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SADRŽAJ / CONTENTS

ORIGINAL ARTICLES / ORIGINALNI ČLANCI

"Power of Music" by Louis Gallait, a Belgian painter (1810–1887), demonstrates capability of music to act as a sedative-hypnotic drug.
Therapeutic effect of music has been known from times immemorial. From 1982 music has been celebrated worldwide on the Summer Solstice (21 June). This day is known as World Music Day. At that time it is an occasion to forget, at the moment, all problems and surrender oneself to the healing effect of music.

„Moć muzike", slika belgijskog slikara Luja Galea (1810–1887), ilustruje sposobnost muzike da deluje kao hipnotik.
Terapijski efekat muzike poznat je od davnina. Od 1982. godine, na dan letnjeg solsticijuma (21. jun) obeležava se Svetski dan muzike. Tog dana, kada se širom sveta održavaju muzičke svečanosti, prilika je da, bar na trenutak, zaboravimo na probleme i prepustimo se njenom blagotvornom dejstvu.

ORIGINAL ARTICLES

UDC: 617-089.5-031.3:617.55
DOI: 10.2298/VSP111011011S

Comparison of analgesic effect of intrathecal morphine alone or in combination with bupivacaine and fentanyl in patients undergoing total gastrectomy: A prospective randomized, double blind clinical trial

Poređenje analgetskog efekta intratekalno primenjenog morfina samog ili u kombinaciji sa bupivakainom i fentanilom kod bolesnika podvrgnutih totalnoj gastrektomiji – prospektivna, randomizovana, dvostruko slepa klinička studija

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Abstract

Background/Aim. Combined spinal-epidural-general anesthesia has several advantages over general anesthesia alone. This study was designed to compare the efficacy of intrathecal (IT) morphine alone, or in combination with bupivacaine and fentanyl, as part of a combined spinal-epidural (CSE) analgesia, in patients undergoing elective total gastrectomy. **Methods.** This prospective, randomized double-blind study included 60 patients undergoing total gastrectomy under general anesthesia and CSE. We compared the analgesic effect of lumbar IT morphine 300 μg (the group M, n = 20) *vs* morphine 300 μg + bupivacaine 2 mg (the group MB, n = 20) *vs* morphine 300 μg + bupivacaine 2 mg + fentanyl 25 μg (the group MBF, n = 20) given after thoracic epidural catheter placement (T6-7) but before general anesthesia induction. Pain visual analogue scale (VAS) at rest (R), with movement (M) and with cough (C), and the number of analgesia requests were assessed for 72 h and after epidural catheter removal. **Results.** Compared to other groups, the MBF group required significantly fewer additional intra-operative epidural bupivacaine doses ($p < 0.001$), whereas the M group required significantly more supplemental intraoperative intravenous fentanyl, compared with the MBF ($p = 0.022$) and MB groups ($p = 0.005$). Postoperative pain relief was satisfactory in all the groups at all the time. VAS-R and VAS-M did not differ significantly among the groups. Compared to the M group, VAS-C scores 30 min postoperatively were significantly lower in the MBF ($p = 0.029$) and MB groups ($p = 0.002$). Duration of analgesia was longer in the MBF and MB groups, but the difference reached no significance. The number of supplemental analgesia requests was similar in all the groups in the first 12 h and during 72 h. Additional analgesia requests after epidural catheter removal were similar in all the groups, and side effects were infrequent. **Conclusion.** Compared to IT morphine alone, triple IT combination administered as part of CSE provided better intraoperative analgesia, but conferred no benefit with regards to postoperative analgesia.

Key words:
anesthesia, epidural; anesthesia, spinal; methods; fentanyl; bupivacaine; morphine; analgesia; gastrectomy.

Apstrakt

Uvod/Cilj. Kombinovana spinalno-epiduralno-opšta anestezija ima nekoliko prednosti nad primenom samo opšte anestezije. Cilj ove studije bio je da uporedi efekat intratekalnog (IT) dodavanja fentanila i bupivakaina morfinu, kao dodatka torakalnoj epiduralnoj analgeziji [kombinovana spinalnoepiduralna analgezija (KSE)], kod bolesnika planiranih za totalnu gastrektomiju. **Metode.** U randomiziranoj dvostruko slepoj studiji poređen je analgetski efekat lumbalno IT primenjenog 300 μg morfina (grupa M, n = 20), 300 μg morfina i 2 mg bupivakaina (grupa MB, n = 20) i trostruke

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kombinacije sa 25 μg dodatog fentanila (MBF, n = 20), primenjenih nakon postavljanja epiduralnog katerera u torakalni segment (T6-7), ali pre uvoda u opštu anesteziju za izvođenje totalne gastrektomije. Za procenu kvaliteta postoperativne analgezije korišćeni su vizuelna analogna skala bola (VAS) u miru (R), pokretu (M) i pri kašlju (C), broj analgetskih zahteva u toku 72 časa i nakon vađenja epiduralnog katetera. **Rezultati.** Intraoperativno, u poređenju sa drugim grupama, u MBF grupi bilo je dodato značajno manje epiduralnog bupivakaina ($p < 0,001$), dok je dodatna količina intravenskog (IV) fentanila bila značajno viša u grupi M u poređenju sa grupom MBF ($p = 0,022$) i MB grupom ($p = 0,005$). Postoperativna analgezija je bila zadovoljavajuća u svim grupama u toku 72 časa. Nije uočena statistički značajna intergrupna razlika za VAS-R i VAS-M ($p > 0,05$). U 30. minutu postoperativno VAS-C bili su značajno niži u grupi MBF ($p = 0,029$) i MB ($p = 0,002$) u poređenju sa M grupom. Trajanje efektivne analgezije bilo je duže u grupama MBF i MB, ali razlika nije bila statistički značajna ($p > 0,05$). Broj zahteva za dodatnom analgezijom bio je sličan u svim grupama u prvih 12 časova i u toku 72 časa, kao i nakon vađenja epiduralnog katetera. **Zaključak.** Trostruka IT kombinacija kao deo KSE obezbeđuje bolju intraoperativnu analgeziju. Ova studija nije dokazala prednosti ove kombinacije za postoperativnu analgeziju u poređenju sa IT morfinom.

Ključne reči:
anestezija, epiduralna; anestezija, spinalna; metode; fentanil; bupivakain; morfin; analgezija; gastrektomija.

Introduction

Total gastrectomy for gastric cancer is a major surgical procedure with significant morbidity and mortality, depending on several perioperative and postoperative variables [1]. Use of intrathecal opioids and local anesthetics in combination with epidural anesthesia/analgesia proved to provide reliable intraoperative and postoperative analgesia in major abdominal surgery, especially colorectal surgery [2–4]. This method is suggested in institutions where pumps designed for continuous epidural local anesthetic infusion are not available [3].

Combined spinal-epidural-general anesthesia (CSE-GA) offers several advantages over general anesthesia alone [5]. Spinal anesthesia provides rapid onset of muscle relaxation and analgesia, the epidural catheter prolongs anesthesia and improves postoperative analgesia, while general anesthesia provides unconsciousness and controlled ventilation, thus providing excellent operating conditions for the surgeon [5].

Subarachnoid lipid-soluble fentanyl, which can provide rapid onset of analgesia, combined with preservative-free water-soluble morphine, which provides analgesia with slower onset but longer duration, combined with a small dose of intrathecal bupivacaine has been shown to synergistically produce rapid and sustained analgesia [6].

Our study was designed to evaluate the potential benefits of intrathecal morphine alone, or in combination with bupivacaine and fentanyl, as part of a combined spinal-epidural analgesia (CSE) regimen, for elective total gastrectomy. Our hypothesis was that the combination of intrathecal bupivacaine, fentanyl and morphine, in addition to thoracic epidural analgesia will improve analgesia in the immediate postoperative period. Postoperative pain at rest, with movement, and with cough was the primary clinical outcome. The need for supplemental intraoperative intravenous (IV) fentanyl and epidural bupivacaine, time to first postoperative analgesia request and postoperative analgesic drug use were designated as secondary outcomes.

Methods

This prospective, randomized, double blind clinical trial was approved by the University Expert Council for Medical Science, and written informed consent was obtained from all patients before they entered the study. In total, 60 American Society of Anesthesiologists (ASA) physical status 1–3 adult patients requiring total gastrectomy for malignancy were recruited. Exclusion criteria were: patient's disapproval, significant comorbidity, preoperative opioid or nonsteroidal antiinflammatory drugs (NSAID) consumption, drug addiction, psychiatric disorders, spinal problems, local infection, coagulation abnormality, neurological problems, allergy to medications used in the study, and any contraindications to neuraxial anesthesia. Preoperative preparation included patient education in order to explain the goals of the study, and familiarize patients with the visual analogue scale (VAS) scale, which was used to quantify pain in the postoperative period. A computer-generated randomization schedule was provided by an independent investigator who prepared and dispensed drugs for the trial. The patients and medical personnel were blinded to group assignment. All surgical procedures were performed by the same surgeon, who specializes in esophagogastric surgery and all anastomoses were hand sewn.

All the patients received midazolam 2.5 mg IV for premedication. Hartman's solution 1 L was given as fluid preload, and antibiotic prophylaxis consisted of ceftriaxone 2 g and metronidazole 500 mg in all cases. All neuraxial blocks were performed in the operating room, with the patients being awake and lying in the right lateral decubitus position. CSE was conducted using the double-space technique: an epidural catheter was inserted at the T6-7 interspace (paramedian approach) (Perifix, Tuohy needle 18 G, catheter 3 lateral eyes), followed by an intrathecal injection at the L2-3 interspace using a 25 G Pencan spinal needle. A test dose of lidocaine 2% and epinephrine 1 : 200 000, total volume 3 mL, was given *via* the epidural catheter to confirm appropriate catheter placement.

The patients were randomized to receive one of three intrathecal solutions (total volume was 1.2 mL in all the groups): morphine 300 μg, fentanyl 25 μg and bupivacaine (0.25%) 2 mg (MBF group), *vs* morphine 300 μg, bupivacaine (0.25%) 2 mg and normal saline 0.5 mL (MB group) *vs* morphine 300 μg and normal saline 0.9 mL (group M). In addition, all the patients received 10 mL of bupivacaine 0.25% through the epidural catheter before anesthesia induction.

General anesthesia was induced with midazolam 2 mg, fentanyl 100 μg and propofol 2 mg kg^{-1}. Suxamethonium 1 mg kg^{-1} was given IV to facilitate tracheal intubation, followed by IV pancuronium 4 mg. Anesthesia was maintained with sevoflurane (1 MAC end-tidal) in O_2/air, supplemented by intermittent epidural bupivacaine boluses and IV fentanyl boluses as needed. Muscle relaxation was maintained using IV pancuronium 1 mg, when requested by the surgeon. A second bolus of epidural bupivacaine 0.25% 5 mL was administered before incision, and was followed by epidural bupivacaine 0.25% 5 mL boluses every hour for the remainder of the case. Intraoperative signs of insufficient analgesia (hypertension, tachycardia, lacrimation, flushing, sweating, swallowing or movement) were treated with additional 3 mL of epidural bupivacaine 0.25%. If signs of insufficient analgesia persisted, IV fentanyl 50 μg was also given.

Crystalloid and colloid solutions were infused as needed to meet fluid maintenance requirements and compensate for blood loss, respectively. Ephedrine 5 mg IV was administered when mean arterial pressure (MAP) fell below 60 mmHg despite adequate fluid infusion. At the end of surgery, residual neuromuscular blockade was reversed with neostigmine 2.5 mg and atropine 0.8 mg, and the patient was extubated. Extubation criteria were recovery from anesthesia, spontaneous breathing, hemodynamic stability and normothermia, but the decision for extubation was at the discretion of the anesthesia and surgery providers. Postoperatively, patients were transported either to the Intensive Care Unit (ICU) (if left on mechanical ventilation) or to a high dependency unit.

Postoperative epidural analgesia started 12 hours after surgery and consisted of intermittent bolus of morphine 2 mg with 0.125% bupivacaine 8 mL every 8 hours. Pain was assessed using the VAS scale (0 = no pain, 100 mm = the worst pain imaginable) at rest (VAS-R), on movement from the supine to the sitting position (VAS-M) and with cough (VAS-C). Tramadol 100 mg was added as slow IV infusion to supplement analgesia if VAS-R score was > 30 mm or VAS-M was > 40 mm. The patients were assessed for technical causes of epidural failure (catheter dislodgment or migration) after each analgesia request. Time to first analgesia request was defined as the time between the application of the surgical dressing (end of surgery) and the first analgesia request. The number of additional analgesics given while epidural analgesia was in use, and after epidural catheter was removed, were recorded. If a patient was asleep, VAS score was recorded as 0. Epidural analgesia was used for 72 hours postoperatively, and then the catheter was removed. In cases where the epidural catheter was dislodged earlier, analgesia was provided with IV tramadol (100 mg) every 6 hours. As we performed statistical analysis based on "Intention to treat", patients with dislodged catheter had data recorded until the time the epidural catheter fell off, and were included in the statistical analysis, in the groups in which they were randomized.

VAS and hemodynamic data were collected, starting at the time of surgery, and continuing for 72 hours postoperatively, at predefined intervals (0 min, 30 min, 1st, 2nd, 3rd, 4th, 6th, 12th, 18th, 24th, 48th and 72nd hour). Postoperative fluids were administered in accordance with hospital protocol. The Bromage scale was used for motor block assessment and the Ramsey scale was used for sedation assessment. Side effects, including nausea, vomiting, pruritus (none, mild, moderate, severe), respiratory depression (defined as respiratory rate < 8 breaths per min), hypotension (MAP < 60 mmHg), VAS mood (0 = the worst mood, 100 mm = the best mood), postoperative complications and complications related to neuraxial anesthesia were recorded. Since all the patients had urinary catheter, urinary retention was not a problem in any patient.

Power analysis was conducted using Graph Pad StatMate 2.00 (GraphPad Software, Inc., La Jolla, California, USA), based on the following assumptions: difference of mean VAS-R between-groups 20 mm, standard deviation 17 mm, power 70%, and significance level (alpha) 0.05 (two-tailed). These assumptions were based on data from previous similar studies [7, 8]. Power analysis estimated that this study would need 18 cases per group. Therefore, we decided to include 20 patients per group, in order to allow for possible patient attrition or missing data. Normality of data distribution was evaluated with the Kolgomorov Smirnov test. Depending on data distribution, data are presented as mean (standard deviation) or median (range). Parametric and non-parametric statistical tests were applied as appropriate. Demographic data were analyzed using analysis of variance or chi-square test as appropriate. Because of their distribution, VAS data were treated as ordinal, and comparisons between the groups were conducted using the Kruskal-Wallis test, whereas the Mann-Whitney test was used for post-hoc comparisons. Nominal data were analyzed using Chi-square. p – values < 0.05 were considered significant for all the tests. All analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, Illinois, USA).

Results

A total of 60 patients were enrolled in the study. Demographic characteristics did not differ significantly among all the groups (Table 1).

The thoracic epidural and lumbar intrathecal spaces were successfully identified in all the patients. There were no differences among the groups with regards to the duration of anesthesia and surgery (Table 2). Compared to all other groups, the MBF group required significantly fewer additional epidural bupivacaine doses ($p < 0.001$). Supplemental IV fentanyl requirements were significantly higher in the group M compared to the groups MBF ($p = 0.022$) and MB ($p = 0.005$) (Table 2). Intraoperative pancuronium use did not differ among the groups ($p = 0.093$; Table 2). Compared to the groups MBF and MB, more patients in the group M were extubated immediately after the surgery, but this difference was not statistically significant (Table 2).

Pain relief was satisfactory in all the groups throughout the entire 72 h postoperative period. Compared to the group M, VAS-C scores were significantly lower in the group MBF ($p = 0.029$; Figure 1) and MB ($p = 0.002$; Figure 1) in the

Table 1

Demographic characteristics of the patients

Preoperative characteristics	Groups of patients			*p*
	MBF (n = 20)	MB (n = 20)	M (n = 20)	
Age (years)	59.8 ± 8.1	58.3 ± 9.9	56.7 ± 10.8	0.602
Weight (kg)	72.1 ± 14.0	66.1 ± 14.1	69.8 ± 14.4	0.419
Height (cm)	172.3 ± 8.9	169.6 ± 10.3	169.8 ± 7.9	0.587
Sex M/F (n)	15/5	13/7	16/4	0.495
ASA 1/2/3 (n)	5/14/1	2/14/3	5/13/2	0.600

Data are presented as mean ± SD or number (n); M – male; F – female; ASA – American Society of Anesthesiologists Physical Status Classification (1 – normal healthy patient; 2 – patient with mild systemic disease; 3 – patient with severe systemic disease); M – the group that received morphine; MB – the group that received morphine and bupivacine; MBF – the group that received morphine, bupivacaine and fentanyl.

Table 2

Intraoperative data

Variables	Groups of patients			*p*
	MBF (n = 20)	MB (n = 20)	M (n = 20)	
GA duration (min)	306.2 ± 99.5	242.53 ± 71.1	265.0 ± 77.4	0.06
Additional epidural bupivacaine (mg)	20.1 ± 4.4 *†	27.9 ± 6.8	30.6 ± 6.5	0.001
Fentanyl (μg)	247.5 ± 237.0†	278.9 ± 161.0‡	475.0 ± 247.9	0.003
Pancuronium (mg)	15.4 ± 4.8	12.6 ± 4.7	12.9 ± 3.5	0.093
Colloids (mL)	955.0 ± 671.6	1052.63 ± 621.3	1337.5 ± 844.0	0.228
Blood (mL)	839.5 ± 451.0	692.6 ± 866.8	1006.5 ± 821.7	0.416
Extubation in OR [n (%)]	8 (40%)	11 (57.9%)	13 (65%)	0.263

Values are presented as mean ± SD, patient number (n), or percentage (%); GA – general anaesthesia; M – the morphine group; MB – the morphine + bupivacine group; MBF – the morphine + bupivacaine + fentanyl group; OR – operating room; *$p < 0.05$ comparing the group MBF *vs* MB; †$p < 0.05$ comparing the group MBF *vs* M; ‡$p < 0.05$ comparing the group MB *vs* M

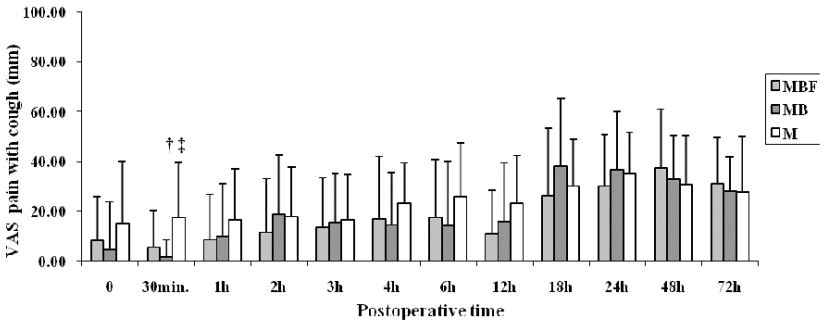

Fig. 1 – Visual analogue scale (VAS) pain scores with cough recorded during the first 72 h.
The values are reported as mean ± SD; *$p < 0.05$ comparing MBF *vs* MB group; ‡$p < 0.05$ comparing MB *vs* M group; M – the morphine group; MB – the morphine + bupivacine group; MBF – the morphine + bupivacaine + fentanyl group.

first 30 minutes after the surgery, but VAS-R and VAS-M pain scores did not differ significantly between the groups at any time ($p > 0.05$; Figure 2 and Figure 3).

Time to first analgesia request was longer in the groups MBF and MB, but the difference did not reach statistical significance (Table 3). The number of supplemental analgesia requests was similar in all the CSE groups in both the first 12 hours and the first 72 hours (Table 3). Similarly, the number of supplemental analgesia requests after epidural catheter removal were similar in all the groups (Table 3).

Intraoperatively, mean pressure was higher in the MBF compared to the MB group at only 90 minute measuring point (Figure 4). Postoperatively, the group MBF had higher mean pressure compared to the group M 30 minutes after the surgery. The group MB had higher mean pressure compared to the group M at 3 hours and 72 hours after the surgery (Figure 5).

The frequency of vomiting, hypotension and sedation was similar in all the groups. VAS mood was significantly higher in the group M compared to the group MB after 18 hours ($p = 0.034$) and 24 hours ($p = 0.002$), and compared to the MBF group after 24 hours ($p = 0.029$) (Figure 6). All the patients were able to walk the morning after surgery. There were no deaths, and there was no nerve injury or central nervous system complication related to neuraxial blockade.

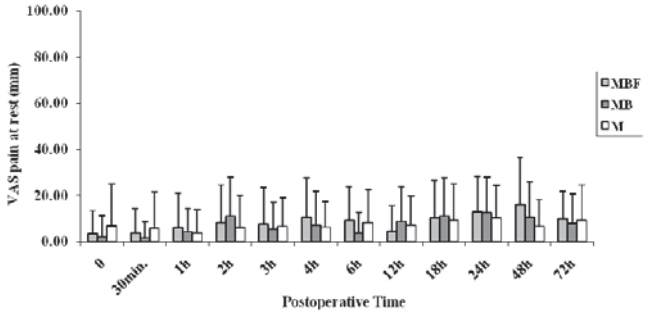

Fig. 2 – Visual analogue scale (VAS) pain scores at rest recorded during the first 72 h
The values are reported as mean ± SD; M – the morphine group; MB – the morphine + bupivacine group; MBF – the morphine + bupivacaine + fentanyl group.

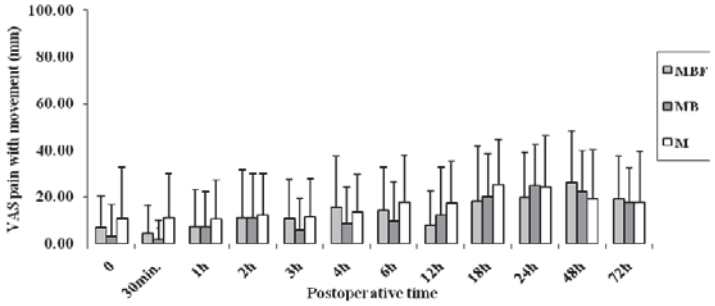

Fig. 3 – Visual analogue scale (VAS) pain scores with movement recorded during the first 72 h
The values are reported as mean ± SD; M – the morphine group; MB – the morphine + bupivacine group; MBF – the morphine + bupivacaine + fentanyl group.

Table 3

Data on postoperative analgesia and hospital stay of patients

Variables	Groups of patients			*p*
	MBF (n = 20)	MB (n = 20)	M (n = 20)	
Time to first analgesia request (min), mean ± SD (min-max)	220.6 ± 206.7 (1–585)	312.3 ± 317.3 (0–1065)	155.2 ± 170.2 (0–570)	0.310
Analgesia requests during 12 hours, n	0–1	0–2	0–2	0.392
Analgesia requests during 72 hours, n	0–4	0–5	0–8	0.707
Analgesia requests after EC removal, n	0–32	0–22	0–30	0.673
Hospital stay (days)	5–30	8–32	9–23	0.321

EC – epidural catheter;
M – the group that received morphine; MB – the group that received morphine and bupivacine; MBF – the group that received morphine, bupivacaine and fentanyl.

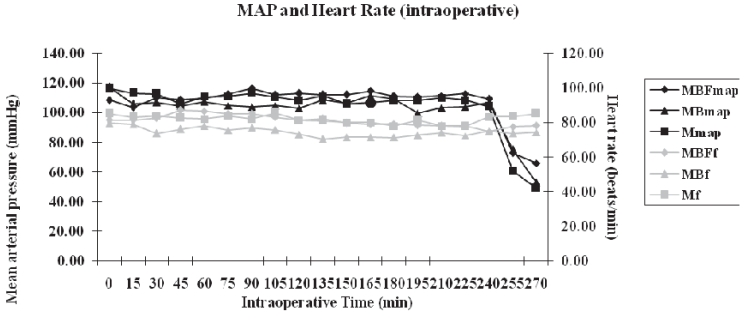

Fig. 4 – Intraoperative heart rate and mean arterial pressure (MAP)
The values are reported as mean ± SD; *$p < 0.05$ comparing the MBF group with the MB group; f –frequency of heart rate; M – the morphine group; MB – the morphine + bupivacine group; MBF – the morphine + bupivacaine + fentanyl group.

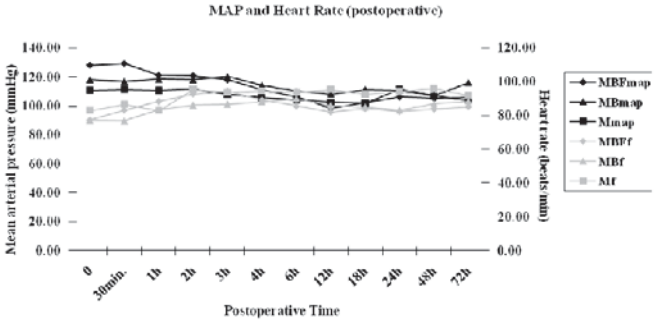

Fig. 5 – Postoperative heart rate and mean arterial pressure (MAP) recorded during the first 72 h.
The values are reported as Mean ± SD; †$p < 0.05$ comparing MBF vs. M group; ‡$p < 0.05$ comparing MB vs. M group; f –frequency of heart rate; M – the morphine group; MB – the morphine + bupivacine group; MBF – the morphine + bupivacaine + fentanyl group.

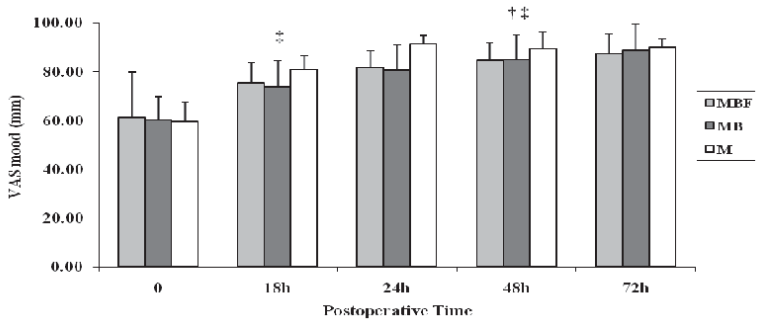

Fig. 6 – Visual analogue scale (VAS) mood scores recorded during the first 72 h
The values are reported as mean ± SD; †$p < 0.05$ comparing MBF *vs* M group; ‡$p < 0.05$ comparing MB *vs* M group; M – the morphine group; MB – the morphine + bupivacine group; MBF – the morphine + bupivacaine + fentanyl group.

Discussion

The study was designed to evaluate the potential benefits of intrathecal morphine alone or in combination with bupivacaine and fentanyl as part of CSE in patients undergoing total gastrectomy. The combination of intrathecal morphine, bupivacaine and fentanyl has been shown to provide useful analgesia in gynecology and obstetrics [9–11]. In this study we investigated whether a similar benefit could be demonstrated for total gastrectomy.

The choice of study medications was based on the concept of anti-nociceptive synergy: intrathecal fentanyl provides rapid (within 5–10 min) analgesia onset, improves surgical blockade quality [12] and enhances the effect of small intrathecal bupivacaine doses [13], whereas intrathecal morphine provides prolonged analgesia [14]. Similarly, intrathecal bupivacaine potentiates epidural bupivacaine [15] and the anti-nociceptive effect of intrathecal morphine [16]. Adequate analgesia after total gastrectomy (at rest, with movement and with cough) was the main study endpoint. Reduction of intraopertive analgesia requirements, time to first postoperative analgesia request and postoperative analgesic drug use, were designated as secondary study endpoints.

Our results suggest that the main benefit of adding fentanyl and bupivacaine to intrathecal morphine were reduced intraoperative IV fentanyl and epidural bupivacaine use in the MBF group. Because the same surgeon performed all procedures, observed differences between groups are probably not related to differences in surgical technique or complications.

Because the visceroperitoneal organs receive multiple innervations by the spinal nerves (T5–T12), the vagus nerve and the phrenic nerve (C3–C5) in the upper abdomen [17], pain perception during gastrectomy is regulated by multiple mechanisms [17]. The complex nature of postoperative pain after gastrectomy was the reason we decided to study the role of CSE analgesia in these patients. Postoperative analgesia was satisfactory in all the patients, and VAS pain scores, time to first analgesia request, number of analgesia requests at 12 hours and 72 hours postoperatively, and after epidural catheter removal did not differ significantly among the groups. Since all the patients had access to adequate pain relief, and pain scores did not differ significantly among the groups, postoperative analgesia requirements are a reasonable way to detect differences in the quality of analgesia among the groups. The absence of a difference with regards to postoperative analgesia between the groups could be due to intrathecal morphine effectively suppressing pain in all the patient groups.

The use of epidural morphine and bupivacaine combination for postoperative analgesia was based on the concept of synergistic anti-nociceptive effect from concurrent administration of pain-inhibiting drugs, which allows a dose reduction for each drug [18]. Moreover, data show that epidural morphine can provide prolonged analgesia [19] and reduce epidural local anesthetic requirements, thereby minimizing

lower extremity motor blockade and facilitating early ambulation. Indeed, all the patients in our study ambulated the morning after the surgery. The widespread worldwide use of epidural morphine for postoperative analgesia suggests that this technique is safe [20]. We opted for the same dose of medications for neuraxial blocks in all the patients, because this approach is simple, less time-consuming and less prone to errors during medication preparation. In order to prevent under-treatment of pain, we instructed the patients to request additional analgesia for VAS-R score > 30 mm or VAS-M > 40 mm. We used IV tramadol for breakthrough pain, because in our experience, IV tramadol is better tolerated than morphine (i.e. causes less nausea) in our patient population. Of note, our study did not have adequate power to assess the impact, if any, of CSE on morbidity and mortality.

Conclusion

Our findings suggest that addition of fentanyl and/or bupivacaine to intrathecal morphine reduces the need for additional intraoperative epidural bupivacaine and IV fentanyl, but does not improve postoperative VAS pain scores after gastrectomy. Well designed prospective clinical studies are needed to further evaluate the potential benefit of adding subarachnoid fentanyl and/or bupivacaine to subarachnoid morphine for total gastrectomy.

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Nutritional and physical activity behaviours and habits in adolescent population of Belgrade

Ponašanje i navike u ishrani i fizičkoj aktivnosti kod beogradskih adolescenata

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Abstract

Background/Aim. Proper nutrition and regular physical activity are essential parts of a adolescent's overall health. The aim of this research was to evaluated eating and physical activity behaviours and habits, nutritional and food knowledge, beliefs and self-efficacy related to diet and health of the adolescents of the city of Belgrade, Serbia. **Methods.** A dietary questionnaire previously constructed and tested in adolescent population from Italy was self-administrated. We evaluated eating habits, physical activity, meaning of healthy and unhealthy dietary habits and food, self-efficacy, barriers affecting food choices, nutritional and food safety, and body mass index (BMI) of the adolescents. The sample included 707 adolescents, the mean age of 15,8 ± 2 years enrolled in the first grade at several high schools in Belgrade. **Results.** Only 27% of the adolescents had satisfactory eating habits; 31% have a very active lifestyle; 7% good nutritional knowledge and 6–12% satisfactory food safety knowledge and hygiene practices. **Conclusion.** Significant deviations from recommendations for healthy lifestyle was noted in adolescents' habits, knowledge and practice. It is therefore necessary to develop and organize programs for promotion of healthy behaviours adapted to the adolescents' needs.

Key words:
feeding behaviour; motor activity; adolescent; questionnaires; life style.

Apstrakt

Uvod/Cilj. Pravilna ishrana i redovna fizička aktivnost su suštinski delovi zdravlja adolescenata. Cilj ovog istraživanja bio je da se ocene ponašanje i navike u ishrani i fizičkoj aktivnosti, znanje o hrani i ishrani, verovanje i samoefikasnost u vezi sa ishranom i zdravljem beogradskih adolescenata. **Metode.** Primenjen je dijetni upitnik za samoprocenu, prethodno konstruisan i proveren na populaciji adolescenata u Italiji. Mi smo ocenili navike u ishrani, fizičku aktivnost, značenje zdrave i nezdrave hrane i dijetnih navika, samoefikasnost, barijere koje utiču na izbor hrane, bezbednost hrane i ishrane i indeks telesne mase (BMI) adolescenata. Ispitanike je činilo 707 adolescenata, uzrasta 15,8 ± 2 godina koji pohađaju prvi razred različitih srednjih škola u Beogradu. **Rezultati.** Svega 27% adolescenata imalo je zadovoljavajuće navike u ishrani, 31% vrlo aktivan stil života, 7% dobro znanje o ishrani i 6%–12% zadovoljavajuće znanje o bezbednosti hrane i higijenske navike. **Zaključak.** Zabeležena su značajna odstupanja od preporuka za zdrav stil života u navikama, znanju i praksi adolescenata, te je potrebno razviti i organizovati programe promocije zdravog ponašanja prilagođenog potrebama adolescenata.

Ključne reči:
ishrana, navike; napor, fizički; adolescenti; upitnici; način života.

Introduction

Nutrition and physical activity are of the greatest importance for health promotion. Eating habits and attitudes towards physical activity shape the lifestyle of an individual to the greatest extent, thus determining health, i.e. the most common diseases of a modern man [1].

Acquiring habits of regular physical exercise from the earliest age is thought to be a significant benefit which will show its positive effects later in life [2–5]. However, adolescence is a period with significantly reduced physical activity, which is particularly characteristic for girls [6–8]. At the same time, during the last decades young people have been spending more and more time in front of TV/computers [6, 9].

Eating habits have been acquired in the family since the earliest childhood, and then they are additionally formed under the influence of social and physical environment and macrosystem [10–12]. Consolidation of nutritional behaviours

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and habits takes place in adolescence. Sweeting et al. [13] concluded that by the age of 15 eating habits consolidate with minimal changes between 15 and 18 years. This is largely in agreement with findings of Kelder et al. [14], who reported consolidation prior to the 6th grade and extension of nutritional and physical activity habits in the adulthood.

Knowledge about healthy and safe food is certainly a precondition for healthy diet, but the motivation for practical application of healthy recommendations is necessary [15]. Shannon et al. [16] and Bandura [17] pointed out that a sense of self-efficacy was crucial for personal change. When a person possesses knowledge and abilities, his or her belief in self-efficacy most probably becomes the main guideline for the choice of actions, their level of making efforts and persistence in their own efforts to achieve goals [18]. Application of behavioural techniques that improve self-efficacy may be effective for weight loss promotion and it may produce positive outcomes related to eating habits in young adults [19].

Recognition of barriers to change behaviour has implications for designing the programs for adolescents' health promotion. Gracey et al. [15] in their study in adolescents aged [15–16] pointed out that barriers to change were related to the availability of healthy food in the surroundings and insufficient knowledge about nutritive food content. These authors pointed out the importance of beliefs and values regarding healthy diets, controlling weight, lowering cholesterol etc. Wardle et al. [20] believe that prevalence of health beliefs in females compared to males, determine better food choices and healthier dietary pattern, which is ultimately attributed to higher morbidity and mortality in men caused by the most common diseases of modern times.

The aim of our research was to get acquainted with the behavioural pattern related to diet and physical activity of the selected group of adolescents of the urban area of Belgrade, as a representative urban area of South-East Europe. It was therefore important to evaluate eating habits, physical activity, meaning of healthy dietary habits and food, self-efficiency, possible barriers affecting healthy food choices, nutritional and food safety knowledge. In addition, on the basis of height and body weight we determined body mass index (BMI) to investigate the possible relationship between BMI and the above mentioned variables.

Methods

The participants for this cross-sectional study represent a convenience sample among the first grade students who attended several high schools in different municipalities in the city of Belgrade, the capital of the Republic of Serbia. Participants were sampled from a variety of school across the spectrum of high-school education, ranging from general college preparatory education to specific vocational education, to account for socioeconomic differences. The study was implemented between March and June of 2010, during regular class hours in the presence of teachers and the study investigators. Participation was voluntary and anonymous. Informed written consent was obtained from each student and their parents. The study was approved by the Ethics Committee of the Faculty of Sport and Physical Education at the University of Belgrade.

Invitations were extended to 900 students, which represents about 2% of the high-school first graders enrolled in the academic year 2009–10 in Belgrade. Of those 900, 733 agreed to participate and mailed back a complete questionnaire. The 26 incorrectly filled out questionnaires were excluded leaving 707 for analysis. The final sample included 377 girls (mean age 15.8 ± 0.2 years) and 330 boys (15.9 ± 0.3 years).

Questionnaire

We used a validated questionnaire [21], which had already been administered to adolescents of the same age in Italy [22]. Giovanna Turconi [21, 22], the author of this questionnaire, gave her written consent for the use of this questionnaire in our study.

The questionnaire consisted of 9 sections referred to: Section 1 – Personal information and parents' education; Section 2 – Eating habits, of 14 questions, the total score being 42; Section 3 – Physical activity, of 6 questions, the total score being 18; Section 4 – Healthy and unhealthy dietary habits and food, of 5 questions, the total score being 15; Section 5 – Self-efficacy, of 8 questions, the total score being 16; Section 6 – Barriers to change, of 9 questions, the total score being 9; Section 7 – Nutritional knowledge, of 11 questions, the total score being 11; Section 8 – Food safety knowledge, of 10 questions, the total score being 10; Section 9 – Food safety and behaviour in hygiene practices, of 8 questions, the total score being 24.

The answers to the questions within the sections 2–9 were scored from 0 to 3, with the most desirable response having the highest score. The exception was section 6 – Barriers to change, where the case was opposite.

The participants were classified as overweight and obese based on Cole's et al. [23] cut-off points.

To derive BMI, body height and mass collected at the time of the last annual check-up were extracted from the students' health records kept by primary care institutions.

Reliability of the sections was checked through Cronbach's alpha coefficient (reliability of internal consistency type). For Eating habits, Physical activity, Self-efficiency, Barriers to change, derived values of coefficients ranged from 0.536 to 0.68. For Healthy and unhealthy dietary habits and food, Nutritional knowledge, Food safety knowledge, Food safety and behaviour in hygiene practices, whose content of the items generally refers to knowledge testing reliability coefficients were low.

Scores for each section in the questionnaire were consolidated by summing subjects' answers and they were shown through arithmetic mean and standard deviation. Scores were transformed into categorical variable with three categories according to the degree of prevalence, so they were divided into tertiles and the percentage distribution of subjects in each category was calculated. The lowest tertile referred to the worst evaluation and the highest to the best evaluation, except for section 6 (Barriers to change) for which the greater barriers to change were related to the highest tertile. To determine a significance of differences between the subjects one sample *t*-test was used, and Pearson's

correlation coefficient was applied to investigate the correlation between BMI and sections scores. Data processing was conducted in SPSS 17.

Results

Sample

The mean BMI was 22.2 ± 3.1 kg/m^2 in boys and 20.5 ± 2.6 kg/m^2 in girls. A proportion of 21.2% of the boys and 7.7 % of the girls were classified as overweight. Obesity was recorded in 3.3% of the boys and 1.9% of the girls. Approximately 3% of males and females can be classified in the group of underweight (calculated as third percentile). The sample characteristics are shown in Table 1. The group mean age was 15.8 ± 0.3 years.

Anthropometric characteristics of the sample with respect to gender are shown in Table 1.

Dietary Questionnaire

Table 2 shows the percentage distribution of subjects according to the scores.

Eating habits

The total score (42) was divided into tertiles (Table 3). The lowest one referred to "inadequate eating habits" and encompassed almost a third of all students. A total of 45% of the subjects had "partially satisfactory eating habits", while only a quarter of the sample (26.7%) showed "satisfactory eating habits". The mean score obtained was 30.5 ± 4.8 with a statistically significant difference (p = 0.000) between males and females (29.4 ± 4.3 for males *vs* 31.5 ± 4.6 for females). The worst eating habits are skipping breakfast (about 36% of the sample). If the subjects who often have breakfast are added to this category, approximately 15% of students skip breakfast. Approximately 30% of the subjects of both genders do not drink milk or yogurt at breakfast, 71.5% of students do not eat at least two portions of fruit and

Table 1

Anthropometric characteristics of the subjects with respect to gender

Variables	Males (n = 330) ($\bar{x}$ ± SD)	% of males	Females (n = 377) ($\bar{x}$ ± SD)	% of females
Age (years)	15.9 ± 0.3		15.8 ± 0.3	
Weight (kg)	73.3 ± 12.5		58.5 ± 8.5	
Height (m)	1.8 ± 0.1		1.7 ± 0.1	
BMI (kg/m^2)	22.2 ± 3.1		20.5 ± 2.6	
Overweight subjects′ BMI (kg/m^2)	25.8 ± 1.3	21.2	25.3 ± 1.2	7.7
Obese subjects′ BMI [(kg/m^2), n%]	31.1 ± 2.2	3.3	30.1 ± 0.7	1.9
Underweight subjects′ BMI (kg/m^2)	17.1 ± 0.6	3.0	16.6 ± 0.6	3.2

BMI – body mass index

Table 2

Percentage distribution of the subjects according to the scores

Questionnaire sections	Scores	Males (n = 330)	Females (n = 377)
2. Eating habits	Lowest	13	12
	Mean ± SD	29 ± 5	31 ± 5
	Highest	41	41
3. Physical activity and lifestyle	Lowest	1	3
	Mean ± SD	12 ± 4	11 ± 4
	Highest	18	18
4. Healthy and unhealthy dietary habits and food	Lowest	4	5
	Mean ± SD	11 ± 2	11 ± 2
	Highest	15	15
5. Self-efficacy	Lowest	4	7
	Mean ± SD	14 ± 2	14 ± 2
	Highest	16	16
6. Barriers to change	Lowest	0	0
	Mean ± SD	2 ± 2	2 ± 2
	Highest	8	9
7. Nutritional knowledge	Lowest	0	0
	Mean ± SD	4 ± 2	5 ± 2
	Highest	10	10
8. Food safety knowledge	Lowest	0	0
	Mean ± SD	4 ± 2	5 ± 2
	Highest	9	10
9. Food safety and behaviour in hygiene practices	Lowest	6	9
	Mean ± SD	17 ± 3	16 ± 4
	Highest	24	24

More than a half of the student's parents had a high school qualification, while about 40% of them had a higher education degree.

Table 3

Percentage distribution of the subjects according to the tertile scores

Questionnaire sections	% (n) of subjects		
	1st tertile	2nd tertile	3rd tertile
2. Eating habits	28.3 (200)	45.0 (318)	26.7 (189)
3. Physical activity and lifestyle	27.4 (194)	41.4 (293)	31.1 (220)
4. Healthy and unhealthy dietary habits and food	39.8 (282)	55.2 (390)	4.9 (35)
5. Self-efficacy	18.5 (131)	47.5 (336)	33.9 (240)
6. Barriers to change	29.3 (207)	54.3 (384)	16.4 (116)
7. Nutritional knowledge	23.7 (168)	69.4 (491)	6.8 (48)
8. Food safety knowledge	44.5 (315)	49.2 (348)	6.2 (44)
9. Food safety and behaviour in hygiene practices	36.8 (260)	50.9 (360)	12.3 (87)

vegetables every day, 32.4% of the subjects have sweets-based snacks.

Physical activity and lifestyle

The total score (18) was divided into tertiles (Table 3). Slightly more than a quarter of adolescents (27.4%) was classified into "sedentary physical level" category. Only a third of the students have a very active lifestyle. Physical activity of the remaining part of the sample corresponded to the category of "partially moderate physical level". The mean score obtained was 11.7 ± 3.8 with a statistically significant difference (p = 0.000) between males and females (12.4 ± 3.7 for males *vs* 11 ± 3.7 for females). A total of 49% of the subjects answered watching television/using computer or other sedentary activities in free time. A statistically significant difference emerged between normal and overweight plus obese boys, with the highest score obtained in normal weight boys (12.5 ± 3.6 score *vs* 12.1 ± 4.2 score) (p = 0.01). A statistically significant negative correlation was found between physical activity score and BMI referred to as overweight and obese (p = 0.012) in boys. No statistically significant correlation was established between physical activity score in students with normal BMI.

Healthy and unhealthy dietary habits and foods

The total score (15) was divided into tertiles (Table 3). Almost 40% of girls and boys could be classified into the first tertile "little comprehension of the meaning of healthy and unhealthy dietary habits and food". The medium one referred to "sufficient comprehension of the meaning of healthy and unhealthy dietary habits and food" and slightly more than one half of the sample belonged to this group. The scores of low percentage of the sample were classified in the last third which indicated "good comprehension of the meaning of healthy and unhealthy dietary habits and food". No statistically significant difference was found in the average score between males and females for this section (Table 2). In response to the question "According to you, which is healthy food?", almost a half of the students (50.7%) reported that "a food rich in protein" is the healthiest one, 3.9% chose "food rich in calories", 2.3% microbiologically tested food" and 42.8% "food without preservatives and additives".

Self-efficiency

The total score (16) was divided into tertiles, where the lowest one referred to "incapacity for using advice aimed at improving one's well-being", the medium one referred to "sufficient capacity for using advice aimed at improving one's well-being", and the highest one referred to "good capacity for using advice aimed at improving one's well-being". The mean score obtained was 14.1 ± 2, with statistically significant differences (p = 0.010) between males and females (13.9 ± 2.3 for males *vs* 14.4 ± 1.8 for females). A large percent of the subjects (81.7%) reported being able to use advice aimed at improving their well-being, while less than one-fifth of the subjects thought themselves not able to do that.

Barriers to change

The total score (9) was divided into tertiles, where the lowest one referred to "no barriers in modifying one's own eating habits with the aim of improving them", the medium one referred to "some barriers in modifying one's own eating habits with the aim of improving them", and the highest one referred to "a lot of barriers in modifying one's own eating habits with the aim of improving them". The mean score obtained was 2.1 ± 1.7, without any statistically significant differences between males and females. Almost a third of the subjects had no barriers to change that would improve eating habits (42.2% of boys and 17.7% of girls), while a half of students had some barrier to change (45.4% of males and 62.0 % of females).

Nutritional knowledge

The total score (11) was divided into tertiles (Table 3). The lowest one referred to "insufficient nutritional knowledge", where almost one-fourth of the students could be classified. Around two-thirds of the subjects had good nutritional knowledge (more females), while only 7% of the sample had quite good nutritional knowledge (highest among females). The mean score obtained was 4.7 ± 1.8 with a statistically significant difference (p = 0.002) between males and females (4.5 ± 1.8 for males *vs* 4.9 ± 1.7 for females). The worst results emerged in the questions related to fat, energy and protein food content. The worst answer was to the question "Which is the nutrient that contains the most energy?", with only 8.6% of the students who answered fat, 64.9% answered proteins and 24% answered carbohydrates.

Food safety knowledge

The total score (10) was divided into the tertiles (Table 3). Over 40% of the subjects belonged to the lowest third which re-

ferred to "insufficient food safety knowledge". Almost half of the subjects manifested "good food safety knowledge", while only 6% of males and females had "quite good food safety knowledge". The mean score obtained was 4.7 ± 1.8 with a statistically significant difference (p = 0.013) between males and females (4.5 ± 1.8 for males *vs* 4.9 ± 1.8 for females).

Food safety and behaviour in hygiene practices

The total score (24) was divided into tertiles (Table 3). The lowest one referred to "inadequate behaviour in hygiene practices" where 38.1% of males and 35.5% of females could be classified. Almost equal number of males and females (49% for males *vs* 52.5% for females) had "partially adequate behaviour in hygiene practices". Only 12.3% of students had the highest one referred to "quite good behaviour in hygiene practices". The mean score obtained was 16.9 ± 3.1 with a statistically significant difference (p = 0.000) between males and females (17.4 ± 2.7 for males *vs* 16.4 ± 3.6 for females). In response to the following questions "If the butcher touches ham with his hands, do you eat it?", "Do you read the instructions for use and for preservation written on package foods?" and "Do you eat canapé lying out for a long time at the bar?", only less than half of the students chose the right answer.

Table 3 summarizes the various scores obtained in each section by males and females, respectively.

Dietary questionnaire and BMI

Scores of all the dietary questionnaire variables were analysed in relation to students' BMI, considering two groups, normal BMI subjects and overweight plus obese subjects. A statistically significant negative correlation emerged between physical activity score (Section 3) and BMI-overweight plus obese (R = -279; p = 0.01), for males, thus normal weight boys had higher score (12.5 ± 3.6 score), and overweight plus obese boys had lower score (12.1 ± 4.2 score). Statistically significant negative correlations emerged between normal BMI boys and scores of the responses to the Section 5 questions – Self-efficacy score (R = 0.13; p = 0.04); Section 6 – Barriers to change score (R = -0.13; p = 0.04); Section 7 – Nutritional knowledge score (R = 0.14; p = 0.02) and Section 8 – Food safety knowledge score (R = 0.19; p = 0.00). Statistically significant negative correlations emerged between normal BMI girls and scores of the responses to the Section 5 questions – Self-efficacy score (R = 0.14; p = 0.01) and Section 8 – Food safety knowledge score (R = -0.12; p = 0.02).

Discussion

In this research on 707, 16-year-old students we evaluated eating habits, physical activity and factors that may affect them. We obtained data that could be used in healthy lifestyle promotion.

In accordance with Cole's et al. [23] reference standards we recorded almost a quarter of boys with excessive BMI, while the number of girls was significantly lower. These data are alarming and call for preventive measures to be taken in order to control and maintain normal body mass. Obesity prevalence was low in both genders. We recorded much greater incidence of overweight males of Belgrade with regard to the data of Pavlović [24] for the North Bačka district – a northern more developed region of Serbia, (together, rural and urban environment; boys – overweight/obesity 12.0% /4.76%; girls – overweight/obesity – 8.72% /3.33%), for the age of 16. The results of analytical study [25] (Institute for Public Health of Serbia) from 2007 show that almost a fifth of adolescents (18%) was moderately obese and obese, which represented the increase in relation to 2000 (11%). When compared to the research of Turconi et al. [22], our study recorded a lower number of females with excessive BMI, while the number of males with overweight was alike.

Data related to eating habits of our adolescents show that the intake of fruit, vegetables, milk and dairy products should be increased, while the intake of sweets, soft drinks and food rich in fat should be lowered. In addition, more frequent meals and regular breakfast should be promoted, which is considered to be important for more qualitative nutritive intake and obesity prevention [26, 27]. The mentioned deviations recorded in our students are contrary to the official recommendations for healthy diet in Serbia [28]. Our results show similarities with those of the research of Turconi et al. [22]. The authors from other countries recorded similar data [10, 29–32] which indicates very similar trends in diet of young people from different regions of developed countries.

Physical activity in boys was higher than in girls, recorded in other researches, as well. Over a quarter of students was classified into sedentary category, which is an alarming information. On the basis of their researches in children of younger adolescent age, D'Addesa et al. [33] and Lazarou et al. [30] attributed the greatest importance for overweight and obesity development to physical inactivity. We recorded a higher physical activity score in normal BMI boys. Almost a half of our subjects spend 3–4 hours a day in front of TV/computer and in other sedentary activities. A similar trend is noted in other researches, as well [4, 30, 34]. These sedentary forms of behaviour are related to higher risk of obesity and higher values of cholesterol [34].

Although a greater number of students showed sufficient meaning of healthy diet and food, almost 40% of subjects showed insufficient meaning. When these results are considered in relation to eating habits, we can say that our students have better eating habits than they are able to understand the meaning of healthy and unhealthy diet and food. In the researches of Turconi et al. [22], Nićiforović-Šurković et al. [35] and Croll et al. [36] the opposite results were recorded. These authors explained their results by the existence of barriers and decreased interest in healthy diet in the period of adolescence. In Serbia, adolescents still have a significant portion of meals at home, so we believe that family has a favourable impact on eating habits to a certain extent. On the other hand, the positive impact of schools on understanding the meaning of healthy diet and food is insufficient. So we believe that in healthy eating promotion apart from focusing on psychological pressure, we should try to increase food and diet knowledge.

Over two thirds of the subjects of our sample perceive their self-efficacy for adoption of attitudes and behaviours that may improve their health status related to diet, as moderate to high. Girls estimate their self-efficacy as higher than boys. The finding is in accordance with the research of Gracey et al. [15], and in opposition to the results of Turconi et al. [22]. Bearing in mind that media, especially television, have the leading role in informing about the significance of adopting healthy lifestyles [17], the obtained findings may indicate the need to formulate different messages to boys and girls.

Barriers to change must be considered when planning the programs for nutrition education. The lack of students' knowledge about how to increase the intake of dietary fibres, how to meet energy needs and how important thing is not to be influenced by peers when choosing food are the greatest barriers recorded in our students. Then follows the lack of knowledge about food less rich in sugar and fat. However, the greatest number of subjects have some or no barriers to change that may positively affect changes in diet. We did not record differences between boys and girls. Turconi et al. [22] also recorded no differences caused by gender, whereas Gracey et al. [15] recorded more different barriers in girls in relation to boys.

Nutritional knowledge is a predisposing factor for good eating habits. Although most of our students have good nutritional knowledge we cannot be satisfied given that almost a quarter of subjects have insufficient knowledge. As in several other studies [15, 22, 35] our students were not sufficiently able to redirect their knowledge about nutrients into good food choices. Contento et al. [37] and Hoppu et al. [29] in their research indicated the fact that just knowledge about diet is not sufficient for adopting healthy eating behaviours. Van Cauwenberghe et al. [32] in their study found limited results of school-based educational interventions to promote healthy diet in children and adolescents. Certainly, our students lack nutrition instruction during their elementary education, but it is obviously necessary to motivate students to change their behaviours related to health. The girls in our research, along with greater nutritional knowledge, showed better eating habits, as well (Section 2) in comparison to the boys. This difference is attributed to their higher interest in weight control and stronger health beliefs [15, 20]. Like the girls in the research of Turconi et al. [22], our girls are traditionally more engaged in preparing and buying food in the family.

Knowledge about food safety was very low, in almost half of the subjects, but students' behaviour and hygiene practices were something better. About a half of students showed good knowledge about food safety, and at the same time nearly as many students showed „partially adequate behaviour in hygiene practices". We believe that experiences gained in the family have the greatest impact on hygiene behaviour and practices, so this explains the recorded difference in favour of practice. The girls showed significantly higher knowledge about food safety, but the boys gave better answers to the questions about hygiene practices.

Adolescence is a period when habits and behaviours related to diet and physical activity are strengthened and as such mostly extended into adulthood. In this research we wanted, in a comprehensive manner, to become familiar with behaviour and knowledge about food/diet and physical activity of the 16-year-olds of Belgrade, a typical urban area of Serbia. At the same time, it was important to become familiar with the factors that greatly affect the mentioned behaviours and BMI as an ultimate outcome related to health. A high level of overweight especially in boys, require interventions that should improve eating habits and increase physical activity. The need for a lower intake of food rich in fat was particularly expressed, which coincided with students' insufficient knowledge about nutritional food composition. Apart from that, it is necessary to stimulate higher intake of fruit, vegetables, milk and dairy products, as well as lower intake of sweets. Better eating habits, greater willingness to use advice for improving health and well-being and higher nutritional knowledge in girls are explained by their stronger beliefs and evaluation of healthy diet and health in general. Therefore, activities for promotion of diet and healthy behaviours among boys should be adapted to their specificities.

Nutrition education programs are very important for children and adolescents. They should be practically applicable and focused on food. We should work on physical activity promotion continuously, as one of the key factors in preventing of various diseases.

Limitations

The first limitation of this study is inherent to its cross-section design. Secondly, the responses are self-reports, which may affect reliability. Finally, height and weight were not measured but rather obtained from medical records at the time of the last check-up.

Conclusion

This study established significant discrepancies from the recommendation for healthy lifestyle in habits, knowledge and practice of adolescents. Almost a third of the students in most of the investigated variables of behaviour and habits was classified in the lowest category, which referred to inadequacy and insufficiency. The difference was manifested between males and females, and the females scored better results except for physical activity. Therefore, it is essential to evaluate the situation previously and determine the significance of all relevant factors affecting the lifestyle of adolescents'. Healthy behaviours promotion should be carried out in a way that is adjusted to the needs of young people.

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ORIGINAL ARTICLE

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Biohumoral and endocrine parameters in assessment of surgical trauma in open and laparoscopic cholecystectomy

Metabolički i endokrini parametri u proceni hirurške traume kod otvorene i laparoskopske holecistektomije

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Abstract

Background/Aim. Every surgical procedure causes metabolic, endocrine, and hemodynamic stress in the organism. The aim of this work was to assess the extent of trauma following each of the two types of cholecystectomy (traditional/open and laparoscopic) by measuring palette of biochemical parameters. **Methods.** This prospective, single- center study included 120 patients subjected to elective cholecystectomy during the period of one year. Sixty patients were treated laparoscopically and 60 traditionally. Biohumoral and endocrine parameters were determined from 24-hour urine and blood. We measured adrenaline, noradrenaline, metabolites of corticosteroid hormones (17-hydroxyl and 17-keto steroids), C-reactive protein (CRP), albumin, glycemia, creatine-phosphokinase (CPK), lactate-dehydrogenase (LDH), red blood cells sedimentation and serum concentration of potassium. **Results.** We observed significantly lower levels of adrenaline ($p < 0.01$), noradrenaline ($p < 0.05$), dopamine ($p < 0.01$), 17-hydroxyl ($p < 0.01$) and 17-keto steroids ($p < 0.01$), glycemia ($p < 0.01$), CPK ($p < 0.01$), LDH ($p < 0.01$) and red blood cells sedimentation ($p < 0.01$) following laparoscopic cholecystectomy compared to traditional one. Significant increase in CRP levels was recorded postoperatively in both groups ($p < 0.05$), as well as significant decrease in serum albumin values ($p < 0.05$). Duration of the hospitalization following laparoscopic cholecystectomy was significantly shorter ($p < 0.01$). **Conclusion.** The intensity of organism response is proportional to the intensity of surgical trauma. Metabolic, tissue and neuroendocrine response of organism to trauma has lower intensity after laparoscopic cholecystectomy.

Key words:
cholecystectomy; cholecystectomy, laparoscopic; intraoperative period; biological markers; blood chemical analysis.

Apstrakt

Uvod/Cilj. Na svaku hiruršku proceduru organizam reaguje u vidu metaboličkog, endokrinog i hemodinamskog odgovora. Cilj ove studije bio je da uporedi intenzitet traume nastale kao posledica dva tipa holecistektomije (otvorene/tradicionalne i laparoskopske) merenjem najšire palete bihemijskih parametara. **Metode.** Ova prospektivna studija uključila je 120 bolesnika podvrgnutih elektivnoj holecistektomiji, od toga 60 operisanih laparoskopski i 60 tradicionalno tokom perioda od jedne godine. Biohumoralni i endokrini parametri određivani su iz 24-časovnog urina i krvi. Mereni su nivoi adrenalina, noradrenalina, metabolita kortikosteroidnih hormona (17-hidroksi i 17-keto steroida) iz 24-satnog urina HPLC metodom, a iz krvi C-reaktivni protein (CRP), albumini, serumska glukoza, kreatin-fosfokinaza (CPK), laktat-dehidrogenaza (LDH), sedimentacija eritrocita i serumska koncentracija kalijuma. **Rezultati.** U poređenju sa tradicionalnom holecistektomijom, posle laparoskopske holecistektomije uočeni su statistički značajno niži nivoi adrenalina ($p < 0,01$), noradrenalina ($p < 0,05$), dopamina ($p < 0,01$), 17-hidroksi ($p < 0,01$) i 17-keto steroida ($p < 0,01$), glikemije ($p < 0,01$), CPK ($p < 0,01$), LDH ($p < 0,01$) i sedimentacije eritrocita ($p < 0,01$). U obe grupe postoperativno zabeležen je značajan porast vrednosti CRP ($p < 0,05$) i značajan pad nivoa albumina ($p < 0,05$). Kod bolesnika lečenih laparoskopski trajanje hospitalizacije bilo je značajno kraće ($p < 0,01$). **Zaključak.** Intenzitet odgovora organizma je proporcionalan intenzitetu hirurške traume. Metabolički, biohumoralni i endokrini odgovor organizma značajno je nižeg nivoa nakon laparoskopske intervencije.

Ključne reči:
holecistektomija; holecistektomija, laparoskopska; intraoperativni period; biološki pokazatelji; krv, hemijske analize.

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Introduction

Every surgical intervention represents a stress and causes a severe metabolic, endocrine and hemodynamic reaction of organism. The intensity of operative trauma can be tracked by monitoring, identification and quantification of various metabolites. Surgical intervention as a stress model causes dynamic exposure to neuroendocrine metabolic and biohumoral substances which are in correlation with duration and intensity of trauma. Negative balance of nitrogen with a consequential catabolism of proteins, glyconeogenesis, reduced utilization of glucose, retention of water and salt and lipolysis are the parameters that determine the organism reaction to trauma.

Neuroendocrine response to trauma is realized in three pathways: *via* the sympathetic nervous system, that influences energy mobilization by stimulating the secretion of adrenalin, noradrenalin, glucagon and inhibiting secretion of insulin using renin-angiotensin system [1–3]; by balancing hormones which are secreted over the hypothalamus, stimulating secretion of ACTH and growth hormone [1] and by stimulating magnocelular nucleus of hypothalamus and releasing vasopressin from the hypophysis. With the increase of endorphine, causing endanalgesia, these reactions confirm the organism adaptation to the stress provoked by surgical intervention [4–6].

Cholelithiasis is the most common disease of hepatobiliary system. According to Schirmer et al. [7], 20 million people in the USA have gallbladder disease and 500,000 cholecystectomies are done per year with the mortality of 3% due to manifested complications. Since the first traditional cholecistectomy, surgeons have tried to use a minimally invasive approach to decrease the intensity of surgical trauma. Since 1985 laparoscopic cholecystectomy has been accepted as the gold standard in the therapy of calculous cholecystitis [5, 6, 8–10]. Laparoscopic cholecistectomy is used instead of traditional one and mini-laparotomic cholecystectomy because better effects, minimal operative traumatism, shorter postoperative recovery and a significant esthetical effect [4, 6, 10–12]. Operative mortality in developed countries is not expected to be higher than 0.2–0.5%.

The reduction of risk connected with the laparoscopic approach can be achieved with the recognition of anatomic variation of hepatobiliary system, intraoperative visualization, an experienced surgical team and a suitable choice of operative approaches. Operative incision being smaller in patients treated laparoscopically contributes to the lower level of surgical trauma. Pneumoperitoneum can be an additional factor of traumatism. Carbon dioxide has a small irritating effect on peritoneum but also causes distension of parietal peritoneum, elevation of diaphragm, reduction of alveolo-capillary membrane and hypoventilation with consequently respiration acidosis as a result [13].

The aim of this study was to asses impact of each of the two types of cholecystectomy (open or laparoscopic) to the extent of operative stress and trauma in the patients being electively operated on due to their chronic calculous cholecystitis, by performing comparative analyze of the pallet of neuroendocrine, metabolic and tissue indicators.

Methods

This prospective, single-center, randomized study included 120 patients with the indication of chronic calculous cholecystitis who were electively operated on during the one year period in the Surgery Department, General Hospital in Berane, Republic of Montenegro, after approval by the local Ethics Committee.

The patients were divided into two groups: the experimental group including the patients treated with laparoscopic and the control group including those treated with open cholecystectomy. The groups were standardized by the number of patients and clinical parameters. The written informed consent was obtained from all the patients included in the study. If patients wanted to reconsider their participation in the trial, they were excluded on the day of admission.

Inclusion criteria implied symptomatic cholecystolithiasis (confirmed by ultrasonography), age of 18 or older at recruitment, ASA (American Society of Anesthesiologists) score of 1 or 2, no known relevant allergies, and signed informed consent letter. Exclusion criteria were less than 18 years, choledocholithiasis (presented with icterus, alcoholic feces, and/or bilirubin level of twice the upper limit of normal), cholangitis, known pregnancy, moderate to severe systemic disease (ASA score of 3 or higher), known cirrhosis of the liver, history of abdominal malignant neoplasm, previous upper abdominal surgery (precluding laparoscopic approach) and psychiatric disease that might make follow-up or completion of questionnaires unreliable.

Assuming no differences in primary outcome measures, sample size calculation was based on differences of costs [6, 14, 15]. For this purpose, the direct costs of the first 30 patients in the trial were calculated to estimate a likely range of differences in costs and their standard deviations. On this basis, we estimated that 120 patients divided in 2 groups would be enough to detect a difference of 10% in direct costs using an α of 0.05 and a β of 0.9.

All the patients were operated under general anesthesia and passed the standard preoperative preparation. In the patients operated on traditionally the length of skin incision was 13 cm, approaching through the right upper quadrant with the standard operative technique.

Biohumoral parameters as a measuring response to trauma were determined from urine and blood samples in a biochemical laboratory in Berane. The levels of adrenaline, noradrenaline, dopamine, metabolites of corticosteroid hormones (17-hydroxy and 17-keto steroids) were determined on the postoperative day 1 in the 24-hour urine by HPLC method. In the postoperative morning 1 and 2, blood samples were taken from every patient to determine C-reactive protein (CRP) and albumin. C-reactive protein was defined by enzyme immunoassay technique. Albumins were determined according to the electrophoresis method on paper. Metabolic and tissue parameters of response to trauma, such as: glycemia, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), red blood cells (RBC) sedimentation and serum concentration of potassium, were determined from the same blood samples.

The obtained values were statistically evaluated using descriptive and analytical statistics (program SPSS version 10 for Windows).

Student's *t*-test, linear correlation, analysis of variance (ANOVA), Mann-Whitney test, *U*-test and logistic regression were used.

Results

In both groups 2/3 of all the patients were females with no statistically significant difference in gender structures among the groups. The average age of the patients in the experimental group was 56 (SD = 9.95) years and in the control group 61 (SD = 8.65) years. The patients in the control group were statistically significantly older than those in experimental one ($p < 0.05$). The average body weight of patients who were traditionally operated on was 73 (SD = 7.91) kg, and of those in the experimental group 69 (SD = 8.06) kg, showing no statistical difference between the groups ($p > 0.05$). There was no significant difference in the operative procedure duration ($p = 0.074$), being 60.7 (SD = 8.25) minutes and 61.68 (SD = 7.31) minutes in the experimental and the control group, respectively. The duration of hospital treatment of patients who were laparoscopically operated was significantly shorter ($p \leq 0.01$), being 3.1 ± 0.9 days comparing with 5.4 ± 1.2 days in the control group.

The average postoperative value of adrenaline in the experimental group was 45.5 nmoL/24h urine, with minimal value of 16.0 and maximum of 66.0 nmol/24h urine. The controls had the average value of adrenaline 167.0 nmol/24h urine, showing statistically highly significant difference ($p < 0.01$). The values of noradrenaline in the experimental group were balancing from minimal value of 150.0 to a maximal one of 640.0 nmol/24h urine, while the values of noradrenalin in the control group were significantly higher (438.9; SD = 146.48 nmol/24h urine; $p < 0.05$) (Figure 1). In the experimental group average value of dopamine was 1,519.4 nmol/24h urine, while the average value in the controls was 2,286.7 nmol/24h urine being statistically significantly higher ($p < 0.01$). The patients from the experimental group have had average value of 17-OH steroids of 83.9 (SD = 20.25) nmol/24h urine, while the average value in the controls was 117.3 (SD = 15.36) nmol/24h urine also showing significant statistical difference ($p < 0.01$) (Figure 1). The average values for 17-keto steroids in the experimental group (169; SD = 45.61 nmol/24h urine) and the controls (284; SD = 42.14 nmol/24h urine) were also statistically highly different ($p < 0.01$) (Figure 1).

The average serum glucose value was also significantly different ($p < 0.01$) being 4.4 (SD = 0.77) mmol/L in the experimental group *vs* 6.6 (SD = 0.73) mmol/L in the control group. Difference between average value of serum CPK in experimental group and controls was highly statistically significant ($p < 0.01$), like the difference between average values of LDH ($p < 0.01$) (Figure 2). The average value of serum potassium in the experimental group was 4.27 (SD = 0.64) nmol/L. The average value serum of potassium in the control group was 4.45 (SD = 0.41) nmol/L and there was no statistically significant difference ($p > 0.05$) between groups. The average value of red blood cells sedimentation in the experimental group was 16.57 (SD = 3.98), while in the control group was 18.97 (SD = 3.24). Comparing the values between the groups a highly statistically significant difference ($p < 0.01$) was proven. There was no significant difference in preoperative CRP ($p > 0.05$) levels. Nevertheless, in both groups significant CRP increase was postoperatively recorded ($p < 0.05$). Preoperative albumin levels showed no significant difference between the groups ($p > 0.05$). Postoperative values in both groups were significantly lower ($p < 0.05$).

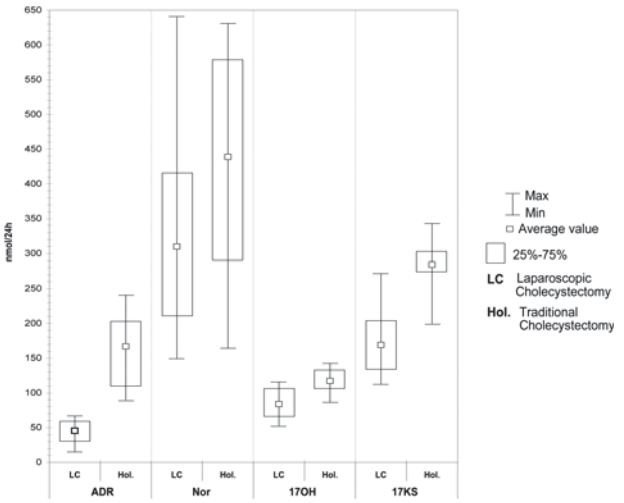

Fig. 1 – Values of adrenaline, noradrenaline, 17-OH and 17-keto steroids in 24-hour urine after cholecystectomy

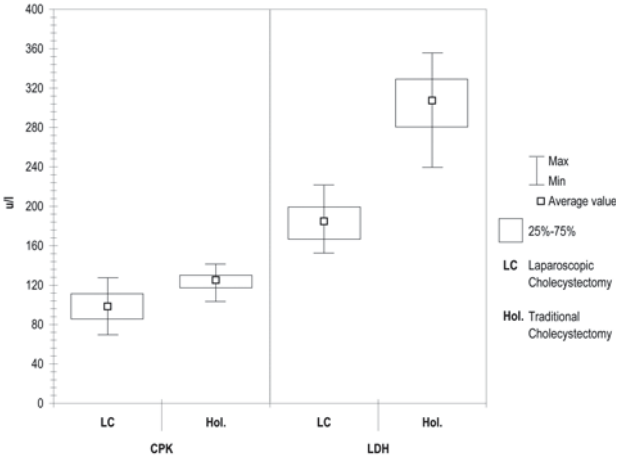

Fig. 2 – Values of creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) in the serum after cholecystectomy

The postoperative course was without complications in all patients. There were no registered intraoperative or postoperative mortality.

Discussion

Surgical trauma entails a complex answer by the organism including releasing of metabolic, tissue, endocrine, and immunologic substances. Tracking these processes we can track the organism answer to trauma as well. The intensity of organism reaction to surgical trauma is proportional to

the trauma intensity. Quantification of some of endocrine, immunologic, tissue, and metabolic parameters was found to correlate with the intensity of surgical trauma itself [16–19].

More specific factors are tissue and immunological factors, which are produced in the initial phase of organism reaction to trauma. The liver synthesizes proteins in increased concentration, *ie* reactants. The reactant group consists among others of haptoglobin and fibrinogen as normal constituents of plasma. The values of albumin, C-reactive protein, α2 acid glycoprotein, transferin and macroglobulin are changing. Tissue mediators (cytokines) including interleukins (IL) IL-1, IL-2, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF) are created by phagocytes and histiocytes [20, 21]. These mediators increase the production of reactant group proteins but also of prostaglandins, which belong to the group of humoral messengers, participating in catabolic processes in the organism. Recently, focus is on thelial cells (oxygen radicals, NO) but also on cellular and humoral answer to trauma, including values of immunoglobulins and T lymphocytes. Analyses show different dynamics of their plasma concentrations after the operation. Tracking these changes can show us how organism reacts to surgical trauma.

Gender structure of the studied patients was expected, considering the prevalence of gall-bladder diseases, recognized to be more frequent in female population. The patients had average weight value above that of general population, being important factor in the etiology of calculous gallbladder [22, 23].

Laparoscopic surgery demands more time, usually leading to longer procedure duration, but after procedure hospitalization is shorter [24–26], as proved in this study as well.

Surgical intervention induces endocrine response of the organism *via* sympathetic nervous system promoting the secretion of adrenalin and noradrenalin. It also stimulates excretion of ACTH and growth hormone *via* hypothalamus [27]. Increase of catecholamine levels in postoperative period verified in this study is expected as previously proven in other studies. It is also interesting that adrenaline values in laparoscopic cholecystectomy are significantly higher in patients who are operated by mini-laparotomy cholecystectomy [1–3]. This difference is explained by the effects of pneumoperitoneum, gastric distension and parietal peritoneum dilatation which lead to stimulating of stress reaction in organism. The phenomenon of stomach gas pillow in abdomen causes compression of portal system and the system of the lower hollow vein leading to lung hypoperfusion, and a disorder of ventilation/perfusion ratio in alveoli by diaphragm uplifting. Absorbed CO_2 also has system effects on the center for respiration and induces respiratory acidosis causing high production of catecholamine as the answer to trauma [13]. It is known that noradrenaline metabolism is different than adrenaline metabolism. Noradrenaline values grow very slowly, but they stay on higher levels for a long period of time [1–3, 27, 28]. A wide range of adrenaline and noradrenaline values detected in both the examined group and the controls can be explained by individual differences in stress reaction. From the other hand, the average noradrenaline level being higher in the controls tells us that stress provoked by procedure is much higher when it is done by traditional way.

Steroid response to trauma can also be objectified by average values of 17-keto steroids and 17-OH steroids in 24-hour urine. Steroid answer of high intensity is a reflection of total stimulus and it represents more reliable and objective parameter than it is the case with catecholamine. The results obtained in this study showing a significant difference between the steroid response to trauma in open and laparoscopic cholecystectomy are going in favor of laparoscopic surgery as a less stress-inducing method.

Although parameters of metabolic and tissue answer do not have high significance as factors of endocrine answer, their tracking in this study gave us a number of statistical importance which is fully in line with the results from previous researches. Hyperglycemia is a phenomenon of surgical trauma. In 1877, Claude Bernard described hyperglycemic reaction in patient who experienced hemorrhagic crisis. It has been proven that every trauma, high infection and sepsis is followed by a hyperglycemic reaction, and that the degree of hyperglycemia is proportional to the intensity of trauma [3, 29]. Just after trauma, the organism wants to secure energy resource and that is the main reason for hyperglycemic reaction happen. At early stages of postoperative procedure, there is a changed metabolism of carbohydrates. There is also an increased splanchnic output (emptying of the deposit in circulation) and decreased utilization of glucose at periphery. During trauma, hyperglycemic reaction is initiated by a catecholamines, cortisol, glucagon, vasopressin, growth hormone and somatostatin as well as insulin [1, 2, 29]. Cortisol and catecholamine increase resistance of periphery tissues on effects of insulin (whose secretion is decreased) and therefore decrease acceptance of glucose at periphery. A high level of glycemia detected in both groups of patients is explained by these processes and higher glycemia values in the patients who were traditionally operated on come as a consequence of more extended trauma during the procedure.

When during surgical trauma comes to an increased destruction of muscle cells, there is a higher level of CPK. Muscle cells contain LDH and aminotransferases as well as CPK. When other muscle diseases are absent, *ie* myocardial infarction or liver disease, then the increase of CPK level shows the degree of muscle damage depending on surgical procedure [30, 31]. Higher CPK in traditionally operated on patients comes from longer incision causing bigger destruction of muscle tissue. Lactate dehydrogenase is an intracellular enzyme which catalyzes processes of carbonhydrate metabolism. After surgical trauma, there is a high LDH level. Increase of LDH level is explained by a release of the enzyme from the tissues which were destroyed during the operation, or local trauma which was done on the liver parenchyma (if operations are done on that anatomic area). Higher LDH measured in the controls group is in favor of less extended tissue destruction in laparoscopic approach being one adventage more.

The increase of serum potassium of operated patients were statistically significantly lower in laparoscopic than in

open operation trauma, but we also have to be aware that other metabolic factors can increase the levels of potassium.

Red blood cells sedimentation as an unspecific parameter of the organism answer to trauma is considered important for tracking patients after operation. Minimal traumatizing effect of laparoscopic operative procedure gives statistically significant lower RBC sedimentation values.

Also, there are changes in metabolism and biosynthesis of liver proteins after trauma. C- reactive protein is the main reactant of acute phase answer, and due to its sensitivity and prompt jump of CRP after trauma, it enables us to track intensity of trauma. C-reactive protein have shown increase in both groups but contrary to RBC sedimentation there were no significant differences in its levels between the groups. As expected in postoperative period average albumin level is proportional to the intensity of trauma [1–3].

Cholecystectomy, as one of the most common abdominal operations, has a small morbidity and mortality. Regardless of well defined technique and standardized procedures precaution is necessary. According to previous studies, during elective operations, percentage of complications is 6-12% and, generally speaking, percentage of complications in this research is in line with the indications and age of patients. Highest number of complications is connected to laparotomic incision. They belong to the group of unspecific complications in which dominate: hematoma, fistulas and granuloma around stitched material, infection and difficulties with wound healing and pain [4–6, 11, 32, 33]. In our patients regardless operative procedure used no postoperative complications were observed.

Conclusion

The intensity of the organism response is proportional to the intensity of surgical trauma. Metabolic, tissue and neuroendocrine response of the organism to trauma has much lower intensity after laparoscopic cholecystectomy. Although the advantages of laparoscopic surgery are well-known these results are speaking in favor of minimal invasive surgery in so far most complete way.

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Declaration of interest

Authors have no conflict of interest to declare.

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Work stress related lipid disorders and arterial hypertension in professional drivers – A cross-sectional study

Povezanost stresa na poslu sa lipidnim poremećajima i arterijskom hipertenzijom kod profesionalnih vozača – studija preseka

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Abstract

Background/Aim. Occupational stress is a term used to define ongoing stress that is related to the workplace. The study was conducted to determine association of occupational stress index (OSI) and its aspects with arterial hypertension and lipid disorders using data from a cross-sectional survey of male professional drivers. **Methods.** The cross-sectional study was performed in 439 professional drivers divided into groups (city- and intercity bus drivers, truck and taxi drivers). The OSI and OSI aspects (high demands, strictness, underload, extrinsic time pressure, noxious exposure, avoidance and conflict) were calculated using the standardized questionnaire. Determination of serum lipids, blood pressure (BP) and cardiovascular risk factors were done. **Results.** A significant difference in prevalence of diagnosed hypertension and dyslipidemia was found along with a difference in total OSI and OSI aspects among examined subgroups of drivers. A total OSI was highest in city, high in intercity bus drivers, and the lowest one in truck and taxi drivers (82.79 ± 3.5, 81.28 ± 3.7, 73.75 ± 3.5, 71.61 ± 4.4, respectively; $p < 0.01$). Similar pattern showed triglycerides (TG), total cholesterol (TC) and LDL cholesterol and BP, while HDL-cholesterol showed reverse order ($p < 0.01$). Logistic regression analyses with multiple OSI aspects adjusted for age and years of exposure showed associations of total OSI with arterial hypertension [OR 5.5; 95% CI (2.24–7.95)] and dyslipidemia [OR 1.43 95% CI (1.09–2.80)]. Underload was the most important OSI aspect associated with the arterial hypertension [OR 1.18; 95% CI (1.04–2.58)] and elevated LDL cholesterol [1.26; 95 CI (1.19–2.1)]. A total OSI had a significant association with elevated LDL cholesterol [2.64; 95% CI (1.19–7.7)], triglycerides [OR 3.27; 95% CI (1.20–5.1)] and low HDL cholesterol [OR 3.29; 95% CI (1.8–5.8)] ($p < 0.01$). **Conclusion.** The study provides the evidence for the significant association of total OSI and underload with lipid disorders and elevated blood pressure in professional drivers, which could be a possible link between job stress and coronary heart disease. Regular periodical examinations and workplace interventions aimed to decrease total OSI and underload are important aspects in primary prevention and additional reduction of cardiovascular risk.

Key words:
occupational exposure; stress, physiological; automobile driving; hypertension; dyslipidemias.

Apstrakt

Uvod/Cilj. Profesionalni stres predstavlja termin koji definiše stres koji je povezan sa radnim mestom. Studija preseka sprovedena kod profesionalnih vozača imala je za cilj da analizira povezanost indeksa profesionalnog stresa (OSI) i njegovih determinanti sa pojavom arterijske hipertenzije i lipidnim poremećajima. **Metode.** U studiji je analizirano 439 profesionalnih vozača podeljenih na grupe (vozači gradskih i međugradskih autobusa, kamiona i vozači taksija). Ukupan OSI i OSI determinante (visoki zahtevi, strogost, podopterećenje, vremenski pritisak, izloženost noksama, izloženost opasnostima i konfliktnost) određeni su pomoću standardizovanog upitnika. Određivanje serumskih lipida, krvnog pritiska i kardiovaskularnih faktora rizika izvršeno je prema protokolu istraživanja. **Rezultati.** Registrovana je značajna razlika u zastupljenosti hipertenzije, dislipidemije i vrednostima ukupnog OSI i njegovih determinanti u ispitivanim grupama vozača. Ukupan OSI bio je najveći kod vozača gradskih, visok kod međugradskih, manji kod vozača kamiona i najmanji kod vozača taksija (82,79 ± 3,5, 81,28 ± 3,7, 73,75 ± 3,5, 71,61 ± 4,4, redom; $p < 0,01$). Sličan nalaz postoji za trigliceride (TG), ukupni holesterol (TC) i LDL holesterol i krvni pritisak, dok je za HDL holesterol ova relacija bila obrnuta ($p < 0,01$). Logistička regresiona analiza sa multiplim OSI determinantama korigovana za starost i ek-

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spozicioni staž pokazala je povezanost ukupnog OSI sa hipertenzijom [OR 5,5; 95% CI (2,24–7,95)] i dislipidemijom [OR 1.43; 95% CI (1,09–2,80)]. Podopterećenje je najvažnija determinanta OSI udružena sa hipertenzijom [OR 1,18; 95% CI (1,04–2,58)] i povišenim LDL holesterolom [OR 1,26; 95% CI (1,19–2,1)]. Ukupni OSI pokazuje značajnu povezanost sa povišenim LDL holestrolom [OR 2,64; 95% CI (1,19–7.7)], hipertrigliceridemijom [OR 3,27; 95% CI (1,20–5,1)] i niskim HDL holesterolom [OR 3,29; 95% CI (1,8–5,8)] ($p < 0,01$). **Zaključak.** Studijom je ustanovljena značajna povezanost vrednosti ukupnog OSI i podopterećenja sa lipidnim poremećajima i povišenim krvnim pritiskom kod profesionalnih vozača, što može predstavljati vezu između profesionalnog stresa i koronarne bolesti srca. Redovno sprovođenje periodičnih pregleda i mera za redukciju ukupnog profresionalnog stresa i podopterećenja predstavlja značajan aspekt u primarnoj prevenciji koronarne bolesti i redukciji kardiovaskualrnog rizika.

Ključne reči:
profesionalna izloženost; stres, fiziološki; vozači automobila; hipertenzija; hiperlipidemija.

Introduction

Occupational stress is a term used to define ongoing stress that is related to the workplace. The stress may have to do with the responsibilities associated with the work itself, or be caused by conditions that are based on the corporate culture or personality conflicts. As with other forms of tension, occupational stress can affect both physical and emotional well being if not managed effectively.

Cardiovascular disease (CVD), and hypertension as one of its major components, is a major cause of morbidity and mortality in modern society [1]. The identification and modification of risk factors associated with cardiovascular disease is the main preventive approach. Work stress is currently not included in the American Heart Association list of established risk factors for CHD, but individual response to stress is acknowledged as a potential contributing factor [2]. The role of occupational stress in the etiology of CVD has recently received considerable attention. Occupation is a major socioeconomic factor that together with a prolonged exposure to stress at workplace may directly affect the autonomic nervous system and neuroendocrine activity contributing to the development of hypertension, lipid disorders and increased incidence of diabetes mellitus [3]. Stress at work has been linked with an increased risk of hypertension and coronary heart disease in retrospective and prospective studies [4, 5].

Although cross-sectional studies have linked work stress with lipid disorders, this association is not consistent [6, 7]. In line with this, mixed results for associations between occupational stressors and blood pressure are found in studies with casual blood pressure measurement [8].

Inconsistency of literature data related to occupational stress and prevalence of cardiovascular diseases implicated development of new tools for occupational stress assessment. Because occupational demands, threats, and conflicts – the most frequent stressors – cannot be identified by direct physical or biological measurement, theoretical concepts, and integrative models have been developed to delineate stressful job characteristics [9].

The occupational stress index *(*OSI) incorporates key aspects of the leading sociological work stress models: Job-Strain and Effort-Reward Imbalance that were developed from the perspective of cognitive ergonomics. Within the OSI the work environment is viewed as a whole, including task level issues, work schedule, physical, chemical and broader organizational factors, which all contribute to total burden [10]. The applications of specific OSI in subsets of professional drivers could be appropriate model for revealing connection between job stress and cardiovascular risk factors.

The study was conducted to determine the association of occupational stress index and its aspects with arterial hypertension and lipid disorders using data from a cross-sectional survey of male professional drivers.

Methods

The prospective cross sectional study was performed during 2008–2010 on a group of 439 working middle-aged male professional drivers (aged 35–60 years) who were divided into four occupational groups (94 city bus drivers, 100 intercity bus drivers, 123 truck drivers and 122 professional taxi drivers). Representativity of the sample size was calculated using data that Niš region worker-based populations is about 30,000. Minimal sample size of 439 ensures confidence level of 95% with 4.6% margin of error. Total number of 460 workers (200 bus drivers, 130 truck drivers and 130 taxi drivers) were randomly selected and invited to participate in the study. Recruitments were done during systematic preventive examinations which are standard work-related procedures organized in specialized dispensaries within the Institute of Occupational Health in Niš. The study was comprised of a standardized questionnaire about working conditions and occupational stressors as well as medical examination and medical record analysis.

The workers included in the study were permanently employed and spent more than 3 years in the current occupation. The workers with acute or chronic inflammatory diseases, immune and major systemic diseases, structural non-ischemic heart disease, history of myocardial infarction, myocardial revascularization procedures, cerebrovascular diseases, secondary hypertension, kidney, liver or other important chronic diseases, were excluded from the study.

The overall participation rate was 95.4% and according to occupational groups there were 97% for bus drivers, 94.6% for truck drivers and 93.8% for taxi drivers. A total of 21 males did not finish examination (16 refused to participate, 3 did not signed informed consent, 2 did not return the questionnaire or did so with incomplete responses, and were excluded).

The research was conducted during working days where the ambulatory blood pressure readings and blood samplings were done.

The occupational stress index

The occupational stress index was calculated using the standardized questionnaire authorized by Belkić [11, 12]. The specific OSI questionnaire for professional drivers was used (Table 1). The questionnaire was anonymously self-rated by participants. If there were inconsistency of the data in questionnaire or weak compliance of participant to help in filling missed data the questionnaire was rejected.

The OSI model is arranged as a 2-dimensional matrix: levels of information transmission and the stressor aspects. The elements are summed into the OSI aspects that are then summed into the total OSI score, reflecting the overall burden from work stressors" [13]. The elements are equally weighted, scored from 0 to 2 (maximum), from absence to strongly present, with higher scores meaning higher level of burden. The elements are summed to yield aspects. The elements were summed by addition with equal weighting. In this study, information was accessible from "medical charts", worksite measurements and expert observations about night-shift work, number of work hours, vacation time, moonlighting, and exposure to physical and chemical toxins.

The OSI questionnaire was used in a cross sectional prevalence study design.

Table 1

The occupational stress index (Version 2003) [11, 12]

Aspects	Information Transmission Level			
	Input	Central decision making	Output/ Task performance	General
Underload	♦ Homogenous signals ♦ Low frequency of incoming signals communication ♦ Works alone-without a need for communication	♦ Decisions automatic from input	♦ Homogenous tasks ♦ Simple Tasks ♦ Nothing to do	♦ Fixed pay ♦ Inadequate pay ♦ No chances for upgrade ♦ Lack of recognition for work
High demand	♦ Several info. sources ♦ Heterogeneous information ♦ Heavy burden on visual system ♦ High frequency of incoming signals ♦ 3 sensory modalities ♦ Communication essentials	♦ Complex decisions ♦ Complicated decisions ♦ Decisions affect work of others ♦ Rapid decision-making	♦ Heterogeneous tasks ♦ Simultaneous task performance ♦ Complex tasks ♦ Rapid task performance	♦ Piece rate work ♦ Long work hours ♦ Holds 2+ jobs ♦ Lack of rest breaks ♦ Night shift/irregular work hours ♦ Lack of paid vacations
Strictness	♦ Strict requirements for signal detection	♦ Strict problem-solving strategy ♦ Strictly defined correct decision	♦ Work must meet a strictly defined standard	♦ Fixed body position ♦ Confined, windowless workspace ♦ Lack of autonomous workspace ♦ Limited in talking time off from work ♦ Low influence over: Schedule; Tasks; Policy; With whom one works
Extrinsic time pressure	♦ No control over speed of incoming signals	♦ Decisions cannot be postponed	♦ No control over rate of task performance	♦ Deadline pressure ♦ Speed-up
Aversiveness / Noxious exposures	♦ Glare ♦ Noise		•Isometric lifting •Vibration	♦ Heat ♦ Cold ♦ Noxious gases, fumes, dust
Avoidance / Symbolic aversiveness	♦ High level of attention (serious consequences of momentary lapse) ♦ Visually-disturbing scenes ♦ Exposed to emotionally disturbing occurrences	♦ Serious consequences of a wrong decision	♦ Hazardous task performance	♦ Work Accident ♦ Witnessed work accident ♦ Suicide occurrence ♦ Work-related litigation/Testifying in court ♦ Lack of emergency system functioning
Conflict / Uncertainty	♦ Signal/noise conflict ♦ Signal/signal conflict	♦ Missing information needed for decision ♦ Contradictory information ♦ Unexpected events ♦ Change of work plan	♦ Conflicting demands ♦ Task performance hampered by: Extrinsic problems ♦ Interruptions from people	♦ Emotionally-charged work atmosphere ♦ Lack of help with work-related difficulties ♦ Opposition to career advancement ♦ Violations of behavior norms/abuses of power ♦ No redress of grievance ♦ Threat of job loss ♦ Job lacks coherence

The use of this questionnaire requires a permission of the author, Dr. Karen Belkic (2003). It is available at http://www.workhealth.org/OSI/Index/Driver/OSI/Index.html

Determination of serum lipids and glycoregulation

Overnight fasting venous blood sample was taken between 8.00 and 9.00 am. After the serum was separated a total amount of cholesterol (TC), triglycerides (TG), high density hipoprotein (HDL) cholesterol and low density hipoprotein (LDL) cholesterol was determined. A total amount of cholesterol and TG was determined enzymatically by Bayer color test, on Technicon-Axon Bayer analyzer. HDL and LDL cholesterol were determined by direct enzyme colori-

metric essay without precipitation by Dade Behring reagens, on a Dimension Expand Dade Behring analyzer.

For this study, the cutoff point for hypertriglyceridemia were TG ≥ 1.7 mmol/L, HDL cholesterol was as low as < 1.04 mmol/L in men or <1.3 mmol/L in women, and the cutoff point for hypercholesterolemia was TC ≥ 5.18 mmol/L and high LDL cholesterol as ≥ 3.37 mmol/L. The workers with history of specific treatment for lipid abnormality or one or more abnormal serum lipid concentrations were classified as dyslipidemic [14].

Blood pressure measurement and hypertension

Upon arrival subjects remained in resting (sitting) position for 5 min before starting measurement. Blood pressure was measured in the left upper arm by auscultation (sphygmomanometer and stethoscope, Becton Dickinson, USA) three times in accordance with the American Heart Association procedure and proposed average values [15]. Hypertension was defined according to WHO criteria: systolic blood pressure greater/equal 140 mmHg and/or diastolic blood pressure greater/equal 90 mmHg. Individuals were also considered hypertensive if they were currently taking antihypertensive therapy [16].

Consent and Data Security

All participants in this study were informed about the purpose and benefits of the project, research methods, potential risks or hazards of participation as well as the right to ask additional information at any time during the research procedure. They were further informed that their choice to participate was on voluntary basis, and that they were free to withdraw from the research project at any time. All phases, testing, and reports of the study were approved by the Institute for Occupational Safety and Health Internal Review Board. Written informed consent was obtained from all participants, and confidentiality was guaranteed for all participants.

Characteristics of the subjects are presented as mean ± SD. Differences between continuous and categorical variables were tested by ANOVA and χ^2-test, respectively. Post hoc multiple comparasion was done by the Tukey's test. Binary logistic regression was used to assess the association of the eight control measures (total OSI and seven OSI aspects) with arterial hypertension and distinctive lipid abnormalities, after controlling for the influence of age and years of exposure. All variables were entered together and enter logistic model were used. Presented data were obtained after the adjustment of odds ratio (OR) with the 95% confidence interval (CI 95%) and corresponding *p*-values. A *p*-value of < 0.05 was considered to be statistically significant. The multicollinearity of variables in logistic regression models is controlled by the examinees Tolerance and Variance Inflation Factor (VIF) for each variable. The value of VIF fewer than 10 were considered as acceptable. SPSS 16.0 software was used for statistical analyses.

Results

The basic characteristics of the examined groups of professional drivers are shown in Table 2. The examined groups were of the similar prevalence in smoking habit and positive family history of any types of atherosclerotic diseases. There were a significant difference in average age, years of service and professional exposition, prevalence of diagnosed hypertension and dyslipidemia. There was also a significant difference in OSI determinants as well as total OSI between occupational groups (ANOVA 218.4, $p < 0.001$) (Table 2).

Table 2

Baseline characteristics according to the worker group

Drivers	City bus driver	Intercity bus driver	Truck driver	Taxi driver	*p* – value by ANOVA or χ^2 test
Number	94	100	123	122	
Age (years), $\bar{x}$ ± SD	50.27 ± 10.1	47.63 ± 9.8	47.7 ± 8.8	42.98 ± 10.5	< 0.05
Years of service, $\bar{x}$ ± SD	26.11 ± 9.4	23.62 ± 9.2	14.08 ± 8.1	18.64 ± 10.0	< 0.001
Years of exposition, $\bar{x}$ ± SD	25.9 ± 9.2	23.2 ± 9.1	13.92 ± 8.1	18.35 ± 10.1	< 0.001
Smoker, n / %	34 / 36.1	47 / 47.0	58 / 47.1	64 / 52.4	NS
Family history of atherosclerotic disease, n / %	60 / 63.8	53 / 53.0	61 / 49.6	56 / 45.9	NS
HTA, n / %	93 / 98.9	94 / 94.0	68 /55.3	26 /21.3	< 0.001
Dyslipidemia, n / %	91 / 96.8	95 / 95.0	99 / 80.4	62 / 50.8	< 0.001
Occupational index determinants, $\bar{x}$ ± SD					
high demand	17.52 ± 1.5	23.38 ± 2.6	11.55 ± 0.9	17.89 ± 2.7	< 0.001
strictness	11.4 ± 1.0	12.69 ± 2.8	9.98 ± 0.5	16.45 ± 1.6	< 0.001
conflict / uncertainty	18.47 ± 1.1	15.93 ± 1.3	11.78 ± 1.9	15.71 ± 1.6	< 0.001
underload	11.87 ± 1.5	7.96 ± 1.3	15.11 ± 2.0	3.11 ± 0.8	< 0.001
avoidance / symbolic aversiveness	9.48 ± 1.0	10.31 ± 0.8	8.92 ± 1.3	7.83 ± 1.5	< 0.001
extrinsic time pressure	6.99 ± 0.8	6.04 ± 0.8	5.27 ± 0.4	6.55 ± 1.1	< 0.001
aversiveness / noxious exposures	7.0 ± 0.8	4.57 ± 0.5	11.15 ± 0.8	4.47 ± 0.5	< 0.001
OSI total, $\bar{x}$ ± SD	82.79 ± 3.5	81.28 ± 3.7	73.75 ± 3.5	71.61 ± 4.4	< 0.001

HTA – arterial hypertension, OSI – occupational stress index.

Figures 1–4 show a boxplot presentation of serum lipids concentration. All the examined lipid parameters showed a significant difference among the examined groups of professional drivers. The value of serum lipids were the highest in bus drivers and the lowest in taxi drivers (Figures 1–4).

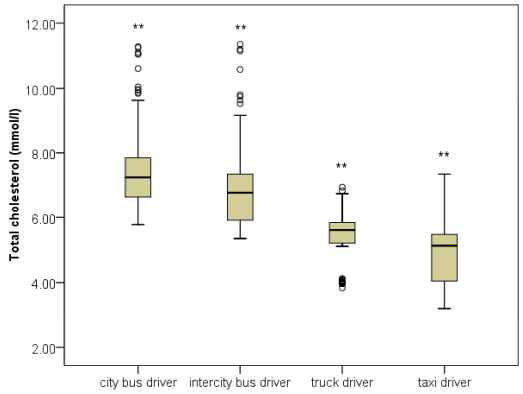

Fig. 1 – Total cholesterol concentration in the examined groups

ANOVA F = 145.9, *Post hoc* **$p < 0.01$ *vs* other groups; a box represents the interquartile range (IQR 25–75. percentiles) computed from Tukey's-hinges, central line represents median, values between 1.5–3 IQR's from the end of the box are labeled as outliers (o).

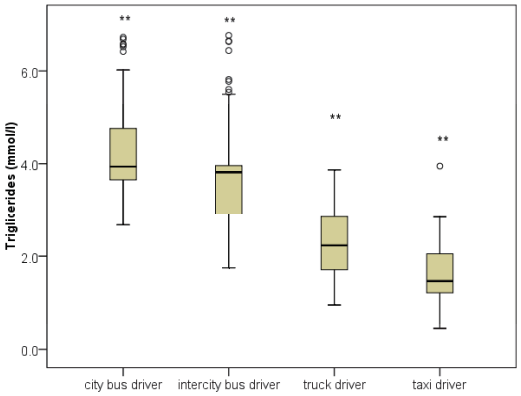

Fig. 2 – Triglyceride concentration in the examined groups

ANOVA F = 202.9,$p < 0.01$ *vs* other groups; a box represents the interquartile range (IQR 25–75. percentiles) computed from Tukey's hinges, central line represents median, values between 1.5–3 IQR's from the end of the box are labeled as outliers (o).**

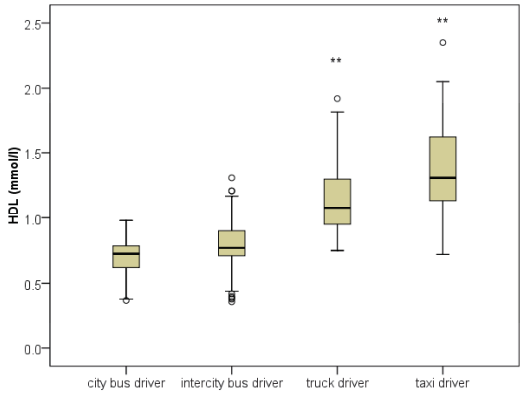

Fig. 3 – HDL cholesterol concentration in the examined groups

ANOVA F = 167.1,$p < 0.01$ *vs* other; a box represents the interquartile range (IQR 25–75. percentiles) computed from Tukey's hinges, central line represents median, values between 1.5–3 IQR's from the end of the box are labeled as outliers (o).**

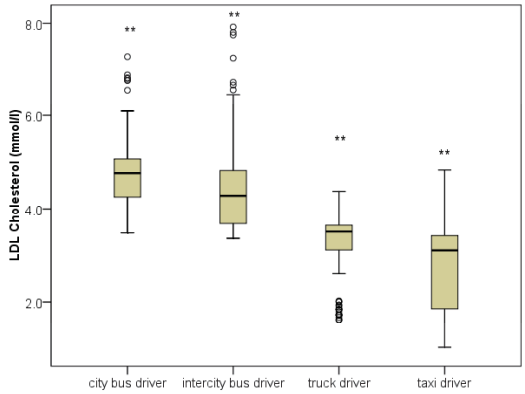

Fig. 4 – LDL cholesterol concentration in the examined groups

ANOVA F = 134.5,$p < 0.01$ *vs* other groups; a box represents the interquartile range (IQR 25–75. percentiles) computed from Tukey's hinges, central line represents median, values between 1.5–3 IQR's from the end of the box are labeled as outliers (o).**

The values of systolic and diastolic BP significantly varied among the examined professional drivers groups. They were significantly higher in city and intercity bus drivers compared to truck and taxi drivers (Figure 5).

The results in Tables 3 and 4 are based on binary logistic regression analyses with multiple OSI aspects entered together adjusted for age and years of exposure. Underload was the most important OSI aspect associated with arterial hypertension (OR 1.18). There were also significant associations between total OSI with arterial hypertension (OR 5.59). The logistic analysis did not show a signifficant association of OSI aspects and total OSI with dyslipidemia after adjusting for age and years of exposure (Table 3).

Table 3

Associations of occupational stress index (OSI) aspects and a total OSI with arterial hypertension and dyslipidemia in professional drivers

Occupational stress index aspects	Arterial hypertension	Dyslipidemia of any type
	Odds ratios (95% CI)	Odds ratios (95% CI)
High demand	0.62 (0.27–1.41)	0.67 (0.01–1.9)
Strictness	0.81 (0.38–1.69)	0.78 (0.01–1.3)
Conflict / Uncertainty	0.91 (0.38–2.14)	0.74 (0.01–1.9)
Under-load	1.18 (1.04–2.58)**	1.01 (0.02–1.8)
Avoidance / Symbolic aversiveness	1.16 (0.43–3.09)	0.84 (0.01–1.1)
Extrinsic time pressure	1.91 (0.60–6.0)	1.20 (0.02–2.4)
Aversiveness / Noxious exposures	0.44 (0.17–1.12)	0.55 (0.01–1.6)
OSI total	5.598 (2.24–7.95)**	1.43 (1.09–2.80)*

Dependent variables: arterial hypertension, binary logistic regression, enter models adjusted for age and years of exposure, CI – confidence intervals; *$p < 0.05$, **$p < 0.01$

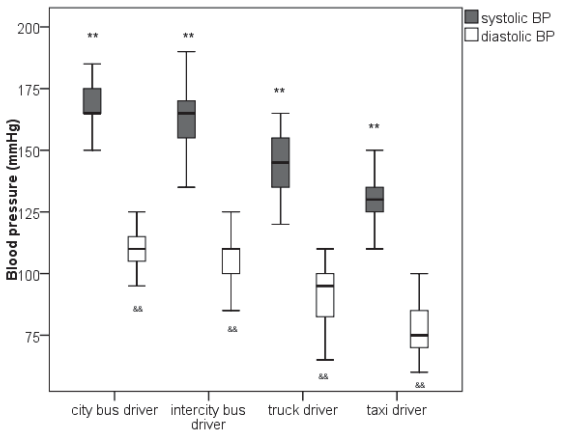

Fig. 5 – Systolic and diastolic blood pressure (BP) values in the examined groups

ANOVA F = 266.3 for systolic BP and ANOVA F = 236.1 for diastolic BP,$p < 0.01$ *vs* other systolic values; $p < 0.01$ *vs* other diastolic values; a box represents the interquartile range (IQR 25-75. percentiles) computed from Tukey's hinges, central line represents median, values between 1.5–3 IQR's from the end of the box are labeled as outliers (o).**

In all the participants including those on lipid-lowering medications underload showed a significant association with elevated concentration of LDL cholesterol, while other occupational stress index aspects did not show any significant association. A total OSI has significant positive association with hypertriglyceridemia, elevated LDL cholesterol and low HDL cholesterol concentration (Table 4).

sional drivers. This could be in part explained by a difference in average age and duration of exposure at current job but also in a significant difference in evaluated OSI aspects and total OSI (Table 2).

According to the Serbian National Registry of Cardiovascular Diseases in 2007 the family history for CAD was presented in 53.8%, smoking 43.9%, dyslipidemia 47.3% and hypertension in 70.8% of population in the district of Niš [17]. Comparing the obtained values we can assume relatively similar prevalence in this study population, but also very unbalanced prevalence among different drivers groups (Table 2). To test the hypothesis that occupational stress has impact on development of arterial hypertension and lipid abnormalities we determined serum lipid concentrations and blood pressure. Taking into account that age and greater lifetime exposure to stressful work environment (for example, job strain) have synergistic effects on cardiovascular diseases [18] we made logisitic regression analysis adjusted for age and exposure.

According to the stress-disequilibrium theory, job stress and particularly low workplace control could lead to chronic disease risk through deregulation processes occurring at several linked levels of cardiac and endocrine system mechanisms [19]. Several plausible mechanisms have been suggested through which long-term work stress may have an impact on the risk of CHD. They include prolonged overactivation and dysregulation of the autonomic nervous system and the hypothalamuspituitary-adrenal (HPA) cortex axis. The COR-

Table 4

Association of occupational stress index aspects and a total occupational stress index (OSI) with lipid disorders

	Low HDL-L	High TC	High TG	High LDL-C
Occupational stress index aspects	Odds ratios (95% CI)	Odds ratios (95% CI)	Odds ratios (95% CI)	Odds ratios (95% CI)
High demand	0.76 (0.44–1.32)	0.63 (0.03–11.5)	0.77 (0.15–3.87)	0.89 (0.26–2.95)
Strictness	0.78 (0.52–1.18)	0.83 (0.04–10.8)	0.84 (0.17–4.08)	0.76 (0.23–2.46)
Conflict / Uncertainty	0.90 (0.50–1.59)	0.61 (0.03–11.0)	0.78 (0.15–3.97)	1.24 (0.37–4.16)
Underl-load	1.10 (0.67–1.79)	1.09 (0.06–2.96)	0.98 (0.2014.85)	1.26 (1.19–2.1)*
Avoidance / Symbolic aversiveness	0.92 (0.46–1.84)	0.85 (0.04–5.6)	0.97 (0.19–4.95)	1.02 (0.29–3.53)
Extrinsic time pressure	1.05 (0.40–2.75)	1.01 (0.05–9.1)	1.12 (0.20–5.06)	1.58 (0.43–5.78)
Aversiveness / Noxious exposures	0.63 (0.33–1.22)	0.41 (0.02–7.8)	0.70 (0.13–3.7)	0.61 (0.17–2.14)
OSI total	3.29 (1.8–5.8)**	4.48 (0.24–12.8)	3.27 (1.20–5.1)**	2.64 (1.19–7.7)**

TC – total cholesterol; TG – triglycerides; HDL – high density hipoprotein; LDL – low density hipoprotein; *$p < 0.05$; **$p < 0.01$; Binary logistic regression models, Odds ratios adjusted for age and years of exposure; CI – confidence intervals.

Discussion

In this cross-sectional study on occupational stressors, hypertension and lipid disorders in professional drivers, the intensity of occupational stressors and their association with serum lipids and blood pressure were examined after controlling for age and years of exposure at current job.

A high prevalence of the examined cardiovascular risk factors (family history of atherosclerotic disease, smoking, presence of arterial hypertension and dyslipidemia) was found in professional drivers. Some of these risk factors (arterial hypertension and dyslipidemia) showed a significant difference in prevalence among different groups of profes-

DIS study [20] demonstrated that some of the pathways linking psychosocial factors (job stress) and cardiovascular disease incidence are: elevation of physiological/hematochemical variables (e.g., blood pressure and serum lipid lipoprotein levels); direct and indirect effects of adverse risk behaviors such as smoking, lack of physical exercise, and poor diet and health care habits; and heightened emotional states, such as anger, tension, and anxiety, implicated in cardiovascular disease development through neuroendocrine mediation [21].

The nature of work has changed over the past two decades, moving towards a 24-hour society with increasing demand for goods and services to be provided around the clock. Consequently, workplace exposures, such as psycho-

social stress, shift-work and long working hours, have been reported to be associated with increased risk of cardiovascular disease (CVD). Chronic stimulation of the HPA axis by depression frequently results in hypercortisolemia, blunted HPA activity, and diminished feedback control, as evidenced by nonsuppression of cortisol secretion following dexamethasone suppression. When present, hypercortisolemia is associated with suppression of growth and sex hormones [22].

Altered adrenocortical function and increased cortisol output can influence hepatic lipoprotein metabolism and insulin sensitivity in target organs. Low concentrations of HDL cholesterol, increased LDL cholesterol and triglycerides have been linked with high basal secretion of cortisol [23]. In the presented study, professional drivers with the highest OSI score have significantly higher serum lipids concentrations (TC, LDL cholesterol and TG) and lower HDL cholesterol than drivers with lower score (Figures 1–4). Binary logisitc regression adjusted for age and duration of job exposure showed that a total OSI has a significant association with dyslipidemia of any type (OR 1.43) (Table 3) and with specific lipid abnormalities such as low HDL cholesterol, high LDL cholesterol and hipertriglyceridemia in the examined professional drivers (Table 4). Underload was the most important OSI aspects associated with specific lipid abnormalities in professional drivers. In all the participants including those on lipid-lowering medications underload showed a significant positive association with elevated LDL cholesterol (Table 4). The obtained results indicate importance of a total OSI and some of its aspects especially underload score, for the development of cardiovascular disease in professional drivers, through lipid abnormalities and hypertension connected with the endothelia function and inflammation [24].

It is unclear whether the development of risk seen in some trials is due in part to the direct effects of chronic stress on insulin resistance, resting blood pressure, and lipoprotein metabolism, or the effects of some specific aspects of occupational stress which is supported by Whitehall II and other studies [25]. Work-related risk factors include both physical and psychosocial elements. It is showed that individuals who suffered more "job strain," i.e. those who were under pressure to work hard or quickly but who had less control over that pressure, had a greater risk of cardiovascular disease. The results based on the job strain model which we used in this study showed that high demands and low control were associated with elevated blood pressure and cardiovascular diseases. Interventions that increase decision-making latitude or diminished psychological demands (e.g., by reducing time pressure) resulted in favorable changes in mediators relevant to the cardiovascular system, such as blood pressure and lipid profile [26].

Binary logistic regression analyses with multiple OSI aspects entered together adjusted for age and years of exposure showed a significant importance of a total OSI for the development of elevated blood pressure. A few-fold increased association of arterial hypertension with total OSI was seen (OR 5.594). Underload (homogenous and simple job tasks, working alone without any communication, automatic decision from input, inadequate pay and no promotion prospects) was the most important OSI aspect associated with the increased blood pressure and hypertension (OR 1.18). Other OSI aspects did not show a significant association with elevated BP (Table 3).

A large body of evidence supports the link between job strain and cardiovascular disease. Ambulatory blood pressure levels have been shown to increase in work situations with high job strain, either directly or through behavior modification and highly contribute to the development of cardiovascular disease [26, 27]. Similar study on occupational stressors and hypertension in transit operators indicate to significant effects of working stressors on hypertension [8].

Strengths and weaknesses of the study

Strengths of the study are reflected in careful assembly of the sample (representative workers` population, high response rate, valid sample size and application of defined exclusion criteria). Assessment of the exposure variable was done using validated occupational specific questionnaire which was anonymous self-rated with control for missing values. Outcome variable were set by explicit diagnostic criteria and medical records organized by professional health workers at worksite clinic. There were also adjustments for age and years of exposure. We were able to test for a potential dose–response relationship between job stressors and levels of blood pressure and lipid parameters.

Potential limitations and potential biases in the present study need to be considered. There are several potential confounders whose data were not available in regression analysis. Obesity/body mass index, socioeconomic level/education and dietary habits were not assessed although they could have impact on distinctive OSI aspect and total OSI association with clinical output (systolic and diastolic BP, serum lipids concentrations). Our study sample does not allow for testing of the association between untreated hypertension and stressors since the majority of hypertensives in our sample were treated.

Much of the occupational exposure data, especially from the longitudinal studies, were gathered from full-time working persons with some degree of occupational stability. The exclusion of temporary workers, such in this study, a group likely to be exposed to job strain, could attenuate risk estimates. Also the cross-sectional design of the study must be accounted for causal inferences and possible confounders. Hence, a predictive role of work stress index aspects was determined by assessment of the potential predictors and its association with disease outcomes of interest. Although the study was conducted with one occupation only, it examined methodological issues that are important to consider for other occupational groups as well. However, the results might not be generalizable in detail to other occupations.

Conclusion

The study provides an evidence for the significant association of occupational stress with lipid disorders and elevated blood pressure in professional drivers, which could be a possible link between job stress and coronary heart disease.

A total OSI and underload as one of its aspects, showing the lack of social communication, simple task prepraration and underestimation of working results, showed the strongest association in this occupational group.

Regular periodical examinations and workplace interventions aimed to decrease a total OSI and underload, are important aspects in primary prevention and additional reduction of cardiovascular risk.

Conflict of interest

The authors declare that they have no conflict of interest.

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Evaluation of telemedicine in the management of dentogenous infections

Procena primene telemedicine u zbrinjavanju dentogenih infekcija

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Abstract

Introduction/Aim. The first written evidence of telemedicine dates back to the times of Sava Nemanjić (the end of 12th and the beginning of 13th century). Nowadays, the use of telemedicine in Serbia gains momentum, and the cause of this lies in the creation of a central telemedicine system XPA3 Online and the establishment of the Center for Telemedicine at the Faculty of Medicine, University of Priština/Kosovska Mitrovica, Kosovska Mitrovica, Serbia. Dentogenous infections are among the most urgent conditions in dentistry, which may have even a fatal outcome. The aim of this study was to assess the possibility of using telemedicine methods in the pathology of dentogenous infections. **Methods.** This experimental randomized study incl uded 414 patients with suspected dentogenous infection. The patients were enrolled at 7 sites, with systematic photograph-taking, collection, and digitalization of the available anamnestic and laboratory data, tests, and x-rays. Together with clinical findings, the data were uploaded on the XPA3 Online central telemedicine system; after that, 10 teleconsultants reviewed the material, set the diagnosis, and gave their opinion about the treatment. The agreement was determined using the Cohen's kappa (κ) coefficient, as well as diagnostic sensitivity (SE), specificity (SP), and efficacy (EFF). Statistical significance and comparisons were done using the χ-test, and testing nonparametric properties using the McNemar's χ^2-test for the significance threshold of $p = 0.05$. **Results.** The results describing agreement of telemedicine diagnosis of the areas primarily involved with infection compared to clinical inspection, indicate an almost complete diagnostic agreement ($\kappa = 0.971$). Diagnostic agreement as to the type of infection was also almost complete ($\kappa = 0.951$), and a similar value was obtained also for the treatment agreement ($\kappa > = 0.892$). **Conclusion.** The method of telemedicine provides us with a tool to make a correct clinical diagnosis of dentogenous infections equally well as in real time, as well as to get a deeper and wider insight into their nature and to suggest adequate treatments.

Key words:
dental informatics; remote consultation; focal infection, dental; treatment outcome.

Apstrakt

Uvod/Cilj. Prvi zapisi o primeni telemedicine u Srbiji datiraju iz vremena Save Nemanjića (kraj XII i početak XIII veka). Danas, primena telemedicine u Srbiji naglo raste, a uzrok leži u stvaranju centralnog telemedicinskog sistema XPA3 *Online* i formiranju telemedicinskog centra Medicinskog fakulteta Univerziteta u Prištini/Kosovska Mitrovica, Srbija. Takođe, dentogene infekcije spadaju u najurgentnija stanja u stomatologiji, koja mogu da imaju i letalan ishod. Cilj rada bio je da se ispitaju mogućnosti primene telemedicine kod patologije dentogenih infekcija. **Metode.** Urađena je eksperimentalna randomizirana studija na 414 pacijenata kod kojih je postojala sumnja na pristustvo dentogene infekcije. Pacijenti su primani na sedam lokacija, pri čemu su rađena sistematska fotografisanja, sakupljanje i digitalizacija dostupnih anamnestičkih i laboratorijskih nalaza, testova, kao i radioloških snimaka. Sve je to zajedno sa kliničkim nalazom prosleđivano na XPA3 *Online* centralni telemedicinski sistem, nakon čega su deset telekonsultanata pregledali materijal, postavljali dijagnozu i davali terapijsko mišljenje. Određena je saglasnost Cohenovim kappa (κ) koeficijentom, dijagnostička senzitivnost (SE), specifičnost (SP) i efikasnost (EFF). Statistička značajnost i poređenja vršena su χ-testom, a testiranje neparametarskih obeležja McNemmar-ovim χ^2 kvadrat testom za prag značajnosti od $p = 0.05$. **Rezultati.** Rezultati slaganja telemedicinskih dijagnoza primarno zahvaćenih prostora infekcijom u poređenju sa kliničkim pregledom ukazuju na postignutu skoro potpunu dijagnostičku saglasnost ($\kappa = 0{,}971$). Slaganje za vrstu infekcije ukazuje takođe na skoro potpunu dijagnostičku saglasnost ($\kappa = 0{,}951$), a o predloženoj terapiji slaganje je slično ($\kappa = 0{,}892$). **Zaključak.** Primenom telemedicine može se podjednako dobro, kao i kliničkim pregledom u realnom vremenu, postaviti ispravna dijanoza infekcija, sagledati njihova problematika i mogućnosti terapije.

Ključne reči:
informatika, stomatološka; konsultacije na daljinu; infekcija, fokalna, zubna; lečenje, ishod.

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Introduction

The first evidence about distant medical consultations (teleconsultations) in the history of Serbia dates back to the time of Sava Nemanjić (the end of 12th and the beginning of 13th century), and writings described two such cases: immediately before the resignation of the Grand Prince Nemanja and succession of the throne by his middle son Stefan, Nemanja fell seriously ill, and his delegation was sent to Sava, his youngest son, a monk in the Hilandar Monastery (Mount Athos, Greece) to come and spend his father's last hours with him. Instead, Sava gave the instructions to the delegates which herbs his father was to take, what to do, and how to pray; in short, he did not come back with them. Firmly believing in the consultation given and praying to the Holy Mountain Protectors, he sent a message to his father to transfer power to the next generation as soon as he was well again, to join Sava on the Holy Mountain and repent for the sins made in worldly life and while in power, and to devote his life to the living God and Holy Mother of God. The success of this teleconsultation was proven by the rapid recovery of Nemanja, his becoming a monk, and permanent moving to the Holy Mountain to join his son Sava. The second distant consultation occurred when Sava's brother, King Stephan fell ill while Sava was abroad. Sava gave his instructions to the brother's messengers, which successfully cured him, accompanied by prayers, and Sava himself came later on to see the healing of the future monk Simon [1–5].

In 2011 telemedicine in Serbia rapidly developed, triggered by the creation and initial use of the central telemedicine system XPA3 Online (XPA3 Online, Niš, Serbia) and by the foundation of the Telemedicine Center of the Faculty of Medicine University of Priština/Kosovska Mitrovica, Serbia. The system is based on one of the most up-to-date forms of application services, with ultra high speed Internet above, and Microsoft Windows 2008 R2 OS and Microsoft SQL Server 2008 R2 data base below. The system manages and co-ordinates provider services for short message service (SMS) information, for rapid and short phone calls, automated receipt and processing of photographs, images, various text formats (.docx,. pdf,. html,. txt), automated conversion of video formats (.mp4,. avi,. mov i dr.) into the widely accepted .flv format, and also receives almost whole patient history. The system is accompanied by a number of ready physicians of almost all specialties, most of which are teachers, associates, principal physicians, and so on. If a physician needs specialist help or interspecialist consultation, he posts a telemedicine request using his/her personal computer or Internet-connected smartphone, and gets the requested consultation in time. In addition to its ability to offer up-to-date and rapid teleconsultation support, the system is equipped with the peripherals for scientific monitoring and analysis, providing researchers with already processed data and access resources. Based on such support of an artificial intelligence system on XPA3 Online, we were able to obtain, in an economical way, a number of results describing the quality of Internet-based telemedicine consultation in different areas of interest.

On the other hand, dentogenous infections represent one of the most urgent conditions in the practice of dentistry, the management of which requires proper training and collaboration of different specialties and which, if managed inadequately or late, can lead even to a fatal outcome [6–8]. Since such patients require a prompt response and permanent specialist observation, the problem of patient management in cases of inaccessibility of adequate specialist services should be resolved. These situations involve e.g. soldiers in the field, bed-ridden or immovable individuals, those with special needs, individuals in remote and unaccessible geographical areas, or physical inaccessibility of specialists from any reason [9]. We have had the situations that, due to restricted traffic in the region of Kosovo and Metohija, the patients with dentogenous infections could not reach the specialists of oral surgery/otorhinolaryngology/maxillofacial surgery, and their management had to be undertaken by untrained dentists/physicians; the posttreatment recovery was controlled from a distance.

Having knowledge about the natural course of inadequately treated or untreated dentogenous infections, and bearing in mind the possibility of infection spread into the adjacent anatomical spaces, thus creating most severe disease forms and complications (such as *Angina Ludovici* or cavernous sinus thrombosis), there is the question of how and to what extent the physicians in distant areas (assisted by modern telemedicine systems) can help in the management of such cases. In particular, there is the question of reliability of distant diagnosis and treatment plan in patients with dentogenous infections.

The aim of this study was, therefore, to assess the possibility of using of modern telemedicine methods in the management of patients with dentogenous infections and find an answer if we can make a clinically reliable diagnosis, and evaluate the primary treatment indicated in such cases (extraction of the causal tooth, incision, need for hospital admission and antibiotic therapy administration).

Methods

This experimental randomized study enrolled 414 patients, aged 12 to 83 years, of both genders, with suspicious dentogenous infection as assessed by the physician in charge. The patients were clinically admitted at 4 main sites in Serbia: Kosovska Mitrovica, Niš, Belgrade, Novi Sad, two sites in Bosnia and Herzegovina (Republic of Srpska: Banja Luka and Trebinje), and in Montenegro (Podgorica). They were all clinically examined by the specialists in oral surgery, otorhinolaryngology, and maxillofacial surgery.

Depending on the quality of equipment, analog ortopans were used with the Tubus 85kV R 76 20 15 mA 50–60 Per, filter 1,2 mm Al image quality, the recordings of which were digitalized into JPEG 2048 × 1536 before transmission, while digital ortopans with the image quality 19 sec/10 mAsex at 63–81 kV were stored in TIFF 998 × 494. Digital cameras and mobile phones are used to photograph. The patients had cameras with the resolution from 3.1 to 8.0 megapixels. The patients were photographed en face and

bilaterally (en face with head turned upwards, bilaterally with head maximally extended backwards and to the side); extraoral edema was also photographed, if present; inner structures of the mouth cavity, and upper and lower jaw teeth were photographed in the extent possible using only digital camera (without any additional tools for intraoral photography); intraoral edema was also photographed, if present. In summary, each of the patients had ortopan taken, as well as the above photographs. The data were stored either in the physicians' PCs or in their smartphones. The physicians accessed the Internet *via* their PCs or smartphones in different ways (ADSL access with 512/128 kbps to 6/1 mbps; global municipal wireless connection at 5 GHz at 2/2 mbps; or *via* standard mobile access, WCDMA 3G and HSDPA 3G UMTS, with EDGE access if there were no 3G networks). After a successful Internet connection, they accessed the application system of the Center for Telemedicine, University of Priština/Kosovska Mitrovica (www.xpa3.com) and passed the authentication and authorization phases (protected with a 128 bit Secure Sockets Layer (SSL) security protocol. Opening a new digital telemedicine request, the physicians entered patient personal information, patient history, as well as the available anamnestic information such as laboratory findings, hospital discharge documents, and other relevant patient data. Attached to the request, there were the files containing photographs and x-rays, together with proper clinical findings, both general and individual, accompanying each of the images. Such a teledentistry request was then sent to the system, and the system in a minute contacted specialist teleconsultants (TCs) with SMS messages, and some of them, additionally, were contacted by phone. Each of the teleconsultants had in the received SMS message the subject of teleconsultation, assessed request urgency (describing the levels as normal or urgent), and expected time to response (30 minutes to 6 hours, depending on the case). Teleconsultants then accessed the Internet system at www.xpa3.com, reviewed the received request, and responded giving their opinion, suggestion, and possible outcome of recommended therapy. Each of the patients was clinically examined, with diagnosis being made in real time (Figures 1, 2).

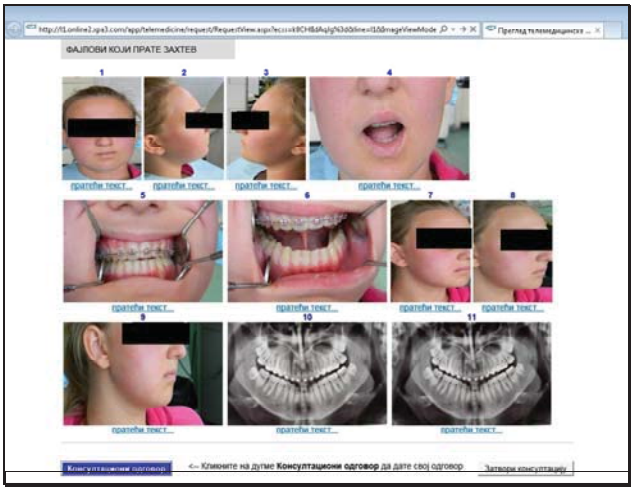

Fig. 1 – A newly received teleconsultation request in the XPA3 Online system.

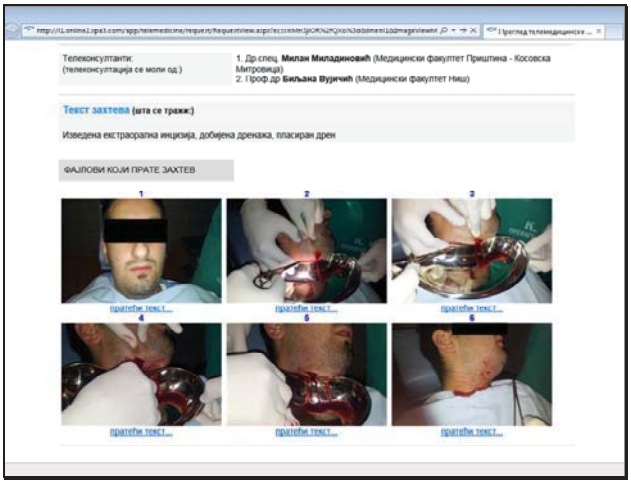

Fig. 2 – XPA3 Online teleconsultation: Dentogenous infection with serious general status of the patient, where teleconsultants unanimously suggested extraoral incision, which was performed and the results of which were sent to them.

The degree of diagnostic accuracy was determined using the following scale: correct – if a telemedicine diagnosis is identical to the primary one, or if it is made and is acceptable as a differential diagnosis, and incorrect – if a telemedicine diagnosis differs completely from the primary one, or if the diagnosis has not been made.

In a similar way, the degree of precision of the treatment plan was determined, too, describing it as correct/incorrect. Statistical data processing and analysis of the obtained results were performed using the Diagnostic and AGreement Statistics (DAG) Software http://www.mhri.edu.au/biostats/DAG_Stat and software package SPSS for Windows version 16.

Agreement among the teleconsultants was obtained dividing the number of patients with the achieved agreement with the total number of examined patients. The following elements were determined: sensitivity (SE), specificity (SP), and efficacy (EFF). The degree of achieved agreement between teleconsultants using the method of telemedicine was expressed as the Cohen's *kappa* (κ) coefficient. The *kappa* coefficient for the confidence interval of 95% was presented according to the Landis and Koch scale (Table 1). Statistical significance of the differences between correct and incorrect diagnoses, planned interventions (yes/no; extractions, incisions, hospitalizations, antibiotic therapy), precision, sensitivity, and specificity, and comparison of all the obtained values were done using the *z*-test, and testing for non-parametric characteristics was done using the McNemar's χ^2-test (contingency table 2 × 2) for the threshold of significance at $p = 0.05$.

Table 1
Kappa coefficient (*k*) and degree of diagnostic agreement (Landis i Koch)

κ	Agreement degree
< 0	No agreement
0.01–0.20	Insignificant agreement
0.21– 0.40	Sufficient agreement
0.41–0.60	Moderate agreement
0.61–0.80	Significant agreement
0.81–0.99	Almost complete agreement
1	Complete agreement

Results

Overall 414 patients were examined and 414 diagnoses were made. All the received photographs, x-rays, and other documents, were declared as usable by the teleconsultants. Out of 414 (100%) patients, teleconsultants stated that there was no dentogenous infection in 18–22 (4.35–5.31%) cases. They established that the most common area involved with dentogenous infection was the submandibular region, in 84–93 (20.29–22.46%) cases, followed by submental, in 63–69 (15.22–16.67%) cases, and buccal space, in 57–66 (13.77–15.94%) cases (Table 2). As the type of infection, teleconsultants identified abscess in 328–333 (79.23–80.43%) cases, while phlegmon was diagnoses in 47–51 (11.35–12.32%) cases (Table 2). Trismus was found in 31–34 (7.49–8.21%) cases (Table 2). Regarding treatment, the teleconsultants suggested 233–241 (56.28–58.21%) extractions, 159–187 (38.41–45.17%) incisions, hospital admission in 42–49 (10.14–11.84%) patients, and antibiotic treatment in 398–406 (96.14–98.07%) cases (Table 2). As the type of necessary incision, intraoral incision was recommended in 113–126 (67.66–67.38%) cases, and extraoral one in 50–60 (31.45–32.62%) cases (Table 2).

The highest degree of teleconsultant agreement as to the primary spaces involved with infection was achieved between TC1 and TC2 ($\kappa = 0.911$), and the lowest degree between TC6 and TC8 ($\kappa = 0.827$). Regarding differential diagnosis abscess/phlegmon, the highest degree of agreement existed between TC8 and TC9 ($\kappa = 0.933$), and the lowest degree between TC5 and TC10 ($\kappa = 0.889$). As for trismus, the best agreement existed between TC1, TC2, TC3, TC4, TC7, TC8, TC9, and TC10 ($\kappa = 1.000$), with the deviation with TC5 and TC6 ($\kappa = 0.600$).

The highest degree of diagnoses agreement of primary infection-involved spaces related to clinical examination was present in TC1 with 2 erroneous diagnoses (0.48%), and the lowest degree was found in TC3 and TC5 with 6 erroneous diagnoses (1.45%), for statistical processing of the results (Table 7 and Table 1), $\kappa = 0.971$, SE = 98.5%, SP = 98.5%, EFF = 98.5%, indicating almost a complete diagnostic agreement. As to the type of infection, the highest agreement with clinical examinations was found for TC1, with 2 erroneous diagnoses (0.48%), and the lowest agreement was observed for TC6, with 10 erroneous diagnoses (4.14%), for statistical processing of the results (Table 1), $\kappa = 0.951$, SE = 97.6%, SP = 97.6%, EFF = 97.6%, indicating almost complete diagnostic agreement. As for trismus, the highest degree of agreement with clinical examination was observed for TC1, TC2, TC3, TC4, TC7, TC8, TC9 and TC10, without any diagnostic errors, and the lowest for TC5 and TC6, with 1 error each (0.41%). Diagnostic differences were not statistically significant.

Regarding the suggested therapy (Table 2), the highest degree of agreement was observed between TC2 and TC10, as well as between TC4 and TC6 ($\kappa = 0.919$), the lowest degree between TC5 and TC7 ($\kappa = 0.892$), while the opinions were somewhat different regarding the choice of intra- or extraoral incision approach (Table 2).

Discussion

The purpose of this paper was to assess the possibility of using modern methods of telemedicine in the management of patients with dentogenous infections, and to answer to the questions: if we can make a clinically reliable diagnosis at a distance, and are we able to assess the primary treatment indicated in such cases (extraction of the causal tooth, incision, need for hospital admission, and antibiotic therapy).

Almost a completely achieved agreement in making the diagnosis of primary involved spaces ($\kappa = 0.971$) and that concerning the type of infection ($\kappa = 0.951$) and the presence of trismus ($\kappa = 1.000$ in 80% of TCs and $\kappa = 0.600$ in 20% of TCs), open the possibility of reliable distant patient management. The results showed that using the method of telemedicine we can make clinically acceptable diagnosis of dentogenous infections, and that the method can replace real time diagnostic evaluation, i.e. direct visual/tactile diagnostic approaches. Regarding the basic therapy assessment, almost a perfect agreement was achieved too ($\kappa >$ or $= 0.892$), indicating the possibility of distant counselling as to the primary treatment approaches, with clear statements about the necessity of hospital admission of patients with dentogenous infections.

One of the striking advantages was the fact that 98% of teleconsultation requests were responded to in the requested time span (30 min to 6 h), which indicated that telemedicine consultation can be a valuable tool for saving the time to specialist examination. Patients were placed in the right management track in a short time, saving valuable time in cases of emergency, which can be of a decisive influence regarding the development and outcome of infection. The results confirmed that "store and forward" telemedicine system resolves the need of physicians for consultation in a cheap, effective, and rapid way, in this case in the pathology of dentogenous infections.

We have not encountered in the literature any studies dealing with the validation of telemedicine diagnosis and assessment of therapy for dentogenous infections, but there were studies which evaluated telemedicine agreements in the related fields. Thus Salazar-Fernandez et al. [10] in their large study of telemedicine consultations in the diseases of temporomandibular joint found that using the store and forward telemedicine method, the opinion of a specialized consultant can be obtained in an effective and efficacious way, suggesting that the method should have been more widely used by oral and maxillofacial surgeons. Herce et al. [11], suggested that using the store and forward telemedicine method the pathology of third molars could be effectively assessed within the pre-surgical treatment, avoiding thus numerous preliminary hospital visits by the patient. Duka et al. [12], comparing the diagnoses of third molar pathology using telemedicine and conventionally, found that the problems could be adequately evaluated by both approaches. Ignatius et al. [13], in their 13 months' study of the possibility of distant diagnosis and distant planning of prosthetic solutions in patients requiring prosthetic or oral rehabilitation treatment, concluded that teleconsultations were successful in 24 out of 27 cases. Brüllmann et al. [14], in their study of recognition of

Table 2

Agreement among teleconsultants (TC) about dentogenous infections parameters

Parameters	TC1		TC2		TC3		TC4		TC5		TC6		TC7		TC8		TC9		TC10	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Involved space																				
sublingual	11	2.66	11	2.66	10	2.43	12	2.90	10	2.42	13	3.14	9	2.17	12	2.90	11	2.66	11	2.66
submental	66	15.94	64	15.46	66	15.94	65	15.70	67	16.18	65	15.70	68	16.43	69	16.67	63	15.22	63	15.22
submandibular	89	21.50	90	21.74	93	22.46	93	22.46	84	20.29	87	21.01	92	22.22	89	21.50	93	22.46	85	20.53
Angina Ludowici	8	1.93	8	1.93	8	1.93	7	1.69	8	1.93	7	1.69	9	2.17	10	2.42	7	1.69	8	1.93
pterygomandibular	24	5.80	22	5.31	22	5.31	24	5.80	26	6.28	23	5.56	22	5.31	25	6.04	25	6.04	22	5.31
buccal	61	14.73	62	14.98	66	15.94	65	15.70	64	15.46	58	14.01	60	14.49	60	14.49	57	13.77	65	15.70
submasseteric	4	0.97	5	1.21	2	0.48	3	0.72	7	1.69	2	0.48	3	0.72	3	0.72	5	1.21	5	1.21
fose canine	41	9.90	41	9.90	39	9.42	43	10.39	39	9.42	42	10.14	44	10.63	38	9.18	37	8.94	44	10.63
abscess of the tongue	2	0.48	2	0.48	1	0.24	2	0.48	2	0.48	2	0.48	2	0.48	2	0.48	1	0.24	2	0.48
palatinal abscess	36	8.70	36	8.70	33	7.97	36	8.70	35	8.45	38	9.18	33	7.97	36	8.70	36	8.70	35	8.45
frontal upper fornix	27	6.52	27	6.52	29	7.00	24	5.80	27	6.52	26	6.28	28	6.77	29	7.00	30	7.25	26	6.28
absence of dentogenous infection	18	4.35	20	4.83	19	4.59	18	4.35	21	5.07	22	5.31	19	4.59	20	4.83	22	5.31	21	5.07
other	27	6.52	26	6.28	26	6.28	22	5.31	24	5.81	29	7.02	25	6.05	21	5.07	27	6.51	27	6.53
Total	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00
Type of infection																				
abscess	330	79.71	332	80.19	332	80.19	333	80.43	329	79.47	328	79.23	330	79.71	331	79.95	331	79.95	330	79.71
phlegmon	50	12.08	48	11.59	50	12.08	47	11.35	51	12.32	49	11.84	48	11.59	49	11.84	51	12.32	51	12.32
absence of infection	18	4.35	18	4.35	19	4.59	18	4.35	21	5.07	22	5.31	19	4.59	20	4.83	18	4.35	21	5.07
cther	16	3.86	16	3.86	13	3.14	16	3.86	13	3.14	15	3.62	17	4.11	14	3.38	14	3.38	12	2.90
Total	414	100.00	414	99.99	414	100.00	414	99.99	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00
Trismus presence																				
yes	33	7.97	33	7.97	33	7.97	33	7.97	32	7.73	34	8.21	33	7.97	33	7.97	33	7.97	33	7.97
no	381	92.03	381	92.03	381	92.03	381	92.03	382	92.27	380	91.79	381	92.03	381	92.03	381	92.03	381	92.03
Total	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00
Therapy																				
required extraction	233	56.28	233	56.28	236	57.00	234	56.52	239	57.73	237	57.25	239	57.73	241	58.21	239	57.73	238	57.49
required incision	167	40.34	168	40.58	183	44.20	159	38.41	173	41.79	187	45.17	173	41.79	178	43.00	181	43.72	177	42.75
required hospitalization	44	10.63	42	10.14	42	10.14	47	11.35	45	10.87	47	11.35	44	10.63	49	11.84	46	11.11	43	10.39
required antibiotic therapy	399	96.38	401	96.86	400	96.62	398	96.14	404	97.58	402	97.10	398	96.14	406	98.07	400	96.62	401	96.86
Incision approach																				
intraoral	113	67.66	114	67.86	124	67.76	109	68.55	118	68.21	126	67.38	120	69.36	124	69.66	122	67.40	122	68.93
extraoral	54	32.34	54	32.14	59	32.24	50	31.45	55	31.79	61	32.62	53	30.64	54	30.34	59	32.60	55	31.07
Total	167	100.00	168	100.00	183	100.00	159	100.00	173	100.00	187	100.00	173	100.00	178	100.00	181	100.00	177	100.00
Diagnosis																				
correct	412	99.52	411	99.28	408	98.55	410	99.03	408	98.55	409	98.79	409	98.79	411	99.28	32	94.12	411	99.28
incorrect	2	0.48	3	0.72	6	1.45	4	0.97	6	1.45	5	1.21	5	1.21	3	0.72	2	5.88	3	0.72
Total	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	414	100.00	34	100.00	414	100.00

open dental root canals using telemedicine, found that distant consultation of experienced dentists can be of great help to younger colleagues regarding the correct diagnosis of open root canal. Kopycka-Kedzierawski and Billings [15] found that using telemedicine the prevalence of caries in children could be successfully assessed, collecting the information from children in the cohort group for 12–60 months and using simultaneously the methods of telemedicine and traditional visual/tactile examination. The results of the respective authors agree with our own results, especially regarding the reliability of used teledentistry methods, with speciall stress on the store and forward telemedicine method.

Since dentogenous infections represent a relatively common pathology, our study offerred a significant insight into the excellent prospects of distant examination and treatment planning in the management of these patients. In contrast, inadequate management and frequent antibiotic treatments of dentogenous infections with only oral antibiotics (which is rather common in the routine dentistry practice), contribute to a rapid development and wide spread of these infections, endangering thus the general health of our patients [16]. In these patients, the first clinical examination is of key importance, since it requires ample clinical knowledge and experience [17]. Using the methods of telemedicine, other specialists or, later, even the patients themselves can seek adequate expert help from oral and maxillofacial surgeons or otorhinolaryngologists, i.e. from subspecialists in the appropriate fields, getting the requested advice regarding proper diagnosis or treatment. Timely teleconsultation can help physicians in charge of the patients to react better, supported by sufficient expertise; in many cases, the spread of infection into deeper regions of the neck can thus be prevented (the origin of such infections is dental in over 40% of cases, and lower molars are most commonly implicated in that regard) [18].

As the precondition of proper telemedicine diagnosis, adequate technical-technologic equipment is required for the collection, analysis, and exchange of diagnostic data between teleconsultants. We achieved excellent results using 3.1 megapixel or stronger cameras (with built in electronic flash units). Since digital photographic equipment is becoming increasingly cheaper, with imaging resolution being on the rise too, we believe that the resolution of 3.1 megapixels constitute the minimum for teledentistry imaging and that there is no need to go below that standard. It is also necessary to adhere to the common procedure of taking intraoral or extraoral photographs, capturing by digital photographs the real situation in the mouth, as well as extraoral changes [12]. The patient should be photographed en face and bilaterally, taking as good as possible images of the region of interest. With dental radiographs, the ideal situation is that the digital image is transported from the radiography center through multiple telemedicine systems in unchanged format. If an analog radiography is to be digitalized using a camera or a scanner device, the minimum requirements a 3.1 megapixel image should be abided by Witherow et al. [19] observed that a large number of patients with dentogenous infections were referred to various other specialists, such as plastic surgeons, dermatologists, emergency services, or general practitioners and general dentists, when a proper diagnosis is commonly a problem. The significance of our study lies in the practical demonstration that in such cases rapid and effective telemedicine consultation is the method of choice, eliminating the need for patient transport to other relevant specialists.

Since the results showed an almost complete agreement between teleconsultants regarding the therapy ($\kappa >$ or $= 0.892$), there is the possibility of help to remote nonspecialists in the decision-making whether to extract the causal tooth at once, or to postpone the extraction and perform only incision. This can have a marked impact on infection subsiding and alleviation of pain, as well as on the general status of the organism [20]. Viewed from the aspect of general intention to reduce microorganism resistance, telemedicine consultation in such patients offers an additional differentiation regarding the decision to include antibiotic therapy or to stick to tooth extraction only, providing surgical debridement, removal of the infection cause, and drainage. In this way, unnecessary increase of resistance to antibiotics can be minimized [21]. An almost complete agreement between the teleconsultants was achieved also regarding the need for hospital admission, eliminating the possibility of inadequate, nonspecialist assessment of the need for constant medical observation, i.e. that the present dentogenous infection had to be seriously considered.

The method of telemedicine used in the study can improve the quality of health care, facilitate the work of doctors, reduce treatment costs, and contribute positively to the budget of the health care system.

Conclusion

Our study showed that using the method of telemedicine it is possible to make correct clinical diagnosis of dentogenous infections equally well as in real time, assess their nature and course, and successfully consider the treatment option.

Telemedicine consultation is a vital patient management tool of key importance to any field doctors or those in geographically remote regions, but also to any other doctors who are not sufficiently specialized in the area of dentogenous infections.

In addition to its being a valuable medical tool, the method of telemedicine significantly contributes to cost-effectiveness of health care and speeds us the process of healing.

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Effects of calcitonin and calcium medication in treatment of edentulous osteoporotic mandible

Efekti primene kalcitonina i kalcijuma u lečenju bezubih pacijenata sa osteoporoznim mandibulama

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Abstract

Background/Aim. In addition to damage of the bones that support the remaining teeth, degradation of osteoporotic oral bones also lead to a consequent reduction of supporting tissues and the loss of dentures retention. The aim of this study was to assess the clinical and radiographic outcomes following injection of a calcitonin and calcium solution into the buccal aspects of edentulous mandibles. **Methods.** The experimental group of 67 edentulous patients diagnosed with osteoporosis, and the control group of 19 nonosteoporotic edentulous patients were treated with the calcitonin and calcium once solution per month. Mandibular bone density was measured from panoramic radiographs, supplemented by T scores of skeletal density in the experimental group. **Results.** After the therapy, measurements showed moderate increases in bone density, compensating for up to 4% of the total loss of minerals and solidity of denture-bearing areas of osteoporotic mandibles. Osteoporosis affected women earlier than men in this study. **Conclusion.** Application of a calcitonin and calcium solution is a suitable method of preprosthetic therapy for edentulous osteoporotic patients.

Key words:
osteoporosis; mandible; mouth, edentulous; calcitonin; calcium; treatment outcome.

Apstrakt

Uvod/Cilj. Degradacija osteoporoznih viličnih kostiju vodi ka oštećenju kostiju koje podržavaju preostale zube, ali i posledičnoj redukciji potpornog tkiva i gubitku retencije proteze. Cilj rada bio je da se procene klinički i radiografski parametri posle tretmana bezubih mandibula rastvorom kalcitonina i kalcijuma ubrizganog u bukalne recesuse. **Metode.** Ispitnu grupu činilo je 67 bezubih pacijenata sa dijagnostikovanom osteoporozom, a kontrolnu grupu 19 bezubih pacijenata sa normalnom gustinom kosti (bez osteoporoze). Svi ispitanici dobijali su rastvor kalcitonina i kalcijuma jednom mesečno. Gustina kosti mandibule merena je na osnovu ortopantomograma svih ispitivanih pacijenata, uz komplementarni T-nalaz gustine skeleta na sistemskom nivou kod pacijenata iz ispitne grupe. **Rezultati.** Po završenoj terapiji utvrđen je umeren porast gustine kosti, čime je nadoknađeno oko 4% ukupnog gubitka mineralnih supstanci u regionima nosećeg tkiva osteoporoznih mandibula. Osteoporoza je zahvatala kosti žena ranije od kostiju muškaraca u ovoj studiji. **Zaključak.** Primena rastvora kalcitonina sa kalcijumom predstavlja kvalitetan metod medikacije i preprotetske pripreme bezubih pacijenata obolelih od osteoporoze.

Ključne reči:
osteoporoza; mandibula; bezubost; kalcitonin; kalcijum; lečenje, ishod.

Introduction

Osteoporotic microstructural degradation and macroscopic reduction of the oral bones have been described in the literature [1]. Degradation of osteoporotic oral bones has been reported not only to damage the bone supporting the remaining teeth, but also to lead to a consequent reduction of supporting tissues and the loss of dentures retention [2]. Analysis of the bone layer appearance on panoramic radiographs was used to assess changes in any parameters of turnover of jaw bones [3–5].

Calcitonin accelerates influx of calcium, improving bone mineral content and bone density [6]. For this research it was speculated that these positive effects on osteopenic and osteoporotic bones could finally be achieved in the mandible, as well as in other human bones.

Considering various data available on multiple drug therapy and possible effects of systemic medication, it could be of particular importance to focus on dependable approaches to oral treatment of osteoporosis.

The aim of this study was to asses the effectiveness of local jaw therapy with calcitonin and calcium, used within

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preprosthetic and prosthetic therapy of selected edentulous osteoporotic patients.

Methods

The study included 67 edentulous patients – 11 men, aged 64–80 (mean age 71 years) and 56 women, aged 55–79 (mean age 56 years) in the experimental group, and 19 edentulous patients – 9 men, aged 55–65 (mean age 59 year), and 10 women, aged 40–55 (mean age 53.5 year), with well-pronounced edentulous ridges and with normal bone density, as the controls. Edentulous patients were selected based on a questionnaire concerning previous medical treatments for osteoporosis, skeletal density, history of fractures of any bones, postmenopausal periods in women, calcium and microelements of blood-plasma, as well as oral status of bone consistency or reduction. No patients had any malignancy or manifest destructions in the mouth.

Skeletal density analysis was performed on a scanner (Lunar DPX-L, GE Healthcare, Piscataway, NJ) and T scores of the patients were obtained, indicating low bone density and systemic osteoporosis in the experimental group. Scanning was done for at least three of lumbar vertebral bodies in the regions of L1-L4 lumbar vertebrae of the experimental group patients. Panoramic radiographs (Orthopantomograph 10-serial no. 01492, Siemens, Germany) of every patient in the experimental group were focused on degradations in the osteoporotic jaw (Figure 1a) at baseline. Panoramic radiographic finding of a patient from the control group before application of calcitonin and calcium solution is shown in Figure 1b). Calcitonin (Miacalcic, Novartis, Switzerland, and Huber, Galenika A.D., Serbia), and calcium (Calcium-gluconate Sterop, Belgium; Calcium-Sandoz, 10% calcium glubionas amp., Sandoz Switzerland) were used locally to improve solidity of edentulous mandibles. After local anaesthetic application, up to 1.5 mL of a solution containing calcitonin and calcium (2 : 1 ration in one dose) was injected submucosally adjacent to the mandibular bone surfaces (Figure 2). A total of 5 doses were applied to both the right and left sides of the osteoporotic mandible at monthly intervals, to each patient of both groups. This treatment was administered over a 4-month period. The final separate dose of calcitonin and calcium solution was applied only to each patient of the experimental group at recalls, after 4 months, but not later than 6 months of follow-up. For the patients of the control group calcitonin and calcium was applied after tooth extractions to consolidate the buccal bone and accelerate recovery of post-extraction wounds, but also for preventing bone loss and to compare increments of bone densities between the control group and the experimental group. Panoramic radiographs of the patients were taken after each application of the solution (Figure 3). Complete dentures (CD) were fabricated for each patient of both groups. All of the patients had complete dentures during the experimental period. During the period of application of the solution containing calcitonin and calcium, a digital densitometer (DT II 05, U.K.) was used to analyse the bone density of mandibu-

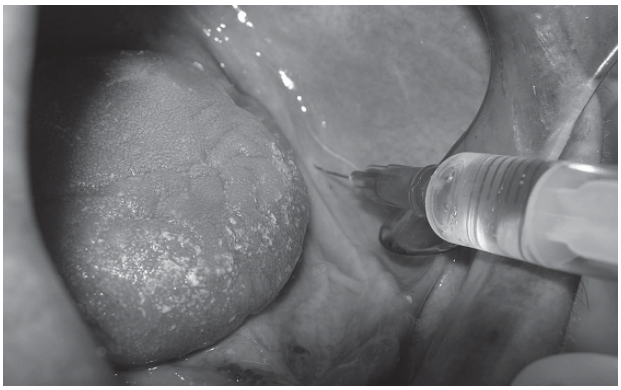
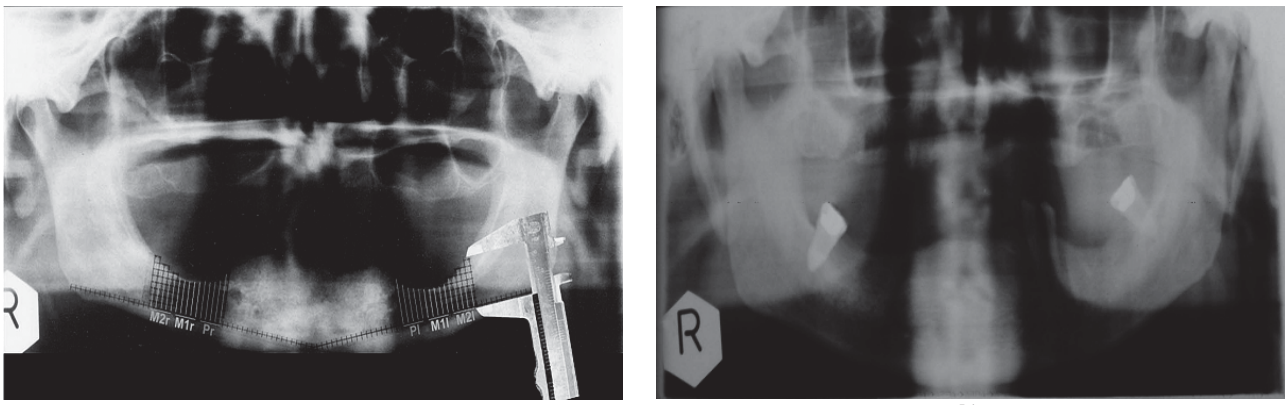
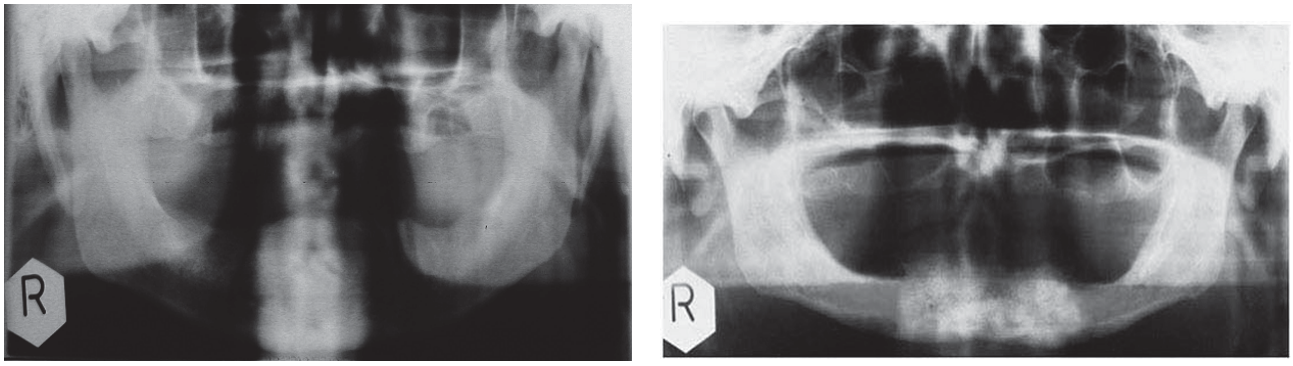


Fig. 2 – Submucosal injection of a calcitonin and calcium solution in the buccal aspect of osteoporotic mandible of the experimental group patient.



a) **b)**

Fig. 1 – a) A grid positioned in the regions of particular interest for panoramic radiograph of osteoporotic edentulous mandible and the determined regions – Pr, M1r, M2r, Pl, M1r, M2r (Pr – region of missing premolar to the right side of the mandible; M1r – region of missing the 1st molar to the right side of the mandible; M2r – region of missing the 2nd molar to the right side of the mandible; Pl – region of missing premolar to the left side of the mandible; M1l – region of missing the1st molar to the left side of the mandible; M2l – region of missing the 2nd molar to the left side of the mandible); b) – Panoramic radiograph of the control group patient before application of calcitonin and calcium solution.



a) **b)**

Fig. 3 – a) Panoramic radiograph of the control group patient after application of calcitonin and calcium solution; b) – Panoramic radiograph of a patient, exposing a local increment in density edentulous mandible (for comparisson see Fig. 1).

lar segments on panoramic radiographs. Measurements of the density of the mandibles were taken over the 4 month period of application of solution. Numerical values of bone density on radiograph were shown on digital densitometer representing intensity of entry of focused beam of light into the determined region of dental panoramic radiographs, and these values were expressed in arbitrary units of U/mm^2.

The Student's *t*-test, ANOVA, Pearson's correlation coefficient and calculation of the incremental trend were used for mandibular density analysis.

Results

Before administration of the calcitonin and calcium solution, T-scores from DPX scanning were up to -2.6.for the men and women of the experimental group.

The results of medication for osteoporotic oral bones were favourable in this study. The incremental changes in density of mandibular segments were compared between the experimental and the control group at each time point, as well as between the experimental group and the control group during the therapy.

When mandibular density was compared in the experimental group at baseline and after the 1st month of the therapy, differences were significant for the segments Pr (p < 0.05) with tempo and range of increment of density (R) 1.0080 (0.80%), for Pl (p < 0.05) with R 1.0078 (0.78%), for M1r (p < 0.05) with R 1.0067 (0.67%), for M1l (p < 0.05) with R 1.0065 (0.65%), M2r (p < 0.05) with R 1.0062 (0.62%) and M2l (p < 0.05) with R 1.0060 (0.60%). Density increased further after the 2nd month of application and was significantly increased for Pr (p < 0.05) with R 1.0087 (0.87%), for Pl (p < 0.05) with R 1.0084 (0.84%), for M1r (p < 0.05) with R 1.0074 (0.74%), for M1l (p < 0.05) with R 1.0073 (0.73%), M2r (p < 0.05) with R 1.0066 (0.66%) and M2l (p < 0.05) with R 1.0065 (0.65%). Mandibular density comparison at baseline and after the 3rd month of application showed that it was a significant change in density for Pr (p < 0.01) with R 1.0117 (1.17%), for Pl (p < 0.01) with R 1.0114 (1.14%), for M1r (p < 0.01) with R 1.007 (1.07%), for M1l (p < 0.01) with R 1.0094 (0.94%), M2r (p < 0.05) with R 1.0083 (0.83%) and M2l (p < 0.05) with R 1.0079 (0.79%).

Following application of the calcium and calcitonin solution, the increase in density was significant for Pr, Pl (p < 0.01), M1r, M1l, M2r and M2l segments (p < 0.05), with respect to the baseline (Table 1), compensating for up to 4% of the total loss of jaw bone mineral in the experimental group. In the control group, a statistically small increase in bone density (p < 0.05) was observed in a premolar and the 1st missing molar regions (Table 1).

Table 1

Mandibular bone density in the control and experimental groups at baseline and after administration of calcitonin with ionized calcium solution

Segment	Control group				Experimental group			
	Digital value of density $\bar{x} \pm SD$			Correlation cofficient	Digital value of density $\bar{x} \pm SD$			Correlation cofficient
	before therapy	after therapy	p		before therapy	after therapy	p	
Pr	1.01 ± 0.6	1.83 ± 0.7	< 0.05	0.578	-1.42 ± 0.8	0.21 ± 0.7	< 0.01	0.814
Pl	1.03 ± 0.8	1.92 ± 0.4	< 0.05	0.542	-1.40 ± 0.9	0.19 ± 0.6	< 0.01	0.793
M1r	0.83 ± 0.8	1.79 ± 0.8	< 0.05	0.528	-1.34 ± 0.8	-0.24 ± 0.9	< 0.05	0.637
M1l	0.79 ± 0.9	1.76 ± 0.9	< 0.05	0.519	-1.33 ± 0.7	-0.21 ± 0.9	< 0.05	0.619
M2r	1.61 ± 0.5	1.83 ± 0.3	> 0.05	0.394	-1.11 ± 0.9	0.61 ± 0.5	< 0.05	0.593
M2l	1.52 ± 0.8	1.85 ± 0.3	> 0.05	0.367	-1.11 ± 0.4	0.62 ± 0.6	< 0.05	0.607

Numerical values of density are in arbitrary units (U/mm^2) showing the intensity of light beam entry through the determined region of dental radiograph.

Discussion

In the dental literature there are few reports on treatment and local medication for osteoporotic oral bones.

Based on convincing medical studies on patients, with systemic osteoporosis, the two foremost treatment approaches

could be described: the first related to application of bisphosphonates [7,8], while the second considered calcitonin as the primary accelerator of calcium influx to the bone [6,7].

Making an allowance for various facts derived from studies conducted on patients undergoing systemic therapy with bisphosphonates, and a few studies on local application of bisphosphonates, as well as for the fact that certain interruptions of immune-response were described as a possible complication of extended bisphosphonate usage [9], therapy with a solution containing only calcitonin and calcium, administered locally to osteoporotic jaws, seemed a rational option.

Calcitonin has been recognized as the primary substance that strongly regulates the influx of calcium [6]. Calcium influx should have been provided by mobilization of calcium ions in plasma, as well as by exchanging calcium ions from pharmacological calcium gluconate and calcium glubionas. Respecting the fact that human osteoporotic mandible may not be very well provided with plentiful blood supply, it was assumed that calcium ions in blood vessels of the mandible would not have provided sufficient calcium influx to prevent osteoporotic degradation. Furthermore, additional sources of calcium ions, directed towards credible local storage in close proximity to the mandible, should have a beneficial effect.

In the dental literature, there were few reports on the local application of calcitonin and calcium to hard oral tissues *in vivo*.

On the strength of the results of this study, it appears that the solution of calcitonin and calcium should be applied to the edentulous ridge prior to positioning of a denture onto supporting tissues.

Careful selection of patients concerning the absence of infectious diseases, malignancy, serious kidney problems or blood diseases, is of ultimate priority when considering local application of calcitonin and calcium solution to oral bones.

Repeated application of calcitonin and calcium solution to jaw bone layers may significantly improve the mineral content of bone, bone density, the condition of the bone and the potential of the area to support dentures. Osteoporotic edentulous mandibles should be the first of all oral bones to benefit from a solution containing calcitonin and calcium. Additionally, respecting the levels of concentration of calcium ions applied, osteoporotic maxillary bone could be treated with a solution of the same kind, over a prolonged period of remedial use.

A probable contraindication for local oral application of calcitonin and ionized calcium in solution should be a very intensive reduction of edentulous ridges with the alveolar crest positioned under the superficial surface of the mylohyoid muscle of the edentulous mouth floor.

Calcitonin accelerated influx of calcium into the mandibles of all the patients in both groups.

Since osteoporosis frequently affects women, mandible osteoporosis is likely to affect women rather than men. The use of calcitonin and calcium for osteoporotic toothless patients should be considered the priority for local oral care. In spite of the limited value of local application, calcitonin and calcium should be the prerequisite foundation for positive bone remodelling and turnover of segments of the jaw. Regardless the absence of macroscopically distinct bone regeneration, local application of calcitonin with calcium could be crucial for success at the first point of positive bone remodelling and turnover, and for laying the foundation for later restorative procedures in oral rehabilitation of osteoporotic patients.

Conclusion

The use of calcitonin and calcium solution should be considered the priority in preprosthetic and prosthetic therapy of edentulous osteoporotic patients. This medication compensated for the loss of mineral from the jaw. Application of a calcitonin and calcium solution seems to be a suitable method of preprosthetic therapy for edentulous osteoporotic patients.

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Pandemic influenza H1N1 outbreak in the Military School

Epidemija pandemijskog gripa H1N1 u Vojnoj gimnaziji

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Abstract

Background/Aim. The first cases of the pandemic pH1N1 influenza virus infection was observed in the Unated States and Mexico in April 2009 and the first laboratory confirmed case in Serbia was registered in June 2009. The aim of this paper was to report on the investigation of the first confirmed outbreak of the 2009 pandemic H1N1 influenza in Serbia and to describe the clinical and epidemiologic findings from this investigation. **Methods.** Descriptive and analytical epidemiological methods were used. Data were collected from medical records of the Military School students and epidemiological questionnaire. Pandemic H1N1 infection was initially confirmed by the RT-PCR assay in nasopharyngeal and oropharyngeal swabs and subsequently by the complement fixation test in serum samples. **Results.** The attack rate of acute respiratory illness was 70.8% (204/288). Pandemic H1N1 virus infection was confirmed in 44 of 82 tested cases of acute respiratory illness (53.7%) The most common clinical manifestations of pandemic influenza H1N1 were fever (88.6%), cough (61.4%), malaise (38.6%), runny nose (36.4%), headache (29.6%), sore throat (20.5%) and muscle pain (15.9%). **Conclusion.** The findings from this investigation suggest that pandemic H1N1 influenza in a high military school was widespread but did not cause severe illness.

Key words:
influenca a virus, h1n1 subtype; disease outbreaks; schools; military personnel; serbia.

Apstrakt

Uvod/Cilj. Prvi slučajevi pandemijske infekcije virusom influence pH1N1 ustanovljeni su u Americi i Meksiku u aprilu 2009. a u Srbiji prvi laboratorijski potvrđen slučaj registrovan je u junu 2009. godine. Cilj rada bio je da se prikaže istraživanje prve dokazane epidemije pandemijske influence H1N1 u Srbiji i opišu klinički i epidemiološki nalazi iz ovog istraživanja. **Metode.** Primenjen je deskriptivni i analitički epidemiološki metod. Izvor podataka bila je medicinska dokumentacija učenika Vojne gimnazije i epidemiološki upitnik. Infekcija pandemijskim virusom H1N1 prvo je dokazana pomoću RT-PCR u nazofaringealnim i orofaringealnim brisevima, a zatim i reakcijom vezivanja komlementa u uzorcima seruma. **Rezultati.** Stopa javljanja akutnog respiratornog oboljenja iznosila je 70.8% (204/288). Infekcija pandemijskim H1N1 virusom potvrđena je kod 44 od 82 testiranih slučajeva akutnog respiratornog oboljenja (53,7%). Najčešće kliničke manifestacije pandemijske influence H1N1 bile su povišena temperatura (88,6%), kašalj (61,4%), malaksalost (38,6%), rinitis (36,4%), glavobolja (29,6%), gušobolja (20,5%) i bol u mišićima (15,9%). **Zaključak.** Rezultati ovog istraživanja ukazuju da je pandemijska influenca H1N1 bila raširena u Vojnoj gimnaziji, ali da nije izazvala teške forme oboljenja.

Ključne reči:
grip a virus, podtip h1n1; epidemije; škole, vojne; srbija.

Introduction

The first cases of the pandemic H1N1 (pH1N1) influenza virus infection were observed in the United States [1] and Mexico [2] in April 2009. Since then virus has spread worldwide to other continents. The first laboratory confirmed case was registered in Serbia on June 24, 2009 [3]. On October 23, 2009 the outbreak of acute respiratory infection (ARI) was declared in a high military school. Based on weekly and monthly reports of the Institute of Public Health of Serbia "Dr Milan Jovanovic Batut", we had information that there was no occurrence of pH1N1 influenza in the epidemic form in Serbia at that time. During the outbreak investigation, pandemic H1N1 virus was confirmed in taken nasopharyngeal and oropharyngeal swabs. This was the first recognized outbreak of the pandemic strain in a boarding school in the Republic of Serbia.

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The aim of this paper was to report on the first recognized outbreak of pH1N1 influenza in a boarding school in Serbia and to describe the clinical and epidemiologic findings from this investigation.

Methods

The Military School (MS) is a high boarding school. The MS is a semi-closed type of school, and the students are allowed to leave the dormitory in the afternoon, after classes. During the outbreak in MS there were 288 students. The students of the 1st grade (n = 87) were located on the ground, 1st and 2nd floors, the 2nd grade students (n = 72) on the 2nd and 3rd floors, the 3rd grade students (n = 81) on the 3rd and 4th floors and the 4th grade students (n = 48) on the 5th floor. Classes were organized so that the students of the 1st and 3rd grades attended school in one shift, while the students of the 2nd and 4th grades in another shift. No one of the students had received vaccine against pH1N1 influenza because vaccine was not available before and during the outbreak.

Descriptive and analytical epidemiological methods were used in this study. The students were divided into groups for comparison based on case definitions, vaccination status, grade and dormitory floor.

Vaccination status was determinated for immunization with the inactivated trivalent influenza vaccine for the 2008–2009 season from the medical records.

The frequency and duration of signs and symptoms, were presented for the groups of cases: ARI cases that had not been laboratory tested, ARI of cases unknown cause (ARI cases that had been laboratory tested but etiologic agent was not found), pH1N1 cases and total ARI cases.

The students health status data (n = 288) were obtained from their medical records and epidemiological questionnaire. Data about symptoms were available for all the affected students (n = 204). Data about the duration of illness were available for 157 students. Vaccination status data was available for 224 of the students. The personnel in the MS was not included in the study.

A clinical case of ARI was defined as a person reporting anyone of the following respiratory symptoms: runny nose, sore throat, or cough.

A case of pH1N1 influenza included the presence of symptoms of ARI with laboratory confirmation by RT-PCR assay or serologic confirmation of a four-fold rise in antibodies to influenza A. Serological confirmation of influenza type A was considered a sufficient evidence for pH1N1 influenza since this strain was predominant at the time of the pandemic and that other strains of influenza type A were present in a very small percentage [3–5].

Nose and throat swabs were taken from 8 ARI cases and tested at the Institute of Vaccines, Virology and Sera, Torlak, Belgrade, Serbia, by the RT-PCR assay for the presence of pH1N1 influenza, seasonal influenza H1N1, influenza H3N2, influence B and Respiratory syncytial virus (RSV).

Serological testing included 94 students divided into two groups: 82 students with symptoms of ARI (including 8 cases tested by RT-PCR assay) and a group of 12 students without ARI symptoms. We tested a group of students without ARI symptoms in order to quantify rates of asymptomatic infection. Paired serum samples were taken at intervals of 4 weeks and tested by complement fixation test (CFT) for the presence of antibodies against influenza virus A and B and adenoviruses. Serological testing of paired sera was carried out at the Military Medical Academy. The results of CFT were considered as positive if there was at least fourfold increase in antibody titers.

After the outbreak we proposed and implemented the following control measures: symptomatic students were placed on the top dormitory floors and asked to stay isolated from the rest of the students and those having high-grade fever were admitted to the school hospital; all the students were advised to strictly follow personal hygiene measures (*eg*, frequent hands and face washing, using a handkerchief while coughing and sneezing); and to avoid group activities and clustering.

To analyze data we used the χ^2 test, Fisher's exact test, calculation of the relative risk and Student's *t*-test. $p < 0.05$ was considered significant. Statistical tests were performed by using Epi Info 6.0 and Statistical Package for the Social Sciences (SPSS) for Windows version 16.0.

Results

The outbreak of this infection in the MS was declared after collecting epidemiological data for 8 students, with symptoms and signs of ARI, who were refered to the Military Medical Academy for medical examination on October 23, 2009. The RT-PCR assay showed the presence of pH1N1 virus. Therefore, epidemiological survey was carried out and control measures proposed.

The collective of the MS were males, aged from 14 to 18 years (16.3 ± 1.1). Body mass index (BMI) of all the students was in normal range (BMI = 21.9 ± 2.3 kg/m^2), with no chronic disease.

This epidemiological investigation showed that the outbreak of ARI began among the students of the 3rd grade on October 12, 2009. Figure 1 presents the number of ARI cases by grades in this outbreak. The number of ARI cases reached the peak among the students of the 3rd grade on October 22, in the students of the 1st grade on October 23, in the students of the 4th grade on October 26 and in the students of the 2nd grade on November 1. The first wave of the epidemic spread among the 1st and 3rd grade students and the 2nd wave involved the students of the 2nd and 4th grades. A total of 204 students were affected by the end of the outbreak.

Table 1 shows that acute respiratory infections spread to all grades of the students even though they were stationed at different floors of the dormitory. The ARI attack rate among the students depending on the grades ranged from 63.9% to 77.1%, and by the dormitory floors from 64% to 83% as shown in Table 1. The total attack rate of ARI was 70.8% (204/288), and the attack rate of pH1N1 influenza was not found because we did not test all the ARI cases for pH1N1.

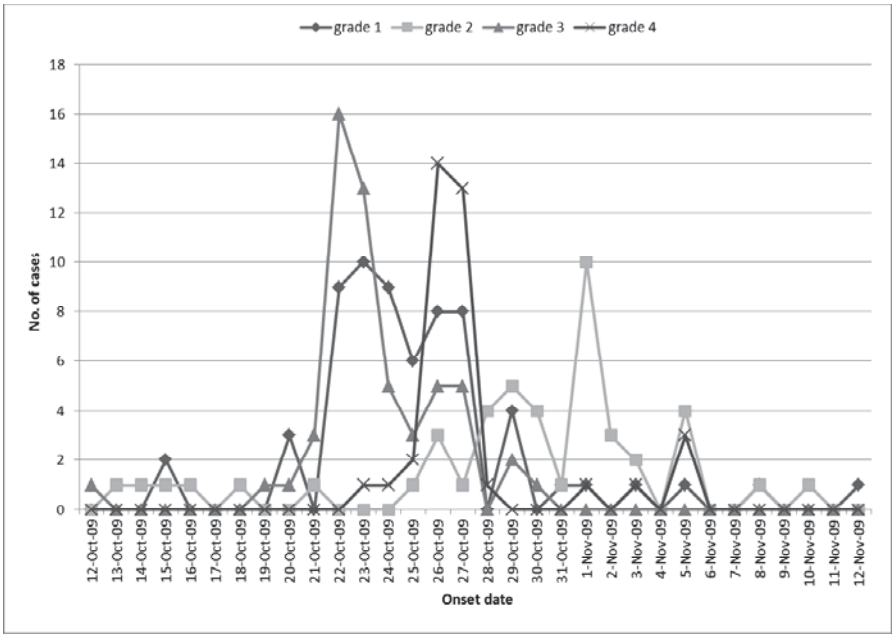

Fig. 1 – The cases of acute respiratory infection (ARI) in the Military School, presented by the school grades.

Table 1

Attack rate of acute respiratory illness (ARI) in the Military School students by floors and grades

Grade	Floor						Total
	0	I	II	III	IV	V	
I	18/22 (82)	33/44 (75)	14/21 (67)	0	0	0	65/87 (75)
II	0	0	24/35 (69)	22/37 (65)	0	0	46/72 (64)
III	0	0	0	15/21 (71)	41/60 (68)	0	56/81 (69)
IV	0	0	0	0	0	37/48 (77)	37/48 (77)
Total	18/22 (82)	33/44 (75)	38/56 (68)	37/58 (64)	41/60 (68)	37/48 (77)	204/288 (71)

Note: Data are presented as the number of ARI cases/students (%).

The results of swabs tested by the RT-PCR assay confirmed the presence of a pH1N1 virus in 4/8 (50%) samples. Nasopharyngeal swabs were also tested for seasonal influenza H1N1, influenza H3N2, influenza type B and respiratory syncytial virus (RSV) and results were negative. The results of serological tests for the presence of influenza type B and adenoviruses were negative in all tested samples. However, in paired sera of 44 persons (including 4 cases that were positive for pH1N1 by RT-PCR assay) a fourfold increase in titer to influenza A was registered. CFT to influenza type A was found positive in 44/82 (53.7%) of the affected individuals. In the remaining 38/82 (46.3%) ARI cases (including 4 cases that remained negative by RT-PCR assay testing) the etiologic agent was not established. In the group of students without ARI symptoms seroconversion was not detected.

The cases of ARI and pH1N1 influenza in the Military School, during October and November 2009, are presented in Figure 2. The first confirmed case of pH1N1 influenza at the Military School developed symptoms on October 12, 2009. Two large peaks can be observed in this outbreak. The first peak occurred on October 22 and the second, higher one on October 26.

In order to determine whether pH1N1 influenza was clinically different from other ARI, the frequencies of signs and symptoms were examined separately (Table 2). Comparison of the representation of certain symptoms and signs of disease revealed that the pH1N1 cases were significantly more frequently malaise ($p = 0.021$) and had dry cough ($p = 0.029$) compared to the cases of ARI of unknown cause. Other signs and symptoms showed no statistically significant differences between the investigated groups of patients.

The most common clinical manifestations of pH1N1 influenza were fever (88.6%), cough (61.4%), dry cough (50%), malaise (38.6%), runny nose (36.4%), headache (29.6%), sore throat (20.5%), muscle pain (15.9), while diarrhea and vomiting were rarely registered. Fever over 39°C was observed in 7 (15.9%) of the patients, while 5 patients had no fever.

Illness duration was determined in 157 patients, and it was in the range from 1 to 6 days. The average illness duration in the ARI outbreak was 2.71 ± 1.35 days (Table 3). By comparing the values of the average duration of illness in patients with pH1N1 influenza (3.00 ± 1.49 days) and in patients with ARI of unknown cause (2.50 ± 1.13), there was no statistically significant difference [Student's *t*-test, $t = 1.636$; $df = 74$; $p = 0.106$; 95% Confidence interval (CI), -0,109–1,109)].

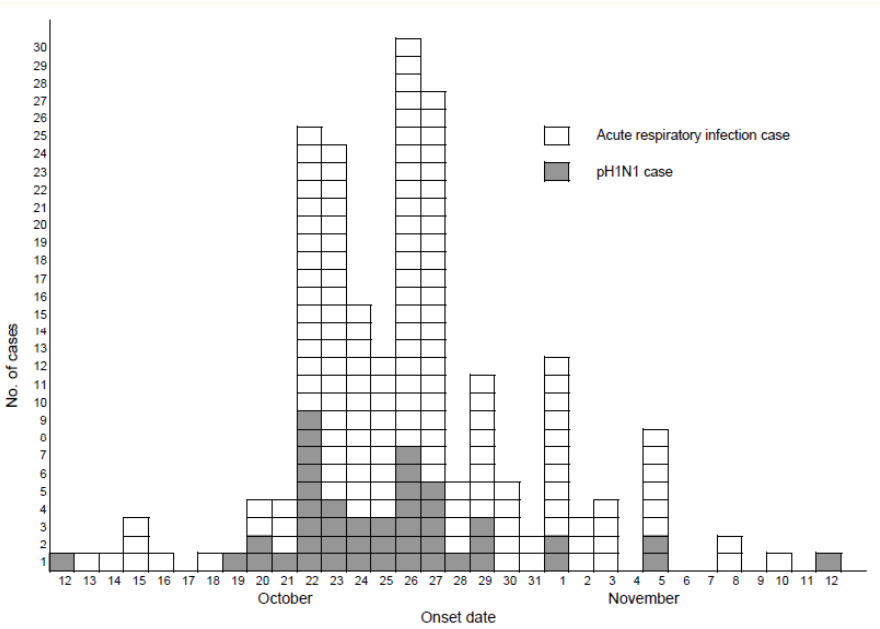

Fig. 2 – Clinical acute respiratory infection and pandemic H1N1 influenza in the Military School, October– November 2009 (n = 204).

Table 2

Symptoms and signs of acute respiratory infection (ARI) in the affected students (n = 204)

Symptoms and signs of ARI	ARI (n = 204) n (%)	ARI of unknown cause (n = 38) n (%)	pH1N1* (n = 44) n (%)	pH1N1 *vs* ARI unknown cause RR (95% CI); *p*
Headache	44 (21.57)	9 (23.68)	13 (29.55)	1.25 (0.60–2.29); 0.550 ‡
Malaise	45 (22.06)	6 (15.79)	17 (38.64)	2.45 (1.07–5.57); 0.021 ‡
Runny nose	75 (36.76)	10 (26.32)	16 (36.36)	1.38 (0.71–2.67); 0.330 ‡
Sore throat	37(18.14)	5 (13.16)	9 (20.45)	1.55 (0.57–4.24); 0.381 ‡
Sneezing	31(15.20)	4 (10.53)	6 (13.64)	1.30 (0.39–4.25); 0.668 †
Dry cough	67 (32.84)	10 (28.95)	22 (50.00)	1.90 (1.03–3.49); 0.029 ‡
Productive cough	19 (9.31)	4 (10.53)	5 (13.64)	1.08 (0.31–3.73); 1.000 †
Shortness of breath	6 (2.94)	1 (2.63)	3 (6.82)	2.59 (0.28–23.88); 0.620 †
Conjunctivitis	4 (1.96)	1 (2.63)	3 (6.82)	2.59 (0.28–23.88); 0.620 †
Diarrhea	9 (4.41)	1 (2.63)	3 (6.82)	2.59 (0.28–23.88); 0.620 †
Nausea	5 (2.45)	0 (0.00)	3 (6.82)	NA; 0.245 †
Vomiting	5 (2.45)	1 (2.63)	2 (4.55)	1.73 (0.16–18.31); 1.000 †
Muscle ache	21 (10.29)	4 (10.53)	7 (15.91)	1.51 (0.48–4.77); 0.476 ‡
Joint pain	7 (3.43)	0 (0.00)	2 (4.55)	NA; 0.497 †
Temp. 37–37.9°C	103 (50.49)	20 (52.63)	18 (40.91)	0.78 (0.49–1.24); 0.288 ‡
Temp. 38–38.9°C	54 (26.47)	6 (15.79)	14 (31.82)	2.02 (0.86–4.73); 0.092 ‡
Temp. > 39°C	20 (9.80)	7 (18.42)	7 (15.91)	0.86 (0.33–2.24); 0.763 ‡

*** – pandemic H1N1 influenza; † – Fisher's exact test; ‡ – χ^2 test;**
RR – relative risk;
CI – confidence interval.

Table 3

Duration of illness in the cases of acute respiratory infections (ARI; n = 157), including the pandemic H1N1 influenza cases (n = 40)

Duration of clinical illness (days)	No laboratory tested ARI (n = 81) n (%)	ARI unknown cause (n = 36) n (%)	pH1N1 (n=40) n (%)	Total ARI (n = 157) n (%)
1	17 (20.99)	7(19.44)	9 (22.50)	33 (21.02)
2	25 (30.86)	11 (30.56)	6 (15.00)	42 (26.75)
3	19 (23.46)	14 (38.89)	11 (27.50)	44 (28.03)
4	11 (13.58)	2 (5.56)	4 (10.00)	17 (10.83)
5	6 (7.41)	1 (2.78)	10 (25.00)	17 (10.83)
6	3 (3.70)	1 (2.78)	0 (0.00)	4 (2.55)
$\bar{x} \pm SD$	2.67 ± 1.351	2.50 ± 1.134	3.00 ± 1.485	2.71 ± 1.345

Clinical manifestations in the majority of the patients could be described as mild. Severe complications were not observed during the outbreak. Only 9 cases of bronchitis were registered, but they required no hospitalization.

We found that the inactivated trivalent influenza vaccine for the 2008–2009 season received more than half of the students, 167/288 (58%), and 57/288 (20%) received no vaccine. For the remaining students, 64/288 (22%), vaccination status was unknown.

Among not vaccinated, 47/57 (82.5%) were those affected by ARI, while among vaccinated that number was significantly lower, 107/167 (64.1%) (RR, 1.29; 95% CI, 1.09 -1.52, $\chi^2 = 6.69$; $p = 0.010$).

For 67 patients there were vaccination status data and the results of serological tests. Of 13 persons who did not received trivalent influenza vaccine 2008–2009, 9 (69.2%) showed seroconversion for influenza A *vs* 21/54 (38.9%) of those that received vaccine (RR 1.78; 95% CI, 1.09–2.91; $\chi^2 = 3.90$; $p = 0{,}048$; Yates corrected: $\chi^2 = 2.77$; $p = 0{,}096$).

Discussion

Influenza virus was easily and rapidly spreading among the MS students. It is likely that the widespread susceptibility of persons under 60 years allowed such a rapid dissemination of the virus [4–6]. The descriptive analysis suggests that household contacts who were under 18 years of age were at significantly higher risk for ARI and influenza-like illness (ILI) than contacts who were 19 to 50 years of age [7]. The first epidemiological reports referring to the pH1N1 influenza in 2009 indicated that the cases of infections and deaths were mostly among adults aged 20–40 years [8]. People born before 1957 were in the lower risk of infection with pH1N1 influenza in 2009. Lower number of infections was not only a reflection of lower testing in this age group. The mechanism that explains this association is not entirely clear but is consistent with the findings of its age related increase in the prevalence of titer of neutralizing antibodies against the pH1N1 virus [9] and may be a reflection of immunity resulting from exposure to a similar virus in life. Maximally effective host immune response to influenza can be arisen by earlier infections throughout life [10]. This is consistent with a high incidence of pH1N1 influenza outbreak in 2009 at schools [11] and reduced the frequency of outbreaks in nursing homes.

Two waves of outbreak were recorded MS. The first wave affected students of 1st and 3rd grades, and the 2nd one the students of the 2nd and 4th grades. This is understandable because the students of the 1st and 3rd grades and the students of the 2nd and 4th grades attended school in the same shift.

A fourfold increase of antibodies against influenza A titer was found in 44 of 82 ARI cases. Based on RTPCR assay findings and epidemiological situation we concluded that influenza A infection was confirmed due to pandemic pH1N1 virus.

The attack rate of ARI was 70.8% in this outbreak. Since the influenza type A was determined by serological test in 53.7% (44/82) of the tested ARI cases it can be estimated that the attack rate of pH1N1 influenza was approximately 38%. During an outbreak at a residential school at Panchgani, Maharashtra, India, the clinical attack rate for ILI in students was 76.4% and the attack rate for pH1N1 influenza cases was 42.4 % [12]. In April 2009 the first recorded school outbreak of pH1N1 influenza occurred in a New York City high school and infected nearly 30% of the student's population [13]. Epidemiological investigation of an outbreak of pH1N1 influenza at a boarding school in China showed a 22.2% attack rate and a 32% infection rate in students aged 15–21 years old. The incidence rate of boarders was higher than day-boarders [14]. On the basis of published data it can be concluded that the incidence of pH1N1 influenza at schools ranged from 8% in England [15] to 42% in India [12]. The higher attack rate of ARI and pH1N1 influenza in this outbreak in comparison to most other described outbreaks at schools can be explained by the specifics of the school where besides traditional classes, students have other additional common activities (sharing dormitory space, sport activities, learning in reading room, meals in the school restaurant etc.)

In the group of students without ARI symptoms seroconversion for influenza was not detected. Our findings suggested no asymptomatic seroconversion. In the outbreak that occurred at a vocational boarding school in Guangzhou, P.R. China, 156 asymptomatic patients (9.9%) of the 1570 participating students were confirmed to be infected by pH1N1 virus [14].

Clinical manifestations in patients during the outbreak in the MS were: fever (88.6%), cough (61%) and malaise (38.6%), runny nose (36.4%), headache (29.6 %), sore throat (20.5%) and muscle ache (15.9%). Fever and cough were the most common symptoms also in investigations of other researchers, and malaise was not taken into consideration by other researchers. Overall, the symptoms were mild and there were no hospitalizations.

In pH1N1 influenza outbreak in a New York City high school, clinical manifestation among confirmed cases were fever 93%, cough 90%, headache 79%, sore throat 76%, muscle ache 76%, runny nose 69%, nausea 46%, diarrhea 26% and vomiting 17% [13].

During the outbreak of pH1N1 influenza in a residential school in India, clinical presentations in ILI cases were fever (100%), cough (100%), nasal discharge (30%), headache (16.9%), sore throat (15.6%), body-ache (9.8%), fatigue (4.4%), vomiting (3.4%) and diarrhea (1.4%). There was no significant difference between clinical presentation of ILI and confirmed pH1N1 influenza cases. Analysis of the pH1N1 influenza in Serbia in 2009/2010 season in general population showed that the leading symptoms were fever (83.0%), dry cough (75.2%) and muscle aches (51.8%) [3]. Clinical manifestations in pandemic flu patients hospitalized in the Clinic for Infectious and Tropical Diseases, Military Medical Academy, were fever (100%), fatigue (95.9%), cough (82.6%), headache (66.3%), while dyspnea and diarrhea were registered in ¼ of the patients [16].

The mean duration of the illness among pH1N1 cases during the outbreak in the MS was 3 days. In an outbreak at a school in New York the mean duration of the illness in the confirmed cases of pH1N1 influenza was 6 days, and 75%

had recovered by 9 days after the onset of symptoms [13]. The mean duration of illness (*ie*, symptomatic period) was 4 days during the outbreak of pH1N1 influenza at a residential school in India [12]. Reasons for a shorter duration of the illness in our patients were probably a good physical fitness of students and the absence of risk factors such as chronic diseases and obesity.

Based on our results we can say that the seasonal influenza vaccine 2008–2009 provided a certain level of protection against pH1N1 virus. The results of the effect of inactivated seasonal influenza vaccination on risk of pH1N1 influenza in a cohort of nurses in Canada who participated in a recent randomized controlled trial suggest a possible positive effect of 2008–2009 trivalent inactivated seasonal influenza vaccine reducing the risk of infection with pH1N1 influenza [17]. In a study of seasonal influenza vaccine and protection against pandemic (H1N1) 2009-associated illness among US military personnel was found moderate association with protection against clinically apparent, laboratory-confirmed pandemic (H1N1) 2009-associated illness for immunization with either TIV or LAIV 2008–2009 seasonal influenza vaccines. This association with protection was found to be especially apparent for severe disease as compared to milder outcome, as well as in the youngest and older populations. Prior vaccination with seasonal influenza vaccines in 2004–2008 was also independently associated with protection [18].

Conclusion

School population was very favorable for pandemic influenza H1N1 virus sprending. In high school population, pandemic influenza had milder clinical manifestations. Clinical manifestations of the 2009 H1N1 influenza virus appear to be similar to those of previously observed in seasonal influenza. Our finding suggests that the seasonal influenza vaccine 2008–2009 provided a certain level of protection against pandemic influenza A (H1N1) virus.

This study illustrates the significance of field epidemiologic investigations for understanding an emergent threat. This was particularly relevant during the emergence of pandemic (H1N1) virus when its pathogenicity was uncertain.

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Comparative radiographic and resonance frequency analyses of the peri-implant tissue after dental implants placement using flap and flapless techniques: An experimental study on domestic pigs

Komparativna radiografska analiza i analiza rezonantne frekvencije periimplantatnog tkiva nakon ugradnje dentalnih implantata primenom hirurške tehnike *flap* i *flapless*: eksperimentalna studija na domaćim svinjama

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Abstract

Background/Aim. Flapless implant surgery has become very important issue during recent years, mostly thanks to computerization of dentistry and software planning of dental implants placements. The aim of this study was to compare flap and flapless surgical techniques for implant placement through radiographic and radiofrequency analyses. **Methods**. The experiment was made in five domestic pigs. Nine weeks following domestic pigs teeth extraction, implants were placed, on the right side using surgical technique flap, and flapless on the left side. Digital dental X-rays were applied to determine primary dental implant stability quality (ISQ). At certain intervals, not later than three months, the experimental animals were sacrificed, and just before it, control X-rays were applied to measure dental implants stability. **Results.** Radiographic analysis showed that peri-implant bone resorption in the first 4 weeks following placement implants with flap and flapless surgical techniques was negligible. After the 3 months, mean value of peri-implant bone resorption of the implants placed using flap tehnique was 1.86 mm, and of those placed using flapless tehnique was 1.13 mm. In relation to the primary dental implant stability in the first and second week there was an expected decrease in ISQ values, but it was less expressed in the dental implants placed using the flapless technique. In the third week the ISQ values were increased in the dental implants placed by using both techniques, but the increase in flapless implant placement was higher (7.4 ISQ) than in flap implant placement (1.5 ISQ). The upward trend continued in a 4-week period, and after 3 months the dental implant stability values in the implants placed using flap technique were higher than the primary stability for 7.1 ISQ, and in the implants placed using flapless technique were higher comparing to the primary stability for 10.1 ISQ units. **Conclusion**. Based on the results of radiographic and resonance frequency analyses it can be concluded that the flapless technique in surgical implants placemat, leads to better results.

Key words:
dental implantation, endosseous; surgical flaps; dental implants; pigs; osseointegration.

Apstrakt

Uvod/Cilj. Implantatna hirurgija *flapless* postaje aktuelna poslednjih nekoliko godina zahvaljujući kompjuterizaciji stomatologije i softverskog planiranja ugradnje implantata. Cilj rada bio je da se radiografskom analizom i analizom rezonantne frekvencije uporede hirurške tehnike *flap* i *flapless* ugradnje implantata. **Metode.** Eksperiment je obavljen na pet domaćih svinja. Devet nedelja nakon ekstrakcije zuba, svinjama su ugrađeni implantati, sa desne strane hirurškom tehnikom *flap*, a sa leve hirurškom tehnikom *flapless*. Urađeni su digitalni radiološki snimci i izmerena primarna stabilnost implantata (ISQ). U određenim vremenskim intervalima do tri meseca, eksperimentalne životinje su žrtvovane, a neposredno pre rađeni su kontrolni rendgenski snimci i merena stabilnost implantata. **Rezultati.** Radiografskom analizom uočeno je da je periimplantatna koštana resorpcija u prve četiri nedelje bila zanemarljiva kod obe hirurške tehnike. Nakon tri meseca srednja vrednost periimplantatne koštane resorpcije kod *flap* implantata bila je 1,86 mm, a kod *flapless* implantata 1,13 mm. U odnosu na primarnu stabilnost implantata, u prvoj i

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drugoj nedelji došlo je do očekivanog pada vrednosti ISQ, ali je taj pad kod *flapless* implantata bio manji. U trećoj nedelji rasle su vrednosti ISQ kod *flap* i *flapless* implantata, ali je porast kod *flapless* implantata bio veći (7,4 ISQ) u odnosu na *flap* implantate (1,5 ISQ). Tendencija rasta se nastavila i u četvrtoj nedelji i nadalje, a nakon tri meseca vrednosti stabilnosti implantata kod *flap* implantata bile su veće u odnosu na primarnu stabilnost za 7,1 ISQ, a kod *flapless* implantata za 10,1 ISQ. **Zaključak.** Na osnovu rezultata radiografske analize i analize rezonantne frekvencije možemo zaključiti da hirurška tehnika ugradnje implantata *flapless* daje bolje rezultate od hirurške tehnike *flap*.

Ključne reči:
stomatološka enosalna implantacija; režnjevi, hirurški; implantati, stomatološki; svinje; oseointegracija.

Introduction

Progress made in the production technology of surface and design of dental implants makes a significant contribution to improving osseointegration characteristics of advanced implants, and the development of radiographic technology has greatly improved preciseness in the process of planning dental implant site.

Many authors suggest that minimally invasive implant surgery additionally improves function, esthetics and comfort [1] promoting early rehabilitation of patients in both functional as well as esthetic implant-prosthetics aspects [2].

Each implant surgery starts with gingival incision, that leads to different results depending on the way of incision [3]. Exposing bone surface for implant placement could be performed in two ways: classical flap method using raising mucoperiostal flap and flapless method, *ie* without raising a mucoperiostal flap.

The flap surgical method means placing an implant in which, after gingival incision is made, a mucoperiostal flap, is raised on the vestibular surface, making the bone on the alveolar ridge exposed. After implant site preparation and implant placement, the flap is sutured [1, 3, 4].

The flapless surgical procedure causes less crestal soft tissue damage. Application of this method – its name is self-explanatory (flap + less), no mucoperiostal flap while placing dental implant, and, therefore, the consequent trauma of peri-implant tissues is smaller. It can be performed in two ways.

The first way is to remove a part of the gingival tissue (size is matching with the implant diameter) above the alveolar bone, exceeded with the punch (round like) knife, then to preparation the bone for implant site starting from the centre of the exposed bone and subsequent dental implant placement (unsubmerged technique). Another way is to perform mini crestal incision and local uplift of mucoperiosteum, only up to the diameter of mini implant that we plan to place (3–5 mm). After implant site preparation and implant placement, mini incisal line is sutured with single suture (submerged technique) [1]. It is worth mentioning that the technique of flapless implant placement 3D panoramic radiograph of the jaw, as well as surgical stent are required.

Radiographic industry development (3D panoramic radiograph and cone beam appliances), as well as computerization of dentistry itself, enabled daily application of flapless dental implant placement technique, therefore researches on comparative analysis of the degree of success of flap and flapless placing implants techniques have been very present in the past few years. In research and comparative analysis of the results on flap and flapless dental implant surgery the parameters used are as follows resonance frequency analysis, radiographic (2D and 3D) analysis, clinical measurement of post operative edema of peri-implant mucosa, probing and determination of sulcus depth around the dental implant, determination of gingival index and gingival bleeding index (GBI) [5, 6], as well as survey testing of patients in terms of anxiety, subjective postoperative discomfort (pain, swelling, recovery) [7].

Resonance frequency analysis is a non-invasive diagnostic method that allows clinical measurement of dental implants stability and monitoring of tissue biological response and osseointergration as a function of time. Resonance frequency analysis (RFA) uses a sophisticated technology with computer-based measurement of resonance frequency (RF), which is determined by two parameters: the degree of bone density on implant-bone interface area and the level of marginal alveolar bone around the transducer [8].

Radiographic procedure in the assessment of peri-implant bone resorption is less invasive and far more practical than direct visualization, although radiography can only analyse proximal bone surfaces.

To reliably estimate peri-implant bone change radiographic images can be repeated at different observational intervals, while rulers, vernier calipers, digital measurers, optical comparators for measuring such changes are used. Computer technique for measuring peri-implant bone is considered to be the most reliable one comparing to another methods and therefore for peri-implant bone resorption analysis intraoral radiography and panoramic radiography supplemented with digital radiography are used nowadays [9, 10].

The aim of this study was to compare of flap and flapless surgical techniques of implant placement through radiographic and radiofrequency analyses.

Methods

In order to achieve the set goals experimental research on 5 domestic pigs, aged 2 and a half to 3 months, weight 20–25 kg were performed. The study included radiography and RFA. The experiment was conducted in 4 phases.

The first phase

During the short-term effect of intravenous anesthesia (ketamine 1 mL/10 kg *iv*) with prior premedication (acepro-

mazine 0.1 mL/kg and 0.5 tramadol *im*) and local application of local anesthetic (lidocaine 2% with adrenaline 2 mL), the second and third premolars in the lower jaw on both sides were extracted. Prior to the surgical procedure animals were deprived of food (24 h) and water (12 h). Teeth were extracted to place implants in the position of premolars following wounds healing. The premolars in the lower jaw belong to the group of chewing teeth with very divergent and gracile roots, that is the reason for separation to precede extraction in order to make a minimum trauma to the surrounding bone and soft tissue.

Extraction wounds were sutured with individual surgical sutures and absorbable surgical sutures (Polysorb 3,0 Braun) and for 9 weeks left to heal secondary. According to the literature [11], a 9-week period is sufficient for bone healing, since the rate of bone healing in domestic pigs is somehow higher comparing to human.

Antibiotics [procaine penicilline with neomycine, (Neopenicillin® 4.000.000 "FM farm", Subotica); dose was 6–12.000 *im*] were administered in the experimental animals after teeth extraction for 5 days. After finishing anesthesia, 12 h later, food and water were given to the study animals. In a post-surgical period they were fed with mushy food during 7 days, kept in a purpose-made, experimental box with daily veterinary supervision. Conditions in the experimental box were in accordance with the current protocol for this kind of work: air temperature 18–24°C (± 2°C), humidity 60–70%, air velocity 0.2 m/s, illuminance 100 lux, with 1.40 kg of diet for finishing pigs (minimum 16% protein) and with automatic watering (flow rate 0.75 L/min, t° of water 18°C).

The second phase

Nine weeks later, anesthesia was given to the experimental animals *iv* with prior premedication and the protocol of implant placement in edentulous segments of the lower jaw with application of local anesthetic in the same manner like in teeth extraction. Each animal got three implants (Bredent, Blue Sky 3.5 × 10 mm) placed on each side as follows: on the right side three implants were placed with flap (submerged) technique (Figure 1); on the left side three implants were placed with flapless, mini-incisional technique (Figure 2).

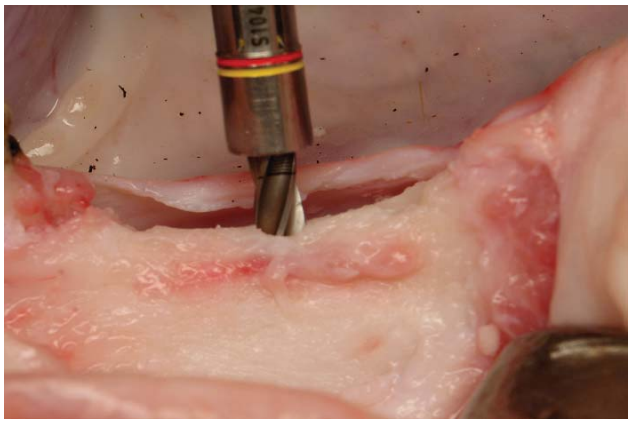

Fig. 1 – The flap tehnique – surgical preparation of the implant site.

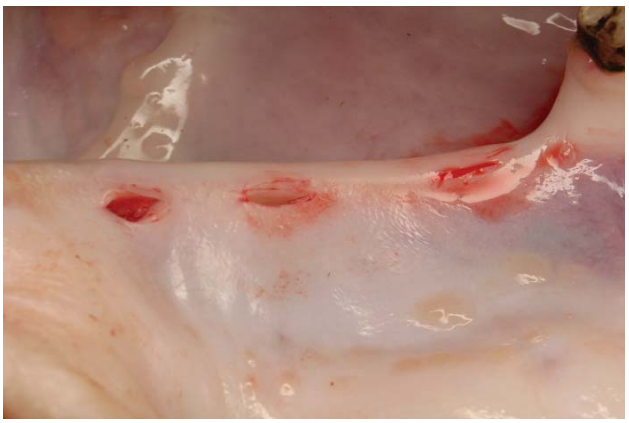

Fig. 2 –The flapless technique (mini incisions).

The incisions were sutured with single resorbable surgical sutures thread (Polysorb 4/0 Braun) and postoperative antibiotics therapy was administered as it was the case in the previous phase with daily veterinary supervision.

Upon completion of implants placement the primary stability of each implant was measured (ISQ – implant stability quality) with resonance frequency analysis using an ostell mentor instrument (Figure 3). Osstell is representative of RFA-technique and was tested first in 1997 [8].

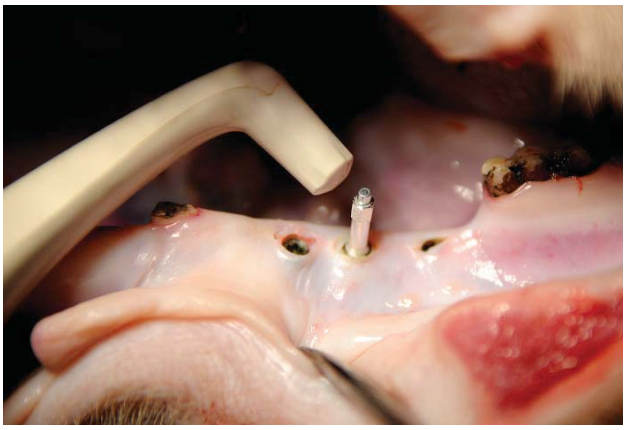

Fig. 3 – Primary stability measuring – flapless implants.

The apparatus consists of an Osstell transducer and Osstell analyzer connected to a PC or independent. The transducer is L-shaped or bolts-like (smartpeg) and firmly screwed in order to be positioned on the implant and its superstructure (4–5 N/cm²) and consists of 2 small voltage-controlled transducers. High-energy pulse-type oscillations of a continuous sinusoidal pulse excite implant, in order to register the mechanical vibration between the implant interconnection zone and the bone. As soon as the apparatus is activated the first electric transducer applies excitation signal of increasing frequency from 5 to 15 kHz to implant. Other voltage-controlled element registers ultrasonic vibrations response, *ie* resonant frequency of the implant-bone interface area and transmits a created signal to the amplifier, which amplifies it and to the analyzer which reads, evaluates and compares it with the frequency of the original signal.

The measured amplitude of resonance frequency is displayed numerically and graphically on the Osstell analyzer, and the maximum amplitude represents the stability of the implant, quantified through the ISQ units. The ISQ value reflects the rigidity of the system transducer-implant-bone and transducer calibration parameters. Measured on a scale from 0 ISQ (3500 Hz) to 100 ISQ units (8500 Hz), a higher ISQ value indicates a greater stability of the implant. After a while ISQ values rise because of osseointegration where implant–bone the connection becomes stronger.

At the same time dento-alveolar digital radiographs were made of parts of the mandible with placed implants using a Gendex device and Dentsply digitilization, with an X-ray distancer (made by the same producer) for standardization of the obtained X-ray images.

The third phase

According to the protocol the animals were sacrificed at various intervals of time (1 week, 14 days, 21 days, 28 days and 3 months upon implants placement) but immediately before it ISQ was measured and retroalveolar radiographic images performed of the parts of the mandible with implants.

Radiographic analysis included bone resorption measuring.

The dimensions of bone tissue loss were measured at certain time intervals and compared with the original height of each bone implants.

Measurements were carried out on the mesial and distal surface of each implant, and then the mean bone tissue loss determined in relation to the periods of sacrificing experimental animals (Figure 4). The error which might occur due to imaging techniques is corrected by determining the index as following:

Measured hight of implant (L') / Actual hight of implant (L = 10 mm) = Measured resorption of bone tissue (R) / periimplant bone resorption (PIBR)

$$PIBR = R \times L / L'$$

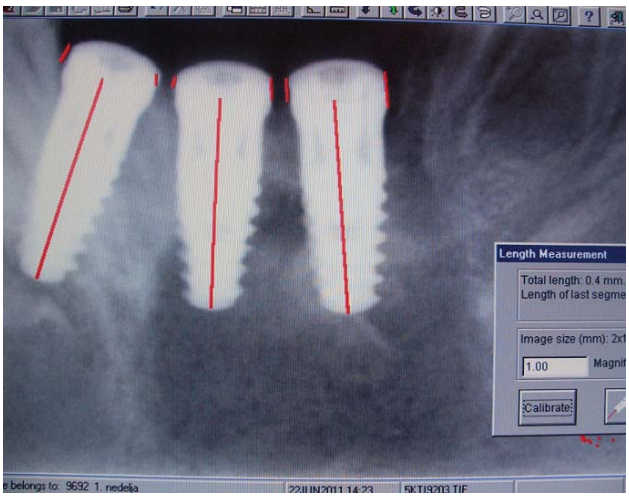

Fig. 4 – Radiographic analysis – peri-implants bone resorption measuring.

The fourth phase

At this stage statistic analysis and processing of the obtained results were performed, using repeated measures ANOVA with time (dependent variable measured after 7, 14, 21, 28 and 90 days) as within-subject factor, and method (flap *vs* flapless) as between-group order to factor.

Results

Embedded implant stability measurements were performed in all 30 implants on 4 sides of the implant (mesial, distal, buccal and lingual) immediately after their placement (primary stability) and immediately after experimental animals sacrifice and then the mean values of implants stability were calculated. The values of the primary stability of implants were taken initially, and then variations in the stability of the implant in relation to the primary stability at certain time intervals were measured, after the sacrifice of experimental animals. Statistically significant difference in stability was found between the methods ($F_{(1,4)} = 9.42$, $p = 0.037$). Neither the main effect of time [the difference in stability between the time points (Wilks lambda = .006, $F_{(4,1)} = 40.74$, $p = 0.117$)], nor the interaction (the difference in the shapes of recovery trajectories) were found to be significant (Wilks lambda = .029, $F_{(4,1)} = 8.43$, $p = 0.252$).

The obtained results are shown in Tables 1 and 2 and figure 5.

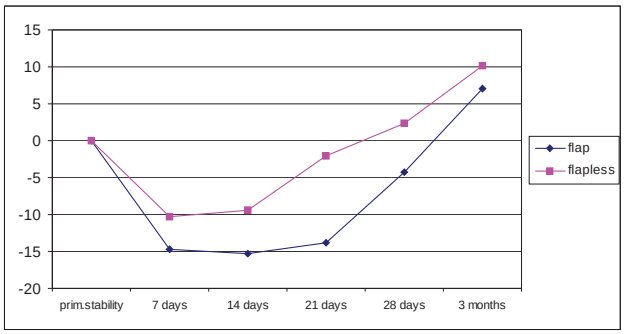

Fig. 5 – The ratio of deviation of implants stability values in relation to the primary stability of flap and flapless surgical techniques.

Radiographic analysis of digital X-ray images was used for measuring peri-implant bone resorption (PIBR) on mesial and distal surfaces of the implant at regular intervals. An error that might occur due to a recording technique was corrected through the appropriate index, thus calculating the mean value of peri-implant bone resorption for certain periods from the time of implants placement. The obtained values are shown in Tables 3 and 4. Bone resorption was analyzed, too. Again, a statistically significant difference between methods was found ($F_{(1,4)} = 32.45$, $p = 0.005$). The main effect of time (the difference in bone resorption between the time points) was significant (Wilks lambda = 0.006, $F_{(3,2)} = 173.06$, $p = 0.006$). The interaction (the difference in the shapes of the bone resorption trajectories) was marginally significant (Wilks lambda = .052, $F_{(3,2)} = 12.21$, $p = 0.077$).

The obtained results are shown in the Figure 6.

Table 1

Deviations of flap implant stability compared to primary stability at certain time intervals shown in Implant Stability Quality (ISQ) units

Experimental animal	Primary stability	After 7 days	After 14 days	After 21 days	After 28 days	After 3 months
No. 1	Implant I 73,73,72,73 Implant II 69,72,69,73 Implant III 63,63,63,65 Mean 69,0	Implant I 52,53,57,52 Implant II 60,56,52,52 Implant III 55,55,55,54 Mean 54,4				
No. 2	Implant I 57,58,58,53 Implant II 63,63,63,63 Implant III 56,56,59,60 Mean 59,1		Implant I 44,44,43,44 Implant II 46,45,46,48 Implant III 42,42,42,41 Mean 43,2			
No. 3	Implant I 78,78,82,71 Implant II 80,78,82,82 Implant III 79,84,84,84 Mean 80,1			Implant I 62,63,62,61 Implant II 62,63,63,63 Implant III 74,74,74,76 Mean 66,4		
No. 4	Implant I 62,62,59,60 Implant II 77,74,77,78 Implant III 63,63,65,63 Mean 66,9				Implant I 58,58,58,58 Implant II 69,69,70,70 Implant III 60,60,60,61 Mean 62,6	
No. 5	Implant I 69,69,71,68 Implant II 59,59,61,59 Implant III 55,55,49,56 M.value 60,0					Implant I 74,74,77,76 Implant II 66,64,64,64 Implant III 62,62,61,61 Mean 67,1
Deviations		- 14,6	- 15,2	- 13,7	- 4,3	+ 7,1

Table 2

Deviations of flapless implant stability compared to primary stability at certain time intervals shown in Implant Stability Quality (ISQ) units

Experimental animal	Primary stability	After 7 days	After 14 days	After 21 days	After 28 days	After 3 months
No. 1	Implant I 69,68,68,66 Implant II 62,64,68,62 Implant III 70,70,66,70 Mean 66,9	Implant I 53,60,57,57 Implant II 58,52,57,57 Implant III 56,58,57,58 Mean 56,6				
No. 2	Implant I 57,57,52,54 Implant II 72,70,69,72 Implant III 73,69,73,70 Mean 65,7		Implant I 49,49,49,51 Implant II 62,60,60,60 Implant III 59,59,59,59 Mean 56,3			
No. 3	Implant I 62,64,62,64 Implant II 72,69,69,72 Implant III 65,61,57,58 Mean 65,1			Implant I 60,60,59,60 Implant II 70,70,70,72 Implant III 57,57,60,62 Mean 63,1		
No. 4	Implant I 76,76,80,77 Implant II 62,62,60,63 Implant III 57,51,57,60 Mean 65,1				Implant I 80,82,82,82 Implant II 63,61,61,65 Implant III 60,57,57,59 Mean 67,4	
No. 5	Implant I 69,69,71,68 Implant II 59,59,61,59 Implant III 55,55,49,56 Mean 60,0					Implant I 79,79,77,76 Implant II 70,,69,69,69 Implant III 64,66,66,66 Mean 70,1
Deviations		- 10,3	- 9,4	- 2,0	+ 2,3	+ 10,1

Table 3

The values of peri-implant bone resorption (PIBR) after application of surgical flap techniques of implants placement

Experimental animal	After 7 days	After 14 days	After 21 days	After 28 days	After 3 months
No. 1	Implant I ms. Ø ds. Ø Implant II ms. Ø ds. Ø Implant III ms. Ø ds. Ø				
No. 2		Implant I ms. Ø ds. Ø Implant II ms. Ø ds. Ø Implant III ms. Ø ds. Ø			
No. 3			Implant I ms. 0,1 ds. Ø Implant II ms. 0,1 ds.0,2 Implant III mz.0,1 ds.0,1		
No. 4				Implant I ms.0,2 ds 0,4. Implant II ms 0,3. ds 0,2. Implant III ms.0,3 ds .0,4	
No. 5					Implant I ms.1,9 ds.1,8 Implant II ms.1,8 ds 2,0. Implant III ms 2,1 ds.1,6
P I B R (mean)	Ø	Ø	0,10 mm	0,30 mm	1, 86 mm

(ms = mesial, ds = distal)

Table 4

The values of peri-implant bone resorption (PIBR) after application of surgical flapless techniques of implants placement

Experimental animal	After 7 days	After 14 days	After 21 days	After 28 days	After 3 months
No. 1	Implant I ms. Ø ds. Ø Implant II ms. Ø ds. Ø Implant III ms. Ø ds. Ø				
No. 2		Implantat I ms. Ø ds. Ø Implantat II ms. Ø ds. Ø Implantat III ms. Ø ds. Ø			
No. 3			Implant I ms.0,1 ds 0,1. Implant II ms. 0,1 ds. Ø Implant III ms.0,1 ds 0,1		
No. 4				Implant I ms.0,3 ds.0,2 Implant II ms 0,1 ds.0,3 Implant III ms.0,3 ds 0,3.	
No. 5					Implant I ms 1,7 ds. 0,9 Implant II ms.0,9 ds 1,0. Implant III ms.1,0 ds.1,3
P I B R (mean)	Ø	Ø	0,08 mm	0,25 mm	1, 13 mm

(ms = mesial, ds = distal)

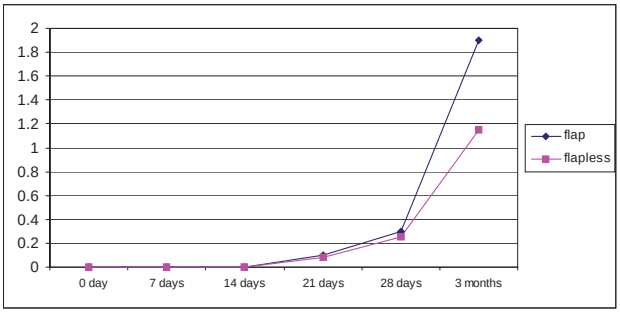

Fig. 6 – The ratio of deviation of peri-implant bone resorption of the flap and flapless surgical techniques.

Discussion

The first scientific research on flapless implant placement techniques dates back a few years ago [1, 12–14]. Evidently, minimally aggressive surgical technique results in less trauma and faster recovery of peri-implant tissue. Technological advances in radiology, use of computed tomography (CT) and cone beam apparatus and the creation of three-dimensional X-ray images of jaw bones, as well as software planning of implants placement enabled clinical application of surgical flapless implants placement techniques in everyday practice.

In a clinical research on comparative analysis of flap and flapless implant surgery, the obtained results in large numbers indicate faster repair of peri-implant tissue and subjectively easier postoperative recovery in patients with flapless technique applied [11].

In experimental flap and flapless implant surgery studies, the local inflammatory response of soft peri-implant tissue, the density of blood vessels in peri-implant tissue, resorption of peri-implant bone tissue and analysis of bone-to-implant-contact (BIC) and the degree of bone density around the implant were compared [5, 6, 15]. Basically advantages of flapless surgical technique are considerably less trauma of soft peri-implant tissue and minimal disruption of peri-implant tissue vascularization that occurs due to mucoperiostal flap raise. This is especially evident in the lower jaw with the most compact bone structure.

In fact, blood circulation within the mandible is centrifugal compared to the normal flow. Inferior alveolar artery is the main arterial supplier of the mandible. The artery passes through the body of mandible and brings blood from the interior of bone, through the cortical bone to the terminal branches of the blood vessels localized in the periosteum. As a result, the circulation of blood within the mandible has a centrifugal flow. When a tooth is extracted, the periodontal plexus is lost, *ie* tooth extraction leads to reduction of blood flow within the inferior alveolar artery [16]. After tooth extraction the alveolar bone heals through reparation or regeneration. Regeneration of tooth socket goes through secondary healing, and tissue in the area of the socket heals with scar tissue which consists of few blood vessels. Based on researches conducted by other authors it can be concluded that there is a reverse direction of blood flow at edentulous mandible from outside to inside the bone, *ie* nutrition is provided by blood vessels of the periosteum and soft tissues, while flap raising additionally reduces and compromises vascularization of the bone [1, 17], and therefore affects the other parameters for assessment of implant placement success. Literature data clearly indicate that bone regeneration almost entirely depends on vascularization, through the periosteum and a small part of bone edges [18]. Periosteum damage leads to rapid bone resorption, that numerous experimental studies report [19]. Choi et al. [1] in their extensive experimental research of flapless implant surgery obtained results, have tested the stability of implants placed in the canine mandible with resonance frequency analysis. During the first week, a decline in the expected value of implant stability in relation to the primary stability was recorded, and the values flapless technique were slightly higher. In the second week the values of implants stability placed with flapless technique remained the same, while the values of implants stability placed with flap method significantly decreased (by about 4 ISQ units). During the third and fourth week there was an expected increase in the value of implants stability, the values of flapless method showed more intensive and significant growth, and the difference in the mean value of implant stability at the end of the fourth week was about 6 ISQ units higher in the implants placed by flapless method. The results of our study on domestic pigs correspond to the results of Choi et al. [1]. The difference is that our values declined more after the first week, while the values of flapless technique were significantly higher (by 4.3 ISQ units). A tendency to decrease in the values of flap method continued in the second week, but with much lower intensity (about 0.6 ISQ), while the values for flapless surgery, increased by 0.9 ISQ units. In the third and fourth week the value of implant stability, placed with flap and flapless method, showed growth, and at the end of the fourth week the difference in the mean value was higher in the implants placed with flapless method by 6.6 ISQ units. Our study took 3 months, and in relation to the primary stability, the values after 3 months increased by 7.1 ISQ units in flap method, and increased by 10.1 ISQ units in flapless method.

Radiographic assessment of PIBR has been used for many years. Analysis of X-ray images has been used in many implant studies to compare different types, sizes, designs, implant surfaces, results of single- and two-phase implant insertion method, and recently flap and flapless techniques of implants placement [20–22].

An important parameter in assessing PIBR is the moment of initial X-ray image shooting. Many studies consider the period after placing implants as initial period [23, 24], while others consider the load period as the reference one [24]. In our study the initial X-ray image was taken immediately after the placement of implants because we opted for the submerged method and loading of implants was not foreseen in this experimental study.

Jeong et al. [20, 21] in their experimental and clinical studies compared peri-implant bone resorption around the implants placed with flap and flapless method, with radiographic analysis for 3-month a period.

Their results showed approximately 1 mm larger peri-implant bone resorption in implants placed with flap method as compared to those placed with flapless method.

Rousseau [22] in his 3-month period clinical studies with radiographic analysis of PIBR obtained no significant difference in the values of bone resorption in implants placed with both flap and flapless techniques.

The results of our research suggest that peri-implant bone resorption in the first 4 weeks was negligible in both flap and flapless method of implants placement, while the measured values 3 months after implants placement showed a difference in the level of the bone around the implant. The mean value of peri-implant bone resorption after 3 months was 1.86 mm in flap technique (ranging from 1.6 to 2.1 mm), and in flapless technique it was 1.13 mm (ranging from 0.9 to 1.7 mm).

The mean value of peri-implant bone resorption was for 0.73 mm less in cases of flapless technique for implants placement compared to the flap technique, which coincides with clinical and experimental researches mainly on dogs conducted by most researchers on this issue [20, 21 25–27].

Conclusion

According to radiographic and resonance frequency analyses of the peri-implant tissue after implant placement the flapless surgical technique has significant advantages over the flap technique.

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Repetitive transcranial magnetic stimulation in bipolar depression: Another puzzle of manic switch?

Repetitivna transkranijalna magnetna stimuacija kod bipolarne depresije: još jedna zagonetka maničnog preokreta?

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Introduction

Bipolar depression is common disorder characterized by substantial comorbidity, mortality, the highest suicide rate among psychiatric illnesses and severe social impairment, but is still often misdiagnosed.

The real prevalence of bipolar depression could be much higher than is thought, because the problem of misdiagnosis.

A percentage of undiagnosed bipolar patients is especially high in population of treatment resistant depression. These patients are most frequently misdiagnosed as having unipolar depression and treated with anitidepressant monotherapy, that result in worsening of the course of the illness and often lead to rapid cycling.

Two independent studies [1, 2] in population of 203 and 250 patients with major depression found 40–49% of bipolar disorder. In 1994, the results of survey of National Depressive and Manic-Depressive Association showed that 73% of 500 bipolar patients were misdiagnosed as having unipolar major depression [3]. Unfortunately, 10 years later the same association survey found that nothing has changed and that 69% of another 600 bipolar patients were misdiagnosed in this period of time [4].

Even when correctly diagnosed, treating bipolar depression can also be challenging, and after many treatment guidelines it is often much more difficult to manage bipolar depression than bipolar mania. In treatment of bipolar depression particularly delicate, often questionable, demanding "art and science", are treatment resistance (require high doses and combinations of antidepressants) *vs* risk of manic switch (sometimes happen even with the lowest doses of antidepressants in a first few days of treatment), but also problems of different treatment strategies in acute and maintenance therapy that often result in the problem of polypharmacy.

Repetitive transcranial magnetic stimulation in affective disorders

During the last decade, the use of repetitive transcranial magnetic stimulation (rTMS) in treatment of pharmacoresistant major depressive disorder (MDD) applies increasingly.

rTMS is a neurostimulative technique, in which a magnetic stimulation coil is placed over a strictly defined positions of subjects head. The passage of current through the copper wire in the coil, leads to induction of magnetic field whose direction is perpendicular to the direction of current flow in the coil. Such a magnetic field leads to the induction of electric field in the surface layers of the cerebral cortex, which causes the activation of interneurons and pyramidal neurons, depending on the intensity of stimulation [5]. The application of a single magnetic pulse, as described above, leads to firing of several descendent potentials along the pyramidal tract, in case of stimulation of the motor cortex, with the possibility of recording short latency EMG response in the contralateral limb muscles (latency between 20 and 25 msec) [6]. However, using a series of magnetic stimuli with precisely defined frequency and intensity (usually lower than the threshold to cause motor response) is feasible to induce neuromodulatory effects, in

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terms of its effects last beyond the duration of stimulation [7]. This led to a concept of purposeful modulation of cortical activity in order to induce plastic changes [8]. This concept was confirmed in humans in the example of motor cortex plasticity induced by motor learning [9]. Low-frequency repetitive TMS (≤ 1 Hz) causes a consistent and lasting decrease in motor cortical excitability in healthy individuals in contrast to the "facilitatory" effects induced by high-frequency repetitive TMS (5–20 Hz) [10, 11]. The knowledge gained in the experiments on the motor cortex, moreover, as an analogy, used in the explanation of mechanisms of therapeutic effects of rTMS treatment of depression [12].

Efficacy of rTMS in major depression has been demonstrated in many randomized controlled trials [13–16]. However, only a few rTMS trials in bipolar depression have been published in the last 10 years with contradictory findings.

In 2002, the first rTMS study in bipolar depression [17] showed a significant rTMS efficacy compared to sham, Nahas [18] did not have a significant response in the group of 23 bipolar patients, but a recent trial showed also rTMS efficacy and safety in mixed episodes of bipolar disorder [19].

rTMS protocols in major depression predominantly used high frequencies of stimulation (≥ 5Hz) of the left dorsolateral prefrontal cortex (DLPFC) and only a few trials used low frequent (≤ 1Hz) rTMS of the right DLPFC [14, 20–22] where antidepressant efficacy was also shown, besides much better safety profile.

Generally, it is considered that major depression is related with the functional insufficiency of both left and right hemispheres, with lower excitability and metabolic hypoactivity in the left prefrontal cortex compared to higher right frontal cortical excitability. Use of rTMS in treatment of major depression is based on the opposite effects of high frequency (≥ 5 Hz) and low frequency (≤ 1 Hz) on cortical excitability [23], where low-frequency rTMS leads to reduced intracortical excitability, regional metabolism and blood flow [10, 24] and *vice versa* for high frequency rTMS [11, 25].

Iatrogenic induction of hypomanic/manic switch – a form of bipolar illness?

Antidepressant associated manic switch was reported to be higher in bipolar type I disorder than unipolar depression [26, 27] or bipolar type II [28], and antidepressant induced mania during the treatment of unipolar depression is considered as a sign of latent bipolar disorder [29].

In unipolar depression, estimates of the rate of antidepressant associated mania have been in the range of 0–25% [26, 27, 30]. Higher rates of antidepressant-associated mania have been reported in bipolar disorder [26].

There is still no general consensus should antidepressant-induced mania be coded as a form of bipolar disorder. The International Classification of Diseases-10 (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) still do not code this state as a specific form of bipolar disorder. However, there is a widespread proposal, strongly supported by clinical evidences and many expert opinions, for antidepressant induced mania to be included in bipolar spectrum, provisionally with the categorization of bipolar III affective disorder [29] and it is also in consideration to be coded as bipolar affective disorder in DSM-V and ICD-11.

As with pharmacotherapy, iatrogenic induction of manic or hypomanic switch may occur during almost all other biological, non-pharmacological treatment for major depression, such as electroconvulsive therapy [31], vagus nerve stimulation [32], phototherapy [33] and therapeutic sleep deprivation [34, 35].

Switch to hypomania/mania during rTMS treatment of major depression

Fitzgerald and Daskalakis [36], in an article addressed the issue of practical guidelines to use rTMS in depression, based both by review of the literature and experience these authors have in rTMS in the treatment of major depression more than 10 years found that the trials specifically of bipolar depression have been too limited at this stage to allow any conclusions, however rTMS could be reasonable treatment option. They suggest concurrent use of mood stabilizers during rTMS in these patients, even the risk of manic switch, in their opinion, is low.

In 2008, Xia et al. [37] published a review focused on treatment-emergent mania/hypomania (TEM) associated with rTMS (37). This review included 53 randomized controlled trials (RCTs) of rTMS in unipolar and bipolar depression, published until 2006, where in 10 trials a total of 13 cases of mania/hypomania were reported (3 cases in RCTs, other 10 in single or multiple case reports). They found that TEM occurrence rate in RCTs, in this sample of both unipolar and bipolar disorders was 0.84% for active treatment group, and 0.73% for sham group, which was not statistically different. In total of 65 bipolar patients the switching rate in the active rTMS group was 3.1% and for unipolar depression was only 0.34%, that all indicate that rTMS does not have a higher risk of manic switch than antidepressants pharmacotherapy.

Furthermore, this comprehensive review evaluated phenomenon of hypomanic/manic switch in rTMS trials in relation with diagnosis (unipolar and bipolar depression), the parameters of stimulation (laterality, frequency, intensity, duration of stimulus train and intertrain intervals, number of pulses per session, frequency of sessions), and found that switch was more often in bipolar depression, in patients who received two rTMS sessions per day, with no specific relation with laterality, frequency and duration of stimulus train.

As was mentioned above, in most rTMS studies of major depression high frequencies of stimulation were used, as in reported cases of switch to hypomania/mania but only a few studies using low frequencies of stimulation in depression reported switch to hypomania/mania. Ella [38] reported two cases of manic switch in patients previously thought to have unipolar depression during slow (1 Hz) rTMS treatment of the right DLPFC where number of stimuli per session they used was 1,200 stimuli/day; in one patient stimulation was applied during 2 weeks (10 sessions) and in another patient 3 weeks (15 sessions); in both cases the switch occurred few days after the last sessions.

In the first placebo-controlled, randomized trial of different frequencies of rTMS in posttraumatic stress disorder two cases of mania were reported – one in group stimulated with 1 Hz, another in group stimulated with 10 Hz, in both cases after only 3 days of stimulation [39]. Hausmann et al. [40] used bilateral rTMS (20 Hz, 100% of the resting motor threshold (RMT) over the left DLPFC and 1 Hz, 120% RMT over the right DLPFC) to enhance antidepressant outcome but patient switched to mania on day 7 of stimulation; in this study citalopram was also started on the first day of stimulation, and it's questionable was switch related to effect of rTMS, citalopram, or both. In this patient bipolar affective disorder was already diagnosed, so it's not clear why she received antidepressants without mood stabilizer.

Fitzgerald et al. [41] 2003 in double-blind, parallel design study reported one switch to mania with stimulation of 1 Hz, 100% RMT, 300 stimuli/session (in group stimulated with 10 Hz was no switch), but that particular patient was the only one diagnosed as bipolar among the unipolar depressed patients, and in 2006, the same author published parallel-crossover study with 1 and 2 Hz, where in 130 stimulated patients only one manic switch occurred (again, in patient with bipolar depression).

In addition, Nedjat and Folkerts [42] also reported transient hypomanic symptoms in a period of a day during high frequency rTMS of the left prefrontal cortex (PFC) in 3 of 50 healthy volunteers.

How to manage hypomanic/manic switch if occurs during rTMS ?

Most of the reported cases of switch to mania occurred within safety guidelines; the problem is that available guidelines focused on prevention of undesired seizures, but still do not consider prevention and management of manic switch.

This explains why the reported methods of managing hypomania/mania switches induced by rTMS in all published case reports were different.

In our case, hypomania has occurred at the end of the treatment when rTMS was already stopped as scheduled and we decided to keep antidepressant and carefully monitor patient during more frequent visits on outpatient clinic. During follow-up period symptoms of hypomania vanished in 2 weeks [43]. In this case, patients also continued to receive fixed dose of velafaxine during rTMS treatment associated with partial sleep deprivation (applied twice during two weeks), so in this patient hypomanic switch could be induced by synergistic effect of antidepressants treatment with rTMS and partial sleep deprivation as add-on therapy. In the above mentioned case reports in one patient after manic switch Ella [38] reported quick switch beck to depressive mood; valproate and sertraline were started and patient recovered in 5 weeks.

Sakkas et al. [44] in their study protocol used more aggressive stimulation with 20 Hz, 110% RMT, 1,600 stimuli/session, two sessions/day and reported one case of hypomania and one case of mania. They firstly tried to manage hypomania symptoms in one patient with discontinuation of antidepressant medication and continued rTMS treatment, but the patient became more manic; when rTMS was stopped the patient became depressed again and finally, euthymic after reintroduction of rTMS, this time stimulation was less aggressive – only once a day with concomitant use of mood stabilizers. This report definitely shows that mania-induced potential of rTMS correlates with the intensity of rTMS, but our protocol and above mentioned previous case reports [38, 40] with less intensive stimulation also result in switch to hypomania, that makes understanding of hypomanic/manic switch during rTMS much more unclear.

In contrary to Sakkas et al,[44] Cohen et al,[39] tried to manage mania during rTMS in posttraumatic stress disorder patients discontinuing firstly rTMS, then also antidepressant medication and manic symptoms abated in a 5 days. Hausmann et al. [40] decided to immediately stop both rTMS and antidepressant medication and started clozapine treatment; manic symptoms vanished in 5 days, but the patient switched back to depression and finally recovered after long lack of response on several antidepressants regimens in the next 3 months; this showed that rTMS treatment had even stronger antidepressant effect than all used medication. These authors also hypothesized that rTMS in combination with antidepressant medications might modulate kindling and sensitization phenomena, which enhance cycle acceleration in bipolar spectrum patients.

Dolberg et al. [45] used more intensive stimulation (10 Hz, 1,200 stimuli/day, 4 weeks) and reported two cases of manic switch that occurred even patients were on valproic acid during rTMS treatment; these authors decided to increase dose of mood stabilizer in one patient and added haloperidol in another patient, but in one of them manic symptoms lasted even 2 months.

In another case of manic switch during rTMS Ella et al. [38] first had enthusiastic try to stabilize a patient who switched during stimulation of right DLPFC with 1 Hz changing frequency of stimulation to 10 Hz at the same side, based on theories and neuroimaging findings of functional laterality of hemispheres in depression and mania and the opposite modulator effect of rTMS. This patient did not recover with this procedure, on the contrary, became more manic and recovered with valproate and risperidone treatment, but in the next few years a few case reports and controlled studies in mania were published, supporting efficacy of high frequency rTMS of right PFC in mania [19, 46, 47].

Use of mood stabilizers during rTMS treatment of bipolar depression was proposed to maximize safety, but that also did not completely stop switches during rTMS [37], similarly to use of mood stabilizers during antidepressant pharmacotherapy.

Conclusion

We want to highlight again that in cases of treatment resistant depression clinicians should always be aware of possible underlying bipolar disorder.

As another antidepressive biological treatments, rTMS also has a potential to induce hypomanic/manic switch.

Considering the fact that studies where hypomanic/manic switches were reported included patients with unipolar and but also bipolar depression, patients also often received antidepressants during rTMS and protocols of stimulation were different, further controlled studies with more specific inclusion criteria should give precise guidelines.

The idea of changing the frequency of stimulation if switch occurs looks elegant and already shown promising results. Further controlled studies of rTMS in mania are needed to answer if the riddle of manic switch during rTMS treatment of depression could be solved in a way with the same therapy.

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Patent law protection of inventions in medicine and pharmaceutical industry

Patentna zaštita pronalazaka u oblasti medicine i farmaceutske industrije

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Ključne reči:
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Introduction

Simply saying, inventions represent new solutions to technical problems. The word "technique" means "restraining of natural forces and controlled use of natural phenomena" [1], that is "human activity in the field of material phenomena characterized by space, time, matter and energy" [2]. Thus, the technique is, actually, human activity to master and control nature. Once the domain of technique was confined to nonliving nature. Yet, today, it has been considered that technique also comprises the activities in the field of living beings, although the patent law protection cannot be realized in domain of those biological processes which cannot be influenced, that is, controlled in a way that their repetition under same conditions would yield the same results [3].

There are two basic types of inventions: inventions of products and inventions of processes. These are the products and processes that have never been comprised in the state of the art (they have never been exposed to public in any form). The particular sorts of inventions are inventions of use. They refer to the technical instructions as for the manner of application of an already existing product, or an already known process, but for a new technical purpose [4].

The inventions are generally protected by patent law, but not all of them. There are three groups of inventions that cannot be legally protected: the commercial application of which would be contrary to "ordre public" or morality; related to methods for treatment of the human or animal body by surgery or therapy and diagnostic methods practiced on the human or animal body; related to plant and animal varieties or essentially biological processes for the production of plants or animals.

The inventions of the products such as substances or compositions used for diagnostic, surgical and healing purposes are not excluded from patent protection. In other words, the inventions related to drugs and medical means can be registered as patents.

A patent constitutes a set of exclusive rights granted to the inventor in a sense that they prevent others to exploit the patented invention without permission – though not for an indefinite period of time, but usually for the period of 20 years. The exceptions are the inventions of human and animal drugs and medicines whose term of protection may be extended if their sale has to be previously authorized and approved by relevant government bodies.

This article is divided in three parts. The first part deals with the inventions of surgery, therapy and diagnostic methods applied to humans and animals. The second part, considers the patents for invention of products (i.e. drugs and medical means) in surgery, therapy and diagnostic to be used in surgery, methods applied on humans and animals. Finally, the third part, reviews the issue of the extended term of patent protection for inventions of drugs for humans and animals.

Exclusion of certain medical methods from patent protection

There is still widely spread opinion that granting the patent rights for inventions in the field of medicine would be inappropriate and against the general concept of health protection. Therefore, it has been decided that patent rights cannot be granted for inventions related to surgical, diagnostic and treatment procedures.

A surgical method, according to the patent law, is a method of cutting and removing a living tissue of the hu-

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man or animal body. Cutting and removing of living tissue can be performed in a classical way, with knife, or by applying lasers, high-frequency electrodes, etc. Therefore, the exclusion of surgery method inventions involving cutting and removing of living tissue from patent protection refers to both invasive and non-invasive procedures. The invention of surgery methods are excluded from patent protection if maintaining life and health of treated subjects is of paramount importance for the performed intervention. Correspondingly, invention of plastic surgical methods such as transplantation of skin burnt in an explosion cannot be patent protected. On the other hand, the invention of methods such as hair cutting, wool sheering, depilation, nail trimming, horseshoeing, although involving cutting and removing of human or animal living tissue, are not excluded from patent protection.

In pursuance of the patent law, a diagnostic method is a method used to identify the health condition of an organism. A diagnostic method includes several phases: examination of health condition, data collecting, data comparison, identification of a disorder and, finally, deductive clinical decision phase. Diagnostic method invention is excluded from patent protection only if all the abovementioned phases are present. If only one of these constituent phases of the diagnostic method is missing, the entire method is not considered to be diagnostic, but only a procedure that can be used in diagnostics [5]. Additionally, the exclusion is only applied where a diagnostic method made it immediately possible to decide on a particular course of medical treatment. But if application of a diagnostic method provides only interim or preliminary results (e.g. methods of internal imaging such as magnetic resonance imaging or methods for measuring temperature), method invention is patentable.

According to the patent law, a therapy method is a method of eliminating or alleviating a disease where the term disease is defined in the patent law as "all, even slight and passing, abnormal activities of the human body which exceeds the standard tolerance level and/or significant and not perishable deviation from standard human experience and perception" [6] (the term disease is differently defined in the patent law, labour law or health insurance law). Therefore, the method of therapy considering the patent law does not refer to the medical treatment that is eliminating or alleviating of some ailments or problems which are not considered to be a disease (e.g. fatigue, blackheads, etc). Also, therapy methods in the sense of the patent law do not include medical treatments undertaken for cosmetic, dietary, hygienic or sanitary purposes (e.g. coloring, straightening/curling, regeneration or promoting hair growth; skin lifting, pregnancy testing, contraception, sweat removing or breath refreshing, etc) [6]. Both preventive and curative treatments fall within the meaning of "therapy" and are therefore excluded [7].

The inventions of surgery, therapy and diagnostic methods are excluded from the patent protection only in cases when the invented method is applied to the living human or animal body. This means that the exclusion does not apply, and the patent rights could be granted for the same method if it is applied to the corpse, or on the living tissue which is separated from the human or animal body (for example, bone marrow, blood, biopsy samples, etc.).

As for the justification of excluding the inventions of surgery, therapy and diagnostic methods from granting the patent rights, legal scholars and theoreticians have contradictory opinions and stands. Some believe that the reasons for this exclusion should be sought in a traditionally reserved stand related to awarding the patent rights for the inventions in domain of health protection, and as such, they are outdated [2]. On the other hand, some believe that the exclusion of the inventions of surgery, therapy and diagnostic methods from patent protection is justifiable since it prevents the patent system to constrain the freedom of doctors in how they treat patients [8]. In other words, this legal solution excludes a possibility that saving a person's life by applying a particular surgical method may depend on the will of the inventor's monopolistic right over that method [9].

Patent protection for inventions of drugs and medical means

Even when all conditions for the exclusion of surgery, therapy and diagnostic methods inventions from awarding the patent rights have been fulfilled, still the inventions of the substances and compositions applied in such methods can be protected by patents. This means that the inventions related to medical equipment, instruments and disability aids, as well as drugs can be the subject of patent protection.

Patent protection for drugs is a newer issue in our patent law history reflecting the interests of leading global pharmaceutical companies to secure profit given their enormous research and development investments. Allowing the possibility of patent protection of drugs and medical means has diminished the significance of ethical reasons for the exclusion of all inventions in the field of medicine from patent protection. This is supported by the fact that out of 56 billion dollars invested annually in medical research, less than 10% of this amount is dedicated to inventing new drugs for treating diseases affecting 90% of global population. In other words, the research and development efforts in pharmaceutical industry are mostly directed into inventing new drugs for the treatment of cardiovascular diseases, the diseases of central nervous system and diuretics [10]. In the period between 1975 and 1997, out of 1,223 newly patented substances or compositions for use in pharmacy, only 11 were related to the treatment of tropical diseases [11].

Although the industrial production of drugs started in 1896 when the first tablet press, a machine compressing powder into tablets was introduced in Germany, it has been considered that modern pharmaceutical industry started with the introduction of sulfonamide in 1935 [12]. The development of pharmaceutical industry has rapidly continued ever since and today this industry is among the most innovative linking chemical industry, biotechnology and medicine. Contemporary pharmaceutical industry is characterized by a large social significance, high quality standards, specially regulated conditions for drugs production and sale and large investments into research and development (sometimes over 100

million dollars need to be invested to develop a new drug) [10]. This was a decisive reason why the pharmaceutical industry put the pressure to obtain the patent protection of newly invented drugs securing the exclusive rights for their commercial exploitation. In this way they could ensure return to investment and profit gain that can further be reinvested into new research cycles. The opponents of the patent protection for pharmaceutical products underline the special purpose of these products – health maintenance and protection. Pointing out that pharmaceutical products assist in maintaining the functions of vital life, they believe that the inventions in this field are for public good and should be made available to entire mankind without patent restrictions. Yet, the opinion of those who advocate the awarding of patent rights for pharmaceutical products has prevailed and possibility of patent protection for these products are envisaged by the 1973 EPC and the 1995 Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS). Patent rights awarding for inventions of drugs has contributed to further boost of this industry and its high profitability. The patented drugs are more expensive, yet it has been estimated that 65% of pharmaceutical substances would have never been developed if the drugs were not allowed patent protection [13].

In our country, in spite of big resistance of the pharmaceutical industry in former Yugoslavia, patent rights were granted to the inventors of pharmaceutical products under the 1990 Law amending and modifying the former federal 1981 Law on the protection of inventions, technical improvements and distinctive signs [14]. However, in order to allow the national pharmaceutical industry to adjust to the new strategic orientation of technological development, granting of patent rights for drug inventions was delayed until December 31, 1992. Despite the advantages offered by this law, our pharmaceutical industry continued to produce mostly generic medical products [15] that cannot be patented. These are the drugs and medicines which contain the same active ingredient as the original medical products, thus having the same effect as the original drugs. However, since the original drug is the subject of patent protection, a generic drug is launched to the market after the expiration of the original drug's patent. Generic drugs are, therefore, cheaper although their quality, efficacy and safety should not lag behind those of the original drugs. From the abovementioned, it can be concluded that since generic medical drugs do not represent newly invented products, they cannot be the subject of patent protection [16].

Curative substances can be obtained from natural sources by applying isolation or purification methods or in a biosynthetic way, that is they can be produced according to previously defined structure of pharmacologically active molecule or designed according to genomics and goals to be attained by these medicines [13]. So far several million compounds have been isolated, synthesized and tested for the purpose of obtaining new drugs [10]. Yet, in modern pharmacology, there is a decreasing number of drugs which are based on a completely new chemical compound, but rather on a new formula, composition, production process or application of the already known active pharmaceutical ingredients (API) [17, 13]. The latter (a new application of known API) means the possibility of obtaining further purpose-related patent protection for the second and any further more specific use of an API in surgery, therapy and diagnostic method since according to article 54 (4) EPC the new use of such substances and compositions for any such method is not comprised in the state of art. It is a significant digression from the corner stone rule of patent law that patents can be granted for inventions upon which the requirement of absolute novelty is fulfilled. This is the result of the fact that during the development of pharmaceutical industry, focus has been placed on the product formulation to secure the optimal exploitation of a curative substance (quantity, composition and form) for treatment and therapeutic purposes [12]. The patent law theory advocates the thesis that the substance itself cannot be the subject of patent protection. Nowadays, it is not difficult to synthesize a new compound, but it is difficult and extremely expensive to find a medical application for such a new compound [14]. Sometimes it is necessary to synthesize hundreds, even thousands of new compounds to create a new drug [10]. Therefore, the invention of the substance itself for the purpose of a drug creation is of a secondary importance, which is not the case with other products. Moreover, in case that the invented substances and not its application was patented, this would be threaten and hinder further enhancement of chemical and pharmaceutical industries [14]. On the other side, a patent protection of the application of the invented curative substance is quite justifiable. Therefore, it can be said that patent law allows the protection of the invented substance or composition in its technical form, to be applied during surgery, therapy or diagnostic methods under the condition that these curative substances are applied for new purposes which have never been indicated before [18].

The extension of the patent protection term for the inventions of human and animal drugs

Human or animal drug is a product which is found on the market in a certain dosage, pharmaceutical form and package containing the substance or a combination of substances which have been proven to be effective in treatment, cure or prevention of disease in humans, or animals, that is the substance or a combination of substances which can be used in humans and animals either for the purpose of establishing diagnosis or for the purpose of restoring, improving or changing a physiological function in humans or animals by means of pharmacological, immunological or metabolic effects of that drug [19]. Patent rights awarded for drug invention fall in domain of property rights, that is, private rights. However, given the general purpose of drugs, this field reflects strong public interest. It is of public interest that substances and compositions declared as drugs are safe for human and animals' health. Obtaining the patent protection for a certain pharmaceutical product means that that product is new, having an inventive step and can be applied in industry. However, granting patent rights for a newly invented drug is not a guarantee that this new, inventive substance or composition, applicable in industry, could not adversely affect one's

health. Since this kind of testing is not performed during the patent administrative procedures, it is necessary to evaluate that the invented human or animal drug (or plant protection product) is "safe for humans, animals and plants before it is launched for sale" [20]. Only after it has been confirmed that the invented product does not represent health hazard, the inventor, that is the holder of patent rights for this drug, is allowed to start its production and sale. In our country the Medicines and Medical Devices Agency of Serbia is the final authority charged for issuing permissions to put of solely quality, safe and efficacious medicinal products and medical devices to market.

Setting up the condition that a human or animal drug needs an authorization issued by a relevant government body before being put into production or sold means that obtaining the patent rights for that product is not enough and that it cannot be commercially exploited before completing the authorization procedure. Sometime this means that, in addition to obtaining a necessary production license, the entire administrative procedure for a new drug to be approved may last for several years. Since the term of patent protection nevertheless elapses, it can expire even before the inventor has gained any profit from the patented drug. This can adversely affect the possibility of profit return on investment and discourage future investment and research efforts in this field. In order to compensate the inventors and the holders of patent rights for this loss of time in the term of patent protection for human and animal drugs, which are the subject of mandatory approval by a relevant state agency, they are entitled to an additional legal protection in the form of a Supplementary Protection Certificate.

The supplementary protection certificate is a *sui generis* industrial property right. The legal powers of supplementary protection certificate and patent are equal, but supplementary protection certificate is not granted for all inventions but only for inventions of drugs for humans and animals and plant protection products such as insecticides and herbicides for which to be put to commercial circulation a prior official authorization has to be secured. Since there are some kinds of these products which do not require a prior regulatory approval (galenic medicines, traditional herbal medicines, active substances used in drug production, etc), the inventions of such products cannot be the subject of additional legal protection by the supplementary protection certificate.

The supplementary protection certificate as a patent law institute was first introduced in the US patent law in 1984, in Japanese patent law in 1987, while in EU it was recognized following the adoption of the Regulation No. 1762/92 by the Council of EU on June 18, 1992. Since originally this Regulation referred only to pharmaceutical products, the Council of EU adopted a new Regulation No. 1610/96 on June 23, 1996 extending the possibility of granting the supplementary protection certificate for inventions of plant protection products [15]. In the Republic of Serbia it was first time laid down by the 1994 Patent Act. According to Serbian law, in order to grant the supplementary protection certificate, it is necessary to fulfill the following preconditions: human or animal drug or plant protection product is covered by a valid patent; there is a valid authorization issued for that product; human or animal drug or plant protection product has never been the subject of a supplementary protection certificate; patent application is submitted after January 1993; first official authorization to put the product into market is issued after January 2005.

The procedures for granting the supplementary protection certificate (as well as for the termination of these rights) are guided by the same provisions for granting the patents. In order to be granted by a supplementary protection certificate, the original inventor, that is the holder of the patent rights for the relevant human or animal drug (or the plant protection product) or his successor in title, needs to file the application within six months from the date of receiving the marketing permission for that product. If this permission was issued before granting the patent rights, then the application has to be filed within 6 months from the date of the official publication of the patent rights. Application includes: a written request for granting the certificate; the data on the person/organization submitting the request (name and address); the data on the legal representative (name and address); the number of the original patent and the full name of the invention; the number and date of issue of the first marketing authorization or the note stating that the product has already been the subject of a supplementary protection certificate; a copy of the marketing authorization issued by a relevant government body; if such a marketing authorization is not the first one, then the proof of the product's identity, the information on the procedures conducted for its granting, and the official journal in which the information on the marketing authorization was published.

The data submitted in the application for granting the supplementary protection certificate are filed in the Register of supplementary protection certificates and published in the Intellectual Property Journal within six months from the application date. This means that within these six months the application has to be formally evaluated, which includes the following checking: that the application was submitted in the required form and contains all required information; that the application was submitted within legally prescribed time frame; that all the required documents were enclosed; that the patent was still valid in time of submitting the application for the supplementary protection certificate.

Having confirmed that application for the supplementary protection certificate has been formally and technically valid, the reviewers proceed with the evaluation of its content to determine: that all the preconditions prescribed by substantial law (valid on the date of the application submission) have been met for granting the supplementary protection certificate; that the product for which the supplementary protection certificate has been filed is patent protected; that the marketing authorization has been issued in an adequate manner; that the product has not been the subject of a supplementary protection certificate.

Having been confirmed that all the preconditions prescribed by substantial law have been met for granting the supplementary protection certificate, a formal decision on accepting the application and granting the supplementary

protection certificate is issued and the data from the granted certificate are published in the Official Journal of the Intellectual Property Office. The holder of the supplementary protection certificate is entitled to supplementary protection of the patent rights for the period which is equal to the period that has lapsed from the date of submitting the patent application to the date of receiving the first approval for product marketing, less five years (even such a shortened period of certificate duration cannot exceed five years from the date of granting the supplementary protection certificate). Since this certificate is valid from the date of the expiring of basic patent rights, it means that the entire patent protection period for inventions of human and animal drugs and products for plant protection can be extended to 25 years.

Granting the supplementary protection certificate favors the interests of pharmaceutical companies which produce original drugs and medicines. In order to decrease negative consequences of introducing the institution of the supplementary protection certificate in the domestic pharmaceutical industry which mostly produces generic drugs and medicines, the application of the legal provisions related to the supplementary protection certificate was postponed July 1, 2013.

Legal considerations

In conformity in classical patent law doctrine, the inventions related to drugs are not appropriate for patent protection. These products are vital for the preservation of basic life functions and therefore, their inventions have been considered as public good which means that the consumption of the good by one individual cannot be exclusive and patent protected, thus reducing availability of the good for consumption by others. The remainders of these traditional patent law theories can be still found in modern patent law provisions related to the exclusion of surgery, therapy and diagnostic methods inventions performed on living people and animals from patent protection. Although one may consider that exclusion from patentability of inventions of surgery, therapy and diagnostic methods is senseless since inventions of substances, compositions and other products used in those medical methods are patentable, this legal solution still may be deemed as justified. There are more reasons for that. The most important is that the legal status of pharmaceutical companies and doctors is very different. Pharmaceutical companies are business operators which produce and sell goods on the market and at the same time compete with other pharmaceutical companies in attracting customers. They have to innovate to survive. Investments in R&D are tremendous and patent protection is necessary to ensure return of invested capital creating monopolistic market position. Doctors are employed or self-employed high educated professionals who devoted themselves to help people. They are not business operators and do not do business to obtain profit but do their job enjoying personal satisfaction, kudos and respect[21]. For mentioned they do not need legally guaranteed market monopoly but conditions to improve their practice and competencies. In contrary doctors would long time ago form a lobby strong enough to procure patentability of inventions of medical methods. There is one more important *rationale pro* exclusion. On the one hand, completely different from the pharmaceutical companies who deal with interposers (drug stores) that is pure commercial activity, doctors perform some kind of public service [22] applying their professional knowledge directly on patients at the time of urgency. On the other hand, patent protection of drugs, medicines and other products used in medical procedures is not of an absolute character. Immediate and individual preparation of medicine in the pharmacy by virtue of presented prescription is excluded from the legal effect of patent. Surgery, diagnostic and therapy are naturally immediate and individual treatment and such exclusionary situation is constantly present in doctors' activity. The fact of emergency also makes acquisition of license uncertain. From the same reason, in the case of patent abuse, acquisition of compulsory license is impossible because the legal procedure for that lasts too long. There are more other argumentation in favor of existing legal solution [23], especially the possibility of moral dilemmas appearing (save life and risk trial or left patient to die).

One may accept exclusion for inventions of surgery, therapy and diagnostic methods performed on the human body but not on animals. Out of any discussion regarding the animals' position in nutrition chain and its meaning for human health, looking strict legally, existing legal solution is in compliance with the ratio of the 2009 Act on Animal Wellbeing and overall trends in legal protection of animals.

Conclusion

Although the introduction of patent protection for drugs, medical means and other products used in medical methods brought in a mess and confusion in the basis of the classical patent law theory, the exception from the patentability of methods for treatment of the human or animal body by surgery or therapy and diagnostic methods practiced on the human or animal body still may be deemed justified.

As for the patent protection of drugs, we could discern two specific characteristics of patent law. The first is related to the possibility to grant patent rights for the new medical application (use) of already known product, and the other is related to the introduction of the supplementary protection certificate which allows the extension of the protection term for up to five years.

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CASE REPORT

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Malakoplakia mimics urinary bladder cancer: A case report

Malakoplakija imitira karcinom mokraćne bešike

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Abstract

Introduction. Malakoplakia is an unusual and very rare chronic inflammatory disease. In bladder especially it can mimic malignancy and lead to serious misdiagnosis. **Case report.** We presented a case of a middle-aged woman with persistent macrohematuria and cystoscopically polypoid bladder mass that resembled a neoplastic process. The final diagnosis was based on cystoscopic biopsy and microscopic findings of acidophilic, foamy histiocytes with the presence of Michaelis-Gutmann inclusions which are characteristic for diagnosis of malakoplakia. Immunohistochemistry confirmed diagnosis by demonstrating CD68-positive macrophages. **Conclusion.** Urinary bladder malakoplakia should be considered in patients with persistent urinary tract infections and tumor mass at cystoscopy. Early identification with prompt antibiotic treatment can be helpful in avoiding unnecessary surgical interventions and in preventing development of possible complications.

Key words:
malakoplakia; urinary bladder; diagnosis, differential; urinary bladder neoplasms; immunohistochemistry.

Apstrakt

Uvod. Malakoplakija je neobična i veoma retka hronična zapaljenska bolest koja može imitirati maligni tumor u mokraćnoj bešici i dovesti do pogrešne dijagnoze. **Prikaz bolesnika.** Predstavljena je bolesnica srednjih godina sa perzistentnom makrohematurijom i cistoskopskim nalazom polipoidne mase nalik na neoplastični proces. Krajnja dijagnoza je postavljena na osnovu cistoskopske biopsije i mikroskopskim nalazom acidofilnih, penastih histiocita u kojima su uočene Michaelis-Gutmann-ove inkluzije, karakteristične za malakoplakiju. Imunohistohemijska analiza potvrdila je dijagnozu, prikazujući prisustvo CD68 pozitivnih makrofaga. **Zaključak.** Malakoplakiju mokraćne bešike trebalo bi uzeti u obzir kod bolesnika sa upornim infekcijama urinarnog trakta i prisutnom tumorskom masom na cistoskopiji. Rano postavljanje dijagnoze i blagovremena primena antibiotika mogu sprečiti nepotrebne hirurške intervencije i razvoj mogućih komplikacija.

Ključne reči:
malakoplakija; mokraćna bešika; dijagnoza, diferencijalna; mokraćna bešika, neoplazme; imunohistohemija.

Introduction

Malakoplakia is an unusual and very rare chronic, inflammatory disease which may be presented as a plaque or a nodule. The clinical presentation of malakoplakia varies and depends on the affected organ. In bladder, especially, it can lead to misdiagnosis of a malignant condition [1–3]. Even though it usually occurs in the genitourinary tract, it has been described in almost all body organs. It should be pointed out that various organs can be affected simultaneously [4–6]. The disease is more frequent in immunocompromised patients [2].

In the past twenty years a single case of urinary bladder malakoplakia has not been seen at the Institute of Pathology in the town of Niš. According to these clinical data, we presented middle-aged woman diagnosed with malakoplakia of the urinary bladder highlighting pathological aspects, since histopathology was the most important in establishing the correct diagnosis.

Case report

A 53-year-old female was presented with general weakness, low-grade fever and difficulties in urination. She was a non-smoker and non-alcoholic. On physical exam she was normotensive, eupneic, cooperative and in good general condition. She reported minor weight loss in the past few weeks. She was not diabetic. No significant medical family history was noted in the patient. In clinical anamnesis, she had a persistent infection with *Esherichia coli* in the last two years. She was sent to the Institute of Urology for further ex-

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amination due to constant changes in urine color, urgency and difficulty in micturition over the past few months.

Urine analysis showed albuminuria, macrohematuria, pyuria and significant bacteriuria. Cystoscopy revealed multiple foci of thickened mucosa of the bladder that resembled a neoplastic mass. Yellowish polypoidal lesions of the bladder, 2 to 3 cm in size, were removed from trigonal area, left ureteric orifice, posterior wall and bladder roof.

Histologically, on hematoxylin and eosin (HE) staining, aggregates of large macrophages with fine eosinophilic granular cytoplasm (von Hansemann cells) (Figure 1A) admixed with basophilic inclusions (Michaelis-Gutmann bodies) (Figure 1B) and infiltrated by dense collections of lymphocytes, and plasma cells were seen in *lamina propria* of urinary bladder. The macrophages were immunohistochemically negative for citokeratin (Figures 1C, 1D) and positive for CD68 (Figures 1E, 1F). Immunohistochemical examination of proliferative activity measured by Ki-67 in malakoplakia was negative. Pearls staining demonstrated deposition of calcium in Michaelis-Gutmann inclusions (Figure 2).

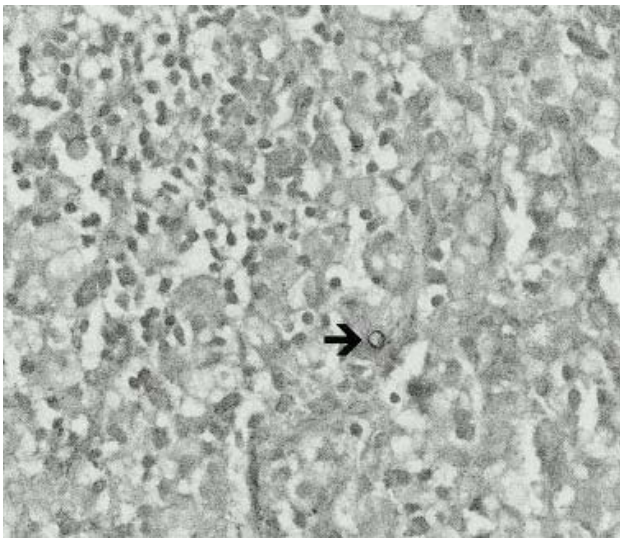

Fig. 2 – Malakoplakia, Michaelis Gutmann inclusion (black arrow) (Pearls staining, × 400).

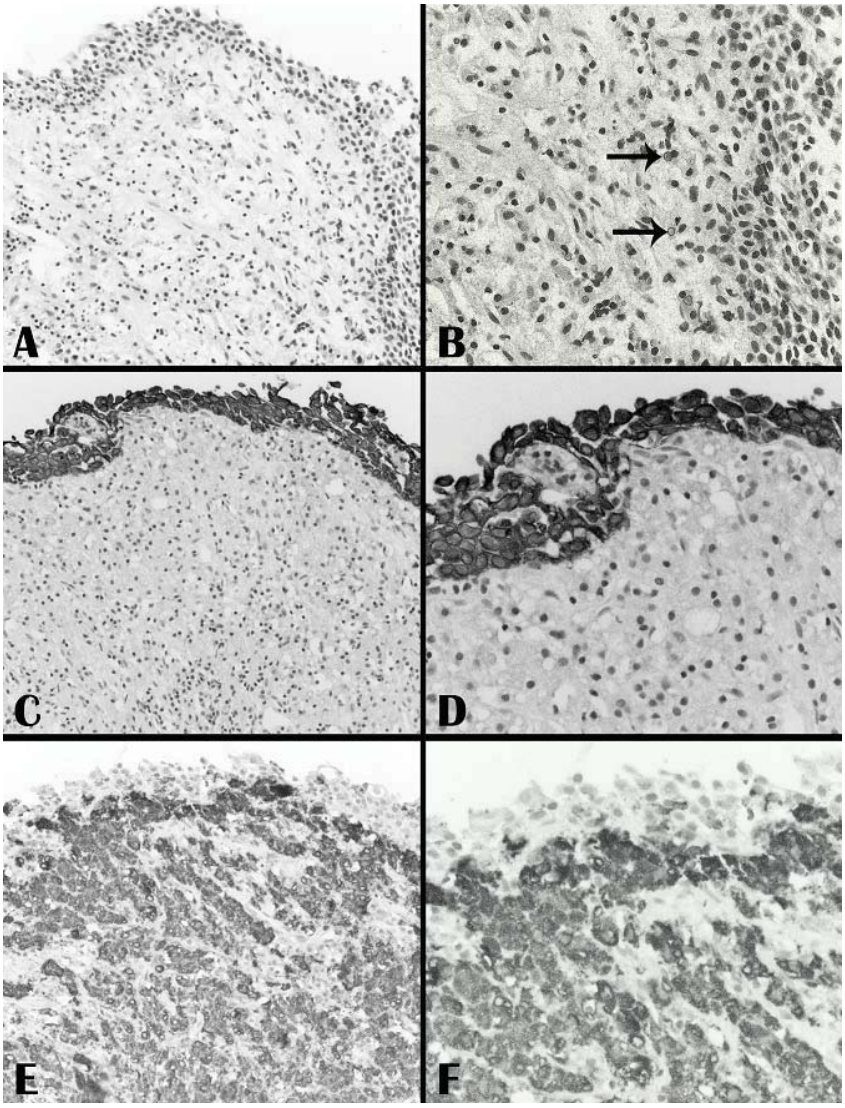

Fig. 1 – A) Malakoplakia. Normal urothelium and von Hansemann's cells (HE, × 200); B) Black arrows show Michaelis-Gutmann bodies (HE, × 400); C) Normal urothelium positive and von Hansemann's cells negative for citokeratin (× 200) and D) (× 400); E) Von Hansemann's cells positive for CD68 (× 200) and F) (× 400).

Discussion

The first case of malakoplakia was described by von Hansemann who introduced the term "malakoplakia" [7]. Malakoplakia is a chronic inflammatory disorder that occurs mostly in the genitourinary tract with a special affinity for bladder. Although malakoplakia in genitourinary tract is four times more common in women, in general, men above the age of 50 years are more frequently affected [5].

The symptoms of bladder malakoplakia are hematuria and irritative voiding symptoms such as frequency, hesitancy and dysuria [1, 2, 5]. Macroscopically, as clinically, malakoplakia can simulate tumors or abscesses, like we presented in this case.

The accurate pathogenesis has not been fully clarified, but it is thought to be a result of chronic infections by coliforms in patients with chronic weariness or immunosupression [1]. The etiopathogenesis of malakoplakia appearance mainly include damaged host defenses and deficient phagocytosis. Inadequate killing of bacteria, most commonly *Escherichia coli*, as a consequence of a defect in monocytes and macrophages phagolysosomal activity, results in an accumulation of bacterial degradation products and a granulomatous reaction [8]. However, partially digested bacteria accumulate in macrophages, eventually become mineralized, forming the pathognomonic calcified intracellular inclusions called Michaelis-Gutmann bodies. Nevertheless, infectious etiology often remains only a suspicion as patients sometimes have scarce symptoms and Gram staining does not always succeed in revealing any bacteria [1, 2]. Malakoplakia can be associated with inflammatory bowel disease which supports the theory that the malakoplakia is a consequence of chronic inflammation and altered regulation of the immune response [9].

Abundant accumulation of macrophages in *lamina propria* of urinary bladder causes the intraluminal protrusion of bladder mucosa, like we presented in this particularl case. Since this clinical entity is very rare and nearly always occurs with dramatic hematuria it can easily be misdiagnosed.

According to the aforementioned we emphasize that the final diagnosis of malakoplakia is based only on cystoscopic biopsy and microscopic findings of characteristic acidofilic, foamy histiocytes with the presence of Michaelis-Gutmann inclusions. Immunohistochemistry demonstrates CD68-positive macrophages.

Conclusion

Urinary bladder malakoplakia should be considered in immunocompromised or patients with neglected, persistent urinary tract infections and tumor mass at the cystoscopy. Early identification with prompt antibiotic treatment can be helpful in avoiding unnecessary surgical interventions and in preventing development of possible complications.

Declaration of interest

The authors declare no conflict of interest.

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CASE REPORT

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Right ventricular myxoma – A case report

Miksom desne komore

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Abstract

Introduction. Myxomas arising from the right ventricle are extremely rare. **Case report.** We presented a 71-year-old patient with worsening symptoms of the exertional dyspnea and atypical chest pains lasting 6 months. A transthoracic and transesophageal echocardiogram revealed a large, 2.6 × 2.2 cm, ovoid, well-circumscribed, echogenic mass in the right ventricle outflow tract attached by small pedicle, partly obstructing the right ventricular outflow tract and protruding through the pulmonic valve during systole. The tumor was completely removed with the stalk and 5 mm of the surrounding tissue. The histopathological findings confirmed the diagnosis of myxoma. **Conclusion.** This case illustrates the usefulness of echocardiography both in diagnosis of patients with atypical symptoms without family history and associated syndromes (like Carney's complex), and in surgical approach planning. It also stresses the importance of surgical excision of tumor as soon as possible following the diagnosis to prevent the complications such are: valvular obstruction, pulmonary embolization and syncopes.

Key words:
heart neoplasms; myxoma; diagnosis; cardiac surgical procedures; echocardiography, transesophageal; treatment outcome.

Apstrakt

Uvod. Miksomi desne komore izuzetno su retki. **Prikaz slučaja.** U ovom radu prikazan je bolesnik star 71 godinu, sa simptomima dispneje u naporu i atipičnim bolovima u grudima koji su se pogoršavali tokom šest meseci. Transtoraksni i transezofagusni ehokardiogram pokazali su prisustvo velike, 2,6 × 2,2 cm, ovalne, dobro ograničene, ehogene mase pričvršćene malom peteljkom za izlazni trakt desne komore, koja delimično opstruiše izlazni trakt i prolazi kroz plućnu valvulu tokom sistole. Tumor je hirurški kompletno odstranjen zajedno sa peteljkom i 5 mm okolnog tkiva. Patohistološkim pregledom je potvrđena dijagnoza miksoma. **Zaključak.** Prikazani bolesnik ilustruje korist ehokardiografije kako u utvrđivanju dijagnoze kod bolesnika sa atipičnim simptomima bez podataka o porodičnoj istoriji miksoma i pridruženih sindroma (kao što je Carney-ev kompleks), tako i u planiranju hirurškog pristupa. On, takođe, ukazuje na značaj hirurške ekscizije tumora, što je moguće ranije po postavljanju dijagnoze, u cilju prevenirannnja komplikacija kao što su valvularne opstrukcije, plućnih embolija i sinkopa.

Ključne reči:
srce, neoplazme; miksom; dijagnoza; hirurgija, kardijalna, procedure; ehokardiografija; transezofagusna; lečenje, ishod.

Introduction

Primary tumors of the heart are rare with the incidence of 0.02% to 0.05% [1]. The majority of them are benign with myxomas accounting for 50%, predominantly from the left and right atrium. Myxomas arising from the right ventricle are extremely rare [2–4].

We reported a patient with myxoma located in the right ventricle outflow tract creating subsequently a partial obstruction and protruding into the pulmonic valve during the systole.

Case report

A 71-year-old retired textile worker with a long history of hypertension and smoking was hospitalized because of worsening symptoms of exertional dyspnea and chest pains (piercing duration of several seconds) atypical for angina pectoris that lasted 6 months. Clinical examination revealed decreased breath sound, regular heart rhythm with resting bradicardia of 48 beats per minute, diminished heart sounds, mild (1/6) systolic murmur at the left upper sternal border (which did not change with position or respiration), and

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hypertension with blood pressure (BP) 160/90 mmHg. Other clinical findings were normal.

The results of routine blood tests (as well as D-dimer) were within the normal range except erythrocyte sedimentation rate (24 mm/h), fibrinogen (4.6 g/L.) and C-reactive protein (11.6 mg/L).

The ECG showed sinus bradycardia and the signs of early depolarization.

The chest x-ray was normal.

The transthoracic echocardiogram revealed a large, 2.6 × 2.2 cm, ovoid, well circumscribed, echogenic mass in the right ventricle outflow tract which was attached by small pedicle, partly obstructing the right ventricular outflow tract and protruding through the pulmonic valve during each systole (Figure 1). The right ventricle was not enlarged; pulmonary artery and its branches were not dilated. Apart from the aortic valve sclerosis, other findings were normal.

Transesophageal echocardiogram clearly revealed mobile tumor with the stalk arising from the right ventricular outflow tract.

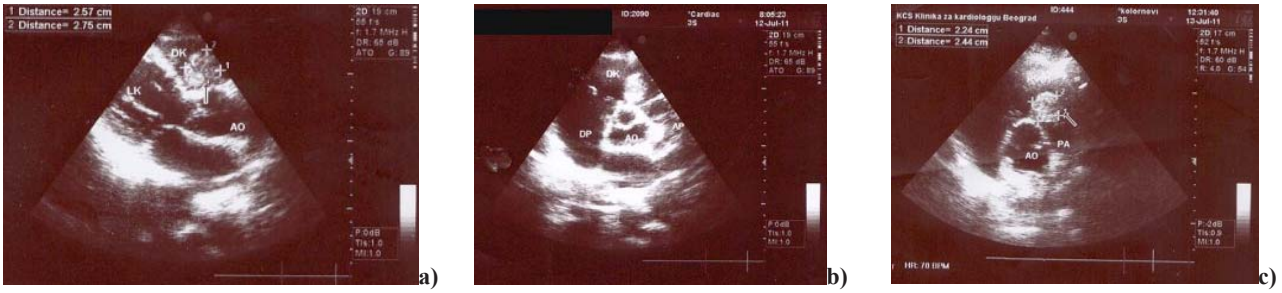
Ventilation/perfusion scanning confirmed pulmonary embolism (hypoperfusion in the apical part of the left lung).

Multidetector Computed Tomography of the pulmonary artery did not show intraluminal thrombotic masses of the main pulmonary artery and its branches.

Color duplex scan of lower extremities veins, the pelvis and abdomen showed normal findings.

Due to the patient's age and the existing chest pain coronary angiography was required, and duly performed. Nevertheless, coronary angiogram was normal.

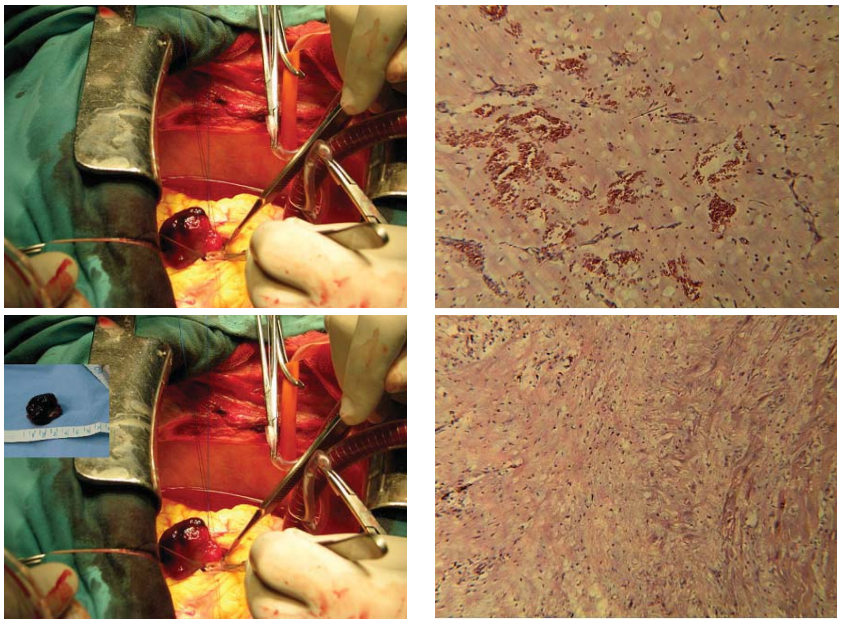
The patient was operated on using the extracorporeal circulation. Cardiopulmonary bypass was instituted with the bicaval cannulation; returning blood to the ascending aorta. Both *venae cavae* were snared, and longitudinal right ventriculotomy was performed. The tumor was excised together with the stalk and 5 mm of the surrounding tissue. Tumor basis was thermocauterized, and thereupon the right ventricular wall was sutured. The patient was weaned from cardiopulmonary bypass and the chest was closed routinely. Postoperative course was uneventful. Pathologically, the macroscopic specimen demonstrated the ovoid mass weighing 12 g, 3.0 × 2.5 × 2.0 cm in diameter, with hemorrhagic areas. The histopathological findings confirmed the diagnosis of myxoma (Figure 2).



a) b) c)

Fig. 1 – Transthoracic echocardiogram

a) Long axis view – showing a large echogenic mass in the right ventricle; b) Short axis view – showing the large, ovoid tumor mass in the right ventricle attached to the outflow tract; c) Short axis view – showing the tumor protruding into the main pulmonary artery in systolic phase.



a) **b)**

Fig. 2 – Right ventricular myxoma

a) Intraoperative photograph during resection; b) Histological findings (HE, ×400).

Discussion

Primary heart tumors are rare and the majority of them are atrial myxomas. Only sporadic cases of myxomas arising from the right ventricular outflow tract have been reported in the literature [5–7]. In a series of 81 myxomas operated in our institution during the period of 29 years, this is the first patient with right ventricular myxoma.

Clinical symptoms of myxomas depend on its position and size, are atypical and vary to a large extent in terms of general symptoms, heart symptoms and embolic events. The majority of patients have a variety of different and atypical symptoms. That is why some authors call myxomas "the great masquerader". Asymptomatic cases are rare. Screening for myxoma is important in cases of family history, in complex myxoma, or Carney disease [8]. Our patient had atypical symptoms and the diagnosis was made by echocardiography. We would like to stress the importance of this diagnostic tool, especially the importance of transesophageal echocardiography in determination of myxomas attachment, anatomical relationship and planning of surgical approach.

Our patient had signs of pulmonary microembolism and partial pulmonary valve obstruction in each systolic phase. Surgical excision of right ventricular myxomas must be accomplished as soon as possible after the diagnosis has been established to prevent serious complications such are valvular obstruction, pulmonary embolization and syncope.

Surgical intervention offers the cure for patients with sporadic intracardiac myxomas. Familial myxomas have greater tendency to recur, even many years after the operation. Although only several cases of recurrence of the right ventricular myxoma have been reported so far [9, 10] routine echocardiography control after the operation is advised.

Conslusion

The presented case illustrates the usefulness of echocardiography both in diagnosing and planning surgical approach in patients with the myxomas. It also stresses the importance of surgical excision of the tumor as soon as possible following the diagnosis establishing to prevent serious complications such are valvular obstruction, pulmonary embolization and syncopes.

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CASE REPORT

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Stent graft infixation after venous dislodgement in a patient with femoral posttraumatic arteriovenous fistula

Fiksacija stent-grafta nakon venske dislokacije kod bolesnika sa femoralnom posttraumatskom arteriovenskom fistulom

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Abstract

Introduction. An arteriovenous fistula (AVF) is an abnormal connection between an artery and a vein which may result from traumatic injury or may occur as congenital abnormality. Stent graft repair through arteriovenous fistula could lead to complications. **Case report.** Endovascular stent graft repair in a 23-year-old patient with posttraumatic superficial femoral arteriovenous fistula was performed to cover a fistula. During the procedure the device migrated through the fistula into the femoral vein. Due to eventual risk of migration to the heart, a prompt decision was made to fix the stent graft with three puncture needles in the common femoral vein region under fluoroscopy guidance. The vascular surgeon was called to perform open surgery. **Conclusions.** The presented way of treating this rare complication in an extreme and uncommon situation is very efficient, safe and inexpensive.

Key words:
arteriovenous fistula; wounds and injuries; femoral artery; femoral vein; stents; dislocations; intraoperative complications; vascular surgical procedures.

Apstrakt

Uvod. Arteriovenska fistula (AV) je abnormalni spoj između arterije i vene i može biti posledica povrede ili se pojavljuje kao kongenitalni defekt. Postavljanje stent-grafta preko AV fistule može dovesti do komplikacija. **Prikaz bolesnika.** Prikazano je postavljanje endovaskularnog stent-grafta kod 23-godišnjeg bolesnika sa posttraumatskom superficijalnom femoralnom arteriovenskom fistulom u cilju pokrivanja fistule. Tokom procedure došlo je do migracije proteze kroz fistulozni kanal u femoralnu venu. Zbog mogućeg rizika migracije u srce, donešena je brza odluka da se stent-graft fiksira punkcionim iglama u predelu femoralne vene pod vođstvom fluoroskopije. Vaskularni hirurg je pozvan da izvrši otvoreni hirurški zahvat. **Zaključak.** Prikazani način rešavanja retke komplikacije u ekstremnoj i neuobičajenoj situaciji je vrlo efikasan, siguran i jeftin.

Ključne reči:
arteriovenska fistula; povrede; a. femoralis; v. femoralis; stentovi; dislokacija fragmenata; intraoperativne komplikacije; hirurgija, vaskularna, procedure.

Introduction

An arteriovenous fistula (AVF) is an abnormal connection between an artery and a vein which may result from traumatic injury or may occur as congenital abnormality. It may be asymptomatic or manifested with symptoms such as pain, edema, varicosities and even heart failure [1]. The treatment of AVFs can be performed by open surgery or using the endovascular approach [2–10]. Stent graft deployment is a widely recognized therapeutic procedure in patients with traumatic lower limb AVF and it could be followed by unwelcome complications such as stent graft migration that requires a rapid decision [1].

Case report

A 23-year-old patient with the positive history of intravenous drug abuse was admitted to the Department of Vascular Surgery due to the injury made by drug needle insertion in the region of the right thigh. After the wound inspection and treatment, the patient was referred to the Department of Radiology for digital subtraction angiography

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(DSA). A posttraumatic AVF was found in the distal third of the right femoral region connecting superficial femoral artery and superficial femoral vein (Figure 1). Due to strong leg pain, leg swelling, palpatory thrill over the fistula and positive laboratory findings for hepatitis C, operative treatment was indicated, but the patient refused it. After consultation with a vascular surgeon, we decided to perform endovascular procedure of covering AVF with stent graft. An 8F introducing sheath was inserted into the right superficial femoral artery over a 180 cm long 0.035" guidewire. As an addition to roadmapping DSA, a metallic marker was placed on the patient`s cover sheath to make sure the AVF entrance is correctly marked. The amount of heparin injected during the procedure was 5,000 IU. A crimped covered Jostent (Abbot vascular, USA), 4–9 mm wide and 49 mm long, was inserted in the right superficial femoral artery and deployed with an insuflation device set to the labelled pressure of 12 atmospheres. The following fluoroscopy showed a loose stent graft positioned 3 cm caudally from the AVF which was still open. After wire manipulation, the stent graft shifted up and was set to the right position. On the following fluoroscopy, right before redilation, the stent graft was not in the field of view but detached from the wire. After moving the table caudally from the lesion nothing was seen, but after moving the table cranially the stent graft was seen in the region of the right femoral head, in the common femoral vein (CFV). It went through the AVF and stopped in the right CFV with possibility to move further up to the heart. Decision was made promptly and the stent graft was pinched with three puncture needles under fluoroscopy guidance in order to infix it in that position (Figure 2). The vascular surgeon was called and right CFV phlebotomy with stent graft extraction performed with surgicall femoral AV fistula repair in the second act.

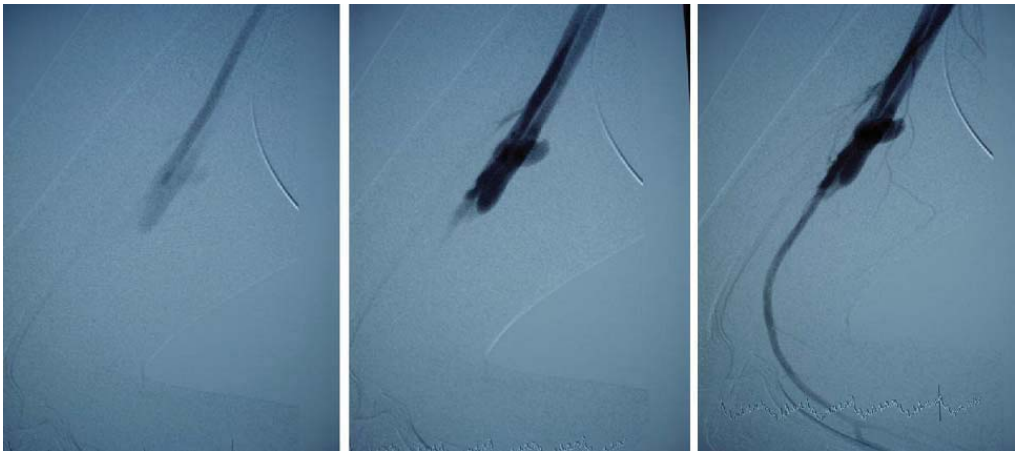

Fig. 1 – Digital subtraction angiography shows an arteriovenous fistula between the femoral artery and femoral vein

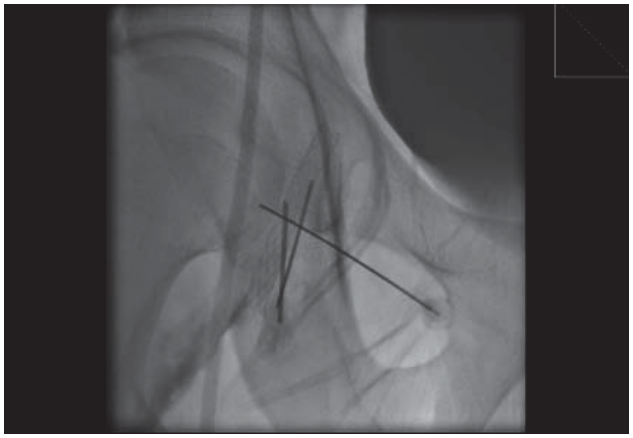

Fig. 2 – A stent graft fixed with puncture needles in the common femoral vein (an introducing sheath is seen in the femoral artery).

Discussion

What goes wrong during this procedure when a stent graft migrate through the fistula into the femoral vein? Some papers describe different causes of migration such as inflation device failure, stent graft production defect and an interventionist`s malpractice in deployment [9]. In our case, the main cause of migration was a stent graft not inflated to its maximal diameter even though the insuflation device did show the labelled pressure of 12 atmospheres. Some authors suggest the use of a self-expandable stent graft rather than a balloon-expandable in this anatomical location, but it is questionable [4, 5].

After the stent graft rapid fixing in the common femoral vein with puncture needles and thus prevention of migration to the heart, we decided not to insert a retrieval device through the femoral vein nor to insert a balloon to expand the stent graft because it could have pushed it cranially. Instead, open surgery was successfully performed by the vascular surgeon.

Our decision to promptly fix the migrated stent graft was based on the fact that it is much easier to do it under fluoroscopy for three reasons. First, stent graft is a metallic foreign body visible with radiography. Second, it is possible to make many projections in all planes (anteroposterior, posteroanterior, lateral and oblique). Third, it is possible to see the movement of the stent graft. On the contrarary, in the operation room with no radiography it is practicaly impossible to localize the stent graft in the blood vessel. Therefore, the decision was to fix stent graft with puncture needles first, and then to perform open surgery.

Finally, the decision to choose endovascular treatment in the healing of iatrogenic femoral AVF in a young patient instead of surgical repair was against usual indications (conservative choices are advised due to the limited knowledge of long-term results of covered stents), because the patient had positive lab findings for hepatitis C and refused surgical repair.

Conclusion

Some papers report on arterial migration but no relevant papers on stent graft migration into veins in patients with AVF, so the real incidence on this complication is still vague. We think the presented unique stent graft fixation with puncture needles in the common femoral region, *ie* vein, in an extreme and complicated situation is efficient, safe and inexpensive approach to prevent further migration of the device.

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Pancreas divisum: Analysis and therapeutic alternatives with a case report

Pankreas divizum – analiza i izbor terapije

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Abstract

Introduction. Pancreas divisum is a relatively common pancreatic duct anatomic variant, firstly described in the 17th century. **Case report**. We reported a 2-year-old child admitted to the Pediatric Clinic with breathing difficulties and abdominal pains. Examination and X-ray image, showed a vast right hydrothorax containing rusty coloured solution with a high degree of amylase. Ultrasound and computed tomography examination revealed pancreatic polycyclic pseudocysts; following magnetic resonance cholangiopancreatography (MRCP), the diagnosis of pancreas divisum was confirmed. The general condition of the patient worsened, requiring an urgent operation. External drainage of the perforated pancreatic pseudocyst was performed. Following external fistula maturation, a change from external to internal drainage was performed using Roux-en-Y fistulojejunostomy. A 3-year postoperative period was uneventful. **Conclusion.** Pancreas divisum cases are unique requiring clinical experience, rational approach, and complex multimodal management. MRCP is a valuable diagnostic method. Amongst therapeutic options, outer and internal drainage can be seen as reliable methods. Further investigations are absolutely required to determine practical and appropriate conclusions.

Key words:
pancreatic pseudocyst; diagnosis; digestive system surgical procedures; treatment outcome.

Apstrakt

Uvod. Pankreas divizum je relativno česta anatomska varijanta kanala pankreasa prvi put opisana u 17. veku. **Prikaz bolesnika.** U radu je prikazano dete uzrasta dve godine koje je primljeno u Kliniku za pedijatriju sa tegobama u vidu otežanog disanja i bolova u trbuhu. Nakon kliničkog pregleda i rendgenskog ispitivanja otkriven je veliki hidrotoraks sa desne strane. Punkcijom i drenažom dobijen je tečni sadržaj boje rđe, sa visokim vrednostima amilaza. Ultrazvukom i kompjuterskom tomografijom otkrivena je policiklična pseudocista pankreasa, a nakon neinvazivne endoskopske retrogradne holangiopankreatografije (ERCP), odnosno holangiopankreatografije izvedene pomoću magnetne rezonance (MRCP), utvrđeno je da se radi o pankreasu divizumu. Zbog fudrojantnog pogoršanja opšteg stanja izvedena je hitna operacija, koja se sastojala u spoljašnjoj drenaži perforirane pseudociste pankreasa. Nakon maturacije spoljašnje kontrolisane fistule pankreasa, urađena je unutrašnja drenaža uz primenu Roux-en-Y fistulojejunostomije. U periodu od tri godine nakon operacije dete nije imalo komplikacija, a rast i razvoj deteta je bio uredan. **Zaključak.** Slučajevi pankreasa divizuma su specifični i kod terapijskog rešavanja karakteristično teških komplikacija potrebno je bogato kliničko iskustvo u oblasti hirurgije pankreasa, kao i krajnje racionalan i multimodalni pristup. Posebno korisna dijagnostička metoda u rešavanju ovih slučajeva je MRCP. Kada su u pitanju terapijske metode, spoljašnja i unutrašnja drenaža imaju značajno mesto i mogu se smatrati pouzdanim terapijskim rešenjima. Za donošenje definitivnih zaključaka neophodna su dalja istraživanja.

Ključne reči:
pankreas, pseudocista; dijagnoza; hirurgija digestivnog sistema, procedure; lečenje, ishod.

Introduction

Pancreas divisum (PD) is a relatively common pancreatic duct anatomic variant, firstly described in the 17th century, attributed to Joseph Hyrtl (anatomist, 1810–1894) [1]. In 1903 Opie [2] precisely described this anatomical variant and later reported in *postmortem* examinations a 10% frequency of PD. Endoscopic retrograde cholangiopancreatography (ERCP) in the 1970's provided radiologic features of pancreas divisum [3, 4]. Nowadays, magnetic resonance cholangiopancrea-

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tography (MRCP) is preferably used as a new and reliable non-invasive imaging technique [5, 6]. Many cases of PD may be asymptomatic, but an association with symptoms of acute or chronic is not rare [1, 3]. The anomaly is explained by the failure of the dorsal (Santorini) and the ventral (Wirsung) pancreatic buds to fuse during the gestational week 6–8, whereby the predominant drainage of the gland is through the duct of Santorini (PD type 1, complete pancreas divisum) [1, 5, 7–9]. The actual hypothesis is that there is an insufficient drainage of the entire dorsal gland through the minor papilla, meaning that there is a relative stenosis resulting in ductal hypertension [7, 10]. Other anomalies of the dorsal and ventral drainage of pancreatic buds include: the absence of the duct of Wirsung (PD type 2), the presence of a filamentous or a very small caliber communication between the dominant dorsal duct of Santorini and the duct of Wirsung (PD type 3, incomplete pancreas divisum) [1], dilation, distortion, contour irregularities, and even existing protein emboli in ducts [9].

Testoni [11] documented the occurrence of pancreas divisum in patients with acute recurrent pancreatitis with a mean of 17.1%, and chronic pancreatitis up to 28.6% [4]. Ultrasonography, CT scans, and conventional MR techniques are commonly used in the work-up of patients with pancreatic symptoms, but are relatively limited in precisely evaluating pancreatic duct anomalies [8]. Different therapeutic treatments have been documented with insufficient success such as: endoscopic stenting procedures, endoscopic or surgical sphincterotomy or sphincteroplasty, surgical pancreaticojejunostomy, and pancreatectomy [9, 10, 12].

Case report

A 2-year old child was admitted to the Pediatric Clinic with difficulties in breathing, mild abdominal pain, and refusal to eat. These symptoms began approximately two months prior to admission. They were mild at first but had worsened over time. Within the period of the onseting symptoms, a bilateral inguinal hernia was also presented and the child was planned for herniectomy. After the admission to the Pediatric Clinic, chest X-rays confirmed vast hydrothorax on the right side (Figure 1). A thoracic drainage was made with a surprised discovery of a rusty coloured solution, with a high degree of amylase. Following an ultrasound and computed tomography examination, the diagnostics revealed polycyclic pseudocysts of the pancreas which ranged from 1 to 4 cm in size. The general condition of the child worsened and presented with a complete clinical picture of severe acute pancreatitis. Standard conservative treatments were initiated (continuous suction with a nasogastric sonde, antibiotics, H_2 antagonists, parenteral rehydration and nutrition, stoppage of the autodigestion with octreotide, and pain control). The abdomen continued to gradually distend, and at one moment clinical signs of incarcerated inguinal hernia were presented. During herniectomy, diagnostic laparoscopy was performed through the inguinal hernial sac. The peritoneal solution was withdrawn containing a high level of amylase. However, we were unable to find other intra-abdominal signs or confirmations for pancreatitis (steatonecrosis or alike). Furthermore, the patient deteriorated from acute pancreatitis to the point of acute abdomen which required another urgent operation. External drainage of the perforated pancreatic pseudocyst was conducted by using a silicon catheter. The progression of the illness was stopped and in this manner the first signs of recovery were seen. Subsequently, pancreas divisum without communication of the Santorini channel and the duodenum was confirmed by MRCP (Figure 2). The child had complications of parenteral nutrition, as well. Afterward, a naso-jejunal sonde was placed to provide adequate enteral nutrition with minimal stimulation of pancreatic secretion. After the fistula's maturation of five weeks, a change of external to internal drainage was performed by using Roux-en-Y fistulojejunostomy (Figures 3 and 4). For this reconstruction, a silicone catheter was used as an endoprosthesis. During the postoperative period of 3 years, the child showed signs of continued clinical improvement and was symptom free. Table 1 shows the results of the oral glucose tolerance test (OGTT) with the obvious preservation of physiological functions of the gland.

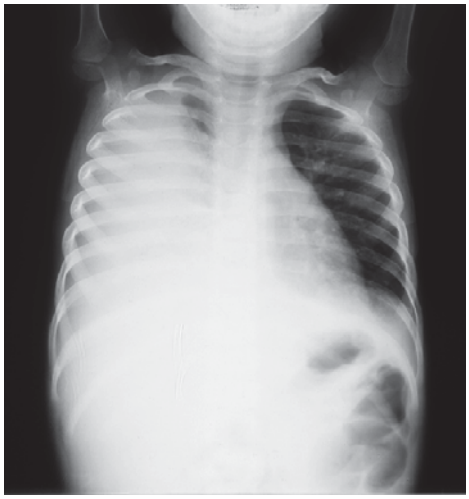

Fig. 1 – X-ray of hydrothorax

Table 1

Preservation and normal secretion of insulin

Time (hour)	Glycemia (mmol/L)	Insulinemia (mU/L)
0	4.5	12
1	5.5	70
2	5.0	35
3	4.0	12

Discussion

The incidence of PD is estimated to be between 3–10% and an additional 0.13–0.9% of patients have incomplete form [1, 4, 5, 11, 13, 14]. Usually, patients have an early onset of recurrent episodic epigastric pain and vomiting at a mean age of 6 years [3]. Many investigators have reported cases of pancreas divisum symptoms arising in late adulthood, as well [1, 3, 15]. It is believed that the earlier onset of the disease represents a greater diagnostic problem even in cases where

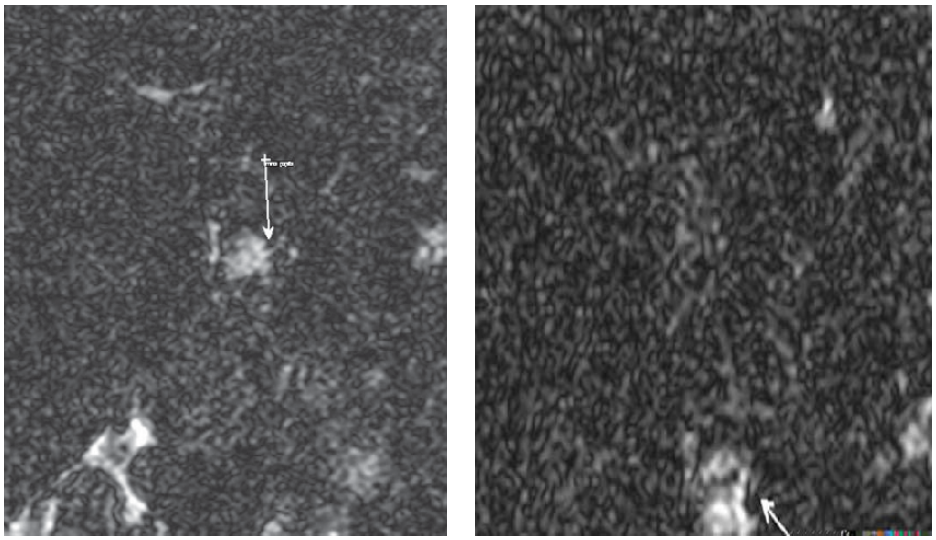

Fig. 2 – Magnetic resonance cholangiopancreatography (MRCP) of pancreas divisum

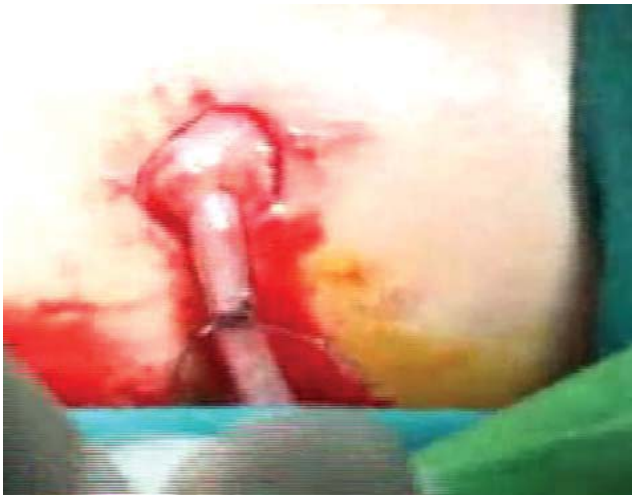

Fig. 3 – Preparation of an external fistula

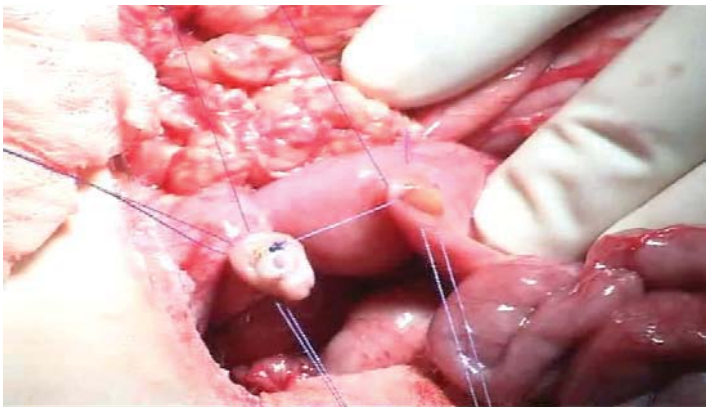

Fig. 4 – Creation of fistulojejunal anastomosis

MRCP, ERCP or other modern diagnostics are used. Usually, dominant abdominal symptoms such as pain, nausea, vomiting and some others are typical signs of acute or chronic pancreatitis. The difficulties in determining the diagnosis is further unfolded as patients may be asymptomatic.

During hospital admission, a number of diagnostics may be performed (standard ultrasound, CT, ERCP, endoscopic ultrasonography, Secretin test, Serum trypsin test, Fecal elastase test, and MRI/MRCP). Some of these diagnostic tests are not widely available (time and labour intensive, risk of pancreatitis, expensive, operator dependent, inaccurate, and may produce false-positive or false-negative results) [7]. A main difficulty in the diagnosis of PD is due to its dimensions especially in younger patients. In our case, PD was discovered by MRCP. The radiologist must be experienced in order to understand and find the anomalies, and must be able

to recognize the Santorini duct which usually has an extremely small diameter as a probable typical sign of PD [6].

When discussing on therapeutical options, the problem may lie in choosing the apropriate procedures. Many studies have shown that these patients have benefits from the decompression of the minor papilla using sphincterotomy, sphincteroplasty or other surgical drainage procedures. In the absence of chronic pancreatitis, PD may be dealt with transpapillary pancreatic duct stent placement through the minor papilla and/or endoscopic sphincterotomy of the minor papilla [13]. These procedures should be taken into consideration that they provide less invasive alternatives [7, 10, 15]. Sherman et al. [16] and Sherman and Lehman [17] suggested the use of needle-knife pancreatic sphincterotomy over a pancreatic stent. The rate of pancreatitis following pancreatic sphincterotomy appears to be approximately 10–12% [18–20]. Prolonged stenting, however, should be avoided due to the risk of inducing pancreatic damage and/or sepsis. It is recommended, thus, to remove a stent within 2–4 weeks [10,15]. Dorsal pancreatic ductography should be applied to those patients who are suspected to have PD. Minor papilla cannulation can be achieved using catheters with tapered or 25-gauge needle tips [9]. In patients with PD, if acute pancreatitis evolves into chronic pancreatitis, there may be an advantage to operate early in the disease by sphincteroplasty [4]. Direct comparison of surgical and endoscopic series is difficult, although complication rates from surgery seem to be less frequent [1]. If the results of sphincteroplasty are not better than endoscopic papillotomy, surgeons are usually recommended to perform pancreaticojejunostomy (Puestow procedure) [10, 15, 21]. A long-term success in the treatment of PD has been reported by performing percutaneous drainage for chronic pseudocysts. However, a persistent communication with the pancreatic duct might prevent its permanent and complete evacuation. It is suggested that only small cyst volumes and low amylase values might be associated with achieving percutaneous drainage [12]. Currently, the experience in the majority of pediatric surgical clinics is limited to relatively few patients [19]. Even with careful patient selection and meticulous surgical techniques to accomplish relief of ductal obstruction, the response to surgery is nevertheless inconsistent [3].

In our case, external drainage as a therapeutical method was shown to be successful. The patient from a near terminal stadium had lastly become stable, even without any signs of multiple-organ dysfunction. This was the reason to plan a permanent therapeutical solution. The patient's recovery from a severe pancreatitis following a perforation of the pseudocyst due to external drainage inspired us to create an inner derivation as a definite therapeutical solution. A silicone catheter which was used as an endo-prosthesis in the external fistula prevented stenosis of the newly created fistulajejunostomy anastomosis, allowing its normal function. The symptom-free period of three years has confirmed this approach, but the experience of other authors made us cautious and to continue with a long term follow-up of this patient. We were aware of alternative solutions whereby a formed external fistula can be completely resected, and that the standard pancreaticojejunal anastomosis can be created. This option comes to mind in the case of the realized derivation (fistulajejunostomy) that could be compromised.

Conclusion

All cases of pancreas divisum are unique and require clinical experience, rational approach, and even a complex multimodal management strategy. For these kinds of patients, a long-term observation is required with an active utilization of a wide range of diagnostic and therapeutical possibilities. Outer drainage showed to be a beneficial therapeutical option Altering outer drainage by permanent inner drainage, as in our case, seems to be successful. Puestow-pancreaticojejunostomy still represents a reliable solution in the management algorithm. A practical and appropriate conclusion requires, a larger series with a careful selection of patients.

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P R I K A Z K N J I G E

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Monografija „Suvo oko“, autorke doc. dr Gordane Stanković-Babić, zaposlene u Klinici za očne bolesti Kliničkog centra Niš i Medicinskom fakultetu Univerziteta u Nišu, predstavlja priređenu doktorsku disertaciju „Analiza kliničkih parametara i morfološkog supstrata u sindromu suvog oka“, odbranjene 8. jula 2010. godine na Medicinskom fakultetu Univerziteta u Nišu. Izdata je u „Biblioteci – Posebna izdanja“, na osnovu Kriterijuma i pravila i stalnog konkursa Zadužbine Andrejević, gde se objavljuju izabrani radovi istaknutih naučnih stvaralaca.

Monografija je napisana na 74 strane i ilustrovana sa 23 slike. Nastala je kao rezultat dugogodišnjeg, marljivog, strpljivog i studioznog proučavanja suvog oka. U monografiji suvo oko sagledava se kao problem, prvenstveno u populaciji starijih osoba, mada se bolest može pojaviti u bilo kom životnom dobu, kod osoba oba pola. Autorka razmatra patogenezu, dijagnostiku i terapiju ove bolesti. Ističe se značaj veštačkih suza u terapiji i ukazuje na rizične grupe u cilju predupređivanja problema ili ublaživanja kliničke slike i posledica bolesti.

Monografija „Suvo oko“ počinje sažetkom napisanim na srpskom jeziku, slede ključne reči, pa apstrakt na engleskom jeziku. U nastavku su poglavlja: Etiopatogeneza, Klasifikacija, Klinička slika suvog oka, Dijagnostika i terapija suvog oka, Analiza kliničkih parametara suvog oka, zatim sledi Zaključak, pa Literatura, a na kraju Prilog, Indeks pojmova, Skraćenice i akronimi.

Recezenti prof. dr Mirjana Aranđelović i prof. dr Maja Nikolić ističu da „.....Monografija predstavlja značajan doprinos razvoju srpske naučne misli iz oblasti oftalmologije i drugih oblasti medicine; predstavlja temelj za dalja istraživanja, doprinosi razumevanju multifaktorijalnog oboljenja kakvo je suvo oko i od koristi je za čitaoce posebno danas kada je povećana upotreba računara i različitih ekrana u svakodnevnom životu. Autorka problem suvog oka rasvetljava sa više strana i pruža novitete u dijagnostičkim i terapijskim procedurama..... “. U nastavku izlaganja recenzenti iznose da „.....U našim uslovima, rad ima karakter pionirskog i predstavlja snažan impuls za pokretanje dalje aktivnosti na ovom naučnom polju. Autorka koristi obimnu literaturu, primenjuje savremenu naučnoistraživačku metodologiju, doprinosi boljem razumevanju oboljenja suvo oko i iznosi korisne rezultate koji daju jasne odgovore primenjiljive u praksi.... “

dr sc. Rade R. Babić,
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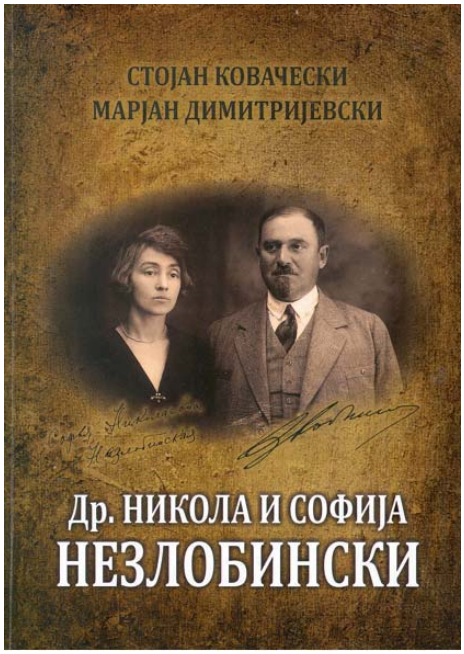
P R I K A Z K N J I G E



Naslov knjige: Dr Nikola i Sofija Nezlobinski
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Knjiga „Dr Nikola i Sofija Nezlobinski“ predstavlja značajno delo iz istorije medicine Makedonije, ali i Srbije i Balkana u celini, u kojoj je prikazan život i delo ruskih emigranata, bračnog para Nezlobinski, koji su živeli i radili u Strugi (Kraljevina Jugoslavija), danas Republika Makedonija.

Knjiga je napisana na 128 strana, bogato je ilustrovana (sadrži 172 slike) i ima 48 navoda iz literature. Napisana je na makedonskom jeziku, uz sažetak na engleskom. Autori ovog dela su dr Stojan Kovačevski i dr Marjan Dimitrijevski (Struga, Makedonija), a recenzenti prof. dr Ljubica K. Jančeva i doc. dr Katerina Mirčevska.

Dr Nikolaj Antonovič Nezlobinski, po nacionalnosti Rus, rođen je 12. maja 1885. godine u Pjantogorsku (Carevina Rusija, danas Ruska federacija). Godine 1912. Nikolaj Antonovič Nezlobinski upisao se na Medicinski fakultet (Императорска војномедицинска академија) u Sankt Petersburgu, i iste godine je diplomirao i stekao akademsko zvanje lekara. Za vreme studija Nikolaj Antonovič Nezlobinski dobija priznanje “залотой медали” za naučnoistraživački i seminarski rad na temu “Анатомо-систематическое описание какой либо группи ленточныхь глисть” (1909). Kako je bio vojni stipendista ostaje pri vojsci i radi kao lekar u ruskoj crnomorskoj floti u Odesi. Za vreme Oktobarske revolucije zajedno sa suprugom Sofijom emigrira u tadašnju Kraljevinu Srba, Hrvata i Slovenaca (1919). U Kraljevini Srba, Hrvata i Slovenaca dr Nikolaj Antonovič Nozlobinski dobija posao u Beogradu, kasnije u Nišu (1919–1920), Krivoj Palanci (1921–1924) i na kraju u Strugi (1924) gde ostaje do kraja života. U Strugi dr Nikolaj Antonovič Nezlobinski radi na suzbijanju malarije, tifusa i drugih zaraznih bolesti, otvara Dom za narodno zdravlje, pa je tako postao osnivač prve javne zdravstvene ustanove u Strugi, učestvuje u higijensko-medicinskom prosvećivanju stanovništva, afirmaciji i prezentaciji prirodnog i kulturnog bogatstva Ohridskog jezera i Struge, osniva Prirodnjački muzej u Strugi, radi na izučavanju flore i faune Ohridskog jezera, naročito crva i dr. Svojim radom dr Nikolaj Antonovič Nezlobinski doprineo je razvoju zdravstvene kulture i zdravstvene zaštite stanovništva Struge, Makedonije, Srbije, Jugoslavije, Balkana i šire. Dr Nikolaj Antonovič Nezlobinski umro je od srčanog udara 17. maja 1942. godine. Sahranjen je na groblju u Strugi.

Knjiga „Dr Nikola i Sofija Nezlobinski“ namenjena je lekarima, stomatolozima, farmaceutima i svima zainteresovanim za istoriju zdravstvene kulture zemalja jugoistočne Evrope, uključujući i bivše jugoslovenske republike.

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ERRATUM

The article „ The influence of the morphometric parameters of the intercondylar notch on rupture of the anterior cruciate ligament“ [Uticaj morfometrijskih osobina međukondilarne jame na povređivanje prednje ukrštene veze], published in Vojnosanit Pregl 2012; 69(7):576–80. (Serbian)

Listed the authors as: Lazar Stijak, Valentina Nikolić, Miloš Mališ, Ružica Maksimović, Milan Aksić, Branislav Filipović

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Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** i **literatura.**

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a) Naslov treba da bude kratak, jasan i informativan i da odgovara sadržaju rada. Podnaslove treba izbegavati.

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Na drugoj stranici nalazi se strukturisani apstrakt sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **uvod i cilj** rada, osnovne procedure - **metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi - **rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt (**250** reči) ima podnaslove: *uvod/cilj, metode, rezultati* i *zaključak*. Za apstrakte na engleskom dozvoljeno je i do **450** reči. Strukturisani apstrakt je obavezan za metaanalize (istog obima kao i za originalne članke) i kazuistiku (do 150 reči, sa podnaslovima *uvod, prikaz slučaja i zaključak*). Ispod apstrakta, pod podnaslovom „Ključne reči“ predložiti 3–10 ključnih reči ili kratkih izraza koji oslikavaju sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Zaključak** može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije. U **uvodu** ponovo napisati naslov rada, bez navođenja autora. Navesti hipotezu (ukoliko je ima) i ciljeve rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo strogo relevantne podatke iz literature i ne iznositi opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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

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

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

Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

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

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

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

Tabele

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

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Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with key words; Text; References**.

1. ***Title page***

a) The title should be concise but informative. Subheadings should be avoided;

b) Full name of each author;

c) Name and place of department(s) and institution(s) of affiliation, clearly marked by standard footnote signs.

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The second page should carry a structured abstract with the title for original articles, metanalyses and case reports. The abstract should state the purposes of the study or investigation, basic procedures (selection of study subjects or laboratory animals; observational and analytical methods), main findings (giving specific data and their statistical significance, if possible), and the principal conclusions. It should emphasize new and important aspects of the study or observations. S t r u c t u r e d abstract should contain typical subtitles: *background/aim, methods, results* and *conclusion*. The abstract for metaanalyses and obrginal papers should have up to 450 words, and up to 150 words for case reports (with subtitles *background, case report, conclusion*). Below the abstract authors should provide, and identify as such, 3–10 key words or short phrases that will assist indexers in cross-indexing the article and will be published with the abstract.

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The text of original articles is divided into sections with the headings: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

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Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and important aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

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References should be superscripted and numbered consecutively in the order in which they are first mentioned in the text. **The references must be verified by the author(s) against the original document.** List all authors, but if the number exceeds 6, give 6 followed by et al. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be designated as ”in press“. Information from manuscripts not yet accepted should be cited in the text as ”unpublished observations“. References are cited according to the ***International Committee of Medical Journal Editors.*** *Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Ann Intern Med 1997; 126: 36–47. Updated October 2001.*

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

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

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

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

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

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Each table should typed double-spaced on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table, using the following symbols, in this sequence: ***, †, ‡, §, ||, ¶, **, ††, ...** Each table has to be mentioned in the text. If you use data from another source, acknowledge fully.

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