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Teodor Grust (1859–1919): sestra iz Crvenog krsta (ulje na platnu; 45,5 × 35,7 cm)
U mesecu maju obeležavaju se dva značajna datuma, oba posvećena humanosti i borbi za očuvanje ljudskih života i zdravlja. Prvi od njih jeste Međunarodni dan Crvenog krsta i Crvenog polumeseca, koji se proslavlja 8. maja, na rođendan Žana Anrija Dinana, osnivača Međunarodnog komiteta Crvenog krsta, a drugi je Međunarodni dan sestrinstva koji se proslavlja 12. maja, na dan rođenja Florens Najtingejl, čuvene britanske humanitarke koja se smatra začetnicom modernog sestrinstva.

Theodor Grust (1859–1919): A Red Cross nurse (oil on canvas; 45.5 × 35.7).
In the month of May two important dates, both dedicated to humanity and the struggle for the preservation of human lives and health are marked. The first of these is the International World Red Cross and Red Crescent Day, celebrated on May 8, the birthday of Jean Henri Dunant, a founder of the International Committee of the Red Cross, and the other one the International Nursing Day that is celebrated on May 12, the birthday of Florence Nightingale, the famous British humanitarian worker considered the originator of modern nursing.

ORIGINAL ARTICLES

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Blood transfusion in cardiac surgery – Does the choice of anesthesia or type of surgery matter?

Transfuzija krvi i operacije srca – mogući uticaji izbora anestezije i tipa operacije

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Abstract

Background/Aim. In spite of the evidence suggesting a significant morbidity associated with blood transfusions, the use of blood and blood products remain high in cardiac surgery. To successfully minimize the need for blood transfusion, a systematic approach is needed. The aim of this study was to investigate the influence of different anesthetic techniques, general *vs* combine epidural and general anesthesia, as well as different surgery strategies, on-pump *vs* off-pump, on postoperative bleeding complications and the need for blood transfusions during perioperative period. **Methods.** Eighty-two consecutive patients scheduled for coronary artery bypass surgery were randomized according to surgical and anesthetic techniques into 4 different groups: group 1 (patients operated on off-pump, under general anesthesia); group 2 (patients operated on off-pump, with combined general and high thoracic epidural anesthesia); group 3 (patients operated on using standard revascularization technique, with the use of extracorporeal circulation, under general anesthesia), and group 4 (patients operated on using standard revascularization technique, with the use of extracorporeal circulation, with combined general and high thoracic epidural anesthesia). Indications for transfusion were based on clinical judgment, but a restrictive policy was encouraged. Bleeding was considered significant if it required transfusion of blood or blood products, or reopening of the chest. The quantity of transfused blood or blood products was specifically noted. **Results.** None of the patients was transfused blood or blood products during the surgery, and as many as 70/81 (86.4%) patients were not transfused at all during hospital stay. No difference in postoperative bleeding or blood transfusion was noted in relation to the type of surgery and anesthetic technique applied. If red blood cells were transfused, postoperative bleeding was the most influential parameter for making clinical decision. **Conclusion.** No influence of off-pump surgery or epidural anesthesia on blood transfusion requirements during a perioperative period was confirmed by this study. It seems, however, that encouraging lower hemoglobin triggers in clinical decision-making could result in less transfusions during surgery or hospital stay.

Key words:
blood transfusion; cardiac surgical procedures; anesthesia; perioperative care.

Apstrakt

Uvod/Cilj. Uprkos postojećim dokazima u literaturi o štetnim efektima i povećanom morbiditetu bolesnika posle transfuzija krvi, primena krvi i krvnih derivata u kardiohirurgiji je česta. Neophodan je sistematski pristup da bi se postigli rezultati u snižavanju potrošnje krvi tokom perioperativnog perioda. Cilj ove studije bio je da se ispita uticaj različitih vrsta anestezije, opšte i kombinovane opšte i epiduralne anestezije, kao i različitih hirurških tehnika, *on-pump* i *off-pump* (sa ili bez primene ekstrakorporalne cirkulacije) na učestalost postoperativnog krvarenja i potrošnju krvi. **Metode.** Ispitivanjem su bila obuhvaćena 82 konsekutivna bolesnika planirana za hiruršku revaskularizaciju miokarda i kompjuterski randomizovana u četiri grupe prema hirurškoj i anesteziološkoj tehnici koja je korišćena: grupa 1 (bolesnici koji se operišu na kucajućem srcu (*off-pump*), bez primene visoke torakalne epiduralne anestezije (TEA); grupa 2 (bolesnici koji se operišu *off-pump*, uz primenu TEA); grupa 3 [bolesnici koji se operišu standardnom tehnikom uz primenu ekstrakorporalne cirkulacije (EKC), bez primene TEA]; grupa 4 (bolesnici koji se operišu standardnom tehnikom uz primenu EKC i primenu TEA). Da li će bolesnici primiti transfuziju krvi ili ne zavisilo je od kliničke procene lekara koji su bili ohrabrivani da primene restriktivne indikacije. Značajno krvarenje je definisano kao potreba da bolesnici

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prime krv ili krvne derivate, odnosno da budu reintervenisani zbog revizije hemostaze. Posebno je evidentirana sva primenjena količina krvi i krvnih derivata. **Rezultati.** Nijedan bolesnik u ispitivanoj grupi nije dobio krv tokom hirurške intervencije, a čak 70/81 (86,4) bolesnika nije primalo transfuzije krvi tokom čitave hospitalizacije. Nije utvrđena razlika u postoperativnom krvarenju ili transfuziji u odnosu na primenjenu hiruršku tehniku i vrstu anestezije. Postoperativna drenaža najviše je uticala na donošenje kliničke odluke o transfuziji. **Zaključak.** Rezultati ove studije ne ukazuju na uticaj *off-pump* hirurgije ili epiduralne anestezije na količinu primenjene krvi tokom perioperativnog perioda. Izgleda da ohrabrivanje lekara da primene i usvoje restriktivni stav i odluče se za transfuziju kod nižih vrednosti hemoglobina može dati rezultate pri štednji krvi tokom operacije i hospitalizacije.

Ključne reči:
transfuzija krvi; hirurgija, kardijalna, procedure; anestezija; perioperativna nega.

Introduction

In spite of the evidence suggesting a significant morbidity associated with blood transfusions, the use of blood and blood products remain high in cardiac surgery. Practice varies among institutions and decision to transfuse patients is still more often based on subjective opinions rather than objective evidence. Patients treated in institutions with liberal transfusion strategies were 6.5 times more likely to receive transfusion than patients treated in institutions with more conservative approaches [1, 2].

It has been shown in clinical studies that regional anesthesia is associated with certain benefits for cardiac surgery patients, such as reduced incidence of atrial fibrillation, myocardial ischemia and myocardial infarction [3]. In addition, superior analgesia during a postoperative period is well proven [4] which also reduces the postoperative stress response and other related complications, including hypertension, tachycardia, increased catabolism, but also influences immune response and coagulation disturbances [5].

In order to avoid complications related to extracorporeal circulation, among them postoperative bleeding, thromboembolic events, organ dysfunction and fluid retention being the most important, interest for off-pump cardiac surgery has increased in recent years [6].

To successfully minimize the need for blood transfusion, a systematic approach is needed.

The aim of this study was to investigate the influence of different anesthetic techniques, general and combine epidural and general anesthesia, as well as different surgery strategies, on-pump *versus* off-pump, on postoperative bleeding complications and need for blood transfusions during the perioperative period.

Methods

From February 2002 to October 2005, after Ethical Committee approval, 82 consecutive patients were included in the study, at the Dedinje Cardiovascular Institute. They were scheduled for coronary artery bypass surgery and were randomized according to the surgical and anesthetic technique into four different groups: the group 1 (patients operated off-pump, under general anesthesia); group 2 [patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA)]; group 3 (patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia), and group 4 (patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA).

In case of conversion of the off-pump procedure into standard operation with the use of extracorporeal circulation, patients were newly assigned to the group according to the surgical technique, matching the anesthetic technique.

Initially, the study was designed for larger number of patients, but for technical reasons enrollment of patients was stopped earlier (82 patients).

The inclusion and exclusion criteria for the study are shown in Table 1.

Table 1

Study inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
More than one graft	Acute infections
Ejection fraction > 30%	Immunological diseases
No contraindications for TEA	Myocardial infarction up to one month before surgery
	Diabetes mellitus type 1
	Acute or chronic renal failure
	Chronic lung diseases
	Stroke or transitory ischemic attack
	Coagulation disorders

TEA – thoracic epidural anestesia

Preoperative assessment and medication was standard for all patients. Induction of anesthesia was done intravenously, with midazolam (up to 5 mg), bolus doses of propofol, fentanyl and pancuronium. To maintain an adequate depth of general anesthesia continuous infusion of propofol together with bolus doses of fentanyl and pancuronium were used.

In patients with TEA, epidural catheter was placed at Th2-Th3 or Th3-Th4 level 30 minutes before the surgery, or

at least 2 h before the first dose of heparin was used. After the test dose, bolus of 10–15 mL of 0.125 or 0.25% bupivacaine with fentanyl was used followed by continuous infusion of the same mixture of local anesthetic at the rate of 5–10 mL per hour. During the cardiopulmonary bypass, rate of continuous infusion was 1–3 mL per hour.

Antithrombotic therapy was stopped before elective surgery, aspirin three, ticlopidine and clopidogrel 10 days before the operation. Aspirin was not stopped in case of emergency surgery.

Coagulation studies, prothrombin time (PT), partial thromboplastin time (PTT), international normalised ratio (INR) and platelet count, were performed in all the patients preoperatively. Any detected abnormality was considered as contraindication for TEA and exclusion criteria for the study.

Heparin was used in standard doses intraoperatively and was neutralized with protamine after weaning from cardiopulmonary bypass or after finishing the last anastomosis in off-pump surgery. Low-molecular weight heparin was given postoperatively. Antithrombotic therapy (aspirin) was started/continued on the first postoperative day.

All the blood from extracorporeal circuit was collected at the end of cardiopulmonary bypass and retransfused to the patients. Cell salvage systems were used in off-pump surgery. Indication to transfuse the patient was based on clinical judgment, but restrictive policy was encouraged: patients were transfused if hemoglobin was less than 7 g/dL, and no transfusion was indicated when hemoglobin concentration was more than 10 g/dL.

Demographics, comorbidity, pre- and intraoperative therapy, operation and cardiopulmonary bypass duration, ischemic time, number of grafts as well as the number and reasons for conversion from off-pump to standard procedure were noted.

Quality of postoperative recovery, outcomes, all postoperative complications, length of intensive care unit (ICU) and hospital stay were also noted.

Bleeding was considered significant if it required transfusion of blood and blood products or reopening of the chest.

Laboratory investigations such as complete blood count, clotting function tests, biochemical tests (serum glucose, electrolytes, and renal and liver function tests) were monitored five times perioperatively: at the induction of anesthesia, 30 min after weaning off cardiopulmonary bypass or finishing the last anastomosis in off-pump coronary artery-by-pass (OPCAB) surgery, and 4, 12 and 48 h after the end of the surgery. Quantity of transfused blood or blood products was specifically noted.

When examining the differences between the defined groups, the *t*-test and analysis of variance (ANOVA) for numeric features and χ^2-square test for attribute characteristics were used. Analysis of variance with repeated measurements (MANOVA) was used to analyze the parameters monitored in five different time pionts during the study.

Results

Eighty-two patients were included in the study. One of the patients had incomplete data and was excluded from further statistical analysis. According to the computer randomization, all other patients (81) were assigned into four previously described groups: group 1 (19 patients); group 2 (17 patients); group 3 (27 patients); group 4 (18 patients).

There were three conversions from off-pump to standard surgery and cardiopulmonary bypass, and these patients were assigned to different groups according to the anesthetic technique applied: 2 patients into the group 3, and 1 patient into the group 4.

Table 2 shows the characteristics of the patients.

Table 2

Demographics of all the patient groups and the average duration of the surgery

	Group 1	Group 2	Group 3	Group 4
Sex (male/female), n	15/19	16/17	24/27	13/18
Age (years), $\bar{x} \pm SD$	51.9 ± 7.6	56.9 ± 8.5	55.4 ± 7.9	55.0 ± 7.8
Weight (kg), $\bar{x} \pm SD$	80.1 ± 11.6	85.9 ± 11.6	84.9 ± 10.6	83.8 ± 15.5
Height (cm), $\bar{x} \pm SD$	173.2 ± 7.1	173.9 ± 6.3	170.7 ± 8.2	170.6 ± 9.2
BMI (kg/m^2), $\bar{x} \pm SD$	26.8 ± 3.1	28.4 ± 3.0	29.2 ± 3.2	28.8 ± 4.7
EF† (%), $\bar{x} \pm SD$	55.3 ± 7.8	53.0 ± 10.1	46.9 ± 9.5	49.6 ± 8.8
NYHA class, $\bar{x} \pm SD$	1.8 ± 0.4	1.9 ± 0.5	1.9 ± 0.5	1.9 ± 0.3
Duration of the surgery (min) $\bar{x} \pm SD$	159.1 ± 36.8	179.4 ± 39.5	184.8 ± 44.8	183.2 ± 51.7

BMI = body mass index; EF = ejection fraction; NYHA = New York Heart Association;
†$p < 0.05$ between groups (ANOVA)
Group 1 – patient operated on off-pump, under general anesthesia; group 2 – patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA); group 3 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia; group 4 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA.

Although ejection fraction (EF) showed statistical difference between the groups, data indicate that the difference is not clinically significant and not represening the difference in severity of illness or the predicted outcome.

Planned coronary artery by-pass grafting (CABG) surgery was done in all patients. Average duration of surgery was not statistically different between the groups (Table 2), $p = 0.225$, ANOVA.

The majority of patients were hemodinamically stable during the procedure.

Table 3 shows the changes in hemoglobin levels that were monitored intra- and postoperatively in five different time points.

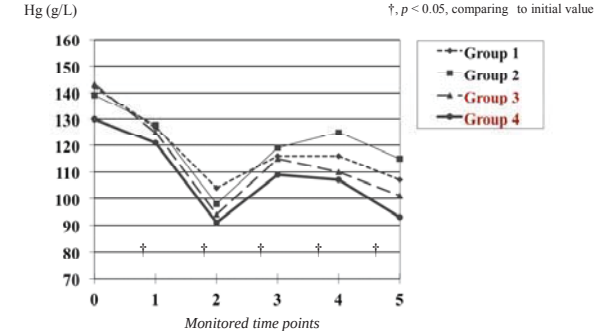
Table 3

Hemoglobin levels in different time points

Time point	Group 1	Group 2	Group 3	Group 4
Hg0, $\bar{x} \pm SD$	141.7 ± 11.9	138.6 ± 34.8	142.6 ± 10.5	129.5 ± 36.1
Hg1†, $\bar{x} \pm SD$	126.7 ± 14.0	128.1 ± 9.6	125.3 ± 10.4	120.7 ± 9.5
Hg2†, $\bar{x} \pm SD$	104.1 ± 15.2	98.1 ± 26.3	94.1 ± 11.5	91.3 ± 8.2
Hg3†, $\bar{x} \pm SD$	116.2 ± 18.4	119.3 ± 13.4	114.9 ± 15.1	108.6 ± 12.0
Hg4†, $\bar{x} \pm SD$	115.8 ± 34.8	125.1 ± 13.2	110.4 ± 13.9	107.4 ± 13.7
Hg5†, $\bar{x} \pm SD$	107.8 ± 13.1	114.9 ± 12	100.5 ± 16.1	92.7 ± 27.5

Hg – hemoglobin levels in g/L; Hg0 – preopretaive hemoglobin; Hg1= hemoglobin at the induction of anaesthesia; Hg2 – hemoglobin 30 min after weaning from cardiopulmonary bypass or finishing last anastomosis in off-pump Coronary artery by-pass (OPCAB); Hg3 – hemoglobin 4 h after the surgery; Hg4 – hemoglobin 12 h after the surgery; Hg5 – hemoglobin 48 h after the surgery; †$p < 0.05$ comparing with the initial value.
Group 1 – patient operated on off-pump, under general anesthesia; group 2 – patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA); group 3 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia; group 4 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA.

Multivariate tests for interaction between various points in time and the groups revealed that hemoglobin values changed significantly in all times comparing with the initial value, with the decrease in hemoglobin concentrations at the first and second monitored time, followed with the increase in the hemoglobin concentrations postoperatively ($p < 0.001$). Hemoglobin concentrations have changed in all the groups in the same manner (Figure 1).

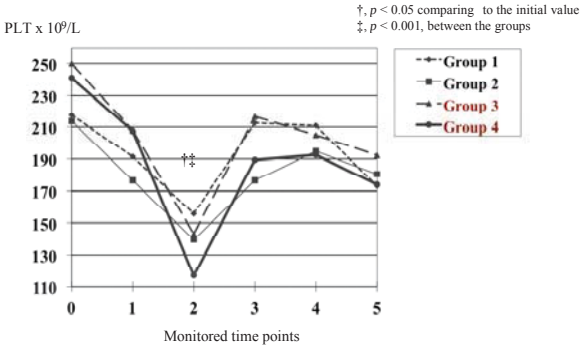


0 = preoperative value, 1 = induction for anesthesia, 2 = 30 min after the induction, 3 = 30 min after cardiopulmonary bypass or the last -pump anastomosis , 4 = 4 h after the surgery, 5 = 12 h after the surgery

Fig. 1 – Hemoglobin (Hg) levels in all the groups
Group 1 – patient operated on off-pump, under general anesthesia; group 2 – patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA); group 3 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia; group 4 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA.

Table 4 shows the changes in platelet count in different time points.

Multivariate tests for interaction between time and group showed that platelet count did change over time, but in a different manner in different groups. There was a decrease in the platelet count in all the groups in the second and third monitored time, with the increase in the fourth and fifth time, and than again slight decrease in the sixth time (Figure 2).



0 = preoperative value, 1 = induction for anesthesia, 2 = 30 min after the induction, 3 = 30 min after cardiopulmonary bypass or the last of -pump anastomosis , 4 = 4 h after the surgery, 5 = 12 h after the surgery

Fig. 2 – Platelet (PLT) count in all the groups
Group 1 – patient operated on off-pump, under general anesthesia; group 2 – patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA); group 3 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia; group 4 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA.

A decrease in platelet count in the third time was statistically significant ($p = 0.006$). Platelet count was signifi-

Table 4

Platelets count in different time points

Time point	Group 1	Group 2	Group 3	Group 4
PLT0, $\bar{x} \pm SD$	217.3 ± 41.3	213.6 ± 56.9	249.9 ± 69.4	241.2 ± 79.2
PLT1, $\bar{x} \pm SD$	191.4 ± 34.5	176.5 ± 33.1	208.1 ± 56.7	207.5 ± 69.6
PLT2†, $\bar{x} \pm SD$	155.9 ± 39.8‡	139.6 ± 32.6	143.2 ± 50.4	116.9 ± 37.8
PLT3, $\bar{x} \pm SD$	212.6 ± 40.2	176.6 ± 42.1	216.6 ± 61.4	189.5 ± 56.8
PLT4†, $\bar{x} \pm SD$	210.9 ± 47.6	195.0 ± 48.6	205.1 ± 52.8	192.6 ± 62.2
PLT5, $\bar{x} \pm SD$	173.1 ± 33.2	180.5 ± 53.5	192.4 ± 48.9	174.2 ± 41.2

PLT – platelet count × 10^9/L; PLT0 – preoperative platelet count; PLT1 – platelet count at the induction of anaesthesia; PLT2 – platelet count 30 min after weaning from cardiopulmonary bypass or finishing last anastomosis in OPCAB; PLT3 – platelet count 4 h after surgery; PLT4 platelet count 12 h after surgery; PLT5 – platelet count 48 h after surgery; †$p < 0.05$ comparing with the initial value in time; ‡$p < 0.05$ compering between the groups
Group 1 – patient operated on off-pump, under general anesthesia; group 2 – patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA); group 3 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia; group 4 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA.

cantly higher in the third time in the group 1, as compared to other groups ($p = 0.030$).

Table 5 shows the INR values in different time points.

Table 5

International normalized ratio (INR) values in different time points

Time point	Group 1	Group 2	Group 3	Group 4
INR1, $\bar{x} \pm SD$	1.1 ± 0.3	1.2 ± 0.2	1.1 ± 0.2	1.1 ± 0.2
INR3†, $\bar{x} \pm SD$	1.4 ± 0.3	1.5 ± 0.4	1.3 ± 0.3	1.4 ± 0.3
INR5, $\bar{x} \pm SD$	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2

INR1 – values at the induction of anaesthesia; INR3 – 4 h after surgery; INR5 – 48 h after surgery; †$p < 0.05$ compared to initial value

Group 1 – patient operated on off-pump, under general anesthesia; group 2 – patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA); group 3 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia; group 4 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA

Multivariate tests for interaction between different points in time and the groups showed that INR values did change over time, but in all the groups in the same manner. INR was significantly higher in the third monitored time compering with the initial value ($p < 0.001$). In the fifth time it decreased again but stayed higher as compared to the initial value ($p = 0.022$).

Table 6 shows postoperative blood drainage.

Table 6

Postoperative blood drainage

Group	$\bar{x} \pm SD$ (range), mL
1	525.6 ± 289.4 (110–1150)
2	580.0 ± 225.8 (300–1100)
3	666.0 ± 408.2 (200–1750)
4	648.7 ± 216.7 (350–1750)

Group 1 – patient operated on off-pump, under general anesthesia; group 2 – patients operated on off-pump with combined general and high thoracic epidural anesthesia (TEA); group 3 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, under general anesthesia; group 4 – patients operated on using standard revascularization technique with the use of extracorporeal circulation, with combined general and TEA

Drainage was not different between the groups ($p = 0.491$).

Reopening the chest due to postoperative bleeding was done in three patients (3.7%). These patients were operated on using standard on-pump surgery with cardiopulmonary bypass and general anesthesia.

None of the patients was transfused blood or blood products during the surgery, and as many as 70/81 (86.4%) patients were not transfused at all during hospital stay. If red blood cells were transfused, postoperative bleeding was the most influential parameter for making clinical decision.

Two of the patients with chest reopening for postoperative bleeding had both transfusion of packed red blood cells and fresh frozen plasma. The third one was returned to the operating theatre shortly after surgery and received only blood from cell saver, without additional blood or blood products. Only three patients were transfused fresh frozen plasma. No patient was given platelet transfusion.

There was only one death in the studied patients. The patient died due to multi-organ failure 53 days after the surgery; he received transfused blood and other blood products according to the clinical indications.

Discussion

Red blood cell transfusion is a common in cardiac surgery patients. Proportion of patients that receive blood transfusion during perioperative period varies in the literature: from up to 95% of patients ten years ago, to 49% of CABG patients more recently [7].

It is well known that red blood cell transfusion can be life-saving intervention. Transfusions improve systemic oxygen capacity, ameliorate vasomotor regulation, improve myocardial oxygen delivery and improve short-term survival in myocardial ischemia [8].

On the other hand, existing literature highlights that the harms of transfusion are probably more serious than has been appreciated and that transfusion is used more frequently than necessary.

Even one unit of transfused blood is associated with significant risk of serious postoperative morbidity and immediate aim should be to avoid transfusing simply to treat low hemoglobin levels, which is usual practice in up to 50% of all transfused patients [7].

Concerns about harms of red blood cells transfusion have traditionally focused on viral and bacterial infection or hemolytic reactions that are very rare [9]. However, immunosuppression, lung injury or organ dysfunction may occur in every recipient [7].

Recently, investigators from the Cleveland Clinic found that administration of red blood cells that had been stored for longer period of time (> 14 days) was independently associated with an increased risk of complications and increased estimated risk of death [10]. The concept that red cells develop storage lesions over time and release cytokines, membrane fragments, free hemoglobin and oxygen radical is well-established and the probability that such injury can contribute to patient morbidity seems real.

Obviously, there is a challenge to determine the circumstances in which the benefits of transfusion outweigh the harms. Unfortunately, the existing evidence is scarce, and existing guidelines and recommendations are based on rather low levels of evidence. Convincing physicians to change their practices is not easy task and appropriate clinical judgment is used as justification for transfusions.

A number of strategies exist to minimize the likelihood that a patient will require a transfusion in the perioperative period.

Some of them are recommended by the existing guidelines [11]: stopping the preoperative use of antiplatelet medications, applying restrictive hemoglobin triggers for transfusing red blood cells, some form of pump salvage and reinfusion of residual pump blood at the end of CPB, use of cell salver systems, but also, off-pump CABG as one form of blood salvage technique.

The main aim of study was to investigate the influence of the type of surgery (on- vs off-pump), and anesthetic technique (TEA vs no TEA) on perioperative blood transfusions. We did use some of the transfusion reduction strategies, which would, otherwise, be able to influence our results and the number of transfused patients.

Antiplatelet therapy was stopped before surgery and was restarted postoperatively in the same manner for the majority of patients. Only the emergency patients did receive aspirin preoperatively.

Reinfusion of residual pump blood at the end of CPB and the use of cell salver systems were applied depending on the surgery technique used.

But most importantly, attending doctors were encouraged to use restrictive hemoglobin triggers to transfuse patients, which is also recommended in recent guidelines [11]: the patients were transfused if hemoglobin was less than 7 g/dL, and no transfusion was indicated to improve oxygen transport when hemoglobin concentration was more than 10 g/dL.

We monitored hemoglobin levels in five different time points perioperatively, and this reveled that they did drop during the procedure, recovered soon after, but did not reach the trigger for transfusion. None of the patients received blood transfusion intraoperatively, independently of the type of surgery and anesthetic technique applied.

About 20% of all CABG surgeries are now performed off-pump [12]. The use of CPB to perform CABG surgery has been associated with multiple deleterious effects, including hemodilution of coagulation factors and platelets, leading to coagulopathy that could result in excessive bleeding and the need for massive blood transfusion. Thus, off-pump would be expected to result in decreased incidence of postoperative complications, and is recommended as one of the transfusion saving strategies [11].

Nevertheless, in our study no difference was noted between the groups in intra- and postoperative blood transfusions. Platelet count was monitored in five different time points, and it differed between the groups. There was a decrease in platelet count intraoperatively, followed by an increase in the immediate postoperative period. Platelet count indeed was higher in the off-pump group of patients in the third time point (30 min after cardiopulmonary bypass or finishing the last anastomosis on off-pump), but with no clinical consequences. Blood drainage after surgery was not different between the groups.

Only 13.6% of the patients received blood transfusion after the surgery and during the course of hospitalization. These decisions were mostly clinical, predominantly influenced by the rate of postoperative drainage. Even so, one of the patients with chest reopening for bleeding did not receive any, but blood from cell saver. Only three patients were back to surgery for bleeding, which is a rather small percentage.

Our study showed no influence of different surgical or anesthetic technique on the incidence of blood transfusion during the perioperative period. It seems that study design itself, with the encouragement of the attending physicians to be restrictive regarding blood transfusion, mostly influenced the number of transfused patients.

Of course, the number of enrolled patients is a limitation of our study. Since only 11 patients did receive blood transfusion, no conclusion of different influences or other correlations could be made.

Conclusion

There are different strategies in cardiac surgery to minimize transfusion requirements in a perioperative period. Our study proved no influence of OPCAB surgery or epidural anesthesia on these requirements. It seems that encouraging lower hemoglobin triggers in clinical decision-making could result in less transfusions during surgery or hospital stay.

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The burden of disease preventable by risk factor reduction in Serbia

Opterećenje bolestima koje se može sprečiti snižavanjem faktora rizika u Srbiji

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Abstract

Background/Aim. Reliable and comparable analysis of health risks is an important component of evidence-based and preventive programs. The aim of this study was to analyze the impact of the most relevant avoidable risk factors on the burden of the selected conditions in Serbia. **Methods.** Attributable fractions were calculated from the survey information on the prevalence of a risk factor and the relative risk of dying if exposed to a risk factor. The population-attributable risks were applied to deaths, years of life lost due to premature mortality (YLL), years of life with disability (YLD) and disability adjusted life years (DALY). **Results.** More than 40% of all deaths and of the total YLL are attributable to cigarette smoking, overweight, physical inactivity, inadequate intake of fruit and vegetables, hypertension and high blood cholesterol. Alcohol consumption has in total a beneficial effect. According to the percent of DALY for the selected conditions attributable to the observed risk factors, their most harmful effects are as follows: alcohol consumption on road traffic accidents; cigarette smoking on lung cancer; physical inactivity on cerebrovascular disease (CVD), ischemic heart disease (IHD) and colorectal cancer; overweight on type 2 diabetes; hypertension on renal failure and CVD; inadequate intake of fruit and vegetables on IHD and CVD, and high blood cholesterol on IHD. **Conclusions.** This study shows that a high percentage of disease and injury burden in Serbia is attributable to avoidable risk factors, which emphasizes the need for improvement of relevant preventive strategies and programs at both individual and population levels. Social preferences should be determined for a comprehensive set of conditions and cost effectiveness analyses of potential interventions should be carried out. Furthermore, positive measures, derived from health, disability and quality of life surveys, should be included.

Key words:
prevalence; risk factors; mortality; epidemiology; smoking; obesity; hypertension; disease.

Apstrakt

Uvod/Cilj. Pouzdana i uporediva analiza zdravstvenih rizika jeste važna komponenta medicine zasnovane na dokazima, kao i preventivnih programa. Cilj rada bio je da se analizira uticaj najbitnijih faktora rizika, koji se mogu izbeći, na opterećenje određenim stanjima u Srbiji. **Metode.** Atributivne frakcije su izračunavane iz istraživanih informacija o prevalenciji faktora rizika i relativnog rizika od umiranja u slučaju izloženosti faktoru rizika. Faktori pripisivi populaciji bili su primenjeni na smrt, godine života izgubljene zbog prerane smrtnosti (YLL), godine života sa invaliditetom (YLD) i invaliditet koji je bio podešen godinama života (DALY). **Rezultati.** Više od 40% svih smrti i ukupni YLL mogu se pripisati pušenju, prekomernoj težini, fizičkoj neaktivnosti, neadekvatnom unosu voća i povrća, hipertenziji i visokom holesterolu u krvi. Konzumiranje alkohola, ukupno ima blagotvoran efekat. U skladu sa procentom DALY za izabrana stanja koj se mogu pripisati posmatranim faktorima rizika, njihov najštetniji efekat je sledeći: konzumiranje alkohola na saobraćajne nesreće; pušenje na karcinom pluća; fizička neaktivnost na cerebrovaskularne bolesti (CVD), ishemijsku srčanu bolest (IHD) i kolorektalni kancer; prekomerna težina na dijabetes melitus tipa 2; hipertenzija na bubrežnu slabost i CVD; neadekvatan unos voća i povrća na IHD i CVD i visok holesterol u krvi na IHD. **Zaključak.** Istraživanje je pokazalo da se visok procenat opterećenja bolestima i povredama može pripisati faktorima rizika koji se mogu izbeći, što naglašava potrebu za poboljšanjem važnih preventivnih strategija i programa na oba nivoa – individualnom i populacijskom. Socijalni prioriteti trebalo bi da budu određeni za opsežnu grupu stanja i trebalo bi da budu sprovedene analize isplativosti potencijalnih intervencija. Nadalje, trebalo bi da budu uključene pozitivne mere proistekle iz istraživanja zdravlja, invalidnosti i kvaliteta života.

Ključne reči:
prevalenca; faktori rizika; mortalitet; epidemiologija; pušenje; gojaznost; hipertenzija; bolest.

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Introduction

Reliable and comparable analysis of risks to health is an important component of evidence-based policies and preventive programs [1]. Understanding the distribution of risk-factor burden is important for targeting specific interventions and programs, and increasing cost-effectiveness [2]. In many studies the major effects from risk factors have been found to be among those at moderately elevated levels, motivating interventions beyond those intended for clinical hypertension [3, 4].

During the last decade of the 20th century, the health status of population in Serbia was harmfully influenced by numerous factors, but especially by the general situation in the country, i.e. the long-lasting economic crisis, the consequences of war in the neighboring countries, a wide range of political and economic sanctions [5, 6].

The aim of this study was to analyze the impact of the most relevant risk factors (cigarette smoking, alcohol, physical inactivity, overweight/obesity, inadequate intake of fruit and vegetables, hypertension, high blood cholesterol) on the burden of the 10 selected conditions as defined by the U-codes in the Global Burden of Disease (GBD) study [7] [lung cancer, cervix uteri cancer, breast cancer, colorectal cancer, ischemic heart disease (IHD), cerebrovascular disease (CVD), type 2 diabetes mellitus, renal failure, road traffic accidents and self-inflicted injuries] in Serbia without Kosovo and Metohia and to determine the relative position of the Serbian Burden of Disease in comparison to the international reference. The main argument for this selection of risk factors was the proportion of related diseases and their public health importance according to Atanasković-Marković et al. [8].

Methods

The data presented in this paper are a part of the Serbian Burden of Disease Study (SBDS)[8], which was conducted in Serbia proper between October 2002 and September 2003. This project was funded by the European Agency for Reconstruction. The SBDS was based on the methods developed for GBD study [7].

Disability adjusted life years (DALY) was used to estimate the burden of disease in population [7, 9, 10]. This indicator is the aggregation of years of life lost because of premature death (YLL) and years of life with disability YLD at the population level. YLL, YLD and DALY were calculated according to standard procedures [7, 11].

The mortality data for the selected conditions for 2000 were used from the Serbian Office of Statistics mortality database. For most conditions the incidence was available directly from disease registers, routine data bases or epidemiology studies [12–15]. The prevalence was used only for diabetes mellitus. When reliable data were not available to run the model (as for injuries), the incidence estimates in other studies were used [16].

YLL was determined by average life expectancy at the age of death while discounting future years by 3%. The life expectancy at birth was fixed at 82.5 years for women and 80.0 years for men [17]. YLL was calculated for all diseases and injuries and YLD was calculated only for the 10 selected diseases and injuries.

In the absence of Serbian specific disability weights, the SBDS adopted GBD 1990/2000 health state evaluation results expressed in the form of disability weights [7, 18]. The GBD study weighted a year of healthy life lived at young ages and older ages lower than for other ages [7]. This approach was used in the SBDS study in order to make possible comparison with other studies.

The population-attributable risk (PAR) has been applied to estimate YLL and YLD, as well as the summary metric of DALYs. The burden of disease and injuries attributable to various health risks can be estimated if the prevalence of exposure to the risk factor in the population and the relative risk of dying if exposed to the risk factor are known [19]. The PAR was calculated using the formula:

$$\text{Population attributable fraction} = \frac{P\,(RR - 1)}{P\,(RR - 1) + 1}$$

P – prevalence of the risk; RR – relative risk of death comparing exposed to non-exposed.

For risk factors with different categories of exposure the next formula was used:

$$PAR = \frac{\sum_{i=0}^{k} P_i\,(RR_i - 1)}{\sum_{i=0}^{k} P_i\,(RR_i - 1) + 1}$$

i – baseline category of risk; P_i – prevalence of the risk factor level; RR_i – corresponding relative risk

Seven risk factors were chosen for the analysis [8]. For the six of them: cigarette smoking, alcohol consumption, physical inactivity, low vegetable and fruit intake, high blood pressure and overweight, the definition of borderlines and the corresponding prevalence data were derived from the 2000 Population Health Survey, which comprised 9,921 persons, aged 20 and more years [20]. The health survey collected self-reported information from the participants for the last seven days. Since there were no recent data on blood cholesterol level in Serbia proper, data from the Population Health Survey Study in the Republic of Srpska, Bosnia & Herzegovina [21] as the most equivalent if not identical neighbouring population were used to estimate the prevalence of that risk factor in Serbia.

Data on cigarette smoking refer only to current smoking. The prevalence of each level of alcohol intake (low, hazardous, harmful) was estimated from weekly consumption by the age group and gender, after conversion to standard drinks per day (10 mL of alcohol equal 7.9 g of alcohol). The consumption of 0–0.25 standard drinks per day was considered as abstinence [20]. The analyzed levels of inactivity were sedentary and low levels defined by estimation based on the frequency and duration of physical activity. More than one serving of fruit and vegetables per day was considered as adequate consumption.

High blood pressure was defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥ 95 mm Hg [8]. "Hypertension" was used as a term referred to those with high blood pressure and/or receiving treatment for high blood pressure.

The body mass index (BMI) (kg/m^2) of 25–29.9 was defined as overweight and BMI > 30 as obesity. A blood cholesterol level > 5.5 mmol/L was defined as elevated [21].

The relative risks associated with the exposure to these seven factors were accepted from the following studies as the best equivalents regarding populations, definitions and borderlines:

– For cigarette smoking, the age adjusted relative risk estimated for persons 35 years and over from the 2nd wave of the American Cancer Society's Cancer Prevention Study [22];

– For alcohol consumption, physical inactivity, hypertension, overweight and high blood cholesterol the relative risk from the Australian Burden of Disease Study [23–25];

–For inadequate fruit and vegetables intake, the relative risk estimated by the New Zealand Ministry of Health [26].

As the prevalence data on risk factors were detailed enough [20], it was possible to make the same category cut-points for the risk factors as those categories used in studies from which the relative risks were accepted. The only exception was fruit and vegetables consumption. Instead of 3–5 servings of fruit and vegetables per day [26], in SBDS more than 1 serving per day was considered as adequate consumption because it better corresponds to the Serbian population diet. For the attribution of the disability burden to risk factors, it was assumed that relative risks apply equally to mortality and morbidity. The relative risks were not fully adjusted to important covariates, and the univariate population attributable risks calculated did not allow for clustering and interaction of risk factors.

Results

In SBDS, the estimations of mortality burden attributable to alcohol harm indicate that 2.5% of total death and 3.4% of total YLL were attributed to alcohol consumption (Table 1). But alcohol benefit to mortality burden indicates that 4.3% of total deaths and 3.3% of total YLL were attributed to alcohol consumption. The most harmful effect of alcohol consumption was on road traffic accidents. The beneficial effect of alcohol on IHD was higher for males (-10.6% of total DALY) than for females (-8.7% of total DALY). Alcohol intake had a harmful effect on CVD in males (10.9% of total DALY) and beneficial effect in females (-19.4% of total DALY).

Cigarette smoking is responsible for 9.8% of total death and 13.7% of total YLL (Table 1). The harmful effect of cigarette smoking was greatest on lung cancer (82.8% of total lung cancer DALY in males and 90.2% in females were attributable to cigarette smoking) (Table 2 and Table 3). The estimated burden of IHD, CVD and cervical cancer attributable to cigarette smoking is higher for males than for females.

Physical inactivity is responsible for 12.0% of total death and 9.8% of total YLL (Table 1). Physical inactivity was most important for IHD and CVD, 22.2% of total IHD DALY in males and 15.1% in females, and 24.7% of total CVD DALY in males and 31.0% in females were attributable to physical inactivity (Tables 2 and 3). The estimated burden of IHD, CVD, colorectal cancer, breast cancer and type 2 diabetes attributable to physical inactivity was higher in females than in males.

Overweight is responsible for 5.4% of total death and 6.1% of total YLL (Table 1). The harmful effect of overweight was greatest for diabetes type 2, 47.3% of total diabetes type 2 DALY in males and 60.2% in females were attributable to overweight (Table 2 and Table 3). The burden of diabetes type 2, IHD, CVD, colorectal cancer and breast cancer attributable to overweight is higher in females than in males.

Hypertension is responsible for 13.3% of all death and 12.0% of YLL (Table 1). The harmful effect of hypertension is greatest on renal failure and CVD, 49.6% of total renal failure DALY in males and 58.9% in females, and 48.7% of total CVD DALY in males and 40.5% in females are attributable to hypertension (Table 2 and Table 3). Hypertension is also an important risk factor for IHD, 19.2% of total IHD DALY in males and 24.7% in females are attributable to hypertension. Burden of IHD, CVD and renal failure attributable to hypertension are higher in females than in males.

High blood cholesterol is responsible for 0.92% of all deaths and 0.99% of all YLL (Table 1). A part of total IHD burden is attributable to high blood cholesterol, 7.0% of total IHD DALY in males and 5.4% in females (Table 2 and Table 3).

Table 1

Number of deaths and years of life lost (YLL) because of premature deaths attributable to the selected risk factors, and their proportions (%) in total number of deaths and total YLL

Risk factor	Attributable deaths number (%)*	Attributable YLL number (%)†
Alcohol consumption	-1,873 (-1.80)	1,044 (-0.13)
Cigarette smoking	10,187 (9.80)	11,196 (13.70)
Overweight	5,701 (5.40)	50,382 (6.10)
Physical inactivity	12,501 (12.01)	79,764 (9.80)
Inadequate intake of fruits and vegetables	2,476 (2.38)	26,084 (3.20)
Hypertension	13,882 (13.30)	97,776 (12.00)
High blood cholesterol	956 (0.92)	8,044 (0.99)

***% of total number of deaths – 104,000; †% of total YLL – 19,758,000**

Table 2

Number of deaths, YLL, YLD and DALY attributable to the selected risk factors for 10 conditions in the male population of Serbia proper, 2002

Risk factors	Conditions	Deaths	YLL	YLD	DALY	% total DALY†
Alcohol (harm)	Road traffic accidents	183	5.227	4.093	9.321	38.8
	CVD	679	6.384	838	7.222	11.0
	Self inflicted injuries	74	1.340	52	1.392	6.9
Alcohol (benefit)	IHD	-1.315	-10.359	-1.050	-11.409	-10.6
Cigarette smoking	Lung cancer	3.502	36.775	1.768	38.543	82.8
	IHD	1.612	19.064	2.570	21.635	22.5
	CVD	1.173	12.348	2.915	15.263	23.2
Physical inactivity	CVD	2.342	14.109	2.126	16.235	24.7
	IHD	2.647	19.332	1.994	21.326	22.2
	Colorectal cancer	475	3.265	243	3.508	20.6
	Type 2 diabetes	92	685	598	1.283	7.5
Overweight	Type 2 diabetes	773	4.916	3.195	8.111	47.3
	IHD	1.042	12.690	1.411	14.101	14.7
	Colorectal cancer	111	1.896	133	2.029	13.1
	CVD	542	6.248	1.356	7.605	11.6
Hypertension	Renal failure	431	3.316	768	4.084	49.6
	CVD	3.367	23.244	3.412	26.656	40.5
	IHD	2.057	16.702	1.727	18.429	19.2
Diet*	IHD	371	4.026	436	4.461	4.7
	CVD	236	2.362	453	2.814	4.3
Cholesterol	IHD	704	6.122	631	6.753	7.0

***Inadequate intake of fruits and vegetables; †Risk factor dependant DALYs as percent of all DALYs generated by the specific disease in question; YLL – years of life because of premature death; YLD – year life with disability; DALY – disability adjusted life years; CVD – cerebrovascular disease; IHD – ischemic heart disease.**

Table 3

Number of deaths, YLL, YLD and DALY attributable to the selected risk factors for 8 conditions in the female population of Serbia proper, 2002

Risk factors	Conditions	Deaths	YLL	YLD	DALY	% total DALY†
Alcohol (harm)	Road traffic accidents	34	972	608	1.580	24.6
	Breast cancer	174	1.820	175	1.995	8.5
	Self inflicted injuries	34	428	18	446	5.8
Alcohol (benefit)	CVD	-3.233	-16.256	-689	-19.945	-19.4
	IHD	-802	-4.732	-524	-5.256	-8.7
Cigarette smoking	Lung cancer	599	11.006	309	11.315	90.2
	CVD	643	7.543	1.617	9.160	13.0
	IHD	470	5.063	1.144	6.208	11.3
	Cervix uteri cancer	45	725	57	782	9.6
Physical inactivity	CVD	3.531	20.035	1.722	21.757	31.0
	Colorectal cancer	460	3.006	241	3.246	31.0
	IHD	2.300	13.576	1.607	15.183	27.7
	Breast cancer	361	3.247	316	3.563	15.1
	Type 2 diabetes	152	981	850	1.831	9.1
Overweight	Type 2 diabetes	1.121	7.920	4.244	12.164	60.2
	Colorectal cancer	199	1.588	125	1.713	16.3
	IHD	809	7.323	1.091	8.414	15.3
	CVD	883	7.800	937	8.737	12.4
	Breast cancer	136	1.427	75	1.501	6.4
Hypertension	Renal failure	537	3.779	394	4.174	58.9
	CVD	5.266	31.700	2.504	34.204	48.7
	IHD	2.223	12.232	1.321	13.554	24.7
Diet*	IHD	180	1.741	251	1.993	3.6
	CVD	220	2.105	256	2.361	3.4
Cholesterol	IHD	251	1.922	1.064	2.986	5.4

***Inadequate intake of fruits and vegetables; †Risk factor dependant DALYs as percent of all DALYs generated by the specific disease in question; YLL – years of life because of premature death; YLD – years of life with disability; DALY – disability adjusted life years; CVD – cerebravascular disease; IHD – ischemic heart disease.**

Inadequate intake of fruit and vegetables is responsible for 2.3% of total deaths and 3.2% of total YLL (Table 1). The disease burden attributable to inadequate intake of fruit and vegetables are calculated only for IHD and CVD (Table 2 and Table 3). Total deaths, YLL and DALY attributable to inadequate intake of fruit and vegetables for IHD and CVD are higher for males than females.

Discussion

Our study can be compared well to the Global and the Australian Burden of Disease (BD) studies [7, 23]. Therefore, some of our key results are discussed in the following section with reference to these two main studies.

Alcohol consumption is a serious public health problem not only in Serbia but worldwide, as it is causally related to more than 60 types of diseases and injuries [27, 28]. On the other hand, it is also protective against some diseases especially as regards to IHD [29]. Whereas in our study the balance is slightly positive, according to the majority of the literature the health impact of alcohol consumption on the whole is negative. It is estimated that 4% of the global burden of disease is attributable to alcohol [1], more than half of it (2.8%) is related to high-risk drinking [30], especially among young males. In 2001, the proportion of DALYs attributable to high-risk alcohol consumption was the highest in Europe and Central Asia (8.3%), and the lowest in South Asia (0.9%) and Sub-Saharan Africa (1.3%) [1, 31, 32]. In Serbia the mortality burden attributable to alcohol is 1.6 times higher than the average of the world population [7]. Also like in other East European countries, e.g. in Russia or Lithuania, there is a high alcohol-attributable mortality due to intentional and unintentional injuries, especially for men [28, 33].

Cigarette smoking is the risk factor associated with the greatest health problems and is responsible for 13.7% of the total YLL in Serbia, similar to the results of the BD study in Australia (13.1%) [23], the greatest proportion of disease burden being associated with lung cancer. According to the World Health Report 2002, active cigarette smoking is after high blood pressure the second leading cause of premature mortality [1]. Cigarette smoking is a causal factor for more than 50 different diseases and causes for example 45% of acute myocardial deaths, 25% of deaths due to CVD and 85% of deaths from chronic obstructive pulmonary disease [34].

In Serbia, 12% of all deaths and 9.8% of total YLL are attributable to physical inactivity, again most similar to the Australian BD study with 10.1% of all deaths and 9.0% of total YLL attributable to physical inactivity [23]. Murray and Lopez [35] estimated that the DALY attributable to physical inactivity are 1% worldwide, 4.8% in established market economies.

Overweight has been acknowledged recently as a key health problem. Worldwide 1.1 billion adults are overweight [36]. In the present study overweight is responsible for 6.1% of total YLL which is higher than in the BD study in Australia (4.6% of all YLL) [23] and with regard to DALYs also considerably higher than the European average [1]. Overweight is one of the strongest lifestyle-related factors for developing type 2 diabetes [1, 37] and associated with IHD, CVD, osteoarthritis, and breast, colorectal, prostate, endometrial, kidney and gallbladder cancer [38]. WHO [1] has estimated that approximately 58% of diabetes mellitus globally and 21% of IHD are attributable to a BMI above 25 kg/m^2.

High blood pressure is the leading cause of global burden of disease, especially also in the developing world [39]. In Serbia, hypertension is responsible for 12.0% of all deaths and 13.3% of total YLLs, more in females than in males. In the Australian BD study 11.2% of total deaths and 8.2% of all YLL were attributable to hypertension, considerably less than in Serbia [23]. Globally, approximately two-thirds of CVD and one-half of IHD were attributable to non-optimal blood pressure, with little variation by sex [1, 39]. It is surprising that the controlled fraction of this key risk factor is usually smaller than a quarter of those with hypertension [40].

Because of differences in the definition of inadequate intake of fruits and vegetables our results are not completely comparable, although they are similar to the results of the Australian BD Study [23]. It has been estimated that globally 2.7 million (4.9%) deaths and 26.7 million (1.8%) DALYs in 2000 were attributable to low fruit and vegetable intake [41]. Ideally to protect against CVD and certain cancers, the WHO recommends an intake of 400 g/day [42]. In the global study, low intake of fruit and vegetables is estimated to cause about 31% of IHD, 19% of ischemic CVD, 20% of esophageal cancer and 19% of gastric cancer worldwide [43].

High cholesterol in the present study is responsible for 7.0% of total DALYs in males and for 5.4% of total DALYs in females. Worldwide approximately 56% of IHD mortality and disease burden is attributable to cholesterol levels of more than 3.8 mmol/L, which correspond to 3.6 million deaths in the year 2000 [44]. Overall, 4.4 million deaths (about 7.9% of the total) and 40.4 million DALYs (2.8% of total) worldwide were estimated to be due to non-optimal cholesterol levels.

Conclusion

More than 40% of all deaths and of the total YLL are attributable to cigarette smoking, overweight, physical inactivity, inadequate intake of fruit and vegetables, hypertension and high blood cholesterol, whereas alcohol consumption in Serbia had overall a slightly positive effect. According to the percent of DALY for the selected conditions attributable to the observed risk factors, their most harmful effects are as follows: alcohol consumption on road traffic accidents; cigarette smoking on lung cancer; physical inactivity on CVD, IHD and colorectal cancer; overweight on type 2 diabetes; hypertension on renal failure and CVD; inadequate intake of fruit and vegetables on IHD and CVD, and high blood cholesterol on IHD. A high percentage of disease and injury burden in Serbia is attributable to avoidable risk factors, which emphasizes the need for improvement of relevant preventive strategies and programs at both individual and population levels. Social preferences should be determined for a comprehensive set of conditions prevalent in Serbia and the burden of disease analysis be linked to marginal cost-effectiveness analysis of potential interventions, e.g. Bjego-

vic et al. [45]. Also in the future the burden of disease assessment could be supplemented by positive measures of health expectancy or health adjusted life expectancy, derived from national health, disability and quality of life surveys.

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The evaluation of malignant astrocytoma score (MAS)

Procena skora za maligni astrocitom (MAS)

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Abstract

Background/Aim. At the moment there are few scoring systems for malignant astrocytoma but they are not widely accepted. The aim of this study was to evaluate malignant astrocytoma score (MAS) on a new group of patients with malignant astrocytoma, to compare MAS with other prognostic tools and to describe the use of MAS in everyday practice in neurooncology. **Methods.** The study was performed on 124 patients with supratentorial malignant astrocytoma grade III or IV. They were operated on and subsequently irradiated with 50–60 Gy. **Results.** The mean age of the patients was 57.3 years. The mean Karnofski performance status (KPS) of the functional inpairment was 54. The removal of the tumor > 90% was done in 59.7% of patients. The mean survival was 9.1 months, and 27.4% of patients had a 12-month survival. The area under receiver operating characteristic (ROC) curve (AUC) of the MAS for predicting 6-, 12- and 18-month survival was 0.754, 0.783 and 0.882, respectively. We compared the MAS with the two mostly cited scoring systems. The AUC for the same prediction for medical research council (MRC) was 0.601, 0.693, 0.772 respectively. For the Radiation Therapy Oncology Group (RTOG) the AUC was 0.732, 0.765, 0.827, respectively. **Conclusion.** MAS represents a useful scoring system for determining illness severity and prognosis in patients with malignant supratentorial astrocytoma. It can be helpful in comparing single patients or groups of patients, as well as results of different treatments and in controlling the quality of hospital treatment and so on.

Key words:
astrocytoma; classification; neurosurgery; radiotherapy; prognosis.

Apstrakt

Uvod/Cilj. Trenutno postoji nekoliko sistema za procenu malignih astrocitoma, ali oni nisu šire prihvaćni. Do sada su objavljeni sistemi: *Medical Research Council* (MRC), *Radiation Therapy Oncology Group* (RTOG) i *Malignant Astrcytoma Score* (MAS). Cilj ovoga rada bio je da se proceni sistem MAS, da se on uporedi sa drugim prognostičkim metodama na novodijagnostikovanoj grupi bolesnika sa malignim astrocitomima velikog mozga, kao i da se opiše upotreba MAS u svakodnevnoj kliničkoj praksi. **Metode.** Ispitivanjem je bilo obuhvaćeno 124 bolesnika sa malignim astrocitomom velikog mozga (gradus III i IV po *World Health Organization* – WHO). Oni su operisani i nakon toga lečeni zračenjem dozom 50–60 Gy. **Rezultati.** Prosečna starost bolestnika bila je 57,3 god. Srednja vrednost indeksa Karnofskog bila je 54. Kod 59,7% slučajeva odstranjeno je više od 90% tumora. Srednje preživljavanje bolesnika bilo je 9,1 mesec, a više od 12 meseci živelo je 27,4% bolesnika. Vrednosti površine ispod krive ROC bile su za MAS procenu preživljavanja od 6, 12 i 18 meseci, 0,754, 0,783 i 0,882, redom. Metoda ROC bila je za iste načine predviđanja za sistem MRC 0,601, 0,693 i 0,772, redom. Za sistem RTOG vrednost ROC bila je 0,732, 0,765 i 0,827, redom. **Zaključak.** Smatramo da je MAS koristan sistem za procenu težine bolesti i prognoze kod bolesnika koji boluju od supratentorijalnog malignog astrocitoma. On je koristan za upoređivanje pojedinačnih bolesnika ili grupa između sebe kao i za upoređivanje rezultata različitih oblika lečenja, za kontrolu kvaliteta lečenja u pojedinim ustanovama i sl.

Ključne reči:
astrocitom; klasifikacioni indeksi; neurohirurgija; radioterapija; prognoza.

Introduction

Malignant astrocytomas are the most frequent glial tumors of the brain in adults. Severity and prognosis of this illness depend on the ratio of the malignant potential of the tumor on one side, and immune defense against tumor cells on the other [1]. Malignant potential depends on the number and types of mutations of three types of genes (oncogenes, tumor

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suppressor genes and DNA repair genes) [2–5]. These factors are primal (direct) prognostic factors and because they are not well known and defined they are not used in the prognostic purpose routinely. Indirect prognostic factors such as: age, histopathological (HP) grade, tumor size, clinical status, Karnofski performance status (KPS) of the functional impairment, magnetic resonance imaging MRI characteristics, p53 inactivation are usually used [6–12].

There are a few prognostic systems that combine these single prognostic factors. The newest scoring system is Malignant Astrocytoma Score (MAS) published in 2007 [1]. Other scoring systems are Medical Research Council (MRC) [13] and Radiation Therapy Oncology Group (RTOG) but they are not widely accepted in the usual practice [14].

A good scoring system should be accurate, easy for use even in undeveloped countries, made of widely accepted prognostic factors, and not to include any treatment option (e.g. extent of resection).

Every scoring system tends to get best results in the group of patients it is designed for. That's why it should be tested on other groups to validate its accuracy and usefulness [15–17].

The aim of this study was to evaluate the MAS on a new group of patients with malignant astrocytoma, to compare is with other prognostic tools and to describe its use in everyday practice in neurooncology.

Methods

This prospective study included 124 patients. It was approved by Ethical Board of Faculty of Medicine, University of Novi Sad. The patients were treated at our clinic in a 5-year period (2003–2007). The enrollment criteria for 131 patients were: supratentorial malignant astrocytoma (HP grade III or IV according to the WHO grade system); patients older than 21 years; complete treatment according to our protocol. The therapeutic protocol included: preoperative antiedematous and symptomatic treatment, surgery (1 or 2) and irradiation. We tried maximal reduction the tumor regardless of the age and KPS of patients. Radical attempts were not made if there was any danger of postoperative neurological or any major clinical deterioration. Irradiation was done by the conformal radiotherapy (Varian 18 MeV) with the dose of 50–60 Gy (25–30 fractions). The zone of irradiation was a tumor location a surrounding 2–3 cm of brain tissue. A total of 7 patients were excluded because not having complete survival data for them, and because two of them suffered from another kind of tumor.

We analyzed the prognostic effect on survival time of three scoring systems – MAS, MRC, and RTOG.

The system MRC uses 4 prognostic factors: age, performance status, extent of surgery and history of fits. According to the score (0–38) the patients are divided into 6 groups [13].

The system RTOG is a descriptive one. It uses 7 factors: HP grade, age, existence of mental status, KPS, duration of symptoms, extent of resection, patient's ability to work. The patients are divided into 6 groups [14].

For calculating MAS we used the program (Serbian version) [1]. The MAS includes: age, KPS, HP grade, initial seizure lasting more than 3 months [1] (Table 1).

Table 1

Malignant astrocytoma score (MAS)

Prognostic factor parameters	Number of points
Age (years)	
15–45	4
46–60	2
> 60	0
KPS (points)	
20–40	0
50–70	3
80–100	6
Histopathological grade	
III	1
IV	0
Initial seizure lasting more than 3 months	
yes	5
no	0
Total	0–16

KPS – Karnofski performance status.

Standard statistical methods were used to analyze data. The influence of a prognostic factor on survival time was tested by Cox proportional hazard model and Stepwise Cox Regression. A *p* value of less than 0.05 was regarded as statistically significant.

With the Receiver Operating Characteristic (ROC) curves we analysed the sensitivity and specificity of scoring systems. The area under the ROC curve (AUC) represents efficacy of a scoring system. In biological systems, if AUC is more than 0.7 (70%) it is considered to be significantly accurate in prognosis (Figure 1).

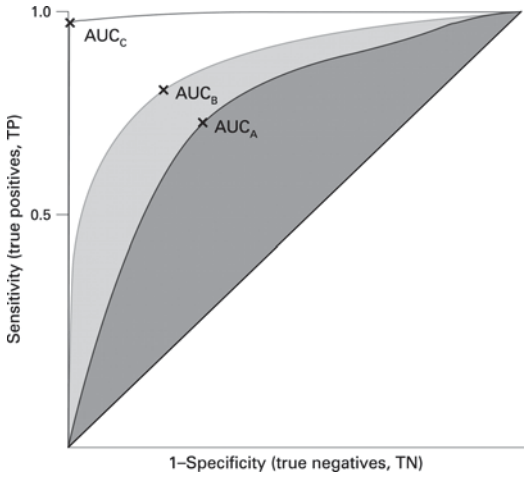

Fig. 1 – The curves for the three prognostic models (A, B and C) that show both their sensitivity and specificity by measuring the area under the curve (AUC).

To compare cumulative survival curves for groups of patients we used the Logrank test.

For statistical analysis we used the PC program MedCalc v. 11.6.1 and S-Plus.

Results

There were 124 patients in this research. The male to female ratio was 57.8 : 42.2% ($p = 0.042$). The mean age was 57.3 years. There were 11.7% of patients with two operations.

The mean duration of illness was 11.4 weeks. The mean KPS was 54.

The mean diameter, measured on T1 Gd MRI was 4.9 cm. In 57.5% of the patients the tumor had a greater diameter than 5 cm.

Postoperative computed tomography with contrast was used to verify the extent of excision. We measured the reduction in the biggest diameter of the tumor. The mean removal of the tumor was 78% (Table 2).

Table 2

Data connected to surgery and histopathology in patients with malignant astrocytoma

Results of surgical treatment	Patients (n) %
Extent of surgical resection	
biopsy	2 (1.6)
10–50%	15 (12.1)
60–80%	33 (26.6)
90–100%	74 (59.7)
Histopathological grade	
III	45 (36.3)
IV	79 (63.7)

Operative mortality was 3.2%, mean survival 9.1 months, while 27.4% of the patients had 12-month survival (Table 3 and 4).

Table 3

Prognostic factors affecting survival time

Prognostic factors	*p*
Age (years)	0.0015
Preoperative KPS (value 10–100)	0.0003
Postoperative KPS (value 10–100)	0.0001
Histopathological grade	0.0319
Epilepsy lasting > 3 months	0.0001

KPS – Karnofski performance status.

According to the MAS we classified patients into 3 groups: G1 0–4; G2 5–10; G3 11–15 (Figure 2).

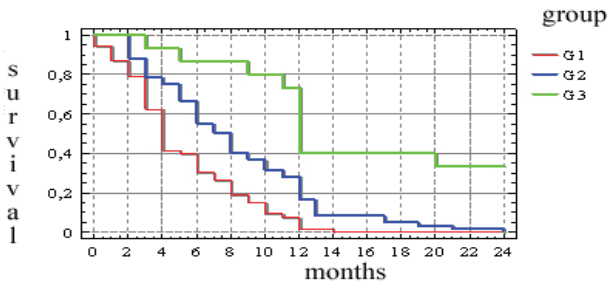

Fig. 2 – Kaplan-Meier survival curves of the groups G1, G2 and G3

G1 – malignant astrocytome score (MAS): 0–4; G2 – MAS: 5–10; G3 – MAS: 11–15.

According to the logrank test there was a significant difference between cumulative survival curves for the groups G1, G2 and G3. These groups were enough distinctive ($p < 0.000001$). For example, 6-month survival in our series was 42% for the group 1, 61% for the group 2, and 87% for the group 3 (Table 5).

Table 5

Patients by the groups G1-G3 distribution

Group of patients	Number of patients	Median survival (months)
G1	52	4
G2	56	8
G3	16	12

G1 – malignant astrocytome score (MAS): 0–4; G2 – MAS: 5–10; G3 – MAS 11–15.

Discussion

The exact prediction of a process (100% accuracy) would be possible if we use factors directly responsible for that process. For malignant astrocytoma there should be two groups of direct prognostic factors. The first group should be a combination of factors affecting the grade of malignancy (probably the types and number of mutated oncogenes and inactivated tumor suppressor genes). The second group should be a combinations of factors influencing human means of antitumor defense

Table 4

The area under the ROC curve (AUC) of nine different prognostic instruments for prediction of 6-,12- and 18-month survival

Prognostic parameters	6 months	12 months	18 months
Age	0.731	0.741	0.810
Histopathological grade	0.532	0.504	0.641
Epilepsy lasting > 3 months	0.643	0.672	0.819
KPS – initial	0.612	0.642	0.731
KPS – at discharge	0.657	0.639	0.761
MAS1	0.727	0.787	0.854
MAS2	0.754	0.783	0.882
MRC	0.601	0.693	0.772
RTOG	0.732	0.765	0.827

KPS – Karnofski performance status; MAS – malignant astrocytoma score; MAS1 – initial values; MAS2 – values at discharge from the hospital; MRC – Medical Research Council; RTOG – Radiation Therapy Oncology Group.

(immunologic and nonimmunologic) [2–5]. Prognostic factors such as age, histopathological grade, labeling index are indirect. They just represent more or less accurately direct prognostic factors which are not until now defined.

Histopathology is the most widely used prognostic factor. There are some researches who facted to prove HP grade as having a prognostic effect in patients older than 60 years (34.2% in our series) [18] According to the classification of malignant glioma by Curran at al. [14], young patients (< 50 years) with a good KPS (> 90) and glioblastoma live longer than older patients with a low KPS and anaplastic astrocytoma. Some study failed to prove the prognostic effect of HP grade at all [13, 19, 20]. Histopathological grading is still subjective to some degree, especially in institutions with less experience [21]. There are two very different methods of grading which are widely accepted (WHO and Daumas-Duport).

New prognotic parameters like detection of genes and tumor markers are not affordable for developing countries. A scoring system that would include these factors would be also inappropriate for them.

Exact prognosis and determination of illness severity are important in a single case for the prediction of survival, quality of life, and cost of treatment. Better prognostic systems are also essential for determination of illness severity of the whole groups of patients with malignant astrocytoma. It enables us to compare different treatment results between these groups. There are many excellent results of new treatments that can be partly influenced by patients selections. There are investigated groups of patents subjected to some treatments of malignant gliomas and for example with mean KPS of 80, or with the mean illness duration of 4 months or with representation of epilepsy more than 20% [22–26]. These data implies some kind of patients selection. The best way to exclude that and to compare different groups of patients are scoring systems [27–28].

The reasons for the MAS to include age, HP grade, KPS and appearance of early long lasting epilepsy, and why certain number of points is assigned to each of them, is explained in the study published earlier [1].

MAS simplicity and feasibility can be described by the following characteristics: it has only 4 parameters (age, HP grade, KPS and appearance of early long lasting epilepsy); these parameters are widely accepted; they are easy to determine even in developing countries; it is numerical, not descriptive. There is a computer program for the easier counting of the MAS, which is possible to incorporate in the Data basis of patients; it has 17 possible results (0–16). That is not too big nor too small for possible numerical descriptions of patients; Patients are, according to MAS, divided into three prognostic groups: 1 point assigned to HP enables to calculate MAS before operation with an acceptable presumption; MAS does not include any type of treatment (e.g. extent of surgical resection of the tumor), so it can be used in unoperated patients; it successfully depicts illness severity and prognosis in a single patients as well as in the groups of patients.

When evaluating illness severity, predicted by the MAS, we can predict factors that depend on it such as: survival time, the quality of life, treatment expenses, etc.

The MAS can help in choosing the extent of treatment concerning benefits for patients on one side and the costs on the other.

The MAS is the most accurate in predicting less severely ill patients (prediction that survival time will be more than 18 months).

According to investigation on a new group of patients the MAS showed better results in prediction than the ways of prediction now available (single prognostic factors and scoring systems RTOG and MRC).

The MAS is a simple and feasible prognostic system.

Conclusion

The MAS is a useful scoring system for determining illness severity and prognosis in patients with malignant supratentorial astrocytoma. It can be useful in comparison of single patients or groups of patients, and subsequently in comparison of results of different treatments and in controlling the quality of hospitals treatment and the like.

The current prognosis, mostly according to the histopathological grade, seems to be similar to a case of making prediction in neurotrauma just according to the type of hematoma and computed tomography findings without the Glasgow Coma Scale (GCS).

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Mutational analysis of ATP7B gene and the genotype-phenotype correlation in patients with Wilson's disease in Serbia

Analiza mutacija ATP7B gena i genetsko-klinička korelacija kod obolelih od Wilson-ove bolesti u Srbiji

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Abstract

Background/Aim. Wilson's disease (WD) is an autosomal-recessive disorder which is characterized with a marked clinical heterogeneity. The gene responsible for WD is located in 13q14.3 chromosome, contains 21 exons and codes for copper specific transporting P-type adenosinetriphosphatase (ATPase) (ATP7B). Mutations in ATP7B gene change biosynthetic and transporting role of ATPase in cell leading to damaged billiary excretion of copper and its accumulation in the liver, brain, cornea and other tissues. Until now, it has been described more than 400 mutations in ATP7B gene with characteristic geographic distribution. The aim of this study was to assess the spectrum of mutations of ATP7B gene on a large number of patients in Serbian population and to make a correlation between particular genotypes and specific phenotypes. **Methods.** Eighty-six consecutive patients with WD from WD Clinical Research programme were included in this study. Genetic analysis was performed by direct gene sequencing method. **Results.** Mutations in ATP7B gene were found in 93% analyzed patients (81.4% of all alleles analyzed). Thirteen mutations were identified, one of which (G998E) was the novel one, so far undescribed in the literature. The most frequent mutation in our population was H1069Q, which was present in 38.4% patients, and the second most frequent mutation was 2304-2305insC (11.6%). Also, a great number of gene polymorphisms of DNA sequences, which do not disturb the ATP7B gene function, was identified. Although neurological form of the disease was more frequent in the group of homozygous for H1069Q and the group of non-H1069Q carriers, there was no statistically significant difference between the groups. **Conclusion.** Our research showed that genetic diagnosis of WD can be done in 80% of cases by analysis of 5 most common mutations in our population, which facilitate diagnosis significantly. There was no correlation between different genotypes and specific phenotypic features of WD, the presence of psychiatric disturbances and cognitive deterioration.

Key words:
hepatolenticular degeneration; diagnosis; genotype; phenotype.

Apstrakt

Uvod/Cilj. Vilsonova bolest (VB) je autosomno-recesivno nasledno oboljenje koje se odlikuje velikom kliničkom heterogenošću. Gen odgovoran za VB je lociran na hromozomu 13q14.3, sadrži 21 egzon i kodira za specifičnu adenozintrifosfatazu (ATP-azu) P-tipa koja transportuje bakar (ATP7B). Mutacije u ATP7B remete biosintetsku i transportnu ulogu ATP-aze u ćeliji, sa posledičnim poremećajem bilijarne ekskrecije bakra i njegovim nagomilavanjem u jetri, mozgu, kornei i drugim tkivima. Otkriveno je preko 400 mutacija u ATP7B genu sa karakterističnom geografskom distribucijom. Cilj rada bio je da se poceni spektar mutacija u genu ATP7B kod velikog broja bolesnika u srpskoj populaciji, kao i da se utvrdi korelacija između pojedinih genotipova i specifičnih fenotipova. **Metode.** Studijom je bilo obuhvaćeno 86 konsekutivnih bolesnika sa VB iz baze Kliničkog programa za istraživanje VB. Genetska analiza vršena je metodom direktnog sekvenciranja gena. **Rezultati.** Mutacije u genu odgovornom za VB (ATP7B) ustanovljene su kod 93% bolesnika (81,4% svih analiziranih alela). Identifikovano je 13 mutacija, od kojih je jedna (G998E), do sada nepoznata u literaturi, prvi put zabeležena u našoj populaciji bolesnika. Najučestalija mutacija u našoj populaciji

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bila je H1069Q, kod 38,4% bolesnika, a druga po učestalosti 2304-2305insC kod 11,6% bolesnika. Takođe, registrovan je i veliki broj genskih polimorfizama DNK sekvenci koji ne narušavaju funkciju ATP7B gena, tj. ne prouzrokuju bolest. Iako je kod homozigota za H1069Q i kod nosilaca ostalih mutacija uočena veća učestalost neurološke forme bolesti, grupe se nisu međusobno statistički značajno razlikovale. **Zaključak:** Naše istraživanje pokazalo je da se analizom 5 najčešćih mutacija u našoj populaciji može postaviti genetska dijagnoza VB kod više od 80% bolesnika, što u značajnoj meri olakšava dijagnozu bolesti. Nije ustanovljena korelacija ispitivanih genotipova sa specifičnim fenotipskim ispoljavanjima VB, prisustvom psihičkih smetnji i kognitivnim osiromašenjem.

Ključne reči:
hepatolentikularna degeneracija; dijagnoza; genotip; fenotip.

Introduction

Wilson's disease (WD) is an autosomal-recessive disorder characterized with marked clinical heterogeneity. Gene responsible for WD is located on chromosome 13q14.3 [1, 2]. It consists of 21 exons and encodes for a copper-transporting P-type adenosinetriphosphatase(ATPase) (ATP7B) [3]. Mutations in ATP7B gene change biosynthetic and transporting role of ATPase in cell, resulting in impaired billiary excretion of copper and its accumulation in liver, brain, cornea and other tissues. The disease incidence is estimated to be 1 in 30,000 to 1 in 50,000 [4, 5], and it is supposed that the prevalence is 30 per million, with frequency of heterozygous mutations carriers of about 1 in 90 to 1 in 150 [6, 7]. Until now, it has been identified more than 400 mutations in ATP7B gene with characteristic geographic distribution [8, 9]. Most mutations are present in particular families, in particular cases. In previous study of our population, molecular gene defect was identified in 80% of alleles of WD patients [10], with 11 different mutations (3 of which were new ones). The most frequent mutations were H1069Q (48.9% of analyzed alleles), 2304–2305insC (11.4%), and A1003T (5.7%) [10].

The disease can be presented through 3 forms: hepatic, neurological and psychiatric [11]. There is no clear evidence of associations between specific genotypes and some clinical features of the disease. Therefore, the aim of this study was to assess spectrum of mutations in ATP7B gene on a larger number of patients in Serbian population, and to make a correlation between particular genotypes and specific phenotypes.

Methods

Eighty six consecutive patients with WD from the WD Clinical Research programme were included in this study. The diagnosis of WD was made based on standard criteria: anamnesis, clinical examination, ceruloplasmin and copper serum level, copper level in 24-hour urine, liver biopsy, "slit"-lamp examination for the presence of Kayser-Fleischer's ring.

Study was approved by the Ethic Committee, Clinical Center of Serbia, Belgrade. Upon signing informed consent, the patients were interviewed in order to obtain demographic and clinical data on disease course, therapy, etc. All the patients passed through neurological and hepatic examination, as well as brain nuclear magnetic resonance (NMR).

Disability level and grading of WD and its multisystem manifestations were measured by the Global Assessment Scale (GAS) for WD [12]. This scale has 2 tiers: tier 1 assesses global disability in 4 domains: liver, cognition and behaviour, motor and osseomuscular functions (each domain is scored independently on a 6-point ascendant scale (0–5) and results are not added), while tier 2 is multidimensional scale for a fine grained evaluation of the neurological dysfunction [14 items are scored on a 5-point ascendant scale (0–4) and their sum (0–56) presents a total score of tier 2]. Psychiatric comorbidity was examined through the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) [13]. The SCID for every patient was performed by the trained psychiatrist. Cognition was estimated by the Mini Mental State Examination (MMSE) scale [14]. Genotype-phenotype analysis was made upon association of specific molecular defect and disease form, age at onset, the presence of psychiatric disturbances, and GAS for WD scores.

DNA extraction and polymerase chain reaction (PCR) analysis were made by standard methods. Mutation detection was performed by the direct sequencing method. Exons 5, 8, 13–15 of ATP7B gene were amplified using primers complementary to DNA sequences flanking the exon-intron boundaries. PCR products were purified using a standard purifying protocol and then sequenced on an automatic sequencer (ABI 310 Genetic Analyzer). Point mutations were detected reliably by means of manual inspection of characteristic double peaks. Mutation analysis was carried out according to following strategy: firstly, exon 14 was analyzed for the most frequent mutation in Serbian WD patients (H1069Q), secondly, if no or only one mutation was found, exons 8, 5, 15 and 13 were analyzed, as locations of other most common mutations.

Data were analyzed using methods of descriptive statistic, analysis of variance (ANOVA) and $\chi 2$ -test.

Results

The study consisted of 86 patients with demographic and clinical characteristics shown in Table 1. A predominant disease form was neurological (54.7%), while hepatic form was present in 41.9% of the patients. Therapy was taken continuously in 71.8% of the patients, mostly d-penicillamine (84.4%).

Molecular defects in ATP7B gene were found in 93% patients (81.4% of all the analyzed alleles). Thirteen mutations were identified: 2 frameshifts, 1splicing and 10 mis-

Table 1

Demographic and clinical characteristics of the patients

Variables	Values
Sex, n (%)	
male	55 (64.0)
female	31 (36.0)
Age (years), $\bar{x} \pm SD$	36.5 ± 11.3
Age at onset (years), $\bar{x} \pm SD$	2.9 ± 8.6
Disease duration (years), $\bar{x} \pm SD$	12.9 ± 9.0
Latency from disease onset to diagnosis (months), $\bar{x} \pm SD$	19.7 ± 29.7
Form of disease, n (%)	
neurological	47 (54.7)
hepatic	36 (41.9)
missing data	3 (3.4)
Therapy, n (%)	
penicillamine	65 (84.4)
Zn-sulphate	23 (29.9)
trientine	1 (1.3)
Continuity of therapy, n (%)	
yes	51 (71.8)
no	20 (28.2)
Psychiatric disorders, n (%)	
present	21 (24)
absent	29 (34)
missing data	36 (42)
MMSE, $\bar{x} \pm SD$	28.6 ± 4.1
GAS liver, $\bar{x} \pm SD$	2.5 ± 1.3
GAS cognition and behaviour, $\bar{x} \pm SD$	1.5 ± 1.5
GAS motor, $\bar{x} \pm SD$	1.3 ± 1.3
GAS osseomuscular, $\bar{x} \pm SD$	0.2 ± 0.7

MMSE – Mini-Mental State Examination scale; GAS – Global Assessment Scale.

sense mutations, one of which (G998E) was the novel one, described for the first time in the literature. Six patients had no known mutations, while three of them had gene polymorphisms. The most frequent mutation in our population, H1069Q, was present in 38.4% of the analyzed alleles, followed by insertion 2304–2305insC in exon 8 (11.63% of analyzed alleles). Two missense mutations, A1003T in exon 13 and R616Q in exon 5 occurred with the frequencies of 9.3% and 8.1%, respectively. Relatively frequently found mutations were also 3402delC (2.32%) and R969Q (1.74%), while G591D and I1102T were individually observed in two heterozygous patients (1.16%). Each of the remaining mutations was present only on one allele of the single patient (Table 2). Furthermore, some DNA polymorphisms that do not disrupt ATB7B gene functions were identified (Table 3).

The patients were divided into three groups according to the found mutations: the group of homozygous for H1069Q, the group of heterozygous for H1069Q, the non-H1069Q patients. H1069Q mutation was identified in 53 patients (13 homozygous and 40 heterozygous). Twenty-seven patients had no H1069Q mutation, but some other ATP7B gene mutation, while 6 patients did not have any of the known mutations. In the group of homozygous for H1069Q more patients had neurological (10) then hepatic (2) disease form. Both clinical presentations had the same frequency (20) in patients heterozygous for H1069Q. There was no significant difference between neurological and hepatic form (13 and 12) in the group of non-H1069Q patients. Although neurological form of disease was more frequent in the groups of homozygous for H1069Q and non-H1069Q patients, there was no statistically significant difference between the examined groups ($\chi 2 = 4.664$; $p = 0.198$). Also, there was no statistically significant difference in frequency of specific mutations in different neurological forms ($\chi 2 = 12.78$; $p = 0.172$).

Age at onset was not statistically significantly different in the examined groups with different genotypes (ANOVA; $F = 1.236$; $p = 0.302$). In group of homozygous for H1069Q dis-

Table 2

Mutations characteristics in the patients with Wilson's disease

Type of mutation	Nucleotide	Frequency (%)	Exon	ATP7B region
		Frameshift		
2304-2305insC	CCCCCCA	20 (11.6%)	8	Tm4
3402delC	TTTCCCGC	4 (2.3%)	15	ATP loop
		Splice- site		
2336G→A		1 (0.6%)		
		Missense		
H1069Q	CAC→CAA	66 (38.4%)	14	ATP loop
A1003T	GCG→ACG	16 (9.3%)	13	Tm6
R616Q	CGG→CAG	14 (8.1%)	5	Cu6
R969Q	CGG→CAG	3 (1.7%)	13	Tm6
I1102T	ATT→ACT	2 (1.2%)	15	ATP loop
G591D	GGC→GAC	2 (1.2%)	5	Cu6
G988E	GGG→GAG	1 (0.6%)	13	Tm6
E1064K	GAG→AAG	1 (0.6%)	14	ATP loop
A994S	GCT→TCT	1 (0.6%)	13	Tm6
T977M	ACG→ATG	1 (0.6%)	13	Tm6

Table 3

DNA polymorphisms in ATP7B gene

Polymorphism	Exon	Intron	ATP7B region
2973G→A (Thr991Thr)	13		Tm6
3009G→A (Ala1003Ala)	13		Tm6
3045G→A (Leu1015Leu)	13		Tm6

ease onset was at the age of 22.6 ± 7.6 years, in heterozygous for H1069Q at 25.6 ± 9.0 years, while in non-H1069Q patients disease started at the age of 23.0 ± 8.9 years (Table 4).

The influence of genotype on severity of clinical presentation was examined through correlations of specific mutations and GAS for WD scores. There was no statistically significant difference among the examined genotype groups and subscores for the assessment of hepatic (ANOVA, F = 0.131; $p = 0.941$), cognitive and behavioural (ANOVA; F = 1.476; $p = 0.231$), motor (ANOVA; F = 0.550; $p = 0.650$) and osseomuscular impairment (ANOVA; F = 0.681; $p = 0.567$). Neither cognitive impairment, estimated by MMSE, (ANOVA; F = 0.428; $p = 0.734$) (Table 5), nor the frequency of psychiatric manifestations (Table 4) ($\chi 2 = 9.109$; $p = 0.168$) differed among various genotype groups.

population. Regarding this, it can be concluded that H1069Q is a very old mutation [16, 17].

The second most frequent mutation in our population, insertion 2304–2305insC in exon 8 (11.6% of examined alleles) is present in 6% of WD patients of the continental part of Italy and in 2.6% of population of Russia and North America [16]. However, the third most common mutation in our group, substitution of alanine with threonine (A1003T) in exon 13 (9.3% of the analyzed alleles) shows no important frequency in other European population. Substitution of arginine with glutamine in the position 616 (R616Q) in exon 5 is the ultimate mutation with significant frequency in our research that was previously described only in one WD patient in Great Britain [18] and Italy [19]. Deguti et al. [20] described deletion 3402delC in exon 15 (2.3% of the analyzed alleles in

Table 4

Influence of genotype of the patients with Wilson's disease on the disease onset

Genotype	n (%)	Age at onset (years)* x̄ ± SD (min–max)	Disease form N (n)	Disease form H (n)	Psychiatric disorders Absent (n)	Psychiatric disorders Present (n)
H1069Q/H1069Q	13 (15.1)*	22.6 ± 7.6 (10–38)	10	2	2	7
H1069Q/other	40 (46.5)	25.6 ± 9.0 (9–49)	20	20	15	8
Other mutations	27 (31.4)*	23.0 ± 8.9 (7–43)	13	12	9	5

N – neurological form; H – hepatic form; *missing data for disease form for the 3 patients; n – number of patients

Table 5

Influence of genotype of the patients with Wilson's disease (WD) on severity of the disease presentation

Scale for clinical assessment	H1069Q/H1069Q x̄ ± SD	H1069Q/other x̄ ± SD	Other mutations x̄ ± SD	*p*
GAS Liver	2.4 ± 1.3	2.6 ± 1.4	2.3 ± 1.4	0.941
GAS Cognition and behaviour	2.4 ± 1.3	1.4 ± 1.6	1.4 ± 1.5	0.231
GAS Motor	1.8 ± 1.3	1.1 ± 1.3	1.2 ± 1.4	0.650
GAS Osseomuscular	0.4 ± 0.9	0.3 ± 0.9	0.1 ± 0.4	0.567
MMSE	28.7 ± 0.9	29.3 ± 1.03	27.6 ± 7.7	0.734

GAS – Global Assessment scale; MMSE – Mini-Mental State Examination scale.

Discussion

Our study identified 13 mutations (12 formerly known, 1 new) in ATP7B gene in 81.3% of the analyzed alleles. Our results confirm that the spectum of mutations in WD consists of a small number of relatively frequent mutations and a large number of rare mutatons.

The most frequent mutatation in our population is substitution of histidine with glutamine on the position 1069 (H1069Q) in exon 14, which is found in 38.4% of the analyzed alleles. Also, this is the most common mutation responsible for WD worldwide, present only in Caucasions [10], and seems to originate from the Central or Eastern Europe [15]. Its frequency is highest in Poland and Eastern Germany (50–80%) [4]. In Europe, south of the Alps, this mutation becomes infrequent and it is totally absent in Sardinia and Sicily, which can bee explained by teritorial isolation and high rate of consanguinity (incidence of WD in these islands is approximately one per 7,000) [16]. This mutation is always found in the same haplotype, haplotype VIII defined by STR loci D13S301, D13S296, D13S297, D13S298 [17] (allele combination 5, 9, 4, 3, respectively) in every analyzed our population) as the most common gene defect in Brazil with the frequency of 30.8%, as well as in Russian population (19%) [4, 10]. It is interesting that this mutation was not found in Mediterranean region, although the dominant immigrant group in the South America was from this region [19, 21]. Substitution R969Q is frequent in Greece (18%) [22], while in our study it was identified only in 1.74% of the analyzed alleles.

Five mutations (splice-site 2336G→A, missense T977M, G988E, E1064K, A994S) were registered in one allele of 13q14.3 locus (0.58%). Mutation G988E in exon 5 has not been identified so far.

Strategy for search of pathological mutations in WD by the direct sequencing method was initially directed towards exons 5, 8, 13, 14 and 15, as loci with majority of the known mutations in our population, as well as in ethnical groups of Central and Eastern Europe.

The main reason for great clinical heterogeneity in WD has not been explained yet, but it is believed that this is caused by different types of mutations in ATP7B gene and their effect on ATP7B function (various mutations cause different impairments of ATP7B protein func-

tions) [23]. Studies that tried to explore influence of ATP7B genotype on clinical presentation and disease course showed no consistent results. Estimation of genotype-phenotype correlation is difficult because of large number of mutations (more than 400) with a marked genetical heterogeneity, small number of patients in the majority of studies, and significant hetererozigocity [23]. The frequency of H1069Q mutations in homozygous state was the only one that met criteria for gentype-phenotype analysis. Some studies showed that homozygous for H1069Q presented first disease symptoms up to 12 years later than compound heterozygotes and non-H1069Q patients, as well as less severe disturbance in copper metabolism [24]. This clinical expression can be explained by partially perserved ATP7B protein function, with residual billiary copper excretion present, resulting in slower accumulation in liver. Our study did not confirm prevously described clinical characteristic of patients with homozygous H1069Q mutation.

It has been suggested that nonsense, splice-site and frameshift mutations are associated with severe phenotypic expression due to the production of non-functional protein product, while missense mutations, that do not disturb protein function totally, are marked as "mild" [24, 25]. Gromadzka et al. [24] demonstrated on 142 WD patients that carry of one or two "severe" mutation have dose-dependent effect on lowering of ceruloplasmin level and younger disease onset. We tried to asses a correlation between genotype and severity of clinical expression using GAS for WD, but no significant associations were observed.

Conclusion

Our study showed that the genetic diagnosis of WD can be made by analysis of the 5 most frequent mutations in our population in more than 80% of cases, which can significantly facilitate diagnosis. Unfortunately, there was no correlation made between the examined genotypes and the specific phenotypic features of WD (disease form and severity of clinical expression), the presence of psychiatric disturbances and cognitive deterioration.

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The use of total ossicular replacement prosthesis after radical tympanomastoidectomy

Upotreba totalne osikularne proteze nakon radikalne trepanacije temporalne kosti

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Abstract

Background/Aim. This paper presents our operative method for hearing recovery after the previous radical tympanomasto-idectomy, radical trepanation of the temporal bone (*trepanatio radicalis ossis temporalis* – TROT) in eight patients submitted to operations for giant cholesteatotoma. **Methods.** All the patients were admitted to our clinic after TROT. There were no signs of cholesteatoma or infection. The patients refused any stent implantations or any hearing aids due to possible aesthetic problems. The described procedure developed in two steps. The first one was to restore the destroyed cavum tympany and to covert with chondroperichondral new membrane with a pin-like "guide" as collumela. The second step was to insert a TORP (total ossicular replacement prosthesis) after guide excision. **Results.** After the first operation (stage one) there were no infections in the operated area nor chondroperichondral graft rejection. Postoperative audiometry (6 to 8 weeks) was done to demonstrate the improvement of air conduction. Three months following the first, the second (stage two) operation was performed and 2.5 to 3 months after this operation even greater audiometry revealed hearing improvement in air- and bone-conduction. The patients were dismissed from the hospital 2 days after each procedure without any complications. They did not experience any dizziness, vomiting nor a severe pain. Three months after the second operative stage, otoscopic findings were very good. The audiometry findings after a 3-months period (after stage one) and 3 months after final TORP insertion was done for each of the patients. After one year, the audiometric curve was the same. Clinical and audiometry follow up demonstrated a hearing recovery and closure of air bone gap (ABG) to values of 5 to 15 dB. **Conclusion.** The use of TORP after radical tympanomastoidectomy is feasible. The first step of the procedure is the fixation of a neomembrane. A stabilized neomembrane is essential for light overpressure on the prosthesis and this is important for optimal or better conductivity. A better hearing recovery is confirmed with audiometric findings and ABG reduction to 5–15 dB. This method could be performed in all patients (with good bone-conduction) after radical tympanomastoidectomy for better hearing.

Key words:
chaolesteatoma; otologic surgical procedures; hearing loss, conductive; ossicular replacement; prostheses and implants; reconstructive surgical procedures.

Apstrakt

Uvod/Cilj. Ovaj rad predstavlja naš operativni metod za oporavak sluha nakon radikalne timpanomastoidektomije ranije, radikalne trepanacije temporalne kosti (lat. *trepanatio radicalis ossis temporalis* – TROT) kod osam bolesnika koji su bili ranije operisani zbog džinovskih holesteatoma. **Metode.** Svi bolesnici primljeni su u kliniku nakon TROT. Nije bilo znakova holesteatoma ni infekcije. Bolesnici su odbijali bilo kakvu implantaciju ili slušno pomagalo koje bi izazvalo estetski problem. Opisana procedura se odvijala u dve faze. Prvo, restauriran je uništeni prostor srednjeg uva i prepokriven hondroperihondralnom neomembranom sa „vodičem" nalik na trn, kao kolumelom. Druga faza bila je insercija totalne osikularne proteze (TORP) nakon ekscizije „vodiča". **Rezultati.** Posle prve operacije (faza 1) nije došlo do inficiranja u zoni operacije, niti do odbacivanja hondroperihondralnog grafta. Posleoperativna audiometrija (6–8 nedelja) pokazala je poboljšanje vazdušne provodljivosti. Tri meseca posle prve, urađena je druga operacija (faza 2), a 2,5–3 meseca posle nje još opsežnija audiometrija pokazala je popravljanje sluha i vazdušne i koštane pokretljivosti. Bolesnici su otpušteni iz bolnice bez ijedne komplikacije, kao ni vrtoglavice, povraćanja niti bola. Tri meseca posle druge operativne faze, nalazi dobijeni otoskopijom bili su veoma dobri. Urađena je i audiometrija tri meseca posle faze 1, ko i tri meseca posle konačnog ubacivanja TORP-a kod svakog bolesnika. Audiometrijska kriva bila je ista i godinu dana kasnije. Klini-

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čkim i audiometrijskim praćenjem ustanovljen je slušni oporavak od 5 do 15 dB i zatvaranje vazdušno-koštanog procepa (air bone gap – ABG). **Zaključak.** Primena TORP posle radikalne timpanomastoidektomije je moguća. Prva faza ovog postupka jeste fiksiranje nove membrane. Stabilna nova membrana važna je za blagi nadpritisak na protezu zbog optimalne ili bolje provodljivosti. Bolji sluh potvrdili su audiometrijski nalazi i smanjenje ABG za 5–15 dB. Ova metoda mogla bi se koristiti kod svih bolesnika sa dobrim koštanim provođenjem posle timpanomastoidektomije za postizanje boljeg sluha.

Ključne reči:
holesteatom; hirurgija, otološka, procedure; sluh, konduktivni gubitak; slušne koščice, proteze; proteze i implantati; hirurgija, rekonstruktivna, procedure.

Introduction

By definition, radical trepanation of the temporal bone (*trepanatio radicalis ossis temporalis* – TROT) includes mastoidectomy, antrotomy, cleansing whole middle ear and closure of the Eustachian tube [1]. This operation is also known as radical tympanomastoidectomy. This procedure is often indicated in patients with huge cholesteatoma processes or tumors in middle ear and mastoid region. After TROT the frequency of hearing impairment was high [2].

There are many attempts to improve hearing thereafter [3–5]. In case of partial defects in ossicular chain, there are different types of tympanoossiculoplasty with partial or total prosthesis in use [6]. If there is no ossicle, a total prosthesis must be used.

The cost of these procedures is very high in developing countries and young individuals experience some aesthetic problems in accepting hearing aids.

The aim of this study was to present our original two-step operative method that can help in hearing recovery in patients who refused a hearing aids, and to demonstrate functional results using this method which is a combination of the known methods in otosurgery.

Method

All the 8 patients were admitted to the University Hospital with poor hearing in the previously operated ear.

The 8 patients were of both sexes, different age (from 13 to 57 years). All the patients had previously been operated on for cholesteatoma and everyone had made radical timpanomastoidectomy, with the epitelized postoperative cavity, and no signs of infection and recurrence of cholesteatoma. All the patients had preserved bone conductivity and conductivity air significantly decreased (about 45 dB air-bone gap-ABG). The studied group of patients refused visible hearing aids or implants, and accepted the proposed surgical treatment, although they had been submited to radical tympanomastoidectomy 3–9 years ago.

Including factors for this type of surgery were: already underwent radical timpanomastoidectomy; no signs of infection and recurrence of cholesteatoma in the postoperative period; good (preserved) bone conductivity (15–20 dB), ABG greater than 40 dB and the wish to improve hearing without visible hearing aids (for aesthetic reasons). The patients with radical timpanomastoidectomy who wanted total assicular replacement prosthesis (TORP) implantation were not treated due to (excluding factors): suspected infection or recurrence of cholesteatoma, the lack of preserved bone conduction and small ABG and/or the existence of sensorineural hypacusis.

Our patients were followed clinically and audiometricaly prior to surgery, after the first phase (about 3 months) and after the set TORP prosthesis (3 months and a year later).

Clinical findings were very good in the years following radical operation. Mastoid CT showed no signs of rest/residual cholesteatoma. There were no recidives of cholesteatoma and no signs of infection. Pure tone audiometric examination showed insufficient air conduction. Audiometrical examination showed a reduction in air conduction. The patients and their families refused any surgical procedure along with the use of visible hearing aids.

At admission otoscopic findings revealed a wide postoperative cavity after radical mastoidectomy with good epithelization. There were no signs of infection, nor residual cholesteatoma. Audiometrical findings showed a satisfied bone-conduction (BC) (approx. 25–30 dB in whole frequencies) and poor air-conduction (AC) (approx. 45–55 dB) in the same range.

Inclusion criteria for this method was good postoperative bone-conduction (BC) (less than 40 dB) and poor air conduction (AC) (more than 65 dB),

After preoperative planning, the operation was performed in two steps. Both operations were performed in general endotracheal anesthesia.

After local infiltration, through retroauricular incision, the whole epithelized cavity was exposed. The first step of operation was performed through several phases.

At the beginning of operation, de-epithelization of the common cavity after radical trepanation of the temporal bone was performed (Figure 1). Special attention was paid not to

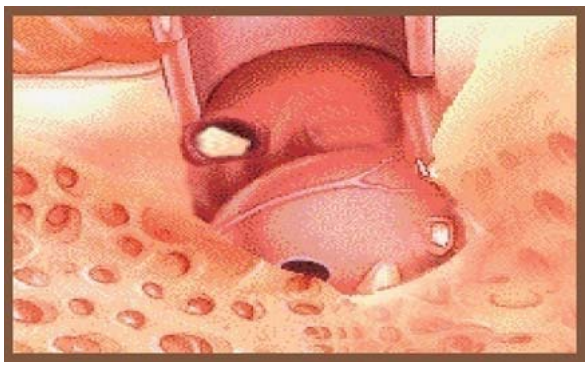


Fig.1 – De-epithelization of the common cavity after radical tympanomastoidectomy.

injure the footplate of stapes, promontorium (the first turn of the cochlea) and the facial nerve. The identification and re-opening of the Eustachian tube was of great importance (Figure 2). The aspiration or probe of the Eustachian tube was necessary. Identification of sticky, transparent secret confirmed the right and proper tube position (Figure 3).

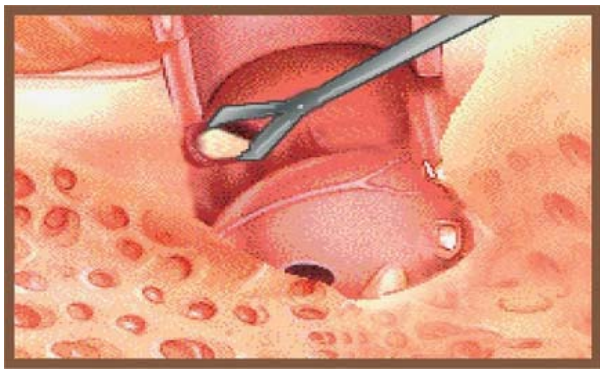


Fig. 2 – Identification of the closed Eustachian tube and its opening.

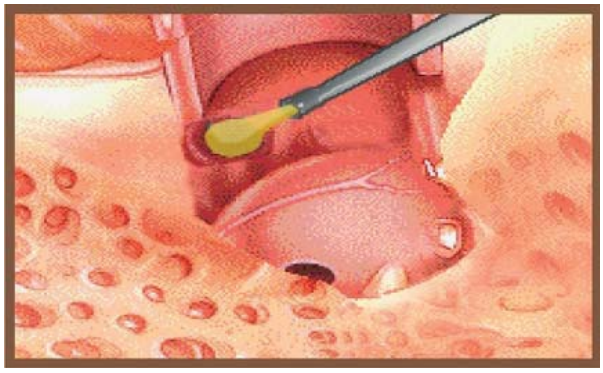


Fig. 3 – Aspiration or a probe into the auditory tube.

Creating a neomembrane was the next step of the procedure to prepare a chondro-peri-chondrial graft from tragus tissue. After the incision on the external ridge of tragus and the exposure of cartilage, a semilunar excision was performed. The excised cartilage was separated in the shape of butterfly wings to be used as a neomembrane. One half of the cartilage was trimmed in order to reduce volume and thickness. On another part a triangular semiexcision was performed where the base of the triangle was fixed to the cartilage. This triangular cartilage part was seen as a "guide" (Figure 4).

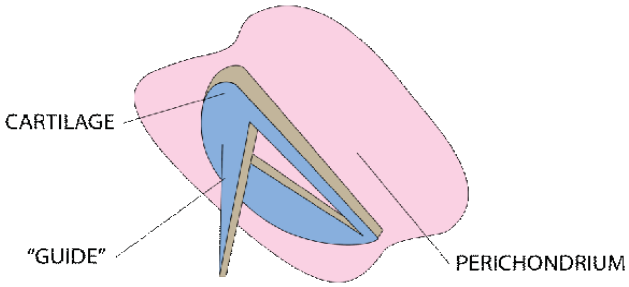


Fig. 4 – Creation of a neomembrane.

It was important to secure an adequate positioning of the neomembrane with the cartilaginous "guide". Two marks should be followed: interiorly, above the tubal orifitium and posteriorly, on the facial ridge of temporal bone. The "guide" was positioned in such a way to touch the base of stapes (Figure 5). This "guide" enabled sound conduction and prevented collapsing of the neomembrane. The "guide" will facilitate the identification of the stapes footplate and easy application of a prosthesis.

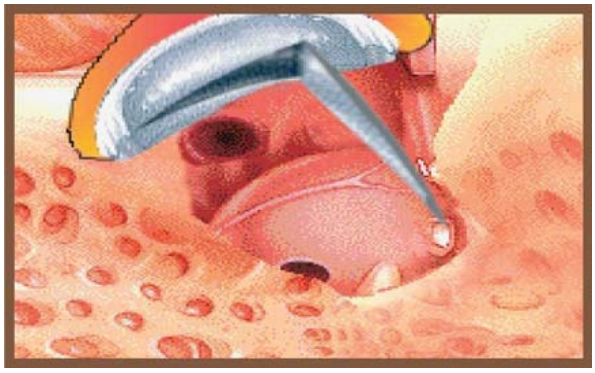


Fig. 5 – A "guide" must touch the base of the stapes.

At the end of the first step we performed the closure of a neocavum tympani and tamponade of the external auditory canal. The closure was very delicate. The peripheral part of the chondro-perichondrial flap must be fixed under the planned excision during deepithelization and the posterior part must lie on the facial ridge on the anterior part (Figure 6). Good fixation of this kind of graft insured a good aeration and good neomembane elasticity.

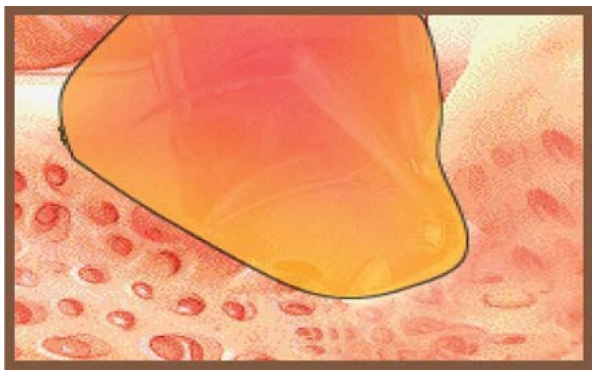


Fig. 6 – Closure of the neocavum.

At the end of this procedure the postoperative course was uneventful. Bandages were changed on the third postoperative day. The patients were dismissed from hospital after 6 days.

Three months postoperatively, audiometry showed better air- and bone-conduction.

Three months after the first step, the second step was performed. Using the transmeatal approach, the neomembrane was identified and opened like a tympanomeatal flap. The cavity below the neomembraine was epithelized with respiratory epithelium. This respiratory-epithelium invasion

probably originates from the Eustachian tube. Also the tip of the "guide" was epithelized at the footplate of stapes. A proximal part of "guide", fixed to the neomembrane, was cut with micro-scissors and totally removed. A small scratch on the stapes footplate was made. A TORP, model Aerial-Vario by "Kurz" (Germany) was used. The TORP was subsequently placed in standard manner.

The second step of the proposed operation was shortly performed in three parts.

The first part comprised of preparing a tympanomeatal flap 5 mm from the fixed neomembrane border which was very slightly pushed up until the "guide" was exposed. The second part was the removal of the "guide" (Figures 7 and 8). The trapezoid space which remained after removing the „guide" was very suitable for the placement of the upper part of TORP. The third part was the insertion of TORP in its place.

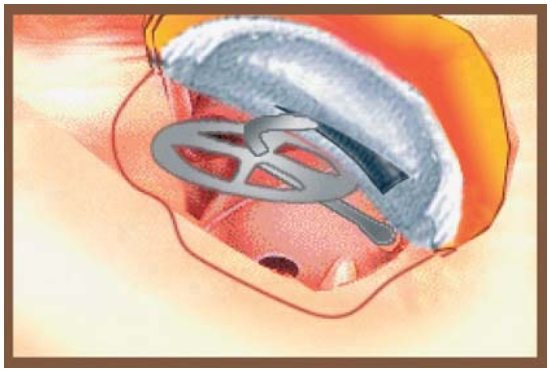


Fig. 7 – A prosthesis in the place.

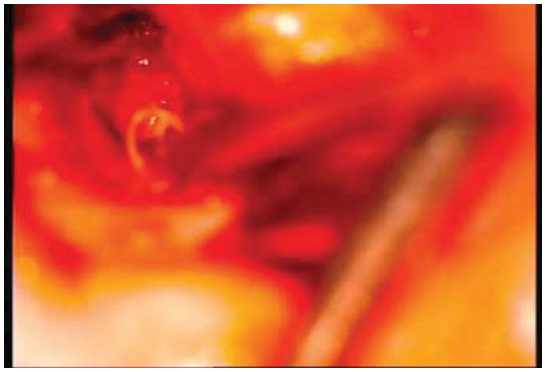


Fig. 8 – A prosthesis in the place (intraoperatively).

The statistical methods used in the study were the ANOVA to the chosen significance level and the Dunnett's test to compare the mean values of the groups. Regression analysis and the significance of differences in the level of air and bone conductivity was performed by ANOVA followed by Dunnett *post hoc* multiple comparison tests using SPSS software Version 11.5.

Results

After the first operation (stage one) there were no infections in the operated area nor chondroperichondral graft rejection. Postoperative audiometry (6 to 8 weeks) was done to demonstrate the improvement of air conduction.

Three months following the first, the second (stage two) operation was performed and 2.5 to 3 months after this operation even greater audiometry revealed hearing improvement in AC and BC (Table 1).

Table 1

Air conduction (AC) and bone conduction (BC) preoperatively (Pre OP) and after total ossicular replacement prosthesis (TORP)

Conductivity	Pre OP (dB)	After TORP (dB)	*p*
AC	68.93 ± 10.21	45.6 ± 8.71	< 0.0001
BC	40.07 ± 8.16	34.28 ± 7.85	< 0.0001

The patients were dismissed from the hospital 2 days after each procedure without any complications. They did not experience any dizziness, vomiting nor a severe pain.

Three months after the second operative stage, otoscopic findings were very good. The audiometry findings after period a 3-months (after stage one) and 3 months after final TORP insertion was done for each of the patients (Figures 9–11).

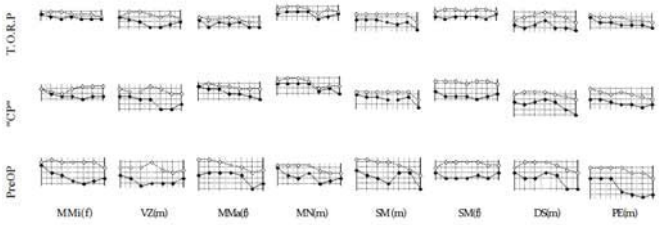


Fig. 9 – Puretone audiometry preoperatively (PreOP), after stage one ("cavum parvum" – "CP") and 3 months after the total ossicular replacemnet prosthesis (TORP) insertion.

After one year, the audiometric curve was the same.

Discussion

For the presented patients, the use of this operative modality was based on their wish to improve their hearing, but specifically without the use of any visible hearing aid. This method was developed out of the need to recover hearing in patients with hearing impairment after radical trepanation of temporal bone. It should not be forgotten that for a patient with childhood cholesteatoma this process has some special characteristics [7].

A different type of prosthesis is used to improve the loss of hearing after tympano-ossiculoplasty [8,9]. A partid ossicular replacement prosthesis (PORP) and TORP are indicated for patients with chronical otitis and damaged ossicular chain. A TORP is currently used in tympanoossiculoplasty as the last resort to preserve hearing abilities [10,11]. In this study TORP was used after radical trepanation of temporal bone. There is no sufficient data describing the use of TORP in this indication.

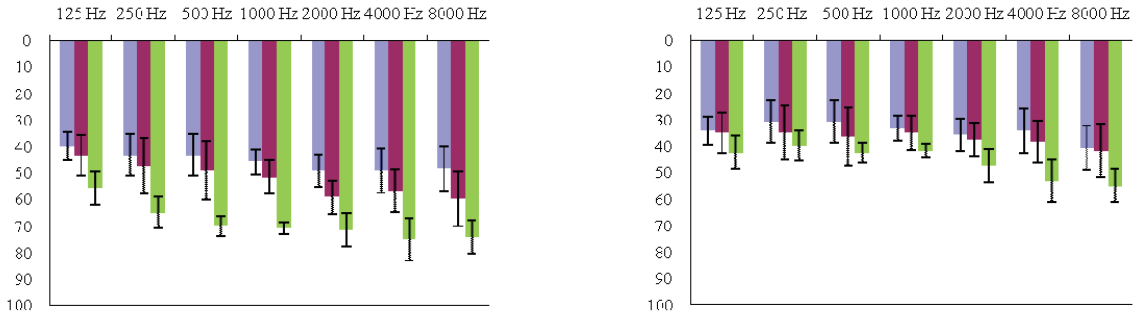
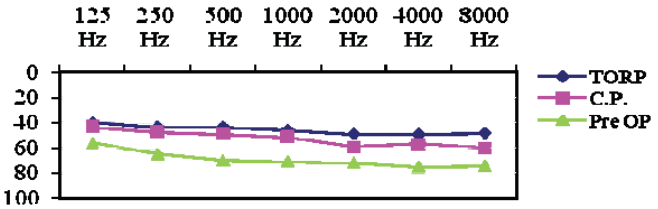
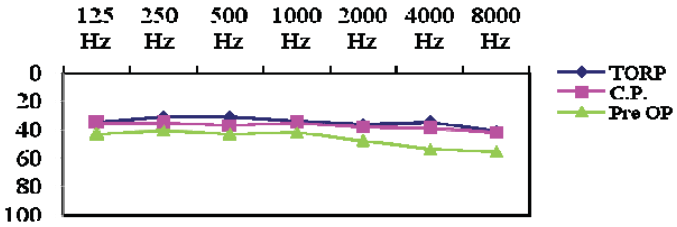


Fig. 10 – Puretone audiometry findings: the average for all the patients for air conduction (AC) and bone conduction (BC) preoperatively ( ), after stage one ( ) and 3 months after the surgery ( )



AC – Air conduction properatively (Pre OP), after stage 1 (*cavum parvum* - CP) and three months after prosthesis insertion (TORP).



BC – bone conduction properatively (Pre OP), after stage 1 (*cavum parvum* - CP) and three months after prosthesis insertion (TORP).

Fig. 11 – Benefit in air conduction and bone conduction (diagrams show a better conduction in all levels even with tympanoplasty with the "guide")

Considering different types of tympanoplasty and reported success [8, 12, 13] with different ossicular prosthesis [14, 15] the right question is: Who are the suitable candidates and why to use this prosthesis? The patients had good BC but poor AC.

The "Aerial-Vario" prosthesis, in comparison with other prothesis [16] has two characteristics which are very important considering its application in this case. First, the usable length (from the footplate up to a neomembrane) can be easily defined by rolling the upper plate up and down intraoperatively in 0.25 mm increments. Second, the elasticity of a neomembrane (chondroperichondral graft) allows better conductivity.

There are several issues that should be resolved in order to perform this procedure. The problem of a closed tube with a small diameter should be solved by using a diamond burr to make it wider. Finding of a "sticky" tubal secret is a proof that the tubal lumen was found. Curettage of tympanal part of tube should support microvascular and epithelial invasion in „cavum parvum" (it is expected after 3 months which is the reason why the second step should be delayed for this period).

Neomembrane creation differs from other cartilage and/or perichondral grafts [17–20]. It uses a tragal cartilage-perichondral "butterfly-like" graft [21]. Its surface and shape are large enough to cover a future neocavum [15]. One half of the cartilage that was orientated posteriorly was of full thickness, and that was orientated interiorly was trimmed to the half of its full thickness. One half of the cartilage had been removed to enable better elasticity due to the weight and volume of the graft.

Considerating that patients with chronical otitis could have a tubal dysfunction, the cartilage "guide" (triangular pin-like cartilage part) was used for two reasons. First, to prevent collapsing of the neomembrane and second, for sound conducting effect (columella effect). In case that auditory tube is blocked (mucous, edema, infection) the pin-like guide preserves a constant distance between a neomembrane and the footplate of stapes, preventing neomembrane collapse in case of negative pressure. Additionally pine-like "guide" serves as a columella to established/reestablish renewed cochlear activity to sound stimulation.

"Cavum parvum" is not the same as the one Wullstein reported on tympanoplasty type IV [1]. In this case it is a space under the neomembrane bordered anteriorly, superiorly and inferiorly on the new ridges (made with 0.5 mm diamond bur), and which lies on the facial ridge posteriorly.

The mastoid cavity was not filled or closed because of the ability to view a mastoid part. Of course, filling of mastoid cavity is possible with bone dust or commercial preparations [22–24] as in any "wall-down" tympanoplasty.

The second stage is simple – to cut out the "guide", remove it and position a TORP in its place. It is very useful to make [25], a small incision on the stapes footplate to prevent dislocation or migration of the prosthesis. For this reason, the upper part of a prosthesis was placed in an empty triangular space inside of a neomembrane.

Before the first stage, the audiometrical findings showed 25–39 dB of BC and 55 dB of AC. The air-bone conduction gap was 30 dB. Two months after the first stage ABG was 10–20 dB (depending of frequencies). It showed a better value range between 250 Hz and 2 kHz. At this time, BC also showed increased values from 5 dB. It could have been a sign that the cochlear activity was getting better. After 3 months, the second stage was performed. Three months after the second stage, audiometry showed BC of 20–25 dB; and AC of 30–35 dB; ABG was 5–15 dB.

With the use of TORP, ABG closure was expected [12, 13, 16]. A surprising fact was that the bone conduction was improving. This study sheds a new light on recovery processes in the cochlea. The reason for it may be found in a better and more permanent stimulation of cochlea. This is a very good procedure for hearing recovery for patients with good bone-conduction. Compared to other implantable systems it is cheaper and it is followed by good audimetrical findings.

In this case no visible hearing aids were used that was a very significant fact. The cost of the procedure (cheaper than other methods) should not be neglected either.

Conclusion

The use of TORP after radical tympanomastoidectomy is feasible. The first step of the procedure is fixation of a neomembrane. A stabilized neomembrane is essential for light overpressure on the prosthesis and this is important for optimal or better conductivity.

A better hearing recovery is confirmed with audiometric findings and ABG reduction to 5–15 dB.

This method could be performed in all patients (with good bone-conduction) after radical tympanomastoidectomy for better hearing.

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The influence of retrobulbar adipose tissue volume upon intraocular pressure in obesity

Uticaj retrobulbarnog masnog tkiva na intraokularni pritisak kod gojaznih osoba

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Abstract

Background/Aim. It is known that glaucoma is associated with elevated intraocular pressure and obesity, yet the precise etiology remains unclear. The aim of this study was to determine whether there is a potential causality between the volume of retrobulbar adipose tissue and the level of intraocular pressure in obese subjects compared with non-obese. **Methods.** A total of 100 subjects were divided according to the body mass index (BMI), into two groups: normal weight (n = 50, BMI = 18–24.9 kg/m^2) and obese (n = 50, BMI ≥ 30 kg/m^2) subjects. Anthropometric measurements, body composition analysis, measurement of intraocular pressure, as well as magnetic resonance imaging (MRI) of the head at the level of the optic nerve, and the derived retrobulbar adipose tissue volume, were undertaken in all subjects. **Results.** The obese subjects, as compared with normal weight ones, had a significantly higher mean retrobulbar adipose tissue volume (6.23 cm^3 vs 4.85 cm^3, $p < 0.01$) and intraocular pressure (15.96 mmHg vs 12.99 mmHg, $p < 0.01$). Furthermore, intraocular pressure correlated positively with retrobulbar adipose tissue volume. **Conclusion.** In obese people, elevated intraocular pressure may be caused by changes in ocular blood flow, affected by the physical pressure exerted by higher retrobulbar adiposity, and/or by internal vascular changes secondary to complications of obesity. These findings indicate the need for more frequent measurement of intraocular pressure in obese individuals to earlier detect glaucoma, and in so doing prevent irreversible blindness.

Key words:
orbit; adipose tissue; intraocular pressure; obesity; magnetic resonance imaging; glaucoma.

Apstrakt

Uvod/Cilj. Zna se da je glaukom povezan sa povišenim vrednostima očnog pritiska i gojaznošću, ipak tačna etiologija je i dalje nepoznata. Cilj ove studije bio je da se utvrdi da li postoji uzročna povezanost između zapremine retrobulbarnog masnog tkiva i visine očnog pritiska kod gojaznih osoba u poređenju sa normalno uhranjenim osobama. **Metode.** Istraživanjem je bilo obuhvaćeno 100 ispitanika podeljenih na osnovu vrednosti indeksa telesne mase [*body mass index* (BMI)] na grupu normalno uhranjenih (n = 50, BMI = 18–24,9 kg/m^2) i grupu gojaznih (n = 50, BMI ≥ 30 kg/m^2) ispitanika. Kod svih ispitanika sprovedena su određena antropometrijska merenja, analiza telesnog sastava, merenje očnog pritiska, kao i snimanje glave u nivou optičkog živca magnetnom rezonancijom (MR), na osnovu kojeg je izračunata zapremina retrobulbarnog masnog tkiva. **Rezultati.** Gojazni ispitanici u poređenju sa normalno uhranjenim imali su značajno više srednje vrednosti zapremine retrobulbarnog masnog tkiva (6,23 cm^3 *vs* 4,85 cm^3, $p < 0,01$) i očnog pritiska (15,96 mmHg *vs* 12,99 mmHg, $p < 0,01$). Takođe, utvrđena je pozitivna korelacija vrednosti visine očnog pritiska i zapremine retrobulbarnog masog tkiva. **Zaključak.** Kod gojaznih osoba povišene vrednosti očnog pritiska mogu biti izazvane promenama u krvotoku oka do kojih dolazi zbog povećanog fizičkog pritiska retrobulbarnog masnog tkiva i/ili zbog vaskularnih promena, kao sekundarnih komplikacija gojaznosti. Ovakvi rezultati ukazuju na potrebu češćeg merenja očnog pritiska kod gojaznih osoba, u cilju ranog otkrivanja glaukoma i sprečavanja pojave ireverzibilnog slepila.

Ključne reči:
orbita; masno tkivo; intraokularni pritisak; gojaznost; magnetska rezonanca, snimanje; glaukom.

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Introduction

The diagnosis of glaucoma and the principles of its treatment rest largely upon the measurement of intraocular pressure (IOP), and an understanding of the anatomy and physiology governing the inflow and outflow of aqueous humor.

In the context of glaucoma, "normal" eye pressure is one that does not lead to glaucomatous damage of the optic nerve head. Unfortunately, such a definition can not be expressed numerically, as not all eyes react identically to the same values of IOP [1]. According to Kanski [2], in a population study, the mean value of IOP was 16 mmHg, with two standard deviations giving a normal range of 11–21 mmHg. These values represent the "normal" level of IOP, yet should be taken with caution, as a normal value of IOP in one person can lead to optic nerve head damage and blindness in another [3]. If there is a damage to the optic nerve head, the state is defined as glaucoma.

Glaucoma is a significant health problem, which if not detected and treated in time may lead to irreversible blindness. After cataract, glaucoma is the second commonest cause of blindness in the world, but is second to none as a cause of irreversible blindness [4].

In recent years there has been a significant progress in methods of both diagnosis and treatment of glaucoma, and yet it remains a disease of both increasing prevalence and unknown etiology. Despite many studies having confirmed that glaucoma is associated with ocular hypertension, insulin resistance, systemic hypertension and hyperlipoproteinemia – all of which are comorbidities of obesity – the exact cause remains unknown [5–9].

By modern definition, obesity represents an increase of fat tissue in the body, deleterious to health and occurring as a result of the imbalance between energy intake and expenditure. Its diagnosis is most reliably based upon the value of body mass index (BMI), as recommended by the World Health Organization (WHO). The WHO classification derived from the incidence of various co-morbidities across the range of body mass index values, and is the same for both sexes and all age categories of adult population [10].

In accordance with the definition of obesity, health risk is related to fat rather than total body mass, and, in addition to measuring weight and height, it is necessary to measure fat mass and determine its share in total body weight [11]. However, reference values for fat mass are not yet fully defined, nor is there an international consensus such as that for BMI, because of the lack of a standardized method of measurement. Nor are there well-defined factors which have an impact on changes in fat mass, such as gender, age, race and ethnicity, or level of physical activity [12].

Obesity as a systemic disease affects multiple organ systems. Hence there is a need to investigate the impact of obesity upon eyes. Different studies have identified links between obesity and ocular hypertension, cataract, age-related macular disease (ARMD), diabetic retinopathy, and diseases of oculomotor nerves [8, 13–18].

As obesity is defined by the increase of fat mass, the consequences of obesity develop as a consequence of morphological and functional changes within the adipose tissue. Morphological changes refer to enlargement of the total mass of adipose tissue with a characteristic distribution which determines the specific complications of obesity. In functional terms, obesity leads to inflammatory changes and changes in basic metabolic and endocrine function of adipose tissue.

In this study we investigated the volume of retrobulbar adipose tissue (RAT). Adipose tissue fills the orbit, being situated between the walls of the orbit and the eyeball and subsidiary organs. It surrounds the eyeball, muscles, nerves and blood vessels within the orbit. It extends from the apex of the orbit to the eyelids, just behind the orbital septum. The adipose tissue of the orbit is divided anatomically into retrobulbar (intraconal) and parabulbar (extraconal) parts. RAT is more developed in the parabulbar area, filling the space behind the eyeball. Orbital adipose tissue is limited by the peripheral muscle cone into a lattice-like structure, the margins of which consist of the four oculomotor muscles (superior, inferior, medial and lateral rectus muscles) which divide the adipose tissue into four sheets [19]. RAT histologically belongs to white (unilocular) fat tissue. This type of fat is found under the skin, in the mesentery, around joints, under the skin of palms and soles, in breasts, tongue, and trachea. White adipose tissue is of a yellowish color and comprises fat cells (adipocytes or steatocytes) which form connective lobuli which are incompletely separated compartments.

The aim of this study was to determine the impact of volume RAT upon IOP in non-obese and obese subjects.

Methods

This cross-sectional study involved a 100 subjects divided into the control and the study group, each of 50 subjects. The criteria for inclusion used to select participants were as follows: age 18–60 years of either gender; stable body weight for the previous 6 months; free from diagnosed malignant disease of any localization, endocrine and metabolic disorders and glaucoma; not in receipt of any drug therapy which may affect body fat composition or IOP; and pacemaker-free, owing to incompatibility with magnetic resonance imaging (MRI) and bioelectrical impedance analysis (BIA). All the subjects were volunteers and a signed consent was obtained from all who took part in the study.

Anthropometric measurements were taken to assess nutritional status and to estimate the distribution of adipose tissue. The following parameters were determined: height, weight, and the derived BMI. For measurement of body height the Harpenden anthropometer (Holtain Ltd, Croswell, UK) was used with 0.1 cm accuracy of measurement. During measurement the subjects were standing with arms hanging by their sides and heels together.

Body weight measurement of the subjects wearing only underwear was performed in a medical decimal scale, with 0.1 kg precision. The obtained values were expressed in kg.

The degree of nutritional status was assessed by BMI, which represents the ratio of body weight (BW) in kilograms by the square of body height (BH) in meters (BMI = BW[kg] / BH[m^2]). The subjects were divided into groups according

to BMI (Table 1). Fifty normal-weight subjects with a BMI = 18–24.9 kg/m^2 represented the control group, while a subjects with BMI ≥ 30 kg/m^2 formed the study group. The classification of nutritional status degree was as recommended by the WHO [20].

BIA was employed for body composition analysis. The equipment used was Omron BF300. It comprises two sensors in the form of electrodes, by the held subjects in their hands. BIA is based on measurements of the body's electrical resistance which provides an indirect estimate of body adipose mass. For this measurement it was necessary to take into account height, weight, age and gender of the subjects'. In order to evaluate as precisely as possible body adipose mass, the following requirements were set: the subjects were not to eat nor drink for at least 4 h before examination, physical activity avoided at least 12 h before measurement; the bladder voided 30 min beforehand; alcohol consumption forbidden 48 h prior to measurement, and not to take diuretic drugs for 7 days leading to examination.

The principle of measurement is as follows: subjects hold the electrodes of the appliance in both hands in a position of abduction in relation to the longitudinal axis of the body. A measurement takes 7–15 sec, after which fat mass is read as a percentage of a total body mass (FAT %) and total body fat mass [FAT (kg)].

The IOP of both eyes of all the subjects was measured 4 times a day (at 8 am, 10 am, 12 pm and 2 pm) using a Goldmann's applanation tonometer. For each individual patient, the measured IOP values of each eye were added together then divided by 2, and so expressed as a mean value in mmHg.

Following anthropometric measurement, MRI axial scanning of the head at the level of optic nerve was undertaken for all subjects. The subjects were imaged on a Magnetom Avanto 1.5T (Siemens, Erlangen, Germany). Each subject had to undergo transverse fluid-attenuated inversion recovery (FLAIR) scanning at the level of optic nerve using predefined technical parameters identical for all the subjects: repetition time (TR) 8,500 ms, echo time (TE) 15 ms, field of view (FOV) 25 × 25 cm, matrix and rectangular FOV factor was 320*220. MRI slice thickness was 0.5 cm. The imaging time was approximately 18 min. During scanning, each subject was placed in an 8-channel head matrix coil in order to achieve a constant signal in the antero-posterior direction.

The images obtained were processed using Adobe Photoshop CS5 software (Waltham, MA) for Microsoft and analyzed by the method introduced by Gronemeyer et al. [21]. Adipose tissue is visually distinguished from other tissues through its density. From the drop-down menu on the initial image (Figure 1) the application threshold was used to select a threshold signal intensity value above which RAT was white and all other tissues were black (Figure 2). These pixels were scored as RAT. Then, using the application histogram, following the prior selection of the lateral and medial half of RAT in relation to the optic nerve, the RATvalue in pixels was obtained (RATpx). The same method was applied to the other eye. The values of the areas of RAT in pixels (RATpx) for both eyes were then inserted into the formula for calculation the real value of the volume in cm^3. The

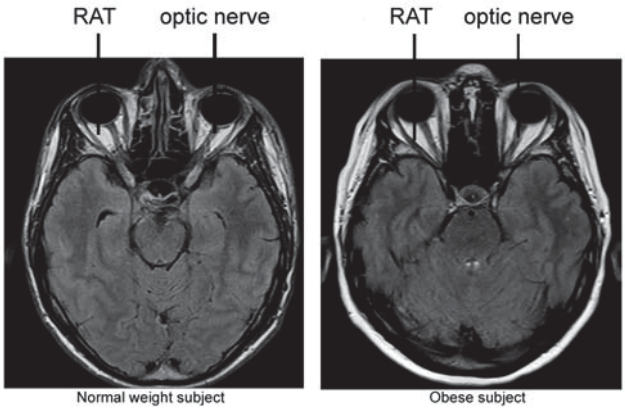

Fig. 1 – Initial magnetic resonance imaging of the head at the level of optic nerve.
RAT – retrobulbar adipose tissue.

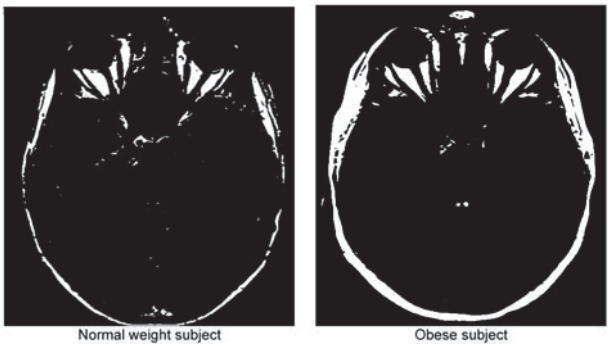

Fig. 2 – Magnetic resonance imaging scans after threshold tool application.

RATpx value was multiplied by the section thickness (0.5 cm) and the value of pixel area (0.0088 cm^2):

$V\ (cm^3) = RATpx \times 0.5\ cm \times 0.0088\ cm^2$

Statistical analysis was performed using Statistica software for Windows, version 10.0 (StatSoft Inc, Tulsa, OK) including the following descriptive parameters: mean ($\bar{x}$), standard deviation (SD), minimum (min), maximum (max) and coefficient of variation (CV). To test the differences between the two groups, we used the paired-samples Student's *t*-test. Pearson's product moment correlation was used for correlation coefficient (*r*). A *p*-value less than 0.05 was considered statistically significant in all the tests. The results are presented in tables and figures.

Results

The group of normal-weight subjects consisted of 50 subjects, aged 19–60 years (mean age, 42.73 ± 11.08 years), of which 26 were male and 24 female.

The obese group consisted of 50 subjects, aged 28–60 years (mean age: 47.5 ± 8.82 years), 23 of whom were male, and 27 female.

The average body weight in the normal-weight subjects was 69.01 ± 8.28 kg, while in the obese subjects the average body weight was significantly higher and reached 91.95 ±

8.93 kg ($p < 0.05$). The mean BMI in the group of normal-weight subjects was 22.83 ± 1.83 kg/m^2, while in obese subjects BMI was significantly higher, 32.62 ± 2.82 kg/m^2 ($p < 0.05$) (Table 2).

RAT volumes in both normal weight and obese subjects ($p < 0.01$) (Table 3).

The next step was to determine the correlation of BMI and body composition with the values of IOP. In the normal-

Table 1

Criteria for assessment of nutritional status based on body mass index (BMI) values

Weight categories	BMI (kg/m^2)
Underweight	< 18.5
Normal	18.5–24.9
Overweight	25.0–29.9
Obese	≥ 30.0

Table 2

Body mass index (BMI) value and body composition analysis in the normal-weight and the obese subjects

Parameters	Normal weight subjects (n = 50)			Obese subjects (n = 50)			p *
	x̄ ± SD	min–max	CV	x̄ ± SD	min–max	CV	
BMI (kg/m^2)	22.83 ± 1.83	18.8–24.82	8.04	32.62 ±2.82	30.04–45.72	8.64	< 0.05
FAT (%)	22.58 ± 7.8	10.6–45.2	34.6	34.50 ±6.68	22.7–49.8	19.37	< 0.05
FAT (kg)	16.32 ± 5.36	7.7–27.5	32.87	30.27 ± 6.42	19.5–49.7	21.19	< 0.05

FAT – body fat mass; x̄ – mean; SD – standard deviation, CV – coeffficient of variation; *Student's *t*-test.

Analysis of the values of certain components of body composition obtained by BIA, confirmed the basic classification of subjects based on the BMI.

BIA showed that the average value of a total body fat mass was significantly higher in the obese compared with the normal-weight individuals ($p < 0.05$). Fat mass as a percentage of total body mass in the obese patients was 34.50 ± 6.68%, while the normal-weight subjects' fat mass was 22.58 ± 7.8% of total body mass.

Fat mass in the obese subjects was 30.27 ± 6.42 kg, while in the normal weight subjects this value was 16.32 ± 5.36 kg. The difference in body fat mass between the control and the subject groups was statistically significant ($p < 0.05$) (Table 2).

In the group of normal weight subjects IOP was found to range from 10 to 18 mmHg (mean value 12.99 ± 2.07 mmHg), while in the obese subjects the IOP range was 11–20.5 mmHg (mean value 15.96 ± 2.3 mmHg). There was a statistically significant difference in the value of IOP between the control and the study group ($p < 0.01$) (Table 3).

In the group of normal-weight subjects the measured values of the volume of RAT ranged from 2.49 to 6.26 cm^3 (mean value 4.85 ± 0.89 cm^3). In the obese subjects the values ranged from 4.47 to 8.26 cm^3 (mean value 6.23 ± 1.02 cm^3). A statistically significant difference was established in

weight and the obese subjects a positive and statistically significant correlation was found between the IOP values and BMI (r = 0.45, $p < 0.05$ *vs* r = 0.31, $p < 0.05$ respectively) (Figure 3). In the normal-weight individuals a positive and highly statistically significant correlation was found for the value of fat mass as a percentage of total body mass (FAT %) (r = 0.55, $p < 0.01$). In the obese subjects the correlation value was also positive, but statistically insignificant (r = 0.16, $p > 0.05$).

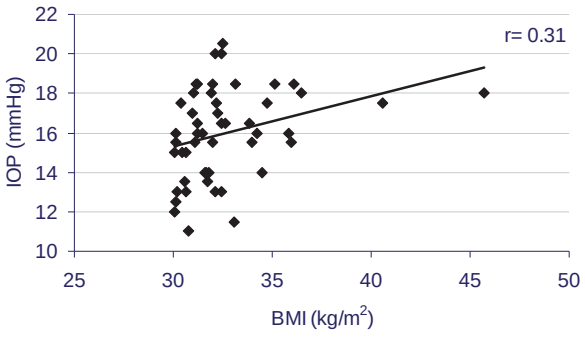

Fig. 3 – Correlation of IOP measurement and BMI in obese subjects.

IOP – intraocular pressure; BMI – body mass index; r – coefficient of correlation.

Table 3

Intraocular pressure (IOP) values and retrobulbar adipose tissue (RAT) volumes in the normal-weight and the obese subjects

Parameters	Normal weight subjects (n = 50)			Obese subjects (n = 50)			p *
	x̄ ± SD	min–max	CV	x̄ ± SD	min–max	CV	
IOP (mmHg)	12.99 ± 2.07	10–18	15.92	15.96 ± 2.3	11–20.5	14.43	< 0.01
RAT (cm^3)	4.85 ± 0.89	2.49–6.26	18.34	6.23 ± 1.02	4.47–8.26	16.37	< 0.01

x̄ – mean; SD – standard deviation, CV – coeffficient of variation; *Student's *t*-test.

The correlation between BMI and RAT in the normal-weight subjects showed insignificantly negative correlation (r = -0.11, $p > 0.05$), while in obese subjects the correlation was positive and highly statistically significant (r = 0.48, $p < 0.01$) (Figure 4). We also investigated the correlation between the fat mass value as a percentage of total body mass (FAT %) and the volume of RAT. In the normal weight subjects, a negative correlation of no statistical significance was found (r = -0.10, $p > 0.05$), while in the obese individuals a positive and statistically insignificant correlation was found (r = 0.25, $p > 0.05$).

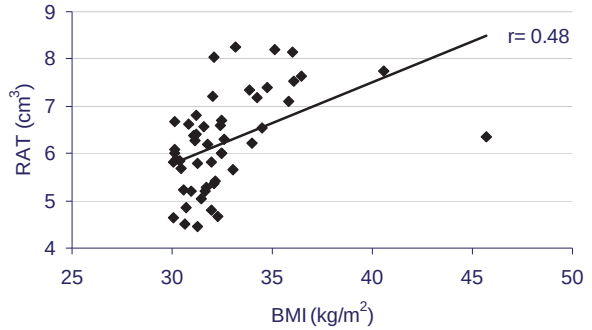

Fig. 4 – Correlation of RAT volume measurement and BMI in obese subjects.
RAT – retrobulbar adipose tissue; BMI – body mass index; r – coefficient of correlation.

We investigated the relationship of IOP values and the volume of RAT in both groups.

In normal-weight subjects a positive correlation was found, but without statistical significance. The value of correlation in this group was r = 0.21 ($p > 0.05$). In the obese subjects a positive and statistically significant correlation was found (r = 0.37, $p < 0.05$) (Figure 5).

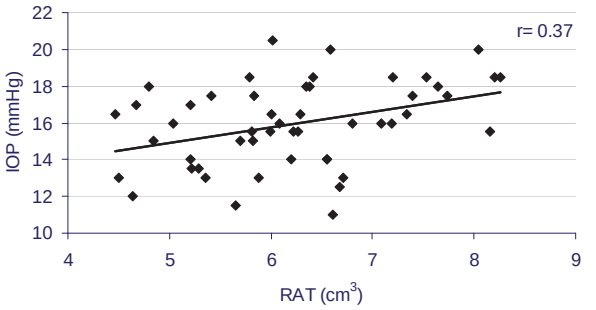

Fig. 5 – Correlation of IOP values and RAT volume in obese subjects.
IOP – intraocular pressure; RAT – retrobulbar adipose tissue; r – coefficient of correlation.

Discussion

Bearing in mind that the exact cause of elevated IOP is still a major scientific challenge, we conducted this research to determine a possible connection between IOP and the volume of RAT and the eventual impact of RAT deposition and obesity upon IOP.

Whilst undertaking the background research, we did not find studies on a possible connection of retrobulbar fat volume and IOP.

Elevated IOP is associated with numerous intraorbital diseases. There are four primary pathophysiological mechanisms that cause elevation of IOP: structural anomalies – congenital and hereditary diseases which lead to disruption of the anatomic integrity of eyeball and orbital tissues; the effect of masses such as tumors and pseudotumors, and of diseases leading to the infiltration of tissues causing compression of ocular and orbital structures; vascular diseases which disrupt eyeball and orbit drainage; inflammatory processes – cellulitis and other forms of orbital inflammation which change the anatomical structure of the orbit and influence vascular function [22]. RAT could be placed into the mechanism of "mass effect", since its presence can directly or indirectly influence episcleral venous pressure which could lead to changes in the Schlemm's canal, thereby inducing IOP elevation. This mechanism is exemplified by orbital space lesions, such as hyperthyroid ophthalmopathy and carotid-cavernous fistula. In support to this theory, a simple example of retrobulbar injection of anesthetic can lead to elevated IOP [22]. Increased intraorbital pressure may have an impact on IOP due to compression of episcleral and orbital venous blood vessels, leading to increased venous pressure [22].

Our findings show that increased RAT volume affects the level of IOP. In the normal-weight subjects the mean IOP was 12.99 mmHg, while in the obese subjects the value was 15.96 mmHg.

Considering that obese people have an increased amount of adipose tissue as compared to normal-weight individuals, we analyzed the relationship between RAT volume in the obese subjects and the controls. In the normal-weight subjects the mean RAT volume was 4.85 cm^3, while in the obese the mean value was 6.23 cm^3. A difference in RAT volume of the normal weight and obese subjects was statistically significant ($p < 0.01$). In available researches, RAT volume was not studied in details, and there are noticeable differences in estimation of the amount of retrobulbar adipose depot.

Tian et al. [23] studied the volumes of intraorbital structures. In their study, the measurement of RAT was performed by MRI. Their results showed that the average total volume of RAT was 21.59 cm^3. According to the results of Forbes et al. [24] the mean RAT volume in men was 11.19 cm^3, while in women it was 10.10 cm^3. Peyster et al. [25] used computed tomography (CT) scan to measure RAT volume. Their results differ from those of Forbes et al. [24]. According to their measurements, RAT volume in the normal-weight group measured 8.16 cm^3, while in the obese subjects it was 11.34 cm^3. Regensburg et al. [26] in their research demonstrated the results of RAT assessment volume based on CT images of 160 orbits. Their results showed statistically significant differences in the volume of adipose tissue in the orbit regarding sex: the mean volume was 16.1 cm^3 in men, and 14.0 cm^3 in women.

Considering the presented results of total RAT volume determation we come to a conclusion that our values correspond to the volume of RAT, because they are related only to a segment of RAT in the thickness of a 5 mm MRI scan slice.

Observing the relationship between IOP value and RAT volume, a positive correlation was found. In the group of normal weight subjects the value of correlation (r) was 0.22 ($p > 0.05$), while in the obese individuals that value was higher and statistically significant (r = 0.37, $p < 0.05$). Unfortunately, there is no available literature data on this kind of comparison, so we cannot compare our results with others.

Due to its simplicity, BMI is commonly used in clinical practice for diagnosing obesity. However, BMI is only an indicator of increasing total body mass, and not of fat mass. Fat from lean body mass cannot be distinguished by using BMI. Being a systemic disease, obesity may increase blood viscosity and episcleral venous pressure, and therefore may lead to obstruction of aqueous humor outflow [27]. The beaver dam eye study showed that the value of IOP increases with BMI values [28]. The same conclusion was reached in a study of over 25,000 subjects in Japan [14]. Cetinkaya et al. [29] indicate that even in children obesity affects IOP elevation, and obesity is considered to be an independent factor for IOP elevation. Zafra Perez et al. [30] also suggest that elevated IOP is associated with obesity.

In our research the mean BMI in the normal-weight subjects was 22.83 kg/m^2, and in the obese 32.62 kg/m^2. The difference was statistically significant ($p < 0.05$). Correlating BMI and IOP in the normal-weight subjects, we found that IOP increases with increasing BMI (r = 0.45, $p < 0.05$). In the group of obese subjects we also found a positive and statistically significant correlation (r = 0.31, $p < 0.05$). Our findings are compatible with data from the literature, and BMI could be considered to be a good predictor of IOP value in normal-weight and obese individuals.

Although it is relatively easy to diagnose obesity, a clear pathophysiological explanation for the links between obesity and IOP remains elusive [8]. To explain this association, mechanical and vascular theories have been proposed.

According to the mechanical theory, it is believed that obesity causes IOP elevation due to the increased volume of retrobulbar fat, increased episcleral venous pressure, increased blood viscosity and aqueous humor outflow disorders [31, 32]. It is evident that ocular hypertension is linked with comorbidities of obesity, such as systemic hypertension, diabetes, lipid disorders and insulin resistance [9, 33–35]. High blood pressure can lead to increased filtration fraction of aqueous humor due to increased pressure in the ciliary artery, while hyperglycemia may lead to disruption of osmotic pressure within the eye which may result in elevated IOP [36]. The vascular theory argues that eyes with genetically poor blood supply to the optic nerve head are more prone to elevated IOP [37]. Recently, more attention has been paid to oxidative stress as a possible cause of elevated IOP [38]. It is known that hyperleptinemia, which occurs in obesity, is associated with oxidative stress [39]. Hyperleptinemia in obesity may be considered as a trigger in the cascade of pathological changes leading to increased IOP [40].

RAT, a fat tissue depot, is clearly limited by the orbital space. Because of this limitation there is no possibility of expansion, as available to other fat tissue depots in the body. According to our results, the correlation between the volume of RAT and BMI in the normal weight subjects was found to be negative and statistically insignificant (r = -0.11, $p > 0.05$), whereas in the obese group the correlation was positive and statistically significant (r = 0.48, $p < 0.01$). Therefore, BMI can be considered as a good predictor of RAT only in obese subjects. These results lead to a conclusion that retrobulbar fat depot correlates with body mass, as do subcutaneous and visceral adipose tissue depots [41].

Janssen et al. [42] clearly point out that BMI is a reliable parameter in predicting visceral, subcutaneous and total adipose tissue. Kamel et al. [43] also consider noted BMI as anthropometric parameter which correlates mostly with intra-abdominal adipose tissue.

BIA is a relatively simple, rapid and noninvasive technique widely used in clinical practice. This method can measure a total fat mass and a percentage of fat mass to total body weight.

According to the results of Srdic [44], fat mass as measured by BIA was significantly higher in obese compared to normal weight subjects, as was the case in our study. Using BIA, our results showed that the average total body fat mass in normal weight subjects was 16.32 kg, compared to 30.27 kg ($p < 0.05$) in the obese group. In addition, BIA also showed a good correlation with the level of IOP, especially in a group of normal weight subjects (r = 0.55, $p < 0.01$). In the group of obese subjects the correlation was also positive, but statistically insignificant (r = 0.15, $p > 0.05$). Our results indicate that the values of IOP may depend on increased volume of adipose tissue in normal-weight subjects and not in the obese.

RAT is a dynamic tissue [45]. RAT volume is different in obese compared to normal-weight subjects. In the obese the volume is far greater, as is the case with other adipose depots of the human organism [46]. Our research confirmed that such adiposity increases in obese individuals. The percentage of body fat mass, measured using BIA, showed a positive correlation with the volume of RAT in obese patients, and this was statistically insignificant (r = 0.25, $p > 0.05$). In the group of normal-weight subjects the correlation was negative and statistically insignificant (r = -0.10, $p > 0.05$). Our findings suggest that the BIA is not valid for determination of enlarged RAT volume in normal weight and obese subjects.

In most studies on orbital soft tissue elements, computed tomography (CT) is widely used [24–26, 31]. MRI used in our study provides safe and accurate measuring of adipose tissue [21, 41]. Different studies state MRI is a gold standard in determination of adipose tissue volume [21, 47]. Although MRI and the special technique of RAT volume determination used in our study provide an accurate assessment of this depot volume, the method is extremely expensive and demanding, in terms of both technical equipment and well-trained staff, and for these reasons is impractical for everyday use in rou-

tine clinical practice. However, MRI can be used to determine adipose tissue depots, the calibration of different techniques for measuring adipose tissue depots and anthropometric parameters for certain population groups, and in cases requiring clinical diagnostics, such as Grave's ophthalmopathy.

Conclusion

According to our results, obese individuals have significantly higher volumes of RAT positively correlated with higher IOP. These results suggest that elevated in obese subjects, IOP may be caused by altered blood flow through ocular vessels, this potentially being mediated by the external physical pressure contingent upon an increased RAT volume, together with internal vascular and metabolic changes occurring as complications of obesity. This therefore recommends a more frequent measurement of IOP in obese individuals, so as to promote early detection and management of any increase in pressure, and in so doing prevent glaucoma and irreversible blindness.

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Improving mechanical properties of flowable dental composite resin by adding silica nanoparticles

Poboljšanje mehaničkih svojstava tečnog kompozita dodavanjem nanočestica silicijum-dioksida

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Abstract

Background/Aim. The main drawback of flowable dental composite resin is low strength compared to conventional composite resin, due to a low amount of filler, neccessary for achieving low viscosity and ease of handling. The aim of this study was to improve mechanical properties of flowable dental composite resin by adding small amount of nanoparticles, which would not compromise handling properties. **Methods.** A commercially available flowable dental composite resin material was mixed with 7 nm aftertreated hydrophobic fumed silica and cured by an UV lamp. Four sets of samples were made: control sample (unmodified), the sample containing 0.05%, 0.2% and 1% nanosilica. Flexural modulus, flexural strength and microhardness were tested. One-way ANOVA followed by Tukey's test with the significance value of $p < 0.05$ was performed to statistically analyze the obtained results. Furthermore, differential scanning calorimetry (DSC) and SEM analysis were performed. To asses handling properties, slumping resistance was determined. **Results.** It was found that 0.05% is the most effective nanosilica content. All the tested mechanical properties were improved by a significant margin. On the other hand, when 0.2% and 1% nanosilica content was tested, different results were obtained, some of the mechanical properties even dropped, while some were insignificantly improved. The difference between slumping resistance of unmodified and modified samples was found to be statistically insignificant. **Conclusions.** Low nanosilica addition proved more effective in improving mechanical properties compared to higher additions. Furthermore, handling properties are unaffected by nanosilica addition.

Key words:
composite resins; surface properties; material testing; nanoparticles; silicon dioxide.

Apstrakt

Uvod/Cilj. Osnovni nedostatak tečnih kompozitnih materijala u odnosu na konvencionalne kompozitne materijale su lošija mehanička svojstva. Ovo je posledica manje količine neorganskog punioca u materijalu u odnosu na standardne kompozitne smole kako bi se dobio materijal manje viskoznosti. Cilj ovog rada bio je poboljšanje mehaničkih svojstava tečnog kompozita dodavanjem male količine nanočestica, čime se ne utiče na način upotrebe ovih materijala. **Metode.** Komercijalni tečni kompozitni materijal umešan je sa modifikovanom hidrofobnom nanosilikom veličine čestica 7 nm, i polimerizovan LED svetlosnim izvorom. Napravljene su četiri grupe uzoraka: kontrolna grupa (nemodifikovana), grupa sa dodatkom 0,05%, 0,2% i 1% nanosilike. Ispitivan je modul elastičnosti, savojna čvrstoća i mikrotvrdoća, dok su rezultati statistički obrađeni jednostrukom analizom ANOVA i Tukey-evim testom sa faktorom značajanosti $p < 0,05$. Takođe, izvršene su i diferencijalnoskenirajuća kalorimetrija (DSC) i SEM analize. Za određivanje mogućnosti rukovanja, iskorišćena je metoda otpornosti na sleganje. **Rezultati.** Ispitivanja su pokazala da najmanja koncentracija nanosilike od 0,05% značajno povećava sva mehanička svojstva. S druge strane, dodatak od 0,2% i 1% daje različite rezultate, gde mehanička svojstva čak opadaju ili se povećavaju, ali ne u značajnoj meri. Otpornost na sleganje kod nemodifikovanih i modifikovanih uzoraka nije bila statistički značajna. **Zaključak.** Nizak sadržaj nanosilike pokazao se kao efikasniji u poboljšanju mehaničkih svojstava u poređenju sa višim sadržajima nanosilike. Način upotrebe uzoraka kod kojih su dodate nanočestice nije bio promenjen u odnosu na uzorke nemodifikovanog tečnog kompozita.

Ključne reči:
smole, kompozitne; površina, svojstva; materijali, testiranje; koloidi; silicijum dioksid.

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Introduction

The first generation of flowable dental resin composites was introduced in the middle of 1990s [1]. They were developed in response to requests for easy handling properties [2]. The low viscosity of the flowable composite simplifies their clinical placement and increases the range of applications in clinical practice [3, 4]. Flowable composites were created by reducing the filler content by 20–25% [1] to reduce the viscosity of the mixture. As a result, these materials are less rigid and have a modulus of elasticity 20–30% lower than conventional hybrid composites [5, 6], but higher volumetric shrinkage and polymerization shrinkage stress [6]. Flowable composites have been proposed as liners [7], fissure sealants and restorative materials for small cavities [8]. Their usage is indicated in non stress bearing areas, because of their low mechanical properties. It was shown that the mechanical properties of flowable composites, such as diametral tensile strength, compressive strength, and fracture toughness, are generally about 60–90% of those of conventional composites [1, 9]. Furthermore, flow composite wear resistance is lower compared to conventional composites and especially highly filled composites [10].

As flowable composites exhibit a relatively low viscosity, one notable way of improving their mechanical properties is adding nanoparticles. Conventional composite mechanical properties, radiopacity and optical properties have been improved by addition of inorganic nano particles. The most common particles are titanium dioxide [11–13] and silica [14], which were added to the composite resin. However, this research may be one of the first studies of flow composites reinforced with nanoparticles.

In this work, an attempt was made to increase flexural strength, flexural modulus of elasticity and hardness of a typical commercially available flowable dental composite resin, by adding silica nanoparticles, without an adverse effect on handling properties. This paper is the result of continuing collaboration program among Faculty of Medicine, Faculty of Technology and Faculty of Technical Sciences of the University of Novi Sad, Serbia.

Methods

In this paper, commercially available Ivoclar Vivadent Te-Econom Flow® composite resin material was used as a basis. This material is based on dimethacrylate paste (Bis-GMA, Triethylene glycoldimethacrylate, Urethane dimethacrylate), with inorganic fillers. In addition to this, initiators, stabilizers and pigments were present, with an overall content of 1% wt [15]. Te-Econom flow was mixed with Evonik AEROSIL® R812 fumed silica aftertreated with hexamethyldisiloxane (HMDS), having hydrophobic properties and particle size of 7 nm [16]. Mixing was done by using a Proxxon FBS12 100 W 3000–15000 min^{-1} precision drill/grinder, with a Dentsply lentulo spiral-paste carrier #4 attached. The lentulo spiral was immersed in composite resin material – nanosilica mixture, poured into a 2 ml predarkened syringe tube, as shown in Figure 1. After one hour mixing, the drill/grinder with lentulo attached was exracted, syringe plunger was inserted into the syringe tube and the mixture was injected into elastomer molds, made from Bego Wirosil®. Curing was done by exposure to LED curing unit (Bluephase C8, 8-mm tip, Ivoclar Vivadent AG). All photopolymerizing steps were performed with a light guide held perpendicularly and within 2 mm of the material surface. The high power curing mode (HIP) was employed throughout the study, while the light output from the curing unit was verified by a built-in radiometer. The intensity of curing light was 1,000 mW/cm^2 and the length of exposure was 20 s (disc samples) and 20 + 20 s (square samples). After curing, 1500 grit SiC paper was used to get the desired shape and dimensions of samples. Dimensions were veryfied by a Hyundai micrometer, accurate to 0.01 mm.

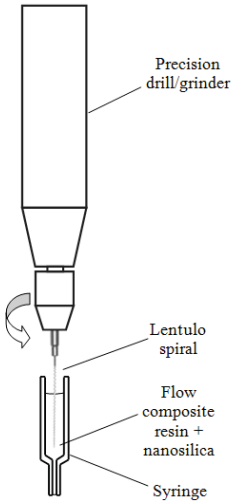

Fig. 1 – Mixing method, by using a precision drill/grinder, lentulo spiral and syringe tube.

Control samples without nanosilica addition were compared to three sets of samples with nanosilica in various concentrations. Three sets of nanocomposite resin material samples were made: with 0.05%, 0.2% and 1% nanosilica. Flexural modulus of elasticity and flexural strenth were determined by using a Toyoseiki AT-L-118B tensile testing machine, with a crosshead speed of 50 mm/min. The 3-point bending method was used with the distance between the supports of 20 mm. Specimens dimensions were 2 × 2 × 22 mm. Flexural modulus of elasticity was calculated in accordance to the following equation:

$$E = \frac{Fl^4}{4dbh^3}$$

where l is the distance between the supports [mm], d is the displacement [mm] for a given load F in [N], b is specimen width [mm] and h is specimen height [mm].

Flexural strength was calculated by using the following equation:

$$\sigma = \frac{3Fl}{2bh^2}$$

where F is maximum force [N], l is the distance between the supports [mm], b is specimen width [mm] and h is specimen height [mm].

Microhardness was determined by using a Huyin HVS-1000 Vickers microhardness tester, by applying 100 g load and 15 s dwell time. Microhardness was measured on 6 mm diameter samples with a height of 2 mm. The common Vickers microhardness and microhardness equation was used:

$$HV = \frac{1.8544\ F}{d^2}$$

where F is applied load [daN] and d is indentation diagonal [mm].

To determine handling properties of unmodified and modified materials, slumping resistance was used. It was determined by using the methodology shown in the work by Lee et al [17]. Namely, an equal amount of flow composite placed into a syringe was extruded onto a slide glass. The resulting bubble was left to slump for 10 s and after that was light – cured. Afterwards, it was measured by a micrometer accurate to 0.01 mm in two aspects, its height and diameter. Height to diameter ratios indicate the slumping resistance: if this ratio is high, slumping resistance is lower and vice versa. These measurements were repeated 10 times for each sample at room temperature.

Mechanical properties and slumping resistance of various samples were statisticaly analyzed using the ANOVA one-way analysis of variance, followed by Tukey's test with the significance value of $p < 0.05$ ($\alpha = 0.05$).

To determine thermal properties of obtained materials, differential scaning calorimetry (DSC) analysis was performed. A TA Instruments Q20 DSC device was used, in the temperature range from 40 to 250^{o} C.

Fracture surfaces were examined by JEOL JSM-6460LV scanning electron microscope (SEM), operating at 25 kV. The specimens were previously coated with gold, using the Balltec SCD-005 device. Per one sample from each flexural strength group was observed. For the purpose of testing, the sample with mechanical properties closest to the average was chosen.

Results

The flexural modulus of elasticity, flexural strength and Vickers microhardness results of the control samples (without nanosilica) and samples with different silica content, as well as the results of one-way ANOVA statistical analysis are shown in Tables 1–3, respectively. The statistical analysis results are represented by the p-parameter. If $p < 0.05$, the difference between the unmodified and nanosilica modified sample is significant.

The flexural modulus of elasticity of nanosilica modified samples was higher than that of the unmodified (control) sample for all the tested samples with various nanosilica content (0.05%, 0.2%, 1%) (Table 1). However, only the samples containing 0.05% and 0.2% nanosilica exhibited statistically significant difference in relation to the unmodified sample. The highest flexural modulus of elasticity was found on the smallest nanosilica content, with a decreasing trend towards higher concentrations. It can be noted that by adding nanosilica, the standard deviation increases, which was the reason why the sample containing the highest concentration of nanosilica (1%) exhibited statistically unsignificant rise in flexural modulus of elasticity.

Table 2 shows the results of flexural strength of unmodified and modified samples. It can be seen, that the flexural strength of samples containing 0.05 and 1% was higher than that of the control samples. However, the flexural

Table 1
Flexural modulus of elasticity of the control sample and nanosilica modified samples, with *p*-values included (derived from ANOVA)

Nanosilica content (%)	Flexural modulus of elasticity E [GPa]		*p*
	mean	standard deviation	
0 (control sample)	3.01	0.18	–
0.05	3.81	0.25	0.00461*
0.2	3.54	0.23	0.00572*
1	3.43	0.24	0.05838

***Values of $p < 0.05$ indicate that the difference between the control sample and nanosilica modified sample is significant with the probability of 95%.**

Table 2
Flexural strength of the control sample and nanosilica modified samples, with *p* -values included (derived from ANOVA)

Nanosilica content (%)	Flexural strength σ_{SM} [MPa]		*p*
	mean	standard deviation	
0 (control sample)	99.48	1.82	–
0.05	103.92	1.75	0.01902*
0.2	94.20	2.45	0.02509*
1	101.58	5.00	0.47356

***Values of $p < 0.05$ indicate that the difference between the control sample and nanosilica modified sample is significant with the probability of 95%.**

Table 3

Microhardness HV0.1 of the control sample and nanosilica modified samples, with *p*-values included (derived from ANOVA)

Nanosilica content (%)	Microhardness HV0.1		*p*
	mean	standard deviation	
0 (control sample)	26.67	0.85	–
0.05	31.40	1.14	0.00024*
0.2	29.80	2.21	0.07095
1	25.40	1.44	0.10959

***Values of $p < 0.05$ indicate that the difference between the control sample and nanosilica modified sample is significant with the probability of 95%.**

strength of the sample with 0.2% nanosilica was lower than that of the unmodified sample. It must be noted that, similarly to the previous results, standard deviations of modified samples were higher than that of unmodified, except from the sample containing 0.05% nanosilica. This particular sample was the only one tested with flexural strength statistically significantly higher than the control sample. On the other hand, sample with 0.2% nanosilica, although its *p*-factor was lower than 0.05, its flexural strength was not significantly higher compared to the unmodified sample.

The microhardness (HV0.1) results are shown in Table 3. The highest microhardness was measured on sample containing 0.05% nanosilica. Microhardness drops as the nanosilica content rised. Furthermore, the microhardness of a sample containing 1% nanosilica was lower than the control sample. Statistically, only the sample with 0.05% nanosilica had a significantly higher microhardness compared to the control sample. Standard deviations of all the modified samples were higher than that of the unmodified samples.

A lateral view of cured flowable composite without and with nanosilica is shown in Figure 2. It can be seen that the shape of the bubbles obtained with various nanosilica concentrations is the same. Slumping resistance represented by bubble height to diameter ratios is shown in Table 4. Although there was a moderate rise in the bubble aspect ratio, the difference between the control sample (non-modified) and nanosilica added samples (0.05 and 0.2%) was not statistically different, as indicated by the factor *p*.

DSC curves of dimethacrylate paste and nanosilica composites showed that a silica content in the examined range, did not have an influence on the glass transition temperature (Tg), (Figure 3), where Tg for all sampless was between 95.22 and 95.88°C. Glass transition temperature

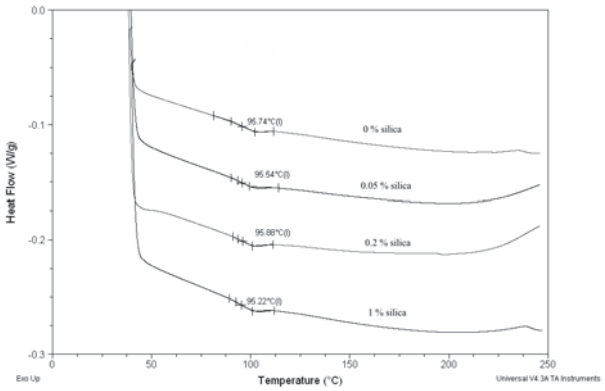

Fig. 3 – Differential scanning of colorimetry (DSC) thermograms of Te-Econom Flow®/silica nanocomposites.

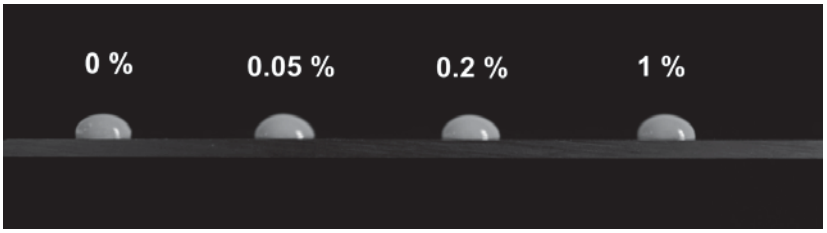

Fig. 2 – A lateral view of cured flowable nonmodified and modified samples after 10 s slumping at room temperature.

Table 4

Aspect ratios of unmodified and modified samples, with *p*-values ncluded (derived by ANOVA)

Nanosilica content (%)	Aspect ratio		*p*
	mean	standard deviation	
0 (control sample)	0.541	0.035911	–
0.05	0.543	0.038449	0.941059*
0.2	0.553	0.038536	0.501769*
1	0.580	0.041927	0.051052*

***Values of $p > 0.05$ indicate that the difference between the control sample and nanosilica modified sample is not significant with the probability of 95%.**

variations are usually correlated to the polymer immobility in interfacial layer[18]. Thus, this indicates that the interfacial layer is relatively thin, meaning that a small amount of polymer is immobilised, having a low influence on Tg.

SEM micrograph showing clustered silica nanoparticles before mixing is shown in Figure 4. Fracture surface micrographs obained by scanning electron microscopy (SEM) are shown in Figure 5. Figure 5 reveals a similar fracture mode for all the samples, unmodified and modified. A brittle type of fracture was noticed, with clearly visible inorganic filler particles. However, the secondary electrone (SE) and especially the backscattering (BS) mode of operation at higher magnifications showed the existance of chemically different particles in comparison to the material matrix (Figure 6). These particles had the size of approximately 100 × 50 nm, which was considerably larger than the average of 7 nm of the Evonik AEROSIL® R812 fumed silica aftertreated with HMDS. Therefore, the detected particles may closely correspond to inorganic particles, fractured in the process of material testing.

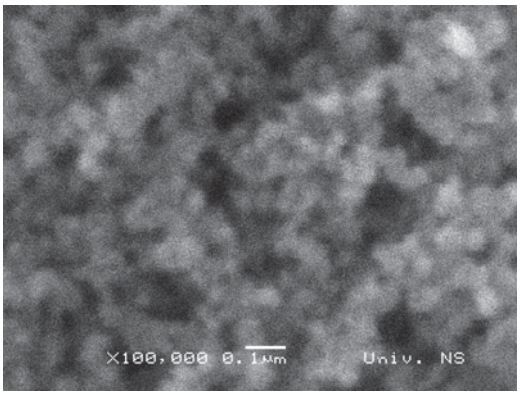

Fig. 4 – Clustered silica nanoparticles before mixing.

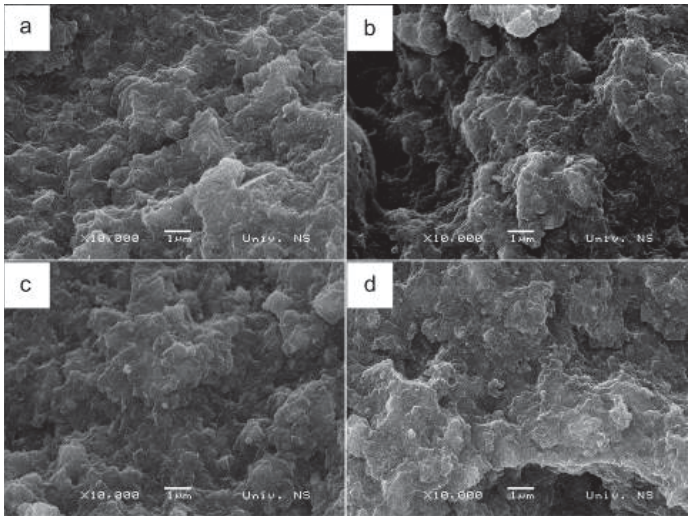

Fig. 5 – Fracture surfaces showing similar morphology:
a) unmodified; b) modified with 0.05%; c) with 0.2%; d) with 1% nanosilica.

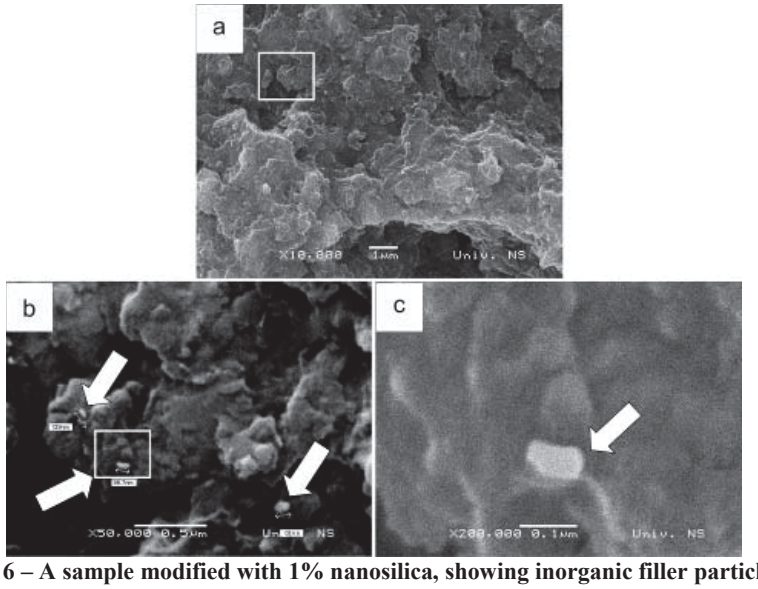

Fig. 6 – A sample modified with 1% nanosilica, showing inorganic filler particles:
a) Fig. 5d fracture surface – detail square is magnified in b); b) Arrows show inorganic particles in BS mode – detail square is magnified in c); c) An inorganic particle, magnified.

Discussion

The presented results of mechanical properties undoubtedly indicate that nanosilica content is not proportional to the benefits of such modification. Namely, even when relatively low nanosilica concentrations are considered, high and and medium nanosilica content (1 and 0.2%) do not offer the highest rise in mechanical properties. Furthermore, samples containing 0.2% and 1% nanosilica have even lower flexural strength and microhardness than the unmodified sample, respectively (Tables 2 and 3). However, flexural modulus of elasticity of all the modified samples is higher in comparison to the unmodified sample. This proves that the most pronounced benefit of adding nanoparticles is the rise in flexural modulus of elasticity. This result has been noticed by other authors as well [19–21]. On the other hand, although average values of flexural modulus of elasticity are higher compared to the unmodified, control sample, the difference is not significant for all the samples. Although 1% nanosilica added composite resin offers 14% higher flexural modulus of elasticity, the difference is not significant, due to the fact that the standard deviation is higher for the modified in comparison to unmodified samples.

Overall, the optimal nanosilica content is the lowest, 0.05%. Such concentration offers significantly higher flexural modulus of elasticity, flexural strength and microhardness in all the tests performed in this study. This result is in accordance with some of the latest published results, where higher nanoparticle concentrations might lead to an uncontrollable and stochastic forming of agglomerates [12]. By agglomerating, a large number of nanosilica particles become joined together, leading to nonhomogenous strengthening due to a nonuniform distribution of nanoparticles [22]. An indirect confirmation of the agglomerate occurrence in higher nanosilica concentrations comes from DCS thermograms. Namely, DSC thermograms show marginally small variations in Tg, which means that the overall interfacial layer volume of all the modified samples is the same, regardless the nanoslilica content. Uniform distribution of nanoparticles of the same size would result in a significant difference in Tg, in favor of the higher nanosilica content, however, this does not occur. The main reason might be agglomeration in the samples containing higher amount of nanosilica. Nevertheless, this theory deserves further attention of researchers, especially by testing low nanoparticle addition in other polymer and composite materials.

All the benefits reflected by mechanical properties were not gained at the cost of handling properties. This was shown by the slumping resistance results, which show statistically insignificant differences between the results obtained by using unmodified and nanosilica modified samples.

Conclusion

According to the presented results obtained by testing the Te-Econom Flow material it could be concluded that low nanosilica addition is more effective in rising mechanical properties of modified composite resin samples, compared to higher nanosilica additions. Also, flexural modulus of elasticity, flexural strength and microhardness may be improved at lower cost than if a higher nanosilica addition is considered. More effective reinforcement of the basic material with lower amount of nanosilica may be the result of agglomeration, which affect more the samples containing a higher amount of nanosilica. Low nanosilica addition of up to 1% does not result in a statistically significant change in slumping resistance, indicating unchanged handling properties. Other basic material and nanoparticle type require careful optimization in term of nanoparticle concentration to achieve optimal mechanical properties.

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ORIGINAL ARTICLE

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Myocardial protection during elective coronary artery bypasses grafting by pretreatment with omega-3 polyunsaturated fatty acids

Zaštita srca tokom operacije revaskularizacije srčanog mišića primenom omega-3 nezasićenih masnih kiselina

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Abstract

Background/Aim. Despite recent advances in coronary artery bypass grafting (CABG), cardioplegic cardiac arrest and cardiopulmonary bypass (CPB) are still associated with myocardial injury. Accordingly, the efforts have been made lately to improve the outcome of CPB by glucose-insulin-potassium, adenosine, Ca^{2+}-channel antagonists, L-arginine, N-acetylcysteine, coenzyme Q10, diazoxide, Na^+/H^+ exchange inhibitors, but with an unequal results. Since omega-3 polyunsatutated fatty acids (PUFAs) have shown remarkable cardioprotection in preclinical researches, the aim of our study was to check their effects in prevention of ischemia reperfusion injury in patients with CPB. **Methods.** This prospective, randomized, placebo-controlled study was performed with parallel groups. The patients undergoing elective CABG were randomized to receive preoperative intravenous omega-3 PUFAs infusion (n = 20) or the same volume of 0.9% saline solution infusion (n = 20). Blood samples were collected simultaneously from the radial artery and the coronary sinus before starting CPB and at 10, 20 and 30 min after the release of the aortic cross clamp. Lactate extraction/excretion and myocardial oxygen extraction were calculated and compared between the two groups. The levels of troponin I (TnT) and creatine kinase–myocardial band (CK-MB) were determined before starting CPB and 4 and 24 h postoperatively. **Results.** Demographic and operative characteristics, including CPB and aortic cross-clamp time, were similar between the two groups of patients. The level of lactate extraction 10 and 20 min after aortic cross-clamp time has shown negative values in the control group, but positive values in the PUFAs group with statistically significant differences (-19.6% *vs* 7.9%; $p < 0.0001$ and -19.9% *vs* 8.2%; $p < 0.0008$, respectively). The level of lactate extraction 30 minutes after reperfusion was not statistically different between the two groups (6.9% *vs* 4.2%; $p < 0.54$). Oxygen extraction in the PUFAs group was statistically significantly higher compared to the control group after 10, 20 and 30 min of reperfusion (35.5% *vs* 50.4%, $p < 0.0004$; 25.8 % *vs* 48.7%, $p < 0.0001$ and 25.8% *vs* 45.6%, $p < 0.0002$, respectively). The level of TnT, 4 and 24 h after CPB, was significantly higher in the control group compared to PUFAs group, with statistically significant differences (11.4 *vs* 6.6, $p < 0.009$ and 12.7 *vs* 5.9, $p < 0.008$, respectively). The level of CK-MB, 4 h after CPB, was significantly higher in the control group compared to PUFAs group (61.9 *vs* 37.7, $p < 0.008$), but its level, 24 h after CPB, was not statistically different between the two groups (58.9 *vs* 40.6, $p < 0.051$). **Conclusion.** Treatment with omega-3 PUFAs administered preoperatively promoted early metabolic recovery of the heart after elective CABG and improved myocardial protection. This study showed that omega-3 emulsion should not be considered only as a nutritional supplement but also as a clinically safe and potent cardioprotective adjunct during CPB.

Key words:
fatty acids, omega-3; myocardial reperfusion; coronary artery bypass; surgical procedures, elective.

Apstrakt

Uvod/Cilj. Uprkos tehnološkom napretku srčani zastoj izazvan kardioplegijom i vantelesni krvotok (*cardiopulmonary bypass* – CPB) tokom operacije revaskularizacije srčanog mišića i dalje dovode do oštećenja srčanog mišića. Napor da se poboljša ishod posle CPB primenom glukoze-insulin-kalijuma, adenozina, blokatora Ca^{2+}-kanala, L-arginina, N-acetilcisteina, koenzima Q10, diazoksida, inhibitora razmene Na^+/H^+ nije dao željene rezultate. Omega-3 nezasićene masne kiseline (*polyunsaturated fatty acids* – PUFAs), u prekliničkim ispitivanjima, pokazale su značajno kardioprotektivno dejstvo. Cilj

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ovog rada bio je ispitivanje efekata primene PUFA u prevenciji ishemijsko reperfuzionih oštećenja srčanog mišića nakon revaskularizacije sa primenom CPB. **Metode.** Ova prospektivna, randomizovana, placebo-kontrolisana studija sprovedena je na paralelnim grupama. Bolesnici sa elektivnim operacijama revaskularizacije srčanog mišića slučajnim izborom podeljeni su u dve grupe. Prva grupa (n = 20) preoperativno je dobijala infuziju PUFAs (PUFAs grupa), dok je kontrolna grupa (n = 20) dobijala istu količinu 0,9% rastvora NaCl. Uzorci krvi su istovremeno uzimani iz radijalne arterije i koronarnog sinusa pre početaka CPB i 10, 20 i 30 minuta posle skidanja kleme sa aorte. Ekstrakcija laktata i kiseonika iz srčanog mišića izvedena je primenom poznatih formula. Nivo troponina I (TnT) i miokardne frakcije kreatin-kinaze (CK-MB) određivana je pre početka CPB i 4 i 24 h posle operacije. **Rezultati.** Demografske i operativne karakteristike, uključujući trajanje CPB i klemovanja aorte, bili su slični u obe grupe. Nivo ekskrecije laktata 10 i 20 min nakon deklemovanja aorte imao je negativne vrednosti u kontrolnoj grupi, dok su vrednosti u PUFAs grupi bile pozitivne, sa statistički značajnom razlikom (-19,6% prema 7,9%; $p < 0,0001$ i -19,9% prema 8,2%; $p < 0,0008$, respektivno). Nivo ekstrakcije laktata 30 min nakon skidanja kleme sa aorte bio je bez statistički značajne razlike između dve grupe (6,9% prema 4,2%; $p < 0,54$). Ekstrakcija kiseonika 10, 20 i 30 min posle skidanja kleme sa aorte bila je veća u PUFAs grupi sa statistički značajnom razlikom u odnosu na kontrolnu grupu (35,5% prema 50,4%, $p < 0,0004$; 25,8 % prema 48,7%, $p < 0,0001$ i 25,8% prema 45,6%, $p < 0,0002$, respektivno). Nivo TnT, 4 i 24 sata nakon CPB, bio je statistički značajno viši u kontrolnoj grupi nego u PUFAs grupi (11,4 prema 6,6 ng/mL $p < 0,009$ i 12,7 prema 5,9 ng/mL $p < 0,008$). Nivo CK-MB, 4 h nakon CPB, bio je statistički značajno viši u kontrolnoj grupi nego u PUFA grupi (61,9 prema 37,7 U/L; $p < 0,008$), dok je nivo CK-MB 24 h nakon CPB bio bez statistički značajne razlike između dve grupe bolesnika (58,9 prema 40,6 U/L; $p < 0,051$). **Zaključak.** Preoperativna primena omega-3 PUFAs pomaže u ranom metaboličkom oporavku srca nakon revaskularizacije tako što štiti srčani mišić. Ovo istraživanje je pokazalo da omega-3 PUFAs nisu samo nutricioni dodatak već i klinički bezbedan i snažan kardioprotektor tokom CPB.

Ključne reči:
masne kiseline, omega-3; miokard, reperfuzija; aortokoronarno premošćavanje; hirurgija, elektivna, procedure.

Introduction

Myocardial protection by using hypothermia and cardioplegia methods during ischemia and reperfusion remains one of the cornerstones of postoperative myocardial function. Coronary artery bypass grafting (CABG) performed with the aid of cardioplegia and cardiopulmonary bypass (CPB) requires a period of cardiac arrest. During this time, myocardial ischemia and necrosis may occur, which is an important determinant of functional and clinical outcome [1]. Despite CPB techniques as well as postoperative intensive care improvement impaired myocardial function is well-documented as a complication of CPB, resulting in increased morbidity and mortality [2–4].

With increasing complexity of adult and pediatric cardiac surgical procedures, complete myocardial protection is proving a challenge. Attempts to provide additive cardioprotection to date has met with little benefit in clinical trials. Most new therapies, such as sodium/hydrogen exchange inhibitors [5], glucose-insulin–potassium [6], adenosine [7], Ca^{2+}-channel antagonists [8], L-arginine [9], N-acetylcysteine (NAC) [10], coenzyme Q10 [11], diazoxide [12], corticosteroids [13], pexelizumab [14] have focused on a single aspect of pathologic ischemia-reperfusion injury, with unequal benefit.

It is experimentally and clinically well established that long-chain omega-3 polyunsaturated fatty acids (PUFAs) protect against and can terminate ischemic arrhythmias [15, 16]. Their anti-inflammatory effects, which include attenuation of leukocyte-endothelial interactions and production of less biologically active prostaglandins and leukotrienes, could also be beneficial in cardiac ischemia-reperfusion injury [17–19]. Furthermore, clinical trials have shown that omega-3 PUFAs pretreatment induces heat shock proteins (HSP) in the myocardium, protecting against ischemia, suggesting a preconditioning phenomenon [20]. One other potential mechanism of action of PUFAs would be that they act as a "sink" to trap free radicals, hence becoming oxidized themselves [21]. The susceptibility of fatty acids to oxidation is thought to be directly dependent on their degree of unsaturation. Docosahexaenoic acid (DHA)-mediated inhibition of interleukin (IL)-1-induced reactive oxygen species (ROS) production, would contribute to the anti-inflammatory actions of omega-3 PUFAs at the endothelial level [22]. Also, omega-3 PUFAs for clinical use and normally given as part of parenteral nutrition, led to myocardial protection administered in the acute setting [23]. In selecting the possible mechanism of observed protection, we focused on the effects of incorporating the PUFAs into the myocardial membrane, a process shown to be important in their antiarrhythmic effect [24, 25].

The aim of this study was to assess the impact of omega-3 PUFAs, as eicosapentaenoic acid (EPA) and DHA, infusion therapy on early metabolic recovery of the heart during elective CABG, leading to better myocardial protection.

Methods

This prospective, randomized, placebo-controlled study was performed with parallel groups. Study enrollment occurred between August 2010 and September 2011. The study protocol was approved by the Ethical Committee of the Military Medical Academy, Belgrade, and all the patients gave written informed consent.

Forty patients scheduled to undergo their first on-pump CABG surgery were included in the study. To do so, the patients needed to be older than 18 years of age, in normal sinus rhythm, and in stable hemodynamic conditions before surgery. The patients were excluded in cases of emergency CABG, redo CABG, combined CABG and any other cardiac procedure, Q-wave myocardial infarction in the last six weeks, un-

stable angina, or poor left ventricular function. All the patients were treated by the same surgical and anesthesiologist team.

Eligible patients were assigned to one of the two study arms according to a computer-generated randomization list: control (placebo) group (usual care), and usual care plus PUFAs.

The PUFAs infusion consisted of 100 mL of a lipid emulsion with a high content of omega-3 PUFAs (Omegaven® 10%, Fresenius Kabi, Bad Homburg, Germany). The same batch of Omegaven® was used throughout the study, and 100 mL of the lipid emulsion contained 1.25–2.82g EPA and 1.44–3.09 DHA. Infusion was given one day before surgery and repeated 4 h before starting CPB *via* the peripheral vein at single doses of 100 mL (25 mL/h). The patients of the control group received an equal volume of 0.9% saline.

Preoperative sedation with 5 mg of intramuscular midazolam was administered to the patients on call to the operating room. All the patients received prophylactic preoperative antibiotics (cefazolin, 2 g preincision and 2 g post-CPB; or if allergic to penicillin, vancomycin, 1 g preincision and 500 mg post-CPB). The same anesthesiologist administered standardized total intravenous anesthesia using sufentanil, midazolam, propofol and pancuronium.

Immediately before CPB, 300 IU/kg heparin was administered intravenously, followed by additional doses as necessary to maintain an activating clotting time exceeding 500 sec. Protamine was administered as 1 mg/100 IU of the heparin dose after complete separation from CPB.

All the patients had CABG with the use of CPB, which was conducted with a roller pump and a membrane oxygenator primed with a solution. During CPB, pump flow was set at 2.4 times the body surface area, and mean arterial pressure maintained between 50 and 60 mmHg. Temperature was allowed to drift with active rewarming at the end of CPB. Myocardial protection was afforded with cold potassium cardioplegia. A single-clamp technique was used, and cardioplegia was given in an anterograde fashion. In all the patients, the left internal mammary artery harvested and anastomosed to the left anterior descending artery. The rest of the grafts were constructed using the great saphenous vein.

After total release of the aortic cross-clamp, epicardial atrial or ventricular pacing wires were placed. Aortic and venous cannulas were removed after thr appropriate test dose of protamine, and the surgery proceeded with closure of the pericardium and sternum.

After the surgery, the patients were followed up in the intensive care unit and were weaned off mechanical ventilation when they fulfilled the following criteria: hemodynamic stability, peripheral temperature of more than 36°C, cooperatively, and no major bleeding.

A retrograde perfusion cannula was used to collect simultaneous blood samples from arterial blood and the coronary sinus just before commencing CPB and at 10, 20 and 30 min after the release of the aortic cross-clamp. These samples were used to determine lactate concentration, hemoglobin (Hb) concentration, and oxygen saturation (O_2 Sat).

Lactate extraction was calculated as the difference between arterial and coronary sinus lactate content [26].

A negative value indicates lactate excretion, while the positive one indicates lactate uptake.

Oxygen content in both arterial and coronary sinus blood was calculated using the formula: $1.38 \times H\ b \times O_2$ Sat.

The arterial – coronary sinus oxygen content difference was calculated and its ratio to arterial oxygen content represented oxygen extraction.

The levels of troponin (TnT) and creatine kinase myocardial band (CK-MB) concentrations were measured 4 and 24 h postoperatively as an indicator of myocardial protection.

The results were presented as mean values with a standard deviation. Significant differences between the study subject groups were analyzed using the *t*-test. Due to a great variability of some data, the Wilcoxon matched pairs test and Mann-Whitney *U*-test were also used. Comparison between more than two groups was done by using the Kruskal-Wallis test.

A *p* value of less than 0.05 was taken to be significant. The obtained data were processed using the Stat for Windows, R.4.5. Software package.

Results

The results of the study are presented in Tables 1 and 2 and Figures 1–4, divided in six parts: demographic and operative characteristics of patients, the influence of PUFAs on lactate and oxygen extraction, a relative relationship between lactate and oxygen extraction, the influence of PUFAs on serum levels of TnT and CK-MB, the coefficient of correlation (r) values, and peri- and postoperative complications.

Demographic and operative characteristics of the patients with CPB

Table 1 shows that the physical characteristics of the patients of the two studied groups (age, weight and height) were very similar, beeing also the case with LVEF and op-

Table 1

Baseline and operative characteristics of the patients in the PUFAs and the control group

Parameter	Control group	PUFAs group	*p*
Age (years) , $\bar{x} \pm SD$	62.4 ± 7	65.3 ± 8	0.56
Gender, male/female (n)	18/2	17/3	0.36
Weight (kg), $\bar{x} \pm SD$	89.8 ± 6	92.1 ± 5	0.48
Height (cm), $\bar{x} \pm SD$	176.4 ± 4	178.5 ± 3	0.06
LVEF (%), $\bar{x} \pm SD$	54 ± 6	53 ± 9	0.10
CPB (min), $\bar{x} \pm SD$	101.4 ± 21	95.5 ± 17	0.29
Aortic cross-clamp time (min), $\bar{x} \pm SD$	42.5 ± 9	38.9 ± 8	0.66
CABG (number), $\bar{x} \pm SD$	2.9 ± 0.8	2.8 ± 0.7	0.65

PUFAs – polyunsaturated fatty acids; LVEF – left ventricular ejection fraction; CPB – cardiopulmonary bypass; CABG – coronary artery bypass grafting; $\bar{x}$ – mean; SD – standard deviation.

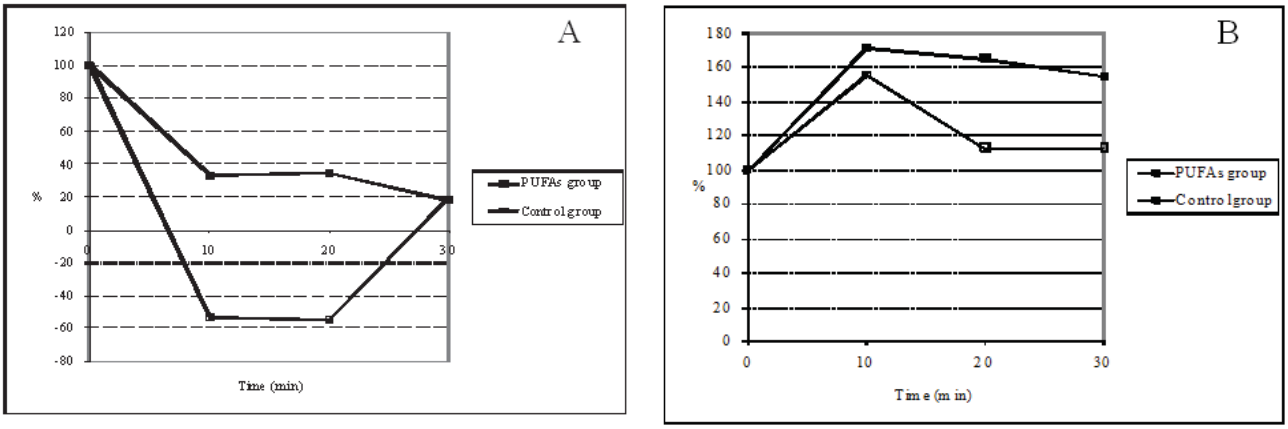

Fig. 1 – Relative percentage versus time-relationship between lactate (A) and oxygen extraction (B) in the control and the polyunsaturated fatty acids (PUFAs) group of patients subjected to cardiopulmonary bypass (CPB) with the initial (basal) values marked as 100%.

erative characteristics (CPB, time of the aortic cross-clamp and CABG number).

The influence of PUFAs on lactate and oxygen extraction in the patients with CPB

Table 2 shows that the results of both studied parameters – lactate and oxygen extraction – were opposite in the two groups of patients. Namely, lactate uptake before ischemia in the patients in the control group was statistically significantly higher compared with the PUFAs group (36.7% *vs* 23.9%; $p < 0.01$). The level of lactate extraction 10 and 20 min after aortic declamping had negative value in the control group compared with positive value in the PUFAs group with statistically significant differences (-19.6% *vs* 7.9%; $p < 0.0001$ and -19.9% *vs* 8.2%; $p < 0.0008$, respectively). A negative value 10 and 20 min after aortic declamping indicated lactate excretion in the control group compared with the positive value in the PUFAs group which indicated lactate uptake. The level of lactate extraction 30 min after aortic declamping was not statistically different between the groups (6.9% *vs* 4.2%; $p = 0.54$) (Table 2). In contrast to this, the level of oxygen extraction increased in both groups of patients. Their peak values ranged from 22.9 ± 16.5% in the control group before CPB to 35.5 ± 8.5% 10 min after aortic declamping and from 29.5 ± 18.6% in the PUFAs group before CPB to 50.4 ± 5.2% 10 min after aortic declamping. The extraction of oxygen in the PUFAs treated patients was highly statistically significant at all the three observed times after CPB in relation to the control group (with p ranging from 0.0001 to 0.0004).

A relative relationship between lactate and oxygen extraction

The relative relationship of lactate and oxygen extraction, based on the values given in Table 2, with their initial values before CPB marked as 100% and the percentage of their respective values at all the three observed time intervals after aortic declamping are presented in Figure 2, and show the time-course of their relative values.

It is self-evident from the results presented in this way that in the case of lactate extraction 10 min and 20 min after aortic declamping they diverge (plate A), and in those of oxygen extracton 10 min after aortic declamping converge, equalizing at 30 min after aortic declamping in the case of lactate, but not in the case of oxygen extraction (plate B).

The influence of PUFAs on the serum level of TnT and CK-MB

Figure 2 shows that the levels of TnT (plate A) and CK-MB (plate B) in both groups of patients were markedly higher at 4 h ($p < 0.009$ and $p < 0.007$). The level of troponin I, 24 h after CPB, was significantly higher in the control

Table 2

Extraction of lactate and oxygen in the patients with CPB treated with omega-3 polyunsaturated fatty acids (PUFAs) in relation to the control group with a standard protocol

Parameter	Extraction (%), $\bar{x}$ ± SD		*p*
	Control group (n = 20)	PUFAs group (n = 20)	
Lactate before CPB	36.7 ± 18.3	23.9 ± 14	0.01
Lactate 10 min after CPB	-19.6 ± 22.8	7.9 ± 20.5	0.0001
Lactate 20 min after CPB	-19.9 ± 22.8	8.2 ± 27.1	0.0008
Lactate 30 min after CPB	6.9 ± 11.8	4.2 ± 21.9	0.54
Oxygen before CPB	22.9 ± 16.5	29.5 ± 18.6	0.3
Oxygen 10 min after CPB	35.5 ± 8.5	50.4 ± 5.2	0.0004
Oxygen 20 min after CPB	25.8 ± 9.7	48.7 ± 8.2	0.0001
Oxygen 30 min after CPB	25.8 ± 9.3	45.6 ± 8.7	0.0002

CPB – cardiopulmonary bypass; $\bar{x}$ – mean; SD – standard deviation.

group as compared with the PUFAs group with statistically significant differences (12.7 versus 5.9 ng/mL $p < 0.008$) (Figure 2A). The level of CK-MB, 24 h after CPB was not statistically different between the groups (58.9 *vs* 40.6 U/L $p < 0.051$) (Figure 2B).

was based on the values of parameters at all the observed time intervals (i.e. 10 to 30 min after aortic declamping in the case of lactate and oxygen extraction or 4 and 24 h after CPB in the case of TnT and CK-MB level) in relation to their initial (basal) values found before CPB.

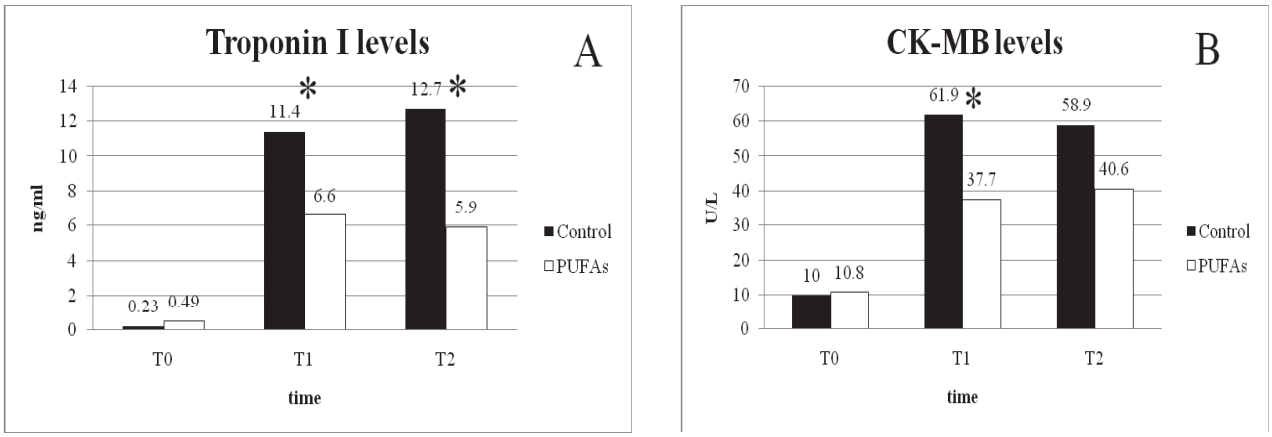

Fig. 2 – Mean values of serum troponin I (A) and creatine kinase myocardial band (CK-MB) (B) in the control and the poly-unsaturated fatty acids (PUFAs) group of patients subjected to cardiopulmonary bypass (CPB); T0 – before the surgery; T1 – 4 h after surgery; T2 – 24 h after surgery; *statistically significant difference ($p < 0.05$).

Concerning the relationship between the two groups, the serum level of TnT (plate A) was statistically significantly lower in the PUFAs treated patients in relation to the

In this respect, Figure 3 shows that the drop of lactate extraction (plate A) was uniform in both studied groups, but it was less pronounced in the the PUFAs group of patients.

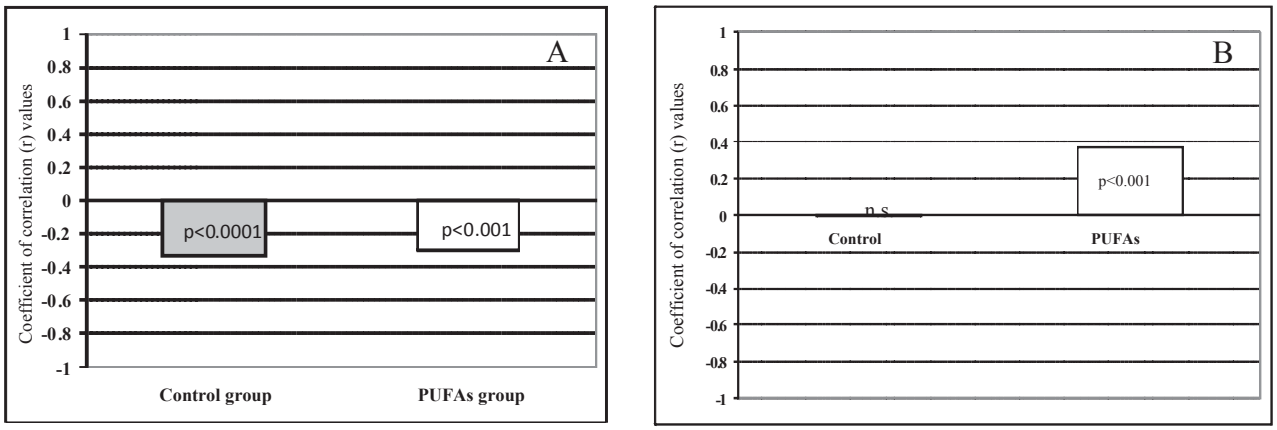

Fig. 3 – Coefficients of correlation between the initial (basal) values and trends in combined values of lactate (A) and oxygen (B) extraction in the control and the polyunsaturated fatty acids (PUFAs) group of patients observed 10 min, 20 min and 30 min after aortic declamping.

control group at both observed times, being 72% (11.4 ng/mL: 6.67 ng/mL, $p < 0.009$) and 115% (12.7 ng/mL: 5.9 ng/mL, $p < 0.008$) lower at 4 hrs and 24 hrs after CPB, respectively. At the same time, the serum level of CK-MB (plate B) was also lower in the PUFAs treated patients at both observed time intervals of 4 h and 24 h after CPB, being 65% (61.9 U/L: 37.7 U/L, $p < 0.007$) and 45% (58.9 U/L : 40.6 U/L $p < 0.051$), respectively, in relation to the control group of patients.

Coefficient of correlation (r) values

Reliablity of described trends of the single values of all four parameters in both groups of patients were additionaly confirmed by the coefficient of correlation. Its calculation

Regarding oxygen (plate B), its level of extraction in the control group did not change from the baseline values in relation to the observed time intervals from 10 to 30 min after aortic declamping. In contrast to this, its level in the PUFAs group of patients steadily increased during that time.

Figure 4 shows the increment tendencies of TnT (plate A) and CK-MB values (plate B) in both groups of patients. However, they were less pronounced in both cases in the PUFAs group of patients.

Peri- and postoperative complicatons

Postoperative complications were similar in both groups of patients. In the control group, one patient died of cardiac failure on the second postoperative day, two patients

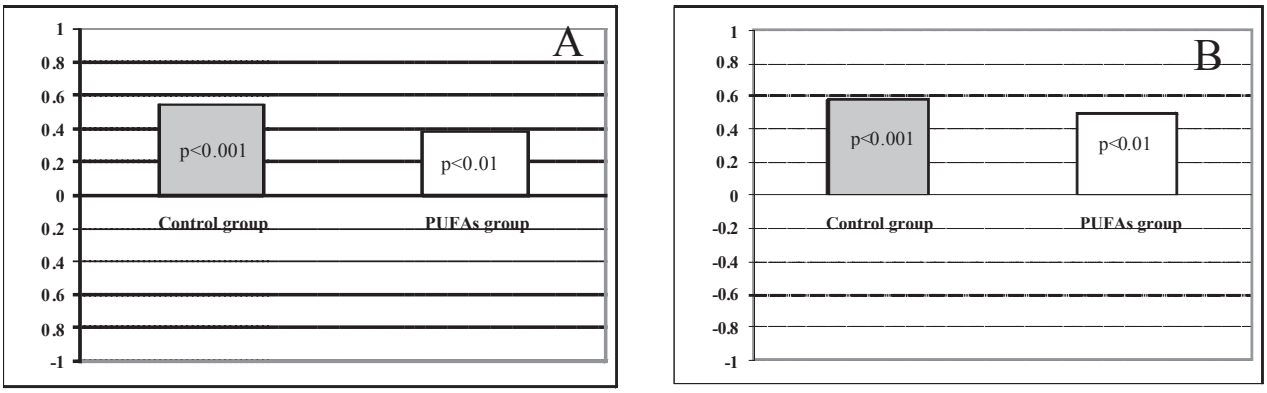

Fig. 4 – Coefficients of correlation between the initial (basal) values and trends in combined values of serum troponin I (A) and creatine kinase myocardial band (CK-MB) (B) in the control and the polyunsaturated fatty acids (PUFAs) group of patients observed 4 h and 24 h after aortic declamping.

had perioperative infarction, three patients needed inotropic support. In the PUFAs group, one patient underwent reexploration for bleeding, one had a respiratory failure and two patients needed inotropic support. Due to the low number of the observed complications, no statistical comparison was performed.

Discussion

The results of our prospective, randomized placebo-controlled study in the two groups of adult patients subjected to CPB showed that the extraction of oxygen and the uptake of lactate were markedly increased in the PUFAs pretreated patients compared to the control group, with the subsequent decrease of serum TnT and CK-MB levels in the PUFAs group, pointing thus to their important cardioprotective effect.

Due to the two separate groups of results dealing with oxygen extraction and lactate uptake from one, to the serum levels of TnT and CK-MB to the other side, discussion is divided in two parts: the influence of PUFAs on oxygen extraction and lactate uptake, and their influence on TnT and CK-MB serum levels.

The influence of PUFAs on oxygen extraction and lactate uptake

Evaluation of myocardial metabolism during cardiac surgery allows the investigator to quantify the degree of physiologic impairment. Direct cannulation of the coronary sinus for coronary sinus blood sampling to measure metabolites or specific biochemical markers of myocardial damage has been shown to be a valid tool to define the degree of such impairment [27–29]. One of the most sensitive markers of inadequate preservation of the myocardium is the development of myocardial tissue acidosis and lactate production [30].

We demonstrated in this study that omega-3 PUFAs intravenous pretreatment prepared the heart metabolically for ischemia and led to an earlier shift to aerobic metabolism during reperfusion, as indicated by earlier lactate uptake. In this respect, the level of lactate extraction 10 and 20 min after aortic declamping had negative values in the control group compared to the positive values in the PUFAs group indicating lactate uptake. The extraction of oxygen in the PUFAs treated patients was highly statistically significant at all three observed times after CPB in relation to the control group, increasing thus metabolic activity enabled by an increased supply of extracted lactate. Early lactate uptake in the PUFAs group is an index of more rapid recovery of aerobic metabolism, pointing to the improved cardioprotection in our patients. Otherwise, a persistent lactate release during reperfusion in the control group suggests a delayed recovery of aerobic metabolism and may be related to intraoperative inadequate myocardial protection [31]. In contrast to this, a significant evidence shows that preserving or enhancing aerobic metabolism, or both, is a key in maintaining cardiac function after ischemia [32–34].

The influence of PUFAs on TnT and CK-MB serum levels

Intraoperative release of TnT and CK-MB has functional significance because it is closely related to ischemia time and reflects a delayed recovery of left ventricular function and oxidative metabolism. Therefore, their measurement can be used as an indicator of myocardial injury sustained during CABG [29, 35–37].

This study demonstrated that the intravenous administration of omega-3 PUFAs before CPB statistically significantly decreased the level of TnT in the PUFAs treated patients in relation to the control group at both observed times, being 58.5% and 46.4% lower at 4 h and 24 h after CPB, respectively. (Figure 2 A and B). At the same time, the serum level of CK-MB was also reduced in the PUFAs treated patients at both observed time intervals of 4 h and 24 h after CPB amounting 60.9% and 68.9 %, respectively, in relation to the control group of patients.

The study demonstrated for the first time that acute intravenous administration of omega-3 emulsion, which is normally used as a part of parenteral nutrition regimens, was associated with a significant reduction in myocardial ischemic-reperfusion injury. At least a part of this effect could be ascribed to the findings that the ischemic preconditioning actually increases the content of the omega-3 fatty acid DHA in the myocardial membrane in advance of a further injury [38], reduces myocardial oxygen demand, and attenuates acidosis and lactate accumulation in the ischemic heart [39].

At molecular level, a special attention in PUFAs cardioprotective effect should be devoted to their high ROS scavenger potential. Namely, the susceptibility of fatty acids to oxidation is thought to be directly dependent on their degree of saturation. Fatty acid micelles scavenge superoxide in an unsaturation dependent manner, up to EPA, which is the most effective fatty acid [40]. From a mechanistic viewpoint, NAD(P)H oxidase is one of the major contributors to endothelial free radical production: its inhibition by DHA [22] and (presumably) other PUFAs, might greatly explain the observed effects on ROS production. DHA-mediated inhibition of IL-1-induced ROS production would also contribute to the anti-inflammatory actions of omega-3 fatty acids at the endothelial level. One additional mechanism of PUFAs action would be that they act as a "sink" to trap free radicals, hence becoming oxidized themselves.

Myocardial reperfusion injury is a complex process with inadequately understood mechanisms and multiple initiating factors. Therefore, instead targeting some specific disturbances, many different pharmacological compounds have been tested not on a genuine, but rather on a screening basis.

All together, the results of such treatments may be divided in two groups: with no or modest outcome and positive findings which deserve further study.

The first group concerns adenosine, Ca^{2+}-channel antagonists, corticosteroids, NAC, diazoxide, L-arginine, cariporide, isoflurane, sevoflurane and nicorandil. So for example, adenosine reduced the levels of TnT, IL-6 and IL-8 release, but was without an effect on the CK-MB level [41]. Corticosteroids had no beneficial effect on mortality and cardiac and pulmonary complications [13], NAC appears to be promising, but increases postsurgical cardiac complications [42], while the levels of TnT and CK-MB were higher in isoflurane compared to sevoflurane group of patients [43]. Glucose-insulin-potassium improved hemodynamic parameters, but no significant effect on plasma TnT levels was demonstrated [44], and may cause severe disturbances in glucose homeostasis [45], which in case of hyperglycemia may enhance oxidative stress and exacerbate myocardial infarction during reperfusion [46, 47].

In the second studied group, encouraging results were reported with the use of pexelizumab, which in the CPB patients decreased the level of CK-MB and protected against myocardial injury [48], and coenzyme Q10, which increased protection of mitochondria and myofilaments against oxidative stress, with a consequent maintenance of energy production and improved contractile recovery of pectinate trabeculae isolated from patients receiving coenzyme Q10 after reoxygenaton stress *in vitro* [11].

According to these results and taking into account the favorable cardioprotective effects of PUFAs in CPB in our patients, it seems appropriate to consider their combination with coenzyme Q10. Coenzyme Q10 is a physiological constituent, declining in synthesis with age. It is an antioxidant and cofactor for mitochondrial ATP generation, basic source of energy in all cells, including myocardial ones. At least, such a combination could fulfill two fundamental requirements for myocardial membrane and cell function after cardioplegia and reperfusion oxygenation in CPB patients: protection by dramatic increase in the omega-3 content of myocardial membrane phospholipids [20], scavenging ROS, and accompanied by direct effects on ion channels modulating the protein kinase C activity by PUFAs [19], and by improving mitochondrial efficiency with coenzyme Q_{10}.

Although we found significantly different results between the PUFAs group and the control group of patients in terms of myocardial injury in the favor of the first, there are still a few limitations of this study: our investigation was performed in a small size sample and with a limited number of clinical events; because of our research fund shortage, we did not use pulmonary artery catheter for monitoring hemodynamics; therefore, we were limited in recording changes between the two groups in myocardial function, and sampling method from coronary sinus does not represent global changes in heart metabolism.

Conclusion

PUFAs therapy administered before CPB promotes early metabolic recovery of the heart during elective CABG and leads to better myocardial protection. This study shows that an omega-3 PUFAs emulsion should not be considered only as a nutritional supplement but also as a potentially clinically safe cardioprotective agent. This strategy warrants further investigation with optimization and shortening of pretreatment regimens to be more clinically applicable. It would be of interest to perform a larger randomized study with a design similar to the present study.

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Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Smoking habits, knowledge about and attitudes toward smoking among employees in health institutions in Serbia

Pušačke navike, znanje i stavovi o pušenju zaposlenih u zdravstvenim institucijama u Srbiji

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Abstract

Background/Aim. According to the number of active smokers, Serbia occupies a high position in Europe, as well as worldwide. More than 47% of adults are smokers according to WHO data, and 33.6% according to the National Health Survey Serbia in 2006. Smoking physicians are setting a bad example to patients, they are uncritical to this habit, rarely ask patients whether they smoke and rarely advise them not to smoke. These facts contribute to the battle for reducing the number of medical workers who smoke, as well as the number of smokers among general population. The aim of the study was to determine the smoking behavior, knowledge and attitudes and cessation advice given to patients by healthcare professionals in Serbia. **Methods.** A stratified random cluster sample of 1,383 participants included all types of health institutions in Serbia excluding Kosovo. The self administrated questionnaire was used to collect data about smoking habits, knowledge, attitudes and cessation advice to patients given by health professionals in Serbia. **Results.** Out of 1,383 participants, 45.60% were smokers, of whom 34.13% were physicians and 51.87% nurses. There were 46.4% male and 45.4% female smokers. The differences in agreement with the statements related to the responsibilities of health care professionals and smoking policy are significant between the "ever" and "never" smokers, and also between physicians and nurses. Twenty-five percent of nurses and 22% of doctors claimed they had received formal training. However, only 35.7% of the healthcare professionals felt very prepared to counsel patients, while 52.7% felt somewhat prepared and 11.6% were not prepared at all. **Conclusions.** According to the result of this survey, there are needs for more aggressive nationwide non-smoking campaigns for physicians and medical students. Experiences from countries where physicians smoke less and more effectively carry out smoking cessation practices need to be shared with Serbian physicians in order to improve their smoking behavior and smoking cessation practices.

Key words:
smoking; health personnel; serbia; habits; preventive health services; smoking cessation.

Apstrakt

Uvod/Cilj. Prema broju pušača Srbija se visoko rangira u evropskim i svetskim razmerama. Prema podacima Svetske zdravstvene orginizacije (SZO) puši 47% stanovništva Srbije, a prema istraživanju zdravlja stanovnika Srbije iz 2006. godine 33,6%. Lekari koju puše daju loš primer drugima, nekritični su prema toj navici i ređe savetuju pacijente u vezi pušenja. Smanjenjem prevalencije među lekarima indirektno utičemo na smanjenje prevalencije u opštoj populaciji. Cilj studije bio je da se definišu pušačke navike, znanja i stavovi o pušenju sdravstvenih radnika u Srbiji. **Metode.** Primenjen je stratifikovani *cluster* uzorak od 1 383 ispitanika na teritoriji Srbije bez Kosova i podaci su dobijeni popunjavanjem upitnika u zdravstvenim ustanovama svih nivoa. **Rezultati.** Od 1 383 ispitanika, pušača je bilo 45,6%. Najmanje ih je bilo među lekarima (34,13%), a najviše među medicinskim sestrama (51,87%). Pušilo je 46,4% muških i 45,4% ženskih ispitanika. Ustanovljena su značajna neslaganja u stavovima o ulozi zdravstvenih radnika u odvikavanju od pušenja kako između nepušača i pušača, tako i između lekara i sestara. Dobro pripremljenih ispitanika za savetovanje pacijenata o pušenju bilo je 35,7%, delimično pripremljenih 52,7% a

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potpuno nepripremljenih 11,6%. **Zaključak.** Kao rezultat studije nameće se potreba za antipušačkom kampanjom na nacionalnom nivou kako kod lekara, tako i kod studenata medicine. Potrebno je primeniti modele iz zemalja u kojima je prevalencija pušača među doktorima niska, sa krajnjim ciljem da se snizi prevalencija pušača među zdravstvenim radnicima u Srbiji, kao i da se unapredi njihovo znanje i stavovi o pušenju.

Ključne reči:
pušenje; zdravstveno osoblje; srbija; navike; preventivno-medicinska zaštita; pušenje, prestanak.

Introduction

Smoking, the most popular and widespread risk factor represents oral inhalation of certain substances, most often tobacco, which releases various materials through burning. The most commonly released material is nicotine, which, in the form of smoke, becomes susceptive for absorption through the lungs.

Throughout centuries, the reputation of smoking had changed from complete discrimination to full affirmation. Fortunately, the "modern world" has perceived smoking as negative and harmful. Therefore, it is classified as a disease in the International Classification of Diseases by the World Health Organization [WHO (ICD-10)], and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) published by the American Psychiatric Association.

According to the number of active smokers, Serbia occupies top positions in Europe, as well as worldwide. Considering the data from the WHO, there are 47% of adult smokers in Serbia.

The final report from National Health Survey Serbia in 2006 shows that 33.6% of population were smokers, with 38.1% male and 29.9% females. Compared to the year 2000, the frequency of smokers has decreased by 6.9% [1].

Although the harmfulness of tobacco is well-known, healthcare professionals do not always represent good examples [2]. Moreover, during the 20th century physicians even used to advertise cigarettes [3, 4]. The prevalence among them was extremely high. Some of the earliest researches show that in the USA 40% of smokers were physicians in 1959 [5]. In the mid-70's this prevalence decreased to 21% [6, 7], while in the '80s it was around 17% including those who smoked pipes or cigars [8]. Between 1987 and 1994, there was a dramatic decrease in the number of smokers with the 10% prevalence [9–12]. Similar trends were registered in the Scandinavian countries during the final 25 years of the 20th century [13–15].

Since 1999, the WHO has taken a stand that a healthcare professional, as one of the most highly esteemed social structure whose model of behavior is respected, should represent a model of healthy life without smoking and should advise their patients on the harmfulness of smoking and smoking cessation. Ever since, special attention has been paid to the analysis and smoking habits of physicians who represent crucial factors in the reduction of the smoking epidemic [16].

Healthcare professionals play the most important role in the creation of health policy of a country, as well as in antismoking campaigns and strategies, and they are the strongest factor in smoking cessation.

A smoking physician setts a bad example to patients; does not have a critical attitude towards this habit, rarely asks patients whether they smoke and rarely advises them not to smoke. This fact is in favor of the battle for reducing the number of healthcare professionals who smoke, thereby decreasing the prevalence in general population [17–21].

The aim of the study was to determine smoking behavior, knowledge and attitudes and cessation advice to patients among health professionals in Serbia.

Methods

This study was organized as an epidemiological multicentric cross-sectional study that included data on smoking habits, knowledge and attitudes of healthcare professionals in Serbia.

The original self administrated questionnaire consists of 48 questions. The first section of the questionnaire included basic demographic characteristics and basic smoking habits of examinees. This part contained 18 questions about the number of smoked cigarettes, starting and cessation of smoking and willingness to quit smoking. The second part included 17 questions about the knowledge and attitudes to smoking, its harmfulness and the role of healthcare professionals in providing advice and help in smoking cessation. The last part contained 13 questions related to trainings, which were available to the examinees and their preparedness to advise patients to quit smoking.

In the present study, smoking is defined as smoking cigarettes. Respondents were classified as current smokers, ex-smokers, occasional or never smokers. Current smokers are those who currently smoke every day at least one cigarette or seven cigarettes per week. The respondents who admit to smoke but not every day, or who smoke fewer than seven cigarettes a week are defined as occasional smokers. Ex-smokers are current non-smokers who used to smoke habitually for 6 months or more. Newer smokers are defined as ones who have never smoked cigarettes at all.

According to profession, they were classified into physicians (teaching physicians and physicians), nurses and staff (technical staff, administrative staff and other professionals).

The research was performed during May and June 2010 on the representative sample of all healthcare institutions in Serbia, excluding Kosovo. A stratified random cluster sample included 4 types of health institutions (primary health centers, clinical centers, clinical-hospital centers, general hospitals and institute for public health). The research was also performed at the Military Hospital in Niš and the Military Medical Academy in Belgrade.

The primary sampling units, or clusters, were departments in health care institutions. A random sampling technique was then used on any relevant clusters to choose which clusters to include in the study. The list of all clusters was stratified by occupation. From each cluster, a sample of study groups was randomly selected.

We distributed 1,773 questionnaires, while 1,383 participants completed it with 78% response rate overall. Four questionnaire samples were not included in the analysis due to the lack of information, so that 1,383 questionnaires were used for further analysis.

We used parametric and non-parametric tests (*t*-test, Man-Whitney U test, χ^2 test, Fisher test and ANOVA) to assess the relationships between variables. The collected data were presented in tables with absolute and relative numbers. For the purpose of some analysis, the respondents were divided into "ever" and "never" smokers, based on their current, past, or non-smoking history, occupation, and age range was determined by the mean age and standard deviation. Any statistical analyses were performed using SPSS (Version 18), and two-tailed $p < 0.05$ was considered statistically significant in all the analyses.

Results

The survey included 1,383 participants (501 physicians, 732 nurses and 150 other staff). The mean age was 40.29 years with the standard deviation of 9.18. The majority of participants were female (1,026), mean age 39.57 ± 9.07, whereas there were 357 male examinees with the mean age of 42.33 ± 9.17.

The prevalence of smokers in our sample was 45.63%, current smokers 34.63% and occasional smokers 11%. There were 18.3% of ex-smokers while the percentage of those who had never smoked was 36.08%.

The highest number of smokers (53.34%), was among the staff (current smokers 48.67% and occasional smokers 4.67%) and nurses 51.78% (current smokers 39.48% and occasional smokers 12.3%). The lowest prevalence of smoking was among physicians (34.13%) (teaching physicians 46.84% and physicians 32.9).

Among the participants, the number of female smokers was 45.41% (34.11% current and 11.30% occasional smokers), whereas there were 46.39% male smokers (36.31% current and 10.08% occasional smokers).

The highest number of smokers, 105 (47.95%), was in the 50–59 age group (Table 1).

From the total number of smokers, 21.2% smoke in front of their patients.

Table 2 shows the characteristics of participants who smoke (starting of smoking and daily number of smoked cigarettes). It can be concluded that the starting of smoking is significantly different among various professions (ANOVA: F = 5.101, $p = 0.002$).

The latest to start smoking were physicians (20.4 years of age) and teaching physicians (20.5 years of age). This is statistically later than nurses who started smoking at 18.9 years of age on average and non-medical staff who started at 18.4 years of age.

Comparing the number of smoked cigarettes with the type of occupation, it can be observed that there is a statisti-

Table 1

Demographic data and smoking rates

Characteristics of participants	Current smokers	Ex-smokers	Occasional smokers	Never smoked	Total
	n (%)	n (%)	n (%)	n (%)	n (%)
All responders					
sex	479 (34.63)	253 (18.30)	152 (11.00)	499 (36.08)	1383 (100)
female	350 (34.11)	170 (16.57)	116 (11.30)	390 (38.02)	1026 (74.2)
male	129 (36.31)	83 (23.25)	36 (10.08)	109 (30.54)	357 (25.8)
Occupation					
teaching physicians	17 (35.42)	11 (22.91)	5 (11.42)	15 (31.25)	48 (3.40)
physicians	100 (22.08)	98 (21.63)	49 (10.82)	206 (45.47)	453 (32.70)
nurses	289 (39.48)	110 (15.02)	90 (12.30)	243 (33.20)	732 (52.80)
staff	73 (48.67)	36 (24.0)	7 (4.67)	34 (22.66)	150 (11.10)
Age (years)					
< 30	69 (34.33)	17 (8.46)	29 (14.42)	86 (42.79)	201 (14.53)
30–39	141 (32.87)	79 (18.41)	51 (11.89)	158 (36.83)	429 (31.02)
40–49	176 (34.04)	106 (20.50)	56 (10.84)	179 (34.62)	517 (37.38)
50–59	92 (42.01)	42 (19.18)	13 (5.94)	72 (32.87)	219 (15.84)
60+	1 (5.88)	9 (52.94)	3 (17.64)	4 (23.54)	17 (1.23)

Table 2

Characteristics of the participants who smoke (ANOVA test)

Characteristics	Teaching physicians		Physicians		Nurses		Staff		Significance
	x̄	SD	x̄	SD	x̄	SD	x̄	SD	
Starting of smoking (years)	20.47	4.48	20.38	5.38	18.91	3.75	18.44	4.06	A/B/C
Number of cigarets/day	22.55	5.16	17.54	6.29	16.24	7.14	19.73	6.05	C/D/E

A – physicians *vs* nurse; B – physicians *vs* staff; C – teaching physicians *vs* nurse; D – teaching physicians *vs* staff; E – nurse *vs* staff.

cally significant difference among the analyzed groups (ANOVA: F = 4.213, $p < 0.006$).

The physicians teaching at medical schools had the highest number of smoked cigarettes, 22.5 a day. This number was significantly high when compared to the physicians (17.5 cigarettes a day), nurses (16.2) as well as other employees in medical institutions (19.7 cigarettes a day).

The data showed that other non-medical staff smoked significantly higher number of cigarettes compared to nurses.

The highest number of smokers (46.5%), smoked out of habit, and slightly less (37.6%) felt pleasure during smoking and cited that as a reason. The lowest number of participants (4.3%) smoked because they were dissatisfied and 11.5% of them listed stress as a reason.

Table 3 presents the results of the percentage agreement with knowledge and attitude statements in the questionnaire compared by smoking status. The highest number of participants, 250 (53.8%), who asked their patients if they were smokers were in the group of those who never smoke which is significantly more compared to those who were smokers 34.2% ($\chi^2 = 13.82$; $p < 0.001$).

Occasional smokers (70 or 47.6%) asked whether their patients smoked more often than smokers ($\chi^2 = 15.07$; $p < 0.001$).

The participants who had quit smoking asked this question in 42% of cases.

Patients were most frequently advised to quit smoking by the participants who had never smoked. The number was significantly higher compared to the smokers (60.5% *vs* 36.4%; $\chi^2 = 17.26$; $p < 0.001$). The same advice was also more often given by the participants who had quit smoking (54.4% *vs* 36.4%; $\chi^2 = 4.23$; $p = 0.04$).

The occasional smokers advised their patients to quit smoking in 39.9% of cases.

A total of 25.2% of non-smokers and 20.9% of occasional smokers followed smoking status of their patients. Statistically, this percentage was significantly higher compared to the group of smokers which formed 10.3% ($\chi^2 = 18.46$; $p < 0.001$ and $\chi^2 = 5.46$; $p = 0.02$). Out of the total number of those who quit smoking, 32 (13.6%) inquired about the smoking status of their patients.

The greatest interest in exposure of patients to cigarette smoke was observed among the participants who had never smoked, namely 158 (34.5%). A statistical significance was considerably higher compared to the smokers (16%); ($\chi^2 = 39.50$; $p < 0.001$). Also, the exposure to cigarette smoke was more often monitored by those who had quit (20.3% at the level of $\chi^2 = 13.93$; $p < 0.001$) as well as by occasional smokers (26.7% at the level of $\chi^2 = 7.80$; $p = 0.005$).

Table 4 represents the results of the questionnaire considering the percentage agreement with knowledge and attitude statements compared by the occupation and by smoking status. All the participants stated that smoking is harmful to health and, in general, all the participants had "appropriate" attitudes considering smoking. However, there were some significant differences between occupation and smoking status regarding the role of healthcare professionals in providing advice to patients.

The participants who smoke did not completely agree that medical workers are role models for patients and employees or that patient's chances to quit smoking are increased as a result of healthcare workers' advise. Moreover, they did not completely agree on whether medical workers should even advise patients about smoking. The differences in attitudes towards smoking between the smokers and non-smokers were noticed with regard to forbidding smoking in enclosed public places, as well as to agreement on whether passive smoking increases risk of lung and heart diseases.

Higher scores were noticed in the groups of physicians compared to nurses and the staff considering whether patients' chances to quit smoking are higher if medical workers advise them to quit, as well as whether indoor smoking should be banned. Compared to other staff, doctors and nurses more often believed that selling cigarettes to children and adolescents should be banned and that passive smoking is harmful to respiratory system.

Table 3

Knowledge and attitudes of healthcare professionals in Serbia according to their smoking status

Questions the patients?	Smoking status	Yes			No		Sometimes		*p*
		n	n	%	n	%	n	%	(*vs* smokers)
Do you ask your patients if they smoke	never	465	250	53.8	96	20.6	119	25.6	< 0.001
	quit	239	98	41.0	63	26.4	78	32.6	
	occasional	147	70	47.6	22	15.0	55	37.4	< 0.001
	smokers	459	157	34.2	144	31.4	158	34.4	
Do you advise patients to quit smoking	never	463	280	60.5	79	17.1	104	22.5	< 0.001
	quit	239	130	54.4	51	21.3	58	24.3	0.04
	occasional	148	59	39.9	25	16.9	64	43.2	
	smokers	459	167	36.4	131	28.5	161	35.1	
Do you follow the smoking status of your patients	never	461	116	25.2	234	50.8	111	24.1	< 0.001
	quit	236	32	13.6	144	61.0	60	25.4	
	occasional	148	31	20.9	80	54.1	37	25.0	0.02
	smokers	457	47	10.3	296	64.8	114	24.9	
Do you ask your patients about their exposure to passive smoking	never	458	158	34.5	177	38.6	123	26.9	< 0.001
	quit	236	48	20.3	114	48.3	74	31.4	0.005
	occasional	146	39	26.7	61	41.8	46	31.5	< 0.001
	smokers	456	73	16.0	271	59.4	112	24.6	

Table 4

Knowledge about and attitude towards smoking of healthcare professionals in Serbia

Knowledge and attitude towards smoking	Group of healthcare professionals	I agree completely	I agree	I am not sure	I do not agree	I disagree completely	Significance
		n (%)	n (%)	n (%)	n (%)	n (%)	
Smoking is harmful to health	Teaching physicians (n = 45)	37 (82.2)	6 (13.3)	1 (2.2)	0 (0.0)	1 (2.2)	
	Physicians (n = 439)	379 (86.3)	48 (10.9)	5 (1.1)	6 (1.4)	1 (0.2)	
	Nurses (n = 719)	572 (79.6)	118 (16.4)	8 (1.1)	18 (2.5)	1 (0.7)	*ns*
Healthcare professionals serve as role models for their patients and the public	Teaching physicians (n = 48)	31 (64.6)	9 (18.8)	2 (4.2)	5 (10.4)	1 (2.1)	
	Physicians (n = 440)	267 (60.7)	104 (23.6)	40 (9.1)	24 (5.5)	5 (1.1)	
	Neurses (n = 717)	386 (53.8)	180 (25.1)	80 (11.2)	64 (8.9)	7 (1.)	
	Staff (n = 145)	66 (45.5)	41 (28.3)	14 (9.7)	18 (12.4)	6 (4.1)	A*
Patient's chances of quitting smoking are increased if they are advised by healthcare professionals	Teaching physicians (n = 48)	23 (47.9)	19 (39.6)	4 (8.3)	0 (0.0)	2 (4.2)	
	Physicians (n = 440)	185 (127)	28.9 (81)	18.4 (44)	44 (10.0)	3 (0.7)	
	Neurses (n = 721)	225 (31.2)	190 (26.4)	173 (24.0)	123 (17.1)	10 (1.4)	
	Staff (n = 144)	44 (30.6)	49 (34.0)	30 (20.8)	19 (13.2)	2 (1.4)	A/B/C*
Healthcare professionals should routinely ask about their patient's smoking habits	Teaching physicians (n =47)	26 (55.3)	15 (31.9)	2 (4.3)	2 (4.3)	2 (4.3)	
	Physicians (n = 437)	180 (41.2)	163 (37.3)	53 (12.1)	2 (4.3)	9 (2.1)	
	Neurses (n = 719)	235 (32.7)	317 (44.1)	77 (10.7)	32 (7.3)	5 (0.7)	
	Staff (n = 143)	44 (30.8)	60 (42.0)	15 (10.5)	85 (11.8)	5 (3.5)	A
Healthcare professionals should routinely advise their smoking patients to quit smoking	Teaching physicians (n = 47)	29 (61.7)					
	Physicians (n = 441)	254 (57.6)	165 (37.4)	9 (2.0)	8 (1.8)	5 (1.1)	
	Neurses (n = 719)	331 (46.0)	297 (41.3)	61 (8.5)	23 (3.2)	7 (1.0)	
	Staff (n = 145)	61 (42.1)	66 (45.5)	11 (7.6)	5 (3.4)	2 (1.4)	E/C*
Smoking in enclosed public places should be prohibited	Teaching physicians (n = 48)	32 (66.7)	10 (20.8)	4 (8.3)	2 (4.2)	0 (0.0)	
	Physicians (n = 438)	260 (59.4)	104 (23.7)	32 (7.3)	25 (5.7)	17 (3.9)	
	Neurses (n = 721)	406 (56.3)	195 (27.0)	61 (8.5)	46 (6.4)	13 (1.8)	
	Staff (n = 145)	67 (46.2)	34 (23.4)	16 (11.0)	19 (13.1)	9 (6.2)	C/A/D*
Tobacco sales to children and adolescents should be banned	Teaching physicians (n = 48)	35 (72.9)	9 (18.8)	2 (4.2)	2 (4.2)	0 (0)	
	Physicians (n = 437)	345 (78.9)	68 (15.6)	10 (2.3)	8 (1.8)	6 (1.4)	
	Neurses (n = 723)	555 (76.8)	142 (19.6)	16 (2.2)	8 (1.1)	2 (0.3)	
	Staff (n = 145)	98 (67.6)	34 (23.4)	5 (3.4)	3 (2.1)	5 (3.4)	C/D
Passive smoking increases the risk of lung disease in non-smoking adults	Teaching physicians (48)	29 (60.4)	14 (29.2)	1 (2.1)	3 (6.3)	1 (2.1)	
	Physicians (n = 440)	279 (62.7)	120 (27.3)	32 (7.3)	7 (1.6)	5 (1.1)	
	Neurses (n = 718)	393 (54.7)	248 (34.5)	50 (7.0)	22 (3.1)	5 (0.7)	
	Staff (n = 146)	70 (47.9)	51 (34.9)	14 (9.6)	5 (3.4)	6 (4.1)	C/D*
Passive smoking increases the risk of heart disease in non-smoking adults	Teaching physicians (48)	26 (54.2)	16 (33.3)	2 (4.2)	3 (6.3)	1 (2.1)	
	Physicians (n = 442)	275 (62.2)	112 (25.3)	41 (9.3)	14 (3.2)	0 (0.0)	
	Neurses (n = 722)	392 (54.3)	256 (35.5)	54 (7.5)	15 (2.1)	5 (0.7)	
	Staff (n = 143)	71 (49.7)	45 (31.5)	19 (13.3)	3 (2.1)	5 (3.5)	C*

A – teaching physicians *vs* staff; B – teaching physicians *vs* nurses; C – physicians *vs* staff; D – nurses *vs* staff; E – physicians *vs* nurses;
***p* < 0.005 between smokers and non-smokers.**

The participants were also asked if they had ever received formal training on strategies for smoking cessation and whether they felt prepared to counsel patients on how to stop smoking. Twenty-five percent of nurses and 22% of doctors claimed they had received formal training. However, as Table 5 shows, only 35.7% of participants felt they were very well prepared to counsel patients, while 52.7% felt they were somewhat prepared. In addition, 11.6% of examinees stated that they were not prepared at all.

In our sample, there were 255 participants who had quit smoking. They said the main reason for smoking cessation was medical (87.6%). Among females, 12.2% quited smoking due to pregnancy. Seven percent of participants quited smoking because of financial reasons. Other reasons for

Table 5

Degree of "feeling prepared" among healthcare professionals in smoking cessation counseling

Degree of preparedness for smoking cessation	Teaching physicians n (%)	Physicians n (%)	Nurses n (%)	Staff n (%)	Total n (%)
Very well prepared	19 (39.6)	159 (35.1)	271 (37.1)	45 (30.0)	494 (35.7)
Somewhat prepared	24 (50.0)	240 (53.0)	380 (51.9)	84 (56.0)	728 (52.7)
Not at all prepared	5 (10.4)	54 (11.9)	81 (11.0)	21 (14.0)	161 (11.6)

quitting smoking were stated in 2.4%. Only 3 participants quited smoking out of awareness that smoking harms health of their children. Among ex-smokers, 73.5% answered they smoked every day and 26.5% said they smoked occasionally.

Out of the total number of smokers, most of them stated that they were not ready to quit smoking in the next 6 months (41.1% physicians and 52.7% nurses). There were 44.2% physicians and 41% nurses who were thinking about quitting smoking, while only 14.7% of physicians and 13.8% of nurses were ready to quit immediately.

Discussion

According to our data, the prevalence of smokers among physicians in Serbia is 34.13%. Out of this percentage, everyday smokers form 23.35% and occasional 10.78%. The prevalence among nurses is 51.78%, out of which 39.48% are everyday smokers and 12.30% are occasional smokers. In our sample, there are 46.4% men and 45.41% women who smoke. These data put Serbia among the top countries according to the number of smokers.

Considering the meta-analysis which included 81 studies conducted in English language over the past 30 years [20, 21], the highest prevalence was recorded in Greece, where as much as 49% of physicians were smokers [22]. The highest prevalence of smokers among physicians in China was 49% (61% men, 12% women) [23]. In Bosnia and Herzegovina, there were 40% of physicians and 51% of nurses who smoke [24]. The lowest prevalence was recorded in the USA, Great Britain and Australia with only 3% of smokers, and New Zealand with only 5% [25–29].

According to the final report of the Ministry of Health of the Republic of Serbia published in May 2006, there were 33.6% smokers in Serbia, of whom 38.1% were men and 29.9% women. Compared to the year 2000, the number is lower by 6.9 %. Our study points out that the prevalence of smokers among the staff is higher than in general population, which is a negative model in anti-smoking campaigns and reduces their efficiency.

In the study conducted in 2008 by Harmon et al. [30] it was established that the prevalence was 38% of men and 37% of women physicians in Serbia, which is slightly more than in our sample.

It is necessary to point out several limitations of the study. First of all, the questionnaire was self reported and not validated, so there is a chance that the prevalence of smokers is higher than shown. As physicians know more about the devastating effects of smoking than the general population, they may be prone to self-deception or understatement, and their underreporting could differ from the general population. The second limitation can be related to 78% of responding rate. There is a possibility that those who did not give any response had different opinions than the participants in the survey, and there is also a chance that they were smokers. In addition, there were no participants from Kosovo, where the prevalence of smokers is expected to be higher than in the rest of Serbia. Since the survey relied primarily on self-reports, there may be a possibility that physicians over-reported their advisory activities concerning smoking cessation.

It is interesting that one of the first epidemiological researches concerning the harmful effects on health caused by smoking were conducted by Doll and Hill [31] on a British Doctors Cohort in 1954 and published 50 years later in the British Medical Journal [32]. It can be said that this was the turning point in public healthcare approach to the treatment and control of smoking.

A smoking status of a physician seems to be a very important determinant of how they address their patient's tobacco use. It appears that doctors who smoke are less willing to inquire about tobacco use, to advise cessation, and to provide evidence-based assistance when compared to their non-smoking colleagues [33, 34]. In countries where anti-smoking strategies have been long in use, such as Canada, the USA, Sweden, Australia, and the UK, this is not a major problem since physicians smoking rates are very low. In many other countries the smoking rate among physicians is similar to that of the general population [35, 20]. In spite of the evident knowledge considering the consequences of smoking, the persistence of healthcare professionals in this detrimental habit may ruin the global efforts to help smokers quit using specific clinical assistance and interventions.

There are at least two reasons why the data of the prevalence of smoking among physicians is useful. First, such information may point to the possibilities of succeeding in anti-tobacco campaigns. In countries where there are high numbers of physicians who smoke, it is difficult to convince the general population that smoking has detrimental effect on their health. Second, the prevalence of smoking among physicians may reflect the 'maturity' of the smoking epidemic in a particular country. As the dangers of smoking become better known, medical profession will give up smoking earlier than the general population. The smoking epidemic of a country may be considered 'mature' when the prevalence of smoking among doctors is lower than that of the general population.

Considering the questions of knowledge and attitude, the differences in agreement with regard to the responsibilities of healthcare professionals and smoking policy could be expected between the "ever" and "never" smokers. However,

such differences were not expected between the physicians and nurses. Regarding the knowledge of harmful effects of smoking, overall results were quite positive.

Counseling by healthcare professionals on smoking cessation is crucial if their patients plan to quit smoking. The relatively high rates of healthcare professionals with formal training reflect the sampling frame of participants. And indeed, almost 90% felt very or somewhat well prepared to counsel their patients on smoking cessation. However, it may be possible that healthcare professionals who are not actively counseling patients on smoking cessation may underestimate the difficulty of successfully supporting their patients through to smoking cessation. More formal training in smoking cessation strategies through continuing education of healthcare professionals in Serbia may be justified, especially if you consider that 21.2% doctors smoke in front of their patients.

Physician training has the potential to reduce the barriers in providing assistance to patients; there is evidence of a dose response relationship between the time spent on training and doctor activity in the promotion of smoking cessation [17, 36].

The results of this study show that there are needs for more aggressive nationwide non-smoking campaigns for physicians and medical students, as well as smoke-free hospital campaigns; however, this is only the first necessary step. Further interventions can target physicians smokers, monitor smoke-free hospitals, and educate about effective smoking cessation practices. Experiences from countries where doctors smoke less and more effectively carry out smoking cessation practices need to be shared with Serbian doctors in order to improve the smoking behavior of Serbian doctors and their smoking cessation practices.

Conclusion

Considering the fact that smoking epidemic is spreading quickly and aggressively, as well as the high level of prevalence among smoking physicians in Serbia, the conclusion is that there is a need for more aggressive and more specific anti-smoking campaigns directed to medical employees. An effort must be made so that medical students do not become smokers. Assisting medical employees in smoking cessation help them achieve self-improvement as well as the improvement of population in general.

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CURRENT TOPIC

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Optimal use of prostate specific antigen for prostate cancer screening

Optimalna upotreba prostata specifičnog antigena za otkrivanje karcinoma prostate

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Key words:
prostate neoplasms; prostate-specific antigen; diagnosis; risk assessment.

Ključne reči:
prostata, neoplazme; prostata, specifični antigen; dijagnoza; rizik, procena.

Introduction

Prostate cancer (PCa) and prostate specific antigen (PSA) screening have been the main focus of discussions among urologists and primary care physicians during the last few years [1]. Ever since its introduction in the 1980s [2–4], PSA screening was implemented as standard of care in many developed countries, but without the supporting level I evidence to justify its initiation (mainly relating to reduction in cancer-specific mortality). Several retrospective reports, such as the Surveillance, Epidemiology, and End Results (SEER) database review suggested a significant decline in mortality rates of 32.5% from PCa due to screening; the Baltimore Longitudinal Study of Aging (BLSA) showed that PSA levels increased years before clinically detectable PCa and that this could lead to an effective early diagnosis and more effective therapy [5]. Similar findings were observed in the study from Tyrol, Austria, where men from this particular region had a notable decrease in mortality after being screened with PSA in comparison to the rest of the country where PSA testing was not freely available [6]. However, in 2009 two large, randomized, prospective studies attempted to answer this decade-long question: The Prostate, Lung, Colorectal and Ovarian Cancer screening trial (PLCO) from the United States which showed no benefit to screening [7, 8], and the European Randomized Trial of Screening for Prostate Cancer (ERSPC) which reported a 20% reduction in mortality from PCa, but at the expense of 1,410 men having to undergo screening and additional 48 men to undergo treatment in order to save 1 life [9–11]. The latter results implied a significant overtreatment and overdiagnosis of clinically insignificant cancers as a result of screening. Although survival rates after radical management of localized PCa are high, the risk of complications including urinary, sexual, and bowel dysfunction is not negligible [12, 13].

Based on these findings, the United States Preventive Services Task Force (USPSTF) after careful review and balance of the risks and benefits – mainly, problems of overdiagnosis and over-treatment – recommended against a population-based screening program [14]. The rationale for screening after inconsistent results from these two large, randomized trials coupled with the inherent PCa treatment complications (including urinary, sexual, and bowel dysfunction) created a conflicting and confusing environment for the world-wide treating physicians and their patients. While many questions still remain unanswered, PSA (although an imperfect test) continues to be the only tool we currently have available to identify patients at higher risk of dying from PCa.

Adaption of new risk stratification strategies may be the solution to minimize patient harms and offset the risk/benefit ratio. In this report we provide evidence in support of early and targeted screening, earlier termination of screening, and prolongation of the screening interval to every 2–4 years.

Early "targeted" screening

Based on the observational data from Sweden [15], PSA screening when performed earlier in patient's life seems to provide strong prognostic risks relating to future development of clinically important PCa. Lilja et al. [15] reported that a single PSA test obtained in the middle-aged men (ages 45–50) had a highly predictive risk of developing subsequent PCa. Specifically, they found that men with total PSA ≤ 0.5

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ng/mL had a cumulative low (10.5%) risk of developing clinically meaningful cancer by age of 75, those with PSA between 0.51 ng/mL and 1.0 ng/mL had a 2.5-fold increased risk, and men with PSA between 2.0–3.0 ng/mL were associated with a 19-fold increased risk of developing cancer [15]. Similarly, Vickers et al. [16] reported that men with PSA ≤ 1.0 ng/mL at age 60 were highly unlikely to possess or develop life threatening PCa by age 85, and even lower risk (0.2%) of dying from disease.

These data are further supported by the findings observed by Stamey et al. [17] regarding correlation between PSA level at radical prostatectomy and final pathology. After a careful evaluation of almost 1,300 specimens, they documented a strong association between the increasing PSA levels and prostate weight, or benign prostate hyperplasia (BPH), but not PCa [17]. These observations imply that as men age and their prostates increase in size (mostly due to BPH), the PSA tends to loose its specificity for diagnosing PCa. As a result, in the current PSA era the main "driver" for PSA rise in older men seems to be mostly related to the amount of BPH. Therefore, "targeted" screening with focus on younger population where BPH represents a minimal confounder should improve the usefulness of PSA testing.

Based on these findings, early PSA elevation could identify a cohort of men who would benefit from early screening and/or preventive measures (pharmacological or lifestyle alterations) [18]. Similarly, in those men without early PSA elevation screening could be potentially deferred until age 50 and obtained less frequently [19]. In cases with early PSA elevation, patients should only undergo biopsy if recognized indications for biopsy are present, such as PSA > 1.0 ng/mL, PSA velocity (v) > 0.75 ng/mL/year, free: total PSA < 10%, and those with strong family history of early PCa.

Early "termination" of screening

There is strong evidence from previous reports that screening men for PCa in the PSA era should be stopped earlier than current guidelines would suggest. According to a population-based study from the pre-PSA era by Albertsen et al. [20], a significant number of PCa identified today (in the PSA era) would unlikely become clinically symptomatic over a patient's lifetime. This was especially true for well-differentiated tumors (Gleason 5–6), in which case the majority of men died from the competing medical conditions during a 15–20 years follow-up [20]. Interestingly, Draisma et al. [21] estimated the impact of PSA testing on lead-time of detecting clinically insignificant disease to be 12 years for 55-year-old men, and estimated that approximately half of the men ages 55–67 diagnosed with PCa would have non-important disease. This suggests that screening should probably be stopped for healthy men with a low-risk of developing PCa around age 60–65, given the long lead-time and natural history of disease. As mentioned earlier, Vickers et al. [16] demonstrated in their study the risk of PCa death for men with PSA < 1.0 ng/mL at age 60 to be negligible. Therefore, termination of screening at later age (like 75) as American Urology Association (AUA) guidelines recommend should be considered only in highly select cases – those who are healthy and have a life expectancy of > 10–15 years.

Risk stratification for prolonged screening interval

The ERSPC [10] study, which clearly showed a reduction in PCa-specific mortality due to screening, was based on a 2–4 year PSA screening interval. However, further subanalyses of the study seemed to support a potential for even further risk stratification and prolongation of screening time interval. For example, Roobol et al. [22] evaluated a group of men with initially low PSA (< 1ng/mL) who underwent every 4–8 years screening interval and found only 0.23% risk of cancer diagnosis during the intervening visit. Another study showed a 0.5% risk of advanced disease and overall 2.5% risk of prostate cancer at 15 years follow-up [23]. On the other hand, van Leeuwen et al. [24] compared men from Gothenburg screening cohort (2-year screening interval) to Rotterdam cohort (4-year screening interval) and found that more frequent screening resulted in a higher proportion of screen-detected cancers (RR:1.18, $p = 0.009$). However, more frequent screening also led to increased incidence of clinically insignificant (low-risk) PCa (RR: 1.46, $p < 0.001$). Interestingly, the Gothenburg cohort also had a lower incidence of advanced cancer during the last follow-up (RR: 0.57, $p = 0.048$), which implied that more frequent screening resulted into more effective way of eliminating high-risk PCa from the population. Importantly, the effect on PCa-related mortality remained uncertain. This study implies that there is a certain population of men that may benefit from a more frequent screening program, but an individualized approach according to patient risks would be the most optimal way of screening. Currently, there is no data that supports annual PSA screening for PCa – the best evidence is based on screening every 4 years [10].

Conclusion

According to recent studies, PSA results obtained during an earlier age may be strongly predictive of long-term risk of clinically important PCa, and as a result a risk-stratification approach should be considered in order to minimize over-diagnosis and overtreatment of this disease. The focus of screening should become on tumors with greater malignant potential in the appropriate age population, instead of clinically insignificant tumors. Such an approach could also reduce associated economic burden of treating PCa in the present health-care system. Until new genomic-based markers are identified, PSA testing will continue to play an important role in identifying patients with high-risk PCa and should not be abandoned.

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PRACTICAL ADVICE
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Preparation for radioiodine therapy: how to increase therapeutic efficacy and accelerate unbound radioiodine excretion

Priprema za radiojodnu terapiju: kako povećati terapijsku efikasnost i ubrzati ekskreciju nevezanog radiojoda

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Key words:
thyroid neoplasms; radiopharmaceuticals; iodine radioisotopes; radiotherapy, dosage; laxatives; diuretics; lithium.

Ključne reči:
tireoidna žlezda, neoplazme; radiopreparati; jod, radioizotopi; radioterapija, doziranje; laksansi; diuretici; litijum.

Introduction

Therapeutic application of radioiodine ^{131}I in postoperative ablation of the remaining thyroid tissue, as well as in treatment of recidives and/or local and remote metastases of differentiated thyroid carcinoma has been a part of clinical practice for over 50 years. It is a regular segment of the standard therapeutic procedure in differentiated thyroid carcinoma treatment and it is recommended by a number of authorities in the field [1–9]. Certain differences in opinion on the subject are concerned only with the dose which is applied, as well as with whether the therapy should be applied in lower risk patients [4, 6, 9–13]. Several decades of experience have shown the indisputable beneficial effects of ^{131}I application as postoperative adjuvant therapy. However, there can be certain adverse effects beside the beneficial ones, which are a consequence of radiation damage to other tissues and organs. The organs most exposed to the harmful radiation effect of ^{131}I in differentiated thyroid carcinoma treatment are the salivary glands, nasolacrimal ducts, stomach mucus, kidneys, bladder wall, colon, gonads, bone marrow, etc.

The question that arises from the aforesaid is how to achieve a good compromise between the beneficial therapeutic effects of radioiodine on one hand, and adverse effects on other tissues and organs on the other. The compromise could be achieved in two ways. The first one is to increase radioiodine uptake in thyroid tissue/tumor tissue by increasing the therapeutic efficiency of ^{131}I. In other words, the aim is to achieve the best therapeutic effect on the target tissue with as low dose of ^{131}I as possible. The second way is to reduce the adverse effects, *ie* the radiation amount of ^{131}I absorbed by other organs and tissues, by accelerating elimination of radioiodine not bound by thyroid/tumor tissue.

There is yet another reason for accelerated elimination of radioiodine from the body of a patient. The reason is legal and concerns regulations set by every country which determine the amount of radioactive iodine that patients are allowed to have in their bodies without being required to receive their treatment in the restricted area. With the doses of radioiodine normally applied in differentiated thyroid carcinoma treatment, most cases require hospitalization of various duration.

For this reason, it is in the best interest of any health system to shorten hospitalization, *ie* isolation of a patient being treated with radioactive iodine, without reducing the therapeutic effect of ^{131}I. In other words, the cost of hospitalization/isolation of such patients should be reduced.

Concerning legal regulations of ^{131}I doses, there are significant variations from country to contry. These variations mostly apply to the upper limit of radionuclide not requiring a patient isolation.

Legal regulations state that anything above that limit requires the therapy to be carried out in hospital premises, or more precisely, in special rooms designed as controlled radiation zones. This limit varies in different countries. For example, the upper limit in Serbia is relatively low and special precautions have to be taken only if the radioactivity of ^{131}I exceeds 400 MBq. In other words, a patient can be released from hospital only when radioactivity in his body is not more than 400 MBq [14]. The limit is significantly higher in the EU and

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USA, where hospitalization is obligatory only if the radioactivity of ^{131}I is more than 30 mCi (1,110 MBq). In this case a patient is hospitalized and kept in isolation until his/her radioactivity level decreases to 30 mci (1,110 MBq) [4, 15].

In cases of differentiated thyroid carcinoma the treatment doses of ^{131}I vary from 30 mCi for the remaining thyroid tissue ablation, to 200 mCi for treatment of metastases, even though there are several records of the doses reaching as much as 333 mCi (9GBq) [10]. With application of these doses, even the largest ones, the permitted radioactivity limit in the body is reached a few days following application of the ablation/therapeutic dose of ^{131}I [16].

The time necessary to reach the limit depends primarily on the dose applied and the condition of kidney function, as well as on the size of the thyroid/tumor tissue being treated.

Methods for increasing radioiodine uptake

Thyrotropin stimulation (endogenous and exogenous thyroid-stimulating hormone stimulation)

In order to achieve a sufficient radioiodine uptake in thyroid remnants/tumor tissue, it is necessary either that a patient has good endogenous thyroid-stimulating hormone-TSH (blood TSH concentration above 30 mIU/mL), or to perform exogenous TSH stimulation by applying recombinant human TSH (rhTSH).

In the first case, sufficient levels of TSH are most commonly achieved if a patient is left without thyroid hormone replacement therapy for 4 to 6 weeks. The primary complication which may arise from this way of increasing TSH levels is hypothyreoidism, which some patients find quite disagreeable. The condition is followed by hypometabolism, constipation, increased cholesterol levels in blood, the risk of cardiovascular disorders, and the most severe one – myxedema.

In the second case, exogenous TSH stimulation of the uptake is achieved by applying rhTSH, available on the market as Thyrogen® (Genzyme). This medication is given to a patient intramuscularly for 2 days, in 0.9 mg doses.

Exogenous stimulation of thyroid minimises the chances of hypothyreoidism, at the same time enabling a better planning of radioiodine therapy. However, application of rTSH increases the cost of treatment significantly, as this medication is relatively expensive.

A number of studies have shown that the final effects of uptake stimulation, both with endogenous TSH, and exogenous rhTSH are equally satisfactory and thus both are equally recommended [6, 17].

Low iodine diet

In order to achieve better uptake in thyroid/tumor tissue, a low iodine diet is recommended, *ie* the daily intake of not more than 25–75 μg of iodine. Patients should be put on the diet for 10 to 30 days prior to the diagnostic or therapeutic application of ^{131}I [18, 19].

The consequence of low iodine intake is iodine depletion, which could result in its increased uptake in thyroid remnants/tumor tissue. Since most countries have legal regulations by which producers are obliged to iodize table salt, this low iodine diet practically presupposes the limitation of table salt intake, which usually proves to be difficult for the patients to put into practice. One teaspoon of iodized table salt contains about 400 μg of iodine. Sea salt is also not recommended due to the fact that it contains a significant amount of iodine. The alternative is uniodized salt, which is often difficult to find. Apart from the limitation on table salt, it is essential that the patients avoid foods with high concentration of iodine (above 20 μg per meal), and these are as following: seafood (fish, shellfish, seaweed, seaweed tablets, kelp). These are all very high in iodine and should be avoided. Food containing sea-based additives, such as carrageenan, agar-agar, algin, alginate and nori should also be avoided; milk and dairy products such as cheese, cream, yogurt, butter, ice cream, milk chocolate, powdered dairy creamers, whey, casein and others which contain significant amounts of iodine (250 mL of milk- 1 cup or 16 tablespoons, contain from 88 to 168 μg of iodine, or an average of 115 μg); egg yolks or whole eggs; bread and pastry; salty processed foods such as potato chips and cured and corned foods such as hot dogs, ham, corned beef, sauerkraut, bacon, sausage, and salami; soybeans and most soy products (soy sauce, soy milk, tofu); red, orange, or brown processed food, pills and capsules, because the artificial colour (erythrosine) used for these foods contains significant amounts of iodine; iodine-containing vitamins and food supplements.

A limited daily intake of food which contains moderate amounts of iodine (5–20 μg per meal) is recommended as follows: fresh meat (meat contains 25–130 μg of iodine per pound), up to 5 ounces a day of fresh meat such as chicken, beef, pork, lamb, and veal are fine on the low-iodine diet; up to 4 servings per day of grains, cereals, pasta, and breads without iodine-containing ingredients are fine for this diet, homemade baked goods and cereals are best for this diet; similar to grains, rices vary in the amount of iodine depending on the region where they are grown, so rice should be eaten only in limited amounts. Some low-iodine diets recommend avoiding rice.

These instructions can often pose a problem because some guidelines only say that certain items or certain food categories should be avoided, and do not give details within categories, or else they just give lists of foods and ingredients that are allowed, without limits on quantities consumed.

Even though most recommendations and guides list iodine diet as an essential part of the preparation for radioiodine therapy application due to the fact that it increases the binding of iodine in thyroid/tumor tissue, there are also other, contrasting data. Some researches have shown that the effects of low iodine diet can include an increased iodine retention, instead of iodine depletion, especially if it is combined with the application of diuretics [20–23].

Lithium

The inhibiting effects of lithium carbonate on the release of iodine from the thyroid tissue are also useful in radioiodine treatment of differentiated thyroid carcinoma, for the purpose of achieving prolonged and increased radioio-

dine retention in the thyroid/tumor tissue [24–27]. Researchers agree that administration of 0.8–1.2 mmol/L of lithium carbonate results in an increased uptake and prolonged retention of radioiodine in thyroid/tumor tissue, which doubles the dose absorbed, without a significant adverse whole-body irradiation. However, the majority of authors urge caution in using this medicine and suggest careful monitoring of its levels in plasma for the purpose of avoiding adverse effects, primarily intoxication, which affects the central nervous system and kidneys, and can potentially be fatal [28].

Retinoids and increased expression of NaI symporter system

Better penetration of ^{131}I into the remnant thyroid/tumor tissue can be achieved through an increased expression of genes which code the synthesis of NaI symporter by means of retinoids or their metabolites, which bind with retinoic A and X receptors (RAR and RXR), resulting in an increased expression of these genes and an increased synthesis of NaI symporters. At the end of this chain is the achievement of the increased iodine uptake in thyroid/tumor tissue. However, there are contradictory data concerning the efficiency of this sort of adjuvant therapy in thyroid iodine uptake. While some researchers [29–31] point out that the administration of 13-cis retinoic acid (in Accutan®, Roche Laboratories, Nutley, NJ, USA) prior to radioiodine application increases its uptake in the tumor tissue, especially in follicular carcinomas, others [32] do not find a significant efficiency in the increase of thyroid iodine uptake, based on researches on large groups of subjects.

However, the latest research on NaI symporter system expression, as well as identification of genes which code its synthesis [33–37] will probably allow for a new approach in radioiodine therapy of thyroid carcinomas, which focuses on the optimisation of the dose administered to patients, *ie* on increasing the efficiency of this therapy.

Methods for increasing unbound iodine excretion from thyroid/tumor tissue

Hydration

The relevant literature suggests that accelerated urinary excretion of ^{131}I can be achieved by extensive hydration. However, there are also data which do not support this. For example, Giebisch et al. [37] concluded in their research on dogs that water diuresis does not induce iodine diuresis, as 95% of the filtered iodine gets reabsorbed by the tubules in proximity to water absorption spots. Even so, extensive hydration is recommended in patients receiving radioiodine therapy, since it can lead to the dilution of radioiodine in urine and a decrease in radioactive urine retention in the urinary tract, which contributes to the decrease in the dose absorbed by the urinary bladder wall and surrounding organs.

Laxatives

In order to accelerate elimination of ^{131}I through stool, some experts prescribe laxatives to expedite bowel evacuation, especially in patients with constipation. Others, however, hold the opinion that only a small, insignificant amount of the applied radioiodine is eliminated in this way, and that therefore laxatives are not of great importance [38]. For these reasons, it is considered that administration of laxatives is not necessary in patients who have at least one stool a day.

Diuretics

For the purpose of reducing the absorbed dosage in critical organs and tissues of patients treated with radioiodine, a simple and efficient method is often recommended for the excretion of unbound ^{131}I from thyroid/tumor tissue – extensive hydration in combination with additional diuretic therapy.

In a study conducted on 49 adult subjects with and without thyroid and kidney function impairment Bricker and Hlad [39] concluded that ^{131}I gets excreted from the body by means of passive filtration in glomeruli and gets partially reabsorbed by the tubuli by means of passive back-diffusion, without any active tubular transport mechanisms.

There are various, often contradictory data in the literature concerning the effects of diuretics on the biokinetics of radioiodine. The majority of researches points to the fact that faster elimination of radioiodine can be achieved by the application of additional diuretic therapy [40–46], but the results of some other researches show that the administration of diuretics leads to increased radioiodine uptake in the thyroid tissue [20–22, 47]. The data concerning the studies of the urinary excretion of iodine and the effects of diuretics on its urinary excretion published so far are in part contradictory. They do not present a clear picture of what kind of benefits, if any, we have from applying this additional therapy to patients suffering from differentiated thyroid carcinoma, treated with radionuclides.

The published data were obtained either from studies performed on animals [41–43], or from studies on patients who did not suffer from differentiated thyroid carcinoma and had not been operated on previously, but who received radioiodine doses far smaller than those given to patients suffering from differentiated thyroid carcinoma [22, 40, 44, 46].

There has been a small number of researches on the effects diuretics have on radioiodine clearance in patients who were treated with therapeutic doses of ^{131}I, but the conditions under which these researches were conducted were to a certain degree different from the ones typical for clinical practice and the way this therapy is normally carried out [45, 47, 48].

The effects of furosemide, hydrochlorotiazide, manitol, ethacrynic acid and acetozolamide on radioiodine urinary excretion have been studied so far. Out of all the diuretics, furosemide has been studied most.

Furosemide

Furosemide is effective, cheap and widely used. Abbott and Kovacic [49] have analysed the data concerning the effects of furosemide from both medical and veterinary literature. Based on a considerable number of analyzed papers, they concluded that one of the chief effects of furosemide includes iodine depletion in the body, which is achieved through a decrease in its reabsorption in the thick ascending

limb of Henle's loop. Furosemide acts as the inhibitor of Na-K-Cl cotransporter 2 (NKCC 2), which is the mechanism present in the majority of other diuretics, excluding spironolactone. The inhibition of co-transporter NKCC 2 is dose-dependent with respect to the concentration of furosemide in lumen, rather than in plasma. The administration of furosemide brings about an increase in sodium, chloride and water in distal collecting ducts, resulting in increased renal excretion of potassium and hydrogen. This can result in some patients developing hypokalemic and hypochloremic alcalosis, which are the most common adverse effects of this diuretic. For the purpose of hypokalemic and hypochloremic alcalosis prevention, it is advised that patients receive potassium chloride together with furosemide in cases of long term therapy.

When it comes to the influence of furosemide on radioiodine excretion, numerous and often contradictory data have been published. Some of them point to the fact that this diuretic influences the acceleration of iodine urinary excretion leading to iodine depletion. However, in one of our previous studies [23] it has been unmistakably shown that this diuretic, in combination with low iodine diet, slows down the elimination of radioiodine in patients treated with this radionuclide.

Our results were somewhat similar to the ones obtained by Maruca et al. [48], who concluded that diuretics mediated iodide depletion is not universally successful and that it is far less effective than it was considered before, therefore casting some doubt on its clinical benefits. Their aim was to achieve iodine depletion with low iodine diet and diuretics (hydrochlorotiazide and furosemide) in patients who had previously undergone thyreodectomy due to differentiated thyroid carcinoma. The results they obtained point to the fact that this low iodine diet and diuretics increase the uptake of iodine in tumor tissue. According to their findings, the total iodine uptake and retention in tumor tissue was mostly the consequence of total body retention, and not some specific mechanism at the cell level of thyroid/tumor tissue.

The presumption that low iodine diet plays an important role in how furosemide affects iodine biokinetics can be supported by the data obtained from a number of researchers, who found that furosemide and other diuretics cause an increase in iodine excretion in those patients who were not put on a prior low iodine diet. A comparison between researches by Seabold et al. [45] and Norfray and Quin [20] provide possible further evidence for this.

Namely, Seabold et al. [45] found that in patients who had not been on a low iodine diet and who had received radioiodine ablation therapy, furosemide as an adjuvant therapy accelerated the excretion of radioactive iodine, which enabled those patients to spend far less time in the hospital premises.

Based on experiments on animals, Norfray and Quinn [20] found that intraperitoneal application of furosemide leads to an increased iodide excretion, which in turn results in a decrease in iodide pool in their bodies. Same authors found that supplemental iodide diet does not reduce this effect of furosemide, even though the thyroid radioiodine uptake increases in comparison to the control group under the influence of diuretic therapy, which reduces the iodide pool. This indirectly points to the fact that an uptake increase in thyroid/tumor tissue can be achieved by administration of diuretics as well. However, they did not measure blood radioactivity, so the possibility that an increase in uptake under the influence of furosemide is at least in part a consequence of increased blood radioactivity, *ie* total body retention, instead of just an increase in the avidity of thyroid tissue for iodine cannot be excluded either.

Other researchers [20–22] also found that furosemide does not increase iodine excretion, but on the contrary, that it decreases it. According to the data provided by Kapucu et al. [46], administration of furosemide results in the loss of iodine from subjects' bodies (iodine depletion). They noticed that after a 5-day furosemide therapy a better penetration of iodine into the thyroid gland was noted in patients who had not previously been on a low iodine diet, than in those who had been on the diet for 14 days, without receiving furosemide. The authors think the explanation for this lies in the loss of sodium from extracellular fluid which is greater when furosemide is administered than when preceded by a low iodine diet alone.

However, Russell and Ingbar [40] state that there is an intrathyroid, pituitary-independent mechanism of increasing thyroid function as an answer to the reduction in iodine concentration in plasma. As far back as 1965 they studied the effect of iodide depletion (with previous low iodine diet and the administration of manitol) on ^{131}I biokinetics and thyroid function, on a group of 8 patients. According to their results, iodide depletion resulted in decreased iodine levels in blood, an increase in thyroid iodine transfer and the speed of thyroid clearance, as well as an increase in thyroid iodine uptake followed by a decrease in absolute iodine accumulation. These authors concluded within the same study that there is no increase in thyroid iodine clearance and ^{131}I uptake if NaI is applied together with manitol.

It should be stressed that in our research on mice [50] we did not note an increased radioiodine retention in thyroid tissue when we applied furosemide, even though they had undergone a low iodine diet. This can point to the fact that iodine biokinetics has certain species specific characteristics, either at the level of kidneys, or at the level of thyroid gland.

In our research, which included patients treated with radioiodine, we did not determine whether there is an increase in thyroid/tumor tissue uptake under the influence of Furosemide therapy, but our results indirectly support the data provided by Maruca et al. [48] that in cases of increase the most likely reason is, up to a certain point, an increase in ^{131}I levels in blood, *ie* an increase in total body retention of this radionuclide under the influence of additional Furosemide therapy.

An important role in this mechanism is played by the preceding low iodine diet, which can be concluded based on the data provided by Hamburger et al. [19]. They determined the uptake in thyroid/tumour tissue in a group of 25 patients with a confirmed diagnosis of inoperable thyroid carcinoma, who had previously been treated with diuretics and a low iodine diet.

What was achieved by a combination of a low iodine diet and diuretics (manitol and ethacrynic acid) was doubled, or even tripled uptake in 16 patients, mild increase in 3, and no increase in 6 patients. According to their data, radioiodine levels in thyroid/tumor tissue remain high 96 hours following the diuretic preparation.

Other diuretics

Based on the results obtained from a controlled study, Tepmonkogol [21] concluded that the binding of radioiodine in the thyroid gland is as many as 7.18 times higher when hydrochlorothiazides are applied together with a low iodine diet. The control group comprised patients who were on a low iodine diet, but who received neither hydrochlorothiazide, nor other diuretics. The control group showed an increase in the uptake as well, even though a significantly smaller one, amounting to 1.33 times the original binding. The study was performed on patients suffering from hyperthyreosis who had been treated with radioiodine. Similar results were obtained by Ding et al. [47], who showed that application of hydrochlorothiazides prior to application of radioiodine can significantly increase the dose absorbed by the thyroid tissue. The study included patients suffering from differentiated thyroid carcinoma who had previously undergone thyroidectomy.

In a study performed on 18 young male subjects following an acute administration of hydrochlorothiazide and acetazolamide, Fregly and McCarthy [44] analysed the fluctuations in urinary excretion of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- and I^-. Based on the results of this study, as well as on the previous studies done on animals [41–43], the authors concluded that hydrochlorothiazide has a significant effect on an increase in iodine excretion, which is closely tied to an increase in chloride excretion, while there was no increase in either iodine, or chloride excretion in those treated with acetazolamide.

Judging by all the aforementioned data, the most probable cause of the decrease in ^{131}I excretion under the influence of diuretics is the state of iodine depletion caused by the prior low iodine diet. For some reason this state is characterised by an absence of iodine reabsorption blockage in the tubuli under the influence of diuretic therapy, and paradoxically, results in increased iodine reabsorption.

Walser and Rahill [51] concluded that the reabsorption of iodine and chlorine is done in the same part of the nephron by means of passive diffusion with a constant ratio of tubular permeability. Since the low iodine diet was at the same time low chloride, as well as a low sodium diet (due to the reduced table salt intake), it is possible that the explanation for this unexpected and paradoxical effect of diuretics on radioiodine excretion lies in that very fact.

Namely, it is possible that in cases where low iodine diet (*ie* low chloride/low sodium diet) was prescribed, the increase in chlorine reabsorption gets followed by an increase in iodine reabsorption at the level of the ascending segment of Henle's loop and the proximal tubules. As a consequence, iodine excretion decreases, instead of increasing, and same goes for its blood levels, which directly influences the prolongation of patient hospitalization in the restricted area after the application of radioiodine therapy, due to the maintenance of high exposition dose. For this reason it is not advisable to use additional diuretic therapy for the purpose of speeding up the urinary excretion of radioiodine, at least not in patients who had previously been on a low iodine diet.

Recommendations and conclusion

With the aim of achieving a satisfactory compromise between high therapeutic efficiency of radioiodine therapy on thyroid/tumor tissue and the need to decrease its adverse effects on other tissues and organs, it is necessary for a patient to be carefully selected and adequately prepared.

Both exogenous and endogenous methods of TSH stimulation are equally valid from the point of view of achieving the uptake, but keeping a patient without substitution for several weeks can be highly disagreeable, and in some patients even dangerous, due to the possible complications. On the other hand, the convenience which comes with the use of rhTSH comes at a higher cost. It is up to a patient and the physician to estimate which method of TSH stimulation to use by evaluating the cost/benefit of exogenous and endogenous TSH stimulation in each individual case.

The low iodine diet comes right after TSH stimulation as the second most important step in the preparation of patients for radioiodine therapy, its purpose being to increase the radioiodine uptake in thyroid/tumor tissue. However, it should be considered that there can be a possible interference of this diet with the potential use of diuretics in patients treated with radioiodine.

In patients who had been on a low iodine diet there is a decrease in excretion of ^{131}I under the influence of diuretics, which results in an increase of its levels in blood, which in turn indirectly prolongs the hospitalisation period. All this also results in a higher dose being absorbed by the patient's critical organs. For this reason, administration of diuretics to accelerate urinary excretion of radioiodine cannot be recommended, at least not in patients who had previously been on a low iodine diet.

When it comes to methods for accelerating excretion of radioiodine which has not been bound to the thyroid/tumor tissue, extensive hydration of patients is recommended, as it reduces the absorption in the critical organs by diluting urine and increasing the number of mictions, even though it does not result in the increased iodine excretion.

Laxative administration in patients who have regular emptying of the bowel can cause certain discomfort to patients, so this is not clinically justified as necessary.

Administration of lithium is an efficient method of increasing the uptake of radioiodine in thyroid/tumor tissue, but it is not recommended in routine clinical practice, since its administration can have serious complications in case of overdose. An increase in NaI symporter system expression in thyroid/tumor tissue, achieved by the application of retinoids, results in the desired increase in radioiodine uptake. Even though it does not belong to the clinical routine, this method can be useful in patients who have lost the ability to

accumulate radioiodine in the tumour tissue. Further research on the identification of the gene responsible for coding NaI symporter system synthesis can provide a new approach to radioiodine treatment of thyroid carcinoma in the prospects of increasing the efficiency of the therapy.

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CASE REPORTS

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Dynamics of electrocardiographic changes, brain-natriuretic peptide and cortisol levels in a patient with stress (takotsubo) cardiomyopathy – a case report

Dinamika elektrokardiografskih promena, nivoa moždanog natriuretskog peptida i kortizola kod bolesnika sa stres (takotsubo) kardiomiopatijom

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Abstract

Introduction. Takotsubo cardiomyopathy is a transient acute heart failure syndrome caused by stress that provokes left ventricular mid-apical akinesis and mimics acute coronary syndrome. **Case report.** A 66-year-old woman had chest pain and dispnoea a few hours before hospitalization. A sudden emotional stressful event preceded the symptoms. Electrocardiographic abnormalities – precordial ST elevation and modest increase of cardiac troponin mimiced acute myocardial infarction. However, echocardiographic examination showed apical ballooning with markedly diminished left ventricle ejection fraction and the obstruction in the outflow tract of the left ventricle. Coronary angiography at admission showed no coronary stenosis and slower blood flow through the left anterior descending artery. According to anamnesis, echocardiography and coronarography finding we established the diagnosis of stress cardiomyopathy – takotsubo cardiomyopathy. We described in details the slow but dynamic electrocardiographic changes, levels of brain natriuretic peptide, cortisol and echocardiography evolution of disease during a 4-month follow-up till the full recovery. **Conclusion.** Stress (takotsubo) cardiomyopathy – became an important differential diagnosis of acute anterior myocardial infarction and it should be reconsidered every time when emotionally stressed patients with transient-apical akinesis or dyskinesis of the LV are present.

Key words:
takotsubo cardiomyopathy; risk factors; diagnosis; diagnosis, diferential; treatment outcome.

Apstrakt

Uvod. Takotsubo kardiomiopatija je sindrom prolazne akutne srčane slabosti izazvan stresom koji dovodi do apikalne akinezije leve komore i podražava akutni koronarni sindrom. **Prikaz bolesnika.** U radu je prikazana 66-togodišnja žena sa bolovima u grudnom košu i gušenjem nastalim nekoliko časova pre hospitalizacije. Iznenadni emocionalni stres predhodio je simptomima. Elektrokardiografske promene ST-segmenta (elevacija) i umereni porast kardiospecifičnih enzima podražavali su akutni infarkt miokarda. Ehokardiografija je pokazala apikalno baloniranje sa značajno sniženom ejekcionom frakcijom leve komore i sa opstrukcijom u izlaznom traktu leve komore. Koronarna angiografija učinjena po prijemu pokazala je odsustvo stenoza krvnih sudova srca i usporeni tok krvi kroz levu koronarnu arteriju. U skladu sa anamnezom, ehokardiografijom i nalazom koronarografije postavljena je dijagnoza: stres kardiomiopatija – takotsubo. Detaljno je opisana spora, ali dinamična evolucija bolesti tokom četiri meseca praćenja sve do potpunog oporavka. **Zaključak.** Stres (takotsubo) kardiomiopatija postala je bitna diferencijalna dijagnoza akutnog infarkta prednjeg zida i treba je uzeti u razmatranje kad god imamo bolesnika izloženog jakom emocionalnom stresu sa apikalnim baloniranjem leve komore srca.

Ključne reči:
kardiomiopatija, takotsubo; faktori rizika; dijagnoza; dijagnoza, diferencijalna; lečenje, ishod.

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Introduction

Stress or takotsubo cardiomyopathy (TCM) is a transient, often severe left ventricle dysfunction caused by stress and mimics acute coronary syndrome. It was first described in Japan in 1990 by Sato et al. [1]. Patients often present with chest pain, have ST-segment elevation and/or T-wave inversion on electrocardiogram, and elevated cardiac enzyme levels consistent with a myocardial infarction. Echocardiography examination typically demonstrates dyskinesis and akinesis of the left ventricular mid-apical segments and hyperkinesis of the basal segments with ballooning appearance of the left ventricle (LV). The regional wall motion abnormalities extend beyond a single coronary artery distribution. Coronary angiography in acute phase of the disease does not show any structural abnormalities of the coronary arteries. The name of the disorder is taken from the Japanese name for octopus trap – takotsubo [2], which has a shape similar to the apical ballooning configuration of the LV in systole in the "typical" form of this disorder. The condition is also referred to as stress cardiomyopathy, stress-induced cardiomyopathy, neurogenic myocardial stunning, ampulla cardiomyophaty, broken heart syndrome or apical ballooning syndrome [1, 2]. The exact etiology of TCM is still unknown, but several theories have been proposed and are being investigated. The most commonly discussed possible mechanism for takotsubo cardiomyopathy is stress-induced catecholamine release, with toxicity to and subsequent stunning of the myocardium. Studies have reported that 1.7–2.2% of patients who had suspected acute coronary syndrome were subsequently diagnosed with TCM [3, 4]. Stress-induced cardiomyopathy is much more common in women than men. Nearly 90% of reported cases involve postmenopausal women [5]. In a review of ten prospective series, women accounted for 80 to 100 percent of cases, with a mean age of 61 to 76 years [6]. Acute complications occur in approximately 20% of patients, including cardiogenic shock, heart failure, pulmonary edema, dysarrhythmias, left ventricular thrombus formation, left ventricular free wall rupture, and death. Estimates of mortality rates have ranged from 1 to 3.2% [5, 7]. Prognosis is excellent, with nearly 95% of patients experiencing complete recovery within 4–8 weeks. Recurrence rate varies but is estimated at 3% [8, 9].

The aim of this case report was to describe the dynamics of electrocardiographic and echocardiography findings, and the curve of brain natriuretic peptide (BNP) and cortisol blood levels in a patient with severe stress induced cardiomyopathy.

Case report

A 66-year-old postmenopausal woman was admitted to the Clinic for Emergency Internal Medicine at the Military Medical Academy, Belgrade, because of sudden onset angina like chest pain occurred after severe emotional stress – she had lost her identity card and had a problem with police. The chest pain was localized substernal with irradiation to the left arm end left scapula. She also felt anxiety and nausea with vomiting. Her cardiovascular risk factors were arterial hypertension, dyslipidemia, borderline hyperglycemia and positive family history of coronary artery disease (her mother died from myocardial infarction). Upon admission her blood pressure was 100/70 mmHg, heart rate 115/min and the electrocardiogram (ECG) showed sinus tachycardia with 3 mm ST-segment elevation in all precordial leads and in DI, DII leads. The results of laboratory analysis showed the elevated serum troponin level of 5.7 ng/ml, creatine kinase 261 IU/L, creatine kinase (CK) MB 42 IU/L, complete blood count (CBC) showed only mild leukocytosis and biochemistry results were in normal range. She was, thus, diagnosed with ST-elevation acute myocardial infarction and sent for emergency transtoracic echocardiogram and coronary angiography. A transthoracic echocardiogram revealed regional systolic dysfunction of the LV walls with dyskinesis-akinesis of the mid-apical segments with apical ballooning phenomena and hyperkinesis of the basal segments generating a LV outflow tract obstruction ejection fraction (EF) of 30%, mild mitral regurgitation 3+, normal right ventricular size and function, the absence of pericardial effusion or pulmonary hypertension (Figures 1 and 2). Coronary angiography showed epicardial coronary arteries with no evidence of significant atherosclerotic changes, spasm or thrombosis (Figure 3). In this moment the patient was diagnosed with stress cardiomyophaty – takotsubo. The patient was then transferred to the Coronary Intensive Care Unit for post-procedure continuous monitoring and treated with oral aspirin, clopidogrel, and subcutaneous low molecular weight

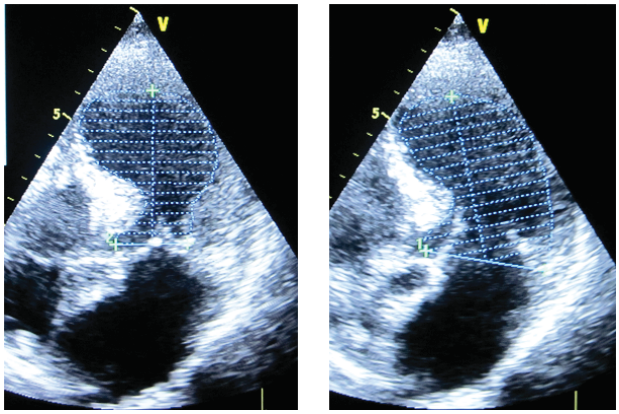


Fig. 1 – Echocardiography performed after admission, a 4-chamber apical view in systole and diastole.

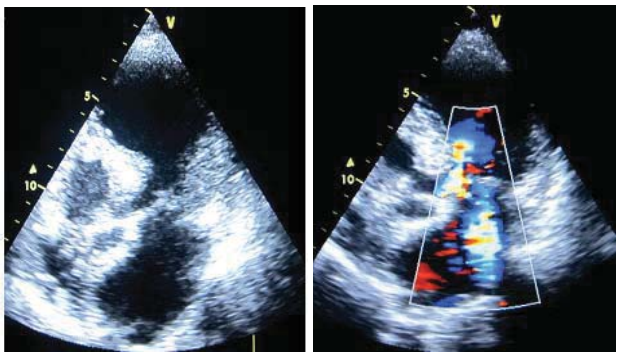
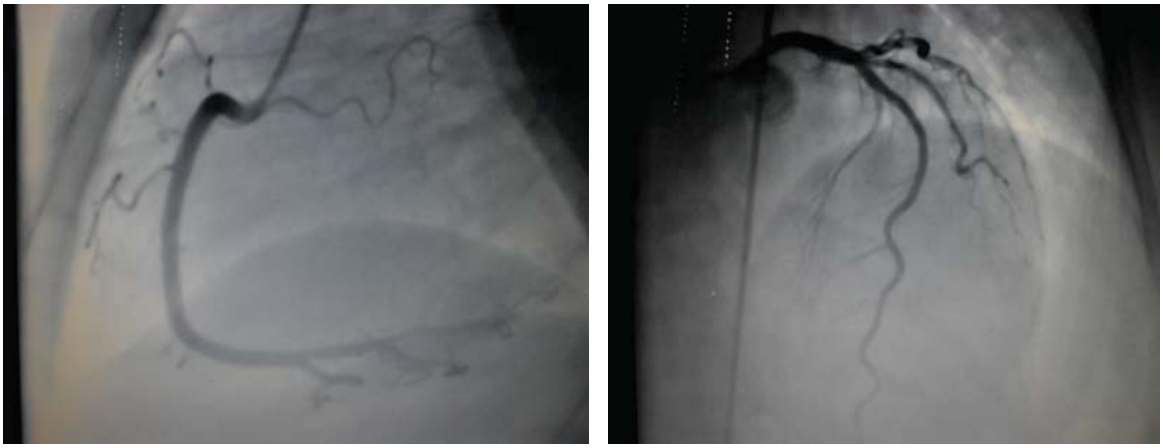


Fig. 2 – Two-dimensional echocardiography in takotsubo cardiomyopathy showing a typical apical ballooning configuration of the left ventricle (LV) in systole, and significant mitral regurgitation.



Right coronary artery (RCA)

Left main coronary artery (LMCA), left anterior descending artery (LAD) and circumflex (ACX)

Fig. 3 – Coronary angiography showing epicardial coronary arteries with no evidence of significant atherosclerotic changes.

heparin. She was also treated with beta blockers and diuretics. Inotropic therapy with medium dosage of dopamine started but caused transient worsening of hemodynamic and was stopped very soon. Twenty four hours after admission troponin level was lower (3.03 ng/ml) and CK achieved the peak of 301 IU/L with the level of CK-MB 28 IU/L. At that time the serum level of BNP was 1209.26 pg/ml (normal range: 0,00–86,10 pg/mL), cortisol level in 08:00 h was 1045,8 nmol/L (normal range 118,6–618,0 nmol/L) and in 16:00 h 1013 pg/L. These results confirmed the existence of severe stress. The rest laboratory analyses were in normal range except increased levels of C-reactive protein (CRP) 22.60 mg/L and fibrinogen 4.51g/L. During the 4-month follow-up, electrocardiography examination was performed very often and the changes of PQRST complex and the extension of the T-wave negativity in the V4 precordial lead were the most prominent changes (Figures 4 and 5). Very deep negative precordial T-waves persisted

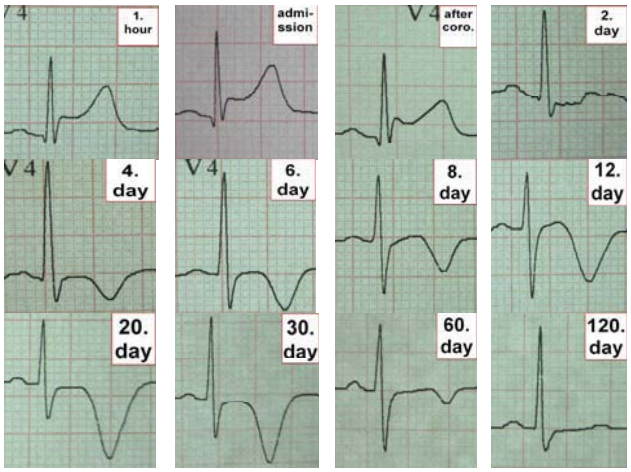


Fig. 4 – Electrocardiography (ECG) changes recorded in the lead V4 from the onset of symptoms to full recovery after 4 months, with the emphasis to changes in T-wave.

for one month with the nadir between 20–30 days after the disease onset. Shallow negative T-waves were present after 4-months. Meticulous echocardiography follow-up was also done. Regional wall motion abnormalities were resolved

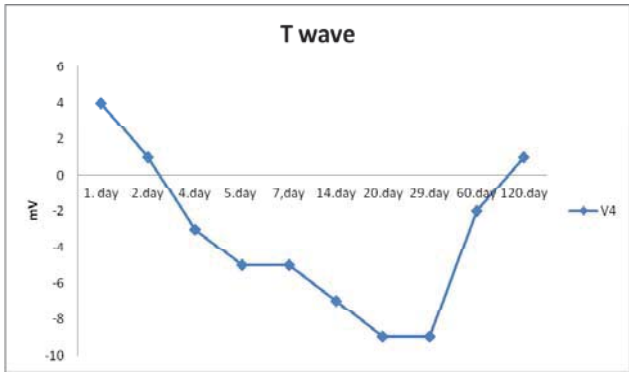


Fig. 5 – T-wave changes from the onset of symptoms until full recovery in the lead V4, showing maximum negativity at the end of the first month and then slow return to isoelectric line in the next 3 months.

slowly with a significant improvement of LVEF after 7 days and the normalization of LVEF after 4 months (Figure 6).

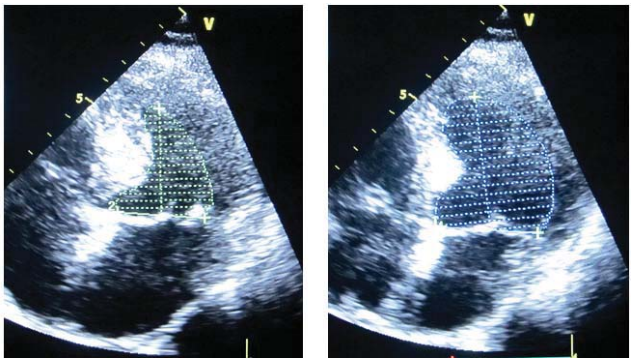


Fig. 6 – Electrocardiography preformed after 4 months, a 4-chamber apical view in systole and diastole, revealing full recovery.

Velocity of the blood flow in the outflow tract of the left ventricle measured by pulse Doppler showed a slow decline from admission to the seventh day when it achieved a plateau (Figure 7).

Mitral regurgitation was very prominent at admission and after 14 days it was trivial (Figure 2). Blood level of BNP was

very high at admission with slow decrease over the next two months when it reached the reference value (Figure 8). Serum cortisol level was elevated at admission with the vanished diurnal variation (it was measured at 8 am and 4 pm for the first several measurements) and it was slowly decreased to the normal values after two weeks (Figure 9).

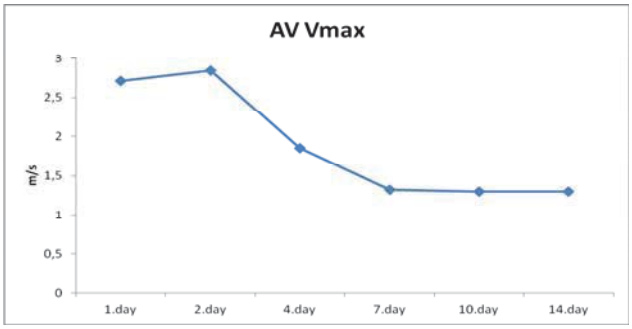


Fig. 7 – Blood flow velocity in the outflow tract of the left ventricle (AV Vmax) measured by pulse Doppler showing slow decline from admission to the 7th day when it achieved a plateau.

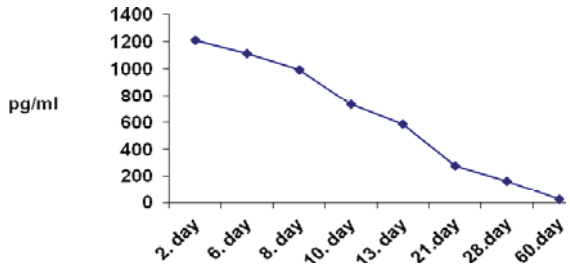


Fig. 8 – Levels of brain natiuretic peptide (BNP) showing a pick value during the first 24 h and slow reduction after, until reaching referent values.

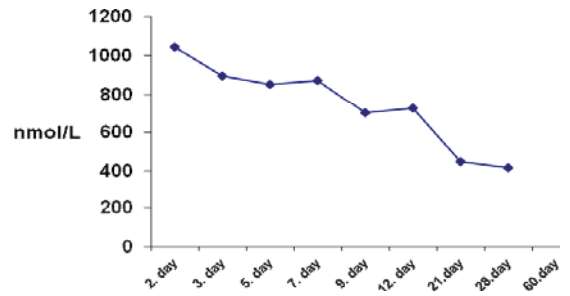


Fig. 9 – Levels of cortisol showing a pick value on the first day and normalization at the end of the first week.

Discussion

The basic aim of this case report was to show dynamic changes of the most prominent electrocardiographic findings, echocardiography parameters, BNP and cortisol levels in a patient with severe stress cardiomyopathy. As far as we know the exact timing of these important parameter changes in takotsubo was not presented. The basic echocardiography measurements were normalized after one month as well as BNP and cortisol levels. ECG changes were persistent for the longer period of time with the most prominent changes at the end of the first months with slow regression through the next 4 months. Most of the published case reports and studies also reported the same dynamics changes in ECG during a follow-up period in TCM. The echocardiography changes that we found in the presented patient are also very similar to other case reports. In our patient the level of BNP was highest in the first 24 hours and then it slowly decreased until the normal range after one month. In the study of Akashi et al. [10], BNP levels in their patients peaked within a first week and then normalized within the next few months. A high serum level of BNP is a marker of poor prognosis [11–13], in our patient very high BNP level was observed in the acute phase when it was hemodinamically compromised with arterial hypotension and significant decrease of global left ventricle EF. Improvement of left ventricular performance and decrease of BNP were parallel during the next month with complete recovery of EF and normalization of BNP. In our patient we measured morning serum cortisol levels as a stress hormone every other day for the first seven days and once weekly for one month and we showed increase of this hormone levels with the peak after 24 h and slow decrease of its level through the next two weeks when it achieved the normal levels. According to the best of our knowledge there is only one study on cortisol levels measured by Madhavan et al. [14], but they compared levels of biomarkers at admission in patients with takotsubo and acute MI, but did not find any difference, and they did not provide dynamics of cortisol levels in patients with stress cardiomyopathy. Thus, more investigations on this point remain to be performed in the future.

Conclusion

Stress (takotsubo) cardiomyopathy is an important differential diagnosis of acute anterior myocardial infarction and it should be reconsidered every time in emotionally stressed patients with transient-apical akinesis or dyskinesis of the LV present themselves. Despite dramatic clinical appearance and significant hemodynamic compromise in a large proportion of patients, the condition has good prognosis. Takotsubo should be discriminated from MI by early coronary angiography. Highly elevated levels of BNP and cortisol could also point out to severe stress and, therefore, to takotsubo. There is no specific treatment for takotsubo, so every patient requires a unique approach.

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CASE REPORT

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Disseminated typical bronchial carcinoid tumor

Diseminovani tipični karcinoidni tumor bronha

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Abstract

Introduction. Bronchial carcinoids belong to a rare type of lung tumors. If they do not expose outstanding neuroendocrine activity, they develop without clearly visible symptoms. They are often detected during a routine examination. According to their clinical pathological features, they are divided into typical and atypical tumors. Typical bronchial carcinoids metastasize to distant organs very rarely. Localized forms are effectively treated by surgery. The methods of conservative treatment should be applied in other cases. **Case report.** We presented a 65-year-old patient with carcinoid lung tumor detected by a routine examination. Additional analysis (chest X-ray, computed tomography of the chest, ultrasound of the abdomen, skeletal scintigraphy, bronhoscopy, histopathological analysis of the bioptate of bronchial tumor, as well as bronchial brushing cytology and immunohistochemical staining performed with markers specific for neuroendocrine tumor) proved a morphologically typical lung carcinoid with dissemination to the liver and skeletal system, which is very rarely found in typical carcinoids. **Conclusion.** The presented case with carcinoid used to be showed morphological and pathohistological characteristics of typical bronchial carcinoid. With its metastasis to the liver and skeletal system it demonstrated unusual clinical course that used to be considered as rare phenomenon. Due to its frequent asymptomatic course and varied manifestation, bronchial carcinoid could be considered as a diagnostic challenge requiring a multidisciplinary approach.

Key words:
carcinoid tumor; lung neoplasms; diagnosis; neoplasm metastasis; liver; skeleton; diagnosis, differential; immunohistochemistry.

Apstrakt

Uvod. Karcinoidi bronha spadaju u retke tumore pluća. Ukoliko nemaju izraženu neuroendokrinu aktivnost, karcinoidi bronha protiču bez jasno uočljivih simptoma. Često se otkrivaju tokom rutinskih ispitivanja. Prema kliničko morfološkim karakteristikama karcinoidi bronha dele se na tipične i atipične. Tipični karcinoidi bronha izuzetno retko daju udaljene metastaze. Lokalizovani oblici se efikasno leče hirurški, a u ostalim slučajevima primenjuju se metode konzervativnog lečenja. **Prikaz bolesnika.** Prikazali smo bolesnike starog 65 godina, kod koga je karcinoid pluća uočen tokom rutinskih ispitivanja. Dodatnim analizama (radiografija pluća, kompjuterizovana tomografija grudnog koša, ultrazvuk abdomena, scintigrafija skeleta, bronhoskopija, patohistološka analiza bioptata bronhijalnog tumora i citologija bronhijlanog brisa, kao i imunohistohemijsko bojenje markerima specifičnim za neuroendokrini tumor) dokazano je da se radilo o morfološki tipičnom karcinodu pluća, sa diseminacijom u jetru i koštani sistem, što se izuzetno retko sreće kod tipičnih karcinoida. **Zaključak.** Prikazani slučaj tumora bronha prema citomorfološkim i patohistološkim karakteristikama odgovarao je tipičnom karcinoidu. Imunohistohemijskim analizama potvrđeno je neuroendokrino poreklo tumora. Ovaj tumor je pokazao izražen metastatski potencijal sa metastazama u jetru i koštani sistem, što se sreće u vrlo malom procentu tipičnih karcinoida. Zbog čestog asimptomatskog toka, kao i neobičnih i raznolikih manifestacija, karcinoidi bronha mogu predstavljati dijagnostički izazov čije rešenje zahteva multidisciplinarni pristup.

Ključne reči:
karcinoid; pluća, neoplazme; dijagnoza; neoplazme, metastaze; jetra; kostur; dijagnoza, diferencijalna; imunohistohemija.

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Introduction

A bronchial carcinoid tumor is a rare neoplasm accounting for 2% of all lung tumors. It belongs to a group of neuroendocrine tumors and arises from cells of the bronchial epithelium. These tumors were earlier classified as benign neoplasms [1–4].

According to the modern conceptions, bronchial carcinoid tumor is considered as malignant neoplasm with neuroendocrine differentiation. It shows low degree of malignancy and its biological nature cannot be precisely assessed only on the basis of its morphological appearance [1, 5–7].

These tumors grow endobronchially in the form of polypoid mass with a smooth surface while at the intersection show a characteristic yellow-brown color with calcification often being present. They often arise in the right lung. In about 68% of cases, they manifest as centrally localized spheral formations sized 0.3–7.5 cm in diameter while in about 30% of all cases they appear as peripheral changes in the form of clearly limited, non-encapsulated nodules [4].

They are classified as central and peripheral, based on their location in lung. Symptoms can vary depending on the location of a tumor. In central tumors symptoms as recurrent pneumonia (41%), cough (35%), hemoptysis (23%) most often occur, while peripheral tumors show significantly fewer symptoms and often develop without any symptoms [4, 8, 9]. Due to this fact, they often develop as undetected and may be revealed during a routine examination [10, 11].

Typical carcinoids are composed of characteristic, uniform population of polygonal cells with fine eosinophil granulocyte cytoplasm and centrally located dark-coloured nucleus. There are rare mitotic figures, no more than two in ten visual fields. The cell can grow as mosaic structures placed around blood vessels, building trabecular and adenopapilar structures with often-present calcifications and amyloid deposits.

Through special, immunohistochemical staining, high positive reaction for chromogranin, synaptophysin and neuron-specific enolase (NSE) is registered which also confirms the carcinoid diagnosis [1, 3, 10, 11].

Unlike typical carcinoids, atypical carcinoids are characterized by nuclear pleomorphism and hyperchromasia, higher degree of cell disorganization, necrosis, as well as by more intensive mitotic activity. Because of their histological characteristics derives greater metastatic potential of atypical carcinoids. In accordance with the above presented facts, there is a difference between clinical courses in these two types of carcinoids. Typical carcinoids rarely develop distant metastases, therefore they have more favorable prognosis. In about 15% of all cases, they metastasize to regional lymph nodes while the metastases to distant organs are very rare and can be registered in about 2% of all patients. A 5-year survival rate for typical carcinoids is 87–100% of all cases. Unlike them, atypical carcinoids in 10% of all cases develop distant metastases while 5-year survival rates in this type of carcinoid approach 35–69% [11–14].

Carcinoids may also arise from other organs. They are often detected at various levels of gastrointestinal tract, yet most commonly in appendix, ileum and rectum. Appendix carcinoids are usually benign and may cause appendicitis. In addition to the above cited characteristics, these tumors can have outstanding neuroendocrine component in cases where, due to the serotonin excess, they exhibit carcinoid syndrome whose main manifestations are present as facial redness, nausea, diarrhea and hypotension [4].

In terms of therapeutic methods, surgical treatment [15] is most effective. In some cases, chemotherapy for non-small cell lung tumors may be applied.

Case report

A 65-year-old man was admitted to the Military Medical Academy (MMA), Belgrade, for medical examination in order to clarify the etiology of infiltrative changes in the lungs and liver, which were accidentally detected during examination, because the patient complained of vague symptoms of discomfort in the area of rib cage and thoracic spine.

The changes in the liver with morphologic features of metastases were detected at initial ultrasound of the abdomen.

Chest X-ray (radiography) showed a change in the left lung, which might be interpreted as tuberculous nodule, nonspecific inflammation, or tumor change.

On the day of admission to our clinic, the patient reported pain in the area of rib cage and thoracic spine. Objective examination below the rib cage revealed an increase of liver size by 3 cm. Physical report on other systems and organs was normal.

Blood and biochemical test results were within the reference ranges.

Control chest X-ray confirmed the existence of an oval shadow 3 × 4 cm in diameter, localized parahilary to the left – on the lower pole of the hilar region (Figure 1).

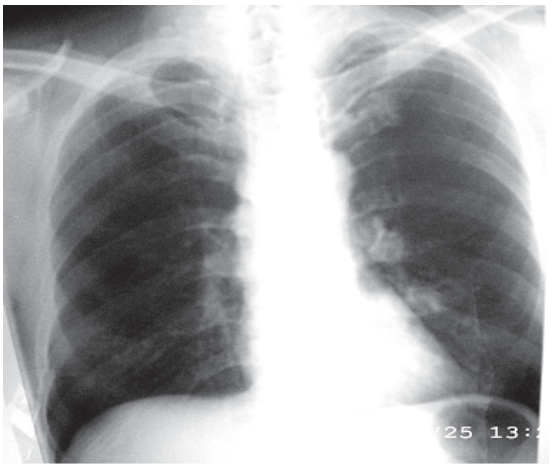

Fig. 1 – Left chest X-ray in the projection of the lower pole of hilar region showing an oval-shaped shaded area of 3 cm × 4 cm.

Computed tomography (CT) scan of the left chest, under the main bronchus, between the hilum and posterobasal segment revealed a solid, oval tumor formation 3 × 4 cm in

diameter (Figure 2). Cross section through the upper abdomen demonstrated a large number of changes in both lobes of the liver that according to their morphological features corresponded to metastatic deposits (Figure 3).

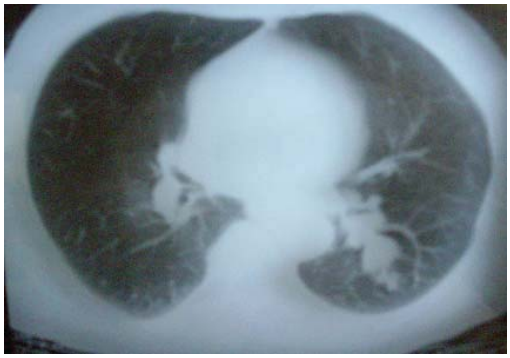

Fig. 2 – Chest computed tomography (CT) scan showing left, a solid, oval tumor formation of 3 × 4 cm, below the main bronchus, between the hilum and the posterobasal segment.

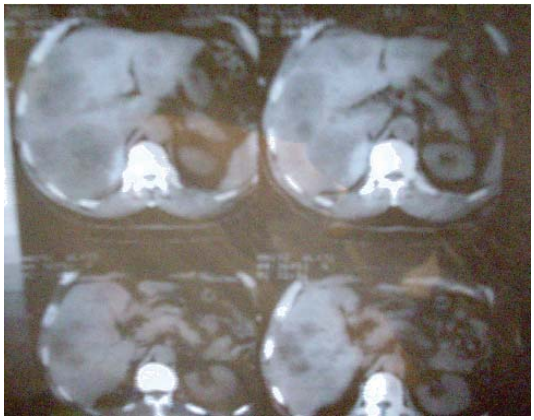

Fig. 3 – In sectional views through the upper abdomen, there are a large number of lesions in both liver lobes, which could, according to their morphological characteristics, represent metastatic deposits.

Ultrasound scan of the abdomen revealed an enlarged liver with several round, solid, diffusely placed lesions. The greatest lesion was 8 cm in diameter with signs of central necrosis (Figure 4). Skeletal scintigraphy showed an increased accumulation of radiotracer in the ribs and in the sternum (Figure 5).

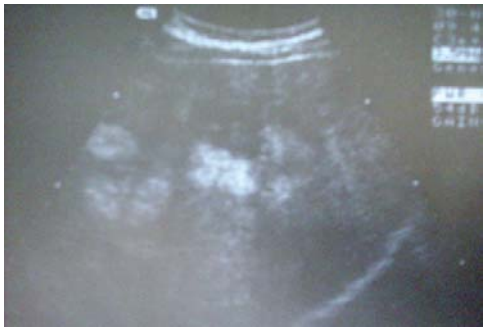

Fig. 4 – Ultrasound examination of the abdomen revealed the enlarged liver with a number of round, solid, diffusely distributed changes (the greatest change was as large as 8 cm, with signs of central necrosis).

Next, bronchoscopy revealed obturation of bronchi LB9 and LB10 by smooth, pink and round tumor change (Figure 6).

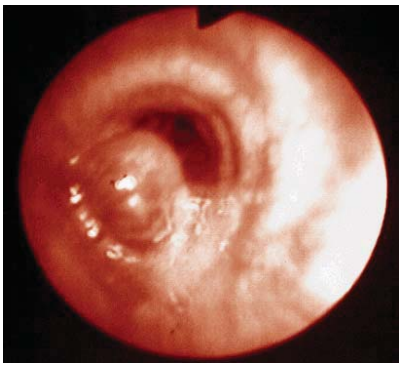

Fig. 6 – Endoscopic finding: an obturation of the bronchi LB9 and LB 10 with a smooth, pinkish, round tumor change.

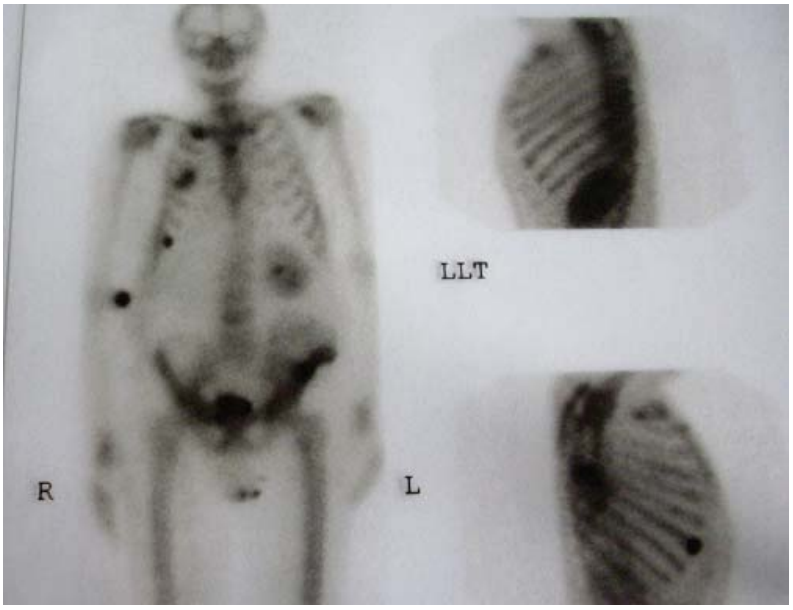

Fig. 5 – Sceletal scintigraphy discovered intensified radiotracer collections in the ribs and the sternal bone.

Histopathological analysis of bronchial tumor change bioptate showed stringy proliferation of uniform, neoplastic cells with nuclei without mitotic figures and visible nucleoli (Figures 7 a and b). Cytological analysis of smear of bronchial brushings detected monomorphic cells with round or oval nuclei, fine chromatin structure, without visible nucleoli and mitotic figures. The nuclei were centrally or eccentrically located in light, basophilic cytoplasm moderate in abundance (Figures 8 a and b). The test results indicated a carcinoid tumor.

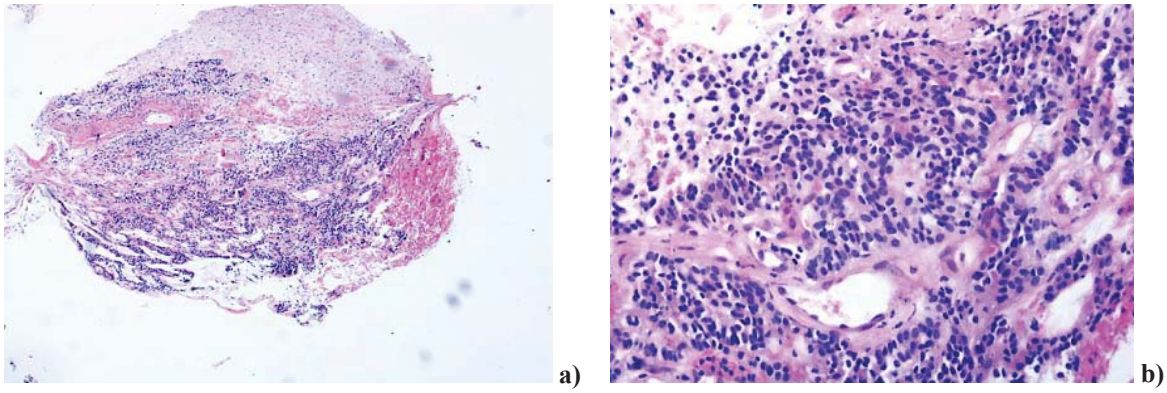

Fig. 7 – Histopathological analysis of the tumor change bioptate: a) stringy proliferation of uniform, neoplastic cells [hematoxylin and eosin (HE), ×200]; b) nuclei without mitotic figures and visible nuclei (HE, ×400).

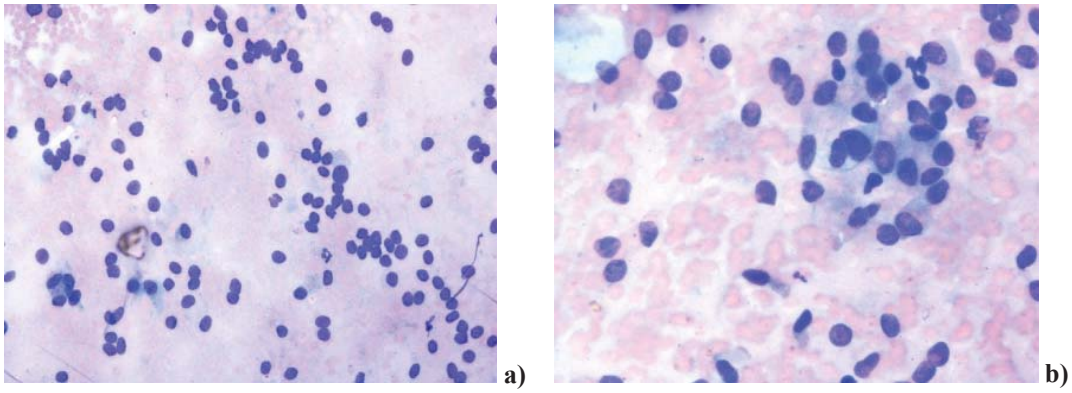

Fig. 8 – Citology analysis of a bronchus swab: a) monomorphic cells with round or oval nuclei of fine chromatin structure, without visible nucleoli and mytotic figures [May-Grünwald-Giemsa (MGG), ×200]; b) nuclei are centrally or eccentrically located in light basophilic and moderate quantity cytoplasms (MGG, ×400).

Immunohistochemical staining performed with markers specific for neuroendocrine tumor demonstrated diffuse a high positivity of tumor cells for chromogranin, neuron specific enolase (NSE), synaptophysin and focal membrane positivity of CD57, which was considered as additional confirmation that it was a carcinoid tumor (Figures 9 a–d).

Cytological analysis of material obtained by a needle biopsy of the liver change showed numerous disperged, separate and grouped tumor cells with fine chromatin structured nuclei, shown as "salt and pepper", without mitotic figures and nucleoli. The parts of capillary loops filled with tumor cells were also found. The obtained results indicated metastatic changes of the bronchial carcinoid (Figures 10 a–d).

Discussion

Bronchial carcinoids are rare neuroendocrine lung neoplasms that are divided into typical and atypical carcinoids according to their pathological and clinical characteristics.

Typical bronchial carcinoids in relation to atypical ones are characterized by increased frequency, milder clinical course and low malignant potential [8, 15, 16].

Typical carcinoids usually appear in the right lung, as solitary endobronchial proliferation localized in the larger airways to the level of the lobar bronchi. They are rarely present as peripheral pulmonary tumors. Most frequently, they metastasize to regional lymph nodes while the occurrence of distant metastases is extremely rare [17].

In our review, bronchial carcinoid showed no typical respiratory symptoms and through additional testing we discovered several unusual features.

The patient was a man aged 65 years, although this type of tumor is more often found in women of 50 years of age [4].

Radiographic and endoscopic examination methods indicated the presence of tumor of the lungs, with present metastatic changes in the liver and bones.

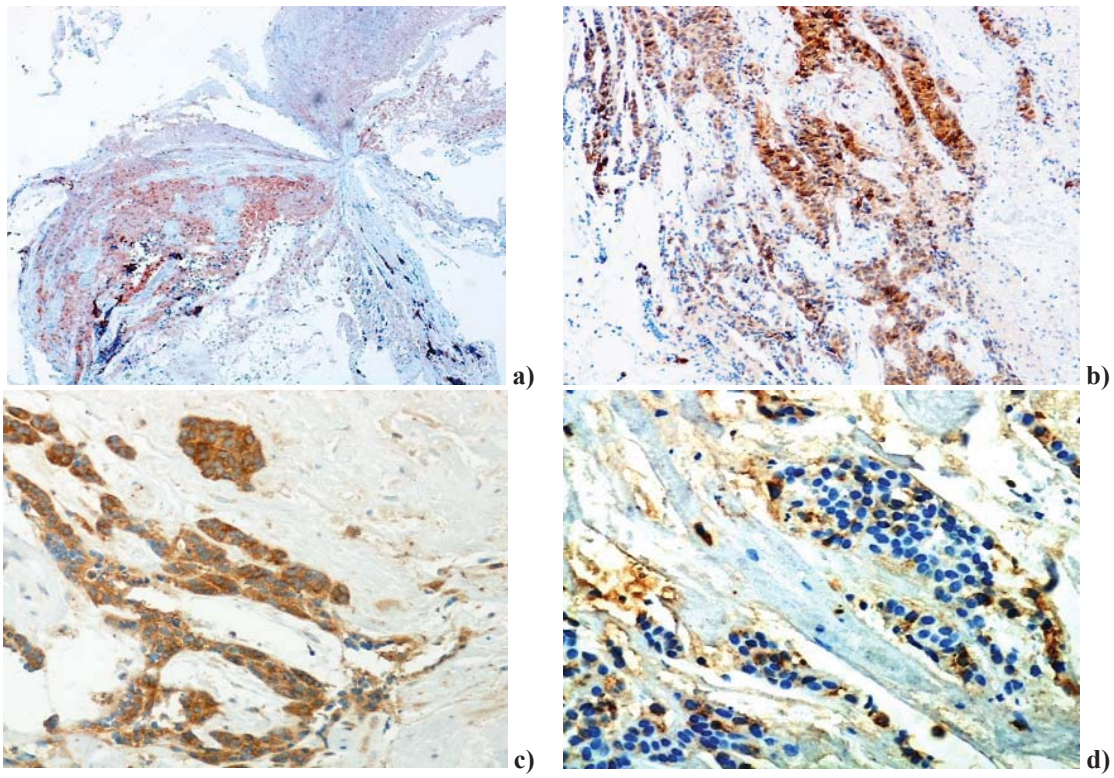

Fig. 9 – Immunohistochemical staining for neuroendocrinal tumor specific markers revealed tumor cells high positive to: a) chromogranin A (×200); b) high positivity of tumor cells for neuron specific enolase (NSE) (×400); c) high positivity of tumor cells to synaptophysin (×400); d) focal membranous positivity of Cd 57(×400).

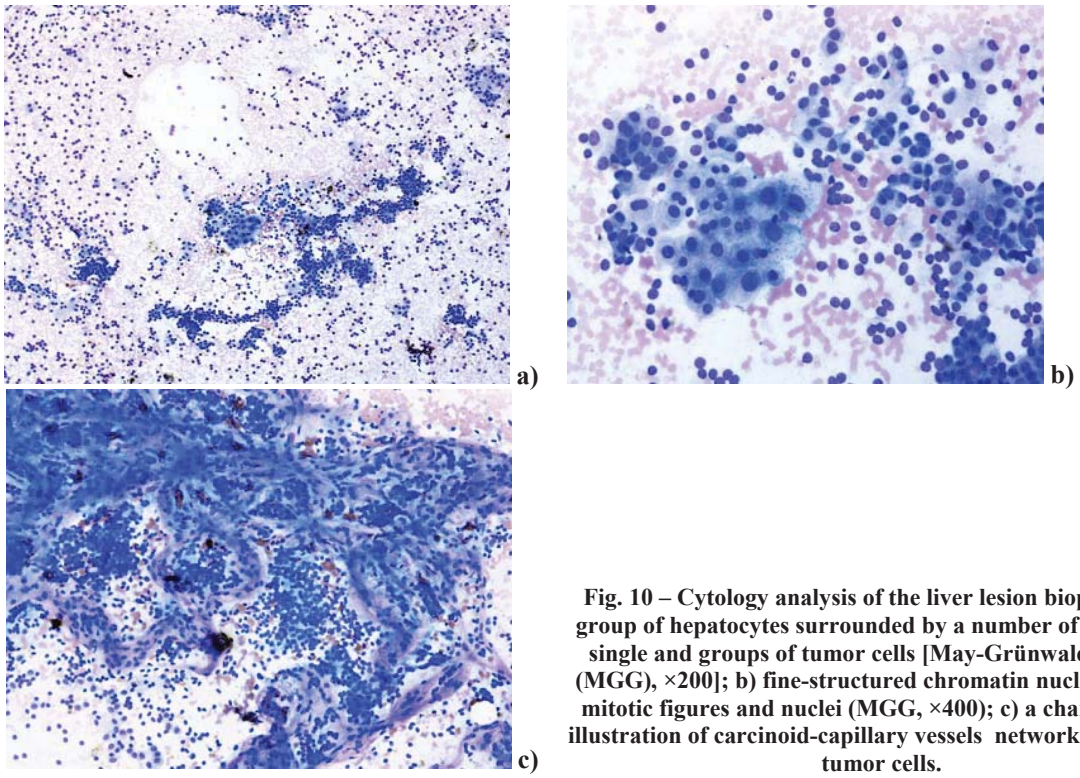

Fig. 10 – Cytology analysis of the liver lesion bioptate: a) a group of hepatocytes surrounded by a number of dispersed single and groups of tumor cells [May-Grünwald-Giemsa (MGG), ×200]; b) fine-structured chromatin nuclei, without mitotic figures and nuclei (MGG, ×400); c) a characteristic illustration of carcinoid-capillary vessels network, filled with tumor cells.

Cytological and histopathological findings suggested a picture of typical carcinoid. It was composed of dispersed cells and bands, trabeculae, papillae and rosettes of uniform, small neoplastic cells with round nuclei and "salt-and-pepper" chromatin without visible nucleoli and mitotic figures.

In liver metastatic change a typical picture of the network of blood vessels with neoplastic cells adhering to its wall was found. With the use of immunocytochemical staining for chromogranin A, NSE, synaptophysin and CD57 the differentiation of neuroendocrine tumor was confirmed.

Despite monomorphism of tumor cells without atypia and mitosis, which classifies the tumor as "low-grade" neuroendocrine neoplasm, it was a disseminated type of typical bronchial carcinoid with metastases in the liver and bones.

However, the appearance of multiple metastases of typical bronchial carcinoid, as described in our work, is very rare. A similar manifestation of this type of carcinoid was described in the work of Suemitsy et al. [18] reporting a case with typical bronchial carcinoid metastasized to the liver.

We presented a rare case of bronchial carcinoid that had cytological and patohistological morphologic features of typical carcinod, while according to its biological behavior and metastatic potential it corresponded to aggressive neoplasms of the lungs, which significantly deviated from the usual picture of typical bronchial carcinoid.

Conclusion

The reported case completely corresponded to a group of typical carcinoids, according to its morphological, cytological and pathohistological features. It differed from typical carcinoids in metastases to the liver and skeletal system that used to be considered extremely rare phenomenon. Due to its asymptomatic course and unusual and diverse manifestations, bronchial carcinoid could present a diagnostic challenge deserving multidisciplinary approach.

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CASE REPORT

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Giant primary retroperitoneal myxoid leiomyoma: a case report

Gigantski primarni retroperitonealni miksoidni lejomiom

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Abstract

Introduction. Leiomyomas are benign smooth muscle tumors that usually arise from the uterus. **Case report.** We present a patient with a 6-month history of vague abdominal discomfort, occasional nausea, vomiting and urinary incontinence. On examination, there was an extremely large firm unpainfull palpable abdominal mass. Laboratory investigation revealed mild leukocytosis and blood creatinine elevation. Abdominopelvic ultrasonography and computed tomography revealed a massive well bordered, encapsulated intraabdominal tumor, extending from the pelvis to epigastrium and almost completely fulfilling the pelvic and abdominal cavity. At laparotomy, tumor arising from the retroperitoneum was excised *in toto*. Histopathological examination disclosed that the tumor was composed mainly of smooth muscle cells and very rare fibrous connective tissue elements with myxomatous alteration and with no mitotic activity. The negative results of numerous additional parameters analyzed (pancytokeratin, epithelial membrane antigen, S100 protein, CD68, CD34, desmin, aktin) ruled out different origin of a tumor. One year after resection the patient had no complaints and no radiological evidence of tumor recurrence. **Conclusion.** Considering current limitations in radiological diagnosis, *in toto* resection of these tumors is necessary to rule out malignancy.

Key words:
leiomyoma; myxoma; retroperitoneal space; digestive system surgical procedures.

Apstrakt

Uvod. Lejomiomi su benigni tumori glatkih mišića koji se najčešće javljaju u uterusu. **Prikaz bolesnika.** U radu smo prikazali bolesnicu sa neodređenim bolovima u trbuhu, povremenim mučninama, povraćanjem i urinarnom inkontinencijom koji su trajali prethodnih šest meseci. Fizikalnim pregledom utvrđeno je prisustvo ekstremno velikog, tvrdog, bezbolnog abdominalnog tumefakta. Laboratorijska ispitivanja otkrila su blagu leukocitozu i povišenu vrednost kreatinina u serumu. Ultrazvučni pregled i kompjuterizovana tomografija abdomena i karlice utvrdili su postojanje masivnog, jasno ograničenog, inkapsuliranog intraabdominalnog tumora koji se prostirao od karlice do epigastrijuma, ispunjavajući skoro u potpunosti karličnu i trbušnu duplju. Nakon laparotomije, tumor poreklom iz retroperitoneuma odstranjen je u celini. Histopatološkom analizom utvrđeno je da se sastojao uglavnom iz glatkomišićnih ćelija i veoma retkih elemenata fibroznog vezivnog tkiva sa miksomatoznom alteracijom i bez mitotske aktivnosti. Negativni rezultati brojnih, dodatno analiziranih parametara (pancitokeratin, epitelijalni membranski antigen, S100 protein, CD68, CD34, dezmin, aktin) isključili su drugačije poreklo tumora. Godinu dana nakon resekcije bolesnica nije imala tegobe niti radiološke pokazatelje recidiva tumora. **Zaključak.** S obzirom na ograničenja radiološke dijagnostike, *in toto* resekcija lejomioma je neophodna da bi se isključio malignitet.

Ključne reči:
leiomiom; miksom; retroperitonealni prostor; hirurgija digestivnog sistema, procedure.

Introduction

Leiomyomas are benign smooth muscle tumors that usually arise from the uterus being the most common benign tumors in women [1]. However, primary leiomyoma of the retroperitoneum without the co-existence of uterine leiomyoma or disseminated disease is very rare [2]. We presented a case of barely symptomatic extremely large primary retroperitoneal leiomyoma (RL).

Case report

A 68-year-old multiparous woman was referred to our department with the 6-month history of vague abdominal discom-

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fort, occasional nausea, vomiting and urinary incontinence. The previous medical history including gynecological was negative. Her general condition and vital signs were normal. On examination, there was an extremely large firm unpainfull palpable abdominal mass with smooth surface, extending from the suprapubic region to the epigastrium and completely fulfilling the abdominal cavity. Laboratory investigation revealed mild leukocytosis (11 000/dL) and blood creatinine elevation (173.0 umol/L). All other parameters including tumor markers (CEA, CA19-9, CA125 and AFP) were normal. Systemic examination, including gynecological, did not reveal any abnormalities. Chest and abdominal radiography examination findings were within normal limits. Abdominopelvic ultrasonography (USG) revealed a large 33 × 28 cm in diameter cystic tumor, located ventrally and fulfilling most of the abdominal cavity with mild hydroureteronephrosis on the right side. Abdominal computed tomography (CT) confirmed USG finding revealing a massive well bordered, encapsulated intraabdominal tumor 40.8 × 36.9 × 19.1 cm in size, extending from the pelvis to epigastrium and almost completely fulfilling the pelvic and abdominal cavity and comprising adjacent organs, mainly the right kidney, aorta, urinary bladder and intestines (Figure 1). There were no signs of ascites and genital pathology.

feeding arteries and hemostasis was achieved without difficulties. The abdomen was closed in layers. The resected tumor weighed 6.3 kg (Figure 2). The postoperative course

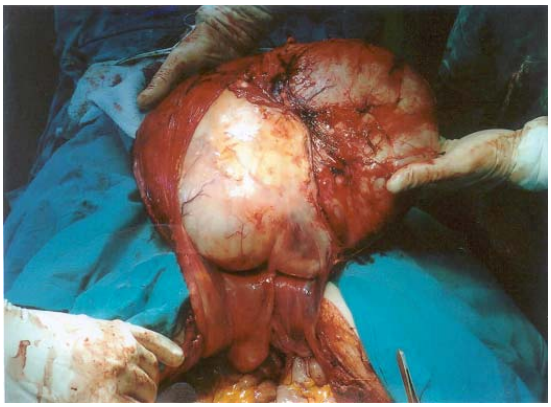

Fig. 2 – Resection and gross appearance of a giant retroperitoneal leiomyoma.

was uneventful. Histopathological examination disclosed that the tumor was RL composed mainly of smooth muscle cells and very rare fibrous connective tissue elements with myxomatous alteration and with no mitotic activity (Figure 3).

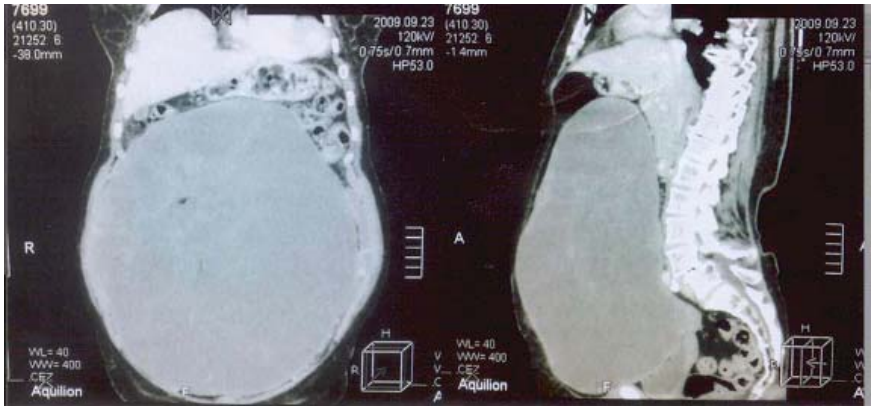

Fig. 1 – Computed tomography (CT) presentation of a giant retroperitoneal leiomyoma.

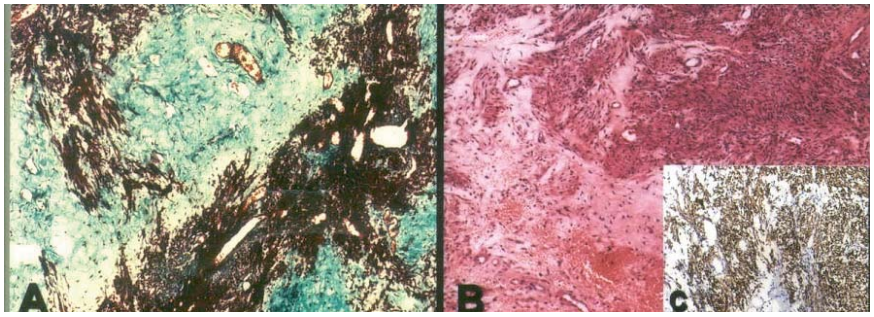

Fig. 3 – Myxoid leiomyoma

[A) Masson′s trichrome stain, 10×; B) Haematoxylin & eosin stain, 10×; C) Desmin stain, 20×].

At laparotomy, a giant encapsulated firm tumor with smooth surface was found, arising from the right retroperitoneal region at the level of paraaortic (interaortocaval) area and extending throughout the whole abdominopelvic cavity. The remainder of the peritoneal cavity was normal. There was no connection of the tumor to the genital tract. The tumor was easily excised *in toto* after ligating the main

The negative results of numerous additional parameters analyzed (pancytokeratin, epithelial membrane antigen, S100 protein, CD68, CD34, desmin, aktin) ruled out a different origin of the tumor. One year after resection the patient had no complaints and no radiological evidence of RL recurrence.

Discussion

Etiology and pathogenesis of leiomyomas are still poorly understood. Since these tumors probably arise from smooth muscle cells, including those in blood vessel walls, they can originate wherever smooth muscle cells exist. The most common site of their location is the uterine corpus during the fourth and fifth decade of life. It has beed estimated that leiomyomas affect 25% of all women during their reproductive life [1]. However, leiomyomas occasionally occur in atypical extrauterine locations like genitourinary tract (vulva, ovaries, urethra, urinary bladder, kidney), lung, rectum etc. [3–5] and may show unusual growth patterns: benign metastasizing leiomyoma, disseminated peritoneal leiomyomatosis, intravenous leiomyomatosis, parasitic leiomyoma etc. [6]. The growth of uterine leiomyomas is most probably hormonally (estrogen) dependent since their frequency is increased after menarche, they enlarge during pregnancy and their regression occur after the menopause [1]. Also, it has been demostrated that a number of different growth factors may be involved in the pathogenesis of leiomyomas: epidermal growth factor, basic fibroblast growth factor, heparin-binding growth factor, transforming growth factor beta, granulocyte-macrophage colony-stimulating factor and insulin-like growth factors [7, 8].

Although there have been reports on various atypical localizations for leiomyomas, their growth in the retroperitoneum is extremly rare. The etiopathogenesis of primary RL is not fully elucidated. It could be related to uterine leiomyomas since more than 40% of patients with RL have a concurrent uterine leiomyoma or a history of hysterectomy for leiomyoma [9]. Zaitoon [10] suggested that large uterine leiomyomas adhere to surrounding structures, acquire an auxiliary blood supply and detache from the uterus – "parasitic" leiomyomas. Also, Stutterecker et al. [11] suggested that RL may arise from embryonal remnants of Müllerian or Wolffian tubes or local blood vessels musculature.

RL may grow very long and become considerably large and still remain asymptomatic. They may be detected incidentally during the examination for other reasons or autopsy [1]. The most frequent clinical feature of these tumors is palpation of abdominal/pelvic mass present in 90% of patients [9]. Rarely, they grow to cause clinically significant symptoms: abdomnal discomfort, fatigue, weight loss and pain radiating to the back. Sometimes, they cause compressive effect on renal collecting system producing hydroureteronephrosis [10], like in the presented case or pressure and displace important retroperitoneal and vascular structures. Since retroperitoneal smooth muscle tumors are more often malignant than benign, prompt and accurate preoperative radiological assessment is necessary. Ultrasonographyc examination provides good initial orientation for retroperitoneal masses. CT and especially magnetic resonance imaging (MRI) are most useful screening tools in evaluating and distinguishing the exact nature of the tumor and its relationship with adjacent organs and vascular structures. However, no radiological diagnostic modality appears highly sensitive or specific in ruling out malignancy and differential diagnosis on the basis of radiological finding alone is difficult [12]. Therefore, the definitive diagnosis of RL requires histopathological examination of the tumor. Sampling of the retroperitoneal mass under USG or CT-guidance preoperatively may allow microscopic examination, although the results may be unreliable due to small histologic specimen. Hence, the final determination of the tumor's nature is to be accomplished with a complete examination of resected specimen. Histologically, the distinction of benign leiomyoma and malignant leiomyosarcoma (especially low grade) may also be difficult. The histopathological parameters used for differential diagnosis include gross tumor size, the presence of nuclear atypia, pleomorphism and necrosis and the mitotic activity as the most useful guide to prognosis [2]. On light microscopy, leiomyoma consists of monomorphic spindle cells arranged in interweaving fascicles which are separated by variable amounts of hyalinized collagen. Smooth muscle cells are elongated with eosinophilic cytoplasm and uniform, cigar-shaped nuclei. Usually, there is no cytologic atypia or necrosis and mitotic index is less than 5/10 in high-power fields. In addition, immunohistochemical staining with estrogen, progesterone receptors, desmin, calponin, h-caldesmon, CD10, CD34, c-kit, ki-67 and p53 may be helpful in differential diagnosis of leiomyoma from leiomyosarcoma[13]. The differential diagnosis of RL includes leiomyosarcomas, nonovarian teratomas, paragangliomas, neurilemmomas-schwannomas, angiomyxomas, hemangiopericytomas, pheochromocytomas, liposarcomas, lymphomas and metastatic tumors [14].

Conclusion

A complete surgical excision is the only curative treatment for retroperitoneal smooth muscle tumors, regardless their benign or malignant nature. Considering current limitations in radiological diagnosis, *in toto* resection of these tumors is necessary to rule out malignancy. However, RL sometimes may be massive, adherent to important adjacent structures and covered with large vessels mimicking malignancy. Therefore, resection of the tumor might be incomplete. Nevertheless, the surgeon should excise the tumor as completely as possible, especially in symptomatic patients. Also, providing a necessary experience, laparoscopic treatment of these tumors is possible.

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CASE REPORT

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Zuclopenthixol decanoate in pregnancy: successful outcomes in two consecutive offsprings of the same mother

Zuklopentiksol dekanoat u trudnoći: uspešan ishod dve uzastopne trudnoće iste majke

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Abstract

Introduction. Almost all individual antipsychotics are classified into the intermediate pregnancy risk category as no or limited data exist about human pregnancy outcomes. We presented the case of zuclopenthixol decanoate using in two successive pregnancies of the same woman, which had not been published in the available peer-reviewed literature. **Case report.** A middle-age female subject who suffered from schizophrenia received zuclopenthixol decanoate injection during her two consecutive pregnancies. About four and a half months before diagnosis of the first pregnancy (~3.5 years after psychosis emergence), zuclopenthixol decanoate (400 mg every other week, *im* injection) was introduced to the treatment protocol (due to previous non-compliance with haloperidol and risperidone). A significant clinical improvement was achieved and the dose during pregnancy was reduced to 200 mg once monthly and maintained to date. In both pregnancies the women gave birth to healthy girls who have been developing normally until now, at their ages of 6 months and of 3.5 years. During pregnancy and after giving birth to children the mothers' psychiatric status and her social functioning were significantly improved and are still stable. Close monitoring of the mother's health, a multidisciplinary approach to both her treatment and the monitoring of pregnancies as well as the complete compliance with the prescribed drug protocol were likely to be crucial for the therapeutic success. **Conclusion.** A favorable outcome of the present case suggests that the zuclopenthixol decanoate is a rational therapeutic option for pregnant women suffering from psychosis when the expected benefit exceed the potential risk, but a definitive evidence for its safety requires large, controlled studies.

Key words:
psychotic disorders; pregnancy; risk factors; psychotropic drugs; treatment outcome.

Apstrakt

Uvod. S obzirom na to da ne postoje ili su podaci o ishodu trudnoće u slučaju njihovog korišćenja oskudni, skoro svi antipsihotici su svrstani u kategoriju sa srednjim rizikom u slučaju trudnoće. Prikazano je korišćenje zuklopentiksol-dekanoata u dve uzastopne trudnoće iste žene, što do sada nije bilo objavljeno u dostupnoj recenziranoj literaturi. **Prikaz bolesnika.** Žena srednjih godina koja je bolovala od šizofrenije primala je injekciju zuklopentiksol-dekanoata tokom svoje dve uzastopne trudnoće. Oko četiri i po meseca pre dijagnoze prve trudnoće (~3,5 godine posle početka psihoze) u terapijski protokol je uveden zuklopentiksol-dekanoat (400 mg svake druge nedelje, *i.m.* injekcija) zbog prethodnog neredovnog uzimanja haloperidola i risperidona. Postignuto je značajno kliničko poboljšanje, pa je doza leka tokom trudnoća snižena na 200 mg jednom mesečno i održavana do sada. U obe trudnoće rođena je po jedna zdrava devojčica i normalno su se razvijale do uzrasta od šest meseci, odnosno 3,5 godine. Tokom trudnoća i posle rođenja dece psihijatrijski status i socijalno funkcionisanje majke bili su značajno poboljšani i do danas stabilni. Pomno praćenje zdravstvenog stanja majke, multidisciplinarni pristup njenom lečenju i praćenju trudnoća kao i potpuna komplijansa sa propisanim medikamentnim protokolom najverovatnije su bili presudni za terapijski uspeh. **Zaključak.** Povoljni ishodi prikazane bolesnice ukazuju da je kod trudnica obolelih od psihoze zuklopentiksol-dekanoat racionalna terapijska opcija, kada očekivana korist prevazilazi potencijalne rizik, ali su za definitivan dokaz o njegovoj bezbednosti potrebne kontrolisane studije sa velikim uzorkom.

Ključne reči:
psihotički poremećaji; trudnoća; faktori rizika; psihotropni lekovi; lečenje, ishod.

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Introduction

Pregnancy in a schizophrenic woman represents a considerable therapeutic challenge as there is a high risk of adverse outcomes [1]. Apart from obstetric complications, the changes in pharmacokinetics and drug response in pregnancy [2] and possible harmful effects of pharmaceuticals on developing fetus [3] could also make using antipsychotic medication difficult. Although clinical experience with the use of antipsychotic drugs in pregnant women is mostly encouraging [4, 5] almost all individual antipsychotics are classified into the intermediate pregnancy risk category as no or limited data exist about human pregnancy outcomes [6]. Zuclopenthixol belongs to an older, thioxanthene drugs but some professionals prefer it for agitated or aggressive patients [7]. Besides, a long-acting parenteral formulation of decanoate ester (depot) is still widely marketed, including many European countries [8]. Zuclopenthixol decanoate, having lower acquisition price in comparison with depot formulations of novel antipsychotics [9], might be convenient choice for decreasing frequency of relapses [10] and when shortages of the older depot antipsychotics happen [11]. However, we were unable to locate the published data dealing with both the use of zuclopenthixol during human or animal pregnancy and the consequent fetal outcomes.

Case report

A 35-old woman, suffered from schizophrenia (F20, ICD-10 code) from November 2003. The disease started with symptoms of disorganized behavior, delusions of control, tactile hallucinations, flat affect and social withdrawal. The patient was hospitalized and psychiatrist prescribed haloperidol, initially with 15 mg per day and then in maintenance daily dose of 10 mg. After four weeks the patient continued ambulatory treatment with the same drug but gradually reduced to 7.5 mg per day. The patient's condition significantly improved and the patient returned to her usual activities. However, in 2005, the second exacerbation appeared, almost immediately after the self-discontinuation of haloperidol due to the perceived adverse effects of the medication (rigidity, bradykinesia and "mind disturbances"). The patient's condition stabilized with risperidone, 4 mg daily, but after a 10-month treatment the patient went abroad, and presented to the psychiatrist again in February 2007 with another exacerbation characterized with delusions of control and persecution, psychomotor agitation and tangential thinking. The patient's status was rated "markedly ill", according to the Clinical Global Impression-Severity Scale (CGI-S).

Six months before the episode, the patient discontinued risperidone on her own, due to amenorrhea perceived by the patient to be "drug-induced". She refused the continuation of treatment with any drugs and intramuscular (*im*) injections of zuclopenthixol acetate and, then, decanoate was prescribed due to the expected compliance problems. The dose of zuclopenthixol acetate was 50 mg every third day, up to 150 mg and then long-acting, *im* depot injection of zuclopenthixol decanoate, 400 mg every two weeks. The psychiatric status significantly improved and rated as "borderline mentally ill" due to minimal residual symptoms (blunted affect, discrete suspiciousness).

The first unintentional pregnancy was diagnosed in the 13th week of gestation, about four and a half months after zuclopenthixol treatment initiation. The team consisting of a psychiatrists, clinical pharmacologists and gynecologists considered the case to be at high risk and took closely further care of both mother and fetus. They explained the risk of using zuclopenthixol during pregnancy to the patient and she freely decided to continue both the pregnancy and the drug treatment. The next drug dose was reduced to 200 mg administered in monthly intervals. The history of fluctuating disease course, noncompliance and frequent exacerbations favored the treatment decision. The same dose continued to be administered throughout further pregnancy.

In 2010 the patient conceived for the second time but again unintentionally and presented to the team from the 12th gestational week. Since the first delivery, the mother continuously received zuclopenthixol decanoate *im* injection, and she was stabilized on 200 mg monthly dose. The scenario from the first pregnancy repeated as both the pregnancy and the antipsychotic were continued.

Hospital pediatricians as well as primary care staff (pediatrician, general practitioner, and nurse) jointed during the end of pregnancies and postnatal periods. Both babies were healthy, mature girls at deliveries (Table 1). The first one was born in January 2008, and the second one in January 2011, without obvious congenital malformations. The brain ultrasound finding in the younger sister was normal and in the first child revealed some clinically-insignificant periven-

Table 1

The clinical and laboratory findings in both babies at deliveries

Variable	The first child	The second child
Gestation, delivery (weeks)	39th	40th
Apgar score (points)	9	9
Body weight, delivery (g)	3,750	3,700
Body weight, discharge (g)	3,650	3,530
Body height (cm)	55	53
Head circumference (cm)	35	35
Thoracic circumference (cm)	35	33
Amniotic fluid	clear	clear
Umbilical cord	normal	normal
Glycemia (mmol/L)	3.2	2.0 and 2.4
C-reactive protein (mg/L)	6.1	not done
Coombs' test	positive	negative

tricular hyperechogenicity. In both girls blood counts were normal and blood groups were ”0“ with positive Rh factor. The babies received vitamin K, BCG and hepatitis B vaccines. Breastfeeding was avoided and they were fed with the artificial alimentary formulas. At discharge, both children had normal general status, skeletal muscle tonus and reflexes as well as respiratory, cardiovascular and gastrointestinal functions. Skin was normal in the older girl and in the younger sister was slightly icteric but otherwise normal. Umbilical cords were dry, but still present, in both cases. The mother recovered uneventfully after both pregnancies.

During the summer 2011 we conducted the follow-up. We first visited the primary care department, discussed with pediatricians in charge of both children and reviewed their medical records. In general, the children were healthy with normal psychomotor developments, somatic, neurological and nutritive status and regularly vaccinated. The younger child had no somatic disease. The older girl experienced several transitory respiratory infections (pharyngitis, bronchitis, laryngitis), and a couple of episodes of mild-to-moderate diarrhea.

The mother’s psychiatric status during both pregnancy, after each delivery and during the follow up period was also favorable (continued to be rated as “borderline mentally ill”), with no exacerbations, receiving regularly 200 mg of zuclopenthixol decanoate *im* injections monthly. She experienced a moderate stressor episode (threat of a possible job loss) without obvious consequences. The mother’s social relationships were very well and pretty stable.

Discussion

Our paper probably represents the first case report on zuclopenthixol decanoate use during entire pregnancy in schizophrenic patients, particularly considering successive events in the same mother. Depot preparations of antipsychotics are rarely prescribed in pregnancy. The case of long-acting risperidone use with a successful outcome was described [12], but a newborn girl, whose mother had received perphenazine decanoate during the second and the third trimester, experienced postnatal extrapyramidal symptoms [13]. Therefore, possible benefits of antipsychotic treatment in pregnant women such as improvement of psychiatric status and social functioning must be carefully balanced against the potential risk from adverse pharmacological effects on the fetus.

It seems that the use of antipsychotics in pregnancy, in general, bears no or little additional fetal risk for congenital malformations. However, many consider the safety of this pharmacological class in pregnancy to be still an unresolved issue. The insufficient amount of sound, evidence-based data, possibility of immediate drug effects after the birth and concern about long-term disturbances of behavioral development make the authorities in the field still vigilant [4].

We counseled the mother to avoid breastfeeding during zuclopenthixol treatment. Some professionals considered that the risk from single antipsychotic agent was less than potential benefits from breastfeeding [5]. Indeed, antipsychotics, in general, enter the mother’s milk with low concentrations but they may have a long half-life and active metabolites. Due to the possibility of entering infant’s brain in measurable amounts and the insufficient data about neurodevelopmental, the delayed the effects American Academy of Pediatrics classified psychotropics, including antipsychotic agents, as “drugs for which the effect on nursing infants is unknown but may be of concern” [14].

The outcomes of the presented pregnancies suggest that the judicious prescribing of antipsychotics in pregnant psychiatric patients could be a safe and effective treatment option. It seems that assuring full drug compliance [9] and close supervision of health status with multidisciplinary approach [15] were crucial for the therapeutic success in our cases. However, the case reports bear many methodological limitations and we were unable to collect some valuable additional data such as zuclopenthixol serum concentrations and the insight into possible hidden malformations of children.

There are no studies in the field with a sufficiently large sample and, therefore, zuclopenthixol and probably any other antipsychotic should be used only if the expected benefits outweigh possible risks. Medical community still waits for the results of incentives based on the widest and the longest possible follow-up of drug use in pregnant women with mental disorders [16]. Until they appear we believe that our paper presents a fair and useful piece of evidence.

Conclusion

The use of zuclopenthixol decanoate in the presented case resulted in therapeutic success for the mother without adverse effects for the children. We need larger studies with the refined research designs in order to confirm our results.

Conflicts of interest and the source of funding

There is no relevant conflict of interest. Dragan Milovanović acknowledges to the Ministry of Education, Science and Technological Development of the Republic of Serbia, partially supporting his scientific work through the research grant No. 175014.

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Poziv za reklamiranje u 2013. godini

U prilici smo da vam ponudimo mogućnost oglašavanja i reklamiranja proizvoda i usluga u časopisu „**Vojnosanitetski pregled**“ (VSP). To je sigurno najbolji vid i najzastupljeniji način upoznavanja eventualnih korisnika sa vašim uslugama i proizvodima.

Časopis „**Vojnosanitetski pregled**“, zvanični organ lekara i farmaceuta Vojske Srbije, naučno-stručnog je karaktera i objavljuje radove iz svih oblasti medicine, stomatologije i farmacije. Radove ravnopravno objavljuju stručnjaci iz vojnih i civilnih ustanova i iz inostranstva. Štampa se na srpskom i engleskom jeziku. Časopis izlazi neprekidno od 1944. godine do sada. Jedini je časopis u zemlji koji izlazi mesečno (12 brojeva), na oko 100 strana A4 formata, a povremeno se objavljuju i tematski dodaci (suplementi). Putem razmene ili pretplate VSP se šalje u 23 zemlje sveta. Radove objavljene u VSP-u indeksiraju: *Science Citation Index Expanded (SCIE)*, *Journal Citation Reports/Science Edition*, *Index Medicus (Medline)*, *Excerpta Medica (EMBASE), EBSCO* (preko ove baze VSP je *on line* dostupan od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

Cene reklama i oglasa u časopisu „Vojnosanitetski pregled“ u 2012. godini su:

1.	Oglas u crno-beloj tehnici A4 formata za jedan broj	20 000,00 dinara
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8.	Oglas u boji na koricama K2 i K4 za celu godinu (11-12 brojeva)	530 000,00 dinara

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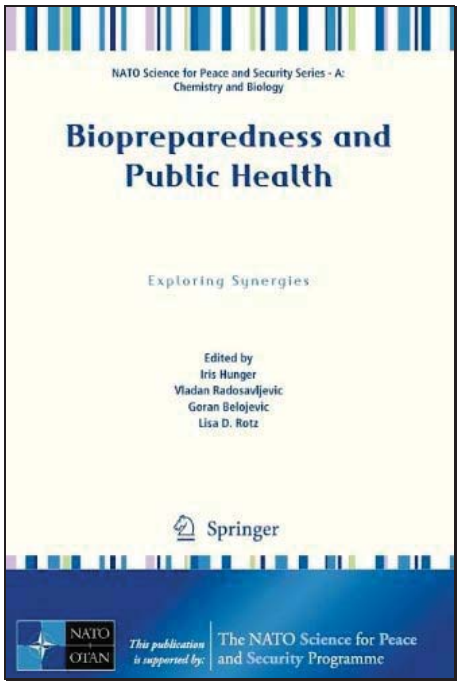
BOOK REVIEW

Biopreparedness and Public Health, Exploring Synergies



Editors: **Iris Hunger, Vladan Radosavljević, Goran Belojević and Lisa Rotz**
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"Biopreparedness and Public Health, Exploring Synergies" edited by Iris Hunger, Vladan Radosavljevic, Goran Belojevic and Lisa Rotz is an up-to-date international monography of highest interest for public health professionals, military experts and wider citizenry. This valuable book unites under the umbrella of a respectable publisher Springer four editors and 31 authors from 11 countries (Republic of Serbia, Germany, United States, Turkey, Israel, Greece, France, Italy, Bulgaria, Poland, and Romania) employed by the institutions which will have a crucial role in the case of a threat for international and national security, such as World Health Organization – WHO; Center for Disease Control and Prevention – CDC, Atlanta, United States of America; European Center for Disease Prevention and Control; Military Medical Academy, University of Defense in Belgrade; Carl Friedrich von Weizsacker Center for Science and Peace Research, University of Hamburg; Center for Biosecurity, University of Pittsburgh, Foundation pour la recherché strategique in France, Center for Biological Security at Robert Koch Institute in Berlin, Germany; Hellenic Army, Emergency and Disaster Management Division and Department for Emergency Medicine; Faculty of Health Sciences of Ben-Gurion University of the Negev in Beer-Sheva, Israel; Italian Army Logistic Branch in Rome, and Clinical Microbiology Laboratory of the Faculty of Medicine and Surgery at "Luigi Sacco" University Hospital in Milan; Military Institute of Hygiene and Epidemiology in Warsaw, Poland; Microbiology at the University of Medicine and Pharmacy "Carol Davila" and National Institute for Infectious Diseases "Prof. Dr. Matei Bals" in Bucharest, Romania; Institute of Hygiene and Medical Ecology at the Faculty of Medicine University of Belgrade.

This book is published in cooperation with the North Atlantic Treaty Organization (NATO) Emerging Security Challenges. That indicates that the authors and the editors jointed efforts to increase our awareness and to deepen our knowledge on biopreparedness that is recognized by an intergovernmental military alliance of 28 member states across Europe and United States. All chapters are written in English, making them accessible to a wider readership. The editors of the book had a challenge not only to coordinate a work of 31 authors from 11 countries, but also to build a network of the most prominent experts in the field of biopreparedness. In case of a real treat this network could be crucial for a prompt organized joint effort across the globe.

The development and outcome of naturally occurring infectious diseases is to a certain degree foreseeable and

easier to control. However, bioterrorist attacks are rare but unpredictable and they have the potential to be mass causality events. The main aim of this book is to describe and compare the relationship between the general public health measures and the measures to prepare for the unlikely but potentially catastrophic event of a bioterrorist attack.

The book is composed of two parts. In part one, after the chapter on the current bioweapons threat, the chapter signed by Vladan Radosavljevic describes a new method for differentiation between a biologic attack and other epidemics. The next two chapters deal with the difference in responding to natural and unnatural outbreaks and managing acute public health events from WHO perspective. Particularly important is the chapter about the recent reforms of public health systems in the countries of the South-Eastern Europe written by Doris Nitzan Kaluski, ex-Head of WHO Country Office in Serbia and Maria Ruseva from WHO Regional Office for Europe. The last chapter of the first part of the book presents the examples of the imminent events, identified through the epidemic intelligence activity in order to illustrate early detection of disease outbreaks and risk management in Europe.

The second half of the book is composed of case report from individual countries with a focus on the countries from the South Eastern Europe. The authors of cases from their countries answer to the questions about the main public health threats in their country, organization of preparedness and response to health emergencies, and the role that the military and civilian agencies play in preparedness and response to natural and human-made health emergencies.

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c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen i mesta u kojima se ustanove nalaze, sa jasnim obeležavanjem odakle je autor, koristeći standardne znake za fus-note.

2. ***Apstrakt i ključne reči***

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.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost etičkog komiteta.

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

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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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