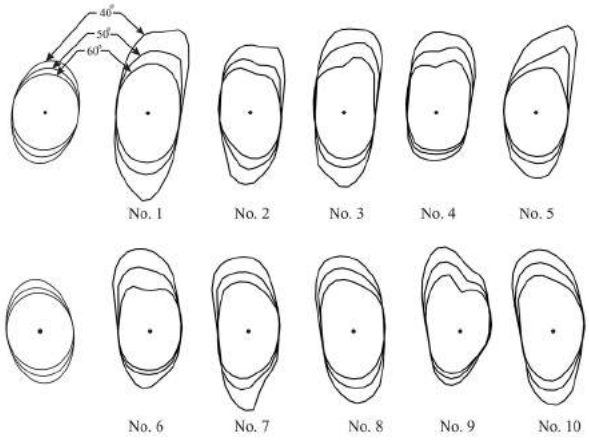

Figure 4. Generated aperture surfaces for sagittal drill angles of 40°, 50° and 60°, transverse angle of 10° and drill-bit diameter of 10 mm for all ten patients are presented. Numbers 1-5 present apertures generated on the left knees and numbers 6-10 present apertures generated on the right knees. Respective ellipse surfaces are shown on the left

Slika 4. *Generisane površine otvora za sagitalne uglove bušenja od 40°, 50° i 60°, transferzalni ugao bušenja od 10° i prečnik burgije od 10 mm za deset pacijenata. Brojevi 1–5 predstavljaju površine otvora generisane na levim kolenima, dok brojevi 6–10 predstavljaju površine otvora generisane na desnim kolenima. Odgovarajuće površine oblika elipse prikazane su sa leve strane.*

the aperture surface is considered to be an ellipse, as in **Figure 5** (right). The length of the generated aperture surface and its centre are another two important parameters of generated tibial tunnel aperture surface that should be taken into consideration and they are given in **Figure 5.** For the ellipse aperture surface, the larger axis length is symmetrical relevant to the centre of tibial insertion (a=b in **Figure 5,** right), but when the real aperture surface is analyzed, it can be seen that the larger axis length is not symmetrical relevant to the centre of tibial insertion and it depends on the ACL insertion site area shape (a≠b in **Figure 5**, middle). This means that the centre of the tibial insertion is not the centre of the generated aperture surface on the tibial bone. It depends on the individual patient's ACL attachment site surface shape.

The "classical" single-bundle (SB) procedure is performed by drilling bone tunnels according to the graft diameter, without considering the relationship between the size of the natural insertion site area and the reconstructed area. This results in a randomized reconstruction of the original ACL tibial footprint [8]. Kopf [7] showed that only 57% of the native tibial insertion was reproduced with standard drilling. Nonanatomic SB techniques were largely successful at a short-term follow-up, but do not completely restore knee kinematics and can lead to long-term degenerative changes [21]. The advantages of anatomic reconstruction in restoring joint kinematics

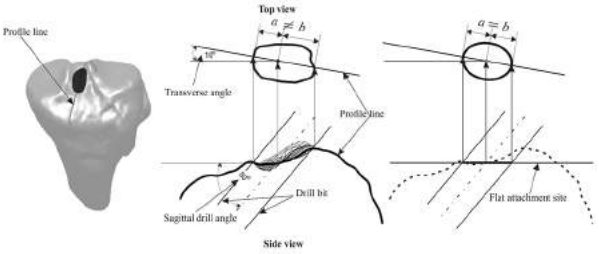

Figure 5. Tibial bone profile line for transverse angle of 10° (left), side and top views of generated real aperture surface (middle) and side and top views of generated ellipse aperture surface if it is assumed that tibial ACL attachment site is flat (right)

Slika 5. *Profilna linija golenjače za transferzalni ugao bušenja od 10° (levo), pogledi sa strane i odozgo na generisanu stvarnu površinu otvora (u sredni) i pogledi sa strane i odozgo na generisanu površinu otvora oblika elipse pod pretpostavkom da je površina pripoja prednjeg ukrštenog ligamenta ravna (desno)*

were described by Bedi et al. [22] in biomechanical cadaveric study, showing that different ACL fibers added to different knee functions, and when a constant central femoral tunnel position was used, tibial tunnel position of a SB ACL graft had a critical effect on the knee stability and impingement. Positioning the tibial tunnel in the anterior aspect of the footprint position (''a horizontal graft'') controls the Lachman and pivot-shift maneuver better than the posterior tibial footprint positioning. The authors' opinion is that the tibial tunnel position in the center of the native ACL footprint may offer the best compromise of favorable knee kinematics with an acceptably low risk of graft impingement after ACL reconstruction. Consequently, by placing the bone tunnels in a defined position, the surgeon defines the biomechanical footprint of the ACL-reconstruction (ACL-R).

The ACL-R has recently focused on moving tunnels from the conventional, nonanatomic position to the native insertion of the ACL to restore the normal knee kinematics and improve the patient's recovery. Anatomic ACL-R is the functional restoration of the ACL to its native dimensions depending on the patient's individual anatomy [23]. Sadoghi et al. [24] compared the anatomic and nonanatomic SB and DB (double-bundle) ACL reconstructions and established that the results in the knee kinematics were not significantly different from the uninjured knee kinematics in anatomic reconstructions.

During the ACL-R, the drill-bit diameter, and sagittal and transverse drill angles affect the size and orientation of the tibial tunnel aperture, and influence the restoration of the native anatomy of the tibial insertion [7, 8]. A great variation of the shape and size of ACL insertion sites is important to consider before the tunnels are drilled. In the 2D projection, the tibial ACL insertion site can vary in shape and size, although 77.8% of specimens had elliptical and 22.2% of them had triangular shaped tibial insertions [25]. The tibial graft position in both SB and DB ACL reconstruction

usually shows as a circle or an ellipse that do not give an accurate representation of the position of graft sites on their insertion. In real situations, they appear as irregular surfaces that are considered as an ellipse in order to simplify calculations [26]. The area of the 2D tibial insertion (projected on the tibial plateau) ranged from 114 mm^2 to 229 mm^2 [18, 27, 28] and in some studies [13, 29] it was concluded that there were critical bony and soft tissue landmarks of the tibial insertion site. The importance of native insertion sites in achieving anatomic ACL reconstruction has motivated several morphometric studies on insertion sites of the ACL [16, 30–32]. These insertion sites, although 3-dimensional, are often reported in 2-dimensions using the system for the tibia by Amis and Jakob [17].

For the anatomical coverage of the original ACL insertion, Rabuck et al. [23] recommended pre-operative measurement of the sagittal magnetic resonance imaging, the patellar tendon, the ACL insertion site and ACL length. Sielbold [8] developed the concept of "insertion site table" based on the idea of a "complete footprint restoration" which makes it necessary to first measure the length of the tibial ACL insertion site with a ruler from anterior to posterior.

Depending on the drill-bit diameter and the angle of drilling, the surface of the ellipse is changed for the anatomical coverage of the original ACL insertion. If the sagittal angle of the drilled tibial tunnel is smaller, the surface of the tibial insertion is larger and closer to its anatomic shape; also, if transverse angle is smaller, the anatomical coverage of the original ACL insertion is larger [7]. An optimal combination of these parameters should be selected during the anatomic reconstruction of the ACL because the decrease of the angle of penetration results in shorter tibial tunnel and a disproportion between the graft length and tunnel length, particularly in bone to bone single bundle techniques. In another cadaveric study, Piasecki et al. [33] found that the use of the more proximal tibial tunnel starting position (smaller sagittal drill angle) allowed more anatomic overlap with the native ACL footprints.

The ACL footprint was usually evaluated with a two-dimensional technique. In recent studies, tibial aperture is analyzed as an ellipse, generated as intersection between a tibial plateau and a tibial bone tunnel [7, 12]. Because of that, real bone geometry is not taken into calculations. Two publications reported measured 3D areas [13, 18]. Understandably, the area

Table 1. Calculated aperture surface areas for ten patients
Tabela 1. *Proračunate površine otvora na golenjači za deset pacijenata*

	Sagittal drill angle											
	40°				50°				60°			
	3D (mm^2)	2D (mm^2)	3D-2D (mm^2) (%)	2D-EA (mm^2) (%)	3D (mm^2)	2D (mm^2)	3D-2D (mm^2) (%)	2D-EA (mm^2) (%)	3D (mm^2)	2D (mm^2)	3D-2D (mm^2) (%)	2D-EA (mm^2) (%)
Patient No.												
Ellipse area (EA)		122.2				102.5				90.7		
1	261.3	230.6	30.7 (13.3)	108.4 (88.7)	177.9	158.3	19.6 (12.4)	55.8 (54.4)	136.2	121.3	14.9 (12.3)	30.6 (33.7)
2	223.2	182.0	41.2 (22.6)	59.8 (48.9)	154.7	130.2	24.5 (18.8)	27.7 (27.0)	123.3	104.2	19.1 (18.3)	13.5 (14.9)
3	253.3	206.5	46.8 (22.7)	84.3 (69.0)	199.7	155.2	44.5 (28.7)	52.7 (51.4)	153.7	119.3	34.4 (28.8)	28.6 (31.5)
4	211.3	167.7	43.6 (26.0)	45.5 (37.2)	175.8	138.1	37.7 (27.3)	35.6 (34.7)	144.3	112.9	31.4 (27.8)	22.2 (24.5)
5	220.4	191.9	28.5 (14.9)	69.7 (57.0)	151.8	136.1	15.7 (11.5)	33.6 (32.8)	113.1	105.1	8 (7.6)	14.4 (15.9)
6	200.8	172.9	27.9 (16.1)	50.7 (41.5)	156.2	132.9	23.3 (17.5)	30.4 (29.7)	117.8	102.9	14.9 (14.5)	12.2 (13.5)
7	221.6	185.0	36.6 (19.8)	62.8 (51.4)	166.8	139.3	27.5 (19.7)	36.8 (35.9)	135.2	112.6	22.6 (20.1)	21.9 (24.1)
8	218.1	191.8	26.3 (13.7)	69.6 (57.0)	173.4	149.2	24.2 (16.2)	46.7 (45.6)	142.2	120.6	21.6 (17.9)	29.9 (33.0)
9	211.7	163.9	47.8 (29.2)	41.7 (34.1)	185.5	134.9	50.6 (37.5)	32.4 (31.6)	154.7	109.8	44.9 (40.9)	19.1 (21.1)
10	234.5	199.7	34.8 (17.4)	77.5 (63.4)	190.5	156.9	33.6 (21.4)	54.4 (53.1)	156.1	124.7	31.4 (25.2)	34.0 (37.5)
mean±SD	225.6±19.0	189.2±19.9	36.4±8.1 (19.6±5.4)	67.0±19.9 (54.8±16.3)	173.2±16.0	143.1±10.7	30.1±11.2 (21.1±8.0)	40.6±10.7 (39.6±10.4)	137.7±15.5	113.3±7.8	24.3±11.1 (21.3±9.6)	22.6±7.8 (25.0±8.7)

3D tibial aperture area (3D), aperture area projected on tibial plateau (2D), ellipse area (EA), difference between 3D aperture area and 3D aperture area projected on tibial plateau (3D-2D) and difference between 3D aperture area projected on tibial plateau and ellipse surface (2D-EA). Drill-bit diameter is set to 10 mm, transverse drill angle of 10°, and sagittal drill angles of 40°, 50° and 60°

data measuring the plane surface of the ACL femoral origin and tibial insertion are smaller than the data resulting from the entire 3D surface. In our analysis, the difference between 3D aperture surface and its projection on the plane surface is 19.6 ± 5.4%, 21.1 ± 8.0% and 21.3 ± 9.6% for the used sagittal-drill angles of 40^0, 50^o and 60^0, respectively. However, there are significant individual differences in aperture surfaces among all the patients, which confirm that during the ACL reconstruction, individual characteristics of every patient should be taken into consideration. Anatomic ACL reconstruction is the restoration of the native ACL insertion site with essential respect to individual patient's characteristics. If ACL restoration and bone tunnel are more anatomically placed, the knee stability and kinematics are better. With respect to the rear entry guide, it is a device that is useful for assisting the surgeon to place the ACL in an anatomic position, but this device is not a perfect one. The patient's anatomy and the inherent variations have to be fully understood to be able to consistently place the ACL in an anatomic position.

Although the importance of anatomic ACL reconstruction is being increasingly appreciated, surgeons can still find it difficult to reliably identify the ACL insertions using arthroscopic techniques alone. Previous studies have shown that computer-aided surgical navigation is effective in tunnel positioning guiding, but performance studies of a fluoroscopic overlay system, as an alternative to improve tunnel positioning, are lacking [16, 34]. Since ACL is attached to the bone three-dimensionally, the improvement of the ACL footprint can be obtained by further anatomic research using 3D camera or computer graphics [13, 15].

Limitation of the study is in a relatively small number of research subjects. In addition, a center of tibial attachment for all patients is selected according to the literature data [16, 18]. It is also necessary to further investigate detailed measuring of the ACL insertion site on cadavers, which is expected to give the real position of the ACL tibial insertion site in space.

Conclusion

Results presented in this study show that there is an individual difference between real 3D aperture surface projected on tibial plateau and ellipse surface which is commonly used in the anterior cruciate ligament reconstruction analysis. This difference is 54.8 ± 16.3%, 39.6 ± 10.4% and 25.0 ± 8.7%, for sagittal drill angles of 40^o, 50^o and 60^o, respectively. The centre of the generated aperture surface can also differ from the centre of the tibial anterior cruciate ligament attachment site. A complex tibial bone anterior cruciate ligament insertion site geometry is different for every patient, so it is required that in the anterior cruciate ligament reconstruction analysis 3D tibial bone geometry is taken into consideration. This can lead to the more precise native anterior cruciate ligament reconstruction on tibial bone.

References

1. Ninković S, Avramov S, Harhaji V, Obradović M, Vranješ M, Milankov M. Influence of different levels of sports activities on the quality of life after the reconstruction of anterior cruciate ligament. Med Pregl 2015;68(3-4):116-21.

2. Ristić V, Ninković S, Harhaji V, Milankov M. Causes of anterior cruciate ligament injuries. Med Pregl 2010;63(7-8):541-5.

3. Abebe ES, Utturkar G, Taylor D, Spritzer C, Kim J, Moorman III C, et al. The effects of femoral graft placement on in vivo knee kinematics after anterior cruciate ligament reconstruction. J Biomech. 2011;44(5):924-9.

4. Moloney G, Araujo P, Rabuck S, Carey R, Rincon G, Zhang X, et al. Use of a fluoroscopic overlay to assist arthroscopic anterior cruciate ligament reconstruction. Am J Sports Med. 2013;41(8):1794-800.

5. Hwang MD, Piefer JW, Lubowitz JH. Anterior cruciate ligament tibial footprint anatomy: systematic review of the 21st century literature. Arthroscopy. 2012;28(5):728-34.

6. Kopf S, Musahl V, Tashman S, Szczodry M, Shen W, Fu FH. A systematic review of the femoral origin and tibial insertion morphology of the ACL. Knee Surg Sports Traumatol Arthrosc. 2009;17(3):213-9.

7. Kopf S, Martin DE, Tashman S, Fu FH. Effect of tibial drill angles on bone tunnel aperture during anterior cruciate ligament reconstruction. J Bone Joint Surg Am. 2010;92(4):88.

8. Siebold R. The concept of complete footprint restoration with guidelines for single-and double-bundle ACL reconstruction. Knee Surg Sports Traumatol Arthrosc. 2011;19(5):699-706.

9. Kopf S, Pombo MW, Szczodry M, Irrgang JJ, Fu FH. Size variability of the human anterior cruciate ligament insertion sites. Am J Sports Med. 2011;39(1):108-13.

10. Milankov MZ, Marcikic A, Gojkovic Z. Tibial insertion is not a circle but an ellipse. Arthroscopy. 2014;6(30):660.

11. Milankov M, Savic D, Milojevic Z. Geometric considerations regarding the surface of the tibial insertion of the ACL graft. Knee Surg Sports Traumatol Arthrosc 2012;20(9):1887-8.

12. Miller MD, Gerdeman AC, Miller CD, Hart JM, Gaskin CM, Golish SR, et al. The effects of extra-articular starting point and transtibial femoral drilling on the intra-articular aperture of the tibial tunnel in ACL reconstruction. Am J Sports Med. 2010;38(4):707-12.

13. Ferretti M, Doca D, Ingham SM, Cohen M, Fu FH. Bony and soft tissue landmarks of the ACL tibial insertion site: an anatomical study. Knee Surg Sports Traumatol Arthrosc. 2012;20(1): 62-8.

14. Hoshino Y, Kim D, Fu FH. Three-dimensional anatomic evaluation of the anterior cruciate ligament for planning reconstruction. Anat Res Int. 2011; 2012.

15. Swami VG, Cheng-Baron J, Hui C, Thompson R, Jaremko JL. Reliability of estimates of ACL attachment locations in 3-dimensional knee reconstruction based on routine clinical MRI in pediatric patients. Am J Sports Med. 2013;41(6):1319-29.

16. Musahl V, Burkart A, Debski RE, Van Scyoc A, Fu FH, Woo SL. Anterior cruciate ligament tunnel placement: comparison of insertion site anatomy with the guidelines of a computer-assisted surgical system. Arthroscopy. 2003;19(2):154-60.

17. Amis A, Jakob RP. Anterior cruciate ligament graft positioning, tensioning and twisting. Knee Surg Sports Traumatol Arthrosc. 1998;6(1):S2-S12.

18. Luites JW, Wymenga AB, Blankevoort L, Kooloos JG. Description of the attachment geometry of the anteromedial and posterolateral bundles of the ACL from arthroscopic perspective for anatomical tunnel placement. Knee Surg Sports Traumatol Arthrosc 2007;15(12):1422-31.

19. Schroeder W, Martin K, Lorensen B. An object-oriented approach to 3D graphics. 3rd ed. New Yersey: Kitware, Inc., Prentice Hall; 2003.

20. Fabri A, Pion S, editors. CGAL: the computational geometry algorithms library. Proceedings of the 17th ACM SIGSPATIAL international conference on advances in geographic information systems; 2009 Apr 11: Seatle, WA, USA, p. 538-9.

21. Ristanis S, Giakas G, Papageorgiou C, Moraiti T, Stergiou N, Georgoulis A. The effects of anterior cruciate ligament reconstruction on tibial rotation during pivoting after descending stairs. Knee Surg Sports Traumatol Arthrosc. 2003;11(6):360-5.

22. Bedi A, Maak T, Musahl V, Citak M, O'Loughlin PF, Choi D, et al. Effect of tibial tunnel position on stability of the knee after anterior cruciate ligament reconstruction is the tibial tunnel position most important? Am J Sports Med. 2011;39(2):366-73.

23. Rabuck SJ, Middleton KK, Maeda S, Fujimaki Y, Muller B, Araujo PH, et al. Individualized anatomic anterior cruciate ligament reconstruction. Arthrosc Tech. 2012;1(1):e23-e9.

24. Sadoghi P, Kröpfl A, Jansson V, Müller PE, Pietschmann MF, Fischmeister MF. Impact of tibial and femoral tunnel position on clinical results after anterior cruciate ligament reconstruction. Arthroscopy. 2011;27(3):355-64.

25. Tállay A, Lim MH, Bartlett J. Anatomical study of the human anterior cruciate ligament stump's tibial insertion footprint. Knee Surg Sports Traumatol Arthrosc. 2008;16(8):741-6.

26. Sahasrabudhe A, Christel P, Anne F, Appleby D, Basdekis G. Postoperative evaluation of tibial footprint and tunnels characteristics after anatomic double-bundle anterior cruciate ligament reconstruction with anatomic aimers. Knee Surg Sports Traumatol Arthrosc. 2010;18(11):1599-606.

27. Harner CD, Baek GH, Vogrin TM, Carlin GJ, Kashiwaguchi S, Woo SL. Quantitative analysis of human cruciate ligament insertions. Arthroscopy. 1999;15(7):741-9.

28. Iriuchishima T, Shirakura K, Yorifuji H, Aizawa S, Murakami T, Fu FH. ACL footprint size is correlated with the height and area of the lateral wall of femoral intercondylar notch. Knee Surg Sports Traumatol Arthrosc. 2013;21(4):789-96.

29. Purnell ML, Larson AI, Clancy W. Anterior cruciate ligament insertions on the tibia and femur and their relationships to critical bony landmarks using high-resolution volume-rendering computed tomography. Am J Sports Med. 2008;36(11):2083-90.

30. Colombet P, Robinson J, Christel P, Franceschi JP, Djian P, Bellier G, et al. Morphology of anterior cruciate ligament attachments for anatomic reconstruction: a cadaveric dissection and radiographic study. Arthroscopy. 2006;22(9):984-92.

31. Forsythe B, Kopf S, Wong AK, Martins CA, Anderst W, Tashman S, et al. The location of femoral and tibial tunnels in anatomic double-bundle anterior cruciate ligament reconstruction analyzed by three-dimensional computed tomography models. J Bone Joint Surg Am. 2010;92(6):1418-26.

32. Pietrini SD, Ziegler CG, Anderson CJ, Wijdicks CA, Westerhaus BD, Johansen S, et al. Radiographic landmarks for tunnel positioning in double-bundle ACL reconstructions. Knee Surg Sports Traumatol Arthrosc. 2011;19(5):792-800.

33. Piasecki DP, Bach BR, Orias AAE, Verma NN. Anterior cruciate ligament reconstruction can anatomic femoral placement be achieved with a transtibial technique? Am J Sports Med. 2011;39(6):1306-15.

34. Kodali P, Yang S, Koh J. Computer-assisted surgery for anterior cruciate ligament reconstruction. Sports Med Arthrosc. 2008;16(2):67-76.

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VARIATIONS IN TIMING OF ELECTIVE ORCHIDOPEXY

VARIJACIJE U VREMENU IZVOĐENJA ELEKTIVNE ORHIDOPEKSIJE

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Summary

Introduction. Undescended testis or cryptorchidism is detected in 3% of full-term male newborns, and in up to 33% of preemies. As the testicular descent may sometimes resolve spontaneously during first months of life, cryptorchidism is found in 1% of boys one year old. According to Consensus of Nordic experts in pediatric urology regarding cryptorchidism the optimal period for surgery is 12-18 months of age. The goal of this study was to identify the age of patients with congenital undescended testis at the time of surgery. **Material and Methods.** A retrospective study included all the cases of cryptorchid patients who had undergone orchidopexy in the period from 2007 to 2014. The patients' age and the place of residence were analyzed. **Results.** A total of 637 patients (722 orchidopexies) underwent the elective operative treatment of undescended testis during the observed period. The analysis revealed that only 144 (22.60%) of cryptorchid infants were operated on within their first 18 months of life. In the group of 359 patients from the urban environment, 101 (28.13%) were operated under the age of 18 months. Among the 278 patients from the rural environment, 43 (15.46%) were 18 months and younger at the time of surgery. **Conclusion.** The timing of surgical treatment of undescended testis in the study period was far from the recommended optimal time. It is evidently necessary to plan and provide additional information for pediatricians and parents about the current view on cryptorchidism and consequences of the late treatment.

Key words: Cryptorchism; Orchidopexy; Congenital Abnormalities; Elective Surgical Procedures; Demography; Age Factors; Child

Sažetak

Uvod. Nespušteni testis se registruje kod 3% novorođene muške dece i čak do 33% kod rođenih pre termina. Embrionalno spuštanje testisa može se spontano nastaviti u prvih nekoliko meseci posle rođenja tako da je incidencija nespuštenog testisa u uzrastu od godinu dana 1%. Prema konceptu Nordijske grupe eksperata za dečju urologiju, optimalni period za operativno lečenje je od 12 do 18 meseci života. Cilj ove studije bio je da se utvrdi uzrast pacijenata kada je hirurško lečenje urađeno i uporedi sa propozicijama Nordijske grupe eksperata. **Materijal i metode.** Retrospektivnom studijom obuhvaćeni su podaci pacijenata podvrgnutih operativnom lečenju nespuštenog testisa u periodu 2007-2014. godine, a analizirani su uzrast pacijenata u vreme operacije kao i mesto stanovanja. **Rezultati.** Tokom analiziranog perioda od osam godina, ukupan broj operisanih pacijenata zbog nespuštenog testisa bio je 637, tj. 722 orhidopeksije. Rezultati analize su pokazali da je samo kod 144 (22,60%) pacijenta operisan nespušten testis unutar prvih 18 meseci koji se smatraju optimalnim vremenom za lečenje. U grupi od 359 pacijenata iz urbanog tipa stanovanja, 101 (28,13%) pacijent je bio operisan do navršenih 18 meseci. U grupi od 278 pacijenata iz ruralnog ambijenta, 43 pacijenta (15,46%) bila su lečena operativnim putem do uzrasta od 18 meseci. **Zaključak.** Uzrast pacijenata koji su u vremenskom periodu 2007–2014. godine bili podvrgnuti operativnom lečenju kongenitalno nespuštenog testisa daleko je od preporučenog optimalnog vremena. Zato se naglašava potreba da se i pedijatri i roditelji pacijenata kontinuirano i planski informišu o savremenim stavovima u lečenju nespuštenog testisa kao i posledicama kasnog lečenja.

Ključne reči: Kriptorhizam; Orhidopeksija; Kongenitalne anomalije; Elektivna hirurgija; Demografija; Starosna dob; Dete

Introduction

As reported in literature, undescended testis (UDT) or cryptorchidism is detected in 3% of full-term male newborns, and in up to 33% of premmies [1]. Congenital cryptorchidism may sometimes resolve spontaneously, the descent occurring mostly during first months of life when endogenous testosterone secretion briefly increases. In some reports this period ranges from three to six (or twelve) months of age [2]. At the age of 1 year UDT is found in 1% of boys [1].

The main therapy for undescended testis is surgical treatment. In order to avoid ongoing testicular degenerative changes the surgery should be carried out before 12–18 months of age [3–5]. The surgical treatment for palpable testis is inguinal exploration

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Abbreviations
UDT – undescended testis

and scrotal orchidopexy. When the testis is non-palpable, laparoscopy plus orchidopexy is the method of therapy. Although pediatricians and parents are aware of the importance of UDT, orchidopexy is not always performed within the recommended period.

The age of boys with UDT at the time of orchidopexy in relation to their urban/rural residence was evaluated in this study.

Material and Methods

Demographic data of the patients subjected to elective orchidopexy in the period from 2007 to 2014 were extracted from the Information System of Pediatric Surgery Department of the Institute for Children and Youth Health Care of Vojvodina in Novi Sad as a tertiary health care institution. The hospital, which renders its service to two and a half million inhabitants living in Vojvodina, can be reached in less than 2 hours from every part. Every settlement in Vojvodina having at least 4000 residents is covered within the primary health care network. All the patients were examined by regional pediatricians before visiting the Pediatric Urology Department.

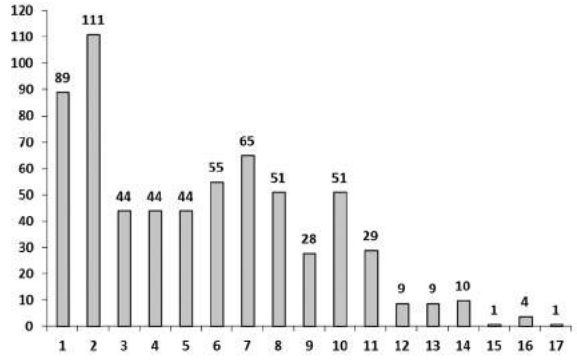
Emergency surgeries such as incarcerated hernia with undescended testis, re-do surgeries and the second stage of orchidopexy were excluded. The patients with bilateral UDT underwent both surgeries at the same time.

All the patients were examined by pediatric urologists and pediatric surgeons. After physical examination, the patients underwent scrotal ultrasound examination in search for any comorbidity (hydroceles, cysts etc.) and testicle volumetry. The patients with bilateral nonpalpable testis underwent the protocol prepared by endocrinologists (hormonal examinations, gonatotropine test, etc). As recommended by Nordic consensus [3] and several medical organizations (European Association of Urology, American Academy of Pediatrics guidelines for the management of cryptorchidism) the surgical treatment has to be finished before the age of 18 months, which was the timing used in our study as well.

Statistical Student's T-test using Microsoft office Excel 2010 system was applied to compare the significance of difference between the urban/rural groups and to measure its significance.

Results

A total of 637 patients (722 orchydopexies) aged from six months to 17 years (mean 5.24), were selected for this study covering the period from 2007 to 2014. No positive trend in the mean age over the years was noticed. There were 542 (85.08%) patients with unilateral orchidopexies and 85 (13.34%) bilateral ones. The right and left sided orchidopexy was performed in 480 (66.48%) and 242 (33.51%) patients, respectively.



Graph 1. Age distribution of boys who underwent orchidopexy in the period from 2007 to 2014
Grafikon 1. *Distribucija pacijenata koji su operisani zbog nespuštenog testisa prema uzrastu u vremenskom periodu 2007–2014. godine*

The analysis revealed that only 89 (13.97%) patients were operated on before the age of 1 year. In the optimal period within 18 months of age, 144 (22.60%) infants with cryptorchidism underwent orchidopexy. Only 200 (31.39%) infants in our sample underwent surgery within 2 years of age, which is the timing also recommended by some authors.

The highest frequency of orchidopexy was noticed in the first two years of age (89+111=200) (31.39%), and at the time of starting the primary education, i.e. 7 years in Serbia (65) (10.20%). Distribution of patients by age is presented in **Graph 1.**

Of all the patients, 359 (56.35%) lived in urban settlements and 278 (43.64%) lived in villages. In the group from the urban environment, 101 (28.13%) were under the age of 18 months. In the rural group, 43 (15.46%) were 18 months of age and younger. Student's test (Microsoft office Excel 2010) revealed a relevant statistical difference between these two groups with $p<0.001$.

Discussion

According to recent knowledge testicular physiology is marked by the transformation of neonatal gonocytes in the period from 3 to 12 months after birth. In UDT this step is disrupted and if left untreated beyond 2 years of age, there is a chance of spermatogenic failure [6,7]. Changes in testis histology in cryptorchid testes are variable depending on the age of the individual at the time of orchidopexy and the position and duration of cryptorchidism. However, some authors believe that this step may be reversible with surgery in infancy [8]. An early surgery, the optimal period being 12-18 (possibly 24) months of age, has been recommended by many authors and many medical organizations in order to prevent temperature-related damages [9-11]. Consensus is that orchidopexy should be done in a medical centre with pediatric specialists in anesthesia and surgical procedures [8].

Table 1. Published data on the age of patients who underwent orchidopexy
Tabela 1. *Publikovani podaci o uzrastu pacijenata koji su operisani zbog nespuštenog testisa*

Study *Studija*	Before age of 1 year *Pre prve godine*	18 months *18 meseci*	Before age of 2 years *Pre druge godine*
Capello, 2006 [9]			37.8%
Bruijmen, 2008 [15]			42.5%
McCabe, 2008 [16]			28.5%
Fouda, 2009 [14]	29.5%		
Kokorowski, 2010 [18]	18%		43%
Türk, 2013 [17]			40.6%
Dobanovacki, 2015 [article]	13.9%	22.6%	31.3%

According to recent literature data, orchidopexy is still performed in patients over 1 year of age despite consistent guidelines and convincing evidence of delay-related risks [12–14] and over 2 years of age [15–17]. As presented in a number of published studies, the percentage of orchidopexies done in the optimal period is about 40% or less **(Table 1)**.

Two periods have been identified by some authors as periods of increased number of surgical treatment of cryptorchidism: the first two years of age and school entry age (6-7 years) as it was noticed in our study as well. The latter peak may partly be explained by secondary testicular ascent [8], but we do not have sufficiently accurate data to support this.

It is very important that cryptorchidism can be detected by pediatricians as early as at birth, so we believe that at regular postnatal checkups during the baby's first months of life pediatricians should pay additional attention to possible spontaneous descent in that period. On any suspicion of an undescended testicle by the age of 6 months the child should be referred to a specialized surgeon for further assessment and follow-up.

The fact that the mean age at orchidopexy is significantly beyond the recommended [5, 14, 18] suggests the need for promoting more awareness among health providers.

Delayed referral of patients with UDT can occur for several reasons. Most often the condition is not identified early enough and in cases when identified, some parents fail to refer their baby timely to a surgeon because they fear surgery or do not understand the importance of appropriate surgical treatment of the condition which is not even accompanied by pain. Some delays are due to misunderstanding of the information given by a doctor that the testicle may descend by itself after the age of 6 months [15, 19]. In our study the available data on the patients' medical history were insufficient for drawing valid conclusions about the reasons of delay, but the indications are clear that the level of health culture is not satisfactory.

Our main finding is that in the period from 2007 to 2014, the patients' age at the time of surgical intervention averaged as high as 5.24 years, which indicates the necessity of raising the health awareness of parents; a number of other studies have also stressed the importance of providing additional education, information and regulation on the importance of timely orchidopexy [15, 16, 19–22].

Since the proper function of UDT depends upon the age at which the testicle descends into its normal position, the current evidence-based recommendation is to perform orchidopexy between 12 and 18 (possibly 24) months of age. Our study shows that the percentage of orchidopexies at the optimal period of the first 18 months (22.60%) and the first two years of age (31.39%) was significantly low. Besides, there is a relevant statistical difference between the patients from urban (28.13%) and rural settlements (15.46%) regarding orchidopexy done in the optimal period.

Conclusion

In our opinion, better pediatric training is necessary, and parents need appropriate information on the current consensus on treatment of undescended testis and its importance regarding the long-term health consequences. We also propose that the timing of orchidopexy for undescended testis should be regulated with the same programs (as immunization for example) in order to achieve a good result.

References

1. Mouriquand PDE. Undescended testes in children: the pediatric urologist's point of view. Eur J Endocrinology. 2008;159(Suppl 1):S83-6.

2. Virtanen HE, Bjerknes R, Cortes D, Jørgensen N, Rajpert-De Meyts E, Thorsson AV, et al. Cryptorchidism: classification, prevalence and long-term consequences. Acta Paediatr. 2007;96:611-6.

3. Ritzén EM, Bergh A, Bjerknes R, Christiansen P, Cortes D, Haugen SE, et al. Nordic consensus on treatment of undescended testis. Acta Paediatr. 2007;96:638-43.

4. Thorup J, Haugen S, Kollin C, Lindahl S, Läckgren G, Nordenskjold A, et al. Surgical treatment of undescended testes. Acta Paediatr. 2007;96:631-7.

5. Springer A, Subramaniam R, Krall C, Fülöp G. Orchidopexy patterns in Austria 1993-2009. J Ped Urol. 2013;9:535-41.

6. Park KH, Lee JH, Han JJ, Lee SD, Song SY. Histological evidences suggest recommending orchiopexy within the first year of life for children with unilateral inguinal cryptorchid testis. Int J Urol. 2007;14:616-27.

7. Virtanen HE, Cortes D, Rajpert-De Meyts E, Martin Ritzén E, Nordenskjöld A, Skakkebaek NE, et al. Development and descent of the testis in relation to cryptorchidism. Acta Paediatr. 2007;96:622-7.

8. Hutson JM. Treatment of undescended testes: time for a change in European traditions. Acta Paediatr. 2007;96:608-10.

9. Capello SA, Giorgi LJ Jr, Kogan BA. Orchiopexy practice patterns in New York State from 1984 to 2002. J Urol. 2006; 176:1180-3.

10. Gapany C, Frey P, Cachat F, Gudinchet F, Jichlinski P, Meyrat BJ, et al. Management of cryptorchidism in children: guidelines. Swiss Med Wkly. 2008;138:492-8.

11. Ritzén EM. Undescended testes: a consensus on management. Eur J Endocrinology. 2008;159(Suppl 1):S87-90.

12. Brown JJ, Wacogne I, Fleckney S, Jones L, Ni Bhrolchain C. Achieving early surgery for undescended testes: quality improvement through a multifacede approach to guideline implementation. Child Care Hlth Dev. 2004;30:97-102.

13. Guven A, Kogan BA. Undescended testis in older boys: further evidence that ascending testes are common. J Pediatr Surg. 2008;43:1700-4.

14. Fouda Neel K. Orchidopexy for undescended testis among Saudi children: is it conducted at the optimal age? Curr Pediatr Res. 2010;14:39-41.

15. Bruijnen CJP, Vogels HDE, Beasley SW. Review of the extent to which orchidopexy is performed at the optimal age: Implications for health services. ANZ J Surg. 2008;78:1006-9.

16. McCabe JE, Kenny SE. Orchidopexy for undescended testis in England: is it evidence based? J Pediatr Surg. 2008;43:353-7.

17. Türk E, Karaca F, Edme Y, Bilen CM. Reasons for delay in undescended testis: the results of two pediatric surgery centers. Turk Arch Ped. 2013;48:44-7.

18. Kokorowski PJ, Routh JC, Dionne A, Nelson G, Nelson CP. Variations in timing of surgery among boys who underwent orchidopexy for cryptorchidism. Pediatrics. 2010;126: e576-82.

19. Bonney T, Souewell B, Donnath S, Newgreen D, Hutson J. Orchidopexy trends in pediatric population of Victoria 1999-2006. J Ped Surg. 2009;44:427-31.

20. Wood R, Blair M. A comparison of Child Health Programmes recommended for preschool children in selected high-income countries. Child Care Hlth Dev. 2014;40:640-53.

21. Kolon TF, Herndon AH, Baker LA, Baskin LS, Baxter CG, Cheng EY, et al. Evaluation and treatment of cryptorchidism: AUA guideline. J Urol. 2014;192(2):337-45.

22. Živković D, Varga J, Grebeldinger S, Dobanovački D, Borišev V. Exsternal genital abnormalities in male schoolchildren: an epidemiological study. Med Pregl. 2004;5-6:275-98.

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SUSCEPTIBILITY OF RESPIRATORY ISOLATES OF STREPTOCOCCUS PNEUMONIAE ISOLATED FROM CHILDREN HOSPITALIZED IN THE CLINICAL CENTER NIŠ

OSETLJIVOST RESPIRATORNIH IZOLATA STREPTOCOCCUS PNEUMONIAE DOBIJENIH IZ MATERIJALA DECE HOSPITALIZOVANE U KLINIČKOM CENTRU NIŠ

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Summary

Introduction. *Streptococcus pneumoniae* is one of the most common causes of respiratory infections. The aim was to study the susceptibility to antimicrobial agents of respiratory isolates of *Streptococcus pneumoniae* obtained from hospitalized children. **Material and Methods.** A total of 190 respiratory pneumococcal isolates obtained from children aged from 0 to 14 years were isolated and identified by using standard microbiological methods. Susceptibility to oxacillin, erythromycin, clindamycin, tetracycline, cotrimoxazole, ofloxacin and rifampicin was tested by disc diffusion method. Minimal inhibitory concentrations for amoxicillin and ceftriaxone were determined by means of E test. The macrolide-resistant phenotype was detected by double disc diffusion test. **Results.** All tested isolates were susceptible to amoxicillin and ceftriaxone. The minimal amoxicillin concentration inhibiting the growth of 50% of isolates and of 90% of isolates was 0.50 μg/ml and 1.0 μg/ml, respectively and the minimal ceftriaxone concentration inhibiting the growth of 50% of isolates and of 90% of isolates was 0.25 μg/ml and 0.50 μg/ml, respectively. Susceptibility to erythromycin and clindamycin was observed in 21.6% and 29.47% of isolates, respectively. The resistence to macrolides - M phenotype was detected in 10.07% of isolates and constitutive macrolide-lincosamide-streptogramin phenotype (constitutive MLS phenotype) was found in 89.93% of isolates. All tested isolates were susceptible to ofloxacin and rifampicin. **Conclusion.** Amoxicillin could be the therapy of choice in pediatric practice. The macrolides should not be recommended for the empirical therapy of pneumococcal respiratory tract infection in our local area.

Key words: Streptococcus pneumoniae; Child; Macrolides; Amoxicillin; Respiratory Tract Infections; Anti-Bacterial Agents; Microbial Sensitivity Tests; Hospitals

Sažetak

Uvod. *Streptococcus pneumoniae* predstavlja jedan od najčešćih uzročnika infekcija respiratornog trakta. Cilj istraživanja bio je ispitati osetljivost izolata *Streptococcus pneumoniae* dobijenih iz materijala hospitalizovane dece sa infekcijom respiratornog trakta. **Materijal i metode.** Istraživanjem je obuhvaćeno 190 pneumokoknih izolata dobijenih iz endotrahealnih aspirata dece uzrasta 0−14 godina. Identifikovanje je izvršeno primenom standardnih mikrobioloških metoda. Primenom disk-difuzione metode ispitivana je osetljivost na oksacilin, eritromicin, klindamicin, tetraciklin, trimetoprim/sulfametoksazol, ofloksacin i rifampicin. Osetljivost na amoksicilin i ceftriakson ispitivana je određivanjem minimalnih inhibitornih koncentracija primenom E-testa. Fenotip rezistencije na makrolide određivan je primenom duplog disk-testa diskovima eritromicina i klindamicina. **Rezultati.** Svi izolati bili su osetljivi na amoksicilin i ceftriakson. Minimalna inhibitorna koncentracija amoksicilina koja inhibira rast 50% izolata iznosila je 0,50 μg/ml dok je za 90% izolata iznosila 1 μg/ml. Minimalna inhibitorna koncentracija ceftriaksona koja inhibira rast 50% izolata iznosila je 0,25 μg/ml dok je za 90% izolata iznosila 0,50 μg/ml. Na eritromicin je bilo osetljivo 21,6% a na klindamicin 29,47% izolata. Kod 10,07% izolata detektovana je rezistencija na makrolide - M fenotip, dok je čak 89,93% izolata pokazalo rezistenciju na makrolide, linkozamide i streptogramine (konstitutivni MLS fenotip). Svi ispitivani izolati bili su osetljjivi na ofloksacin i rifampicin. **Zaključak.** Ampicilin predstavlja lek izbora u empirijskoj terapiji respiratornih infekcija kod dece. U našoj sredini makrolide ne bi trebalo primenjivati u empirijskoj terapiji infekcija izazvanih pneumokokom.

Ključne reči: Streptococcus pneumoniae; Dete; Makrolidi; Amoksicilin; Infekcije respiratornog trakta; Antibiotici; Mikrobni test senzitivnosti; Bolnice

Introduction

Streptococcus pneumoniae (*S. pneumoniae*) is the cause of respiratory tract infections, otitis media, sinusitis, bacteriemia, and meningitis. Children, old people, persons with functional or anatomic asplenia and immunocompromised adults represent the most sensitive population for the development of infections caused by this bacterium.

Penicillin used to be the treatment of choice for pneumococcal infections for very long. However, the information about an ever increasing resistance

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Abbreviations

MICs – minimal inhibitory concentrations
CLSI – Clinical and Laboratory Standards Institute
MIC_{50} – minimal inhibitory concentrations which inhibits 50% of isolates
MIC_{90} – minimal inhibitory concentrations which inhibits 90% of isolates

to penicillin has caused the changes in the empirical therapeutic approach in community-acquired respiratory tract infections [1, 2]. A previous study has demonstrated that the resistance to penicillin is detected in 65% of invasive isolates obtained from pediatric patients [3]. Pneumococcal isolates demonstrating a high level of resistance to cephalosporins (minimal inhibitory concentration, (MICs ≥ 4 μg/ml)) are rare; nevertheless, they have been reported in some countries [4].

Macrolides have frequently been used and when overused, the resistance of pneumococci to these antimicrobial agents has been reported. A more recent study of the resistance of respiratory isolates of *S. pneumoniae* obtained from pediatric patients performed in Serbia (the territory of Belgrade) has shown the resistance to macrolides in 36.6% of the cases [5].

The data on regional or local susceptibility patterns can be very significant in the selection of empirical therapy. Limited data are available about the susceptibility of respiratory isolates in hospitalized children. Therefore, this study was aimed at examining the susceptibility pattern of pneumococcal isolates obtained from the respiratory tract of children hospitalized in the Clinical Center of Niš, south-eastern Serbia.

Material and Methods

The study sample consisted of isolates of *S. pneumoniae* obtained from hospitalized children**.** Pneumococcal isolates were obtained from the tracheal aspirates of 190 pediatric patients admitted with respiratory tract infections. Duplicate isolates from the same patient were excluded from the analysis. The aspirates were collected in the period from January 2012 to July 2014. The patients were between 1 month and 14 years of age (median age being 3.31 years). The children were divided into three age groups: 0-1 year; 1-2 years; and 2-14 years. *S. pneumoniae* was identified according to the colony morphology, alpha hemolysis, Gram staining, optochin susceptibility and commercial agglutination test (bioMerieux, Marcy l'Etoile, France).

Antibiotic susceptibility testing was performed using the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines and criteria for interpretation [6]. Susceptibility of all isolates was tested using the discs of oxacillin 1 μg for screening penicillin resistance, erythromycin, clindamycin, tetracycline, cotrimoxazole, ofloxacin and rifampicin (Neo-Sensitabs, Rosco Diagnostica, Taastrup, Denmark). Minimal inhibitory concentrations for amoxicillin and ceftriaxone were determined by using the E test (bioMerieux, Marcy l'Etoile, France) and the results were interpreted according to the CLSI breakpoints for non-meningeal isolates.

The macrolide resistance phenotype was detected by means of the double disc diffusion test, using the erythromycin and clindamycin discs placed 15 mm apart from each other. Isolates resistant to erythromycin and showing a circular zone around clindamycin were defined as the M phenotype; isolates showing a D-shaped zone of inhibition around the disc of clindamycin were defined as the MLS inducible phenotype; isolates with a circular zone of inhibition whose diameter was smaller than the diameter in the sensitive isolates around both erythromycin and clindamycin or without the zone of inhibition were defined as the constitutive (macrolide-lincosamide-streptogramin) MLS phenotype.

S. pneumniae ATCC 49619 strain was used for the purpose of quality control.

X^2 or Fischer's exact tests were used to compare susceptibility rates. P value < 0.05 was considered statistically significant.

Results

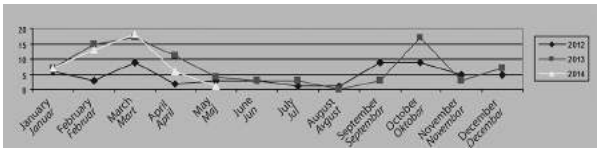
A total of 190 isolates of *S. pneumoniae* were included in the study. The seasonal distribution of respiratory isolates of *S. pneumoniae* is given in **Graph 1**.

The isolates were collected from 113 (59.47%) boys and 77 (40.53%) girls. Regarding the age group distribution, 62 (32.63%) isolates were obtained from the children in the group from 0 to 1 year, 39 (20.52%) and 89 (46.84%) were taken from the children between 1 to 2 years and 2 to 14 years of age, respectively.

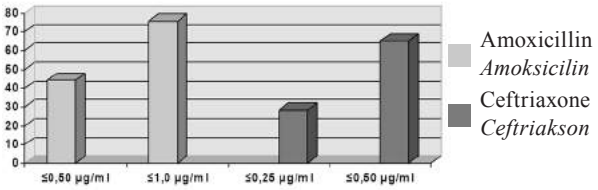
A total of 176 (92.63%) isolates were resistant to oxacillin (**Table 1**). For all isolates, the MICs to amoxicillin and ceftriaxone were determined in order to interpret the susceptibility to beta lactam

Table 1. Resistance of *S. pneumoniae* to oxacillin
Tabela 1. *Resistencija izolata S. pneumoniae na oksacilin*

Oxacillin resistant/*Rezistentni na oksacilin*				
Total No %/*Ukupno Br. %*		Age group/*Starosne grupe*		
		0 - 1 year/*godine*	1 - 2 years/*godine*	2 - 14 years/*godina*
		No of isolates (%)/*Br. izolata (%)*		
176	92.63	58 (32.95)	35 (19.88)	83 (47.16)



Graph 1. Seasonal distribution of respiratory isolates of *S. pneumoniae*
Grafikon 1. *Sezonska distribucija respiratornih izolata Streptococcus pneumoniae*



Graph 2. Isolates of *S. pneumoniae* with MICs below MIC50 and MIC90 for amoxicillin and ceftriaxone
Grafikon 2. *Prikaz izolata S. pneumoniae čije su minimalne inibitorne koncentracije ispod vrednosti MIC50 i MIC90 za amoksicilin i ceftriakson*

antibiotics. All tested isolates were susceptible to amoxicillin and ceftriaxone. For amoxicillin, minimal inhibitory concentrations inhibiting 50% of isolates (MIC_{50}) and 90% of isolates (MIC_{90}) were 0.50 µg/ml and 1.0 µg/ml, respectively (ranging from 0.016 µg/ml to 2.0 µg/ml). For ceftriaxone, MIC_{50} and MIC_{90} were 0.25 µg/ml and 0.50 µg/ml, respectively (ranging from 0.016 µg/ml to 1.0 µg/ml). Out of all tested isolates, 84 (44.21%) had an amoxicillin MIC ≤ 0.50 µg/ml and 144 (75.79%) MIC ≤ 1.0 µg/ml. Fifty-four (28.42%) isolates had a ceftriaxone MIC ≤ 0.25 µg/ml and 124 (65.26%) a MIC ≤ 0.50 µg/ml (**Graph 2**).

The susceptibility to other tested non beta-lactam antibiotics is presented in **Table 2**. Out of all tested isolates, 41 (21.6%) were susceptible to erythromycin and 56 (29.47%) to clindamycin. A low rate of susceptibility to erythromycin was detected in all age groups (14.51%; 25.64%; 24.72%). There were two different phenotypes of macrolide resistance among 149 isolates of *S. pneumoniae*: the M phenotype was detected in 15 (10.07%) isolates and constitutive MLS (resistant to erythromycin and clindamycin) in 134 (89.93%) isolates. There were 54.21% and 36.02% of isolates susceptible to tetracycline and cotrimoxazole, respectively. The susceptibility to tested antibiotics did not reveal significant differences among age groups. All tested isolates were susceptible to ofloxacin and rifampicin.

Discussion

S. pneumoniae is an organism which can be a colonizer in healthy children, but it is also one of the most common causes of respiratory infections. In case of severe respiratory tract infection, children often have to be hospitalized. The resistance of *S. pneumoniae* to antimicrobial agents could delay adequate treatment, which could lead to a higher risk for development of an invasive infection [7].

Detection of *S. pneumoniae* isolates resistant to penicillin in the 1960s, as well as the growth of resistance rates with time, caused an ever decreasing use of penicillin. Amoxicillin and cephalosporins are increasingly used in the therapy of pneumococcal infections, in addition to antibiotics from other classes [4].

The Alexander Project is the surveillance study that examined the susceptibility of bacteria causing community-acquired respiratory tract infections. The data from this study demonstrated that 95.1% of pneumococcal isolates collected in 26 countries were susceptible to amoxicillin. Only 11.5% of isolates resistant to penicillin were resistant to amoxicillin as well [8]. Moreover, orally administered amoxicillin exhibited a better pharmacokinetic profile than orally administered penicillin [9]. According to these findings, amoxicillin could be an appropriate agent for the treatment of respiratory tract infection. Since the resistance to antimicrobial agents shows geographical variations, it is essential to know the susceptibility of the etiological agent present in the local hospital. Monitoring of the resistance trends makes it possible to introduce adequate initial therapy.

In this study, we evaluated the susceptibility of respiratory isolates of *S. pneumoniae* isolated from hospitalized children. These are the first data on the susceptibility of non-invasive *S. pneumoniae* in hospitalized patients. The susceptibility to the following commonly used antibiotics was evaluated: two beta lactam antibiotics (amoxicillin and ceftriaxone), erythromycin, clindamycin, tetracycline, ofloxacin, cotrimoxazole and rifampicin.

All tested isolates were sensitive according to the CLSI breakpoints for amoxicillin for non-invasive *S. pneumoniae* (S ≤ 2 µg/ml to R 8 ≥ µg/ml). The data from the study performed by Lismond et al., which evaluated the sensitivity of respiratory isolates of *S. pneumoniae* obtained from children and adults, have shown that only 3.2% of isolates are non-susceptible to amoxicillin. However, MIC_{50} and MIC_{90} in the isolates evaluated in this study were 0.06 µg/ml and 0.125 µg/ml, respectively, which was significantly lower than minimal inhibitory concentrations detected in our study. Similarly, Lismond et al. demonstrated that MIC_{50} and MIC_{90} for ceftriaxone were 0.03 µg/ml and 0.125 µg/ml, respectively, while MIC_{50} and MIC_{90} were 0.25 µg/ml and 0.50 µg/ml, respectively for the isolates in our study [10].

The previous study showed the antimicrobial susceptibility of the total of 2279 invasive and non-invasive isolates of *S. pneumoniae* collected in eight European countries. For isolates tested in this study, MIC_{50} and MIC_{90} to amoxicillin were 0.016 µg/ml and 1.0 µg/ml, respectively, and to cefotaxime 0.03 µg/ml and 1.0 µg/ml, respectively. The lowest values of MIC_{50} and MIC_{90} for amoxicillin, being 0.016 µg/ml and 0.03 µg/ml, respectively were detected in isolates obtained in Austria, Belgium and Germany. No isolates non-sensitive to amoxicillin were detected among the isolates from Austria, Belgium and Switzerland [11].

Table 2. Susceptibility of *S. pneumoniae* to other tested non beta-lactam antibiotics by age groups
Tabela 2. *Osetljivost izolata S. pneumoniae na antibiotike kod pojedinih starosnih grupa*

Antibiotic agents *Antibiotik*	No. of isolates *Br. izolata*	Total susceptibility *Ukupno osetljivi*		Susceptibility by age group *Osetljivost po starosnim grupama*					
				0–1 years/*godine*		1–2 years/*godine*		2–14 years/*godina*	
		No	%	No.	%	No.	%	No.	%
Erythromycin/*Eritromicin*	190	41	21.6	9	14.51	10	25.64	22	24.72
Clindamycin/*Klindamicin*	190	56	29.47	15	24.19	14	35.89	27	30.33
Tetracycline/*Tetraciklin*	190	103	54.21	37	59.68	19	48.72	47	30.33
Ofloxacin/*Ofloksacin*	190	190	100	62	100	39	100	89	100
Cotrimoxazole/*Kotrimoksazol*	161	58	36.02	23	41.07	8	25	27	36.98
Rifampicin/*Rifampicin*	29	29	100	5	100	7	100	17	100

MIC_{50} and MIC_{90} for pediatric isolates collected from sterile body sites in China were higher than in our study. Ma et al. demonstrated that MIC_{50} and MIC_{90} for amoxicillin-clavulanic acid were 0.75 μg/ml and 2.0 μg/ml, respectively, and for ceftriaxone 1.0 μg/ml and 2.0 μg/ml, respectively. In non-meningeal isolates, the resistance to ceftriaxone was detected in 3.8% [12]. A rather good sensitivity of *S. pneumoniae* to amoxicillin suggests that this antibiotic can be used as an initial therapy of respiratory tract infections. However, a study performed in France has suggested that strains may appear with high-level resistance to amoxicillin as the result of selective pressure [4].

Macrolides are antibacterial agents frequently used for the treatment of respiratory tract infections in children. Moreover, since it is difficult to differentiate the pneumococcal infections of the respiratory tract from the infections caused by atypical organisms, macrolides are often used to treat these infections in pediatric outpatients. Due to simple administration, azithromycin (once a day) is commonly used as an empirical therapy of infections of the respiratory tract in children [13]. The resistance of pneumococci to these antimicrobial agents has been reported together with overuse of macrolides [1].

Horvat et al. showed increase of resistance of pneumococcal isolates obtained from outpatients of all ages, to erytromycin in the period from 2008. to 2013 [14]. The susceptibility of *S. pneumoniae* to macrolides in children has already been evaluated in Serbia, and this study included the isolates collected from various body sites. A high rate of resistance to macrolides was observed, ranging from 22.2% in 2004 to 44.9% in 2009. Macrolide resistant pneumococcal isolates showed M phenotype less frequently (27.3%) compared to constitutive MLS phenotype (72.7%) [5]. We detected a much higher rate of resistance to erythromycin and clindamycin in our isolates than in a recent study, that was 78.4% and 70.5% respectively. In our study, M phenotype was detected in only 10.07% of isolates. The majority of tested isolates in this study showed the constitutive MLS phenotype. The detected resistance rates to erythromycin and clindamycin in the studied age groups were similar. There were several possible explanations for this finding: first, our study took place a couple of years later (from January 2012 to July 2014); second, in the present study we evaluated the susceptibility of isolates obtained from the children in another part of the country, south-eastern Serbia; and third, the isolates originated only from aspirates.

A high rate of resistance to macrolides has been detected in some Asian countries, such as China (96.6%), Japan (87.9%), and Taiwan (91.6%) [12, 15, 16]. In contrast to those findings, the data from the studies in India have shown that the resistance of pneumococcal isolates to erythromycin was very low. The resistance of invasive pneumococcal isolates to erythromycin was only 4.2% in India, whereas there were no pneumococcal isolates from children with severe pneumonia that showed resistance [17, 18].

Reinert et al. have reported the susceptibility to clarithromycin of pneumococcal isolates collected in 31 centers of eight European countries and the resistance in 28% of isolates. The resistance rates showed a degree of variability among the countries. Lower resistance rates were detected in Austria, Germany and Portugal (10.0%; 10.6%; and 10.3%, respectively), in Belgium it was 23.7%, in Switzerland 17.3%, while the highest resistance rates were detected in Italy, Spain and France (35.5%; 43.6%; and 46.1%, respectively). In macrolide resistant isolates of *S. pneumoniae,* the constitutive MLS phenotype was more common than M phenotype [11].

In our study, resistance rates to cotrimoxazole and tetracycline (63.7% and 45.8%, respectively) were lower than those to erythromycin and there were no isolates resistant to ofloxacin and rifampicin. In the study of Reinert et al., susceptibility testing of *S. pneumoniae* to twelve antibiotics was performed and the highest rate of resistance was detected to cotrimoxazole in isolates collected in Austria (11.3%), Germany (14.5%), Italy (41.1%), Portugal (24.1%), Spain (66.5%) and Switzerland (28.9%) [11].

Reynolds et al. have reported that the largest increase of treatment costs was observed for the patients hospitalized with pneumococcal isolates resistant to

erythromycin. The authors estimated that 32% of pediatric outpatients under 18 years of age treated with erythromycin for pneumococcal pneumonia had to be hospitalized due to inadequate treatment. In order to reduce the impact of macrolide treatment failure, the Pediatric Infectious Disease Society has suggested the use of amoxicillin in the treatment of community-acquired pneumonia, while macrolides should be administered only in case of infection with atypical microorganisms [19].

Conclusion

The susceptibility of non-invasive pneumococcal isolates obtained from hospitalized children was reported in this study. The high rate of resistance of *S. pneumoniae* isolates detected in our study indicated that macrolides should not be recommended as an empirical therapy of pneumococcal respiratory tract infection in our territory. Opposite to this finding, the susceptibility of all tested isolates to amoxicillin indicated that this antibiotic could be the therapy of choice in pediatric practice. Further research is warranted in order to monitor the trends of sensitivity of *S. pneumoniae.* It would also be valuable if we could ascertain whether the hospitalization of pediatric outpatients with pneumococcal respiratory tract infection is the consequence of treatment failure.

References

1. Linares J, Ardanuy C, Pallares R, Fenoll A. Changes in antimicrobial resistance, serotypes and genotypes in Streptococcus pneumoniae over a 30-year period. Clin Microbiol Infect. 2010;16:402-10.

2. Jones RN, Jacobs MR, Sader HS. Evolving trends in Streptococcus pneumoniae resistance: implications for therapy of community-acquired bacterial pneumonia. Int J Antimicrob Agents. 2010;36:197-204.

3. Gajić I, Mijač V, Ranin L, Andjelković D, Radičević M, Opavski N. Invasive isolates of Streptococcus pneumoniae in Serbia: antimicrobial susceptibility and serotypes. Srp Arh Celok Lek. 2013;141:48-53.

4. Doit C, Loukil C, Fitoussi F, Geslin P, Bingen E. Emergence in France of multiple clones of clinical Streptococcus pneumoniae isolates with high-level resistance to amoxicillin. Antimicrob Agents Chemother. 1999;43:1480-3.

5. Mijac V, Opavski N, Markovic M, Gajic I, Vasiljevic Z, Sipetic T, et al. Trends in macrolide resistance of respiratory tract pathogens in the paediatric population in Serbia from 2004 to 2009. Epidemiol Infect. 2015;143:648-52.

6. Clinical and Laboratory Standards Institute (CLSI): Performance Standards for Antimicrobial Susceptibility Testing; Nineteenth Informational Supplement. M100-S19. Wayne, USA 2009, 29.

7. Hammerschmidt S, Paterson GK, Bergmann S, Mitchell TJ. Pathogenesis of Streptococcus pneumoniae infections: adaptive immunity, innate immunity, cell biology, virulence factors. In: Suttorp N, Welte T, Marre R, editors. Community-Acquired Pneumonia. Basel: Birkhauser Verlag; 2007. p. 139-81.

8. Jacobs MR, Felmingham D, Appelbaum PC, Gruneberg RN, and the Alexander Project Group. The Alexander Project 1998-2000: susceptibility of pathogens isolated from community-acquired respiratory tract infections to commonly used antimicrobial agents. J Antimicrob Chemother. 2003;52:229-46.

9. Garau J. Both penicillin and amoxycillin should be tested in antimicrobial surveillance for Streptococcus pneumoniae. Clin Microbiol Infect. 2005;11:422-3.

10. Lismond A, Carbonelle S, Verhaegen J, Schatt P, De Bel A, Jordens P, et al. Antimicrobial susceptibility of Streptococcus pneumoniae isolates from vaccinated and non-vaccinated patients with a clinically confirmed diagnosis of community-acquired pneumonia in Belgium. Int J Antimicrob Agents. 2012;39:208-16.

11. Reinert RR, Reinert S, van der Linden M, Cil MY, Al-Lahham A, Appelbaum P. Antimicrobial susceptibility of Streptococcus pneumoniae in eight European countries from 2001 to 2003. Antimicrob Agents Chemother. 2005;49:2903-13.

12. Ma X, Zhao R, Ma Z, Yao K, Yu S, Zheng Y, et al. Serotype distribution and antimicrobial resistance of Streptococcus pneumoniae isolates causing invasive diseases from Shenzhen Chidren's Hospital. PloS One. 2013;8(6):e67507.

13. Ovetchkine P, Rieder MJ. Azithromycin use in paediatrics: a practical overview. Paediatr Child Health. 2013;18:311-3.

14. Horvat O, Mihajlović-Ukropina M, Mijatović V, Sabo A. Susceptibility of common bacterial respiratory pathogens to antimicrobial agents in outpatients from South Backa District. Med Pregl. 2014;67(3-4):71-7.

15. Yokota S, Sato K, Yoshida S, Hayashi T, Matsuda K, Kuwahara O, et al. Macrolide-resistant Streptococcus pneumoniae clinical isolates that occur in Hokkaido prefecture, Japan. J Infect Chemother. 2004;10:284-7.

16. Lin W, Lo W, Chou C, Chen Y, Tsai S, Chu M, et al. Antimicrobials resistance pattern and serotype distribution of invasive Streptococcus pneumoniae isolates from children in Taiwan from 1999 to 2004. Diagn Microbiol Infect Dis. 2006;56:189-96.

17. Invasive Bacterial Infection Surveillance (IBIS) group, International Clinical Epidemiology Network (INCLEN). Prospective multicentre hospital surveillance of Streptococcus pneumoniae disease in India. Lancet. 1999;353:1216-21.

18. Bansal A, Singhi SC, Jayashree M. Penicilin and gentamicin therapy vs amoxicillin/clavulanate in severe hypoxemic pneumonia. Indian J Pediatr. 2006;73:305-9.

19. Reynolds CA, Finkelstein JA, Ray GT, Moore MR, Huang SS. Attributable healthcare utilization and cost of pneumonia due to drug-resistant Streptococcus pneumoniae: a cost analysis. Antimicrob Resist Infect Control. 2014;21:3-16.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
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LOWER BACK PAIN – SILENT SYMPTOM OF CHRONIC INFRARENAL ABDOMINAL ANEURYSM RUPTURE

LUMBALNI BOL – „TIHI“ SIMPTOM HRONIČNE RUPTURE ANEURIZME INFRARENALNE ABDOMINALNE AORTE

Mirela JUKOVIĆ[1], Tijana KOKOVIĆ[1], Dragan NIKOLIĆ[2], Dalibor ILIĆ[1] and Viktor TILL[1]

Summary

Introduction. The rupture of infrarenal abdominal aortic aneurysm is a surgical emergency condition with a high rate of mortality before the patients arrive at hospital. The signs and symptoms of abdominal aortic aneurysm rupture into the retroperitoneal cavity are pulsatile mass, abdominal pain, hypotension and shock, but sometimes silent symptoms also hide a dangerous and life threatening condition, such as chronic aneurysm rupture of abdominal aorta into the retroperitoneal cavity. **Case Report.** We present a patient having had the lower back pain for 4 months, which had been recognized and treated as *lumbar ischialgia* but which was eventually diagnosed to be chronic infrarenal abdominal aortic aneurysm rupture by computed tomography angiography . The surgical intervention was successful and the patient was discharged from hospital after 6 days without any clinical complications. Preoperative imaging by computed tomography angiography of ruptured abdominal aortic aneurysm is highly sensitive for detection of several specific signs for rupture. This condition leads to urgent vascular surgery.

Key words: Low Back Pain; Aortic Aneurism, Abdominal; Aortic Rupture; Signs and Symptoms; Emergency Treatment; Tomography, X-Ray Computed; Diagnosis, Differential

Sažetak

Uvod. Ruptura aneurizme infrarenalnog segmenta abdominalne aorte urgentno je kliničko stanje sa visokim procentom smrtnosti pre nego što pacijent dospe u bolnicu. Simptomi i znaci rupture aneurizme infrarenalnog segmenta abdominalne aorte u abdominalnu duplju jesu: masa koja pulsira, bol u trbuhu, hipotenzija i šok, ali nekada „tihi“ simptomi kriju preteće i po život ozbiljno stanje, kao što je hronična ruptura abdominalne aorte u retroperitonealni prostor. **Prikaz slučaja.** Prikazujemo pacijenta sa istorijom četvoromesečnog bola u leđima koji je prepoznat i lečen kao lumboishijalgija, a koji je imao hroničnu rupturu aneurizme infrarenalnog segmenta abdominalne aorte, dijagnostikovanu kompjuterizovanom tomografskom angiografijom. Hirurška intervencija bila je uspešna i pacijent je otpušten iz bolnice šest dana kasnije, bez kliničkih komplikacija. Preoperativni imidžing kompjuterizovanom tomografskom angiografijom rupturirane aneurizme infrarenalnog segmenta abdominalne aorte visoko je senzitivan za detekciju specifičnih znakova hronične rupture. Ovakvo stanje pacijenta vodi prema urgentnom vaskularnom tretmanu.

Ključne reči: Lumbalni bol; Aneurizma abdominalne aorte; Ruptura aorte; Znaci i simptomi; Urgentno lečenje; CT; Diferencijalna dijagnoza

Introduction

The abdominal aorta is a complex retroperitoneal organ with normal diameter less than 3 cm. Focal dilatation more than of 50% of diameter of abdominal aorta is recognized as aneurysm [1]. Many pathophysiological mechanisms and inherited disorders are recognized as contributing factors for aneurysm formation [2]. In general population an abdominal aneurysm has the prevalence between 2 and 5% [3]. The rupture of abdominal aortic aneurysm (AAA) is a surgical emergency condition with a high rate of mortality before the patients arrive at hospital. The literature reports the following four types of abdominal aortic aneurysm rupture: “open” rupture, “sealed” or chronic-contained aortic aneurysm rupture, “closed” rupture and rupture in the surrounding abdominal cavity tissues [4]. The signs and symptoms of acute abdominal aortic rupture into the retroperitoneal cavity are usually pulsatile mass, abdominal pain and hemodynamically unstable patients leading to hypotension and shock [5]. Because of unusual clinical presentation and silent symptoms, chronic abdominal aortic rupture could be overlooked.

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Abbreviations

AAA – abdominal aortic aneurysm
CT – computed tomography
CTA – computed tomography angiography
EVAR – endovascular aortic repair

Case Report

We present a patient having had the lower back pain for 4 months who had been recognized as a patient with *lumbar ischialgia* with underlying chronic infrarenal abdominal aneurysm rupture. On admission to hospital, a 60 year-old man, a smoker, was in good condition, hemodinamically stable, with high blood pressure, who had had myocardial infarction 15 years before. Since his laboratory analysis findings were normal, he was referred to computed tomography angiography (CTA) of abdominal aorta and common iliac arteries. On this exam, the abdominal aorta was 10 cm in diameter with discontinuity of circumferential wall calcification, with a large left sided retroperitoneal hematoma into the psoas muscle, without a post contrast enhancement of hematoma and without a contrast extravasation in the abdominal cavity **(Figure 1)**. An abdominal aortic sack eroded the frontal aspect of adjacent L3 vertebral body **(Figure 2)**, which is not common in daily clinical and radiological practice and speaks in favor of chronic aneurysm rupture. Having undergone diagnostic procedure, the patient was subjected to vascular surgery treatment. The xyphopubic laparotomy was performed. After bowel lateralization, the proximal and distal clamps were set, with prepa-

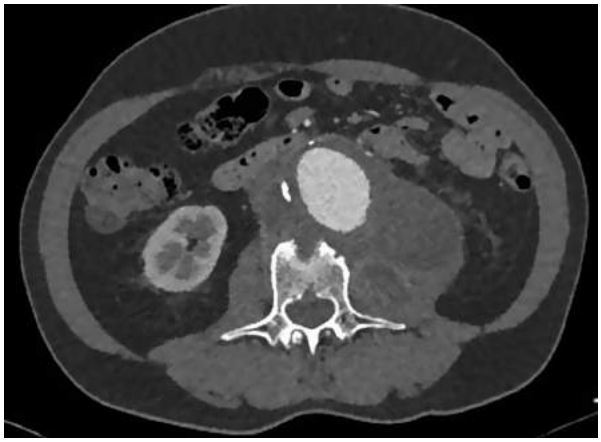

Figure 1. Axial CT scan of chronic ruptured abdominal aortic aneurysm with retroperitoneal hematoma of the left side in the psoas muscle
Slika 1. *Kompjuterizovana tomografija - aksijalni presek hronične rupture aneurizme trbušne aorte sa retroperitonealnim hematomom sa leve strane smeštenom u mišiću iliopsoasu*

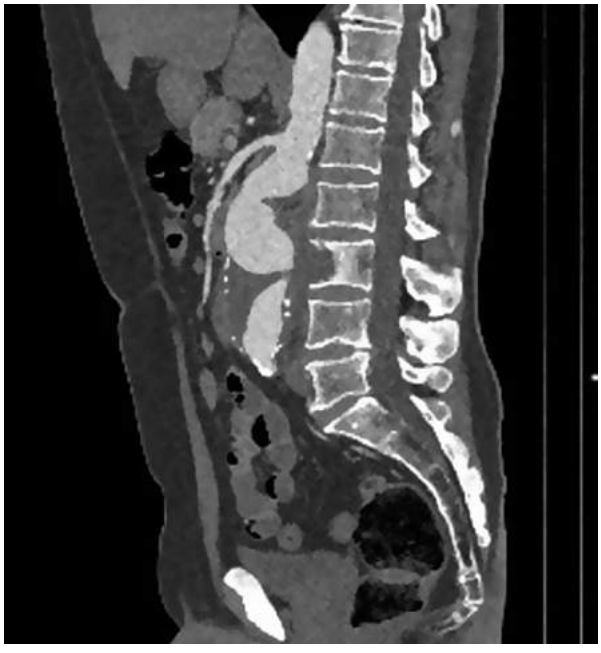

Figure 2. Sagital CT scan - erosion area of L3 vertebral body by abdominal aortic aneurysm
Slika 2. *Kompjuterizovana tomografija - sagitalni presek − erozija prednjeg segmenta tela trećeg slabinskog pršljena, izazvana aneurizmom trbušne aorte*

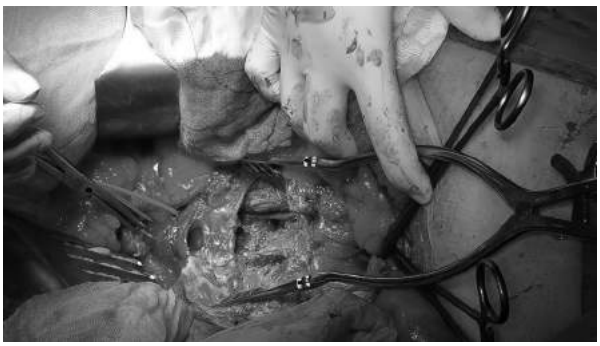

Figure 3. Intraoperative finding: chronic abdominal aortic ruptured aneurysm after setting of proximal and distal clamps, aneurysm neck and both common iliac artery preparation, erosion of anterior vertebral bodies
Slika 3. *Intraoperativni nalaz: hronična ruptura aneurizme trbušne aorte; preparacija vrata aneurizme trbušne aorte i bedrenih arterija; erozija prednjeg aspekta tela pršljena*

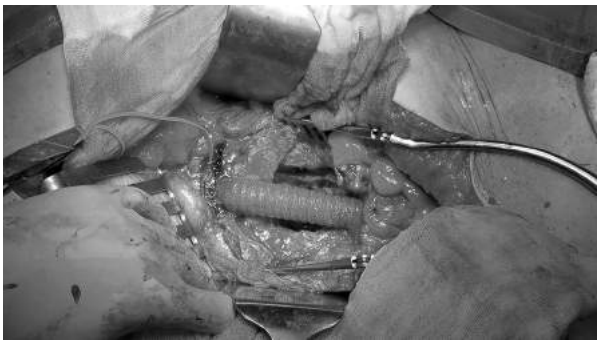

Figure 4. Aneurysmectomy of infrarenal ruptured AAA and interposition with Dacron Silver tubus 16 mm prosthesis
Slika 4. *Aneurizmektomija infrarenalnog segmenta rupturirane aneurizme trbušne aorte sa postavljenom Dakron Silver protezom od 16 mm*

ration of aneurysm neck and both common iliac arteries. Intraoperative findings corresponded to CT angiography report **(Figure 3)**. Aneurysmectomy of infrarenal ruptured AAA was done as well as interposition with Dacron Silver 16 mm prosthesis **(Figure 4)**. The surgical intervention was successful and the patient was discharged from hospital after 6 days without any clinical complications.

Discussion

According to the location of process, there are several computed tomography signs of impending and total rupture of AAA. If the aneurysm is larger in size, the risk for rupture is higher [1]. The precise mechanism of chronic abdominal aneurysm rupture has not been clarified. A large aneurysmal sac triggers a strong local reaction around the aneurysm that provides high resistance to blood extravasation if AAA ruptures. The psoas muscle and vertebral body resist the pressure of retroperitoneal hemorrhage [6] as was the case in our patient. In addition, a small aneurysmal tear, fibrosis and organized thrombus are considered to be the mechanism for chronic AAA rupture [7]. CTA is a very sensitive diagnostic imaging for complete and sovereign diagnosis of chronic AAA retroperitoneal rupture. It could detect signs for chronic AAA rupture such as discontinuity of calcification of the aortic wall, soft peri-aneurysmal tissue density, and non-enhanced retroperitoneal hematoma in the psoas muscle and sometimes eroded area of adjacent vertebral body [8]. Having undergone diagnostic testing, the patients are planned for vascular treatment with two approaches- an abdominal aortic aneurysm open repair and endovascular aortic repair (EVAR) [9].

Conclusion

The lower back pain without neurological deficit should be considered a potential silent symptom of chronic infrarenal abdominal aortic aneurysm rupture and use of computed tomography angiography in this situation is justified. Quick preoperative diagnostic using computed tomography angiography for suspected rupture of infrarenal abdominal aortic aneurysm accelerates better planning for correct vascular treatment and consequently increases the survival rate of patients.

References

1. Nhien Vu K, Kaitoukov Y, Morin-Roy F, Kauffmann C, Giroux MF, Thérasse E, et al. Rupture signs on computed tomography, treatment, and outcome of abdominal aortic aneurysms. Insights Imaging. 2014;5:281-93.

2. Erbel R, Eggebrecht H. Aortic dimensions and the risk of dissection. Heart. 2006;92:137-42.

3. Martínez Pérez R, Marenco De La Fuente JL, Rodríguez Montero S, Escudero C. Chronic back pain as the first symptom in the rupture of an abdominal aortic aneurism: presentation of 2 cases. Reumatol Clin. 2010;6(5):273-4.

4. Davidović LB, Lotina SI, Cinara IS, Zdravković ĐM, Simić TA, Đorić PL. Chronic rupture of abdominal aortic aneurysms. Srp Arh Celok Lek. 1998;126(5-6):177-82.

5. Assar AN, Zarins CK. Ruptured abdominal aortic aneurysm: a surgical emergency with many clinical presentations. Postgrad Med J. 2009;85:268-73.

6. Erdogan A, Gilgil E, Demircan A. Vertebral erosion resulting from a chronic retroperitoneal rupture of an abdominal aortic aneurysm. EJVES Extra. 2005;9:113-5.

7. Dobbeleir J, Fourneau I, Maleux G, Daenens K, Vandekerkhof J, Nevelsteen A. Chronic contained rupture of an abdominal aortic aneurysm presenting as a grynfeltt lumbar hernia: a case report. Acta Chir Belg. 2007;107:325-7.

8. Ando M, Igari T, Yokoyama H, Satokawa H. CT features of chronic contained rupture of an abdominal aortic aneurysm. Ann Thorac Cardiovasc Surg. 2003;9:274-8.

9. Šarac M, Marjanović I, Zoranović U, Jevtić M, Mišović S, Rusović S. Embolizacija donje mezenterične arterije-prevencija endoleaka tipa II. Med Pregl. 2012;65(5-6):255-8.

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Case report
Prikaz slučaja
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TRANEXAMIC ACID IN SURGICAL TREATMENT OF SCOLIOSIS IN CHILDREN: A CASE REPORT

TRANEKSAMIČNA KISELINA U HIRURŠKOM TRETMANU SKOLIOZE KOD DECE: PRIKAZ SLUČAJA

Goran RAKIĆ[1], Danica STANIĆ[1], Anna URAM BENKA[1], Marina PANDUROV[1], Jovana M. SIMIN[1] and Biljana DRAŠKOVIĆ[1,2]

Summary

Introduction. Children who are subjected to surgical treatment for scoliosis usually end up receiving a lot of blood transfusions since they tend to lose one or more blood volumes during the surgery. Tranexamic acid is an antifibrinolytic agent, increasingly used in children to reduce perioperative blood loss in various settings, including corrective surgery of scoliosis. **Case Report.** A 12-year-old girl, weighing 44 kg, was admitted to our hospital for scoliosis correction. She had congenital scoliosis caused by congenital malformation of vertebrae. The surgery was performed under balanced general anesthesia. Two central and one peripheral line were cannulated in case massive transfusion would be required. Invasive monitoring was used, as well as prevention of hypothermia. Since massive blood loss was expected, bolus of tranexamic acid had been administered prior to the surgery. Tranexamic acid was given continuously in an intravenous infusion during the surgery. Blood loss was only 10 ml/kg, and since the hemoglobin value was borderline (89 g/l) during the surgery, the patient received 10 ml/kg of packed red blood cells. The child was hemodynamically stable throughout the surgery. After the completion of surgery, which lasted for 5 hours, the patient was extubated in the operating room. Postoperatively, the patient was transferred to the surgical ward. Hemoglobin values were stable and there was no need for additional blood replacement. **Conclusion.** Extensive blood loss is common in pediatric scoliosis correction surgery, transfusion being unavoidable in the majority of cases. In our patient, tranexamic acid proved safe and effective in reducing perioperative blood loss and transfusion requirement.

Key words: Tranexamic Acid; Antifibrinolytic Agents; Scoliosis; Child; Perioperative Period; Blood Loss, Surgical

Sažetak

Uvod. Deca kojoj se radi korektivna operacija skolioze često dobiju obimne transfuzije krvi jer se tokom ove procedure često gubi jedan ili više cirkulišućih volumena krvi. Traneksamična kiselina je antifibrinolitik, koji se sve češće koristi kod dece sa ciljem smanjenja perioperativnog krvarenja u različitim situacijama, pa i u hirurškom zbrinjavanju skolioze. **Prikaz slučaja.** Dvanaestogodišnja devojčica, telesne mase 44 kg hospitalizovana je na našem institutu radi hirurške korekcije kongenitalne skolioze. Operacija je izvedena u opštoj balansiranoj anesteziji. Plasirane su dve centralne i jedna periferna venska linija u slučaju potrebe za masivnom transfuzijom krvi. Sproveden je invazivni hemodinamički monitoring kao i prevencija hipotermije. Pošto je veliki gubitak krvi unapred očekivan, pre početka operacije ordiniran je bolus traneksamične kiseline. Tokom operacije traneksamična kiselina je data u kontinuiranoj intravenskoj infuziji. Gubitak krvi bio je samo 10 ml/kg i, s obzirom da su vrednosti hemoglobina intraoperativno bile granične, ordinirano je 10 ml/kg resuspendovanih eritrocita. Devojčica je bila hemodinamički stabilna tokom cele operacije. Nakon petočasovne operacije, devojčica je ekstubirana u operacionoj sali. Postoperativno je smeštena na Odeljenje ortopedije. Vrednosti hemoglobina sve vreme su bile stabilne i nije bilo potrebe za dodatnom transfuzijom. **Zaključak.** Obiman gubitak krvi je česta pojava u rekonstruktivnoj hirurgiji skolioze, a masivna transfuzija krvi se u najvećem broju slučajeva ne može izbeći. Kod našeg pacijenta traneksamična kiselina se pokazala efikasnom u smanjenju perioperativnog krvarenja kao i potrebe za transfuzijom krvi.

Ključne reči: Traneksamična kiselina; Antifibrinolitički agensi; Skolioza; Dete; Perioperativni period; Krvarenje u hirurgiji

Introduction

Children who are subjected to surgical treatment of scoliosis usually end up receiving a lot of blood transfusions since they tend to lose one or more blood volumes during the surgery [1]. Therefore, extensive blood transfusion as well as transfusion-related morbidity and mortality [2] are common in these patients.

Since the lost blood volume is replaced with crystalloid, colloid solutions and packed red blood cells, coagulation factors are diluted, fibrinolysis increased and surgical bleeding increases [3].

Tranexamic acid (TXA) is an antifibrinolytic agent, increasingly used in children to reduce perioperative blood loss in various settings, including corrective surgery of scoliosis [4–6].

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Abbreviations
TXA – tranexamic acid

Case Report

A 12-year-old girl, weighing 44 kg, was admitted to our hospital for scoliosis correction. She had congenital scoliosis caused by congenital malformation of vertebrae (Hemivertebra L1).

The patient was premedicated with 4 mg intravenous Midazolam, 30 minutes before induction. The induction was intravenous with bolus of Fentanyl (1 μg/kg), Propofol (2.5 mg/kg) and Cisatracurium (0.15 mg/kg). The patient was intubated with 6.5 sized cuffed endotracheal tube. The right internal jugular vein and the right femoral vein were cannulated with 17 Gauge and 18 Gauge central venous catheter, respectively. Invasive arterial blood pressure was monitored via the right radial artery, cannulated with 22 Gauge cannula. The peripheral venous access included 20 Gauge cannula in the right upper limb. A urinary catheter was inserted for measuring the urine output. The anesthesia was maintained with oxygen, air, sevoflurane, cisatracurium and continuous infusion of remifentanyl. The patient was positioned very carefully in the prone position and all intravenous fluids were warmed, as well as the operating room and the patient herself in order to prevent hypothermia.

Since massive blood loss was expected, bolus of 50mg/kg of TXA had been administered prior to the surgery. During the surgery, TXA was given continuously at the rate of 10 mg/kg/h. Blood loss was only 15 ml/kg, and since the hemoglobin value was borderline (89 g/l) during the surgery, the patient received 10ml/kg of packed red blood cells. The girl was hemodynamically stable throughout the surgery. After the surgery, which lasted for 5 hours, the patient was extubated in the operating room. Postoperatively, the patient was transferred to the surgical ward. Hemoglobin values were stable and there was no need for additional blood replacement.

Discussion

Posterior spinal fusion surgery for scoliosis correction is often complicated by massive perioperative bleeding, which can lead to severe hypotension, metabolic acidosis, coagulopathy, acute lung injury, postoperative ventilation, and even cardiac arrest and death. A long-lasting surgery, as well as the substantial soft tissue and bone preparation are responsible for such a vast amount of bleeding [7].

Since increased fibrinolysis has also been deemed responsible for enhancing bleeding, antifibrinolytic drugs, such as TXA, might prove beneficial in reducing bleeding in patients undergoing surgical treatment of scoliosis [8].

Tranexamic acid has been studied in different fields of surgery, including the spine surgery. However, the exact dose of TXA has not been determined yet, especially for surgical treatment of scoliosis in pediatric patients [9]. Optimal dosage regimen remains controversial, with wide variations in reported loading doses (10–100 mg/kg) and infusion rates (1–10 mg/kg/h) [7, 8].

In a study of patients undergoing posterior spinal fusion for scoliosis, Sethna et al. observed a 41% decrease in intraoperative blood loss after the administration of TXA. In another study examining the difference in blood loss between scoliosis patients undergoing posterior spinal fusion receiving TXA versus placebo, Neilipovitz et al. found no significant differences in intraoperative blood loss, but observed a 28% decrease in transfusions. The major difference in these two studies was in the dose of TXA used. Sethna et al. used a higher dose of TXA (100 mg/kg loading dose, followed by 10 mg/kg/h) in comparison to Neilipovitz et al. (10 mg/kg loading dose, followed by 1 mg/kg/h).

Higher doses of TXA proved to be more effective in reducing the need for red blood cell transfusion in comparison to the lower doses [10]. In order to balance the antifibrinolytic effect with possible side effects, we opted for pre-emptive administration of a medium loading dose of TXA (50 mg/kg). Since gradual but significant blood loss can occur throughout the whole procedure and extend into the postoperative period, we decided to give TXA as an intravenous infusion in higher dose of 10 mg/kg/h during surgery. Blood loss in our case was only 15 ml/kg and transfusion (only 10 ml/h of packed ed blood cells) was administered to keep the hemoglobin above 80 g/l.

The child's hemodynamic parameters, blood gas analysis as well as hemoglobin and hematocrit values were stable throughout the surgery, and there was no need for further blood transfusion intra or postoperatively.

The primary concern when using antifibrinolytic agents, such as TXA, is the increased risk of thromboembolic events [11]. There were no adverse events recorded in our patient.

Conclusion

Extensive blood loss is common in pediatric scoliosis correction surgery, transfusion being unavoidable in the majority of cases. In our patient tranexamic acid proved safe and effective in reducing perioperative blood loss and transfusion requirement.

References

1. Neilipovitz D, Murto K, Hall L, Barrowman N, Splinter W. A randomized trial of tranexamicacid to reduce blood transfusion for scoliosis surgery. AnesthAnalg [serial on the Internet]. 2001 [cited 2015 March15];93:82-7. Available from: http://journals.lww.com/_layouts/OAKS.Journals/ePDF.aspx

2. Sethna N, Zurakowski D, Brustowicz R, Bacsik J, Sullivan L, Shapiro F. Tranexamicacid reduces intraoperative blood loss in pediatric patients undergoing scoliosis surgery. Anesthesiology [serial on the Internet].2005 [cited 2015 March15]; 102:727-32. Available from: http://www.docencianestesia.com/uploads/1/3/1/6/13162488/tranexamic_acid_reduces_intraoperative_blood_loss_in_pediatric_patients_undergoing_scoliosis_surgery.pdf

3. Murray DJ, Pennell BJ, Weinstein SL, Olson JD: Packed red cells in acute blood loss: Dilutional coagulopathy as a cause of surgical bleeding. Anesth Analg [serial on the Internet]. 1995 [cited 2015 March16]; 80:336–42. Available from: http://www.researchgate.net/profile/David_Murray2/publication/15381331_Packed_red_cells_in_acute_blood_loss_dilutional_coagulopathy_as_a_cause_of_surgical_bleeding/links/5421858f0cf203f155c6e01f.pdf

4. Tzortzopoulou A, Cepeda MS, Schumann R, Carr DB. Antifibrinolyticagents for reducing blood loss in scoliosis surgery in children. Cochrane Database Syst Rev. 2008;(3):6883.

5. Schouten ES, van de Pol AC, Schouten AN, Turner NM,Jansen NJ, Bollen CW. The effect of protinin, tranexamic acid, andaminocaproic acid on blood loss and use of blood products in majorpediatric surgery: a meta-analysis. Pediatr Crit Care Med. 2009;10:182-90.

6. Kozek-Langenecker S, Afshari A, Albaladejo P, Santullano CAA, DeRobertis E, Filipescu D, et al. Management of severe perioperative bleeding, Guidelines from the European Society of Anaesthesiology. Eur J Anaesthesiol. 2013;30:270-382.

7. Lykissas M, Crawford A, Chan G, Aronson L, Al-Sayyad M. The effect of tranexamic acid in blood loss and transfusion volume in adolescent idiopathic scoliosis surgery: a single-surgeon experience. J Child Orthop [serial on the Internet]. 2013 [cited 2015 March16];7:245-9. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3672458/pdf/11832_2013_Article_486.pdf

8. Neilpovitz D. Tranexamic acid for major spinal surgery. Eur Spine J [serial on the Internet]. 2004 [cited 2015 March16];13:62-5. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3592185/pdf/586_2004_Article_716.pdf

9. Grant J, Howard J, Luntly J, Herder J. Perioperative blood transfusion requirements in pediatric scoliosis surgery: the efficacy of tranexamicacid. J Pediatr Orthop. 2009;29:300-4.

10. Yagi M, Hasegawa J, Nagoshi N, Iizuka S, Keneko S. Does the intraoperative tranexamic acid decrease operative blood loss during posterior spinal fusion for treatment of adolescent idiopathic scoliosis? Spine. 2012;37:1336-42.

11. Murkin JM, Falter F, Granton J, Young B, Burt C, Chu M. High-dose tranexamicacid is associated with nonischemicclinical seizures in cardiac surgical patients. Anesth Analg [serial on the Internet]. 2010 [cited 2015 March16]; 110:350-3. Available from: http://journals.lww.com/_layouts/OAKS.Journals/ePDF.aspx

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VALPROATE, BIPOLAR DISORDER AND POLYCYSTIC OVARIAN SYNDROME

VALPROAT, BIPOLARNI POREMEĆAJ I SINDROM POLICISTIČNIH JAJNIKA

Milana OKANOVIĆ[1,2] and Olga ŽIVANOVIĆ[1,2]

Summary

Introduction. Polycystic ovarian syndrome is a syndrome of ovarian dysfunction with the principal features of hyperandrogenism and polycystic ovary morphology. A large number of studies conducted on this topic have suggested a possible role of anticonvulsants, particularly valproate, in the pathogenesis or risk factors associated with polycystic ovarian syndrome. Bipolar treatment guidelines from Canada and the United States of America recommend valproate as the first line strategy in the acute treatment of bipolar disorder. **Discussion**. Most persons with bipolar disorder require maintenance treatment. Long–term administration of valproate in women with bipolar disorder or epilepsy is believed to result in the increased risk of hyperandrogenism, menstrual abnormalities and polycystic ovaries. Valproate may also increase the risk of infertility and other associated symptoms of polycystic ovarian syndrome. Therefore, particular caution is indicated in the use of valproate in women of reproductive age. **Conclusion.** The treatment of the female patients with bipolar disorder presents various challenges for the clinician. Every woman of reproductive age needs to know the risk and benefits of her pharmacologic treatment options. Bipolar disorder should be considered chronic disorder, whose development is largely affected by hormonal changes and reproductive cycle in women. These issues should be researched more thoroughly in order to opt for the most appropriate treatment in women with bipolar disorder.

Key words: Polycystic Ovary Syndrome; Bipolar Disorder; Epilepsy; Valproic Acid; Risk Factors; Anovulation; Menstrual Cycle; Gonadal Hormones; Obesity; Hyperandrogenism

Sažetak

Uvod. Sindrom policističnih jajnika je ovarijalna disfunkcija čije su osnovne karakteristike hiperandrogenizam i policistična struktura jajnika. Veliki broj do sada sprovedenih studija ukazuje na moguću ulogu antikonvulzivnih lekova, prevashodno valproata, u patogenezi sindroma policističnih jajnika ili je primena valproata još jedan faktor rizika u razvoju pomenutog sindroma. Smernice za lečenje bipolarnog poremećaja iz Kanade i Amerike preporučuju valproat kao lek prvog izbora u akutnom tretmanu bipolarnog poremećaja. **Diskusija.** Veliki broj osoba sa bipolarnim poremećajem zahteva tretman održavanja. Smatra se da produžena terapija valproatom kod žena obolelih od bipolarnog poremećaja i epilepsije doprinosi povećanom riziku od pojave hiperandrogenizma, poremećaja menstrualnog ciklusa i sindoma policističnih jajnika. Valproat takođe može povećati rizik od neplodnosti i drugih simptoma udruženih sa sindromom policističnih jajnika. Iz ovih razloga potreban je poseban oprez ukoliko se valproat upotrebljava u periodu reproduktivnih godina žene. **Zaključak.** Tretman žena obolelih od bipolanog poremećaja predstavlja veliki izazov za kliničare. Svaka žena u reproduktivnim godinama mora biti upoznata sa koristima i potencijalnim rizicima od upotrebe različitih farmakoterapijskih mogućnosti u lečenju bipolarnog poremećaja. Bipolarni poremećaj je potrebno posmatrati kao hronični poremećaj na čiji tok u velikoj meri utiču hormonske promene i reproduktivni ciklus kod žena. Neophodna su dalja istraživanja ove teme kako bi se obezbedio najadekvatniji tretman ženama obolelim od bipolarnog poremećaja.

Ključne reči: Sindrom policističnih jajnika; Bipolarni poremećaj; Epilepsija; Valproična kiselina; Faktori rizika; Anovulacija; Menstrualni ciklus; Polni hormoni; Gojaznost; Hiperandrogenizam

Introduction

Since polycystic ovary syndrome (PCOS) affects 2-7% of women of reproductive age, it ranks among the most common endocrine disorders. PCOS is characterized by both hormonal and metabolic disorders, and the disorder itself could be characterized as hyperandrogenism and chronic anovulation in the absence of the pituitary and adrenal pathology. Given that PCOS is defined as a clinical syndrome, no diagnostic criterion is sufficient for clinical diagnosis [1].

Polycystic ovary syndrome is characterized by co-occurrence of increased androgen levels and menstrual cycle disorder. Androgens are subject to aromatization in fatty tissue. The product of this aroma-

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Abbreviations

PCOS – polycystic ovary syndrome
LH – luteinizing hormone
FSH – follicle-stimulating hormone
GnRH – gonadotropin-releasing hormone
IGF-1 – insulin-like growth factor
VPA – valproate
CYP – cytohrome P450 oxidase
AAPs – atypical antipsychotics

tization, which is referred to as extraglandular, is *estrone*. A characteristic of this weak estrogen is to sensitize the pituitary gland, produce luteinizing hormone (LH) intensively under the influence of gonadotropin-releasing hormone (GnRH), while on the other hand hindering it in the creation of follicle-stimulating hormone (FSH). The high level of LH stimulates the cells of the stroma and theca to produce androgens, mainly *androstenedione* and *testosterone*. These androgens join the adrenal androgens thus closing the circle from which there is no spontaneous escape. A consistently low level of FSH continuously stimulates the development of follicles, which are subject to atresion, because it is insufficient for their proper growth and maturation. The end result is anovulation with other clinical manifestations, such as acne and hirsutism.

Although the etiology of PCOS is not determined and defined in a satisfactory way, there has recently been more and more data pointing to insulin resistance as an important component. Hyperinsulinemia is a side effect of peripheral insulin resistance (a probable absence or blockage of the insulin receptors), and this phenomenon is also known to be more common in obese patients. Insulin, which is excessive and does not find its receptors, binds to insulin-like growth factor (IGF-1) receptors, which are structurally similar to insulin receptors. Since IGF-1 is known to make theca cells particularly sensitive to the effects of LH, it is clear then that the ovaries of these patients are the major source of androgens. Furthermore, insulin has inhibitory effect on the synthesis of sex hormone-binding globulin (SHBG) in the liver; hence free androgens in serum further deteriorate the patient's condition [2].

Elevated concentrations of insulin may initiate a cascade of events which may eventually result in metabolic syndrome. Metabolic syndrome is a collection of physical symptoms and signs that represent the most important risk factors for cardiovascular and cerebrovascular diseases, and it includes obesity, diabetes mellitus type II, hypertension, hypertriglyceridemia and low levels of high-density lipoprotein (HDL) cholesterol [3].

Bipolar treatment guidelines from Canada and the United States of America recommend valproate as the first line strategy in the acute treatment of bipolar disorder [1]. A large number of studies conducted so far suggest a possible role of anticonvulsant drugs (primarily VPA) in the pathogenesis of PCOS in patients suffering from bipolar disorder and epilepsy. These studies found an association between the long-term application of VPA and an increased risk of hyperandrogenism and menstrual cycle disorders and the consequent development of PCOS [4]. Higher prevalence of overweight or obesity was also detected among women suffering from bipolar disorder compared to the general population. Adverse effect of VPA in the form of an increase in body weight has long been proven. Obesity itself may predispose or be one of the risk factors in the development of numerous endocrine and metabolic disorders including PCOS, which in addition to the previously mentioned, results in infertility due to anovulation. Given that VPA may increase the risk of infertility causing PCOS, it is necessary to be particularly careful when applying it in the treatment in women of reproductive age suffering from bipolar disorder [5].

This paper has been aimed at reviewing the current literature that examines the association between bipolar disorder, administration of VPA and the subsequent development of PCOS in order to provide women suffering from bipolar disorder the most optimal treatment. To achieve this, the impact of endocrine and reproductive events on the course of the disorder, as well as the implications of psychiatric treatments upon the course of reproductive events must be understood.

Discussion

A large number of studies conducted on the association between bipolar disorder, application of VPA and the occurrence of PCOS have found a higher prevalence of PCOS in women suffering from bipolar disorder [6]. The theories which tried to explain this increased prevalence included the effects of PCOS or effects of the application of VPA that could cause the development of PCOS directly or indirectly. Metabolic disorders were confirmed both in patients suffering from bipolar disorder and in those suffering from PCOS. Insulin resistance, hyperglycemia and obesity are common to patients suffering from both bipolar disorder and PCOS, thus suggesting that there is a certain degree of pathophysiological overlap between these two disorders. Due to the fact that both bipolar disorder and PCOS are complex polygenetic diseases, endophenotypic overlap between these two disorders may be a result of common genes. Future studies to be conducted on this issue should provide definitive confirmation of genetic overlap between these two disorders [7].

Studies that compared the long-term use of lithium as opposed to long-term treatment with VPA in women suffering from bipolar disorder pointed to a significantly higher rate of menstrual cycle disorders in patients receiving prolonged VPA therapy compared to women who had been treated with lithium for a long time [1]. The increased rate of hyperandrogenism and development of the metabolic syndrome was also confirmed in women

treated for long time with VPA compared to long-term treatment with lithium [8].

The most frequently cited mechanisms of impact of VPA on the development of PCOS are:

1. Increased androgen synthesis in the ovaries
2. Impact of GnRH on intensive production of LH
3. Increase in body weight and insulin resistance
4. Absence of induction of hepatic microsomal enzyme cytochrome P450 oxidase (CYP)
5. Use in vulnerable populations (e.g. patients suffering from epilepsy, young people, irregular menstrual cycles)

Preliminary data suggest a possible direct effect of VPA on an increased androgen production in the ovaries, but there is still no reliable evidence of its influence on the development of the symptoms of PCOS. The manner in which VPA potentially achieves a direct effect on the ovaries is the impact on GnRH which stimulates the intensive production of LH resulting in increased ovarian androgens synthesis - mainly testosterone [9].

The risk of overweight and obesity was higher in women with bipolar disorder than in the healthy controls, whereby psychopharmaceuticals used in the treatment of bipolar disorder increased central obesity, and thus contributed to the increased cardiovascular morbidity [10]. The increase in body weight may impair the compliance and the positive doctor – patient relationship, and also lead to a number of other medical complications such as high blood pressure, high triglycerides, diabetes, etc. The increase in body weight may potentially cause an increased risk of PCOS, probably because overweight causes the development of insulin resistance [11]. It has been found that women treated with VPA are more likely to be obese than women treated with some other mood stabilizers used in the treatment of bipolar disorder [12]. Loss of body weight after discontinuation of VPA has contributed to the improvement of the symptoms of PCOS which could indicate that obesity may increase the risk of developing PCOS. In case reports on this subject, the replacement of VPA with lamotrigine resulted in a decline in serum testosterone levels with improved ovarian ultrasonographic findings in the following period [13]. It was also shown that the women who had developed obesity with amenorrhea while being treated with VPA, lost weight after replacing the above psychostabilizers (VPA replaced by lamotrigine) with the restabilization of the menstrual cycle [14]. Being an adverse effect of the application of VPA, obesity may potentiate the development of symptoms of PCOS; therefore, many authors advise regular monitoring of body weight and prevention of obesity as a risk factor in the possible development of PCOS [15].

Apart from leading to an increase in body weight and elevated insulin levels in the blood, VPA also contributes to the increased concentration of leptin in the blood. Leptin, a peptide synthesized primarily by the adipocytes, acts at the level of the hypothalamus modulating the neuroendocrine axis, energy expenditure and appetite. Leptin levels correlate with the body mass index (BMI) and may reflect the size of the peripheral adipose depot. Elevated leptin levels may be a critical effector system in the initiation of reproductive hormone changes [1].

A pharmacodynamic property of VPA that might contribute to an association with PCOS is its lack of induction of hepatic microsomal CYP oxidases (contrary to barbiturates, carbamazepine and phenytoin). The induction of CYP isoenzymes facilitates clearance of gonadal steroids and reduces the circulating testosterone levels. Since VPA does not induce CYP enzymes, it lacks this mitigating action against hyperandrogenemia – an action that might otherwise limit the risk of PCOS indirectly [9].

If the administration of VPA contributes to the risk for PCOS, it is possible that the effect may be seen only in the women who already have other risk factors for this disorder, such as epilepsy, obesity, or preexisting PCOS morphology. Women with epilepsy may be particularly susceptible to PCOS because they have high rates of anovulatory cycles, reproductive-endocrine disorders, and neuroendocrine abnormalities, such as altered release of GnRH and LH [16]. Based on the above mentioned findings, it has been concluded that reproductive disorders in women suffering from epilepsy may be caused by the use of anticonvulsant medications as well as by neuroendocrine effects induced by the disease itself [17]. The situation is similar in women with bipolar disorder because the neuroendocrine system plays a central role both in disorders of the reproductive function and the occurrence of mood disorders.

Women with bipolar disorder are often treated with combinations of psychotropic medications including atypical antipsychotics (AAPs), a class of medication that has been associated with weight gain, central adiposity, and the development of insulin resistance and type 2 diabetes. All of these side effects can be potential risk factors in the development of PCOS symptoms [18].

A meta-analysis of 11 studies performed on a sample of 556 women with epilepsy treated with VPA, 593 women treated with some other anticonvulsant, 120 women in whom epilepsy was not treated and 329 healthy controls showed an increased incidence of PCOS in the women treated with VPA compared to the women who did not use VPA. The results differed because of various definitions of the diagnostic criteria for PCOS. Treatment with VPA was associated with an increased incidence of PCOS in the criteria that related to hyperandrogenism, oligoovulation with two of the following three conditions fulfilled: ultrasonography of polycystic ovarian structure, the increased serum testosterone levels and menstrual cycle disorder (oligo/amenorrhea). In contrast, in the criteria of PCOS that included ovulatory dysfunction (polymenorrhoea, amenorrhea or oligomenorrhea), clinical and/or biochemical evidence of hyperandrogenism was not confirmed (VPA did not increase the incidence of PCOS compared to other anticonvulsants) [19].

The fact that should not be overlooked is that irregular menstrual cycle and amenorrhea may be a physiological phenomenon. Menstrual irregularities are common in the first few years after menarche but are usually transient and non-pathological. Menstrual cycles also stop during pregnancy and are suppressed during lactation. Women over 40 years of age may experience oligomenorrhea associated with the perimenopause. In athletic women or those with eating disorders or extreme stress, common pathological causes of irregular or absent menses include hyperprolactinemia and hypothyroidism. Abnormal elevation of prolactin can induce oligo/amenorrhea and galactorrhea and it is often seen if the female patients have been using certain types of antipsychotics such as risperidone. Without evaluation of other common causes of menstrual dysfunction, PCOS cannot be assumed to be the cause of menstrual irregularities [9, 20]. Clinicians who prescribe VPA should be aware of the contradictory data describing the relationship between VPA use and PCOS [21]. Since an increasing number of young patients with epilepsy or bipolar disorder are exposed to long-term VPA maintenance therapy, it is important to define any risk associated with its long-term use [22]. Definitive data on the putative association between VPA and PCOS are imperative since PCOS is associated with infertility, diabetes mellitus, and possibly cardiovascular disease and endometrial carcinoma [9]. Most authors recommend the compulsory control of symptoms of PCOS before VPA is introduced into the treatment of women of reproductive age as well as mandatory monitoring of possible development of symptoms of PCOS after the initiation of VPA therapy [23].

The female reproductive cycle greatly affects the course of bipolar disorder (menstrual cycle, childbirth, menopause, use of hormone therapy) which makes the treatment of patients suffering from bipolar disorder even more complex. A large number of women without any psychiatric disorders experience mood changes during the menstrual cycle, and according to a great number of studies, women suffering from bipolar disorder represent a particularly vulnerable population group [24]. The differences in the manifestation of bipolar disorder also depend on the gender. A higher number of depressive episodes, rapid cycling, mixed episodes as well as medical and psychiatric co-morbidity were observed in women with bipolar disorder than in men treated for the same diagnosis. In addition, antidepressant-induced rapid cycling and the risk of progressing to mania are more often described in women with bipolar disorder than in men treated for the same diagnosis [5]. Differences in the expression of side effects also vary depending on the gender (e.g., hypothyroidism, and weight gain are more often described in women treated with lithium than in men). A study conducted in 2005 which included 17 women treated with VPA, 15 women whose therapy did not include VPA (treated for the diagnosis of bipolar disorder) and 22 healthy women without any psychiatric diagnoses, pointed to a significantly higher percentage of menstrual cycle disorders (47%) in the group of women treated with VPA than in the women not using this drug (13%) and healthy women (0%). Bipolar disorder needs to be considered a chronic disorder whose course is largely influenced by hormonal changes and the female reproductive cycle [1].

An increased rate of menstrual cycle disorders in women with bipolar disorder as well as the associated menstrual irregularities due to long-term application of VPA and the consequent increase in the level of free testosterone in the blood has been shown in numerous papers. However, no study has managed so far to compare these results with the controls. The studies to tackle this issue and menstrual cycle disorders in the future must take into account the fact that a large number of women who subjectively report oligomenorrhea or amenorrhea have two out of three, if not all three positive ovulations during the period of monitoring ovulation (provided ovulation is monitored using biochemical parameters). All these findings suggest that more objective ways of monitoring menstrual cycle and ovulation are necessary. In some studies, psychotropic drugs were not associated with biochemical markers of menstrual cycle disorders although drugs from AAP group showed a slightly higher association with the increased rate of the present or menstrual irregularities in the past [18, 25].

In summary, reports describing a relationship between VPA use and PCOS among women with either epilepsy or bipolar disorder are contradictory. There are several possible explanations for the inconsistent findings in studies investigating the relationship between VPA use and PCOS:

- small study size
- non-randomized study design
- patients' characteristics (ethnicity, body weight)

Because of all this, well-controlled, prospective, multicenter studies are needed to confirm the correlation between the use of VPA and development of PCOS (9). If women taking VPA to be treated for bipolar disorder meet two or more of the following criteria:

1. menstrual cycle disorders
2. obesity
3. hyperandrogenism (hirsutism, alopecia)
4. anovulation (infertility),

they should check the level of free testosterone in the blood. In case of elevated findings, it would be necessary to consult a gynecologist and endocrinologist [1, 26]. In women of reproductive age who are treated with VPA, the function and structure of the ovaries (ultrasound examinations) should be evaluated regularly, especially if there is a disorder of the menstrual cycle during the treatment. Because of all these findings, clinicians must inform the patients on the possibility of a disorder of the menstrual cycle and other metabolic disorders before introducing VPA into therapy [1, 27]. The increasing number of young patients who are treated long-term with VPA for bipolar disorder necessitates further research on this issue in order to clarify the relationship between

long-term administration of VPA and other mood stabilizers and potential development of metabolic and endocrine disorders. Some studies have suggested that regardless of psychopharmaceuticals used in the treatment of bipolar disorder, women suffering from bipolar disorder are more likely to have disorders related to the menstrual cycle, which would all point to the compromised hypothalamic-pituitary-gonadal axis in bipolar patients. A study conducted in female adolescents showed that the level of androgens and LH was greater in those female adolescents who were diagnosed with bipolar disorder as compared to other psychiatric disorders [28].

Considering the aforementioned, an early development of bipolar disorder may be associated with certain symptoms of PCOS. It is believed that the early exposure to VPA administration makes female adolescents increasingly susceptible to the development of PCOS as a consequence of changes at the level of hypothalamic-pituitary-gonadal axis in the early years after menarche [9, 29]. Studies on the safety and monitoring of these medications in adolescent girls have shown high probability of development of gynecologic and reproductive adverse effects [30].

The psychiatric disorders accompanied with the clinical symptoms and hormonal abnormalities are very important although underestimated aspects in PCOS. Obesity, hirsutism, acne, menstrual disturbances and infertility play important roles in decreasing the quality of life in women with PCOS [31]. The authors discuss adverse events which are often overlooked but clinicians should pay attention to them in order to preserve the patient's quality of life [32]. The safe and effective treatment of bipolar disorder requires the cooperation between medical care providers and patients, including routine discussions on therapeutic and adverse medication effects and shared decision-making regarding changes in the therapeutic regimen. Monitoring of adverse effects is an essential component of therapeutic management [33]. One must be aware of possible endocrine side effects of antiepileptic drugs because they can have a major impact on the quality of life, and are, at least partly, reversible after antiepileptic drugs discontinuation [34].

The presence of psychiatric co-morbidity has a negative influence on the outcome of PCOS and vice versa [35]. Both endocrinologists and gynecologists, who treat PCOS patients most frequently, should be aware of the potential presence of psychiatric disorders and should have a proactive approach to the treatment of not only physical but also psychiatric co-morbidity. Timely management of psychiatric co-morbidity can improve the outcome significantly and enhance the quality of life of these patients. In fact, a multidisciplinary team approach not only involving a gynecologist and endocrinologist but also a psychiatrist would be the most optimal way to provide adequate treatment of these patients [36].

Conclusion

Patients suffering from bipolar disorder may be of different ages and each age group for itself represents an additional risk of developing certain diseases.

Treatment of bipolar disorder in women has always been, and still is a great challenge for clinicians, the more so because the female reproductive cycle makes the treatment even more complex. The course of bipolar disorder in women is greatly influenced by menstrual cycle, childbirth, menopause and use of hormone therapy. It is essential that clinicians assess the benefits and potential risks of introducing certain mood stabilizers used in the treatment of bipolar disorder, particularly in the reproductive years of women.

Co-morbidity of bipolar disorder with physical diseases is higher than in the general population (some physical diseases such as diabetes, thyroid diseases, cardiovascular diseases, dyslipidemia, obesity and others are 30-50% more common in bipolar patients than in the general population). Inadequate monitoring of bipolar disorder and associated physical conditions result in inadequate treatment of both bipolar disorder and the associated physical diseases. Additional stigma of concomitant physical diseases in patients with bipolar disorder further undermines the patient's compliance. A psychiatrist is more successful if he/she has a "broad" view of bipolar disorder and its possible effects on the physical health of bipolar patients.

The knowledge on possible co-morbid psychiatric and/or physical conditions, as well as the knowledge on the course of bipolar disorder makes the treatment of bipolar disorder far more successful.

References

1. McIntyre RS, Mancini DA, McCann S, Srinivasan J, Kennedy SH. Valproate, bipolar disorder and polycystic ovarian syndrome. Bipolar Disord. 2003;5:28-35.

2. Plećaš D, Stanimirović B, Stanković A, Vasiljević M. Ginekologija i akušerstvo. Beograd: Medicinski fakultet; 2006.

3. Scarpitta AM, Sinagra D. Polycystic ovary syndrome an endocrine and metabolic disease. Gynecol Endocrinol. 2000;14:392-5.

4. O Donovan C, Kusumakar V, Graves GR, Bird DC. Menstrual abnormalities and polycystic ovary syndrome in women taking valproate for bipolar mood disorder. J Clin Psychiarty. 2002;63: 322-30.

5. Freeman MP, Gelenberg AJ. Bipolar disorder in women: reproductive events and treatment considerations. Acta Psychiatr Scand. 2005;112:88-96.

6. Genton P, Bauer J, Duncan S, et al. On the association between valproate and polycystic ovary syndrome. Epilepsia. 2001; 42:295-304.

7. Jiang B, Kenna HA, Rasgon NL. Genetic overlap between polycystic ovary syndrome and bipolar disorder: the endophenotype hypothesis. Med Hypotheses. 2009;73:996-1004.

8. Rasgon NL, Altshuler LL, Gudeman D, et al. Medication status and polycystic ovary syndrome in women with bipolar disorder: a preliminary report. J Clin Psychiatry. 2000;61:173-8.

9. Joffe H, Hall JE, Cohen LS, Taylor AE, Baldessarini RJ. A putative relationship between valproic asid and polycystic ovarian syndrome: Implications for treatment of womwn with seizure and bipolar disorders. Harv Rev Psychiatry. 2003;11:99-108.

10. Elmslie JL, Silverstone JT, Mann JI, Williams SM, Romans SE. Prevalence of overweight and obesity in bipolar patients. J Clin Psychiatry. 2000;61:179-84.

11. Davari-Tanha F, Hosseini Rashidi B, Ghajarzadeh M, Noorbala AA. Bipolar disorder in women with polycystic ovarian syndrome. Acta Med Iran. 2014;52:46-8.

12. Chappell KA, Markowitz JS, Jackson CW. Is valproate pharmacotherapy associated with polycystic ovaries Ann Pharmacohter. 1999;33:1211-6.

13. Isojarvi JI, Tapanainen JS. Valproate, hyperandrogenism and polycystic ovaries: a report of 3 cases. Arch Neurol. 2000;57: 1064-8.

14. Isojarvi JI, Rattya J, Myllyla VV, Knip M, Koivunen R, Pakarinen AJ, et al. Valproate, lamotrigine and insulin-mediated risks in women with epilepsy. Ann Neurol. 1998;43:446-51.

15. Bowden CL, Singh V. Valproate in bipolar disorder: 2000 onwards. Acta Psychiatr Scand. 2005;111:13-20.

16. Isojarvi JI, Laatikaainen TJ, Knip M, Pakarinen AJ, Juntunen KT, Myllyla VV. Obesity and endocrine disorders in women taking valproate for epilepsy. Ann Neurol. 1996;39:579-84.

17. Joffe H, Taylor AE, Hall JE. Polycystic ovarian syndrome – relationship to epilepsy and antiepileptic drug therapy. J Clin Endocrinol Metab. 2001;867:2946-9.

18. Reynolds-May MF, Kenna HA, et al. Evaluation of reproductive function in women treated for bipolar disorder compared with healthy controls. Bipolar Disord. 2014;16:37-47.

19. Hu X, Wang J, Dong W, Fang Q, Hu L, Liu C. A meta – analysis of polycystic ovary syndrome in women taking valproate for epilepsy. Epilepsy Res. 2011;97:73-82.

20. Lobo RA, Carmina E. The importance of diagnosing the polycystic ovary syndrome. Ann Intern Med. 2000;132:989-93.

21. Isojarvi JI, Laatikaainen TJ, Pakarinen AJ, Juntunen KT, Myllyla VV. Polycystic ovaries and hyperandrogenism in women taking valproate for epilepsy. N Eng J Med. 1993;329:1383-8.

22. Irwin M, Masand P. Valproate and polycystic ovaries. J Am Acad Child Adolesc Psychiatry. 1998;37:9-10.

23. Freeman MP, Smith KW, Freeman SA, et al. The impact of reproductive events on the course of bipolar disorder in women. J Clin Psychiatry. 2002;63:284-7.

24. Rasgon N, Bauer M, Glenn T, Elman S, Whybrow PC. Menstrual cycle related mood changes in women with bipolar disorder. Bipolar Disord. 2003;5:48-52.

25. Rasgon NL, Altshuler LL, Fairbauks L, Elman S, Bitran J et al. Reproductive function and risk for PCOS in women treated for bipolar disorder. Bipolar Disord. 2005;7:246-59.

26. Soares JC. Valproate treatment and risk of hyperandrogenism and polycystic ovaries. Bipolar Disord. 2000;2:34-41.

27. Bilo L, Meo R. Polycystic ovary syndrome in women using valproate: a review. Gynecol Endocrinol. 2008;24:562-70.

28. Geller B, Luby J. Child and adolescent bipolar disorder: a review of the past 10 years. J Am Acad Child Adolesc Psychiatry. 1997;36:1168-76.

29. Garland EJ, Behr R. Hormonal effects of valproic asid? J Am Acad Child Adolesc Psychiatry. 1996;35:1424-5.

30. Talib HJ, Alderman EM. Gynecologic and reproductive health concerns of adolescents using selected psychotropic medications. J Pediatr Adolesc Gynecol. 2013;26:7-15.

31. Krepula K, Bidzinska-Speichert B, Lenarcik A, Tworowska-Bardzinska U. Psychiatric disorders related to polycystic ovary syndrome. Endokrynol Pol. 2012;63:488-91.

32. Watanabe K, Kikuchi T. Adverse events of psychotropic drugs. Seishin Shinkeigaku Zasshi. 2014;116:323-31.

33. Kemp DE. Managing the side effects associated with commonly used treatments for bipolar depression: a review. J Affect Disord. 2014;169:34-44.

34. Svalheim S, Sveberg L, Mochol M, Taubll E. Interactions between antiepileptic drugs and hormones. Seizure. 2015;28:12-7.

35. Franks S. Polycystic ovary syndrome. N Eng J Med. 1995; 333:853-61.

36. Hussain A, et al. Prevalence of psychiatric disorders in patients with a diagnosis of polycystic ovary syndrome in Kashmir. Indian J Psychol Med. 2015;37:66-70.

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Propratno pismo:

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– autori svojim potpisom preuzimaju odgovornost da rad ispunjava sve postavljene uslove i da ne postoji sukob interesa i

– autor mora navesti kategoriju članka (originalni rad, pregleni rad, prethodno saopštenje, stručni rad, prikaz slučaja, rad iz istorije medicine, itd.).

Rukopis

Opšta uputstva

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

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

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

2. Sažetak

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

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

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

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

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

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

3. Tekst članka

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

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

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

Uvod

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

Materijal i metode

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

Rezultati

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

Diskusija

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

Zaključak

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

4. Literatura

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

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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

∗ Organizacija kao autor

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

∗ Bez autora

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

∗ Volumen sa suplementom

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

∗ Sveska sa suplementom

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

∗ Sažetak u časopisu

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

Knjige i druge monografije

∗ Jedan ili više autora

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

∗ Urednik (urednici) kao autor (autori)

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

∗ Poglavlje u knjizi

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

∗ Zbornik radova sa kongresa

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

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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

∗ Kompjuterska datoteka

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

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

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

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

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

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

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

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

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

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

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

6. Dodatne obaveze

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

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

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

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

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

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

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

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

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

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

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

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

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

Preparation of the manuscript

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

The covering letter:

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

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

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

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

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

2. Summary.

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

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

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

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

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

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

3. The text of the paper.

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

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

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

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

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

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

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

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

Articles in journals

∗ *A standard article*

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

∗ *An organization as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

∗ *A dissertation and theses*

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

Electronic material

∗ *A journal article in electronic format*

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

∗ *Monographs in electronic format*

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

∗ *A computer file*

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

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

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

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

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

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

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

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

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

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

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

6. Additional requirements

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