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**Adresa redakcije**: Vojnomedicinska akademija, Institut za naučne informacije, Crnotravska 17, poštanski fah 33–55, 11040 Beograd, Srbija. Telefoni: glavni i odgovorni urednik 3609 311, glavni menadžer časopisa 3609 479, pretplata 3608 997. Faks 2669 689. E-mail (redakcija): **vsp@vma.mod.gov.rs**

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# CONTENTS / SADRŽAJ


Since 1992 the International Day of Disabled Persons has been marked on December 3 with aims to promote an understanding of disability issues and mobilize support for the dignity, rights and well-being of persons with disabilities. It also seeks to increase awareness of the need and gains to be derived from the integration of persons with disabilities in every aspect of political, social, economic and cultural life.
The theme of this year's commemoration is: "Sustainable Development: The Promise of Technology" emphasizing the power of technology to achieve the full and equal participation of persons with disabilities in society and shape the future of sustainable development for all.

Od 1992. godine, 3. decembra svake godine obeležava se Međunarodni dan osoba sa posebnim potrebama, sa ciljem isticanja njihovih problema i potreba i obezbeđenja podrške za ostvarenje njihovih prava, dostojanstva i dobrobiti. Takođe, cilj je podizanje svesti o neophodnosti i društvenoj koristi od integracije osoba sa posebnim potrebama u sve aspekte političkog, društvenog, privrednog i kulturnog života.
Tema ovogodišnjeg obeležavanja Međunarodnog dana osoba sa posebnim potrebama jeste: „Održivi razvoj: obećanje tehnologije". Akcent je stavljen na mogućnosti korišćenja tehnoloških dostignuća u ostvarivanju punog i ravnopravnog uključenja osoba sa posebnim potrebama u sve društvene tokove i oblikovanje budućnosti održivog razvoja za sve.

Poštovani autori, urednici, recenzenti i čitaoci Vojnosanitetskog pregleda,

Opraštajući se od 2014. godine u kojoj smo proslavili 70. rođendan našeg časopisa, zahvaljujem vam na plodnoj saradnji i podršci uz želje da nam nastupajuća 2015. godina bude u svemu još bolja i uspešnija!

SREĆNA NOVA GODINA I BOŽIĆNI PRAZNICI!

Srdačno,
prof. dr Silva Dobrić


Dear Authors, Editors, Peer Reviewers, Readers of the *Vojnosanitetski pregled*,

On saying farewell to 2014 in which we celebrated the 70th anniversary of our Journal, I thank you for our fruitful cooperation and support with my best wishes that the coming New Year 2015 be even better and more successful at everything!

MERRY CHRISTMAS AND A HAPPY NEW YEAR!

Cordially,
Prof. Dr. Silva Dobrić
Editor-in-Chief

**VOJNOSANITETSKI PREGLED**
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd, Srbija**
Tel/faks: +381 11 2669689
vsp@vma.mod.gov.rs
vmavsp@hotmail.com

## Poziv za reklamiranje u 2015. 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 dostupan *on line* od 2002. godine u *pdf* formatu) i *Biomedicina Serbica*.

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# Comparison of efficacy of local hemostatic modalities in anticoagulated patients undergoing tooth extractions

## Poređenje efikasnosti različitih metoda lokalne hemostaze kod pacijenata na oralnoj antikoagulantnoj terapiji posle ekstrakcije zuba

**Branislav V. Bajkin, Srećko D. Selaković, Siniša M. Mirković, Ivan N. Šarčev, Ana J. Tadić, Bojana R. Milekić**

**Dental Clinic of Vojvodina, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia**

**Abstract**

**Background/Aim.** Patients receiving long-term oral anticoagulant therapy pose a clinical challenge during invasive dental procedures. The goal of this study was to compare different local hemostatic modalities after tooth extraction in patients receiving chronic Vitamin-K antagonist therapy. **Methods.** Totally 90 patients with International Normalized Ratio (INR) ≤ 3.0 requiring simple extraction of one or two teeth were randomized into three groups, 30 patients in each group. The patients with the mean INR value of 2.35 ± 0.37, in whom extraction wound was sutured comprised the group A. In the group B with the mean INR of 2.43 ± 0.4, local hemostasis was achieved by placing absorbable gelatin sponges into the wound without suturing. The group C consisted of the patients with the mean INR of 2.36 ± 0.34 in whom neither gelatin sponge nor suturing were used for providing local hemostasis. Bleeding was registered as an event if other than initial hemostatic measure was needed or additional oral surgeon intervention required. **Results.** The obtainded results show that 1 (3.3%) patient in the group A, 2 (6.7%) patients in the groups B and C manifested post-extraction bleeding. All cases of hemorrhage were easily solved with local hemostatic measures and all, except one case, were registered in the first two hours after the procedure until the dismissal. A difference between the groups was not statistically significant ($\chi^2 = .42$, $p > 0.05$). **Conclusion.** In therapeutically anticoagulated patients tooth extractions can be safely performed without altering the dose of anticoagulant medication if efficient local hemostasis is provided. In most cases, in patients with INR ≤ 3.0 after extraction of one or two teeth postoperative bleeding can be controlled with local pressure, without any additional local hemostatic measures.

**Key words:**
**tooth extraction; antiacoagulants; administration, oral; drug therapy; hemostasis.**

**Apstrakt**

**Uvod/Cilj.** Pacijenti koji duže vremena primaju antikoagulantnu terapiju predstavljaju poseban izazov prilikom invazivnih dentalnih procedura. Cilj rada bio je da se uporede različite metode lokalne hemostaze kod pacijenata na oralnoj antikoagulatnoj terapiji nakon ekstrakcije zuba. **Metode.** Ukupno 90 pacijenata na oralnoj antikoagulantnoj terapiji sa *International Normalized Ratio* (INR) vrednostima ≤ 3,0 kojima je bila potrebna ekstrakcija jednog ili dva zuba, bilo je podeljeno u tri grupe, po 30 ispitanika u svakoj grupi, zavisno od primenjenog metoda lokalne hemostaze. Grupu A činili su pacijenti čija je ekstrakciona rana ušivena, a zabeležena prosečna INR vrednost u ovoj grupi iznosila je 2,35 ± 0,37. U grupi B za postizanje lokalne hemostaze u ekstrakcionu ranu su postavljani resorptivni želatinski sunđeri, bez ušivanja. Prosečna INR vrednost u ovoj grupi iznosila je 2,43 ± 0,4. U grupi C (pacijenti sa prosečnom INR vrednošću 2,36 ± 0,34) posle ekstrakcije zuba nisu korišteni ni lokalni hemostatici, niti je rana ušivana. Produženo postekstrakcijsko krvarenje definisano je kao događaj u slučaju da početne mere hemostaze nisu bile dovoljne ili je bila potrebna dodatna oralnohirurška intervencija. **Rezultati.** Kod 1 (3,3%) pacijenta u grupi A i po 2 (6,7%) pacijenta u grupi B i grupi C javilo se produženo postekstrakcijsko krvarenje. Nije utvrđeno postojanje statistički značajne razlike u učestalosti krvarenja po grupama ($\chi^2 = .42$; $p > 0.05$). Svi pacijenti sa produženim krvarenjem bez poteškoća zbrinuti su merama lokalne hemostaze i svi su, izuzev jednog, registrovani u prva dva časa nakon intervencije. **Zaključak.** Kod pacijenata na oralnoj antikoagulantnoj terapiji moguće je bezbedno izvršiti ekstrakciju zuba bez izmene terapijskog režima uz primenu adekvatnih mera lokalne hemostaze. Kod pacijenata sa INR ≤ 3,0 nakon jednostavnih ekstrakcija zuba kompresija rane gazom u većini slučajeva dovoljna je mera za postizanje lokalne hemostaze.

**Ključne reči:**
**zub, ekstrakcija; antikoagulansi; oralna primena; lečenje lekovima; hemostaza.**

**Correspondence to:** Branislav V. Bajkin, Dental Clinic of Vojvodina, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 12, 21 000 Novi Sad, Serbia. Phone: +381 62770665; fax: +381 21526120. E-mail: bajkinb@eunet.rs

## Introduction

Patients receiving long-term oral anticoagulant therapy (OAT) pose a clinical challenge during invasive dental procedures. The important question is whether to continue, modify or interrupt OAT before dental treatment. Cessation or reduction of anticoagulant intake for several days prior to dental procedure may expose these patients to the risk of thromboembolism [1], especially in high risk patients, i.e. those with artificial heart valves and atrial fibrillation with related risk factors. An increasing number of authors state that tooth extraction in anticoagulated patients within the therapeutic International Normalized Ratio (INR) values (INR $\leq$ 4.0) can be safely done without changing OAT regimen if using local hemostatic measures which stabilize or enhance clot formation at the surgical site [2–19]. Hemostatic agents used for bleeding control in patients on OAT are: oxidized cellulose [2–7], absorbable gelatin sponges [5–8], absorbable collagen sponges [9, 19], fibrin glue [10], cyanoacrylate glue [11], platelet rich plasma gel [12] and topical thrombin [17]. The importance of antifibrinolytic solutions for mouthwash in post-extractional bleeding prevention has also been noted in several articles [4, 20]. None of these hemostatic agents has been proved to be superior compared to others [21–23].

The aim of this study was to compare some local hemostatic modalities in anticoagulated patients without interruption of OAT, including a group of patients in whom local hemostatic agents were not used.

## Methods

The study was initiated after it had been approved by local Ethics Committee and all study participants were provided with a written informed consent. The study lasted for ten months and included patients on OAT who were referred to the Department of Oral Surgery, Dental Clinic of Vojvodina by their general dental or medical practitioners in the need for tooth extractions.

The study included patients on long-term OAT whose INR was $\leq$ 3.0 on the day of the procedure and in whom extraction of one or two teeth could be done using local anesthesia without the need to raise a mucoperiosteal flap. The following patient categories were excluded from the study: patients with liver disease or bleeding disorders, patients taking medications that affect hemostasis, patients with severe bleeding after dental extractions even before starting OAT and patients who did not agree to participate in the investigation.

During a patient's first visit, retroalveolar or ortopantomographic x-ray and fulfilling of the standard form on the basis of a patient's prior medical documentation were done. The underlying diagnosis which was the reason for starting OAT was noted, as well as the duration of OAT, type and dosage of oral anticoagulant used, recent INR values and every other medication a patient had been using.

The first 30 patients were randomized in the group A, next 30 patients into the group B and the remaining 30 patients comprised the group C. The INR value was determined for each patient on the day of the procedure. Antibiotic prophylaxis was given for patients at risk of endocarditis in accordance with the American Heart Association guidelines [24].

Group A patients underwent suturing of the extractional wound with "figure of eight" nonresorbable suture (black silk 3–0) without using of any local hemostatic agent. In group B absorbable gelatin sponge was used as local hemostatic agent without wound suturing. In the group C no additional local hemostatic measures were used, except local pressure with gauze.

All extractions were done by the same surgeon, on an outpatient basis. Lidocaine hydrochloride 2% with 1/80,000 adrenaline was used as local anesthetic. Local infiltration method and intraligamentary anesthesia were mostly used, although regional blocks of the inferior alveolar and lingual nerve were also used [25]. Extractions were as atraumatic as possible. Local pressure was applied in the patients of all the three groups afterwards, i.e. the patients were asked to hold sterile gauze in a firm bite for thirty minutes. The patients were observed for the next two hours. The first measure in case of post-extraction bleeding was a superficial gauze tamponade of the wound for ten minutes. This was repeated, if needed, twice at the most. It was planned, in cases of unsuccessful superficial gauze tamponade in the patients of the group A, to put a hemostatic agent, gelatin sponge, into the wound along with suturing, while in the same situation in the group B the wound would be sutured. In the group C the insertion of gelatine sponge was to be the first measure after which suturing of the wound would be done if necessary. Repeated hemorrhage in all the three groups of patients would be treated by insertion of a new hemostatic agent – oxidized regenerated cellulose into the wound. Should there be hemorrhage that could not be controlled with repeated local measure, the attending physician would be consulted, and if needed vitamin K or fresh frozen plasma would be administered.

All the patients were advised to continue OAT after the procedure. Paracetamol was suggested for pain relief. Each patient was given a telephone number to contact the surgeon in case of hemorrhage.

All the subjects were examined thirty minutes, two hours, on the first, second and fifth day after the extraction, when sutures in the patients in the group A were removed. The hemorrhage was registered as an event if other than initial local hemostatic measure was needed or an additional oral surgeon intervention was required. Hemorrhages that occurred in the first two hours after the procedure, i.e. during the observational period until the dismissal of the patient were characterized as "immediate bleeding". Hemorrhages after this period were considered as "late bleeding".

Data were analyzed using $\chi^2$-test and analysis of variance (ANOVA) as appropriate, and the probabilities of less than 0.05 were accepted as significant.

## Results

Initially, 98 patients fulfilled criteria to participate. Six patients who did not come to control examinations were ex-

cluded, but were contacted by telephone to exclude postextractional hemorrhage. Two patients who were on combined oral anticoagulant and aspirin therapy were also excluded. Finally, the study included 90 patients, 30 in each group. Indications for OAT are shown in the Table 1.

**Table 1**

**Indications for anticoagulant treatment**

| Indications | Group A (n) | Group B (n) | Group C (n) |
|---|---|---|---|
| Prosthetic valve replacement | 11 | 4 | 7 |
| Cardiac arrhythmia (atrial fibrillation) | 6 | 14 | 11 |
| Atrial fibrillation and valvular diseases | 3 | 2 | 1 |
| Atrial fibrillation and cerebrovascular accident | 1 | 5 | 3 |
| Deep vein thrombosis/pulmonary embolus | 6 | 2 | 7 |
| Ischemic heart disease | 1 | none | 1 |
| Cerebrovascular accident | 1 | 2 | none |
| Dilated cardiomyopathy | none | 1 | none |
| Thrombophilia | 1 | none | none |

**n – number of patients; group A – patients underwent suturing of the extractinal wound; group B – patient with absorbable gelatin sponge used for local hemostasis; group C – patients with no local hemostatic measures except local pressure with gauze.**

The mean INR values on the day of procedures were 2.35 ± 0.37 in the group A, 2.43 ± 0.4 in the group B and 2.36 ± 0.34 in the group C. The most common anticoagulant drug in all the three patient groups was acenocoumarol (28, 26 and 28 patients in the group A, B and C, respectively). All the other patients were taking warfarin. Basic characteristics of all the three groups of patients are shown in Table 2. The groups did not differ significantly in INR-values ($p = 0.662$), number of tooth extractions ($p = 0.708$), gender ($p = 0.543$) and age ($p = 0.868$).

## Discussion

Most authors suggest that the vast majority of oral surgical procedures in anticoagulated patients can be safely done without alteration of their regular OAT, thus avoiding the risk of thromboembolic complications [1–20]. The need for efficient local hemostasis is emphasized. Local hemostatic measures usually mean the use of certain hemostatic agent such as oxidized regenerated cellulose, gelatine sponges, collagen sponges, fibrin glue and antifibrinolytic mouthwash.

**Table 2**

**Characteristics of patients under coumarin treatment undergoing tooth extraction**

| Characteristics | Group A | Group B | Group C |
|---|---|---|---|
| Gender: (male/ female), n | 19/11 | 16/14 | 20/10 |
| Age (years), $\bar{x}$ ± SD (range) | 65.5 ± 11.1 (23–78) | 66.9 ± 9.8 (41–81) | 65.8 ± 11.2 (36–85) |
| Mean INR on the day of procedure, $\bar{x}$ ± SD (range) | 2.35 ± 0.37 (1.74–3) | 2.43 ± 0.4 (1.76–2.95) | 2.36 ± 0.34 (1.75–3) |
| Single/double extractions, n | 18/12 | 20/10 | 21/9 |
| Causes for extraction, n | | | |
| periodontal disease | 22 | 19 | 18 |
| deep caries | 20 | 21 | 21 |
| Patients with postoperative bleeding, n (%) | 1 (3.3) | 2 (6.7) | 2 (6.7) |
| Immediate / late bleeding, n | 1/none | 1/1 | 2/none |
| Procedure to control bleeding, n | | | |
| local pressure | 1 | none | none |
| sponge | none | none | 1 |
| suturing | none | 2 | 1 |

**INR – International normalized ratio; Immediate bleeding – hemorrhage that occurred in the first two hours; Late bleeding – hemorrhage that occurred after the first two hours; $\bar{x}$ ± SD – mean ± standard deviation; group A – patients underwent suturing of the extractinal wound; group B – patient with absorbable gelatin sponge used for local hemostasis; group C – patients with no local hemostatic measures except local pressure with gauze.**

Postoperative bleeding was noted in 1 (3.3%) patient in the group A, and 2 (6.7%) patients in the groups B and C (Table 2). Except one patient in the group B with "late bleeding", which occurred several hours after the intervention and whose wound had to be sutured, all the other cases of postextractional bleeding were noted in the first two hours after the procedure and characterized as "immediate bleeding". All cases of hemorrhage were easily solved only with local hemostatic measures and none of the patients had serious bleeding that would require systemic therapy. No statistically significant difference between these two groups of patients was found ($\chi^2 = .42$; $p = 0.811$).

Studies that compare different local hemostatic measures do not confirm advantages of certain agents [21–23]. Nevertheless, having in mind limited possibilities in the use of some preparations (such as fibrin glue since it costs a lot and carries the risk of viral transmission; tranexamic acid in the form of mouthwash is not available commercially in many countries and may have an effect only on the superficial clot and not on bleeding from the depth of the socket), there is still a need

to find the safest method for local hemostasis in these patients.

In most studies that dwell on tooth extractions in anticoagulated patients, the suturing of the extractional wound had been done [2–8]. Ferrieri et al. [26] point out that only suturing of the wound with local application of antifibrinolytics in cases of hemorrhage is sufficient for successful local hemostasis. Recent studies, however, suggest that suturing of the extractional wound is not always necessary [9, 19, 27].

Up to now there is only one study by Campbell et al. [28] in which tooth extractions in anticoagulated patients were performed without additional local hemostatic measures. The authors found no difference in blood loss among groups of patients who continued, stopped and have never been on OAT during oral surgery. However, the number of patients who continued OAT was small and only 12 patients with INR < 3 were included in the study.

The results of our study show that minor oral surgical procedures, such as extraction of one or two teeth, could be safely done without alteration of OAT and without the use of any topical hemostatic agent. Wound suturing is as efficient in local hemostasis as the use of topical hemostatic agent, but it is not necessary measure in each patient. However in patients on OAT in whom adequate primary local hemostasis cannot be achieved, suturing is a procedure of a great importance.

This study has some potential drawbacks and limitations: its relatively small sample size; only oral surgery procedures with low bleeding risk, simple extraction of one or two teeth, were performed; only patients with INR ≤ 3.0 were included although the current recommendation is that oral surgery can be safely done if INR values are ≤ 4.0. The reason for this is our intention to check safety of dental extractions without the use of any local hemostatic agents.

**Conclusion**

In therapeutically anticoagulated patients tooth extractions can be safely performed without altering the dose of anticoagulant medication, provided efficient local hemostasis. In most cases, in patients with INR ≤ 3.0 after extraction of one or two teeth postoperative bleeding can be controlled with local pressure, without any additional local hemostatic measures.

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## REFERENCES

1. *Wahl MJ*. Dental surgery in anticoagulated patients. Arch Intern Med 1998; 158(15): 1610–6.
2. *Evans IL, Sayers MS, Gibbons AJ, Price G, Snooks H, Sugar AW*. Can warfarin be continued during dental extraction? Results of a randomized controlled trial. Br J Oral Maxillofac Surg 2002; 40(3): 248–52.
3. *Devani P, Lavery KM, Howell CJ*. Dental extractions in patients on warfarin: Is alteration of anticoagulant regime necessary. Br J Oral Maxillofac Surg 1998; 36(2): 107–11.
4. *Carter G, Goss A*. Tranexamic acid mouthwash: A prospective randomized study of a 2-day regimen vs 5-day regimen to prevent postoperative bleeding in anticoagulated patients requiring dental extractions. Int J Oral Maxillofac Surg 2003; 32(5): 504–7.
5. *Karaca I, Simşek S, Uğar D, Bozkaya S*. Review of flap design influence on the health of the periodontium after mandibular third molar surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104(1): 18–23.
6. *Zanon E, Martinenelli F, Bacci C, Cordioli G, Girolami A*. Safety of dental extraction among consecutive patients on oral anticoagulant treatment managed using a specific dental management protocol. Blood Coagul Fibrinolisis 2003; 14(1): 27–30.
7. *Salam S, Yusuf H, Milosevic A*. Bleeding after dental extractions in patients taking warfarin. Br J Oral Maxillofac Surg 2007; 45(6): 463–6.
8. *Blinder D, Manor Y, Martinowitz U, Taicher S*. Dental extractions in patients maintained on oral anticoagulant therapy: Comparison of INR value with occurrence of postoperative bleeding. Int J Oral Maxillofac Surg 2001; 30(6): 518–21.
9. *Bajkin BV, Popovic SL, Selakovic SD*. Randomized, prospective trial comparing bridging therapy using low-molecular-weight heparin with maintenance of oral anticoagulation during extraction of teeth. J Oral Maxillofac Surg 2009; 67(5): 990–5.
10. *Bodner L, Weinstein JM, Baumgarten AK*. Efficacy of fibrin sealant in patients on various levels of oral anticoagulant undergoing oral surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 86(4): 421–4.
11. *Al-Belasy FA, Amer MZ*. Hemostatic effect of n-butyl-2-cyanoacrylate (histoacryl) glue in warfarin-treated patients undergoing oral surgery. J Oral Maxillofac Surg 2003; 61(12): 1405–9.
12. *Della Valle A, Sammartino G, Marenzi G, Tia M, Espedito di Lauro A, Ferrari F,* et al. Prevention of postoperative bleeding in anticoagulated patients undergoing oral surgery: use of platelet-rich plasma gel. J Oral Maxillofac Surg 2003; 61(11):1275–8.
13. *Morimoto Y, Niwa H, Minematsu K*. Risk factors affecting postoperative hemorrhage after tooth extraction in patients receiving oral antithrombotic therapy. J Oral Maxillofac Surg 2011; 69(6): 1550–6.
14. *Rodríguez-Cabrera MA, Barona-Dorado C, Leco-Berrocal I, Gómez-Moreno G, Martínez-González JM*. Extractions without eliminating anticoagulant treatment: A literature review. Med Oral Patol Oral Cir Bucal 2011; 16(6): 800–4.
15. *Jiménez Y, Poveda R, Gavaldá C, Margaix M, Sarrión G*. An update on the management of anticoagulated patients programmed for dental extractions and surgery. Med Oral Patol Oral Cir Bucal 2008; 13(3): 176–9.
16. *Scully C, Wolf A*. Oral surgery in patients on anticoagulant therapy. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94(1): 57–64.
17. *Marjanović M*. Use of thrombin powder after tooth extraction in patients receiving anticoagulant therapy. Vojnosanit Pregl 2002; 59(4): 389–92. (Serbian)
18. *Mihailović B, Duka M, Miladinović M, Vujičić B, Mačukanović-Golubović L*. Conditions causing copious bleeding important for dental medicine practice. Vojnosanit Pregl 2010; 67(1): 59–64. (Serbian)

19. *Bajkin BV, Bajkin IA, Petrovic BB*. The effects of combined oral anticoagulant-aspirin therapy in patients undergoing tooth extractions: a prospective study. J Am Dent Assoc 2012; 143(7): 771−6.
20. *Sindet-Pedersen S, Ramstrom G, Bernvil S, Blomback M*. Hemostatic effect of tranexamic acid mouthwash in anticoagulant-treated patients undergoing oral surgery. N Engl J Med 1989; 320(13): 840−3.
21. *Blinder D, Manor Y, Martinowitz U, Taicher S, Hashomer T*. Dental extractions in patients maintained on continued oral anticoagulant: Comparison of local hemostatic modalities. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 88(2): 137−40.
22. *Carter G, Goss A, Lloyd J, Tocchetti R*. Tranexamic acid mouthwash versus autologous fibrin glue in patients taking warfarin undergoing dental extractions: A randomized prospective clinical study. J Oral Maxillofac Surg 2003; 61(12): 1432−5.
23. *Halfpenny W, Fraser JS, Adlam DM*. Comparison of 2 hemostatic agents for the prevention of postextraction hemorrhage in patients on anticoagulants. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 92(3): 257−9.
24. *Wilson W, Taubert KA, Gewitz M, Lockhart PB, Baddour LM, Levison M*, et al. Prevention of infective endocarditis: Guidelines from the American Heart Association: A guideline from the American Heart Association Rheumatic Fever, Endocarditis and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. J Am Dent Assoc 2008; 139(1): 3−24.
25. *Bajkin BV, Todorovic LM*. Safety of local anaesthesia in dental patients taking oral anticoagulants: is it still controversial. Br J Oral Maxillofac Surg 2012; 50(1): 65−8. PubMed PMID: 21130546
26. *Ferrieri GB, Castiglioni S, Carmagnola D, Cargnel M, Strohmenger L, Abati S*. Oral surgery in patients on anticoagulant treatment without therapy interruption. J Oral Maxillofac Surg 2007; 65(6): 1149−54.
27. *Al-Mubarak S, Al-Ali N, Rass M, Al-Sohail A, Robert A, Al-Zoman K*, et al. Evaluation of dental extractions, suturing and INR on postoperative bleeding of patients maintained on oral anticoagulant therapy. Br Dent J 2007; 203(7): E15.
28. *Campbell JH, Alvarado F, Murray RA*. Anticoagulation and minor oral surgery: Should the anticoagulation regimen be altered. J Oral Maxillofac Surg 2000; 58(2): 131−5.

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# Factors influencing antibiotic treatment cost and outcome in critically ill patients: A "real-life" study

## Faktori koji utiču na cenu antibiotske terapije i ishod kod kritično obolelih pacijenata: "*real-life*" studija

**Aneta Perić*†, Maja Šurbatović†‡, Sandra Vezmar Kovačević§, Mirjana Antunović*†, Milić Veljović†‡, Dragan Djordjević†‡, Tamara Andjelić‖, Snježana Zeba†‡, Silva Dobrić†¶**

***Sector for Pharmacy, ‡Clinic of Anesthesiology and Intensive Therapy, ‖Institute of Medical Biochemistry, ¶Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; §Department of Pharmacokinetics and Clinical Pharmacy, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia**

**Abstract**

**Background/Aim.** Critically ill patients are at very high risk of developing severe infections in intensive care units (ICUs). Procalcitonin (PCT) levels are eleveted in the circulation in patients with bacterial sepsis and PCT might be useful in guiding antibiotic treatment. The aim of this study was to estimate factors influencing patients survival and treatment cost in ICU with special emphasis on the impact of PCT serum levels use in guiding antimicrobial therapy. **Methods.** The study was conducted from August 2010 to May 2012 in the Intensive Therapy Unit, Clinic of Anesthesiology and Intensive Therapy, Military Medical Academy (MMA), Belgrade, Serbia. All adult critically ill patients with sepsis and/or trauma admitted in the ICU were included in the study. This study included only the cost of antimicrobial therapy in the ICU and the cost for PCT analysis. We used prices valid in the MMA for the year 2012. PCT in serum was measured by homogeneous immunoassay on a Brahms Kryptor analyzer. **Results.** A total of 102 patients were enrolled. The mean patients age was 55 ± 19 years and 61.8% of patients were male. The mean length of stay (LOS) in the ICU was 12 ± 21 days. There was a statistically significant difference ($p < 0.001$) between the sepsis and trauma group regarding outcome (higher mortality rate was in the sepsis group, particularly in the patients with peritonitis who were mostly women). The patients younger than 70 years had better chance of survival. LOS, the use of carbapenems and PCT-measurement influenced the cost of therapy in the ICU. **Conclusions.** The obtained results show that age, the diagnosis and gender were the main predictors of survival of critically ill patients in the ICU. The cost of ICU stay was dependent on LOS, use of carbapenems and PCT-measurement although the influence of these three factors on the outcome in the patients did not reach a statistical significance.

**Key words:**
**critical illness; sepsis; anti-bacterial agents; cost and cost analysis; biological markers.**

**Apstrakt**

**Uvod/Cilj.** Kritično oboleli pacijenti imaju veliki rizik od razvoja teških infekcija u jedinicama intenzivne terapije (JIT). Nivo prokalcitonina (PCT) u cirkulaciji je povišen kod bolesnika sa bakterijskom sepsom, tako da PCT može biti koristan u praćenju antibiotske terapije. Cilj ove studije bio je da se ustanove faktori koji utiču na ishod i troškove lečenja u JIT u našoj ustanovi sa posebnim naglaskom na uticaj korišćenja serumskog nivoa PCT u vođenju antimikrobne terapije. **Metode.** Studija je sprovedena od avgusta 2010. godine do maja 2012. godine u Jedinici intenzivne terapije Klinike za anesteziologiju i intenzivnu terapiju Vojnomedicinske akademije (VMA) u Beogradu, Srbija. Svi kritično oboleli sa sepsom i/ili traumom koji su primljeni u JIT bili su uključeni u studiju. Studijom su obuhvaćeni samo troškovi antimikrobne terapije u JIT i troškovi PCT analize. Koristili smo cenovnik VMA za 2012. godinu. PCT u serumu je meren tehnikom homogenog imunoeseja na Brams Kriptor analizatoru. **Rezultati.** Studijom su bila obuhvaćena 102 bolesnika. Prosečna starost bolesnika iznosila je 55 ± 19 godina, a 61,8% bolesnika bili su muškarci. Prosečna dužina boravka u JIT (*lenght of stay* – LOS) iznosila je 12 ± 21 dana. Postojala je statistički značajna razlika ($p < 0.001$) između ishoda lečenja u grupi sa sepsom u odnosu na grupu sa traumom. Bolesnici mlađi

**Correspondence to:** Aneta Perić, Sector for Pharmacy, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia.
Phone: +381 11 3609 225, E-mail: aneta.peric@gmail.com

od 70 godina imali su bolju šansu da prežive. Dužina boravka, upotreba karbapenema i merenje PCT uticali su na cenu terapije u JIT. **Zaključak.** Dobijeni rezultati pokazuju da su godine života, dijagnoza i pol bili glavni prediktori preživljavanja kritično obolelih u JIT. Cena terapije zavisila je od dužine boravka u JIT, upotrebe karbapenema i merenja PCT, ali uticaj ovih faktora na ishod lečenja nije dostigao statističku značajnost.

**Ključne reči:**
**kritična stanja; sepsa; antibiotici; cene i analize cena; biološki pokazatelji.**

## Introduction

Severe infections with multiresistant bacteria represent a medical challenge and a financial burden for hospitals. Sepsis is a frequent cause of intensive care unit (ICU) admission and may also develop in patients admitted to the ICU for other reasons. Critically ill patients are at very high risk of developing severe nosocomial infections with the incidence rate about 5–10-fold higher than in general medical wards [1, 2]. The recent Sepsis Occurence in Acutelly Ill Patients (SOAP) Study across Europe reported that more than 35% of ICU patients had sepsis at some point during the ICU stay, with the mortality rate of 27%. In the USA, approximately 750,000 cases occur each year, at least 250,000 of which are fatal. Septic patients are generally hospitalized for extended periods, sometimes 2–3 weeks [3–6].

The pathophysiology of sepsis is complex and comprises diffuse endothelial and epithelial injury, increased capillary permeability, impaired hemodynamics, microvascular thrombosis, tissue ischemia, apoptosis and multiorgan failure [7–9].

Critically ill patients with sepsis are commonly treated with antimicrobials. Selecting the appropriate initial antimicrobial is most important, since the inappropriate choice may be responsible for therapeutic failure and higher mortality rate in ICU [2]. The use of inappropriate initial antibiotics may occur in 34.3% of cases involving nosocomial-acquired bacteremia. The risk of death increased from 30–60% in ICU bacteremia and 70–100% in gram-negative shock when the initial antimicrobial therapy was inappropriate [7].

The choice of initial empirical anti-infective therapy should be broad enough to cover any likely patogens and guided by local prevalence of microorganisms. Appropriate intravenous antibiotics (e.g. carbapenems, fluoroquinolones) should be initiated as rapidly as possible, preferably within the first hour of establishing diagnosis of sepsis [7, 10, 11]. The most common pathogens that cause sepsis in hospitalized patients are gram-positive bacteria, followed by gram-negative and mixed bacterial microorganisms. Once blood culture profile results become available, de-escalation to the most appropriate single-agent therapy should be performed as soon as possible. This practice reduces the prevalence of antimicrobial resistance or the risk of antibiotic related diarrhea from *Clostridium difficile*, as well as pharmaceutical expenditure [10, 11]. However, conventional microbiology cultures, despite their specificity and accuracy, are time consuming, and a negative result in many cases of bacterial sepsis (50% or more) does not exclude an infective etiology [10, 12, 13]. Procalcitonin (PCT), a prohormone of calcitonin, was shown to be a marker of sepsis. Its levels are elevated in the circulation in patients with bacterial sepsis, due to the failure of suitable proteolysis. PCT has a longer half-life of 24 to 30 hours in circulation, in contrast to other markers of sepsis such as tumor necrosis factor (TNF) or interleukin (IL)-6 [2, 14–16]. The use of PCT is useful in guiding antibiotic treatment, but with some limitations. The results derived from a multicentre randomised controlled trial PRORATA, show that despite lower antibiotic exposure in the PCT group compared to the control group, there was no difference between emerging multidrug-resistant bacteria [17]. The results from a study conducted across Denmark, with 1,200 critically ill patients, show that PCT-guided antimicrobial strategy does not improve a 28-day survival. The authors observed deleterious effects on organ function and length of stay (LOS) in the ICU in the PCT-guided group [15]. Another limitiation for the use of PCT is associated with the cost of analysis.

Pharmacoeconomics is a scientific discipline that evaluates pharmaceutical interventions, taking into account both the cost and the value of health benefits. When performing pharmacoeconomic evaluations of ICU expenditure, it is customary to consider only the direct price of medication. The single most important factor determining the magnitude of cost is the LOS in the ICU, which is influenced by the high mortality in severe sepsis and septic shock patients and the high incidence of nosocomial infections in critically ill patients [18, 19].

The aim of this study was to provide data about the cost and outcome of critically ill patients admitted to our ICU. We analyzed factors that influence survival of critically ill patients and the cost of treatment in the ICU. Moreover, the impact of PCT measurement on the patient survival and cost of treatment was analysed. Our data are derived from real-life clinical population of critically ill patients in the ICU.

## Methods

The observational study was conducted from August 2010 to May 2012 in the Intensive Therapy Unit of the Clinic of Anesthesiology and Intensive Therapy of the Military Medical Academy (MMA), tertiary university hospital in Belgrade, Serbia. All adult critically ill patients with sepsis and/or trauma admitted to the ICU were included in the study.

The study was approved by the Ethic Committee in the MMA and performed in accordance with the Declaration of Helsinki. Sepsis, severe sepsis and septic shock were diagnosed according to the criteria proposed by the American College on International Sepsis Definition Conference [20]. Complete medical data for all patients were recorded until their discharge or death.

This study included only the cost of antibacterial therapy in the ICU. We analyzed cost-related expenditures such as a total drug cost and the cost for PCT analysis. Costs related to equipment usage, estates (e.g. cost related to infra-

structure, electricity, etc) and non-clinical support services, as well as indirect cost (productivity loses), were not included. The enrolled patients were assessed during ICU stay. After completion of data collection, all costs were priced. We used prices valid in the MMA for the year 2012. All the costs are presented in RSD (Serbian currency). As we collected data from 2010, we adjusted all values using a 10% average inflation rate to 2012 values, according to Serbian indexes for that period. In order to compare our data with others, we presented costs in euro (€), as well. The exchange rate of 1€ was considered as 115 dinars for the year 2012.

PCT in serum was measured by homogeneous immunoassay (sandwich principle) using time resolved amplified cryptate emission (TRACE) technology on Brahms Kryptor analyzer.

The results are expressed as mean ± standard deviation (SD) for variables that exhibit normal distribution. All costs are reported as median, with the interquartile range (IQR) and 95% confidence interval (CI) [21]. Statistical analyses were conducted using PASW 18.0 (SPSS Inc., Chicago, IL, USA). Binary logistic regression was used to determine the predictors of survival and linear regression was used to determine the predictors of treatment cost. The results were presented with odds-ratio (OR) or *p*-value. Both models were obtained using a stepwise approach, variables were excluded at the selection threshold of 0.1. A probability value of < 0.05 was considered to be statistically significant.

## Results

A total of 102 patients were enrolled. The mean patients age was 55 ± 19 years and 61.8% of patients were male. The reasons for ICU admissions were severe trauma, severe trauma and secondary sepsis, severe sepsis due to peritonitis, pancreatitis and other causes. The mean length of ICU stay was 12 ± 21 days (Table 1).

Regression analysis revealed that gender, the diagnosis and age influenced the survival of critically ill patients with sepsis. There was a difference between males and females regarding the diagnosis. In the sepsis group (regardless of underlying cause) there were 49 (58.3%) males *vs* 35 (41.7%) females, whereas in the trauma group (with or without secondary sepsis) males strongly dominated with 14 (77.8%) *vs* 4 (22.2%) of females. In the sepsis group with peritonitis females were dominant. Regression analysis showed that gender influenced the outcome. The mortality rate in males was 42.9% and they had better chances to survive (OR = 2.13). Consequently, there was a statistically significant difference ($p < 0.001$) between the sepsis and the trauma group regarding the outcome; in the trauma group the mortality rate was 17.6% while in the sepsis due to peritonitis, for example, the mortality rate raised to 48%. The outcome, among other parameters, was related to age of the studied population. In survivors, the mean age was lower (44 ± 16 years), compared to non-survivors (66 ± 15 years) ($p < 0.001$). The younger patients (< 70 years) had better chance of survival (Figure 1).

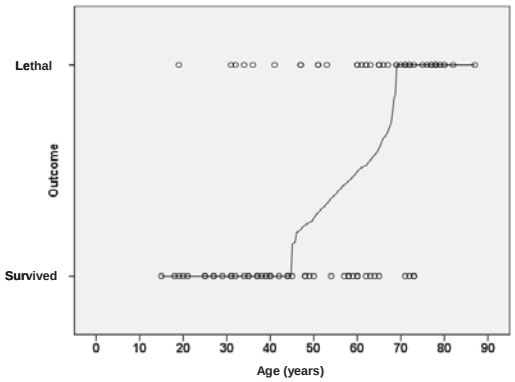


**Fig. 1 – Correlation of age and outcome of survivors and non-survivors.**

**Table 1**

**Demographic and clinical characteristics of critically ill patients**

| Characteristics | Value |
|---|---|
| Total number of patients (n) | 102 |
| Age (years), mean ± SD (range) | 55 ± 19 (18 - 87) |
| Sex, n (%) | |
| male | 63 (61.8) |
| female | 39 (38.2) |
| SAPS II score, mean ± SD | 56.82 ± 9.83 |
| APACHE II score, mean ± SD | 21.87 ± 4.21 |
| SOFA score, mean ± SD | 7.56 ± 2.30 |
| Length of ICU stay in days, mean ± SD (range) | 12 ± 21 (2–169) |
| Severe trauma (ISS 28.73 ± 9.40), n (%) | 18 (17.6) |
| Severe trauma and secondary sepsis, n (%) | 17 (16.7) |
| Severe sepsis due to peritonitis, n (%) | 49 (48) |
| Pancreatitis, n (%) | 13 (12.7) |
| Other causes, n (%) | 5 (4.9) |
| Blood cultures, n (%) | |
| gram-positive | 16 (15.7) |
| gram-negative | 4 (3.9) |
| mixed | 47 (46.1) |
| fungi | 1 (1) |
| sterile | 34 (33.3) |
| Mortality, n (%) | 51 (50) |

**APACHE II – Acute and Physiology and Chronic Health Evaluation II; SAPS – Simplified Acute Physiology Score II; SOFA – Sequential Organ Failure Assessment; ICU – Intensive Care Unit; ISS – Injury Severity Score.**

Examining costs and the outcome in 102 patients in the ICU, irrespective of the underlying cause of admission, we found that the median cost *per* patient was higher in 51 non-survivors compared to 51 survivors (€ 488 *vs* € 358, respectively), but regression analysis showed that survival did not influence ICU cost significantly. In contrast, LOS, the use of carbapenems and PCT-measurement influenced the cost of therapy in the ICU (Figures 2–4 and Table 2).

consultants from the Clinic for Infectious and Tropical Diseases of the MMA, and not attending physicians from the ICU. Our study showed that several combinations of antibiotics were used in the studied period. Some of them included carbapenems and vancomycin or carbapenems and aminoglycosides (gentamycin or amikacin). The other usual combination was cephalosporins with vancomycin.

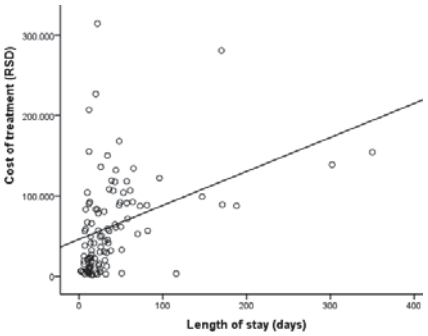


**Fig. 2 – Length of stay in Intensive Care Unit.**

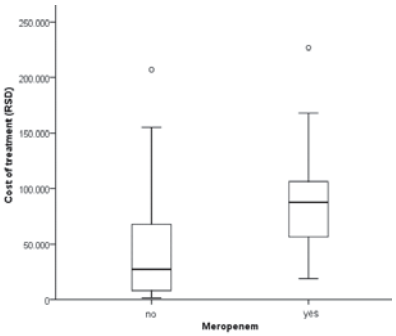


**Fig. 3 – Cost of treatment with meropenem.**

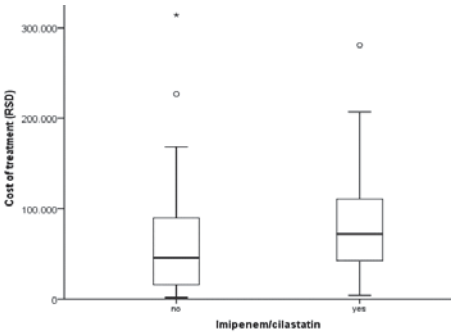


**Fig. 4 – Cost of treatment with imipenem/cilastatin.**

**Table 2**

**Comparison of cost and outcome in critically ill patients with and with no procalcitonin (PCT) guided antibiotic therapy**

| Parameter | Survivors (median; IQR; 95% CI) | Non-survivors (median; IQR; 95% CI) |
|---|---|---|
| PCT-guided therapy | | |
| - in RSD | 75,729.15; 33,984.60-107,941.06; 59,385.51-98,001.47 | 91,278.23; 56,797.17-125,294.68; 71,138.43-126,056.18 |
| - in € | 658.51; 343.97-888.09; 516.40-852.19 | 793.72; 493.89-1,089.52; 618.60-1,096.14 |
| Non PCT-guided therapy | | |
| - in RSD | 71,729.15; 29,984.60-103,941.06; 55,385.51-94,001.47 | 87,278.23; 52,797.17-121,294.68; 67,138.43-122,056.18 |
| - in € | 623.73; 322.78-800.03; 510.20-893.87 | 758.94; 405.46-989.79; 598.98-1,001.12 |
| LOS in the PCT group (days) | 34; 19-57; 28.01-58.57 | 26; 11-57; 22.29-94.11 |
| LOS in non-PCT group (days) | 22; 14-39.50; 18.57-42.67 | 15; 12-31.5; 13.48-41.16 |

**LOS – lenght of stay in Intensive Care Unit; IQR – interquartile range; CI – 95% confidence interval.**

Antibiotics were administered to all patients during ICU stay. Monotherapy was administered to 32 (31.4%) patients. Our analysis showed that the most prescribed antibiotics were carbapenems (58.8%). In the carbapenem group, 56.9% of patients survived. In non-carbapenem group, 43.1% of patients survived. Although there was an obvious trend of increased survival in the carbapenem group, it did not reach a statistical significance. On the other hand, the use of carbapenems (meropenem or imipenem) significantly increased the cost of ICU therapy ($p < 0.001$).

The majority of patients had combined antimicrobial therapy (68.6%). The combination of antibiotics depended on clinical and microbiological data, and antimicrobial therapy was introduced and managed by the infectious disease (ID) specialists. Those ID specialists were

PCT measurement was introduced on the proposal of ID specialists, in order to control the length of antimicrobial therapy, bacterial resistance, and to reduce the use of antimicrobials as well as the cost of antimicrobial therapy. During the follow-up period, the observed patients were divided into two groups: the PCT-guided group of 56 (54.9%) patients and the non-PCT-guided group of 46 (45.1%) patients. Our results show that in the PCT-guided group the cost of antimicrobial therapy in the ICU was significantly higher than in the non-PCT-guided group (761.56 € *vs* 329.98 €, respectively; $p < 0.001$). The differences between the cost and the outcome are shown in Table 2. There was no significant difference between the outcome and the length of use of antibiotics in the two groups. Our results show that 75% of the patients in the PCT-

guided group had combined antibiotic therapy, and 40.5% of them died. In the PCT-guided group, carbapenems were administered in 69.6% of the patients and 56.4% of them survived. The most prescribed was meropenem. In the PCT-guided group, 41.1% of the patients had mixed bacteria in blood culture and 23.2% of them died. Sterile blood cultures were found in 33.9% of the patients and 10.7% of them died.

In the non-PCT guided group, 60.9% of the patients had combined antibiotic therapy and 50% of them died. Carbapenems were administered in 45.7% of the patients in the non-PCT guided group and 33.3% of them survived. In this group, 52.2% of the patients had mixed bacteria in blood cultures, out of whom 45.6% died. The differences between PCT-guided group and non-PCT guided group did not reach a statistical significance.

## Discussion

In the present observational study, three factors were identified as the major cost drivers: duration of ICU-LOS; cost of antimicrobial therapy; PCT-measurement.

In our population of critically ill patients, the mortality rate was 50%. This outcome is comparable with that found previously in larger studies, where high mortality rates, between 40% and 70%, were common in ICU if septic shock developed [6, 18, 22, 23]. Moreover, ICU patients had a significantly increased mortality risk and a decrement in the quality of life and continued to die in the months and years after hospital discharge [20, 24, 25].

Patients who stay longer in the ICU are at increased risk of infection and probably would have higher cost since LOS is a major determinant of cost. A prolonged ICU stay consumes a large part of ICU resources. According to the literature, predominantly because of the long ICU-LOS, the cost of treatment for patients with sepsis is considerably higher than treatment for other ICU patients. The ICU direct costs *per* day are generally three to seven times higher than for non-ICU care [26, 27]. A multicenter, prospective pharmacoeconomic study of septic patients, showed that the cost of non-survivors increased day by day, while the cost of survivors decreased after the first few days. These findings suggest that patients who developed less organ dysfunction would have consequent reduced cost [17, 18, 27]. In the United States, the mean hospital cost per patient was estimated at $ 22,100 with higher cost in infants, patients who died, ICU patients, surgical patients and patients with multiple organ dysfunction [5]. In three ICUs in Germany similar results were found and again, total direct hospital costs were higher in non-survivors, surgical patients and patients requiring emergency procedures [26]. Our results also show the tendency of increased ICU cost in non-survivors. The lack of statistical significance may be attributed to the relatively small number of patients and great variability of cost.

Our costs were determined according to the pricing of drugs used during ICU-LOS in the hospital and those values are smaller than mentioned in other studies. This may be explained by the difference in pricing of drugs as well as the difference of the type of costs which were considered in comparing studies and included cost of diagnostic methods, surgical procedures, laboratories tests, microbiological tests, hospital fee, salaries and workload in ICU.

The most frequently used antibiotics in our study were carbapenems, which is in accordance with guidelines for antibotic treatment of sepsis [10, 12]. Carbapenems, especially meropenem, are antibiotics typically used in ICUs worldwide. The results of different studies show that meropenem is a cost-effective alternative to imipenem/cilastatin or piperacillin/tazobactam – the preferred carbapenem unless other factors affect this decision (such as local pathogen resistance) [18, 19, 28–30]. However, in our study carbapenems increased the cost of therapy in the ICU. As mentioned before, our population of patients was not analyzed in controlled environment, so our results were observational and from a follow-up period and may vary from the results derived from predefined study and the control groups. This might be one of the possible explanations of the fact that although there was the obvious trend of increased survival in the carbapenem group, it did not reach a statistical significance. Further controlled studies should be conducted in order to get additional data about clinical and economic benefits of the use of carbapenems in our ICU.

PCT measurement is also important in guiding duration of antibiotic therapy in ICU patients and in differentiating infective and non-infective inflammatory conditions [12, 14, 15, 31]. Since the levels of PCT rise in response to infection, its utility for the diagnosis of infection has been extensively investigated with conflicting results depending on the setting and population studied [17, 32]. The existing literature supports the position that PCT-guided therapy is associated with the average of 2 days of reduction in antibiotic use [33]. We did not have a reduction in antibiotic use in PCT-guided group, despite the fact that the ID specialist was in charge of antibiotic treatment. We are currently missing tools to facilitate the discontinuation of antibiotics in the ICU. In standard practice, duration of antibiotic courses in ICU vary greatly. In critically ill patients on prolonged therapy with broad-spectrum antibiotics, superinfections may occur and should be carefully monitored as possible infectious complications. The duration of antimicrobial therapy should be limited to 7 to 10 days. According to the literature, combination therapy should be used in *Pseudomonas* infections and should be discontinued in 3 to 5 days [7]. The severity of presenting symptoms correlate with the mortality in ICU patients and is used to justify more prolonged therapy. In one recent survey, critical care and infectious disease specialists were not even completely swayed by the evidence that limiting antibiotics attenuates the emergence of resistant Gram-negatives in ICU [5, 34–36]. A small number of antibiotic-free days (e.g. 3 days in a PRORATA trial) might not be sufficient to record a decreased resistance-emergence rate, especially for some ICUs with high cross-transmission rates [17]. Our results show that PCT did not affect the LOS in ICU and that LOS depended on diverse clinical characteristics and reasons for admissi-

ons to ICU. In our population of patients, antibiotic treatment was independent on PCT use and the mean duration was 7 days. These findings are similar to others [35,36].

The price of PCT analysis is about € 35 in our laboratory which is higher than in France, where PCT analysis costs € 10–15, and is comparable to the expenditure of unnecessary antibiotics. Nevertheless, Vandjick et al. [37] reported that the acquisition cost of antibiotics used to treat nosocomial bloodstream infections in ICU in adults was € 114 daily. Clearly, we need more data to confirm the value of PCT as a diagnostic parameter to guide antibiotic therapy.

The main limitation of our study lies in the number of patients and the use of direct medication pricing cost only. Despite this limitation, our results are the real-life results, obtained in population of critically ill patients and those results represent valuable data about clinical and economic aspects of antibiotic usage.

**Conclusion**

The obtained results show that age, the diagnosis and gender were the main predictors of survival of critically ill patients in the Intensive Care Unit. However, the cost of Intensive Care Unit treatment was not significantly influenced by the survival of patients possibly due to a relatively small number and large variability of treatment cost. In contrast, cost of Intensive Care Unit stay was determined by the lenght of stay, use of carbapenems and procalcitonin-measurement, although the influence of these three factors on the outcome in patients did not reach statistical significance. To our knowledge, this type of cost analysis is rarely, if ever, performed routinely. With direct connecting economic analysis to routine data collections, as we did in our real-life study, the results would be better and more applicable in everyday practice.

## R E F E R E N C E S


1. *Wilke MH*. Multiresistant bacteria and current therapy: The economical side of the story. Eur J Med Res 2010; 15(12): 571−6.
2. *Pea F, Viale P, Furlanut M*. Antimicrobial therapy in critically ill patients: a review of pathophysiological conditions responsible for altered disposition and pharmacokinetic variability. Clin Pharmacokinet 2005; 44(10): 1009−34.
3. *Marik PE*. Surviving sepsis: going beyond the guidelines. Ann Intensive Care 2011; 1(1): 17.
4. *Martin GS, Mannino DM, Eaton S, Moss M*. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003; 348(16): 1546−54.
5. *Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR*. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001; 29(7): 1303−10.
6. *Vincent J, Sakr Y, Sprung CL, Ranieri MV, Reinhart K, Gerlach H,* et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006; 34(2): 344−53.
7. *Sharma S, Kumar A*. Antimicrobial management of sepsis and septic shock. Clin Chest Med 2008; 29(4): 677−87.
8. *Surbatovic M, Jevdjic J, Veljovic M, Popovic N, Djordjevic D, Radakovic S*. Immune Response in Severe Infection: Could Life-Saving Drugs Be Potentially Harmful. ScientificWorldJournal 2013; 2013: 961852.
9. *Cavaillon J, Annane D*. Compartmentalization of the inflammatory response in sepsis and SIRS. J Endotoxin Res 2006; 12(3): 151−70.
10. *Delinger PR, Levy MM, Rhodes A, Annane D, Gerlach H, Opal S,* et al. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock: 2012. Crit Care Med 2013; 41(2): 580−620.
11. *Dellinger PR, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R,* et al. Surviving Sepsis Campaign: international guidelines for management for severe sepsis and septic shock: 2008. Crit Care Med 2008; 36(1): 296−327.
12. *Patil VK, Morjaria JB, de Villers F, Babu SK*. Associations between procalcitonin and markers of bacterial sepsis. Medicina (Kaunas) 2012; 48(8): 383−7.
13. *Maki DG*. Microbiologic diagnosis of blood culture-negative sepsis by hemofiltration. Crit Care Med 2004; 32(4): 1075−7.
14. *Tang BM, Eslick GD, Craig JC, McLean AS*. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet Infect Dis 2007; 7(3): 210−7.
15. *Jensen JU, Hein L, Lundgren B, Bestle MH, Mohr TT, Andersen MH*, et al. Procalcitonin-guided interventions against infections to increase early appropriate antibiotics and improve survival in the intensive care unit: a randomized trial. Crit Care Med 2011; 39(9): 2048−58.
16. *Schuetz P, Christ-Crain M, Müller B*. Procalcitonin and other biomarkers to improve assessment and antibiotic stewardship in infections: hope for hype. Swiss Med Wkly 2009; 139(23−24): 318−26.
17. *Bouadma L, Luyt C, Tubach F, Cracco C, Alvarez A, Schwebel C*, et al. Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicentre randomised controlled trial. Lancet 2010; 375(9713): 463−74.
18. *Brun-Buisson C, Roudot-Thoraval F, Girou E, Grenier-Sennelier C, Durand-Zaleski I*. The costs of septic syndromes in the intensive care unit and influence of hospital-acquired sepsis. Intensive Care Med 2003; 29(9): 1464−71.
19. *Edwards SJ, Campbell HE, Plumb JM*. Cost-utility analysis comparing meropenem with imipenem plus cilastatin in the treatment of severe infections in intensive care. Eur J Health Econ 2006; 7(1): 72−8.
20. *Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D*, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Crit Care Med 2003; 31(4): 1250−6.
21. *Spiegel MR*. Theory and problems of probability and statistics. New York: McGraw-Hill; 1992.
22. *Edbrooke DL, Hibbert CL, Kingsley JM, Smith S, Bright NM, Quinn JM*. The patient-related costs of care for sepsis patients in a United Kingdom adult general intensive care unit. Crit Care Med 1999; 27(9): 1760−7.
23. *Kumar A, Ellis P, Arabi Y, Roberts D, Light B, Parrillo JE,* et al. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. Chest 2009; 136(5): 1237−48.
24. *Lee H, Doig CJ, Ghali WA, Donaldson C, Johnson D, Manns B*. Detailed cost analysis of care for survivors of severe sepsis. Crit Care Med 2004; 32(4): 981−5.
25. *Winters BD, Eberlein M, Leung J, Needham DM, Pronovost PJ, Sevransky JE*. Long-term mortality and quality of life in sepsis: A systematic review. Crit Care Med 2012; 38(5): 1276−83.
26. *Burchardi H, Schneider H*. Economic aspects of severe sepsis: a review of intensive care unit costs, cost of illness and cost effectiveness of therapy. Pharmacoeconomics 2004; 22(12): 793−813.

27. *Sogayar AM, Machado FR, Rea-Neto A, Dornas A, Grion CM, Lobo SM*, et al. A multicentre, prospective study to evaluate costs of septic patients in Brazilian intensive care units. Pharmacoeconomics 2008; 26(5): 425−34.
28. *Edwards SJ, Wordsworth S, Clarke MJ*. Treating pneumonia in critical care in the United Kingdom following failure of initial antibiotic: a cost-utility analysis comparing meropenem with piperacillin/tazobactam. Eur J Health Econ 2012; 13(2): 181−92.
29. *Hsueh P, Liu C, Shi Z, Lee M, Chang F, Yang M*. Cost minimisation analysis of antimicrobial treatment for intra-abdominal infections: a multicentre retrospective study from Taiwan. Int J Antimicrob Agents 2010; 35(1): 94−6.
30. *Attanasio E, Russo P, Carunchio G, Basoli A, Caprino L*. Cost-Effectiveness Study of Imipenem/Cilastatin versus Meropenem in Intra-Abdominal Infections. Dig Surg 2000; 17(2): 164−72.
31. *Cheval C, Timsit JF, Garrouste-Orgeas M, Assicot M, de Jonghe B, Misset B*, et al. Procalcitonin (PCT) is useful in predicting the bacterial origin of an acute circulatory failure in critically ill patients. Intensive Care Med 2000; 26(Suppl 2): S153−8.
32. *Honh A, Schroeder S, Gehn A, Bernhardt K, Bein B, Wegscheider K*. Procalcitonin-guided algorithm to reduce length of antibiotic therapy in patients with severe sepsis and septic shock. BMC Infect Dis 2013; 13: 158.
33. *Heyland DK, Johnson AP, Reynolds SC, Muscedere J*. Procalcitonin for reduced antibiotic exposure in the critical care setting: a systematic review and an economic evaluation. Crit Care Med 2011; 397(7): 1792−9.
34. *Adrie C, Alberti C, Chaix-Couturier C, Azoulay E, de Lassence A, Cohen Y,* et al. Epidemiology and economic evaluation of severe sepsis in France: age, severity, infection site, and place of acquisition (community, hospital, or intensive care unit) as determinants of workload and cost. J Crit Care 2005; 20(1): 46−58.
35. *Kollef MH, Golan Y, Micek ST, Shorr AF, Restrego MI*. Apprasing contemporary strategies to combat multidrug resistant gram-negative bacterial infections-Proceedings and data from the Gram-Negative Resistance Summit. Clin Infec Dis 2011; 53(Suppl 2): 33−55.
36. *Fraimow HS*. Chipping away at unnecessary antibiotic use in the ICU, one day and one study at a time. Crit Care Med 2013; 41(10): 2447−8.
37. *Vandijck DM, Depaemelaere M, Labeau SO, Depuydt PO, Annemans L, Buyle FM,* et al. Daily cost of antimicrobial therapy in patients with Intensive Care Unit-acquired, laboratory-confirmed bloodstream infection. Int J Antimicrob Agents 2008; 31(2): 161−5.

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# Heart rate variability and increased risk for developing type 2 diabetes mellitus

## Varijabilitet srčane frekvencije i povišen rizik od razvoja dijabetesa melitusa tipa 2

**Biljana Penčić-Popović*, Vera Ćelić*, Zoran Ćosić*, Milena Pavlović-Kleut*, Zorica Čaparević*, Nada Kostić*, Branislav Milovanović†, Aleksandra Šljivić*, Biljana Stojčevski***

***Clinical Hospital Center "Dr Dragiša Mišović-Dedinje", Faculty of Medicine, University of Belgrade, Belgrade, Serbia; †Clinical Hospital Center "Bežanijska Kosa", Faculty of Medicine, University of Belgrade, Belgrade, Serbia**

**Abstract**

**Background/Aim.** To our knowledge there are no data about the relationship between elevated risk for developing type 2 diabetes mellitus (DM2) and altered cardiac autonomic function. The aim of this study was to evaluate the association between heart rate variability (HRV) and slightly increased risk for DM2. **Methods.** We evaluated 69 subjects (50.0 ± 14.4 years; 30 male) without DM2, coronary artery disease and arrhythmias. The subjects were divided into two groups according to the Finnish Diabetes Risk Score (FINDRISC): group I (n = 39) included subjects with 12 > FINDRISC ≥ 7; group II (n = 30) subjects with FINDRISC < 7. HRV was derived from 24-h electrocardiogram. We used time domain variables and frequency domain analysis performed over the entire 24-h period, during the day (06–22 h) and overnight (22–06 h). **Results.** Standard deviation of the average normal RR intervals was significantly lower in the group with increased risk for DM2 compared to the group II (127.1 ± 26.6 ms *vs* 149.6 ± 57.6 ms; $p = 0.035$). Other time domain measures were similar in both groups. The group I demonstrated significantly reduced frequency domain measures, total power – TP (7.2 ± 0.3 ln/ms$^2$ *vs* 7.3 ± 0.3 ln/ms$^2$; $p = 0.029$), and low frequency – LF (5.9 ± 0.4 ln/ms$^2$ *vs* 6.3 ± 0.6 ln/ms$^2$; $p = 0.006$), over entire 24 h, as well as TP (7.1 ± 0.3 ln/ms$^2$ *vs* 7.3 ± 0.3 ln/ms$^2$; $p = 0.004$), very low frequency (6.2 ± 0.2 ln/ms$^2$ *vs* 6.3 ± 0.2 ln/ms$^2$; $p = 0.030$), LF (5.9 ± 0.4 ln/ms$^2$ *vs* 6.2 ± 0.3 ln/ms$^2$; $p = 0.000$) and high frequency (5.7 ± 0.4 ln/ms$^2$ *vs* 5.9 ± 0.4 ln/ms$^2$; $p = 0.011$) during the daytime compared to the group II. Nocturnal frequency domain analysis was similar between the groups. The low diurnal frequency was independently related to elevated risk for diabetes mellitus (beta = -0,331; $p = 0.006$). **Conclusion.** The obtained results suggest that even slightly elevated risk for developing diabetes mellitus may be related to impaired HRV.

**Key words:**
**heart rate; electrocardiography, ambulatory; diabetes mellitus, type 2; risk factors; predictive value of tests.**

**Apstrakt**

**Uvod/Cilj.** U nama dostupnoj literaturi nismo našli podatke o povezanosti povišenog rizika od nastanka dijabetesa melitusa tipa 2 (DM2) i poremećaja srčane autonomne funkcije. Cilj rada bio je da se utvrdi povezanost između varijabiliteta srčane frekvencije i blago povišenog rizika od DM2. **Metode.** Ispitivano je 69 osoba (50,0 ± 14,4 god; 30 muškaraca) bez DM2 i koronarne bolesti, kao i bez poremećaja ritma. Ispitanici su bili podeljeni u dve grupe prema finskom skoru rizika od dijabetesa melitusa tipa 2 (*Finnish Diabetes Risk Score* – FINDRISC): grupu I (n = 39) činili su ispitanici sa 12 > FINDRISC ≥ 7 i grupu II (n = 30) sa FINDRISC < 7. Varijabilitet srčane frekvencije ustanovljen je na osnovu 24-h elektrokardiograma. Korišćene su vremenska i spektralna analiza za vreme od 24 h, u toku dana (06–22 h) i noći (22–06 h). **Rezultati.** Standardna devijacija prosečnih vrednosti normalnih RR intervala bila je značajno niža u grupi sa povišenim rizikom od DM2, nego u grupi II (127,1 ± 26,6 ms *vs* 149,6 ± 57,6 ms; $p = 0,035$). Tokom 24h u prvoj grupi primećena je značajno smanjena ukupna snaga – TP (7,2 ± 0,3 ln/ms$^2$ *vs* 7.3 ± 0.3 ln/ms$^2$; $p = 0.029$) i niska frekvencija – LF (5,9 ± 0,4 ln/ms$^2$ *vs* 6,3 ± 0,6 ln/ms$^2$; $p = 0,006$), a tokom dana značajno smanjenje TP (7,1 ± 0,3 ln/ms$^2$ *vs* 7,3 ± 0,3 ln/ms$^2$; $p = 0,004$), vrlo niske frekvencije (6,2 ± 0,2 ln/ms$^2$ *vs* 6,3 ± 0,2 ln/ms$^2$; $p = 0,030$), LF (5,9 ± 0,4 ln/ms$^2$ *vs* 6,2 ± 0,3 ln/ms$^2$; $p = 0.000$) i visoke frekvencije (5,7 ± 0,4 ln/ms$^2$ *vs* 5,9 ± 0,4 ln/ms$^2$; $p = 0,011$) u odnosu na grupu II. Nije bilo značajne razlike između grupa u spektralnoj analizi za noćni period. Niska frekvencija tokom dana bila je nezavisno povezana sa povećanim rizikom od DM2 (beta = -0,331; $p = 0,006$). **Zaključak.** Dobijeni rezultati ukazuju da čak i blago povišen rizik od razvoja DM2 može biti povezan sa izmenjenim varijabilitetom srčane frekvencije.

**Ključne reči:**
**srce, frekvencija; elektrokardiografija, holter; dijabetes melitus, insulin-nezavisni; faktori rizika; testovi, prognostička vrednost.**

**Correspondence to:** Biljana Penčić-Popović, Clinical Hospital Center "Dr Dragisa Mišović-Dedinje", Milana Tepića 1, 11 000 Belgrade, Serbia. Phone: +381 11 3630700. E-mail: pencicbiljana@gmail.com

## Introduction

Sympathetic and parasympathetic modulation of the autonomic nervous system is commonly assessed by heart rate variability (HRV) [1]. Impaired HRV reflecting autonomic dysfunction is related to many cardiovascular risk factors, especially glucometabolic abnormalities [2]. Many studies have previously revealed cardiac autonomic neuropathy in diabetic patients[1]. Parasympathetic dysfunction is associated with complex pathophysiological mechanisms in obesity, insulin resistance, as well as increased glucose production from the liver leading to atherosclerosis and cardiovascular morbidity [3].

The Finnish Diabetes Risk Score (FINDRISC) has been accepted for predicting 10-year risk of type 2 diabetes mellitus (DM2) in adults in the setting of known risk factors for cardiovascular diseases [4].

To our knowledge there are no data about the relationship between elevated risk for developing DM2 and altered cardiac autonomic function.

The aim of this study was to estimate the association between altered HRV and slightly elevated risk for DM2.

## Methods

We evaluated 69 subjects (age 50.0 ± 14.4 years; 30 male) admitted to the Clinical Hospital Center in order to be evaluated for coronary artery disease. After excluding coronary disease (according to the medical history, 24-h electrocardiogram, echocardiography, exercise stress test), they entered the study. Other excluding criteria were: 30 > age > 70 years, pulmonary and renal diseases, arrhythmias, current medical treatment with beta blockers, calcium antagonists and antiarrhythmics.

All of the participants were asked to complete a form on age, medical history (antihypertensive drug treatment), history of high blood glucose level (discovered during medical examination, during an illness, or during pregnancy), health behavior (daily physical activity, daily intake of vegetables and fruits) and the family history of diabetes. Clinical examination included measurements of weight, height, body mass index (BMI) calculated by dividing the weight (kg) by the height squared ($m^2$), waist circumference (bellow the ribs, usually at the level of the navel). The total score for each subject was composed as the sum of the scores according to the questionnaire, BMI and waist circumference [4]. According to FINDRISC (Table 1), which considers several variables (age, BMI, waist circumference, physical activity, eating vegetables every day, using antihypertensive drugs, high blood glucose level and heredity for DM) we divided subjects into two groups [4].

**Table 1**

**Type 2 diabetes mellitus risk assessment form**

| FINDRISC (p-points) | Questions (p-points) |
|---|---|
| Age | Using antihypertensive drugs regularly |
| 0 p. Under 45 years | 0 p. no |
| 2 p. 45–54 years | 2 p. yes |
| 3 p. 55–64 years | High blood glucose level ever been found |
| 4 p. Over 64 years | 0 p. no |
| Body mass index | 5 p. yes |
| 0 p. < 25 kg/$m^2$ (%) | Heredity (diabetes type 1 or 2) |
| 1 p. 25–30 kg/$m^2$ (%) | 0 p. no |
| 3 p. > 30 kg/$m^2$ (%) | 3 p. yes – grandperent, aunt, uncle or first cousin |
| Waist circumference | 5 p. yes – parents, brother, sister or own child |
| 0 p. Men (women) < 94 (80) cm | Eating vegetables, fruit or berries every day |
| 3 p. Men (women) < 94–102 (80–88) cm | 0 p. every day |
| 4 p. Men (women) < 102 (88) cm | 2 p. not every day |
| Physical activity > 30 min | |
| 0 p. yes | |
| 2 p. no | |

**FINDRISC – Finnish Diabetes Risck Score:**
**Lower than 7 (low): estimated one in 100 will develop disease; 7–11 (slightly elevated): estimated one in 25 will develop disease; 12–14 (moderete): estimated one in 6 will devolop disease; 15–20 (high): estimated one in three will develop disease; Higher than 20 (very high): estimated one in two will develop disease.**

The group I included subjects with slightly increased risk (12 > FINDRISC ≥ 7) predicting that one in 25 will develop DM2. The group II enrolled subjects with low risk (FINDRISC < 7), one in 100 might develop DM2 [4].

HRV was obtained from 24 h electrocardiogram Holter recordings (Argusys) using three channels (V1, V5, aVF). Electrocardiogram signals were digitalized, stored and analyzed using standard software program. According to the Task Force of the European Society of Cardiology we used time domain and frequency domain variables as markers of HRV [1].

Time domain measures included: standard deviation of all normal RR intervals (SDNN) that were considered as an estimate of overall HRV; standard deviation of the average normal RR intervals for all 5-minute segments (SDANN); average of the standard deviation of normal RR intervals for all 5-minute segments (ASDNN); percent of differences between adjacent normal RR intervals ≥ 50 ms (pNN50), root mean square of successive RR interval differences (RMSSD) [1].

Frequency domain analysis of RR intervals were carried out by Fast Fourier Transformation. The analysis of RR intervals were performed over the entire 24-h period, as well as

overnight (22–06 h) and during the day (06–22 h). The high frequency (HF) (0.15–0.40Hz) oscillation of HRV reflected mostly parasympathetic modulation of heart rate, the low frequency (LF) region of the power spectra (0.04–0.15 Hz) included the influence of both parasympathetic and sympathetic function. A very low frequency component (VLF) (0.015 - 0.04 Hz) was considered to reflect the mixture of neuroendocrine and parasympathetic modulation. The LF to HF ratio (LF/HF) was considered as an index of sympathicovagal balance. We used a logarithmic transformation of total power (TP) ($ln/ms^2$), VLF ($ln/ms^2$), LF ($ln/ms^2$) and HF ($ln/ms^2$) values [1].

Mean, minimal and maximal heart rates were also analyzed and compared among the groups.

The Ethics Committee approved the study protocol and consent procedures.

Statistical differences were considered significant when $p < 0.05$. Continuous variables were presented as mean ± standard deviation (SD) and were compared by using the Student's *t*-test for two independent samples since they showed the normal distribution. The differences in proportions were compared by using the $\chi^2$-test. Pearson's correlation coefficient was used for determining the correlation between FINDRISC and HRV parameters. The variables which showed *p*-value < 0.050 were included into linear regression analyses, stepwise method. Regression analysis was used to determine independent predictors of elevated risk for DM2. The statistical method for evaluating the diagnostic accuracy of HRV parameters was receiving operating characteristics (ROC).

## Results

There were 39 subjects in the group I (FINDRISC, mean 9.1 ± 1.3) and 30 individuals in the group II (FINDRISC, mean 4.1 ± 1.7). The individuals with elevated risk for DM2 were significantly older ($p = 0.000$). They had higher BMI ($p = 0.000$) and used antihypertensive drugs more frequently ($p = 0.000$). We observed more subjects in the group II with normal BMI ($p = 0.001$) and normal waist circumference ($p = 0.000$) compared to those in the group I. Both groups were similar in physical activities longer than 30 min daily, eating vegetables, heredity, and high blood glucose level (Table 2).

No significant differences were observed in the mean, maximal and minimal heart rate between the two groups (Table 3).

Time domain variables are presented in Table 4. SDNN was slightly shorter ($p = 0.057$) and SDANN ($p = 0.035$) was significantly shorter in subjects with increased risk for DM2.

Frequency domain measures over the entire 24 h differed significantly in TP ($p = 0.029$) and LF ($p = 0.006$), between the groups. Individuals in group I compared to the group II had significantly lower TP ($p = 0.004$), VLF

**Table 2**

**Demographic characteristics of the studied subjects according to the FINDRISC**

| Parameters | Group I (n = 39) | Group II (n = 30) | *p* |
|---|---|---|---|
| Age (years), $\bar{x} \pm SD$ | 57.9 ± 9.6 | 39.4 ± 11.8 | 0.000 |
| Male, n (%) | 19 (48.7) | 11 (36.7) | 0.340 |
| Body mass index ($kg/m^2$), $\bar{x} \pm SD$ | 26.2 ± 3.4 | 22.7 ± 3.7 | 0.000 |
| < 25 $kg/m^2$, n (%) | 10 (25.6) | 20 (66.7) | 0.001 |
| 25–30 $kg/m^2$, n (%) | 26 (66.6) | 10 (33.3) | 0.008 |
| > 30 $kg/m^2$, n (%) | 3 (7.7) | 0 | 0.252 |
| Waist circumference (cm), (%) | | | |
| men (women) < 94 (80) | 1 (2.6) | 18 (60.0) | 0.000 |
| men (women)< 94–102 (80–88) | 21 (53.8) | 8 (26.7) | 0.029 |
| men (women) <> 102 (88) | 17 (43.6) | 4 (13.3) | 0.008 |
| Physical activity > 30 min, n (%) | | | |
| yes | 7 (17.9) | 9 (30.0) | 0.264 |
| Eating vegetables every day, n (%) | | | |
| yes | 17 (44.1) | 16 (53.3) | 0.631 |
| Using antihypertensive drugs, n (%) | | | |
| yes | 19 (48.7) | 2 (6.7) | 0.000 |
| High blood glucose level, n (%) | | | |
| yes | 3 (7.7 ) | 0 | 0.252 |
| Heredity, n (%) | | | |
| yes (grandparent, aunt, uncle) | 1 (2.6) | 3 (10.0) | 0.297 |
| yes (parents, brother, sister) | 3 (7.7) | 0 | 0.252 |

**FINDRISC – Finnish Diabetes Risck Score; Group I – subjects with 12 > FINDRISC ≥ 7; Group II – subjects with FINDRISC < 7.**

**Table 3**

**Mean, minimal and maximal heart rate in study groups**

| Heart rate (HR), bpm | Group I (n = 39) $\bar{x} \pm SD$ | Group II (n = 30) $\bar{x} \pm SD$ | *p* |
|---|---|---|---|
| HR | 72.1 ± 11.5 | 77.2 ± 9.4 | 0.052 |
| HR min | 50.8 ± 14.2 | 53.9 ± 12.0 | 0.464 |
| HR max | 126.1 ± 30.3 | 138.7 ± 22.9 | 0.063 |

**bpm – beats *per* minute; Group I – subjects with 12 > FINDRISC ≥ 7; Group II – subjects with FINDRISC < 7.**

**Table 4**

**Time domain and frequency domain variables in study groups**

| Variables | Group I (n = 39) $\bar{x} \pm SD$ | Group II (n = 30) $\bar{x} \pm SD$ | *p* |
|---|---|---|---|
| SDNN (ms) | 141.6 ± 28.3 | 157.9 ± 41.4 | 0.057 |
| SDANN (ms) | 127.1 ± 26.6 | 149.6 ± 57.6 | 0.035 |
| ASDNN (ms) | 61.2 ± 21.9 | 65.7 ± 20.8 | 0.389 |
| PNN50 (%) | 10.9 ± 2.2 | 10.8 ± 8.4 | 0.942 |
| RMSSD (ms) | 46.3 ± 31.2 | 41.5 ± 22.9 | 0.484 |
| 24h-TP (ln/ms$^2$) | 7.2 ± 0.3 | 7.3 ± 0.3 | 0.029 |
| 24h-VLF (ln/ms$^2$) | 6.3 ± 0.2 | 6.4 ± 0.2 | 0.154 |
| 24h- LF (ln/ms$^2$) | 5.9 ± 0.4 | 6.3 ± 0.6 | 0.006 |
| 24h- HF (ln/ms$^2$) | 5.9 ± 0.5 | 6.0 ± 0.4 | 0.259 |
| 24h-LF/HF | 1.1 ± 0.3 | 1.2 ± 0.3 | 0.128 |
| Daytime TP (ln/ms$^2$) | 7.1 ± 0.3 | 7.3 ± 0.3 | 0.004 |
| Daytime VLF (ln/ms$^2$) | 6.2 ± 0.2 | 6.3 ± 0.2 | 0,030 |
| Daytime LF (ln/ms$^2$) | 5.9 ± 0.4 | 6.2 ± 0.3 | 0.000 |
| Daytime HF (ln/ms$^2$) | 5.7 ± 0.4 | 5.9 ± 0.4 | 0.011 |
| Daytime LF/HF | 1.03 ± 0.0 | 1.04 ± 0.0 | 0.293 |
| Night-time TP (ln/ms$^2$) | 7.3 ± 0.4 | 7.4 ± 0.3 | 0.189 |
| Night-time VLF (ln/ms$^2$) | 6.4 ± 0.2 | 6.4 ± 0.2 | 0.580 |
| Night-time LF (ln/ms$^2$) | 6.0 ± 0.4 | 6.2 ± 0.4 | 0.122 |
| Night-time HF (ln/ms$^2$) | 5.9 ± 0.5 | 6.1 ± 0.4 | 0.167 |
| Night-time LF/HF | 1.0 ± 0.0 | 1.0 ± 0.0 | 0.979 |

**SDNN – standard deviation of all normal RR intervals; SDANN – standard deviation of the average normal RR intervals for all 5-minute segments; ASDNN – average of the standard deviation of normal RR intervals for all 5-minute segments; pNN50 – percent of differences between adjacent normal RR intervals ≥ 50 ms; RMSSD – root mean square of successive RR interval differences; TP – total power; VLF – very low frequency; LF – low frequency; HF – high frequency; Group I – subjects with 12 > FINDRISC ≥ 7; Group II – subjects with FINDRISC < 7.**

($p = 0.030$), LF ($p = 0.000$) and HF ($p = 0.011$) during the daytime. However, both groups were similar in all nocturnal frequency domain parameters (Table 4).

We found a significant negative correlation between the FINDRISC and TP (r = -0.263; $p = 0{,}029$); LF (r = -0.249; $p = 0.039$) over the entire 24 h hours. Diurnal variables such as: TP (r = -0.294; $p = 0{,}014$); LF (r = -0.331; $p = 0.006$); HF (r = -0.272; $p = 0{,}024$) and nocturnal HF (r = -0.279; $p = 0.020$) also inversely correlated with the FINDRISC (Table 5).

According to linear regression analysis, the stepwise method, which included independent HRV variables, that previously had expressed $p < 0.05$ and the FINDRISC as dependent variable, only daytime LF was independently related to elevated risk for DM2 (beta = -0.331, $p = 0.006$). A model summary is presented in Table 6 and the linearity of the association between the FINDRISC and daytime LF is presented in Figure 1. Other time domain and frequency domain variables did not reach a statistical significance as independent predictors.

**Table 5**

**Correlation between the FINDRISC and measures of heart rate variability in the study subjects**

| Variables | r | *p* |
|---|---|---|
| SDNN (ms) | -0.210 | 0.084 |
| SDANN (ms) | -0.270 | 0.065 |
| ASDNN(ms) | -0.188 | 0.121 |
| PNN50 (%) | -0.083 | 0.500 |
| RMSSD(ms) | -0.045 | 0.715 |
| 24h- TP (ln/ms $^2$) | -0.263 | 0.029 |
| 24h-VLF (ln/ms $^2$) | -0.131 | 0.283 |
| 24h-LF (ln/ms $^2$) | -0.249 | 0.039 |
| 24h-HF (ln/ms $^2$) | -0.170 | 0.162 |
| 24h-LF/HF | -0.076 | 0.537 |
| Daytime TP (ln/ms $^2$) | -0.294 | 0.014 |
| Daytime VLF (ln/ms $^2$) | -0.204 | 0.093 |
| Daytime LF (ln/ms $^2$) | -0.331 | 0.006 |
| Daytime HF (ln/ms $^2$) | -0.272 | 0.024 |
| Daytime LF/HF | -0.069 | 0.571 |
| Night-time TP (ln/ms $^2$) | -0.198 | 0.103 |
| Night-time VLF (ln/ms $^2$) | -0.038 | 0.758 |
| Night-time LF (ln/ms $^2$) | -0.174 | 0.152 |
| Night-time HF (ln/ms $^2$) | -0.279 | 0.020 |
| Night-time LF/HF | 0.189 | 0.121 |

**SDNN – standard deviation of all normal RR intervals; SDANN – standard deviation of the average normal RR intervals for all 5-minute segments; FINDRISC – Finnish Diabetes Risk Score; ASDNN – average of the standard deviation of normal RR intervals for all 5-minute segments; pNN50 – percent of differences between adjacent normal RR intervals ≥ 50 ms; RMSSD – root mean square of successive RR interval differences; TP – total power; VLF – very low frequency; LF – low frequency; HF – high frequency; r – Spearman's correlation coefficients.**

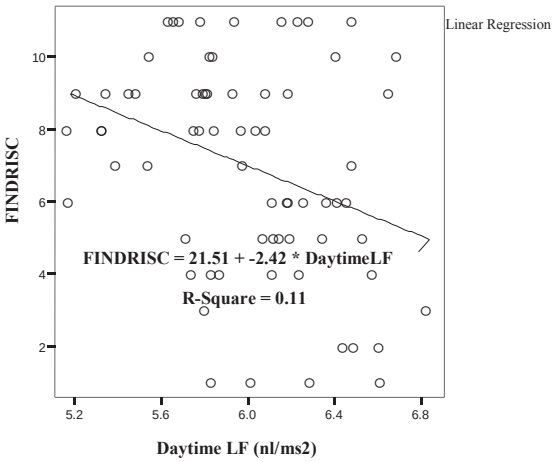


**Fig. 1 – Linear regression model: Correlation between the FINDRISC and daytime low frequency (LF).**
**(FINDRISC – Finnish Diabetes Risk Score)**

The area under the ROC curve show the significant average sensitivity of the daytime LF over the range of specificity in the group with FINDRISC < 7 (AUC = 0.72; $p = 0.002$) but not in the group with FINDRISC ≥ 7 (AUC = 0.251; $p = 0.000$) (Figure 2, Table 7).

## Discussion

Previous investigations about HRV have reported autonomic failure caused by diabetes mellitus, arterial hypertension and/or metabolic syndrome [5]. Ten year risk of diabetes type 2 could easily be predicted by FINDRISC which is accepted as a noninvasive and reliable tool for screening for individuals who are at increased risk for DM2 [4].

To our knowledge this is the first study dealing with altered heart rate variability in subjects with elevated risk for DM2 assessed by the FINDRISC.

Our main findings were: shorter time domain measure (SDANN) and decreases in frequency domain parameters especially during the day (TP, VLF, HF, LF) in subjects with higher risk for DM2; negative correlation between the FINDRISC and frequency domain measures over entire 24 h (TP, LF); diurnal TP, HF, LF and nocturnal HF; the independent predictor for increased risk for DM2 was daytime LF.

Our study demonstrated a decrease in SDANN in subjects with elevated risk for DM2. Al-Hazimi et al. [6] also reported that all time domain parameters were lower in diabetic patients with and without diabetic neuropathy compared to normal controls [6]. A significant difference in the time domain measures among diabetic patients and healthy volunteers were also found in the study by Seyd and al. [7].

We found a significantly reduced TP and LF during 24 h as well as a decrease in TP and LF during daytime in subjects with a higher FINDRISC. There were also strong inverse correlation between TP, LF over entire 24 h; daytime TP, LF and FINDRISC.

It was daytime LF that was found to be independently related to increased risk for diabetes mellitus type 2. Although we did not confirm a significant sensitivity of daytime LF for subject with FINDRISC ≥ 7, we obtained significant diagnostic accuracy for daytime LF in the group with lower risk for DM2 (FINDRISC < 7).

It has been accepted that the power in the low frequency band is commonly influenced by sympathetic oscillatory modulation, although a significant vagal component has been recognized, too [8].

**Table 6**

**Linear regression model: R Square Change statistics (model summary)**

| Model | R | $R^2$ | Adjusted $R^2$ | | Std. error of the estimate | |
|---|---|---|---|---|---|---|
| 1 | 0.331[a] | 0.109 | 0.096 | | 2.793 | |
| | Change statistics | | | | | |
| | $R^2$ Change | F Change | df1 | df2 | Sig. | Durbin-Watson |
| | 0.109 | | 1 | 67 | 0.006 | 2.226 |

**Predictor – Daytime low frequency (LF); b) Dependent variable – Finnish Diabetes Risk Score (FINDRISC).**

**Table 7**

**Area under the curve (AUC): test results for daytime low frequency (LF)**

| Area | Std. error [a] | Asymptotic Sig.b | Asymptotic 95% Confidence Interval | |
|---|---|---|---|---|
| | | | lower bound | upper bound |
| 0.720 | 0.063 | 0.002 | 0.597 | 0.843 |

**Predictor – daytime LF.**

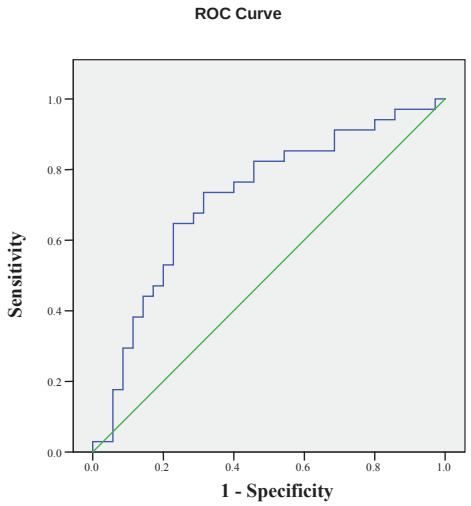


**Fig. 2 – Sensitivity of daytime low frequency (LF) in the subjects with FINDRISC < 7.**
**FINDRISC – Finnish Diabetes Risk Score;**

In our study a significant decrease in daytime HF and negative correlation between daytime and nocturnal HF with the FINDRISC also suggested the impairment of the vagal component.

It seems that the decrease in HF and LF (both reflecting parasimpathetic oscillation) might present the beginning of impairment in the vagal fibre conduction in subjects with elevated risk for DM2. Although altered LF and HF were observed in our subjects with a higher FINDRISC, no change in sympathovagal balance was found. A reduced power in all spectral bands, as well as unchanged LF/HF ratio, are some of the most common manifestations related to diabetic autonomic neuropathy [1, 9].

The early complication of diabetes mellitus is autonomic neuropathy that is characterized by degeneration in small fibres. The pathophysiological mechanisms of altered HRV due to disturbances in small fiber conduction in patients with diabetes mellitus have already been reported [1].

A decrease in the power of LF and HF associated with diabetic patients even without evidence of autonomic neuropathy has been also reported [9].

Recent studies have suggested that even subjects with impaired glucose tolerance may show slower nerve conduction due to distal small fiber neuropathy [10]. According to Cardiovascular Health Study diminished HRV was also related with increased fasting glucose levels in non-diabetic subjects [11].

No previous research has investigated the relationship between HRV and higher risk for DM2. Our subjects with increased risk for DM2 according to the FINDRISC were older, had higher BMI, larger waist circumference and used antihypertensive drugs more frequently. It was reported that each of these risk factors is likely to alter HRV. A decrease in HRV could occur due to increasing age alone [12]. A significant increase in sympathovagal balance was revealed in obese subjects [13]. It was suggested that obesity especially abdominal visceral fat may significantly contribute to the sympathetic over-activity [14].

It has been also demonstrated that arterial hypertension is related to impaired HRV [15]. A decreased HRV associated with arterial hypertension was shown in the Framingham Heart Study [16]. It was also published that cardiac autonomic function was impaired even in white-coat hypertensive patients [17]. According to some investigators low HRV, demonstrating a relative sympathetic over-activity, may be associated with the development of metabolic syndrome and its components [18].

Therefore alteration in HRV in our subjects with the increased FINDRISC including higher mean age, BMI and the higher rate of arterial hypertension could be explained by the influence of age alone and other risk factors contributing to the pathogenesis of atherosclerosis that might also pay a role in the development of small nerve conduction disturbances. It has previously been demonstrated that lower heart rate variability is associated with the development of coronary artery disease in individuals with diabetes [19, 20]. There has been considerable discussion regarding the meaning and interpretation of LF/HF. Reduced LF/HF has also been recognized as a risk factor for cardiovascular disease [21, 22]. On the other hand a strong correlation was shown with each 1-point increase in the FINDRISC and 16–23% increase in the likelihood of cardiovascular disease and mortality [23–26].

Limitations of our study were the small study group, the fact that we excluded subjects with coronary artery disease, according to noninvasive diagnostic procedures, without performing selective coronary angiography. Also, self reported responses might not be absolutely exact. Hence, further studies on a larger sample are necessary to prove the relationship between the FINDRISC and impaired HRV.

## Conclusion

Abnormal glucoregulation is associated with altered heart rate variability and cardiovascular autonomic diabetic neuropathy. The FINDRISC is an inexpensive, noninvasive and reliable tool to identify individuals at high risk for diabetes type 2. Our study is unique in that it deals with heart rate variability in subjects with slightly increased risk for diabetes type 2.

We found that subjects with higher risk for diabetes type 2 had also impaired heart rate variability, especially decreased standard deviation of the aberage normal RR intervals and reduced diurnal frequency domain measures, without significant changes in sympathicovagal balance.

It has long been known that impairment of heart rate variability is also closely related to cardiovascular mortality and morbidity.

Thus early detection of impaired heart rate variability may be the first sign of autonomic dysfunction suggesting further clinical evaluation of subjects with slightly increased risk for development of diabetes type 2. Evaluation of heart rate variability in subjects with increased risk for diabetes type 2 (FINDRISC $\geq$ 7) and *vice versa* might be useful for early detection of autonomic dysfunction related to altered glicoregulation.

Further larger studies would be also necessary to assess the risk for cardiovascular disease in subjects with higher risk for diabetes type 2.

## REFERENCES

1. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996; 93(5): 1043−65.

2. *Aso Y, Wakabayashi S, Nakano T, Yamamoto R, Takebayashi K, Inukai T.* High serum high-sensitivity C-reactive protein concentrations are associated with relative cardiac sympathetic overactivity during the early morning period in type 2 diabetic patients with metabolic syndrome. Metabolism 2006; 55(8): 1014−21.

3. *Szelag B, Wroblewski M, Castenfors J, Henricsson M, Berntorp K, Fernlund P,*et al. Obesity, microalbuminuria, hyperinsulinemia, and increased plasminogen activator inhibitor 1 activity associated with parasympathetic neuropathy in type 2 diabetes. Diabetes Care 1999; 22(11): 1907−8.
4. *Rydén L, Standl E, Bartnik M, Van den Berghe G, Betteridge J, de Boer MJ*, et al. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). Eur Heart J 2007; 28(1): 88−136.
5. *Gottsäter A, Ahmed M, Fernlund P, Sundkvist G.* Autonomic neuropathy in Type 2 diabetic patients is associated with hyperinsulinaemia and hypertriglyceridaemia. Diabet Med 1999; 16(1): 49−54.
6. *Al-Hazimi A, Al-Ama N, Syiamic A, Qosti R, Abdel-Galil K.* Time-domain analysis of heart rate variability in diabetic patients with and without autonomic neuropathy. Ann Saudi Med 2002; 22(5−6): 400−3.
7. *Seyd PTA, Ahamed VI, Jacob J, Joseph PK.* Time and Frequency Domain Analysis of Heart Rate Variability and their Correlations in Diabetes Mellitus. Int J Biol Life Sci 2008; 4(1): 24−7.
8. *Kuch B, Hense HW, Sinnreich R, Kark JD, von Eckardstein A, Sapoznikov D,* et al. Determinants of short-period heart rate variability in the general population. Cardiology 2001; 95(3): 131−8.
9. *Pagani M, Malfato G, Pierini S, Casati R, Masu AM, Poli M.* A Spectral analysis of heart rate variability in the assessment of autonomic diabetic neuropathy. J Auton Nerv Syst 1988; 23(2): 143−53.
10. *Grandinetti A, Chow DC, Sletten DM, Oyama JK, Theriault AG, Schatz IJ*, et al. Impaired glucose tolerance is associated with postganglionic sudomotor impairment. Clin Auton Res 2007; 17(4): 231−3.
11. *Stein PK, Barzilay JI, Domitrovich PP, Chaves PM, Gottdiener JS, Heckbert SR*, et al. The relationship of heart rate and heart rate variability to non-diabetic fasting glucose levels and the metabolic syndrome: The Cardiovascular Health Study. Diabet Med 2007; 24(8): 855−63.
12. *Jensen-Urstad K, Storck N, Bouvier F, Ericson M, Lindblad LE, Jensen-Urstad M.* Heart rate variability in healthy subjects is related to age and gender. Acta Physiol Scand 1997; 160(3): 235−41.
13. *Muscelli E, Emdin M, Natali A, Pratali L, Camastra S, Gastaldelli A*, et al. Autonomic and Hemodynamic Responses to Insulin in Lean and Obese Humans. Endocrinol Metab 1998; 83(6): 2084−90.
14. *Alvarez GE, Beske SD, Ballard TP, Davy KP.* Sympathetic neural activation in visceral obesity. Circulation 2002; 106(20): 2533−6.
15. *Pavithran P, Mithun R, Jomal M, Nandeesha H.* Heart rate variability in middle-aged men with new-onset hypertension. Ann Noninvasive Electrocardiol 2008; 13(3): 242−8.
16. *Singh JP, Larson MG, Tsuji H, Evans JC, O'Donnell CJ, Levy D.* Reduced heart rate variability and new-onset hypertension: insights into pathogenesis of hypertension: the Framingham Heart Study. Hypertension 1998; 32(2): 293−7.
17. *Madsen LB, Rasmussen JK, Møller DS, Nyvad O, Pedersen EB.* Heart rate variability in white-coat hypertension. Blood Pressure Monit 2008; 13(2): 65−71.
18. *Resnick HE, Jones K, Ruotolo G, Jain AK, Henderson J, Lu W,* et al. Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic american indians: the Strong Heart Study. Diabetes Care 2003; 26(3): 861−7.
19. *Gasic S, Winzer C, Bayerle-Eder M, Roden A, Pacini G, Kautzky-Willer A.* Impaired cardiac autonomic function in women with prior gestational diabetes mellitus. Eur J Clin Invest 2007; 37(1): 42−7.
20. *Liao D, Carnethon M, Evans GW, Cascio WE, Heiss G.* Lower heart rate variability is associated with the development of coronary artery disease in individuals with diabetes. The Atherosclerosi Risk in Communities (ARIC) Study. Diabetes 2002; 51(12): 3524−31.
21. *Balanescu S, Corlan AD, Dorobantu M, Gherasim L.* Prognostic value of heart rate variability after acute myocardial infarction. Med Sci Monit 2004; 10(7): CR307−15.
22. *Bigger JT, Fleiss JL, Rolnitzky LM, Steinman RC.* The ability of several short-term measures of RR variability to predict mortality after myocardial infarction. Circulation 1993; 88(3): 927−34.
23. *Lindström J, Tuomilehto J.* The diabetes risk score: A practical tool to predict type 2 diabetes risk. Diabetes Care 2003; 26(3): 725−31.
24. *Silventoinen K, Pankow J, Lindström J, Jousilahti P, Hu G, Tuomilehto J.* The validity of the Finnish Diabetes Risk Score for the prediction of the incidence of coronary heart disease and stroke, and total mortality. Eur J Cardiovasc Prev Rehabil 2005; 12(5): 451−8.
25. *Sztajzel J, Jung M, Bayes de Luna A.* Reproducibility and gender-related differences of heart rate variability during all-day activity in young men and women. Ann Noninvasive Electrocardiol 2008; 13(3): 270−7.
26. *Vesterinen V, Häkkinen K, Hynynen E, Mikkola J, Hokka L, Nummela A.* Heart rate variability in prediction of individual adaptation to endurance training in recreational endurance runners. Scand J Med Sci Sports 2013; 23(2): 171−80.

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ORIGINAL ARTICLE

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# Examination of the bioelectrical activity of the masticatory muscles during Angle's Class II division 2 therapy with an activator

## Ispitivanje bioeleketrične aktivnosti mastikatornih mišića kod strmog zagrižaja tokom terapije aktivatorom

**Djordje Petrović, Sanja Vujkov, Branislava Petronijević, Ivan Šarčev, Igor Stojanac**

**Dental Clinic of Vojvodina, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia**

**Abstract**

**Background/Aim.** The muscles of the orofacial region have great influence on the development of dentition and occlusion formation. It is known that improper function of these muscles is one of the major etiological factors in malocclusion. A correlation between function disorders of orofacial muscle and occlusion disorders has been confirmed, as well as a correlation between the bioelectric activity of the masticatory muscles, recorded by electromyography, and bite force upon maximal voluntary contraction of these muscles. The aim of the study was to analyze the bioelectriacal activity of temporal and masseter muscles. **Methods.** The sample consisted of 100 subjects of both sexes, divided into the control group (n = 30) with neutral and complete dental arches, and the study group (n = 70) of patients with distal occlusion. Electromyographic measurement of bioelectric potentials in all the subjects was conducted for the examined muscles in the physiologic rest position, central mandible occlusion, and during maximal voluntary contraction of muscles and saliva swallowing, in Angle Class I and II/2 occlusal relationships, prior to treatment, after one year of the orthodontic treatment and after the treatment with an activator. **Results.** Comparing the values of thebioelectrical activity in the control and the study group before the treatment, a decreased muscle activity was established in all the three positions in the study group. After the first year of orthodontic treatment the results showed an elevation in the bioelectrical activity in both muscles. After treatment with an activator, the bioelectrical activity in both muscles in the study group was higher than before the treatment, as it is confirmed by a positive highly significant coefficient of correlation. **Conclusion.** In all the three measured positions of the mandible with Angle Class II/2 malocclusion, bioelectrical activity was lowest at baseline and increased during the first year of treatment, and at the end of the treatment it partially reduced close to the approximate values in normal occlusion. Research on electromyographic activity of masticatory muscles is useful in everyday clinical practice, especially in present distinctive skeletal discrepancy before, during and after orthodontic treatment, if on the bases of the results we can evaluate the treatment, but also determine the start and duration of the retention period and retention device type.

**Key words:**
**electromyography; masticatory muscles; malocclusion, angle class I; malocclusion, angle class II; activator appliances; treatment outcome.**

**Apstrakt**

**Uvod/Cilj.** Mišići orofacijalne regije imaju veliki uticaj na razvoj zubnih nizova i formiranje okluzije. Poznato je da je nepravilna funkcija mišića jedan od značajnih etioloških faktora u nastanku malokluzija. Takođe, potvrđena je uzajamna povezanost poremećaja funkcije orofacijalnih mišića i poremećaja okluzije, kao i korelacija između bioelektrične aktivnosti mastikatornih mišića, registrovane elektromiografskom metodom, i ispoljene sile zagrižaja pri maksimalnoj voljnoj kontrakciji ovih mišića. Cilj studije bio je analiza bioelektrične aktivnosti temporalnog i maseteričnog mišića. **Metode.** Uzorak je činilo 100 osoba oba pola, podeljenih u kontrolnu grupu (n = 30) sa neutrookluzijom i potpunim zubnim nizom, i ispitivanu grupu (n = 70) sa distookluzijom. Kod svih ispitanika sprovedeno je elektromiografsko merenje bioelektričnog potencijala za ispitivane mišiće u položaju fiziološkog mirovanja, centralne okluzije mandibule, i pri maksimalnoj voljnoj kontrakciji mišića i gutanju pljuvačke, kod klase I i II/2 okluzalnih odnosa po Anglu, i to pre lečenja, nakon jedne godine ortodontskog lečenja i po završetku lečenja sa aktivatorom. **Rezultati.** Poređenjem vrednosti biolektričnog potenicijala pre lečenja utvrđena je smanjena aktivnost u sva tri položaja praćenih mišića u ispitivanoj grupi u odnosu na kontrolnu. Nakon prve godine ortodontskog tretmana utvrđeno je povišenje bioelektrične

**Correspondence to:** Djordje Petrović, Dental Clinic of Vojvodina, Hajduk Veljkova 12, 21000 Novi Sad, Serbia.
Phone: +381 21 661 22 22. E-mail: petrdj@yahoo.de

aktivnosti oba mišića. Po završetku tretmana aktivatorom, bioelektrična aktivnost oba mišića u ispitivanoj grupi bila je viša u odnosu na vrednosti pre tretmana, što dokazuje pozitivan, veoma značajan koeficijent korelacije. **Zaključak.** U sve tri merene pozicije, pri različitim položajima mandibule kod strmog zagrižaja, bioelektrična aktivnost je bila najmanja na početku terapije i povećavala se tokom prve godine lečenja, da bi se na kraju terapije delimično smanjila na vrednosti približne vrednostima normalne okluzije. Istraživanja elektromiografske aktivnosti mastikatornih mišića imaju smisla u svakodnevnoj kliničkoj praksi, kod izrazitih skeletnih diskrepanci pre, u toku i nakon ortodontske terapije, ukoliko na osnovu njih možemo vrednovati rezultate lečenja, ali i odrediti početak i dužinu trajanja retencionog perioda i vrstu retencionog aparata.

**Ključne reči:**
**elektromiografija; mišići, mastikatorni; malokluzija, klase I; malokluzija, klase II; ortodontski aparati; lečenje, ishod.**

## Introduction

Normal occlusion (*eugnathia*) presents a morphologically and functionally balanced bite. Disruption of this balance leads to the formation of malocclusion, with expected change in force and electromyographic (EMG) activity of the muscles of the orofacial region [1, 2].

The role of the basic functions of the orofacial region in the etiology of malocclusion is relatively unknown, because it is observed that the normal occlusion is often accompanied by markedly disturbed functions of the orofacial musculature. On the other hand, according to the Moss and Chalmers [3] theory, the skeleton is formed in response to the soft tissue. Therefore, it is very important to estimate the functional part in the etiology of certain malocclusion. EMG recording of orofacial muscle activity is important in scientific research, but even more in clinical practice [2, 4]. Craniofacial region is a part of the organism which consists of organs that perform many different functions by motor muscle activity. Jaws and teeth in the rest position are under the constant influence of external and internal muscles of the orofacial region [5]. The muscles of the orofacial region together with other factors (shape and position of the tooth bud, craniofacial skeleton form, etc.) play an important role in teeth setting, dental arch shape modifications and other dentoalveolar structures, establishing jaw relationships, etc. [6]. Morphology of these muscles at the same time depends on the type of diet, sex, age, and state of development of teeth and jaws [7]. Angle has emphasized that muscles have great influence on the development of dentition and occlusion formation. It is well-known that improper function of muscles is one of the major etiological factors in malocclusion [8]. A correlation between function disorders of orofacial muscle and occlusion disorders has also been confirmed. Furthermore, a correlation between the bioelectric activity of the masticatory muscles, recorded by EMG, and bite force upon maximal voluntary contraction of these muscles has been established [9].

The improper function of muscles is one of the major etiological factors in malocclusion. Malocclusions present a common pathological condition of masticatory system. They are characterized by an irregular contact between the maxillary and mandibular teeth that prevent the necessary effectiveness of jaw movement [10]. Malocclusion is usually defined as a consequence of orofacial system growth disorder, trauma, poor oral habits, genetic factors and disorders of masticatory forces balance. Of orthodontics importance are mandible elevating muscles: m. masseter, *m. temporalis, m. pterygoideus medialis*, and mandible depressants such as *m. pterygoideus lateralis* and *m. genioglossus*, which play an important role in facial morphology and tongue function. Also important are the facial muscles such as *m. mentalis* and m. *orbicularis oris* [4, 11].

A specific orofacial anomaly is classified by Angle's sagittal occlusion irregularities as a second class malocclusion (Class II). This malocclusion is also known as degbis [12]. In people with temporal degbis unfavorable type of chewing prevails with a significantly reduced abrasion of posterior teeth. Regarding the function of the orofacial musculature, there is an increased temporal muscle function with masseteric muscle limitation, which is related to the type of chewing [13].

The orofacial muscles region consists of muscles of the head, face and jaws. They differ in origin, structure and physiological properties, and are accordingly divided into: masticatory muscles, facial muscles and muscles of the cranial vault [14].

The group of the masticatory muscles is built of 4 pairs of muscles: temporal, masseteric, outer and inner pterygoid. *M. temporalis* is a broad, flat, fan-shaped muscle that fills temporal pit. Contraction of its anterior and middle fibers elevates the mandible and closes the mouth. Contraction of isolated anterior fibers involves in propulsion, and isolated contraction of posterior muscle fibers in lower jaw retropulsion. Unilateral muscle contractions perform mandible lateral pulsion. Temporal muscle is especially sensitive to occlusal interference and is responsible for the position of the jaw in the vertical direction. Muscle inervation originates from the deep temporal nerves (*temporales profundi*), lateral branches of the mandibular nerve [15]. M. masseter is a short, strong, and thick rectangular muscle, which extends from the zygomatic bone arch, lower to the mandible angle, covering the mandible laterally. *M. masseter* strongly elevates the lower jaw and closes the mouth. Isolated contraction of superficial muscle fibers involves in propulsion, while isolated deep muscle fibres contraction is involved in retropulsion of lower jaw. Unilateral muscle contractions perform mandible lateral pulsion. By its strong action it is involved in food crushing [16]. The muscle innervation originates from masseteric nerve, a lateral branch of the mandibular nerve.

The aim of the study was to analyze the bioelectrical activity (BA) of m. masseter superficialis and *m. temporalis anterior* in different jaw positions both in Angle Class I and

Class II/2 occlusal relations, periodically – before treatment, after one year of orthodontic treatment and after orthodontic treatment with activator.

## Methods

The sample consisted of 100 subjects of both sexes, who were treated at the Dental Clinic of Vojvodina, Novi Sad, divided into the control and the study groups. The control group (30 subjects, aged 8–12 years) were subjects with neutroocclusion and complete dental arches. The study group (70 subjects, aged 8–12 years) consisted of patients with distal occlusion and retrusion of upper incisors (Angle's Class II/2), with complete dental arches, randomly selected from the current Dental Clinic casuistry, with previously signed consent.

In both groups EMG analysis was performed for *m. temporalis anterior* and m. masseter superficialis in the physiologic rest position (PR), mandibular central occlusion (CO), during maximal voluntary contraction of muscles and saliva swallowing (MVC), in normal occlusion (Angle Class I malocclusion) and distal occlusion (Angle Class II/2 malocclusion), before the treatment, after one year of treatment and after orthodontic treatment with the activator. Measurement was done at the constant temperature of 25˚C, using the Medelec Synergy® device. For recording action potentials of the orofacial muscles we used facial intramuscular coaxial electrodes, set according to the Greenfield scheme. Before the testing two lines were determined: horizontal (representing the Frankfurt horizontal line) and vertical (passing through the frontal edge of the external acoustic hole). Then we determined the muscles positions according to their lines: m. masseter 3 cm forward relative to the vertical line and 6 cm below the horizontal line; *m. temporalis* 3.6 cm in front of the vertical line and 5.6 cm above the horizontal line. EMG was performed symmetrically on the left and the right side, for both muscles for 20 seconds. Upon registration EMG activity, EMG device filters are set to 100 Hz–2 KHz. For evaluation of EMG activity in relaxation (physiological rest) registered activity was 10 μV oscilloscope amplification, and the mandibular central occlusion and maximum voluntary muscle contraction was 250–500 μV, depending on subject's EMG activity. To incorporate the summary of muscle bioelectric activity integrator is used, and the mean value of three consecutive measurements were analyzed. Survey results were analyzed visually with an oscilloscope and graphical paper representation, in milivolt (mV), as mean cumulative action potential voltage amplitude.

We used orthodontic records of: history, clinical findings, functional analysis, study models, cephalometric orthopantomographic and profile shots; before, one year after the treatment and after the treatment was complited. To all the study group subjects, lower lip activator pelota and upper incisors proclination padel spring were set. Construction bite brought the lower jaw forward toward class I jaw relationships, with vertical opening of 2–3 mm beyond vertical resting dimension.

The registered values of the orofacial muscles action potential changes in determined positions were compared. We analyzed the correlation between orofacial muscle EMG activity and occlusal relationships. BA data of the muscles were analyzed quantitatively describing the results, classified according to the parameters set out in the study protocol, and presented in tables and figures.

Statistical analysis consisted of standard statistical methods, while the significance of the results was determined by Student's *t*-test ($p < 0.05$). For the analysis of the significance of Pearson's correlation factor ($r$), the scale values for $r$ were used: ± 0.2 – ± 0.01 – no significance, ± 0.4 – ± 0.2 – weak significance, ± 0.7 – ± 0.4 – significant, ± 0.7 – ± 1.0 – very significant correlation.

## Results

All the subjects included in this study were divided into the normal occlusion – Angle Class I (the control group) (n = 30) and the distocclusion group – Angle Class II (the study group) (n = 70). In both groups subjects were 8–12 years old (average age in the control group – 11.23 ± 1.56 years, and in the study group 11.68 ± 1.21 years). The age difference was statistically insignificant ($p > 0.05$).

During the research BA was measured for the selected masticatory muscles in three positions: PR, CO, and MVC.

At the beginig BA was recorded for both groups. According to the results, there was no significant difference in the left temporal muscle between the two groups, while in the right temporal muscle the significance was noted in all three positions ($p < 0.05$) (Table 1). BA analysis of m. mas-

**Table 1**

**Bioelectrical acitivity of *m. temporalis* in the subjects with normal bite (Class I) and distocclusion (Class II)**

| *M. temporalis* (measured position) | Bioelectrical activity (μV) | | | | *r* | *p* |
|---|---|---|---|---|---|---|
| | Class I (n = 30) | | Class II (n = 70) | | | |
| | x̄ ± SD | CV (%) | x̄ ± SD | CV (%) | | |
| Left | | | | | | |
| PR | 8.06 ± 1.94 | 24.09 | 6.8 ± 0.62 | 9.1 | -0.4 | > 0.05 |
| CO | 64.94 ± 19.74 | 30.4 | 52.4 ± 13.97 | 26.66 | -0.25 | > 0.05 |
| MVC | 653.15 ± 109.13 | 16.71 | 535.56 ± 137.73 | 25.72 | -0.39 | > 0.05 |
| Right | | | | | | |
| PR | 7.70 ± 1.79 | 23.28 | 7.06 ± 2.63 | 37.28 | -0.35 | < 0.05 |
| CO | 70.38 ± 32.86 | 46.7 | 57.81 ± 26.0 | 44.98 | -0.36 | < 0.05 |
| MVC | 635.26 ± 252.6 | 39.76 | 559.63 ± 125.92 | 22.5 | -0.39 | < 0.05 |

**PR – physiologic rest position; CO – central occlusion; MVC – maximal voluntary contraction; CV – coefficient of variation; *r* – Pearson's correlation factor.**

seter showed statistical significance ($p < 0.05$) in both left and right muscle in all positions, except CO (Table 2).

(535.56 μV *vs* 663.2 μV, $p > 0.05$), but the correlation was positive and very significant in all the positions, except CO

**Table 2**

**Bioelectrical acitivity of m. masseter in the subjects with normal bite (Class I) and distocclusion (Class II)**

| M. masseter (measured position) | Bioelectrical activity (μV) | | | | *r* | *p* |
|---|---|---|---|---|---|---|
| | Class I (n = 30) | | Class II (n = 70) | | | |
| | x̄ ± SD | CV (%) | x̄ ± SD | CV (%) | | |
| Left | | | | | | |
| PR | 5.77 ± 2.73 | 47.36 | 4.9 ± 1.75 | 35.78 | -0.22 | < 0.05 |
| CO | 81.69 ± 10.63 | 13.01 | 44.97 ± 6.17 | 13.73 | -0.37 | > 0.05 |
| MVC | 604.21 ± 234.63 | 38.83 | 510.12 | 154.50 | 30.29 | -0.26 |
| Right | | | | | | |
| PR | 6.1 ± 2.21 | 36.16 | 5.57 ± 3.08 | 55.3 | -0.31 | < 0.05 |
| CO | 79.66 ± 28.36 | 35.6 | 63.74 ± 19.77 | 31.02 | -0.22 | > 0.05 |
| MVC | 626.12 ± 191.72 | 30.62 | 565.66 ± 179.39 | 31.71 | -0.34 | < 0.05 |

**PR – physiologic rest position; CO – central occlusion; MVC – maximal voluntary contraction; CV – coefficient of variation; *r* – Pearson's correlation factor.**

BA was measured in the study group subjects after the first year of the activator therapy. In both temporal and masseteric muscles there was a statistically significant difference between BA ($p < 0.05$) in all the three positions (Tables 3 and 4). A significant positive correlation ($r$) was established in all measuring positions for temporal muscle, while in the masseteric muscle it was positive and very significant in CO and MVC position for the left, and PR and MVC positions for the right muscle.

in the right muscle ($r = 0.6$), where it was significant (Figure 1).

In the masseteric muscle a statistically significant difference was present only in PR and MVC positions for both left and right muscle ($p < 0.05$), while a correlation was positive and of a very high significance for all the positions in the left and the right masseteric muscle ($r > 0.7$) (Figure 2).

**Table 3**

**Bioelectrical acitivity of *m. temporalis* in the subjects with distocclusion (Class II), before and after a year of the therapy**

| *M. temporalis* (measured position) | Bioelectrical activity (μV) | | | | *r* | *p* |
|---|---|---|---|---|---|---|
| | before therapy | | after year of the therapy | | | |
| | x̄ ± SD | CV (%) | x̄ ± SD | CV (%) | | |
| Left | | | | | | |
| PR | 6.8 ± 0.62 | 9.1 | 7.46 ± 2.79 | 37.32 | 0.85 | < 0.05 |
| CO | 52.40 ± 13.97 | 26.66 | 63.6 ± 16.95 | 26.66 | 0.71 | < 0.05 |
| MVC | 535.56 ± 137.73 | 25.72 | 593.97 ± 147.83 | 24.89 | 0.83 | < 0.05 |
| Right | | | | | | |
| PR | 7.06 ± 2.63 | 37.28 | 7.58 ± 4.39 | 57.92 | 0.79 | < 0.05 |
| CO | 57.81 ± 26.0 | 44.98 | 64.22 ± 28.89 | 44.98 | 0.8 | < 0.05 |
| MVC | 559.63 ± 125.92 | 22.5 | 601.97 ± 205.21 | 34.09 | 0.83 | < 0.05 |

**PR – physiologic rest position; CO – central occlusion; MVC – maximal voluntary contraction; CV – coefficient of variation; *r* – Pearson's correlation factor.**

**Table 4**

**Bioelectrical acitivity of m. masseter in the subjects with distocclusion (Class II), before and after a year of therapy**

| M. masseter (measured position) | Bioelectrical activity (μV) | | | | *r* | *p* |
|---|---|---|---|---|---|---|
| | before therapy | | after year of the therapy | | | |
| | x̄ ± SD | CV (%) | x̄ ± SD | CV (%) | | |
| Left | | | | | | |
| PR | 4.9 ± 1.75 | 35.78 | 5.37 ± 1.92 | 35.78 | 0.66 | < 0.05 |
| CO | 44.97 ± 6.17 | 13.73 | 54.9 ± 12.83 | 23.36 | 0.81 | < 0.05 |
| MVC | 510.12 ± 154.50 | 30.29 | 549.12 ± 166.31 | 30.29 | 0.7 | < 0.05 |
| Right | | | | | | |
| PR | 5.57 ± 3.08 | 55.3 | 5.81 ± 3.21 | 55.30 | 0.75 | < 0.05 |
| CO | 57.88 ± 19.77 | 31.02 | 65.47 ± 20.31 | 31.02 | 0.66 | < 0.05 |
| MVC | 565.66 ± 179.39 | 31.71 | 588.0 ± 186.47 | 31.71 | 0.78 | < 0.05 |

**PR – physiologic rest position; CO – central occlusion; MVC – maximal voluntary contraction; CV – coefficient of variation; *r* – Pearson's correlation factor.**

At the end of research we compared BA in all the positions for both muscles before and at the end of the therapy treatment. In the temporal muscle a statistically significant difference was noted in all positions of the left and the right muscle, except for the right muscle in MVC position

**Discussion**

This electromyographic activity (EA) study on chewing muscles at Angle Class II/2 confirmed the basic starting point that improper orofacial muscle function has an impact

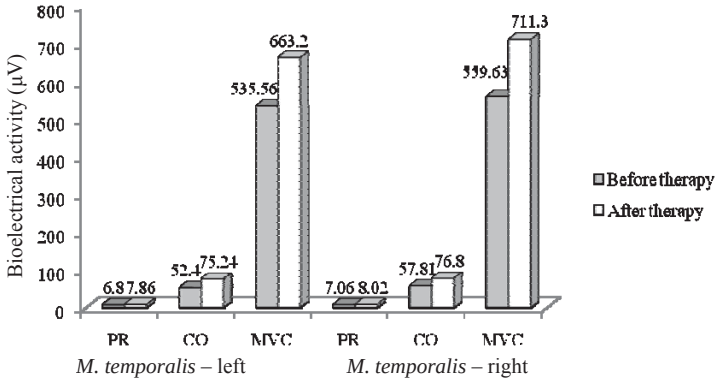


**Fig. 1 –Bioelectrical acitivity of *m. temporalis* in the subjects with distocclusion (Class II), before and at the end of the therapy (PR – physiologic rest position; CO – control occlusion; MVC – maximal voluntary contraction).**

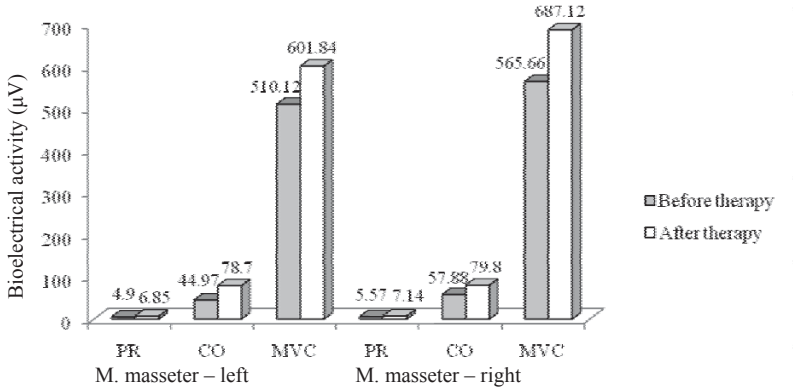


**Fig. 2 – Bioelectrical acitivity of m. masseter in the subjects with distocclusion (Class II), before and at the end of the therapy (PR – physiologic rest position; CO – control occlusion; MVC – maximal voluntary contraction).**

on occlusion and that reeducation of modified function is important in the treatment, affecting treatment results stability.

The results show that in the control group with normal occlusion, Angle Class I malocclusion had a higher mean bioelectrical potential (BP) of *m. temporalis* compared to the m. masseter in the physiologic rest position of the mandible (15.76 μV *vs* 11.87 μV) and at maximum voluntary muscle contraction (1288.41 μV *vs* 1230.33 μV). Deviations are defined only in central occlusion, where m. masseter showed higher BP compared to *m. temporalis* (161.35 μV vs. 135.32 μV), while during maximal voluntary contraction m. masseter showed BP close to temporal muscle activity, but did not reach it. These results are consistent with results of studies by other authors and the cause may be the temporal chewing type which dominates in this group. However, the results of the study by Shi and An [17] indicate a higher activity in the masseteric muscle in normal occlusion compared to distocclusion. These variations can be explained as a consequence of stress and psychological factors, which are reported in findings of Ingervall and Bitsanis [18], and are followed by the increase in impulses coming into the muscle from the central nervous system in children, during the first registration of BA of muscles.

Ma et al. [19] noted that independantly on the occlusion temporal muscles were more active in the postural position of the mandible, and the masseter in central occlusion during maximal voluntary contraction of masticatory muscles.

Comparing the results of Castroflorio et al. [20] with the results of this study, we can conclude that all of the registered values of masticatory muscles action potentials in our study, are proportionally higher, which is directly related to the increased EMG calibration of our device.

The subjects with Angle Class II/2 malocclusion showed a greater BA of *m. temporalis* compared to m. masseter in all the measured positions, which can be the consequence of the dominant type of chewing in this group.

Comparative analysis of the BA of the masticatory muscles in physiological rest, central occlusion of the mandible and at maximal voluntary muscle contraction in normal and distal occlusion, showed that all the registered nominal values of action potential increased with a statistical significance in central occlusion of the mandible, in the control group compared with the experimental group of subjects ($p < 0.05$). These results may be the consequence of the decrease in muscle tone of the disturbed occlusal relationship, and as the result of reducion in the number of active muscle fibers and impulses coming from the central nervous system,

and the consequential reduction in activated motor units, or a combination of both. Our results are compatible with this results of other authors [3, 21, 22]. Antonarakis et al. [23] point out that unlike the Class I malocclusion, where the muscle function is normal, except in cases of open bite, in Angle Class II malocclusion there is abnormal muscle activity [23]. Lowe et al. [21] suggests that there is a dependent relationship between skeletal morphology (size of the mandibular angle and the degree of parallelism in jaw bases) and EMG amplitude of the anterior temporal and masseter muscles during maximal voluntary contraction [21]. According to Tuncer et al. [24], damaged muscle activity at Angle Class II/2 can be attributed to different dentofacial morphology and unstable conditions of occlusal contact.

The EA of the masticatory muscles in Angle Class II/2 malocclusion significantly increased during the first year of the treatment with the activator compared to the start of the therapy. Increasing potential action is approximately equal for both observed muscles. At the end of the therapy both muscles activity decreased approaching the values of the action potentials of muscles in normal occlusion, but stil not reaching it. The bioelectric potential values of masticatory muscles at the end of the treatment were significantly higher compared to their values before the treatment ($p < 0.05$). These results are compatible with the results of Ingervall and Bitsanis [18] and Stavridi and Ahlgren [25].

Uner et al. [26] have showed the effects of activators on the masticatory muscles in distal occlusion and found an increased activity in the masseter muscle in the physiologic rest position of the mandible at the beginning of treatment, and the reduction of the activity at the end of treatment, while in the control group there was no change in the recorded EMG actions of the muscles examined.

Ingervall and Bitsanis [18] have observed the function of the masticatory muscles in the first six months and in the first year after the initiation of the activator therapy in patients with distal occlusion, and concluded that the increased muscle activity of the temporal muscle, after starting the treatment, gradually decreased as the result of adaptation to the new position of the mandible. Activity of masseteric and temporal muscle during maximal voluntary contraction and chewing was influenced by the incisal instability, and the changes in the activity of these muscles were slight.

Stavridi and Ahlgren [25] have reported an increase of EA in the masseter muscle during swallowing in patients with distal occlusion treated with an activator.

Pancherz et al. [27] concluded, by measuring the action potentials of masseter and temporal muscles in patients with distal occlusion, that the reduced activity of these muscles after treatment with activator and Herbst appliance, gradually increased, approaching the values obtained with normal occlusion, which is compatible with the results of our study.

The BA of the masticatory muscles certainly has an impact on clinical status of malocclusions, but also on performance of their functions and coordination during the various mandible movements.

## Conclusion

The bioelectrical activity of the masticatory muscles in the position of physiologic mandible rest and in maximal voluntary muscle contraction, in Angle Class I malocclusion, was higher in *m. temporalis*, while in the central occlusion position m. masseter showed a higher activity. In Angle Class II/2 malocclusion occlusal relationships, prior to be therapy, the bioelectric activity was higher in *m. temporalis*. Bioelectrical activity of both muscles in all the three measured positions was nominally higher in Class I occlusal relationships when compared to Angle Class II/2 malocclusion. In all the three different mandible positions with Angle Class II/2 malocclusion, bioelectrical activity was lowest at the beginnig of the therapy, and increased during the first year of treatment, and by the end of the treatment it was partially reduced to the value of normal occlusion. Masticatory muscles electromyographic activity research is useful in everyday clinical practice, especially in the present distinctive skeletal discrepancy before, during and after the orthodontic treatment, if on the bases of the results we can evaluate the treatment, but also determine the start and the duration of a retention period and retention device type.

## Conflict of Interest

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

## REFERENCES


1. *Kahl-Nieke B.* Einführung in die Keiferortopädie. 2nd ed. München-Jena: Aufl. Urban & Fischer; 2001.
2. *Cuevas MJ, Cacho A, Alarcón JA, Martín C.* Longitudinal evaluation of jaw muscle activity and mandibular kinematics in young patients with Class II malocclusion treated with the Teuscher activator. Med Oral Patol Oral Cir Bucal 2013; 18(3): 497−504.
3. *Moss JP, Chalmers CP.* An electromyographic investigation of patients with a normal jaw relationship and a Class III jaw relationship. Am J Orthod 1974; 66(5): 538−56.
4. *Meenakshi I, Ashima V.* Electromyography and its application in orthodontics. Curr Sci 2001; 80(4): 503−7.
5. *Cuccia AM.* Interrelationships between dental occlusion and plantar arch. J Bodyw Mov Ther 2011; 15(2): 242−50.
6. *Marquezin M, Gavião M, Alonso M, Ramirez-Sotelo L, Haiter-Neto F, Castelo P.* Relationship between orofacial function, dentofacial morphology, and bite force in young subjects. Oral Dis 2013; doi: 10.1111/odi.12174. (In Press)
7. *Marquezin MC, Kobayashi FY, Montes AB, Gavião MB, Castelo PM.* Assessment of masticatory performance, bite force, orthodontic treatment need and orofacial dysfunction in children and adolescents. Arch Oral Biol 2013; 58(3): 286−92.
8. *Dolce C, Mansour DA, McGorray SP, Wheeler TT.* Intrarater agreement about the etiology of Class II malocclusion and

treatment approach. Am J Orthod Dentofacial Orthop 2012; 141(1): 17−23.
9. *Lin LH, Huang GW, Chen CS*. Etiology and Treatment Modalities of Anterior Open Bite Malocclusion. J Experiment Clin Med 2013; 5(1): 1−4.
10. *Sciote JJ, Raoul G, Ferri J, Close J, Horton MJ, Rowlerson A*. Masseter function and skeletal malocclusion. Rev Stomatol Chir Maxillofac Chir Orale 2013; 114(2): 79−85.
11. *Uysal T, Yagci A, Okkesim KS*. Influence of Pre-Orthodontic Trainer treatment on the perioral and masticatory muscles in patients with Class II division 1 malocclusion. Eur J Orthodont 2012; 34(1): 96−101.
12. *Proffit WR, Fields HW, Sarver DM*. Contemporary orthodontics. 4th ed. St. Louis, Mo: Mosby Year Book; 2006.
13. *Ambrosio AR, Trevilatto PC, Martins LP, Santos-Pinto AD, Shimizu RH*. Electromyographic evaluation of the upper lip according to the breathing mode: a longitudinal study. Braz Oral Res 2009; 23(4): 415−23.
14. *Tuijt M, Koolstra JH, Lobbezoo F, Naeije M*. Differences in loading of the temporomandibular joint during opening and closing of the jaw. J Biomech 2010; 43(6): 1048−54.
15. *Tecco S, Crincoli V, Di Bisceglie B, Caputi S, Festa F*. Relation between facial morphology on lateral skull radiographs and sEMG activity of head, neck, and trunk muscles in Caucasian adult females. J Electromyogr Kinesiol 2011; 21(2): 298−310.
16. *Linsen S, Schmidt-Beer U, Fimmers R, Grüner M, Koeck B*. Craniomandibular pain, bite force, and oral health-related quality of life in patients with jaw resection. J Pain Symptom Manage 2009; 37(1): 94−106.
17. *Shi CS, An Y*. Observation of proportionality of myoelectrical activity of anterior temporalis to masseter muscle during clenching at varied jaw positions. J Oral Rehabil 1992; 19(5): 539−43.
18. *Ingervall B, Bitsanis E*. Function of masticatory muscles during the initial phase of activator treatment. Eur J Orthod 1986; 8(3): 172−84.
19. *Ma SY, Whittle T, Descallar J, Murray GM, Darendeliler MA, Cistulli P*, et al. Association between resting jaw muscle electromyographic activity and mandibular advancement splint outcome in patients with obstructive sleep apnea. Am J Orthod Dentofacial Orthop 2013; 144(3): 357−67.
20. *Castroflorio T, Falla D, Tartaglia GM, Sforza C, Deregibus A*. Myoelectric manifestations of jaw elevator muscle fatigue and recovery in healthy and TMD subjects. J Oral Rehabil 2012; 39(9): 648−58.
21. *Lowe AA, Takada K, Taylor LM*. Muscle activity during function and its correlation with craniofacial morphology in a sample of subjects with Class II, Division 1 malocclusions. Am J Orthod 1983; 84(3): 204−11.
22. *Miralles R, Berger B, Bull R, Manns A, Carvajal R*. Influence of the activator on electromyographic activity of mandibular elevator muscles. Am J Orthod Dentofacial Orthop 1988; 94(2): 97−103.
23. *Antonarakis GS, Kjellberg H, Kiliaridis S*. Bite force and its association with stability following Class II/1 functional appliance treatment. Eur J Orthod 2012; 35(4): 434−41.
24. *Tuncer BB, Ozogul B, Akkaya S*. Differences in opening and protrusive mandibular movements between Class I and II malocclusions in healthy adolescents. Korean J Orthod 2011; 41(2): 127−37.
25. *Stavridi R, Ahlgren J*. Muscle response to the oral-screen activator. An EMG study of the masseter, buccinator, and mentalis muscles. Eur J Orthod 1992; 14(5): 339−49.
26. *Uner O, Darendeliler N, Bilir E*. Effects of an activator on the masseter and anterior temporal muscle activities in Class II malocclusions. J Clin Pediatr Dent 1999; 23(4): 327−32.
27. *Pancherz H, Anehus-Pancherz M*. Muscle activity in class II, division 1 malocclusions treated by bite jumping with the Herbst appliance. An electromyographic study. Am J Orthod Dentofacial Orthop 1980; 78(3): 321−9.

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# Subclinical hypothyroidism in children and adolescents after hematopoietic stem cells transplantation without irradiation

## Supklinički hipotireoidizam posle transplantacije matičnih ćelija hematopoeze kod dece i adolescenata koji nisu dobijali radioterapiju

**Tatjana Milenković*, Dragana Vujić†‡, Rade Vuković*, Željko Zečević†, Ivan Soldatović§, Katarina Mitrović*, Sladjana Todorović*, Dragan Zdravković*‡**

***Department of Endocrinology, †Pediatric HSCT Center, Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić", Belgrade, Serbia; §Institute of Medical Statistics, Faculty of Medicine, University of Belgrade, Belgrade, Serbia; ‡Faculty of Medicine, University of Belgrade, Belgrade, Serbia**

**Abstract**

**Background/Aim.** Although total body irradiation (TBI) was considered to be the primary cause of thyroid dysfunction following hematopoietic stem cells transplantation (HSCT), a significant prevalence of subclinical hypothyroidism after HSCT with chemotherapy-only conditioning regimens has been observed in several studies. The aim of this study was to assess changes in thyroid stimulating hormone (TSH) levels in children after HSCT, without the use of irradiation at any time in the course of the treatment. **Methods.** Our cohort consisted of 41 children and adolescents who underwent autologous or allogeneic HSCT and were available for follow-up for at least one year after transplantation. Irradiation was not performed in any of the subjects, neither during pretransplatation therapy, nor during conditioning. The median duration of follow-up was 2.9 years. The indications for HSCT were hematologic malignancy (41.5%), solid malignant tumor (34.1%), and other disorders (24.4%). The thyroid status of all the subjects was assessed prior to HSCT and after follow-up period. **Results.** Thyroid dysfunction after HSCT was present in 27 (65.8%) subjects. Subclinical hypothyroidism was the most common abnormality, presenting in 23 (56.1%) patients, primary hypothyroidism was present in one (2.4%) patient, while 3 (7.3%) subjects had low free T4 with normal TSH values. Significantly ($p < 0.01$) higher elevations in TSH levels were present in the patients who received chemotherapy for the underlying disease prior to HSCT. **Conclusion.** Our findings emphasize the need for long-term monitoring of thyroid function following HSCT, regardless of whether or not irradiation was used.

**Key words:**
**hematopoietic stem cell transplantation; child; adolescent; hypothyroidism; radiotherapy.**

**Apstrakt**

**Uvod/Cilj.** Iako se smatra da je radioterapija posle transplantacije matičnih ćelija hematopoeze (TMĆH) glavni uzrok poremećaja tireoidne funkcije, u više istraživanja utvrđena je značajna prevalencija supkliničkog hipotireoidizma posle TMĆH kada u sklopu kondicioniranja nije korišćena radioterapija, već isključivo hemioterapija. Cilj ovog istraživanja bio je procena promene u nivoima tireostimulišućeg hormona (TSH) posle TMĆH kod dece koja nisu zračena tokom lečenja. **Metode.** Ispitivana grupa dece sastojala se od 41 deteta i adolescenta kojima je učinjena autologna ili alogena TMĆH i koji su praćeni najmanje godinu dana posle transplantacije. Radioterapija nije primenjivana kod ispitanika, ni tokom pretransplantacione terapije, niti tokom kondicioniranja. Prosečno vreme praćenja iznosilo je 2,9 godina. Indikacije za TMĆH bile su: hematološko maligno oboljenje (41,5%), solidni maligni tumori (34,1%) i druga oboljenja (24,4%). Tireoidna funkcija svih ispitanika procenjena je pre TMĆH i na kraju perioda praćenja. **Rezultati.** Tireoidna disfunkcija posle TMĆH utvrđena je kod 27 (65,8%) ispitanika. Najčešći poremećaj funkcije bio je supklinički hipotireoidizam, kod 23 (56,1%) ispitanika, primarni hipotireoidizam kod jednog (2,4%) bolesnika, dok su 3 (7,3%) ispitanika imala niske nivoe slobodnog tiroksina i normalne vrednosti TSH. Značajno ($p < 0.01$) veća tendencija povećanja koncentracija TSH uočena je kod bolesnika koji su dobijali hemioterapiju u sklopu lečenja osnovne bolesti pre TMĆH. **Zaključak.** Nalazi učinjenog ispitivanja ukazuju na neophodnost dugoročnog praćenja tireoidne funkcije posle transplantacije matičnih ćelja hematopoeze, bez obzira na primenu radioterapije.

**Ključne reči:**
**transplantacija hematopoeznih matičnih ćelija; deca; adolescenti; hipotireoidizam; radioterapija.**

**Correspondence to:** Rade Vuković, Mother and Child Health Care Institute of Serbia "Dr Vukan Čupić", Radoja Dakića 8, 11 070 Belgrade, Serbia. Phone: +381 11 3108 193. E-mail: radevukovic9@gmail.com

## Introduction

The increasing number of performed autologous and allogeneic hematopoietic stem cells transplantations (HSCT) in the treatment of acquired and inborn disorders, along with a substantial decrease in early patient mortality has resulted in an increased frequency and importance of late effects of HSCT, especially in children [1–7]. The endocrine system is well-known to be highly sensitive to both cytotoxic drugs and radiation used in pretransplant conditioning regimens [8–10]. Disorders of thyroid function are the commonest endocrine complications of HSCT in childhood, most notably subclinical hypothyroidism which usually presents within one year after HSCT with reported incidence of up to 40% [11–13]. Although total body irradiation (TBI) was considered to be the primary cause for thyroid dysfunction following HSCT, these disorders have also been observed after HSCT with chemotherapy-only conditioning regimens [14–17]. The role of other factors associated with the development of hypothyroidism following HSCT remains unclear, including the influence of the underlying disorders, graft *versus* host disease or post-HSCT immune reconstitution [11, 18]. Data regarding this matter are scarce, and although several studies investigated hypothyroidism as a late complication of childhood HSCT without the use of TBI in conditioning, most of them were cross-sectional in design, with no record of the thyroid status of subjects prior to HSCT, and the patients who received TBI or thyroid irradiation treatment before conditioning for HSCT were not excluded [11, 15].

The aim of this study was to assess the frequency of hypothyroidism and the factors associated with changes in thyroid stimulating hormone (TSH) levels in children and adolescents after HSCT, without the use of TBI or thyroid irradiation at any time in the course of the treatment.

## Methods

We studied a group of 41 children and adolescents who underwent autologous (n = 24) or allogeneic (n = 17) HSCT at the Institute of Mother and Child Health Care of Serbia "Dr Vukan Ćupić", and who were available for follow-up for at least one year after transplantation. There were 10 female and 31 male subjects, aged 2.7–20.5 years (median age 11.4 years). HSCT was performed at the mean age of 8.2 ± 5.3 years, and median duration of follow-up was 2.9 (range 1.0–6.0) years. The mean age at the diagnosis of the underlying disease was 7.2 ± 5.3 years, and the indications for HSCT were: hematologic malignancy (41.5%), namely acute myeloid leukemia (n = 7), chronic myelogenous leukemia (n = 2), acute biphenotypic leukemia (n = 1), Hodgkin lymphoma (n = 3) and Burkitt lymphoma (n = 4); solid malignant tumor (34.1%), namely neuroblastoma (n = 10), medulloblastoma (n = 2) and Ewing's sarcoma (n = 2); and other disorders (24.4%), namely aplastic anemia (n = 4), Wiskott–Aldrich syndrome (n = 2), β-thalassemia major, hemophagocytic lymphohistiocytosis, mixed connective tissue disease and Omenn syndrome (n = 4).

All the patients were clinically euthyroid and none were treated with L-thyroxin prior to HSCT. The thyroid status of all the subjects, namely TSH and T4/ free T4 (in regards to the analysis used at the time of HSCT), was assessed prior to HSCT and after the follow-up period. Normal thyroid status was defined by low or normal TSH with normal T4 or free T4, subclinical hypothyroidism by elevated TSH with normal T4 or free T4, primary hypothyroidism by high TSH concomitant with low T4 or free T4 and central hypothyroidism by low T4 or free T4 and low or normal TSH. T4 was measured by radioimmunoassay (RIA T4, INEP; laboratory normal range: 55.0–160.0 nmol/L), free T4 was measured by electrochemiluminescence immunoassay (ECLIA Cobas e411, Roche; laboratory normal range: 12.0–22.0 pmol/L) and serum TSH was measured using electrochemiluminescence immunoassay (ECLIA Cobas e411, Roche; laboratory normal range 0.27–4.20 mIU/L). Although for descriptive purposes TSH concentrations higher than 4.0 mIU/L were considered as elevated in our study, due to different physiologic reference intervals in children in regards to age and gender, changes in TSH levels pre- and post-HSCT were also calculated and analyzed [19].

Irradiation was not performed in any of the subjects, neither during pretransplatation therapy, nor during conditioning. The therapy for underlying disease prior to HSCT in 34 (82.9%) patients consisted of chemotherapy, while the remaining seven (17.1%) patients did not receive chemotherapy prior to HSCT conditioning. All subjects received conditioning regiments without irradiation. As part of pretransplantation regimen, 14 (34.1%) patients received corticosteroids for more than 4 weeks, 10 (24.4%) received antithymocyte globulin. Nine (21.9%) patients were given methotrexate for prophylaxis of graft *versus* host disease.

The differences in the means of variables between the groups were tested using both parametric and nonparametric tests depending on the distribution of the variables. Comparisons were performed using Student's *t*-test in the case of normally distributed continuous variables or Mann–Whitney *U* test for non-normally distributed continuous variables. Paired samples *t*-test, Wilcoxon signed-rank test and McNemar's test were used to test the differences before and after the treatment. Spearman's correlation analysis was used to test the relationship between the TSH change and other numerical variables. Probability values of less than 0.05 were considered to be statistically significant, and values were expressed as frequencies or means ± SD unless otherwise stated. SPSS version 15.0 (SPSS, Chicago, IL) was used for statistical analysis.

## Results

We found clinical and/or laboratory evidence of thyroid dysfunction after HSCT in 27 (65.8%) subjects. Subclinical hypothyroidism was the most common abnormality, presenting in 23 (56.1%) patients, primary hypothyroidism was present in one (2.4%) patient, while 3 (7.3%) subjects had low free T4 with normal TSH values. Among the 24 subjects with elevated TSH levels after HSCT (range 4.08–9.34

mIU/L), six had TSH > 4.0 mIU/L prior to HSCT (range 4.05–5.55 mIU/L).

According to McNemar's test, a statistically significant difference ($p < 0.001$) was observed regarding the number of subjects with elevated TSH levels prior to HSCT (n = 8) and after HSCT (n = 24), and as shown in Table 1, mean TSH levels of all subjects after HSCT were significantly higher than mean TSH levels prior to HSCT.

No significant differences were observed in the changes of TSH levels in regards to gender, underlying disease or HSCT type (Table 1). Correlation analysis showed no correlation between the changes in TSH levels after HSCT and the age at diagnosis ($\rho$ = 0,053; $p$ = 0,753), age at HSCT ($\rho$ = 0,135; $p$ = 0,411) or the follow up period ($\rho$ = -0.034; $p$ = 0,839). Also, no correlation was found between TSH levels prior to HSCT and these parameters.

No significant differences were observed between all the groups shown in Table 1 in regards to TSH levels before HSCT, while higher post-HSCT TSH levels were observed with a borderline significance ($p$ = 0.061) in subjects who received chemotherapy prior to HSCT.

Although a greater elevation in the mean TSH levels after HSCT was observed in the patients who underwent autologous HSCT and those who received corticosteroids for more than four weeks, as a well as lower TSH rise in the subjects who received methotrexate and antithymocyte globulin (Table 1), these findings were not statistically significant. In nine patients who developed graft *versus* host disease, no significant differences were observed in either post-HSCT TSH levels or in relative changes in TSH levels after HSCT. However, analysis showed significantly ($p$ = 0.003) higher elevations in TSH levels in the patients who received chemotherapy for the underlying disease prior to HSCT (Table 1, Figure 1).

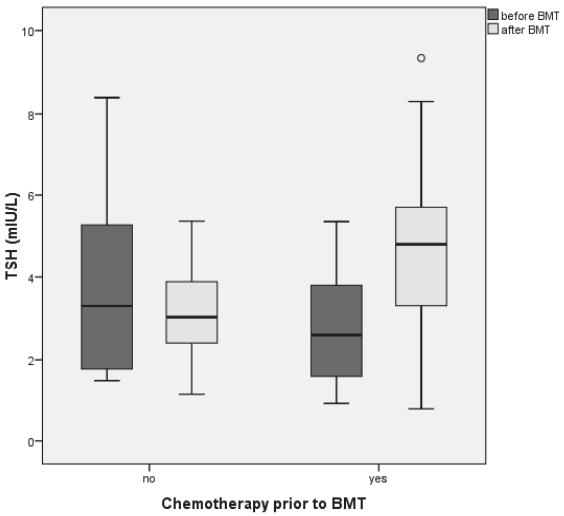


**Fig. 1 – Thyroid-stimulating hormone (TSH) levels before and after hematopoietic stem cells transplantation (HSCT) in regards to chemotherapy prior to HSCT.**

## Discussion

Compensated hypothyroidism defined as an elevation of TSH levels in the presence of normal thyroid hormone concentrations, is recognized as one of the most frequent complications of HSCT [12, 20]. According to the results of previous studies, the occurrence of hypothyroidism in children after HSCT has been observed in up to 58% of patients [21]. In

**Table 1**

**Thyroid-stimulating hormone (TSH) levels before and after hematopoietic stom cell transplantation (HSCT) in regards to selected clinical parameters**

| Parameters | n | TSH (mIU/L) [a] | | |
|---|---|---|---|---|
| | | before HSCT | after HSCT | *p*-value |
| Total | 41 | 2.96 ± 1.58 | 4.32 ± 1.88 | < 0.001[a] |
| Gender | | | | NS [b] |
| male | 31 | 3.15 ± 1.58 | 4.46 ± 1.90 | 0.001[a] |
| female | 10 | 2.36 ± 1.57 | 3.92 ± 1.87 | 0.015[c] |
| Indication for HSCT | | | | NS[b] |
| hematologic malignancy | 17 | 3.03 ± 1.39 | 4.77 ± 2.23 | 0.001[c] |
| solid tumors and other | 24 | 2.92 ± 1.75 | 4.00 ± 1.58 | 0.024[c] |
| Type of HSCT | | | | NS[b] |
| autologous | 24 | 2.77 ± 1.20 | 4.52 ± 2.00 | < 0.001[c] |
| allogeneic | 17 | 3.28 ± 2.08 | 4.04 ± 1.74 | NS[c] |
| Chemotherapy prior to HSCT | | | | 0.003[b] |
| no | 7 | 3.93 ± 2.57 | 3.16 ± 1.36 | NS[c] |
| yes | 34 | 2.79 ± 1.33 | 4.56 ± 1.91 | < 0.001[a] |
| Corticosteroid use > 4 weeks | | | | NS[b] |
| no | 27 | 3.14 ± 1.73 | 4.29 ± 1.73 | 0.005[c] |
| yes | 14 | 2.57 ± 1.18 | 4.39 ± 2.23 | 0.005[a] |
| Methotrexate | | | | NS[b] |
| no | 32 | 2.95 ± 1.63 | 4.51 ± 1.96 | < 0.001[a] |
| yes | 9 | 2.68 ± 1.28 | 3.51 ± 1.50 | NS[c] |
| Antithymocyte globulin | | | | NS[b] |
| no | 31 | 2.79 ± 1.29 | 4.39 ± 1.90 | < 0.001[a] |
| yes | 10 | 3.52 ± 2.34 | 4.11 ± 1.94 | NS[c] |

**All the values expressed as mean ± SD; NS – no significant difference ($p > 0.05$); [a]analysis performed by *t*-test; [b]analysis performed by Mann-Whitney *U* test for group comparison of TSH change; [c]analysis performed by Wilcoxon signed-rank test.**

our cohort, subclinical hypothyroidism as the most common abnormality of thyroid function after HSCT was discovered in 56.1% of the patients. Although this finding correlates well and is simple to compare to the results of other studies, having in mind that physiologic reference intervals for TSH in children differ significantly in regards to age and gender [19], we focused on the dynamics of TSH change after HSCT in the present study. These analyses confirmed a significant increase in TSH in our cohort after HSCT, which is an important finding having in mind that previous studies in the prevalence of thyroid dysfunction after HSCT without irradiation investigated only post-HSCT TSH levels, with no data regarding thyroid status prior to HSCT [11]. Thus, the results of our study complement and further strengthen the results of other studies regarding thyroid dysfunction after HSCT with chemotherapy-only conditioning regimens.

Although thyroid dysfunction after HSCT was mainly linked to the use of TBI [12, 22, 23], the results obtained in our cohort from subjects that were not exposed to any kind of irradiation therapy during treatment, show a high percentage of thyroid abnormalities after HSCT with chemotherapy-only conditioning regimens. This correlates with other reports that indicate that TBI or thyroid irradiation is not the only cause of thyroid dysfunction following HSCT [11, 12, 14, 15, 24]. Other proposed factors inducing thyroid injury either directly or indirectly by modifying immune processes include the underlying disease, the process of HSCT, immune reconstitution and chemotherapy [12, 15, 18, 25]. In our cohort, the children who received chemotherapy prior to HSCT conditioning had a significant rise in TSH levels after HSCT compared to other children, who had a slight decrease in TSH levels after HSCT. Since these groups did not differ significantly in regards to TSH levels before HSCT, a statistically significant difference in both the change and the post-HSCT TSH levels between these groups, indicates that the previous chemotherapy represents a significant risk factor for hypothyroidism following HSCT with chemotherapy-only conditioning regimens.

The present study is partially limited by a relatively small number of subjects in our cohort. However, our results are strengthened by a well-defined sample of children and adolescents who underwent HSCT without the use of TBI or thyroid irradiation at any time in the course of the treatment. Also, to our knowledge, this is the first study to evaluate the pre- and post-HSCT dynamics of TSH levels following HSCT with chemotherapy-only conditioning regimens.

## Conclusion

Our results demonstrate a substantial rise in thyroid stimulating hormone levels following hematopoietic stem cells transplantation without the use of total body irradiation or thyroid irradiation. The most common thyroid disorder was subclinical hypothyroidism, and chemotherapy prior to hematopoietic stem cells transplantation conditioning was the most significant factor associated with the increase in thyroid stimulating hormone levels after hematopoietic stem cells transplantation. These findings emphasize the need for long-term monitoring of thyroid function following hematopoietic stem cells transplantation, regardless of whether or not irradiation was used during conditioning.

## REFERENCES


1. *Mulcahy LJM, Tello T, Giller R, Wilkening G, Quinones R, Keating AK,* et al.. Late effects of total body irradiation and hematopoietic stem cell transplant in children under 3 years of age. Pediatr Blood Cancer 2013; 60(4): 700−4.
2. *Perkins JL, Kunin-Batson AS, Youngren NM, Ness KK, Ulrich KJ, Hansen MJ,* et al.. Long-term follow-up of children who underwent hematopoeitic cell transplant (HCT) for AML or ALL at less than 3 years of age. Pediatr Blood Cancer 2007; 49(7): 958−63.
3. *Giri N, Davis EA, Vowels MR.* Long-term complications following bone marrow transplantation in children. J Paediatr Child Health 1993; 29(3): 201−5.
4. *Sanders J, Sullivan K, Witherspoon R, Doney K, Anasetti C, Beatty P,* et al. Long term effects and quality of life in children and adults after marrow transplantation. Bone Marrow Transplant 1989; 4(Suppl 4): 27−9.
5. *Sanders JE.* Endocrine complications of high-dose therapy with stem cell transplantation. Pediatr Transplant 2004; 8(Suppl 5): 39−50.
6. *Bresters D, van Gils IC, Kollen WJ, Ball LM, Oostdijk W, van der Bom JG,* et al. High burden of late effects after haematopoietic stem cell transplantation in childhood: a single-centre study. Bone Marrow Transplant 2010; 45(1): 79−85.
7. *Baker KS, Ness KK, Weisdorf D, Francisco L, Sun CL, Forman S,* et al. Late effects in survivors of acute leukemia treated with hematopoietic cell transplantation: a report from the Bone Marrow Transplant Survivor Study. Leukemia 2010; 24(12): 2039−47.
8. *Bailey HK, Kappy MS, Giller RH, Gralla J.* Time-course and risk factors of hypothyroidism following allogeneic hematopoietic stem cell transplantation (HSCT) in children conditioned with fractionated total body irradiation. Pediatr Blood Cancer 2008; 51(3): 405−9.
9. *Shalitin S, Phillip M, Stein J, Goshen Y, Carmi D, Yaniv I.* Endocrine dysfunction and parameters of the metabolic syndrome after bone marrow transplantation during childhood and adolescence. Bone Marrow Transplant 2006; 37(12): 1109−17.
10. *Shalet SM.* Endocrine consequences of treatment of malignant disease. Arch Dis Child 1989; 64(11): 1635−41.
11. *Slatter MA, Gennery AR, Cheetham TD, Bhattacharya A, Crooks BN, Flood TJ,* et al. Thyroid dysfunction after bone marrow transplantation for primary immunodeficiency without the use of total body irradiation in conditioning. Bone Marrow Transplant 2004; 33(9): 949−53.
12. *Al-Fiar FZ, Colwill R, Lipton JH, Fyles G, Spaner D, Messner H.* Abnormal thyroid stimulating hormone (TSH) levels in adults following allogeneic bone marrow transplants. Bone Marrow Transplant 1997; 19(10): 1019−22.
13. *Katsanis E, Shapiro RS, Robison LL, Haake RJ, Kim T, Pescovitz OH,* et al. Thyroid dysfunction following bone marrow transplantation: long-term follow-up of 80 pediatric patients. Bone Marrow Transplant 1990; 5(5): 335−40.
14. *Afify Z, Shaw PJ, Clavano-Harding A, Cowell CT.* Growth and endocrine function in children with acute myeloid leukaemia after bone marrow transplantation using busulfan/cyclo-

phosphamide. Bone Marrow Transplant 2000; 25(10): 1087−92.
15. *Toubert ME, Socié G, Gluckman E, Aractingi S, Espérou H, Devergie A*, et al. Short- and long-term follow-up of thyroid dysfunction after allogeneic bone marrow transplantation without the use of preparative total body irradiation. Br J Haematol 1997; 98(2): 453−7.
16. *Fisher LV*. Term follow-up in hematopoietic stem-cell transplant patients. Pediatr Transplant 1999; 3(Suppl 1): 122−9.
17. *Borgström B, Bolme P*. Thyroid function in children after allogeneic bone marrow transplantation. Bone Marrow Transplant 1994; 13(1): 59−64.
18. *Sherer Y, Shoenfeld Y*. Autoimmune diseases and autoimmunity post-bone marrow transplantation. Bone Marrow Transplant 1998; 22(9): 873−81.
19. *Zurakowski D, di Canzio J, Majzoub JA*. Pediatric reference intervals for serum thyroxine, triiodothyronine, thyrotropin, and free thyroxine. Clin Chem 1999; 45(7): 1087−91.
20. *Matsumoto M, Ishiguro H, Tomita Y, Inoue H, Yasuda Y, Shimizu T,* et al. Changes in thyroid function after bone marrow transplant in young patients. Pediatr Int 2004; 46(3): 291−5.
21. *Sanders JE, Hoffmeister PA, Woolfrey AE, Carpenter PA, Storer BE, Storb RF*, et al. Thyroid function following hematopoietic cell transplantation in children: 30 years' experience. Blood 2009; 113(2): 306−8.
22. *Sanders JE*. Late effects in children receiving total body irradiation for bone marrow transplantation. Radiother Oncol 1990; 18(uppl 1): 82−7.
23. *Leiper AD, Stanhope R, Lau T, Grant DB, Blacklock H, Chessells JM,* et al. The effect of total body irradiation and bone marrow transplantation during childhood and adolescence on growth and endocrine function. Br J Haematol 1987; 67(4): 419−26.
24. *Michel G, Socié G, Gebhard F, Bernaudin F, Thuret I, Vannier JP,* et al. Late effects of allogeneic bone marrow transplantation for children with acute myeloblastic leukemia in first complete remission: the impact of conditioning regimen without total-body irradiation: a report from the Société Française de Greffe. J Clin Oncol 1997; 15(6): 2238−46.
25. *Brennan BM, Shalet SM*. Endocrine late effects after bone marrow transplant. Br J Haematol 2002; 118(1): 58−66.

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# Retrobulbar hemodynamic parameters in men and women with open angle glaucoma

## Parametri retrobulbarne cirkulacije kod muškaraca i žena sa glaukomom otvorenog ugla

**Ivan Marjanović*†, Marija Marjanović‡, Ranko Gvozdenović§, Dušica Risović†‖**

**†Faculty of Medicine, University of Belgrade, Belgrade, Serbia, *Institute of Ophthalmology, ‡Cardiology Clinic, Clinical Center of Serbia, Belgrade, Serbia; ‖Eye Clinic, University Medical Center Zvezdara, Belgrade, Serbia; §Ophtalmology Clinic "Perfect Vision", Subotica, Serbia**

**Abstract**

**Background/Aim.** Several factors may have influence on systemic circulation. Additionally, peripheral circulation also demonstrates sex differences, in young women presenting significantly lower finger blood flow in comparison to men of the same age, a finding that disappears in women after menopause. The aim of this study was to compare the retrobulbar hemodynamic parameters measured by means of color Doppler imaging in women and men with open-angle glaucoma and elevated intraocular pressure. **Methods.** A total of 52 eyes from 52 open-angle glaucoma (OAG) patients, with elevated intraocular pressure (IOP), were included in this cross-sectional study. Peak-systolic velocity (PSV), end-diastolic velocity (EDV), and Pourcelot resistivity index (RI) were assessed in the ophtalmic artery (OA), central retinal artery (CRA), and posterior cilliary arteries (PCA). IOP was measured both with Goldmann Applanation tonometer (GAT) and with the dynamic contour tonometer (DCT), three times respectively. Ocular pulse amplitude (OPA) appeared during the DCT measurement. **Results.** The retrobulbar hemodynamic parameters did not show any difference between men and post-menopausal women. **Conclusion.** The results of our study did not find any difference between sexes in patients with open-angle glaucoma and elevated intraocular pressure.

**Key words:**
**glaucoma, open-angle; men; women; intraocular pressure; ischemia; eye; diagnosis.**

**Apstrakt**

**Uvod/Cilj.** Postoji nekoliko faktora koji utiču na sistemsku cirkulaciju. Osim toga, periferna cirkulacija pokazuje razlike među polovima, čineći znatno nižim protok krvi u prstu kod mladih žena nego kod muškaraca istih godina. Ova razlika nestaje kod žena u menopauzi. Cilj ovog rada bio je upoređivanje parametara retrobulbarne cirkulacije izmerenih kolor doplerom kod žena i kod muškaraca sa glaukomom otvorenog ugla. **Metode.** Istraživanjem je bilo obuhvaćeno 52 oka od 52 bolesnika (50 i više godina starosti) sa glaukomom otvorenog ugla i povišenim intraokularnim pritiskom (IOP). Vršni sistolni (PSV), završni dijastolni (EDV) i Pourcelotov indeks rezistencije (Ri) mereni su u oftalmičkoj arteriji (OA), centralnoj retinalnoj arteriji (CRA) i zadnjim kratkim cilijarnim arterijama (PCA). IOP je meren i Goldmannovim aplanacionim (GAT) i dinamičkim konturnim tonometrom (DCT), po tri puta uzastopno. Okularna pulsna amplituda je dobijana tokom merenja dinamičkim konturnim tonometrom. **Rezultati.** Analizom parametara retrobulbarne hemodinamike nije ustanovljena razlika između muškaraca i žena. **Zaključak.** Prema rezultatima ove studije ne postoji razlika u retrobulbarnoj cirkulaciji između muškaraca i žena sa glaukomom otvorenog ugla i povišenim IOP.

**Ključne reči:**
**glaukom, otvorenog ugla; muškarci; žene; intraokularni pritisak; ishemija; oko; dijagnoza.**

### Introduction

The term glaucoma covers a wide range of chronic, multifactorial, and progressive optic neuropathies in which elevated intraocular pressure (IOP) is an important risk factor [1–3].

Nevertheless, there is increasing evidence suggesting that ocular blood flow disturbances are involved both in the

**Correspondence to:** Ivan Marjanovic, Institute of Ophthalmology, Clinical Center of Serbia, 11 000 Belgrade, Serbia.
E-mail: Ivanmarjanovic007@gmail.com

pathogenesis of glaucoma [4–9] and in progression of glaucomatous damage [10–12].

Several factors may have influence on systemic circulation. Additionally, peripheral circulation also demonstrates sex differences, in young women presenting significantly lower finger blood flow in comparison to men of the same age, a finding that disappears in women after menopause [13–14].

Many different methods are used to measure directly or calculate indirectly the ocular hemodynamic parameters in humans. Among them, the color Doppler imaging (CDI) combines B-scan grey scale imaging of tissue structure, color representation of blow flow based on Doppler shift and pulsed Doppler measurement of blood flow velocities. This method is used, in ophthalmology, to measure blood flow velocities in retrobulbar vessels [6, 15–17].

The aim of this study was to compare the retrobulbar hemodynamic parameters measured by means of color Doppler imaging in women and men (age 50 and more) with open-angle glaucoma and elevated intraocular pressure.

## Methods

This prospective cross-over study was conducted on consecutive recruited patients who met inclusion and exclusion criteria, seen at the University Eye Clinic, Clinical Center of Serbia in Belgrade, from December 2009 to December 2010.

This study was approved by the Ethics Committee of the University Eye Clinic, Clinical Center of Serbia, and was conducted in accordance with Good Clinical Practice and the tenets of the Declaration of Helsinki. The patients signed an informed consent form before inclusion.

All the participants were required to meet the following inclusion criteria: age equal to or higher than 50 years, clinical diagnosis of open-angle glaucoma in early to moderate stage [18], IOP equal or higher than 25 mmHg without treatment and willingness to comply with the investigators and protocol indications.

The patients were excluded if they had other type of glaucoma different than open-angle glaucoma, previous treatment with ocular filtering surgery, the history of previous refractive surgery, any hormonal therapy, acute myocardial infarction or stroke within the past three month, diabetes, the history of progressive retinal or optic nerve disease of any cause, and asthma or any other obstructive pulmonary disease.

All the patients underwent complete ophthalmologic examination, Goldmann applanation (GAT) (Goldmann tonometer; Haag Streit AG, Koeniz, Switzerland) and dynamic contour tonometry (DCT) (Dynamic Contour tonometer; Ziemer Ophthalmic Systems, Port, Switzerland), central corneal thickness (CCT) with ultrasound pachymetry (Palm Scan AP 2000, Ophthalmic Ultrasound, Micro Medical Devices, Inc., Clabasas, CA, USA), visual field examination (Humphrey VFA, Carl Zeiss Meditec) and confocal scanning laser retinal tomography (HRT II) (Heidelberg Engineering Inc. Heidelberg, Germany). IOP was measured both with GAT and DCT, three times respectively. After a decrease of elevated IOP (IOP < 20 mmHg), both by medications or by surgery, we repeated GAT and DCT. Ocular pulse amplitude (OPA) appeared during the DCT measurement.

Hemodynamic parameters were measured in the ophthalmic artery (OA), central retinal artery (CRA), and posterior cilliary arteries (PCA). Peak systolic (PSV) and end-diastolic (EDV) velocities were measured. Peak systolic velocity and EDV were used to calculate the Pourcelot resistivity index (RI) using the following equation: RI = PSV - EDV/PSV [19]. All color Doppler imaging (CDI) examinations (model Antares; Siemens, Munich, Germany) were performed by the same experienced observer, who was masked to the diagnosis. Evaluations of blood pressure and radial pulse were obtained in a supine position after 10 min of rest. Systolic (SBP) and diastolic blood pressure (DBP) were measured in the upper right arm using a mercury sphygmomanometer and heart rate (HR) was measured by palpation of the radial pulse. These parameters were obtained every 10 min, during Doppler examination.

Descriptive statistics (mean and standard deviation) and 95% confidence intervals (95% CIs) were used to report demographic and ocular baseline characteristics. Data were tested for normal distribution using a D'Agostino-Pearson test [20]. As data were normally distributed, a two-tailed, independent samples Student's *t*-test was used to evaluate the IOP and the hemodynamic parameters by intergroup comparisons. Because of the large number of tests, simultaneous inference using the Bonferroni correction was used to correct the $p$ -value ($\alpha/9$). Statistical significance was accepted for $p < 0.0055$.

Statistical analysis was performed using MedCalc11.5.1.0 (MedCalc software, Mariakerke, Belgium).

## Results

Of the 60 patients who were screened, 52 (22 women and 30 men) fulfilled the respective demands of the inclusion and exclusion criteria. The mean (SD) [95% confidence interval (CI)] age was 70.7 (9.9) [66.6 to 74.8] and 68.3 (12.9) [63.7 to 72.9] years in women and men, respectively. The main clinical and demographic characteristics are summarized in Table 1.

Regarding the retrobulbar hemodynamic parameters, there were no significant differences between women and men (Table 2).

## Discussion

The results of our study suggested no differences in the retrobulbar hemodynamic parameters between the women of 50 years and more and age-matched men with open-angle glaucoma and elevated IOP.

There are relatively few studies that evaluate possible differences in the retrobulbar hemodynamic parameters between sexes in open-angle glaucoma patients.

In a cross sectional study Harris et al. [21] evaluated the influence of age on retrobulbar circulation assessed with color Doppler. Based on the results of this study, it seemes

**Table 1**

**Baseline demographic and clinical characteristics of the patients**

| Characteristics | Women (n = 22) mean (SD) [95% CI] | Men (n = 30) mean (SD) [95% CI] | *p*-value |
|---|---|---|---|
| Age (years) | 70.7 (9.9) [66.6 – 74.8] | 68.3 (12.9) [63.7 – 72.9] | 0.367 |
| CCT (μ) | 558.5 (36.3) [543.3 – 573.7] | 550.3 (40.3) [535.9 – 564.7] | 0.447 |
| IOP (mm Hg) | 30.8 (7.9) [27.7 – 33.9] | 31.2 (9.2) [27.4 – 35.0] | 0.850<br>0.715 |
| OPA (mm Hg) | 4.2 (1.4) [3.6 – 4.8] | 4.1 (1.2) [3.7 – 4.5] | 0.515 |
| MD (dB) | -3.2 (3.3) [-4.6 – -1.8] | -2.8 (3.2) [-3.9 – -1.7] | 0.681 |
| PSD (dB) | 2.6 (1.9) [1.8 – 3.4] | 2.6 (2.1) [1.8 – 3.4] | 0.958 |

**SD – standard deviation; 95% CI – 95% confidence interval; CCT – central corneal thickness; IOP – intraocular pressure; OPA – ocular pulse amplitude; MD – mean defect; PSD – pattern standard deviation.**

***p*-values were calculated comparing the parameters at baseline between the two study groups (one-way ANOVA test); *p*-values were considered statistically significant if lower than 0.05.**

**Table 2**

**Comparison of the retrobulbar hemodynamic variables between the women and men**

| Parameter | Women (n = 22) | | Men (n = 30) | | *p* value |
|---|---|---|---|---|---|
| | Mean (SD) | 95% CI | Mean (SD) | 95% CI | |
| PSV OA | 54.9 (29.9) | 42.4 – 67.4 | 57.5 (21.4) | 48.6 – 66.4 | 0.733 |
| EDV OA | 16.6 (11.9) | 11.6 – 21.6 | 17.5 (12.2) | 12.4 – 22.6 | 0.785 |
| RI OA | 0.72 80.12) | 0.67 – 0.77 | 0.71 (0.10) | 0.67 – 0.75 | 0.903 |
| PSV CRA | 24.7 (9.5) | 20.9 – 28.5 | 28.6 (10.2) | 24.3 – 32.9 | 0.266 |
| EDV CRA | 7.3 (2.4) | 6.3 – 8.3 | 9.9 (3.9) | 8.3 – 11.5 | 0.377 |
| RI CRA | 0.68 (0.14) | 0.62 – 0.74 | 0.67 (0.11) | 0.63 – 0.71 | 0.878 |
| PSV PCA | 27.7 (12.9) | 22.3 – 33.1 | 33.1 (13.3) | 27.5 – 38.7 | 0.173 |
| EDV PCA | 9.0 (3.7) | 7.5 – 10.5 | 9.4 (4.5) | 7.5 – 11.3 | 0.713 |
| RI PCA | 0.64 (0.13) | 0.59 – 0.69 | 0.68 (0.14) | 0.62 – 0.74 | 0.294 |

**SD – standard deviation; 95% CI – 95% confidence interval; PSV – peak systolic velocity; EDV–end-diastolic velocity; RI – resistivity index; OA – ophthalmic artery; CRA – central retinal artery; PCA – posterior cilliary artery.**

**Unpaired Student's *t*-test (*p*-values were considered statistically significant if lower than 0.05).**

that women and men show different behavior. In women not receiving estrogen replacement therapy older age is associated with reduced EDV, constant PSV, and elevated resistivity index in posterior cilliary arteries. Although our study did not evaluate the impact of age on the retrobulbar hemodynamic, we did not find any difference between men and women in these parameters.

It is well-known that peripheral and central circulations respond differently to the status of sexual hormones. Harris-Yitzhak et al. [22] examined the role that estrogen may play on retrobulbar arteries. This study reports that estrogen-replacement therapy in postmenopausal women apparently helps to reduce vascular resistance distal to the ophthalmic artery to the levels matching those of young women. Similarly, Centofanti et al. [23] and Kavroulaki et al. [24], observed higher choroidal blood flow in premenopausal women. In our study women did not take estrogen replacement therapy and that fact might justify the lack of differences in retrobulbar circulation among them.

In our study we did not evaluate hormonal involvement in men and women, nor its correlation with retrobulbar circulation. Hormonal therapy was one of the exclusion criteria for our study.

## Conclusion

The results of our study show no difference between sexes in patients with open-angle glaucoma and elevated intraocular pressure. Further investigations are needed in this field to clarify these results.

## REFERENCES


1. *Gordon MO, Beiser JA, Brandt JD, Heuer DK, Higginbotham EJ, Johnson CA,* et al. The Ocular Hypertension Treatment Study: baseline fac–rs that predict the onset of primary open-angle glaucoma. Arch Ophthalmol 2002; 120(6): 714−20; discussion 829−30.
2. *Kass MA, Heuer DK, Higginbotham EJ, Johnson CA, Keltner JL, Miller PJ*, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that –pical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. Arch Ophthalmol 2002; 120(6): 701−13.
3. *Heijl A, Leske CM, Bengtsson B, Hyman L, Bengtsson B*, et al. Reduction of Intraocular Pressure and Glaucoma Progression: Results from the Early Manifest Glaucoma Trial. Arch Ophthalmol 2002; 120(10): 1268−79.
4. *Carter CJ, Brooks DE, Doyle LD, Drance SM*. Investigations in– a Vascular Etiology for Low-tension Glaucoma. Ophthalmology 1990; 97(1): 49−55.
5. *Hayreh SS*. Progress in the understanding of the vascular etiology of glaucoma. Curr Opin Ophthalmol 1994; 5(2): 26−35.
6. *Flammer J, Orgül S, Costa VP, Orzalesi N, Krieglstein GK, Serra LM, Stefánsson E*. The impact of ocular blood flow in glaucoma. Prog Retin Eye Res 2002; 21(4): 359−93.
7. *Harris A, Kagemann L, Ehrlich R, Rospigliosi C, Moore D, Siesky B*. Measuring and interpreting ocular blood flow and metabolism in glaucoma. Can J Ophthalmol 2008; 43(3): 328−36.
8. *Werne A, Harris A, Moore D, BenZion I, Siesky B*. The Circadian Variations in Systemic Blood Pressure, Ocular Perfusion Pressure, and Ocular Blood Flow: Risk Fac–rs for Glaucoma. Surv Ophthalmol 2008; 53(6): 559−67.
9. *Weinreb RN, Harris A*. Ocular Blood Flow in Glaucoma: The 6th Consensus Report of the World Glaucoma Association. Section II: Clinical Measurement of Ocular Blood Flow. Amsterdam: Kugler Publications; 2009. p. 59
10. *Galassi F, Sodi A, Ucci F, Renieri G, Pieri B, Baccini M*. Ocular hemodynamics and glaucoma prognosis. Arch Ophthalmol 2003; 121(12): 1711−5.
11. *Satilmis M, Orgül S, Doubler B, Flammer J*. Rate of progression of glaucoma correlates with retrobulbar circulation and intraocular pressure. Am J Ophthalmol 2003; 135(5): 664−9.
12. *Martínez A, Sánchez M*. Predictive value of colour Doppler imaging in a prospective study of visual field progression in primary open-angle glaucoma. Acta Ophthalmol Scand 2005; 83(6): 716−22.
13. *Bartelink ML, de Wit A, Wollersheim H, Theeuwes A, Thien T*. Skin vascular reactivity in healthy subjects: influence of hormonal status. J Appl Physiol (1985) 1993; 74(2): 727−32.
14. *Bollinger A, Schlumpf M*. Finger blood flow in healthy subjects of different age and sex and in patients with primary Raynaud's disease. Acta Chir Scand 1976; 465(Suppl): 42−7.
15. *Weinreb RN, Harris A*. Ocular Blood Flow in Glaucoma: The 6th consensus report of the World Glaucoma Association. Section II: Clinical Measurement of Ocular Blood Flow. Amsterdam: Kugler Publications; 2009. p. 21−2.
16. *Lieb WE*. Color Doppler ultrasonography of the eye and orbit. Curr Opin Ophthalmol 1993; 4(3): 68−75.
17. *Butt Z, O'Brien C, McKillop G, Aspinall P, Allan P*. Color Doppler imaging in untreated high- and normal-pressure open-angle glaucoma. Invest Ophthalmol Vis Sci 1997; 38(3): 690−6.
18. *Hodapp E, Parrish R, Anderson D*. Clinical Decisions in Glaucoma. St Louis, Missouri: Mosby-Year Book, Inc; 1993.
19. *Pourcelot L*. Indications de l'ultrasonographie Doppler dans l'etude des vaisseaux peripheriques. Rev Prat 1975; 25: 4671−80.
20. *Sheskin DJ*. Handbook of parametric and nonparametric statistical procedures. 5th ed. Boca Ra–n: Chapman & Hall /CRC; 2011.
21. *Harris A, Harris M, Biller J, Garzozi H, Zarfty D, Ciulla TA,* et al. Aging affects the retrobulbar circulation differently in women and men. Arch Ophthalmol 2000; 118(8): 1076−80.
22. *Harris-Yitzhak M, Harris A, Ben-Refael Z, Zarfati D, Garzozi HJ, Martin BJ*. Estrogen-replacement therapy: effects on retrobulbar hemodynamics. Am J Ophthalmol 2000; 129(5): 623−8.
23. *Centofanti M, Bonini S, Manni G, Guinetti-Neuschüler C, Bucci MG, Harris A*. Do sex and hormonal status influence choroidal circulation. Br J Ophthalmol 2000; 84(7): 786−7.
24. *Kavroulaki D, Gugleta K, Kochkorov A, Katamay R, Flammer J, Orgul S*. Influence of gender and menopausal status on peripheral and choroidal circulation. Acta Ophthalmol 2010; 88(8): 850−3.


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# Vascular changes in the retina in patients with chronic respiratory insufficiency

## Vaskularne promjene mrežnjače kod bolesnika sa hroničnom respiratornom insuficijencijom

**Antoaneta Adžić-Zečević*, Biljana Milojko†, Mirjana A. Janićijević-Petrović‡**

***Eye Clinic, Clinical Center of Montenegro, Podgorica, Montenegro; †Special Hospital for Lung Diesease "Dr Jovan Bulajić", Brezovik, Montenegro; ‡Clinic of Ophthalmology, Clinical Center Kragujevac, Kragujevac, Serbia**

**Abstract**

**Background/Aim.** Chronic respiratory insufficiency is a pathological state which occurs as a result of respiratory system inability to maintain normal gas exchange between the outside air and circulating blood. For the purposes of human organism's proper functioning, it is necessary that a certain amount of air in the lungs comes into contact with a certain amount of blood within a unit of time, so that an adequate hemoglobin oxygenation could be achieved. Then, hemoglobin from erythrocytes in the blood supply delivers oxygen to all the tissues and cells of the body including the eye. Direct impact of hypoxemia and hypercapnia on the wall of arterioles, venules and capillaries results in a severe vasodilatation along with the increased permeability of the walls causing clinically evident changes in the retina. The aim of this study was to determine the degree of ocular changes in retina with patients suffering from chronic respiratory insufficiency. **Methods.** A prospective study was conducted on 80 patients, 40 patients with respiratory failure and 40 patients with chronic obstructive pulmonary disease an and bronchial asthma (the control group). In all the patients direct and indirect ophthalmoscopy and fluorescein angiography was performed. Clinically visible fundus and retina changes in patients suffering from chronic respiratory failure were categorized as mild (dilatation and retinal veins and arteries tortosion up to the mid-periphery), moderate (retinal hemorrhage) and severe (optic nerve edema, macular edema, superficial and deep retinal hemorrhages and venous occlusion). **Results.** In the patients suffering from respiratory insufficiency the changes in retinal blood vessels were found [in 18 (45%) mild, in 13 (32.5%) moderate, and in 9 (22%) severe], while in the patients with chronic obstructive pulmonary disease and bronchial asthma (without respiratory insufficiency) no changes were recognized. **Conclusion.** The results of this study indicate the need for ophthalmologic examination in patients with chronic respiratory insufficiency. It is important to recognize, identify and quantify the changes on retinal blood vessels which are clinically significant. It is necessary to provide their monitoring and to prescribe proper therapeutic treatment in order to preserve visual functions.

**Key words:**
**respiratory insufficiency; pulmonary disease, chronic obstructive; anoxia; retinal disease; optic nerve.**

**Apstrakt**

**Uvod/Cilj.** Hronična respiratorna insuficijencija je patološko stanje koje nastaje kao posljedica nesposobnosti respiratornog sistema da održava normalnu razmjenu gasova između spoljnog vazduha i cirkulišuće krvi. Za potrebe organizma neophodno je da određena količina vazduha u plućima dođe u kontakt sa određenom količinom krvi u jedinici vremena, radi adekvatne oksigenacije hemoglobina. Dalje, hemoglobin u eritrocitima krvnom strujom doprema kiseonik do svih tkiva i ćelija organizma, pa i oka. Direktnim dejstvom hipoksemije i hiperkapnije na zid arteriola, venula i kapilara, nastaje izrazita vazodilatacija uz povećanu permeabilnost zidova usled čega nastaju klinički vidljive promjene na retini. Cilj ovog rada bio je da se utvrdi stepen oftalmoloških promjena na retini kod bolesnika sa hroničnom respiratornom insuficijencijom. **Metode.** Sprovedena je prospektivna studija koja je obuhvatila 80 bolesnika, 40 sa respiratornom insuficijencijom i 40 oboljelih od hronične opstruktivne bolesti pluća i bronhijalne astme (kontrolna grupa). Oftalmološki pregledi su vršeni direktnom i indirektnom oftalmoskopijom, a po potrebi rađena je i fluoresceinska angiografija. Klinički vidljive promjene na očnom dnu, odnosno retini, kod bolesnika sa hroničnom respiratornom insuficijencijom, klasifikovane su kao blage (dilatacija i tortoznost retinalnih arterija i vena do srednje periferije), umjerene (retinalne hemoragije) i teške (edem optičkog nerva, makularni edem, površna i duboka retinalna krvarenja i venske okluzije). **Rezultati.** Kod bolesnika sa respiratornom insuficijencijom nađene su promjene na retinalnim krvnim sudovima

**Correspondence to:** Antoaneta Adžić-Zečević, Eye Clinic, Clinical Center of Montenegro, Podgorica, Montenegro.
Phone: +00382 20 280 469, E-mail: miaz@t-com.me

[kod 18 (45%) lake, kod 13 (32,5%) umjerene i 9 (22,5%) teške], dok kod bolesnika sa hroničnom opstruktivnom bolešću pluća i bronhijalnom astmom (bez respiratorne insuficijencije) promjene nisu nađene. **Zaključak.** Rezultati ovog istraživanja ukazuju na potrebu oftalmološkog pregleda bolesnika sa respiratornom insuficijencijom. Važno je da se uoče, prepoznaju i kvantifikuju klinički značajne promjene na krvnim sudovima retine. Neophodno je njihovo praćenje i odabiranje adekvatnog terapijskog lečenja u cilju očuvanja vidne funkcije.

**Ključne reči:**
**respiratorna insuficijencija; pluća, opstruktivne bolesti, hronične; anoksija; retina, bolesti; n. opticus.**

## Introduction

Respiratory insufficiency is an ill condition that is the result of respiratory system inability to transmit normal exchange of respiratory gases between the outside air and circulating blood [1–4]. It is characterized by a decreased partial pressure of oxygen ($PaO_2$) (hypoxemia) in arterial blood and reduced saturation (hyposaturation) with hemoglobin oxygen ($SaO_2$), with normal or elevated partial pressures of carbon dioxide ($PaCO_2$) (hypercapnia).

If the breathing process is properly conducted, arterial values $PaO_2$, $PaCO_2$ and pH will be in the physiological range. Hypoxemia includes a reduced $PaO_2$ in arterial blood below 9.3 kPa on average (normal pressure depends on age), hemoglobin saturation below 0.94, while the occurrence of hypercapnia is the elevated partial in arterial blood over 6 kPa. [5, 6]. Respiratory insufficiency may be acute or chronic.

The natural course of chronic respiratory failure is characterized by occasional acute deteriorations, which is called respiratory decompensation. Acute deterioration of chronic respiratory failure is defined through finding of respiratory gases in arterial blood when $PaO_2 < 50$ mmHg and/or $PaCO_2 > 50$ mmHg [2, 6, 7].

Signs and symptoms of pulmonary insufficiency are manifested at the stage of pulmonary decompensation. Severe hypoxemia and hypercapnia lead to many metabolic, circulatory, respiratory, enzymatic, endocrine and hematologic disorders. Facies of patients get polyglobulic, pletoric and cyanotic look.

Hyperemia of the conjunctiva and sclera is fully shown, often exophthalmia, bloatedness and sweating over the entire face, particular glow in the eyes that get brilliant, and tearful appearance called "frog faces" can be observed.

Ophthalmologic examination shows dilated and visible, tortoise blood vessels of retina, superficial and deep retinal hemorrhages with macular and papillary edema [8, 9].

Three basic changes in arterial blood resulting in chronic pulmonary insufficiency are hypoxemia, hypercapnia, changes in acid-base balance in the direction of acidity. They affect retinal blood vessels in terms of alterations in blood flow.

The eye allows visualization of its circulation [10–14]. The retina is translucent, and the visible part of the retina is intraretinal vascular stem. Alterations that attack the intraretinal vascular stem caused by arterial hypertension, arteriosclerosis, diabetes mellitus, sickle cell disease, and conditions such as hyperviscosity, embolic and thrombotic phenomena, can be observed [15]. The eye is a complex organ that has a number of different microcirculation systems to help its needs. Structural, neurological and permeability differences can be found in different intravascular microcircular structures. Micro blood vessels in every type of tissue continuously monitor the needs of tissue, such as nutrition and accumulation of harmful products in the tissues, which in turn controls local blood flow with great precision at a level appropriate to the activities of tissue. Also, the neural control provides additional attributes for controlling the flow of blood [11, 16]. The vasodilator theory of blood flow local regulation indicates that the higher metabolic activity and the lower blood flow or lesser delivery of oxygen and nutrients into the tissue substrate, the more quickly vasodilator substances are generated. It is considered that the vasodilator matters could be: adenosine, $CO_2$, lactate, ADP, histamine, $K^+$ ions, $H^+$ ions. They diffuse to precapilar structures (meta-arterioles and arterioles) and cause vasodilatationm [17]. The theory of metabolic control of the local flow is based on tissue requirements for oxygen and other nutritive substances. When the delivery of oxygen and other nutritive substances is inadequate, blood vessels dilate themselves. Thus, in the kidneys and the brain local control of blood flow depends on tissue concentration $O_2$, $CO_2$ and $H_2$ [18].

The aim of this study was to determine the degree of retinal vascular changes in patients with chronic pulmonary insufficiency, in order to estimate any correlation between the severity of clinical signs on the retina and the severity and deterioration of hypoxemia and hypercapnia, as well as to clearly differentiate clinical and morphological changes of the retina caused by hypoxemia and hypercapnia.

## Methods

The study included a total of 80 patients, 40 patients with chronic pulmonary disease and 40 patients with chronic obstructive pulmonary disease (COPD) and bronchial asthma (the control group). The survey was conducted at the Ophthalmology Clinic in Podgorica, Specialized Hospital for Lung Diseases in Brezovik, Department for Pulmonology and Clinical Center of Montenegro, Podgorica, in the period from January 2009 to January 2011, and in patients who also were treated in the Pulmonary and Ophthalmology Institutes of Clinical Center of Serbia, Belgrade. The survey was conducted with the approval of the Ethic Committee of the above mentioned institutions.

The study consisted of laboratory and clinical phases testing. The subjects with chronic respiratory failure were in different conditions, from those with stable conditions to those with acute deterioration. The participants of the control

group had COPD and bronchial asthma. The laboratory phase included analysis of $PaCO_2$ and $PaO_2$ in arterial blood. The measurement of these parameters was performed by taking a blood from brachial and radial arteries or peripheral parts of the body (fingertip and earlobe). Clinical-stage testing included biomicroscope eye bottom scan with lenses, indirectly; ophthtalmological examination, and examination of the eye contact Goldmann bottom glass with three mirrors, and, finally, fluorescein angiography (FA) – a diagnostic method that is used for the interpretation of pathological conditions of the eye. FA is a method to show the vascular structures of various layers of retinal tissue and analyze the dynamics of circulation. FA is not only diagnostic technique, but also a way of controlling the evolution of lesions on the retina, in the same time being a help in the therapeutic indication, especially in retinal laser photocoagulation. By ophthalmologic eye examination, we classified the bottom changes to mild, moderate and severe. Retinal-vascular changes are the result of interaction of hypoxemia, hypercapnia, and clinically are visible as: segmented, tortuous, large and dilated retinal blood vessels (mild changes) retinal hemorrhages (moderate changes) and optic nerve papilloedema, macular edema, superficial and deep retinal hemorrhages and venous occlusion of blood vessels (severe changes).

The subjects were divided into three groups based on the presence of retinal-vascular changes.

Statistical data analysis was performed using $\chi^2$ test to estimate the statistical significance of differences in the frequency of occurrence of certain characteristics in the study and the control group and between them. The value of $p < 0.05$ was considered statistically significant.

**Results**

This study included a group of 40 patients with chronic pulmonary insufficiency (the study group) and the control group (40 patients) with COPD and bronchial asthma.

The analysis of demographic data shows that in the study group most patients were aged 60 to 69, and in the control group between 40 and 49 (Table 1), while in terms of gender in both groups the majority were men (Table 2).

**Table 1**

**Demographic characteristics by the groups**

| Age (years), range | Men (n) | Women (n) | Total (n) |
|---|---|---|---|
| Study group | | | |
| 50–59 | 9 | 2 | 11 |
| 60–69 | 11 | 6 | 17 |
| 70–79 | 8 | 4 | 12 |
| Control group | | | |
| 30–39 | 6 | 4 | 10 |
| 40–49 | 14 | 8 | 22 |
| 50–59 | 3 | 3 | 6 |
| 60–69 | 1 | 1 | 2 |
| 70–79 | 0 | 0 | 0 |

**Study group – patients with chronic respiratory insuficiency;**
**Control group – patients with chronic obstructive pulmonary disease and asthma.**

**Table 2**

**The subjects of the study and the control group by gender**

| Groups of patient | Sex, n (%) | | Total |
|---|---|---|---|
| | male | female | n (%) |
| Study | 28 (53.85*; 70†) | 12 (42.86*; 30†) | 40 (50*; 100†) |
| Control | 24 (46.15*; 60†) | 16 (57.14; 40†) | 40 (50*; 100†) |
| Total | 52 (100*; 65†) | 28 (100*; 35†) | 80 (100*; 100†) |

**Study group – patients with chronic respiratory insuficiency;**
**Control group – patients with chronic obstructive pulmonary disease and asthma.**
*** – vertical; † – horizontal.**

There were no statistically significant differences between the study and the control group in relation to gender ($p > 0.05$). The ratio of men and women between the study and the control group was 53.85%: 46.15% and 42.86%: 57.14%, respectively.

Analysis of respiratory gases in arterial blood in the study group showed that the value of the partial pressure of oxygen ($PaO_2$) less than 50 mmHg was present in 77.5% of the subjects, and $PaCO_2$ values of more than 60 mmHg in 85% of participants which corresponds to acutisation of chronic respiratory failure. Only 15% of the participants of the study group had $PaCO_2$ values < 60 mmHg (Table 3).

In the control group, the average values of $PaO_2$ were 65.24 mmHg in men and 65.03 mmHg in women, and average values of $PaCO_2$ 36.11 mmHg in men and 36.40 mmHg in women.

Therefore, we analyzed the changes found in blood vessels of the retina in the 40 patients of the study group. The analysis showed that 45% of the patients in the study groups had mild forms of changes, 32.50% moderate changes, and 22.50% severe changes (Table 4).

**Table 3**

**The values of partial pressure of oxygen ($PaO_2$) and partial pressure of carbon dioxide ($PaCO_2$) of the study group participants according to gender**

| Partial pressure of $O_2$ and $CO_2$ in arterial blood (mmHg), range | Sex, n (%) | | Total n (%) |
|---|---|---|---|
| | male | female | |
| $PaO_2$ | | | |
| 35–39 | 12 (42.86*; 100†) | 0 (0*; 0†) | 12 (30*; 100†) |
| 40–44 | 6 (21.43*;60†) | 4 (33.33*; 40†) | 10 (25*; 100†) |
| 45–49 | 5 (17.86*; 55.56†) | 4 (33.33*; 44.44) | 9 (22.15; 100†) |
| 50–54 | 3 (10.71*; 60.5†) | 2 (16.67*; 40†) | 5 (12.5; 100†) |
| 55–59 | 2 (7.14*; 66.67†) | 1 (8.33*; 33.33†) | 3 (7.5; 100†) |
| 60–64 | 0 (0*; 0†) | 1 (8.33*; 100†) | 1 (2.5; 100†) |
| 65–69 | 0 (0*; 0†) | 0 (0*; 0†) | 0 (0*; 0†) |
| Total | 28 (100*; 70†) | 12 (100*; 30†) | 40 (100; 100†) |
| $PaCO_2$ | | | |
| 30–39 | 0 (0*; 0†) | 0 (0*; 0†) | 0 (0*; 0†) |
| 40–49 | 0 (0*; 0†) | 0 (0*; 0†) | 0 (0*; 0†) |
| 50–59 | 3 (10.71*; 50†) | 3 (25*; 50†) | 6 (15*; 100†) |
| 60–69 | 5 (17.86*; 45.45†) | 6 (50*; 54.55†) | 11 (27.5*; 100†) |
| 70–79 | 2 (7.14*; 66.67†) | 1 (8.33*; 33.33†) | 3 (7.5*; 100†) |
| 80–89 | 9 (32.15*; 81.82†) | 2 (16.67*; 18.18†) | 11 (27.5*; 100†) |
| 90–99 | 4 (14.29*; 100†) | 0 (0*; 0†) | 4 (10*; 100†) |
| 100–109 | 3 (10.71*; 100†) | 0 (0*; 0†) | 3 (7.5*; 100†) |
| 110–119 | 2 (7.14*; 100†) | 0 (0*; 0†) | 2 (5*; 100†) |
| Total | 28 (100*; 70†) | 12 (100*; 30†) | 40 (100*; 100†) |

**Study group – patients with chronic respitratory insufficiency.**
*** – vertical; † – horizontal**

**Table 4**

**The degree of changes in the retina of the patients in the study group**

| Degree of retinal changes | Sex, n (%) | | Total n (%) |
|---|---|---|---|
| | male | female | |
| No | 0 (0*; 0†) | 0 (0*; 0†) | 0 (0*; 0†) |
| Mild | 9 (31.24*; 50†) | 9 (75*; 50†) | 18 (45*; 100†) |
| Moderate | 10 (35.72*; 76.92†) | 3 (25*; 23.08†) | 13 (32.5*; 100†) |
| Severe | 9 (32.14*; 100†) | 0 (0*; 0†) | 9 (22.5*; 100†) |
| Total | 28 (100*; 70†) | 12 (100*; 30†) | 40 (100*; 100†) |

**Study group – patients with chronic respitratory insufficiency.**
*** – vertical; † – horizontal**

## Discussion

Our study showed that in patients with chronic respiratory insufficiency there were changes in retinal blood vessels. These changes are the result of hypoxemia and hypercapnia in retinal blood vessels. Hypoxemia and hypercapnia occur as a result of permanent damage of lung function in chronic respiratory insufficiency [7].

Small changes in oxygen concentration cause changes in blood flow in large diameter retinal blood vessels. Hypercapnia has larger impacts on the macular flow increasing it to 24%. The similar happens in large blood vessels, but it does not change the diameter of blood vessels. Chronic hypoxemia causes slight changes in the retina, which are reflected in segmented dilatation and tortuosity of large retinal blood vessels [15, 17].

The interactive action of hypoxemia and hypercapnia causes moderate changes in the retina (retinal hemorrhage) when sudden outbreak of hypoxemia and hypercapnia, papilloedema of the optic nerve and macular edema appear, together with superficial and deep retinal hemorrhage and thrombosis.

Our study included 80 patients, 40 of whom were chronic pulmonary insufficiency, and 40 with COPD and bronchial asthma and in stable condition without a significant respiratory insufficiency. The research showed that pulmonary disease may in the course of its evolution lead to permanent damage of lung function, and in the terminal stage to chronic pulmonary failure.

The study group was dominated by men (70%). In the control group there was 60% of men, and 40% of women. These relationships do not indicate any statistically significant differences between the groups in relation to gender. The data provided correspond to those listed in our bibliography [1, 2, 6].

Lung diseases presented in our patients are widespread in both industrialized countries and in developing countries. Smoking habits, environmental pollution, substandard housing conditions and working environment, rapid urbanization, contribute to causes of these lung diseases. These diseases are undoubtedly more common with adult men.

However, in recent years more and more women suffer because of the widespread habit of smoking.

In the study group the patients were mainly adults between 50 and 79. Most of them were in the age group between 60 and 69, 17 (42.50%), and the fewest in the age group between 50 and 59, 11 (27%). In the control group there were adults of the age of 30 and 70. Most sufferers were in the age group between 40 and 49, 22 (55%), the fewest were in the age group between 60 and 69, 2 (5%). This finding is analogous to the findings in the bibliography [17, 19].

Chronic pulmonary insufficiency occurs in adults and elderly population, together with COPD, while bronchial asthma is prevalent in younger population.

Examining $PaO_2$ values in relation to sex, it can be concluded that the distribution of respondents by $PaO_2$ was very uneven. In the study group the values of $PaO_2$ up to 50 mm Hg were present in 77.5% of respondents, and more than 50 mm Hg in 22.5% of them.

In the control group subjects had no significant respiratory failure, and the average value of $PaO_2$ for men was 65.24 mm Hg and for women 65.03 mm Hg.

In the study group $PaO_2$ was less than 50 mm Hg in 77.5% of the patients, while in the control group none of the participants had the $PaO_2$ lower than 65 mmHg. This explains the nature and course of lung disease.

Chronic pulmonary insufficiency is a condition with severely impaired lung function, varying degrees of hypoxemia, and/or hypercapnia, whereas in COPD and bronchial asthma, there is a slight disturbance in arterial blood gases in stable condition [5, 20, 21].

In the study group $PaCO_2$ values up to 60 mmHg were present in only 15% of the patients. In the control group, all subjects had lower $PaCO_2$ of 45 mmHg (the average values of $PaCO_2$ were 36.11 mmHg for men and 36.40 mmHg for women).

It was observed that in the study group due to hypoxemia and hypercapnia there were changes in the retinal blood vessels in all patients. Most of the respondents had a mild form of changes, 18 or 45%, followed by the moderate changes, 13 or 32.5%, while heavy changes had 9 (22.5%) of the patients. In the control group, since there were no significant disturbances of respiratory gases, no respondent showed any changes in the retina. Our findings are similar to those of American authors [10, 11].

The results of this study on the changes in blood vessels of the eye bottom in patients with chronic respiratory failure indicate the need for the same ophthalmologic examination, monitoring and selecting the appropriate therapeutic procedure [22, 23].

With the correct interpretation of clinical findings, an ophthalmologist should act timely to: recognition, control and monitoring of retinal vascular changes in patients with chronic respiratory insufficiency, setting the indications for laser photocoagulation, and setting the indication for fluorescein angiography [24, 25].

Treating patients with severe changes is a particular problem because of the threat visual function. It is possible to maintain a satisfactory visual retinal function with an adequate laser photocoagulation applied timely.

## Conclusion

The results of this study indicate the need for ophthalmologic examination in patients with chronic respiratory insufficiency.

Early detection, monitoring and selection of patients for active treatment is of a major importance in the treatment of severe retinal-vascular changes in patients with chronic respiratory insufficiency in the stage of decompensation.

## REFERENCES


1. *Bogdanovic M*. Chronic obstructive insufficiency In: *Stefanovic S*, editor. Internal medicine. Belgrade: Medicinska knjiga; 1994. p. 523−32. (Serbian)
2. *Durie O, Sekulic S*. Disease organs for respiration. In: *Manojlovic D*, editor. Internal medicine. Belgrade: Zavod za udzbenike i nastavna sredstva; 1998. p. 335−46. (Serbian)
3. *Miladinovic D*. Pharmacotherapy chronical respiratory failure and chronic lungs heart. In: *Varagic V, Stevanovic M*, editors. Pharmacotherapy in pulmology. Belgrade: Elit Medica; 1998. p. 407−23. (Serbian)
4. *Sato T, Oku H, Tsuruma K, Katsumura K, Shimazawa M, Hara H*, et al. Effect of hypoxia on susceptibility of RGC-5 cells to nitric oxide. Invest Ophtalmol Vis Sci 2010; 51(5): 2575−86.
5. *Gidday JM, Maceren RG, Shah AR, Meier JA, Zhu Y*. KATP channels mediate adenosine-induced hyperemia in retina. Invest Ophtalmol Vis Sci 1996; 37(13): 2624−33.
6. *Hill SN*. Chronic respiratory failure and noninvasive ventilation. In: *Baum GL*, editor. Textbook of pulmonary diseases. Philadelphia: Lipincott-Raven; 1998. p. 969−86.
7. *Mitic Milikic M*. Chronical respiratory failure. In: *Manojlovic D*, editor. Internal medicine. Belgrade: Zavod za udzbenike i nastavna sredstva; 2009. p. 494−505. (Serbian)
8. *Economopoulou M, Langer HF, Celeste A, Orlova VV, Choi EY, Ma M*, et al. Histone H2AX is integral to hypoxia-driven neovascularization. Nat Med 2009; 15(5): 553−8.
9. *Fisher OA, Romanchikov IuN, Ignat'eva LP*. Effect of local revasularization of choroid on ocular tissues and lipid peroxidation in experimental atherosclerotic chorioretinopathy. West Oftalmol 1998; 114(4): 32−5.
10. *Alm A*. Ocular circulation. In: *Hart WM*, editor. Adler's physiology od the eye. St Louis, USA: Mosby Year Book; 2011. p. 198−227.
11. *Duker J, Weiter II*. Ocular circulation. In: *Tasman W, Jaeger EA*, editors. Duane's foundations of clinical ophtamology. New York: JB Lippincott Co; 1991. p. 1−34.
12. *Tayyari F, Venkataramen ST, Gilmore ED, Wong T, Fisher J, Hudson C*. The relationship between retinal vascular reactivity and arteriolar diameter in response to metyabolic provocation. Invest Ophthalmol Vis Sci 2009; 50(10): 4814−21.
13. *Thylefors J, Piitulainen E, Havelius U*. Dark adaptation during systemic hypoxia induced by chronic respiratory insufficiency Invest Ophthalmol Vis Sci 2009; 50(3): 1307−12.
14. *Chen B, Guber A, Marom Z*. Advance long-term oxygen therapy. RT Intern 1995; 4(1): 55−60.
15. *Schweitzer D, Hammer M, Kraft J, Thamm E, Königsdörffer E, Strobel J*. In vivo measurement of the oxygen saturation of retinal vessels in healthy volunteers. IEEE Trans Biomed Eng 1999; 46(12): 1454−65.
16. *Dunskyj MJ, Eriksen E, Dore CJ, Kohner EM*. Autoregulation in the human retinal circulation: Assessment using isometric ex-

ercise. Laser doppler velocimetry and comuter-assisted image analysis. London: Raven Press; 1996.
17. *Liu X, Wang W, Wang AR, Ning Q, Luo XP*. Pathogenesis of retinal neovascularization in a rat model of oxygen fluctuations-induced retinopathy. Zhonghua Er Ke Za Zhi 2007; 45(1): 7−13. (Chinese)
18. *Fulton AB, Akula JD, Mocko JA, Hansen RM, Benador IY, Beck SC,* et al. Retinal degenerative and hypoxic ischemic disease. Doc Ophthalmol 2009; 118(1): 55−61.
19. *Grippi MA*. Pulmonary pathophysiology. Philadelphia: JB Lippincott Co; 1998.
20. *Sekulić S*. Pulmonary diseases. Belgrade: Elit Medica; 2009. p. 373−84.
21. *Slavkovic V*. Pulmonary function. In: *Stefanovic S*, editor. Internal medicine. Belgrade: Medicinska knjiga; 1994. p. 454−9. (Serbian)
22. *Mitrouska I, Tzanakis N, Siafakas NM*. Oxygen therapy in chronic obstructive pulmonary disease. Eur Respir Mon 2006; 11(38): 302−12.
23. *Venkataraman ST, Hudson C, Fisher JA, Rodrigues L, Mardimae A, Flanagan JG*. Retinal arteriolar and capillary vascular reactivity in response to isoxic hypercapnia. Exp Eye Res 2008; 87(6): 535−42.
24. *Williams CD, Rizzolo LJ*. Remodeling of functional complexes during the development of the outer blood reinal barrier. Anat Rec 1997; 249(3): 380−8.
25. *Zeng XX, Ng YK, Ling EA*. Labelling of retinal microglial cells following an intravenose injection of a fluorescent dye into rats of different ages. J Anat 2000; 196(Pt 2): 173−9.

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# The assessment of electromagnetic field radiation exposure for mobile phone users

## Određivanje izloženosti korisnika mobilnih telefona zračenju elektromagnetnog polja

**Raimondas Buckus*, Birute Strukcinskiene*, Juozas Raistenskis†**

***Faculty of Health Sciences, Klaipeda University, Klaipeda, Lithuania; †Faculty of Medicine, Vilnius University, Vilnius, Lithuania**

**Abstract**

**Background/Aim.** During recent years, the widespread use of mobile phones has resulted in increased human exposure to electromagnetic field radiation and to health risks. Increased usage of mobile phones at the close proximity raises questions and doubts in safety of mobile phone users. The aim of the study was to assess an electromagnetic field radiation exposure for mobile phone users by measuring electromagnetic field strength in different settings at the distance of 1 to 30 cm from the mobile user. **Methods.** In this paper, the measurements of electric field strength exposure were conducted on different brand of mobile phones by the call-related factors: urban/rural area, indoor/outdoor setting and moving/stationary mode during calls. The different types of mobile phone were placed facing the field probe at 1 cm, 10 cm, 20 cm and 30 cm distance. **Results.** The highest electric field strength was recorded for calls made in rural area (indoors) while the lowest electric field strength was recorded for calls made in urban area (outdoors). Calls made from a phone in a moving car gave a similar result like for indoor calls; however, calls made from a phone in a moving car exposed electric field strength two times more than that of calls in a standing (motionless) position. **Conclusion.** Electromagnetic field radiation depends on mobile phone power class and factors, like urban or rural area, outdoor or indoor, moving or motionless position, and the distance of the mobile phone from the phone user. It is recommended to keep a mobile phone in the safe distance of 10, 20 or 30 cm from the body (especially head) during the calls.

**Key words:**
**electromagnetic fields; cellular phone; risk assessment; health.**

**Apstrakt**

**Uvod/Cilj.** Tokom zadnjih godina rasprostranjeno korišćenje mobilnih telefona ima za posledicu povećano izlaganje ljudi zračenju elektromagnetnog polja, a time i rizicima za svoje zdravlje. Povećano korišćenje mobilnih telefona na malom rastojanju nameće pitanja i izaziva sumnju u bezbednost njihovih korisnika. Cilj ove studije bio je da utvrdi izloženost korisnika mobilnih telefona zračenju elektromagnetnog polja merenjem jačine elektromagnetnog polja u različitim okruženjima na rastojanju od 1 do 30 cm od korisnika telefona. **Metode.** U ovoj studiji vršena su merenja izloženosti korisnika raznih marki mobilnih telefona zračenja elektromagnetnog polja mobilnih telefona pomoću faktora koji se odnose na poziv: gradska/seoska zona, zatvoren/otvoren prostor i kretanje/mirovanje tokom poziva. Razni tipovi mobilnih telefona postavljani su okrenuti prema sondi za merenje na rastojanja od 1 cm, 10 cm, 20 cm i 30 cm. **Rezultati.** Najjače elektromagnetno polje zabeleženo je kod poziva u seoskoj zoni (zatvoren prostor), dok je najslabije zabeleženo za one u gradskoj zoni (otvoren prostor). Pozivi sa telefona iz kola koja se kreću pokazali su slične rezultate kao pozivi iz zatvorenog prostora; međutim, pozivi sa telefona iz kola koja se kreću izlažu korisnika duplo jačem električnom polju nego pozivi iz mirovanja (bez kretanja). **Zaključak.** Zračenje elektromagnetnog polja zavisi od snage mobilnog telefona i faktora kao što su gradska ili seoska sredina, otvoren ili zatvoren prostor, kretanje ili mirovanje, i rastojanje mobilnog telefona od korisnika telefona. Preporučljivo je držati mobilni telefon na bezbednom rastojanju – 10, 20 ili 30 cm od tela (naročito glave) tokom razgovora.

**Ključne reči:**
**elektromagnetna polja; mobilni telefon; rizik, procena; zdravlje.**

**Correspondence to:** Birute Strukcinskiene, Faculty of Health Sciences, Klaipeda University, H. Manto str. 84, Klaipeda, LT-92294, Lithuania. Phone: +370 698 03097, E-mail: birutedoctor@hotmail.com

## Introduction

During recent years, the widespread use of mobile phones has resulted in increased human exposure to electromagnetic field and radiofrequency field. Although national and international agencies have established safety guidelines for exposure to these fields, concerns remain about the potential adverse health risks and health outcomes from power-frequency fields [1, 2].

Adverse effects investigated by various clinical trials include the possible link to increased risk of leukaemia, sleep disturbances and brain tumours [1, 3]. Health endpoints reported to be associated with electromagnetic and/or radiofrequency fields include genotoxic effects, neurological effects and neurodegenerative diseases, immune system deregulation, allergic and inflammatory responses, breast cancer, miscarriage and some cardiovascular effects. It was stated that a reasonable suspicion of risk exists based on clear evidence of bioeffects at environmentally relevant levels, which, with prolonged exposures may reasonably be presumed to result in health impacts [1].

There are reports stating that an intensive use of the mobile phone can cause headache, fatigue, insomnia, muscle pains, hearing and eyesight defects, failures of memory, neck and facial skin redness, and can increase stress [4, 5]. The mentioned symptoms can short-termed arise either during or sometime after a phone conversation [6]. Some uncertainties concerning possible carcinogenic effects should also be considered. According to epidemiological studies of mobile phones and cancer, it was concluded that the possibility of an enhanced cancer risk cannot be excluded [2]. The use of mobile phones is associated with an increased risk for brain tumour after 10 years [1]. The International Agency for Research on Cancer stated overall evaluation that radiofrequency electromagnetic fields are possibly carcinogenic to humans [7].

Mobile communication is a technology that enables data exchange with the help of radio signals [8]. A mobile network nowadays covers the whole world and electromagnetic waves are lingering around us but we cannot smell, see or touch them [9, 10]. Mobile network has received mass application, but nobody gives a thought to the principles of its operation and to the possible damage caused by what we even do not feel [11]. Electromagnetic radiation emitted by mobile phones and antennas of their base stations affects a human being at the cell level and causes damage to health [12, 13].

At radiofrequencies, electromagnetic field penetrates into human body. The exposure to radiofrequency radiation is usually described by the "Specific absorption rate" (SAR). It is the amount of energy absorbed per mass of tissue and has units of watts per kilogram (W/kg) [14, 15]. As the mobile phone is always very close to its owner, it necessarily has some effect on him/her [16]. When speaking over the phone an electromagnetic field exposure is targeted directly to the brain. The strongest electromagnetic field is generated at a distance of 1 to 10 centimetres from the phone antenna and the largest amount of electromagnetic radiation is absorbed in skin, at a depth of 1 cm [17]. When the phone is in a standby mode, the levels of emitted radiation are particularly low and nearly insensible. However, the power of radiation is largely dependent on a distance from the base station. The shorter the distance is the lower radiation is [18].

Mobile phones can radiate very strong electromagnetic fields. Analogous communication generates stable, while digital-pulsed electromagnetic fields. Electromagnetic fields and waves are generated during the change of electric charges [19]. These are turbulent electric and magnetic fields, invisible to eye, and propagating in the space at the speed of light. Biological effects of electromagnetic radiation depend on the power of its energy, impact duration and individual characteristics of the organism. Live organisms either reflect or absorb electromagnetic waves [20]. Absorption of electromagnetic radiation by tissues leads to the changes in the spatial arrangement of water and protein molecules, which become positioned in accordance with a certain axis, i.e. electrify themselves. The transformation of this radiation into thermal energy produces a thermal effect [14, 21].

The larger the number of people speaking over mobile phones is the higher environmental electromagnetic pollution is [22, 23]. The electromagnetic field safety of base stations and mobile phones has been broadly investigated and discussed worldwide. More and more scientific data are obtained on harm to human health caused by electromagnetic field emitted from the base stations and mobile phones [24].

The aim of the study was to assess an electromagnetic field radiation exposure for mobile phone users by measuring electromagnetic field strength in different settings at the distance of 1 to 30 cm from the mobile user: in urban/rural area, indoor/outdoor setting and at moving/stationary position.

## Methods

The exposure of electric field strength radiated from the sampled mobile phones of different brands and models was measured. For the study, the measurements from Global System for Mobile Communications (GSM) 900 and GSM 1800 cells mobile phones were conducted in different settings (urban/rural area, indoor/outdoor setting and moving/stationary), and in both the worst case was recorded.

A broadband electromagnetic field meter NBM-550 with isotropic probe EF 0392 (electronic field, flat) was used for investigations. The operating frequency range of the broadband electromagnetic field meter NBM-550 with an isotropic probe was 100 kHz – 3000 MHz. It corresponds to the range in which a possible radiation sources that can cause hazard (for instance, base stations of mobile communications, mobile communication antennas and mobile phones) can operate. The broadband electromagnetic field meter NBM-550 with an isotropic probe is distinguished by its high sensitivity: it measures electric field strength from 0.01 V/m, a magnetic field strength from 0.01 mA/m, and electromagnetic field energy flux density from 0.001 $mW/m^2$ or 0.1 $nW/cm^2$.

Dynamic coverage of the device: for electric field strength 0.01 V/m – 100 kV/m; for magnetic field strength 0.01 mA/m – 250 A/m; for electromagnetic field energy flux density 0.001 $mW/m^2$ – 25.00 $MW/m^2$; and for electromag-

netic field energy flux density 0.1 nW/cm2 – 2.5 kW/cm$^2$. A larger dynamic coverage of the broadband electromagnetic field meter NBM-550 with an isotropic probe means that the measurement of electric field strength covers a wider interval.

The measurements were performed at 1cm, 10 cm, 20 cm and 30 cm away from the probe during calls (Figure 1).

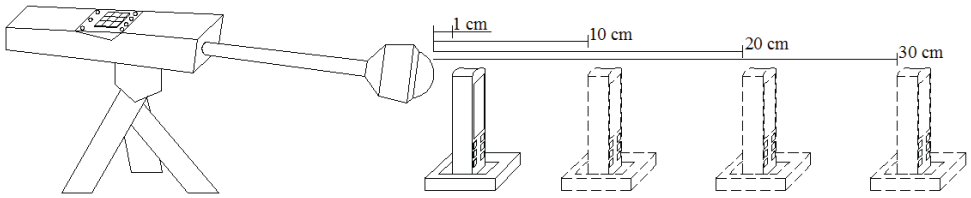



**Fig. 1 – Measurement scheme of mobile phone's electric field strength**

The first point of the measurements was the main one, because the mobile phones were placed facing the field probe at the similar position as of the ear. The duration of one measurement took 6 minutes. Measurements were taken during an outgoing call from a mobile phone.

The different models of the GSM mobile phones, with different SAR types, and different technical characteristics were used for the study (Table 1). Not all mobile phones have the same maximum power output level, so we have chosen traditional mobile phones with maximum power output up to 2 W. There are mobile phones with maximum power output of 4 W, 5 W or 8 W, but these mobile phones are used for the special purposes (like car phones and so on), and it is difficult to find them (they are not ordinary in our country).

The measurements for the study were taken in urban and rural settings. Urban areas (central Vilnius) included 1800 MHz microcells. Rural areas (Lavoriskes) had only 900 MHz macrocells. Outdoors measurements were taken in the yard (stationary), and indoors measurements – in the room (stationary). The distance between the antenna of the base station and the measuring points in the urban area was 200 m and in the rural area – 1000 m. Moving measurements were taken in the car driving around antenna in the 200 m beam (urban area), and in the 1000 m beam (rural area). The antenna of macrocell base station in rural area was mounted relatively high – on 70 m freestanding tower in order to cover a larger surrounding geographical area. The output power was between 10 and 500 W. The antenna of microcell base station in urban area was mounted on the roof of building (15 m) and was used to add additional capacity for a high number of users. The output power from the antenna of the microcell base station was between 10 W and 50 W. Calls were made during two days at 8 a.m. to 5 p.m.

## Results

The study results revealed the electromagnetic field strength measured in four different distances during outgoing call mode in urban/rural area, indoor/outdoor setting and in moving car. These results are plotted by 10 brand mobile phones, shown in Figures 2–5.

**Table 1**

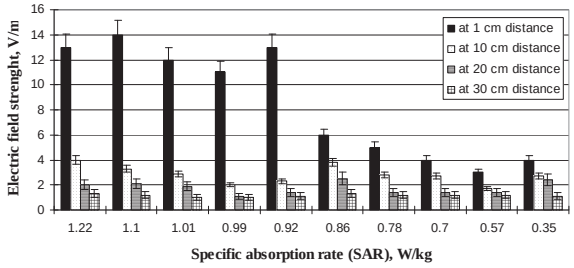
**Technical characteristics of the mobile phones**

| Specific absorption rate (SAR) | GSM 900 maximum power output | GSM 1800 maximum power output |
|---|---|---|
| 1.40 | 2 W | 1 W |
| 1.31 | 2 W | 1 W |
| 1.16 | 2 W | 1 W |
| 1.01 | 2 W | 1 W |
| 0.99 | 2 W | 1 W |
| 0.82 | 0.8 W | 0.25 W |
| 0.78 | 0.8 W | 0.25 W |
| 0.6 | 0.8 W | 0.25 W |
| 0.44 | 0.8 W | 0.25 W |
| 0.37 | 0.8 W | 0.25 W |

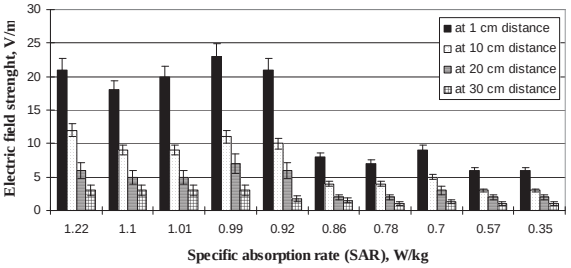
**GSM – Global System for Mobile Communications**

Figure 2a shows that the highest electric field strength emitted by the phones during call mode in urban area (GSM -1800) outdoors obtained with brand 1 (SAR-1.31) was 14 V/m and the lowest with brand 9 (SAR-0.44) was 3 V/m. The experiments revealed that the phone transmitted electric field strength depending on mobile phones SAR. Mobile phones with high SAR have much more higher maximum power output when compare with mobiles phones with low SAR. The higher SAR of mobile phone led to the higher electric field strength, which mobile phone had to emit. The electric field strength values of the mobile phones with SAR from 1.4 W/kg to 0.99 W/kg vary from 11 V/m to 14 V/m while mobile phones with SAR from 0.82 W/kg to 0.37 W/kg decreased by 2 to 5 times and vary from 3 V/m to 6 V/m.

Figure 2b shows that the highest electric field strength emitted by the phones during call mode in urban area (GSM-1800) indoors obtained with brand 4 (SAR-1.01) is 23 V/m and the lowest with brand 9 (SAR-0.44) is 5 V/m. The electric field strength values of the mobile phones with SAR




**(a)**



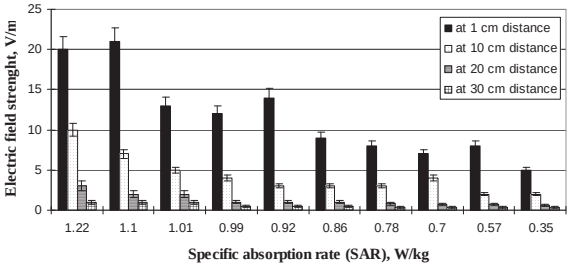

**(b)**

**Fig. 2 – Electric field strength values of the mobile phones measured in urban area (GSM–1800) during outgoing call mode at different distances from the probe: (a) outdoors and (b) indoors.**

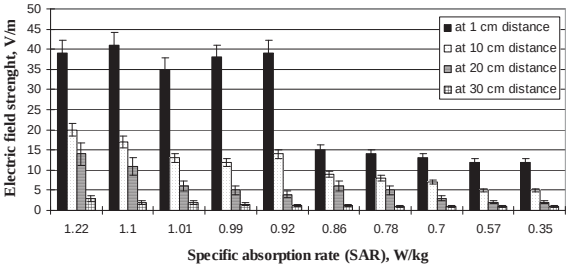
from 1.4 W/kg to 0.99 W/kg vary from 18 V/m to 23 V/m while mobile phones with SAR from 0.82 W/kg to 0.37 W/kg decreased by 2 to 5 times and vary from 5 V/m to 9 V/m. The experiments revealed that the electric field strength is about twice as large in the urban area indoors when compared with the urban area outdoors. The electric field strength values depends not only on SAR, they depends on electromagnetic signal intensity in exploring environment too. The electromagnetic signal intensity inside was 85 dBm and outside was 75 dBm. The lower intensity of electromagnetic signal led to the higher transmitting electric field strength, which mobile phone had to emit.

Figure 3a shows that the highest electric field strength emitted by the phones during call mode in rural area (GSM-900) outdoors obtained with brand 2 (SAR-1.31) is 21 V/m and the lowest with brand 10 (SAR-0.37) is 5 V/m. The electric field strength values of the mobile phones with SAR from 1.4 W/kg to 0.99 W/kg vary from 21 V/m to 12 V/m while mobile phones with SAR from 0.82 W/kg to 0.37 W/kg decreased by 2 to 4 times and vary from 5 V/m to 9 V/m.

Figure 3b shows that the highest electric field strength emitted by the phones during call mode in rural area (GSM-900) indoors obtained with brand 2 (SAR-1.31) is 41 V/m and the lowest with brands 9 and 10 (SAR-0.44 and SAR-0.37) is 12 V/m. The electric field strength values of the mobile phones with SAR from 1.4 W/kg to 0.99 W/kg vary from 41 V/m to 35 V/m while mobile phones with SAR from 0.82 W/kg to 0.37 W/kg decreased by 3 times and vary from 12 V/m to 15 V/m.




**(a)**




**(b)**

**Fig. 3 – Electric field strength values of the mobile phones measured in rural area (GSM-900) during outgoing call mode at different distances from the probe: (a) outdoors and (b) indoors.**

The experiments revealed that the higher exposition of the electromagnetic field radiation during calls was observed in rural area when compared with urban settings (Figures 2 and 3). It is because of different operating bands: in rural area, mobile phones are working at the 900 MHz band, while in urban settings – at the 1800 MHz band. The maximum powers that GSM mobile phones are permitted to transmit in rural area by the present standards are 2 W (900 Hz), while in urban area – 1 W (1800 Hz). Because of that, the electric field strength was about two times as high for rural area when compared with urban calls.

The electromagnetic signal intensity indoors was 100 dBm, while outdoors was 85 dBm. The lower intensity of electromagnetic signal in rural area compared with urban area led to the higher transmitted electromagnetic field strength.

Figure 4 shows that the highest electric field strength emitted by the phones during call mode in urban area (GSM-

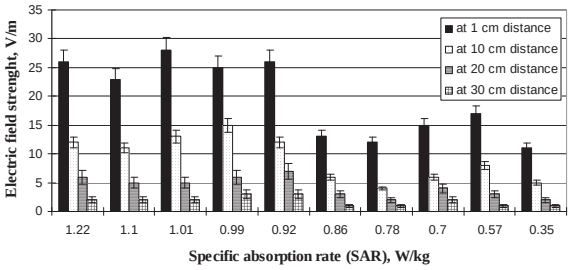



**Fig. 4 – Electric field strength values of the mobile phones measured in urban area (GSM-1800) during outgoing call mode moving (at different distances from the probe).**

1800) moving obtained with brand 3 (SAR-1.16) is 28 V/m and the lowest with brand 10 (SAR-0.37) is 11 V/m. The electric field strength values of the mobile phones with SAR from 1.4 W/kg to 0.99 W/kg vary from 23 V/m to 28 V/m while mobile phones with SAR from 0.82 W/kg to 0.37 W/kg decreased by 2 times and vary from 11 V/m to 17 V/m.

Figure 5 shows that the highest electric field strength emitted by the phones during call mode in rural area (GSM-

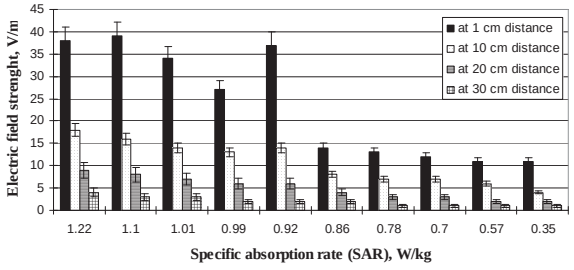



**Fig. 5 – Electric field strength values of the mobile phones measured in rural area (GSM -900) during outgoing call mode moving at different distances from the probe.**

900) moving obtained with brand 2 (SAR-1.31) is 39 V/m and the lowest with brands 9 and 10 (SAR-0.44 and SAR-0.37) is 11 V/m. The electric field strength values of the mobile phones with SAR from 1.4 W/kg to 0.99 W/kg vary from 28 V/m to 39 V/m while mobile phones with SAR from 0.82 W/kg to 0.37 W/kg decreased by 2 to 3 times and vary from 11 V/m to 14 V/m.

The factors influencing the electric field strength level while mobile phone was in the moving car were: the distance between mobile phone and the base station, the attenuation of the electromagnetic signal, and change of connecting base station "handover". The electromagnetic signal intensity was decreasing along with the longer distance, and the signal was very poor at the end of the base station cell. The signal was worse especially when mobile phone was used in the car, and when the car was mowing. At that case in the car the intensity of electromagnetic signal was 95 dBm to 105 dBm in rural area, and 80 to 90 dBm in urban area. The lower intensity of the electromagnetic signal in rural area when compared with urban area led to the higher transmitted electric field strength. Handovers (process when the mobile phones temporarily increase electric field strength while they are connecting to a new base station) could be made as well when the mobile phones were moving closely to the boundary of the main cell covered by one base station to another cell. However, we could not evaluate that fact.

## Discussion

The electric field strength of a mobile phone was found to depend on a mode of the phone, geographical factors, electromagnetic signal intensity from the antenna of the base station, shadowing, SAR, operating frequency and on a distance.

This work demonstrates that mobile phones emitted higher electric field strength in rural area, when compared with urban area. The electric field strength was about twice as large indoors, when compared with outdoors. This is because of the attenuation of the electromagnetic signal by houses. Mobile phones with high SAR have much more higher maximum power output, when compared with mobile phones with low SAR. The maximum powers that GSM mobile phones are permitted to transmit in rural area by the present standards are 2 W (900 Hz) while in urban area they are 1 W (1800 Hz). Because of that, the electromagnetic field strength of rural calls was about two times more than that of urban calls. Calls made from a mobile phone in a moving car gave a similar result like for indoor calls concerning the exposure of the electromagnetic field strength. However, calls made from a phone in a moving car exposed electric field strength two times more than that of calls in a standing (motionless) position.

Our measurements showed that in order to reduce risk for health and enhance safety it is of importance to keep safe distance between mobile telephone and human body. The experiments revealed that the electric field strength values of all mobile phones at distance of 10 cm decreased by more than 2 times, at the distance of 20 cm decreased by more than 4 times and at the distance of 30 cm decreased by more than 10 times when compared with the distance of 1 cm. Other authors underline similar solution that to make electromagnetic field safe it is important to ensure safe distance from the electromagnetic

field radiation source [25]. This could protect mobile phone users from biologic effects and health problems. In addition, safe distances could prevent mobile phone users from the possible carcinogenic effects in a long run.

Various guidelines exist for limiting exposure to radio frequency electromagnetic field by different countries. The most common one is *The Council Recommendation* on electromagnetic field exposure limits (1999/519/EC). Those are the guidelines, where standards of 41 V/m and 58 V/m are set as the limits (at 900 MHz and 1800 MHz). In many others countries the guidelines are far below this limit due to a complaint and scepticism demonstrated by public. Lithuania does not have limitations for electric field strength at 900 MHz and 1800 MHz (the only limitation for electromagnetic field power density is 10 μW/cm²). According to the formulas: $S = E^2/377*100$, where E is the electric field in V/m and S is the power density in $\mu W/cm^2$, we can translate electromagnetic field power density to electric field strength: 10 μW/cm² is about 6.1 V/m. If we can do such comparison, many mobile phones are far above the guidelines set in our country.

The strength of the study was to objectively measure and to assess the electromagnetic field radiation exposed by the mobile phone to the phone user in the different settings and at the different distances. The results of the study could be used for health risk and hazard prevention of population.

## Conclusion

Electromagnetic field radiation depends on mobile phone power class and factors, like urban or rural area, outdoor or indoor setting, and the distance of the mobile phone from the phone user. It is recommended to keep a mobile phone at the safe distance of 10, 20 or 30 cm from the body (especially head) during calls. This is necessary due to the uncertainties concerning mobile user safety and the lack of evidence on the direct harmful impact of mobile phone to human health.

## REFERENCES


1. *Hardell L, Sage C.* Biological effects from electromagnetic field exposure and public exposure standards. Biomed Pharmacother 2008; 62(2): 104−9.
2. *Krewski D, Glickman BW, Habash RW, Habbick B, Lotz WG, Mandeville R,* et al. Recent advances in research on radiofrequency fields and health: 2001-2003. J Toxicol Environ Health B Crit Rev 2007; 10(4): 287−318.
3. *Munshi A, Jalali R.* Cellular phones and their hazards: the current evidence. Natl Med J India 2002; 15(5): 275−7.
4. *Valuntaite V, Girgzdiene R.* Investigation of ozone emission and dispersion from photocopying machines. J Environ Eng Landsc Manage 2007; 15(2): 61−7.
5. *Vaisis V, Janusevicius T.* Investigation and evaluation of noise level in the Northern part of Klaipeda city. J Environ Eng Landsc Manage 2008; 16(2): 89−96.
6. *Usman A, Wan Ahmad W, Ab Kadir M, Mokhtar M.* Wireless Phones Electromagnetic Field Radiation Exposure Assessment. Am J Eng Appl Sci 2009; 4 (2): 771−4.
7. *IARC Working Group on the Evaluation of Carcinogenic Risks to Humans.* Non-ionizing radiation, Part II: Radiofrequency electromagnetic fields. Lyon, France: International Agency for Research on Cancer; 2011.
8. *Akbal A, Kiran Y, Sahin A, Turgut-Balik D, Balik H.* Effects of electromagnetic waves emitted by mobile phones on germination, root growth, and root tip cell mitotic division of Lens culinaris Medik. Pol J Environ Stud 2012; 21(1): 23−9.
9. *Baltrenas P, Fröhner K, Puzinas D.* Investigation of noise dispersion from seaport equipment on the enterprise territory and residential environment. J Environ Eng Landsc Manage 2007; 15(2): 85−92.
10. *Damian C, Foşalău C.* Sources of indoor noise and options to minimize adverse human health effects. Environ Eng Manage J 2011; 10(3): 393−400.
11. *Bahr A, Dorn H, Bolz T.* Dosimetric assessment of an exposure system for simulating GSM and WCDMA mobile phone usage. Bioelectromagnetics 2006; 27(4): 320–7.
12. *Januseviciene I, Venckus Z.* The numerical modeling of nitrogen oxides and coal monoxide in the atmosphere when applying PHOENICS programme. J Environ Eng Landsc Manage 2011; 3(19): 225–33.
13. *Paulauskas L, Klimas R.* Modelling of the spread of motor transport noise in Siauliai city. J Environ Eng Landsc Manage 2011; 1(19): 62–70.
14. *Hillert L, Ahlbom A, Neasham D, Feychting M, Järup L, Navin R,* et al. Call-related factors influencing output power from mobile phones. J Expo Sci Environ Epidemiol 2006; 16(6): 507−14.
15. *INTERPHONE Study Group.* Brain tumour risk in relation to mobile phone use: results of the INTERPHONE international case-control study. Int J Epidemiol 2010; 39(3): 675−94.
16. *Bednarek K.* Electromagnetic Action of Heavy-Current Equipment Operating With Power Frequency. Int J Occup Saf Ergon 2010; 16(3): 357−68.
17. *Baltrenas P, Buckus R, Vasarevicus S.* Modelling of the Computer Classroom Electromagnetic Field. Electron Electric Eng 2011; 109(3): 75–80.
18. *Baltrenas P, Buckus R.* The exploration and assessment of electromagnetics fields in duplicators. . J Environ Eng Landsc Manage 2009; 17(2): 89−96. (Lithuanian)
19. *Dolan M, Rowley J.* The precautionary principle in the context of mobile phone and base station radio frequency exposures. Environ Health Perspect 2009; 117(9): 1329–32.
20. *Grigoriev J.* Electromagnetic Fields and the Public: EMF Standards and Estimation of Risk. Earth Environ Sci 2010; 10(1): 1–6.
21. *Lin JC.* Cellular mobile phones and children. IEEE Antennas and Propagation Magazine 2002; 44 (5): 142-5.
22. *Mousa A.* Electromagnetic Radiation Measurements and Safety Issues of some Cellular Base Stations in Nablus. J Eng Sci Technol Rev 2011; 1(4): 35−42.
23. *Loughran SP, Wood AW, Barton JM, Croft RJ, Thompson B, Stough C.* The effect of electromagnetic fields emitted by mobile phones on human sleep. Neuroreport 2005; 16(17): 1973−6.
24. *Psenakova Z, Hudecova J.* Influence of Electromagnetic Fields by Electronic Implants in Medicine. Electron Electric Eng 2009; 95 (7): 37–40.
25. *Ahlbom A, Cardis E, Green A, Linet M, Savitz D, Swerdlow A.* Review of epidemiologic literature on EMF and health. Environ Health Perspect 2001; 109(Suppl 6): 911–33.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# Fractures of the humerus during arm wrestling

## Prelomi humerusa nastali obaranjem ruke

**Marko Ž. Bumbaširević*†, Aleksandar R. Lešić*†, Sladjana Z. Andjelković*†, Tomislav D. Palibrk*, Suzana M. Milutinović***

***Orthopedic and Traumatology Clinic, Clinical Center of Serbia, Belgrade, Serbia;**
**†Faculty of Medicine, University of Belgrade, Belgrade, Serbia**

**Abstract**

**Background/Aim.** Humeral shaft fractures may occur as a result of arm wrestling. The aim of this study was to present our treatment of humerus fracture sustained during arm wrestling. **Methods.** A total of six patients, aged 22 to 48, were treated at our department form January 2008 to January 2010 with open reduction and internal fixation and with hanging arm casts. A review of all the relevant literature on the subject was also presented. **Results.** In all the cases, the fractures healed and function returned to normal. No patient had any neural or vascular compromise. **Conclusion.** Closed and operative treatments were equally successful in all reported cases.

**Key words:**
**humeral fractures; athletic injuries; orthopedic procedures; treatment outcome.**

**Apstrakt**

**Uvod/Cilj.** Prelom dijafize humerusa može nastati kao rezultat „obaranja ruke“. Cilj rada bio je prikaz načina lečenja preloma humerusa zadobijenih prilikom obaranja ruke. **Metode.** U periodu od januara 2008. do januara 2010. godine lečili smo na našem odeljenju šest pacijenata, starosti od 22 do 48 godina, otvorenom repozicijom i unutrašnjom fiksacijom ili visećim gipsom. Takođe, pregledana je i analizirana postojeća literatura vezana za ovu vrstu povrede. **Rezultati.** Kod svih bolesnika došlo je do srastanja preloma i potpunog funkcionalnog oporavka. Pacijenti nisu imali neurološke, ni vaskularne povrede. **Zaključak.** Operativno i neoperativno lečenje jednako su uspešni kod ove vrste povreda.

**Ključne reči:**
**humerus, prelomi; povrede, atletske; ortopedske procedure; lečenje, ishod.**

### Introduction

Humeral shaft fractures may occur as a result of arm wrestling, also known as “iron arm”, “wrist wrestling” or “Indian wrestling”. In this game (contest) two opponents sit face-to-face with their elbows placed on a surface, gripping their hands and trying to force opponent's arm down. According to the literature, different types of fractures can occur. Spiral fracture of the humeral shaft with or without butterfly fragment [1], fracture of the medial humeral epicondyle [2], radial head fracture with anterior dislocation, even a radial shaft fracture [3]. However, most of the injuries from arm wrestling are soft tissue injuries as muscular strain and sprain of the shoulder, elbow and wrist joints.

The aim of this study was to present our experience with treatment of humerus fracture sustained during arm wrestling.

### Methods

Within a 2-year period (from January 2008 to January 2010) we treated 6 patients with humeral shaft fractures. The treatment included open reduction and internal fixation and hanging arm casts.

### Results and discussion

All treated patients were males and all of them wrestled with their right arm. The patients ranged in age from 22 to 48 years old, average 31.2 years.

Their humerus fractures were spiral in nature and usually located between the middle and distal third of the humerus. One case had a medial butterfly fragment. Three patients were operated primarily (Figure 1). The fracture was fixed with an AO compression plate, and the postoperative course was regular. During surgical procedure we found a muscle interposed between the fracture fragments. Three patients were treated with a hanging arm cast following closed reduction (Figure 2).

All the fractures united. The average union time of the humeral shaft fracture was ten weeks. No patient had any neural or vascular compromise. There was no shoulder, elbow, nor finger stiffness. The patients were re-

**Correspondence to:** Tomislav D. Palibrk, Koste Todorovića 26, 11 000 Belgrade, Serbia. Phone: +381 11 366 2330, +381 63 280 633. E-mail: tpalibrk@gmail.com

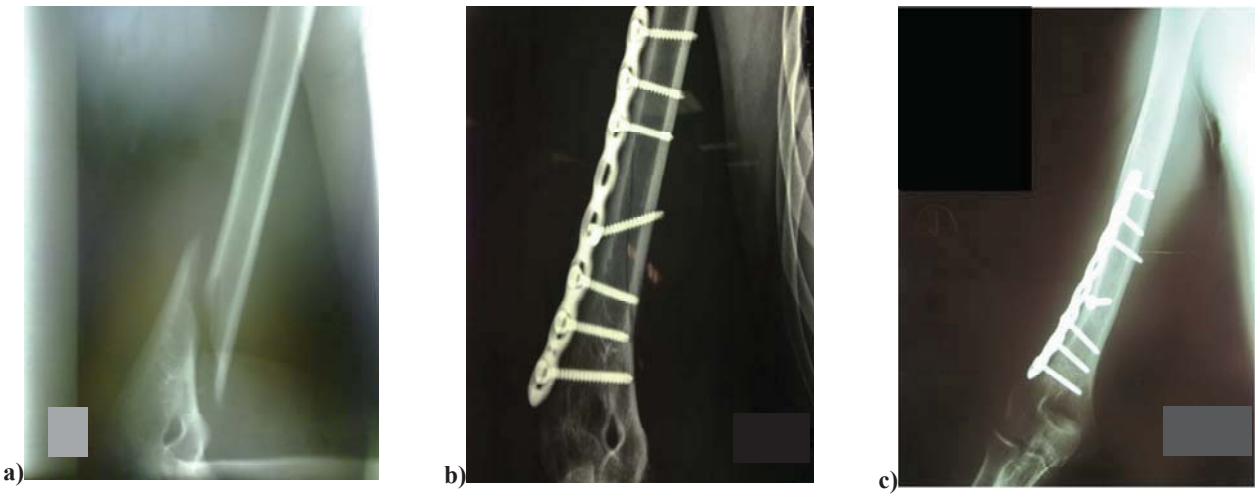

**Fig. 1 – Patient A: a) preoperative radiography done at the Emergency Room Department on the day of injury; b) and c) postoperative radiography.**

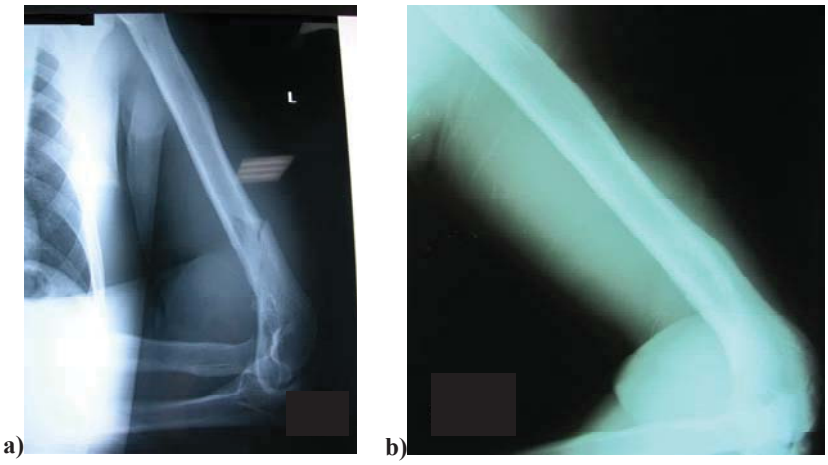

**Fig. 2 – Patient B: a) initial radiography done at the Emergency Room Department on the day of injury; b) radiography done six months after the injury (treatment with a hanging arm cast).**

turned into their previous job after an average time period of 16 weeks.

Fractures of the shaft of the humerus as a result of muscular violence are uncommon. In the literature we found articles with a small number of patients (mostly from two to ten cases) [4–6]. The largest series we found in a work of Ogawa and Ui [7] from Japan. They analyzed 30 patients with humeral shaft fracture sustained during arm wrestling.

It is obvious that arm wrestling can be connected with a powerful muscle activity, especially in the shoulder joint. While the elbow joint is fixed in flexion by the biceps and brachialis muscle, the shoulder joint is actively internally rotated against the opponent by pectoralis major, subscapularis and teres major muscle. It results in strong violent torque forces across the humeral shaft. The possible mechanism was discussed in many articles [2, 6, 8, 9]. Humerus shaft fractures are spiral with or without butterfly fragment. According to some authors, pure rotator force without axial load on the humerus causes spiral fracture only, while axial load and rotator force can cause a butterfly fragment [1].

Fractures will unite regardless of the type of treatment, but we believe that open reduction and internal fixation shortens the immobilization time and functional recovery is faster. In our cases there was no nerve involvement, but we found that some authors recorded radial nerve palsy [7, 8, 10].

Some authors also mention other factors like hypertrophy of muscles, fatigue and kinetic forces of body weight, which may contribute to fracture of the humerus, because they create of unbalanced forces [8]. Some also believe that position of the arm during competition determine the fracture location and type [11].

In Citak's series of cases with only muscle strain, patients had regular sport activity. These factors may be considered as important for the intensity of injury [3]. On the other hand, many believe that these clinical entities may occur in anyone of any age who engages in this type of sport [7, 12].

## Conclusion

Arm wrestling may cause severe injuries. Different types of fractures after arm wrestling have been reported in

the literature, but the most common is humeral shaft fracture. These fractures are the result of torsion forces and axial compression applied to the humerus. Closed and operative treatments were equally successful in all reported cases.

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## REFERENCES

1. *Moon MS, Moon YW, Sihn JC, Kim SS, Sun DH, Kim SS.* Arm wrestler's injury-A report of thirteen cases. J. Orthopedic Surg 1997; 5(2): 29−34.
2. *Ogawa K, Ui M.* Fracture-separation of the medial humeral epicondyle caused by arm wrestling. J Trauma 1996; 41(3): 494−7.
3. *Citak M, Backhaus M, Seybold D, Muhr G, Roetman B.* Arm wrestling injuries: report on 11 cases with different injuries. Sportverletz Sportschaden 2010; 24(2): 107−10. (German)
4. *Moon MS, Kim I, Han IH, Suh KH, Hwang JD.* Arm wrestler's injury: report of seven cases. Clin Orthop Relat Res 1980; (147): 219−21.
5. *Peace PK.* Fractures of the humerus from arm wrestling. Injury 1977; 9(2): 162−3.
6. *Brismar B, Spangen L.* Fracture of the humerus from arm wrestling. Acta Orthop Scand 1975; 46(4): 707−8.
7. *Ogawa K, Ui M.* Humeral shaft fracture sustained during arm wrestling: report on 30 cases and review of the literature. J Trauma 1997; 42(2): 243−6.
8. *Low BY, Lim J.* Fracture of humerus during arm wrestling: report of 5 cases. Singapure Med J 1991; 32(1): 47−49.
9. *Khashaba A.* Broken arm wrestler. Br J Sports Med 2000; 34(6): 461−2.
10. *Heilbronner DM, Manoli A, Morawa LG.* Fractures of the humerus in arm wrestlers. Clin Orthop Relat Res 1980; (149): 169−71.
11. *Whitaker JH.* Arm wrestling fractures: a humerus twist. Am J Sports Med 1977; 5(2): 67−77.
12. *Ahčan U, Aleš A, Završnik J. Završnik J.* Spiral fracture of the humerus caused by arm wrestling. Eur J Trauma 2000; 26(6): 308−11.

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# Biological pollutants in indoor air

## Biološki zagađivači u zatvorenom prostoru

**Sonja S. Radaković*, Milan Marjanović†, Maja Šurbatović*, Gradimir Vukčević‡, Milena Jovašević-Stojanović§, Elizabeta Ristanović***

***Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia; †Galenika, Belgrade, Serbia; ‡Sector of Logistics, Military Medical Academy, Belgrade, Serbia; §Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia**

**Key words:**
**air pollution; air pollution, indoor; humidity; bacteria; fungi; protozoa.**

**Ključne reči:**
**vazduh, zagađenje; vazduh, zagađenje u zatvorenom prostoru; vlažnost; bakterije; gljivice; protozoa.**

### Introduction

Interest in indoor air quality emerged in the USA in the 1970, when "sick building syndrome" was first described [1]. During those years, due to energy crisis, building environments substantially changed – namely, ventilation, air-conditioning, and other energy-demanding maintenance processes were sparingly used, particularly in public office buildings. Many office workers reported headache, mucous membrane irritation, and difficulty in concentrating during working hours. All symptoms disappeared at home. Understanding of risk factors underlying this epidemic led to improvement of legislation regarding ventilation rates and maintenance, so the number of complaints decreased in the 1990s.

Indoor air pollution is present in virtually each and every indoor space, with the exception of strictly controlled and sterile spaces in pharmaceutical, medical and research facilities. Biological pollutants may originate from human activity, building materials and carpets; they may also penetrate from outdoor environments by forced ventilation, diffusion or infiltration. Humans are inevitably exposed to such pollutants, considering the amount of time spent indoors, but the influence of the pollution on human health may vary, depends on age, health condition, and individual predisposition. Historically, moulds were the very first recognized and recorded contaminants in indoor environment. In Bible, there are several passages where the moulds are mentioned, together with possible health aspect of exposure to them [2].

Interest in indoor air monitoring is growing worldwide every year, considering the indoor pollution ranged among the first five leading causes of illness in the world [1]. The World Health Organization (WHO) Regional Office for Europe prepared in 2006 the Guidelines for indoor air quality [3], according to previously formulated postulate "The right to healthy indoor air". In these Guidelines, the WHO presents public health risks due to dampness, associated microbial growth and contamination of indoor air. This organization recognized problems of indoor air quality as important risk factors for human health all over the world, regardless the annual income of given country. The importance of this problem is emphasized by the fact that people, particularly vulnerable populations such are children, pregnant women, elderly, ill and disabled, spend a substantial amount of time indoors.

There is a wide range of possible biological contaminants in indoor air, with different origin and patterns of spreading. For example, pollen and spores of plants are predominantly emitted from outside the building, transferring through doors and windows, or by personal contacts. Various species of bacteria, fungi, algae, and protozoa can originate both from outside space and from materials inside the buildings. According to conclusions of a WHO working group [3], there are no specific microorganisms that can be specifically associated with indoor air pollution; rather they represent common allergens and other pathogens. However, some agents such as house dust mites and pet hairs are predominantly present in indoor air. Considering the variety of microorganisms and their characteristics, it is virtually impossible to quantify their concentrations in a form of tolerable levels of exposure.

Many studies have found that health risks are increased by exposure to microorganisms, but there is growing evidence that exposure in early life to endotoxins and/or fungal agents protects against atopy and allergic disease. A prospective birth cohort study suggested an inverse relation

**Correspondence to:** Sonja S. Radaković, Sector of Preventive Medicine, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone/Fax: +381 11 266 3368. E-mail: sonja.radakovic@vma.mod.gov.rs

between the levels of these pollutants and wheezing problems in 4-year-old children [4]. These results are in agreement with findings obtained from several studies of reduced incidence of hay fever, eczema and asthma in children who grew up on farms compared to urban children, and thus supported the "hygiene hypothesis" which suggests the protective role of microbial exposure.

**Effects of dampness on indoor exposure to biological pollutants**

Besides the WHO Guidelines mentioned above, another major reviews published in 2004 by the Institute of Medicine (IOM) report on a wide range of health effects of which there were sufficient evidence for associating the presence of pathogens in damp buildings with following diseases and symptoms: nasal and throat symptoms, cough, wheezing, asthma exacerbation, and hypersensitivity pneumonitis [5]. The IOM committee concluded that limited or suggestive evidence existed for associating the same exposure with shortness of breath, asthma development, and lower respiratory disease. Building dampness and mould are present even in high-income countries. Estimations of dampness and mould presence vary from 20% buildings in Scandinavia [6] to 50% buildings in United States [7]. Fewer studies were conducted in low-income countries; nevertheless, they suggest that the problem of indoor dampness is even greater [8]. The dampness and mould are traditionally related to overcrowded accommodations without adequate heating, ventilation and insulation, hence, the lower income is – these problems are more evident. Climatic changes such as global warming with more frequent occurrence of storms and heavy rains lead to gradual increase in sea level. Together with more frequent floods, it results in the increase in the percentage of buildings affected by dampness and mould, particularly in the areas near the rivers. Increased indoor dampness provides optimal conditions for increased growth of dust mites, fungi and bacteria. Furthermore, chemical contamination is promoted too, because dampness accelerates the degradation of building materials releasing their particles into the air. Finally, excess moisture in indoor spaces creates optimal conditions for insects and rodents. These animals release their own allergens into indoor environments, but can also be the reservoir of contagious diseases agents.

Indoor air contains numerous microorganisms of very different types. For example, house dust mites are small arachnids. Among numerous various species, few are of major importance for indoor air contamination, and their growth is directly related to relative humidity. Moreover, in house dust mites living in mild and temperate climatic conditions, moisture represents a major factor of their increased growth. For survival, development and multiplication, they require a relative humidity in excess of 45–50%, but their activity, including feeding and maturation is more rapid at higher rates of relative humidity which was confirmed in field studies [9, 10]. The common foods for house dust mites are skin scales, but they are adapted to use other food sources, as well. House dust mite allergens are commonly produced by *Dermatophagoides pteronyssinus* (proteolytic allergens Der p I and Der p II), and *Dermatophagoides farina* (Der f I). The faecal particles containing these allergens are predominantly found in house dust, mattresses and pillows [11].

Other common indoor air pollutants are fungi. Their presence in indoor air is a result of transportation from outside environment *via* building materials, carpets, furniture, wallpapers, etc. Ventilation and air-conditioning systems are another common ways of penetrating of fungi into the buildings. The rate of further growth, spreading and multiplication depends exclusively on moisture content in indoor air, regardless the type of surface. Even the primary colonizers, or xerophilic fungi, which may grow on less moisture surfaces, require relative humidity in excess of 50%. Secondary colonizers require more humidity in their substrates, while tertiary colonizers, or hydrophilic, need sheer water content in liquid phase for their germination and mycelia growth [12]; hence, they are present only in buildings with severe condensation problems. Natural food source for fungi vary from plant, animal and human particles in house dust, to fragments of construction materials such as floor and wall textile coverings, furniture, residue of cooking traces, food storage, paper materials. Since these materials are in ample in every building, and considering that optimal temperature for fungi growth ranges from 10–35 ºC, the only limiting factor for development of fungi and mould contamination is dampness. Fungi may be extremely harmful for human health, but may also destruct the building itself, particularly wooden parts, such as roofs, timbers, and other materials.

Some fungi species produce strong allergens, which initiate immune reaction type I (IgE mediated). For example, the indoor contamination with *Alternaria, Penicillium, Aspergillus* and *Cladosporium* spp., is related to asthma and other allergic respiratory diseases. Some of these species, such as *Penicillium* and *Aspergillus* can also induce type III allergy (IgG mediated), while at high concentrations, may also initiate combined type III and IV reaction manifested as hypersensitivity pneumonitis. Major fungal allergens are isolated and identified (such as Cla h I from *Cladosporum herbarum*, Alt a I and Alt a II from *Alternaria alternata* and Asp f I and Asp f III from *Aspergillus fumigatus*). Most of them are glycopeptide enzymes, produced during germination and released through spores and hyphae, i.e. live particles [13]. Nevertheless, even dead particles carry substantial health risk, because they may contain possibly harmful (1→3)-β-D-glucans with the potential to impair respiratory functioning [14], and mycotoxins. The harmful effect of mycotoxins is manifested by interference with RNA synthesis leading to DNA damage. Sometimes this toxicity is beneficial – e.g. penicillin, a strong bactericidic antibiotic, is a mycotoxin produced by fungi *Penicillium*. But, in general, fungi mycotoxins have strong genotoxic, cancerogenic, and immunotoxic potential. The cancerogenic effects of aflatoxin (mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*) are well known. The most important mycotoxins related to indoor air contamination are trichotechenes, generated by fungi *Stachybotrys chartarum* (macrocyclic trichotechenes, trichodermin, sterigmatocystin and satratoxin G) [15].

Several fungi also produce volatile organic compounds as the result of their metabolic processes, but their effects on human health are yet to be investigated. The assessment of fungi contamination in indoor air is very difficult. In a study conducted by Pietarinen et al. [16] culture methods identified only few of species that were recognized and quantified by quantitative polymerase chain reaction (qPCR). *Penicillium, Aspergillus* and *Streptomyces* were predominantly indentified by both methods. But, culture method successfully indentified *Aspergillus fumigates* only in samples containing the amount of total viable fungi more than $10^6$ cfu/g. Likewise, culture method was able to detect *Stachybotrys chartarum* only in samples with a very high level of fungi contamination, contrary to qPCR method. These results are in agreement with another Finnish study which confirmed the highest prevalence of *Penicillium/Aspergillus* species in house dust, with more precise results obtained by qPCR method [17]. The same authors indicated that concentrations of fungi differ significantly between the seasons with the highest concentrations of *Aspergillus* in winter (more than 10,000 cells/mg of dust).

Numerous species of bacteria are also common contaminants of indoor air. Contrary to relatively harmless saprophytic species originated from people, the species that actively grow in indoor substrates may be potentially harmful. Although the health aspects on moulds and fungi in indoor air are extensively studied, similar investigations of bacteria influence have been of little interest so far. The common feature for both types of microorganisms are requirements for water and temperature ranges for optimal growth and development. Hence, we can fairly assume that bacteria grow in the same sites as fungi, preferably on damp substrates. This suggestion is confirmed by evidences that species such as *Streptomycetes*, which are not normally present in indoor environments, easily grow on wet surfaces, so their presence is used in screening for moisture problems in buildings [18]. Very few studies were conducted so far regarding this problem, apart from several investigation conducted by Finnish authors who identified *Sreptomyces* and *Mycobacteria* in indoor surfaces [16, 19–21]. The latter bacteria have particularly strong immunogenic potential originated from cell wall components. The majority of culturable bacteria in indoor dust and air are Gram-positive *Micrococcus, Staphylococcus* and *Bacillus* strains. Similarly to fungi, there is a certain doubt regarding the method for determination of bacterial load in house dust. Culture method is relatively simple, but only 1% of airborne bacteria in indoor air are culturable. Culturable bacterial concentrations range from $7.3 \times 10^4$ to $1.85 \times 10^7$ cfu/g (public buildings) and $1.1 \times 10^4$ to $2.1 \times 10^7$ cfu/g in samples of house dust [15]. Chemical markers analysis, i.e. detection of chemical compounds that build the bacterial cell wall (3-hydroxy fatty acids for Gram-negative bacteria and muramic acid for Gram-positive bacteria), has limited value, since these compounds are non-specific, and the gas chromatography-mass spectrometry method requires complex sample preparation. Simultaneous usage of all the three methods, as reported by Karkkainen et al. [21], reveals only a moderate correlation between them. Another study conducted in Finland also indicated that culture method failed to detect *Aspergillus fumigates*, while qPCR in the same samples detected the average of $2.21 \times 10^3$ cells/g. The average concentrations of *Penicillium spp.* and *Aspergillus spp*. were significantly lower when detected by culture method than qPCR ($9.01 \times 10^3$ cfu/g *vs* $1.96 \times 10^5$ cells/g and $1.35 \times 10^4$ cfu/g *vs* $5.44 \times 10^6$ cells/g, respectively) [19].

Finally, protozoa may also be present in indoor air in damp buildings. Yli-Pirila et al. [22] detected amoebae in 22% of 124 samples of various materials collected from buildings with evident moisture damage; among them there were 11 samples (collected from the most severely damaged surfaces) contained ciliates and flagellates. Field studies on the presence and concentrations of protozoa in indoor air, as well as health aspects of these microorganisms in given conditions are still lacking, with the exception of one *in vitro* study conducted by the same authors, who suggested that amoebae act synergistically with certain bacteria, enhancing their cytotoxic and proinflammatory potential [23].

## Conclusion

Epidemiological, clinical and toxicological evidences suggest that microbiological contamination of indoor air may be related to numerous diseases and health conditions. Damp and humid environments are obligatory factors for growth, development and multiplication of microbes, hence, the main public health goal should be targeting these problems. Considering the variety of microorganisms, possible synergistic effects, the fact that the most endangered populations are children, women, elderly (who spent relatively substantial time indoors), disadvantages of determination techniques and lack of evidence-based risk assessment, it should be concluded that further investigations are needed.

In Serbia, the very first study on the presence and concentrations of biological pollutants in indoor air is ongoing, financially supported by the Ministry of Education, Science and Technological Development. The first results expected to be available within 2014.

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## REFERENCES

1. *Cox-Ganser J, Park JH, Kanwal R.* Epidemiology and health effects in moisture-damaged damp buildings. In: *Goldstein WE*, editor. Sick Building Syndrome and Related Illness. Boca Raton: CRC Press, Taylor & Frances Group; 2011. p. 11−23.

2. *Anyanwu EC*. Advances in Environmental Health Effects of Toxigenic Mold and Mycotoxins. New York: Nova Science Publishers, Inc; 2011.
3. *World Health Organization (WHO)*. Guidelines for indoor air quality: Dampness and mould. Copenhagen: WHO Regional Office for Europe; 2009.
4. *Douwes J, van Stien R, Doekes G, Smit J, Kerkof M, Gerritsen J*, et al. Can bacterial endotoxin exposure reduce the risk of asthma?, The Prevention and Incidence of Asthma and Mite Allergy birth cohort study. J Allerg Clin Immunol 2006; 117: 1067−73.
5. *Institute of Medicine (IOM)*. Damp Indoor Spaces and Health. Washington, DC: The National Academies Pres; 2004.
6. *Gunnbjörnsdóttir MI, Franklin KA, Norbäck D, Björnsson E, Gislason D, Lindberg E*, et al.. Prevalence and incidence of respiratory symptoms in relation to indoor dampness: the RHINE study. Thorax 2006; 61(3): 221−5.
7. *Mudarri D, Fisk WJ*. Public health and economic impact of dampness and mold. Indoor Air 2007; 17(3): 226−35.
8. *Tham KW, Zuraimi MS, Koh D, Chew FT, Ooi PL*. Associations between home dampness and presence of molds with asthma and allergic symptoms among young children in the tropics. Ped Allerg Immunol 2007; 18(5): 418−24.
9. *Arlian LG*. Water balance and humidity requirements of house dust mites. Exper Appl Acarol 1992; 16(1−2): 15−35.
10. *Zock JP, Heinrich J, Jarvis D, Verlato G, Norback D, Plana E*, et al. Distribution and determinants of house dust mite allergens in Europe: the European Community Respiratory Health Survey II. J Allergy Clin Immunol 2006; 118(3): 682−90.
11. *Simpson A, Simpsom B, Custovic A, Cain G, Craven M, Woodcock A*. Household characteristics and mite allergen levels in Manchester, UK. Clin Exp Allergy 2002; 32(10): 1413−9.
12. *Grant C, Hunter CA, Flannigan B, Bravery AF*. The moisture requirements of moulds isolated from domestic dwellings. Int Biodeterior 1989; 25(4): 259−84.
13. *Green BJ, Tovey ER, Serkombe JK, Blachere FM, Beezhold DH, Schmechel D,* et al. Airborne fungal fragments and allergenicity. Med Mycol 2006; 44(suppl 1): 245−55.
14. *Douwes J*. (1: >3)-Beta-D-glucans and respiratory health: a review of the scientific evidence. Indoor Air 2005; 15(3): 160−9.
15. *Bloom E, Bal K, Nyman E, Must A, Larsson L*. Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by Stachybotrys and Aspergillus spp. in indoor environments. Appl Environ Microbiol 2007; 73(13): 4211−7.
16. *Pietarinen VM, Rintala H, Hyvatinen A, Lignell U, Karkkainen P, Nevalainen A*. Quantitative PCR analysis of fungi and bacteria in building materials and comparison to culture-based analysis. J Environ Monit 2008; 10(5): 655−63.
17. *Kaarakainen P, Rintala H, Vepsalainen A, Hyvarinen A, Nevalainen A, Meklin T*. Microbial content of house dust samples determined with qPCR. Sci Tot Environ 2009; 407(16): 4673−80.
18. *Solomon GM, Hjelmroos-Koski M, Rotkin-Ellman M, Hammond KS*. Airborne mold and endotoxin concentrations in New Orleans, Louisiana, after flooding, October through November 2005. Environ Health Perspect 2006; 114(9): 1381−6.
19. *Lignell U, Meklin T, Rintala H, Hyvarinen A, Vepsalainen A, Pekkanen J*, et al. Evaluation of quantitative PCR and culture methods for detection of house fungi and streptomycetes in relation to moisture damage of the house. Lett Appl Microbiol 2008; 47(4): 303−8.
20. *Rintala H, Nevalainen A*. Quantitative measurement of streptomycetes using real-time PCR. J Environ Monit 2006; 8(7): 745−9.
21. *Karkkainen PM, Valkonen M, Hyvarinen A, Nevalainene A, Rintala H*. Determination of bacterial load in house dust using qPCR, chemical markers and culture. J Environ Monit 2010; 12(3): 759−68.
22. *Yli-Pirilä T, Kusnetsov J, Haatainen S, Hänninen M, Jalava P, Reiman M,* et al. Amoebae and other protozoa in material samples from moisture-damaged buildings. Environ Res 2004; 96(3): 250−6.
23. *Yli-Pirilä T, Huttunen K, Nevalainen A, Seuri M, Hirvonen M*. Effects of co-culture of amoebae with indoor microbes on their cytotoxic and proinflammatory potential. Environ Toxicol 2007; 22(4): 357−67.

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CASE REPORTS

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# Double heart rupture after acute myocardial infarction: A case report

## Dupla ruptura srca nakon akutnog infarkta miokarda

**Igor Ivanov*, Aleksandra Lovrenski†, Jadranka Dejanović*, Milovan Petrović*, Robert Jung*, Violetta Raffay‡**

***Clinic of Cardiology, Institute for Cardiovascular Diseases of Vojvodina, Sremska Kamenica, Serbia; †Pathology Department, Institute for Lung Diseases of Vojvodina, Sremska Kamenica, Serbia; ‡Municipal Institute for Emergency Medicine, Novi Sad, Serbia**

**Abstract**

**Introduction.** Double heart rupture is a rare complication of acute myocardial infarction with high mortality. **Case report.** We presented a 67-year-old female patient with symptoms and signs of myocardial infarction, diagnosed with echocardiography, rupture of the septum, the presence of a thrombus and a small pericardial effusion. Soon after admission the patient died. Autopsy revealed tamponade and double myocardial rupture, free wall rupture and ventricular septal rupture, as a cause of death. **Conclusion.** This case highlights the need to evaluate patients with myocardial infarction, recurrent chest pain, echocardiographic signs of effusion and the presence of thrombus in the pericardium in terms of double rupture of the heart.

**Key words:**
**myocardial infarction; acute disease; heart rupture, post-infarction; echocardiography.**

**Apstrakt**

**Uvod.** Dupla ruptura srca retka je komplikacija akutnog infarkta miokarda sa visokom stopom smrtnosti. **Prikaz bolesnika.** U radu je prikazana 67-godišnja bolesnica sa simptomima i znacima akutnog infarkta miokarda, kojoj je ehokardografski postavljena dijagnoza rupture septuma i prisustvo tromba i manjeg perikardnog izliva. Ubrzo po prijemu bolesnica je umrla. Obdukcijom je ustanovljena tamponada i dupla ruptura srca, ruptura slobodnog zida leve komore i ventrikularnog septuma, što je bilo uzrok smrti. **Zaključak.** Prikaz ove bolesnice ukazuje na potrebu procene bolesnika sa infarktom miokarda, ponavljanim bolovima u zoni grudi, ehokardiografskim znakovima izliva i prisustva tromba u perikardu u smislu dvostruke rupture srca.

**Ključne reči:**
**infarkt miokarda; akutna bolest; srce, postinfarktna ruptura; ehokardiografija.**

### Introduction

There are three manifestations of heart rupture which include the left ventricle (LV): free wall rupture (FWR), ventricular septal rupture (VSR) and papillary muscle rupture (PMR). Double heart rupture (DHR) implies the combined presence of the two of the above forms. DHR is a very rare complication, present in about 0.3% of acute myocardial infarction, with the most common combination of FWR and VSR [1]. In a relatively small study, it was revealed that FWR in half of the cases occurred after VSR [1]. It was shown that primary percutaneous coronary interventions (pPCI) are protective factor for FWR compared to thrombolysis [2]. Older age, female gender, and a prolonged time from the onset of symptoms to treatment are independent risk factors for rupture [3].

Echocardiography is a reliable method for the diagnosis of mechanical complications in acute myocardial infarction in terms of localization and size, but it is also crucial in the decision about treatment and postoperative follow-up [4].

We presented a patient with late presentation of inferior myocardial infarction involving the right ventricle, complicated with a combination of FWR and VSR. Caution is obligatory in such patients because of their high mortality, and also the necessity of prompt diagnosis and treatment.

### Case report

A 67-year-old female was admitted in the intensive care unit because of prolonged chest pain, shortness of breath, and electrocardiographic signs of acute myocardial infarction with ST-segment elevation in the inferior and right ventricle leads. Symptoms occurred three days before admission, with epigastric pain, nausea and vomit, which were initially diagnosed as gastritis. The next day the pain spread to the chest, and increased in intensity two hours before admission. The

**Correspondence to:** Igor Ivanov, Clinic of Cardiology, Institute for Cardiovascular Diseases of Vojvodina, Radnička 35b, 21 000 Novi Sad, Serbia Serbia. Phone: +381 21 420 155. E-mail: ivanovigor05@gmail.com

patient was previously treated for angina pectoris, with risk factors for coronary disease of a positive family history and high blood pressure.

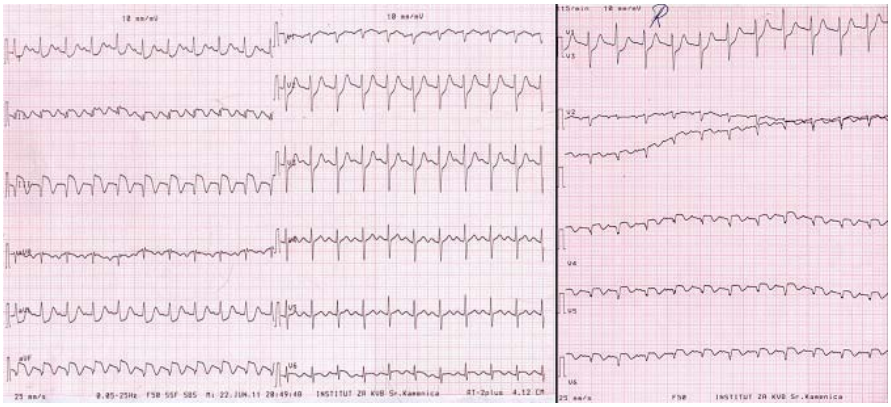
Objective examination on admission revealed somnolent state of the patient, diaphoretic skin, dyspnea, tachycardia, low blood pressure, bilateral crackles and strong precordial holosystolic audible murmur. The laboratory findings reported elevated levels of cardiac enzymes.

The 12-lead electrocardiogram (ECG) recorded sinus tachycardia with signs of myocardial infarction of inferior localization (Figure 1a). In the right precordial leads, ST segment elevation was seen in VR4 lead as a sign of right ventricle myocardial infarction (Figure 1b).

discontinuity in the ventricular septum was recorded, measuring up to 1.6 cm, with the occurrence of left-right shunt, which was verified by two-dimensional echocardiography and with color and continuous Doppler (Figure 2a). Due to the large dimensions of rupture, the gradient at the shunt was not high. The other walls of the left ventricle were moved hyperkinetically. No significant valvular heart disease was found.

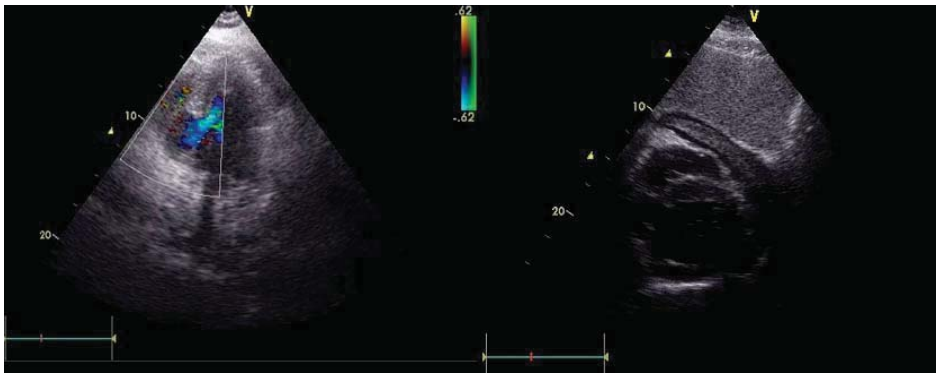
Around the heart a large thrombus was observed, that has partially compromised the free wall of the right ventricle (Figure 2b). There was no sign of the free wall rupture in the lower part of the left ventricle.

The cardiothoracic surgeon was immediately consulted due to life treating condition, in the first place state of shock.



a) b)

**Fig. 1 – a) Electrocardiograph (ECG) registered sinus tachycardia, frequency of about 120/min, normogram, Q wave and ST elevation till +3.5 mm in D2, D3, aVF leads, ST segment denivelation of -2 mm in DI, aVL,V2 and V3 leads, without rhythm and conduction disorders; b) Right precordial leads with ST segment elevation in VR4 lead as a sign of right ventricle myocardial infarction.**



a) b)

**Fig. 2 – a) Doppler registered ventricular septum defect; b) Large thrombus in the pericardium.**

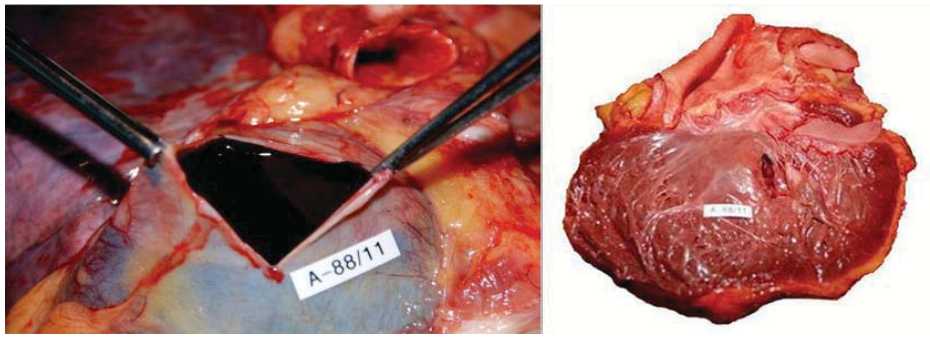
Soon after admission due to acute left ventricular failure, hypoxemia was recorded in blood gases and the patient developed anuria and was intubated and mechanically ventilated. The patient was treated with double antiplatelet therapy, statins, and inotropes. Volume and acid-base status were also corrected according to blood gases. In order to adequately monitor the patient a central venous catheter was introduced (central venous pressure was 15 mm$H_2O$).

Immediately upon admission echocardiography was performed. There were normal left ventricular dimensions, with akinesia of all segments of the inferior and basal inferoseptal wall. In the region of the basal part of the inferoseptal wall

He indicated preparation of the patient for surgical intervention. During the entire hospitalization patient`s condition was unstable and critical, and despite all the implemented measures cardiac arrest developed. Cardiopulmonaly resuscitation did not restore spontaneous circulation and the patient died about 10 hours after admission.

Autopsy revealed the presence of 200 mL of blood and 250 mL of blood clot beneath the pericardium, which was smooth and shiny (Figure 3a).

The left ventricle was enlarged, with left ventricular thickening. In the upper third of the muscular part of the ventricular septum, a defect with torn edges, 2 cm in its larg-




a) b)

**Fig. 3 – a) Pericardial effusion with the presence of blood clot; b) Ventricular septum rupture.**

est diameter, was found (Figure 3b). At the posterior wall of the left ventricle, in the projection of the upper third of the ventricular septum, a small stellate defect, with blood permeated edges, 0.7 cm in its largest diameter that communicated with the left ventricular cavity was found (Figure 4).

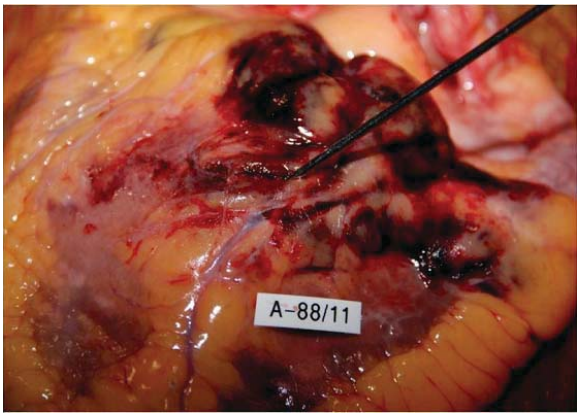



**Fig. 4 – Left ventricle free wall rupture.**

Histologically, in the cardiac muscle slices taken from the area of the septum and posterior wall of the left ventricle, the muscle fibers were torn, mainly without visible nucleoli. The cytoplasm of muscle cells was extremely acidophilic, homogeneous, without lines which are characteristic for muscle fibers. Interstitium was unevenly dilated, edematous and filled with neutrophils of which many were in close contact with the muscle fibers (Figure 5). Described areas corresponded to the coagulation type of necrosis, i.e. myocardial infarction.

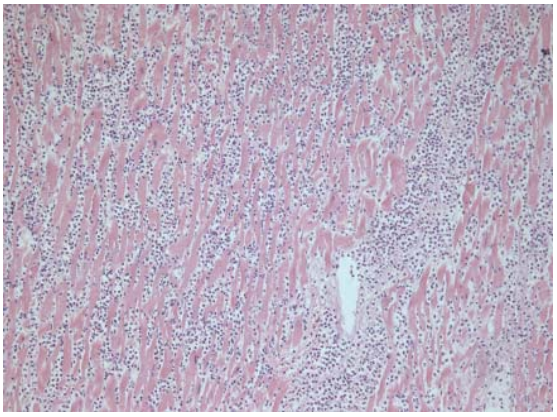


**Fig. 5 – Myocardial infarction about four days old (HE, ×10).**

Coronary arteries had a narrowed lumen due to the presence of yellowish thickening of the intima, and serial sections of the right coronary artery found a fresh thrombus which completely closed the artery.

Macroscopic examination of other organs revealed significant atherosclerosis which mainly affected the thoracic and abdominal aorta and picture of shock in the kidneys. In the pleural cavities, on both sides, 200 mL of serous effusion was found, and examination of the lungs showed acute pulmonary edema.

## Discussion

Despite the application of fibrinolysis and pPCI in the treatment of acute myocardial infarction, heart rupture remains a complication that is difficult to predict and heal. Many papers have studied the factors that may indicate the possibility of rupture in the era before pPCI and they were: one vessel coronary heart disease, previously myocardial infarction, older age, female gender and hypertension [5–7].

In the era after PCI incidence of FWR decreased, and immediate PCI showed that can prevent development of abrupt rupture following acute myocardial infarction [8].

In relation to the time of occurrence, rupture of the free wall of the left ventricle can be named as early rupture when it occurs within 48 hours. It is usually associated with delayed hospitalization, persistent chest pain and persistent ST segment elevation on ECG. Acute rupture of the free wall of the left ventricle is usually fatal within a few minutes due to the development of tamponade, and do not respond to standard cardiopulmonary resuscitation. Clinically, it manifests with cardiovascular collapse and pulseless electrical activity. In 30% of the cases rupture of the free wall of the left ventricle is late (subacute) and occurs after the second day [9]. The clinical picture includes recurrent chest pain and re-elevation of ST on ECG. Usually, this results in rapid deterioration of hemodynamic status of patients with permanent hypotension. Late rupture is thought to be less dependent on hypertension, but physical activity such as persistent vomiting and coughing can trigger it [9].

Echocardiography can visualize the place of rupture of the free wall, which is accompanied by the presence of blood in the pericardium. Echocardiography cannot register this complication, and the presence of pericardial effusion is not diagnostically enough for the diagnosis of rupture. Typical finding in subacute rupture of the free wall of the left ventricle is echo mass in the pericardium, which indicates the

presence of blood clot, although it is not enough for a complete diagnosis without direct view of the rupture. This emphasizes the need for better education and best echo machines in intensive care units for better and faster diagnosis of acute myocardial infarction complications.

VSR as a mechanical complication after acute myocardial infarction in patients who did not receive reperfusion therapy occurs late, within five days, in contrast to patients who received fibrinolysis when it occurs within the first few days [9]. Risk factors for this complication are older age, infarction of anterior localization and one vessel coronary heart disease [10].

Emergency operation in patients with this complication is vital. In the literature there are just single case reports which show successful operations [11–13]. We have to emphasize that even when they are operated, mortality is extremely high [8].

It should be noted that the operative mortality in patients with inferior infarction, as in our case, is much higher and reaches 58% as compared to patients with anterior infarction (25%) [14].

DHR represents a serious and usually fatal complication, and often is primary found at surgery or autopsy in these patients. Risk factors for DHR are: older age, female gender, first myocardial infarction, anterior localization and hypertension [1].

DHS after acute myocardial infarction occurs rarely, so in the literature there are only single case reports. The only study on 10 patients with clinicopathological features of DHS is the study of Tanaka et al. [1]. This is why each new case report is of some significance to this issue.

According to anamnesis, clinical findings and cardiac enzymes (creatine phosphokinase, myoglobin and troponin I) can be assumed that the myocardial infarction of inferior localization associated with right ventricular myocardial infarction in our example took about three days before admission, but that was misdiagnosed. In relation to this it was defined as a subacute rupture, as evidenced by the presence of blood clot in the pericardium, which probably in the first stage prevented acute tamponade. Elevation of ST segment on ECG and the presence of thrombus and small effusion in the pericardium was probably a sign of rupture of the free wall of the left ventricle. In our case reperfusion therapy (fibrinolysis or PCI) was not applied, myocardial infarction was three days old with hypertension, it was first myocardial infarction, and the autopsy showed one vessel coronary artery disease, so this patient had all the risk factors for myocardial rupture, which are described in the literature.

## Conclusion

This case points out the need for early reporting after symptoms of acute myocardial infarction in order to prevent complications, the need for early reperfusion, and the necessity of detailed noninvasive tests in patients with acute myocardial infarction if the facilities are available. Echocardiography should be routinely performed in patients with acute myocardial infarction at admission to the intensive care unit and is always required in cases of hemodynamic instability of the patient.

## REFERENCES


1. *Tanaka K, Sato N, Yasutake M, Takeda S, Takano T, Ochi M,* et al. Clinicopathological characteristics of 10 patients with rupture of both ventricular free wall and septum (double rupture) after acute myocardial infarction. J Nippon Med Sch 2003; 70(1): 21–7.
2. *Moreno R, López-Sendón J, García E, Pérez IL, López SE, Ortega A*, et al. Primary angioplasty reduces the risk of left ventricular free wall rupture compared with thrombolysis in patients with acute myocardial infarction. J Am Coll Cardiol 2002; 39(4): 598–603.
3. *Markowicz-Pawlus E, Nozyński J, Sedkowska A, Jarski P, Hawranek M, Streb W,* et al. Cardiac rupture risk estimation in patients with acute myocardial infarction treated with percutaneous coronary intervention. Cardiol J 2007; 14(6): 538–43.
4. *Yuan SM, Jing H, Lavee J*. The mechanical complications of acute myocardial infarction: echocardiographic visualizations. Turkish J Thorac Cardiovasc Surg 2011; 19(1): 36–42.
5. *Becker RC, Gore JM, Lambrew C, Weaver WD, Rubison RM, French WJ,* et al. A composite view of cardiac rupture in the United States National Registry of Myocardial Infarction. J Am Coll Cardiol 1996; 27(6): 1321–6.
6. *Becker RC, Hochman JS, Cannon CP, Spencer FA, Ball SP, Rizzo MJ*, et al. Fatal cardiac rupture among patients treated with thrombolytic agents and adjunctive thrombin antagonists: observations from the Thrombolysis and Thrombin Inhibition in Myocardial Infarction 9 Study. J Am Coll Cardiol 1999; 33(2): 479–87.
7. *Nakamura F, Minamino T, Higashino Y, Ito H, Fujii K, Fujita T*, et al. Cardiac free wall rupture in acute myocardial infarction: ameliorative effect of coronary reperfusion. Clin Cardiol 1992; 15(4): 244–50.
8. *Tanaka K, Sato N, Yasutake M, Takeda S, Takano T, Tanaka S*. Clinical course, timing of rupture and relationship with coronary recanalization therapy in 77 patients with ventricular free wall rupture following acute myocardial infarction. J Nippon Med Sch 2002; 69(5): 481–8.
9. *Figueras J, Cortadellas J, Soler-Soler J*. Left ventricular free wall rupture: clinical presentation and management. Heart 2000; 83(5): 499–504.
10. *Crenshaw BS, Granger CB, Birnbaum Y, Pieper KS, Morris DC, Kleiman NS,* et al. Risk factors, angiographic patterns, and outcomes in patients with ventricular septal defect complicating acute myocardial infarction. GUSTO-I (Global Utilization of Streptokinase and TPA for Occluded Coronary Arteries) Trial Investigators. Circulation 2000; 101(1): 27–32.
11. *Takahashi S, Uchida N, Imai K, Sueda T*. New infarct exclusion repair using cohesive double-patch closer. Interact Cardiovasc Thorac Surg 2012; 14(3): 353–5.
12. *Wozakowska-Kapłon B, Dabkowski P, Pietrzyk E, Sadowski J*. Ventricular septum and free wall rupture in a 56-year-old male with myocardial infarction. A case report with follow-up of 7 years. Kardiol Pol 2009; 67(6): 651–5.
13. *Takeuchi K, Morishige N, Iwahashi H, Hayashida Y, Teshima H, Ito N,* et al. Posterior ventricular septal perforation successfully repaired through right ventricular approach. Kyobu Geka 2006; 59(13): 1177–80. (Japanese)
14. *Jones MT, Schofield PM, Dark JF, Moussalli H, Deiraniya AK, Lawson RA*, et al. Surgical repair of acquired ventricular septal defect. Determinants of early and late outcome. J Thorac Cardiovascular Surg 1987; 93(5): 680–6.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CASE REPORT

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# Atypical case of Wilson's disease with psychotic onset, low 24 hour urine copper and the absence of Kayser-Fleischer rings

## Atipični primer Vilsonove bolesti sa psihotičnim početkom, niskim bakrom u 24-satnom urinu i odsustvom Kajzer Flajšerovih prstenova

**Dragan Krstić*, Jadranka Antonijević*, Željko Špirić*†**

***Clinic of Psychiatry, Military Medical Academy, Belgrade Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

**Abstract**

**Introduction.** Wilson's disease is typically manifested in two clinical forms, neurological and hepatic and in rare cases it starts with psychiatric symptoms exclusively. We presented a rare atypical case of Wilson's disease with psychotic onset. **Case report.** A 22-year-old male patient was initially presented with predominant signs and symptoms of psychiatric disorder and then later with the development of neurological signs and symptoms. Neuroimaging, detected metal deposits in central nervous system (CNS) but not in peripheral organs, while serum analysis excluded pantothenate-kinase associated neurodegeneration and aceruloplasminemia. In favor of the diagnosis of Wilson's disease there were reduced concentrations of copper and ceruloplasmin concentrations and metal deposits in CNS, but other pathognomonic signs and symptoms were absent: increased copper in urine, Kayser-Fleischer rings in Descemet's corneal membrane and deposits of copper in liver. Introduction of penicillamine treatment resulted in improvement in mental and general health of the patient. Molecular genetic analysis definitely confirmed the diagnosis of Wilson's disease. **Conclusion.** Wilson's disease can remain undetected for a long period of time if masked with dominant or exclusive psychiatric symptoms. If clear clinical symptoms and signs, and unambiguous laboratory findings are not present, it is necessary to perform molecular genetic analysis to confirm the definitive diagnosis.

**Key words:**
**hepatolenticular degeneration; diagnosis; mental disorders; copper; molecular biology; genetic diseases, inborn; treatment outcome.**

**Apstrakt**

**Uvod.** Vilsonova bolest se karakteristično ispoljava kroz dva klinička oblika, neurološki i heparni, a ređe počinje isključivo sa psihijatrijskim simptomima. Prikazan je redak, atipični slučaj Vilsonove bolesti sa psihotičnim početkom. **Prikaz bolesnika.** Muškarac od 22 godine imao je u početku, tokom nekoliko godina, dominantne znakove i simptome psihijatrijskog poremećaja, a tek kasnije neurološke znakove i simptome. Neuroradiološkim analazima su detektovani depoziti metala u centralnom nervnom sistemu (CNS), ali ne i u perifernim organima, a laboratorijskim analizama krvi isključeni su *pantothenate-kinase associated neurodegeneration* i aceruloplazminemija. U prilog dijagnoze Vilsonove bolesti bili su snižena koncentracija bakra i ceruloplazmina u serumu i depoziti metala u CNS-u, ali su bili odsutni drugi patognomonični znaci i simptomi: povišen bakar u urinu, Kajzer-Flajšerovi prstenovi u Descemetovoj membrani korneje i depoziti bakra u jetri. Na terapiju penicilaminom došlo je do poboljšanja psihičkog i opšteg zdravstvenog stanja bolesnika. Dijagnoza Vilsonove bolesti definitivno je potvrđena molekularno genetskim analizama. **Zaključak.** Vilsonova bolest može dugo ostati neprepoznata, ukoliko je maskirana dominantnim ili isključivo psihijatrijskim simptomima. Ako nisu prisutni jasni klinički simptomi i znakovi i nedvosmisleni laboratorijski nalazi, neophodno je uraditi molekularno genetsku analizu radi konačne potvrde dijagnoze.

**Ključne reči:**
**hepatolentikularna degeneracija; dijagnoza; mentalni poremećaji; bakar; biologija, molekulska; nasledne bolesti; lečenje, ishod.**

### Introduction

Wilson's disease is a progressive autosomal recessive disorder characterized by disruption of transport and excretion, as well as excessive accumulation of copper in liver, eyes, central nervous system (CNS) and other organs. It is clinically manifested in childhood or adolescence, usually before the age of 40, most often in the second decade. Siblings of affected persons have risk of 25% of developing this disease. The name of the disease dates back from 1912, when Alexander Kinnier

**Correspondence to:** Dragan Krstić, Clinic of Psychiatry, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: suzanark@sbb.rs

Wilson [1] first described family neurological disorder manifesting with extensive tremor and parkinsonism-like symptoms, all associated with liver cirrhosis. Synonyms for this disease are "hepatolenticular degeneration", "Westphal's pseudosclerosis" and "Strümpel's disease".

Wilson's disease is caused by mutations in the ATP7B gene locus 13q14.3 - q21.1 on the second arm of the 13th chromosome. This gene controls binding of copper for transport protein ceruloplasmin (alpha-2 globulin). 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 [2]. The disease incidence is estimated to be 1 in 30,000 to 1 in in 50,000 [3, 4], and it is supposed that the prevalence is 30 *per* million, with the frequency of heterozygotous mutations carriers of about 1 in 90 to 1 in 150 [5, 6]. Until now, it has been identified more than 400 mutations in ATP7B gene with characteristic geographic distribution [7, 8]. In a study on Serbian population, molecular gene defect was identified in 80% of alleles of Wilson's disease patients, with 11 different mutations, and the most frequent mutations were H1069Q (48.9% of analyzed alleles), 2304–2305insC (11.4%), and A1003T (5.7%) [9].

Copper is an essential oligoelement absorbed in duodenum, deposited in liver in form of cuproprotein, and in plasma 95% binds to aceruloplazmin, building ceruloplasmin. The remaining 5% of copper binds to albumin and erythrocytes and is excreted through biliary excretion in a form that is not reabsorbed [10, 11].

In cases with a reduced binding of copper for ceruloplasmin which leads to an increase of free circulating copper and its deposit in tissues. The precise nature of biochemical abnormalities is not known, but studies of mitochondrial function indicate the occurrence of free radicals and oxidative stress, due to copper accumulation in mitochondria.

The most common non-neurological manifestations are ocular and hepatic abnormalities. The most prominent ocular sign is Kayser-Fleischer ring – a bilateral green-brown granular deposit of copper in Descement's membrane around corneal limbus which is observed in nearly 100% patients, but not in all [12]. Involvement of the liver leads to chronic liver cirrhosis which can be complicated with splenomegaly, esophageal varices, haemolytic anemia and thrombocytopenia.

The major symptoms of neurological forms of the disease are tremor, dystonia, dysarthria, rigidity, bradykinesia, horeiform dyskinesia and ataxia.

Phenomena associated with this form may be changes in mental status that are often manifested in the form of dementia as a psychomotor retardation, impaired concentration, mnestic deficit, personality disorders, behavioral and affective disorders. Psychotic phenomena, including hallucinations appears very rarely. The psychiatric form of the disease is relatively rare and it is estimated to about 10% of all cases [13–15].

Typical changes in blood laboratory tests point to damage to the functioning of liver and kidneys (aminoaciduria). The levels of serum copper and ceruloplasmin are low: copper < 11 mmol/L (reference values 11.9–20.4 mmol /L), ceruloplasmin < 0.2 g / L (0.2–0.6 g/L), and 24-hour urine copper is increased (finding > 0.1 mg/24 h confirms Wilson's disease, and finding of 0.04 mg/24 h is strongly indicative of Wilson's disease). Liver biopsy reveals cirrhosis and copper deposition. Common findings in computerized tomography (CT) and magnetic resonance imaging (MRI) are cerebrocortical atrophy and the abnormalities in the basal ganglia [15].

The combination of neurological symptoms, Kayser-Fleisher's rings and a low ceruloplasmin level is considered sufficient for the diagnosis of Wilson's disease.

In general, the approach to treatment is dependent on whether there is clinically-evident disease or laboratory or histological evidence of aggressive inflammatory injury, whether neurologic or hepatic, or whether the patient is identified prior to the onset of clinical symptoms. The recommended initial treatment of symptomatic patients or those with active disease is with chelating agents, though there are some reports showing primary treatment with zinc may be adequate for some individuals. The largest treatment experience worldwide is still with D-penicillamine; however, there is now more frequent consideration of trientine for primary therapy [17].

**Case report**

A male patient, aged 22 was admitted to the Clinic of Psychiatry, Military Medical Academy (MMA), Belgrade, Serbia, after two years of altered behavior in the form of social withdrawal, reduction of verbal communication, increased hostility towards family members, subjective sensation of "vibration" in epigastrium and with occasional verbalization of suicidal ideas. Over the past two years, he was treated as an outpatient in regional psychiatric institution where he was initially diagnosed as adolescent crisis, and was treated with anxiolytics and psychotherapy, and later he was rediagnosed as paranoid schizophrenia, with, prior to hospitalization in MMA, a 12- months-long treatment with atypical antipsychotics (risperidone and clozapine), and antidepressants (paroxetine, mirtazapine, bupropion) and then with typical antipsychotic haloperidol. During outpatient psychiatric treatment, despite the usage of these antipsychotic drugs, there were not any reduction of psychotic phenomena. In that period an electroencephalography (EEG) and transcranial ultrasonography (TCD) were performed – all findings were described as normal. A few months before current hospitalization the patient was in Africa and there he had a brief febrile episode.

On admission, complete physical examination showed practically normal somatic finding: the patient was afebrile, eupnoic, normotensive, cardipulmonally compensated, without the presence of organomegaly. Psychiatric examination revealed changed behavior, hostility, negativism, reduction of verbal communication, poor control of aggressive impulses, proprioceptive hallucinations and excessive sensitivity. Extrapyramidal symptoms – acynetic-rigid syndrome with a significant anteflexion of toes of his left foot were observed by neurological examination. Taking into account that patient had been receiving antipsychotic treatment for one year, extrapyramidal symptoms could be explained as a manifestation of iatrogenic (medicament) parkinsonism.

During hospitalization, additional diagnostic procedures were done. Findings of laboratory blood analysis, urine biochemical analysis, peripheral blood smear, serum iron and iron binding data [Fe – 8 μmol/L (11–31 μmol/L); unsaturated iron binding capacity (UIBC) – 32 μmol/L (35–54 μmol/L) total iron binding capacity (TIBC) – 40 μmol/L (45–80 μmol/L); ferritin – 299 μmol/L (22–561 μmol/L)] were normal, as also additional serological blood tests (for echinococcosis, cysticercosis borreliosis, plasmodium, viral hepatitis, syphilis and HIV). Lumbar puncture was done and cerebrospinal fluid analysis showed normal findings. The values of ceruloplasmin and copper in serum and urine were signifficantly different from the reference values: serum ceruloplasmin was markedly reduced – < 0.077 g/L (reference values: 0.2–0.6 g/L); copper concentrations were significantly decreased 3.77 mmol/L (11.90–20.41 mmol/L) as 24-hour urine copper < 0.01 mg/24 h (reference value < 0.05 mg/24 h). Control EEG was normal. When auditory brainstem evoked potentials (AEPMS) was done, bilateral dysfunction at the level of the rostral part of the brainstem was found. The finding of visual evoked potential (VEP) was normal. CT scan showed secondary deposits of metal in basal ganglia. Endocranium MRI showed atrophic changes in the brain parenchyma with a metal deposition in the nucleus *lentiformis* and *pars compacta* of the substantia nigra (Figure 1).

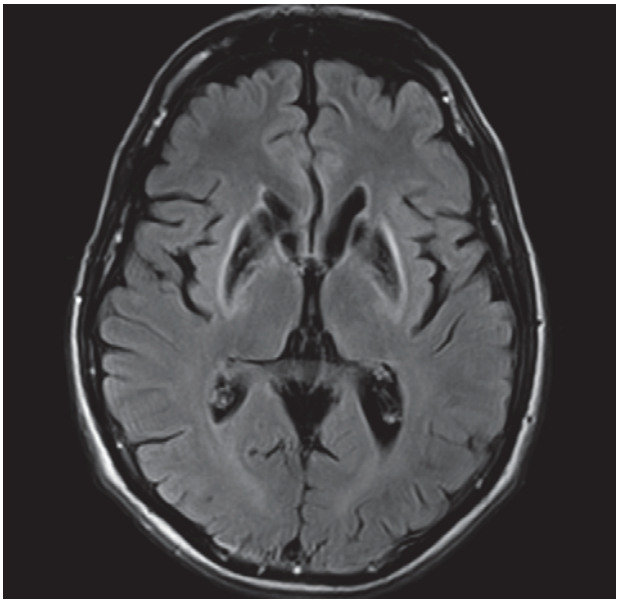

**Fig. 1 – Magnetic resonance imaging of the endocranium shows atrophic changes in the brain parenchyma with deposition of metal in the nucleus *lentiformis* and *pars compacta* of *substantia nigra.***

Transcranial Doppler (TCD) discovered a decreased flow. Multislice computerized tomography (MSCT) of the liver was normal: homogenous structure without focal changes. The possibility of liver biopsy was considered, but it was not approved by the patient. Ophthalmological examination was performed in the first week after admission, then a month later and in both cases the results were normal. Control ophthalmological examination 3 months after admission to the hospital, demonstrated for the first time the occurrence of Kayser-Fleischer's rings.

Regardless of the lack of confirmation of the existence of Wilson's disease 4 weeks after admission to the hospital, penicillamine therapy was administered with concomitant symptomatic polyvitamin and sedative therapy. Regular monitoring of urine copper concentration showed a significantly positive response to the therapy, which resulted in increased excretion of copper in urine (400 times). The definitive diagnosis of Wilson's disease was established 3 months later, after receiving the results of molecular genetic analysis. Genetic testing which was conducted in 2 independent laboratories confirmed heterozygous carrier of mutations for Wilson's disease: in exon 8 (229 Ins C and c.236G-A) and small insertion in exon 8 (c.insC2304-2305), as also in exon 16 (A 1140 V).

## Discussion

The presented case of Wilson's disease has several specific features that distinguish it from most other patients with the same diagnosis. The first peculiarity consists primarily of the dominant clinical psychiatric disorder, and because of that, the patient was diagnosed with a psychotic disorder, and for nearly 6 months was treated accordingly, with antipsyhotic therapy, and during that period he manifested theraporesistance. Similar cases were described elsewhere [18, 19].

With psychopathological phenomena manifested on admission which had a character of non-specific psychotic disorder, neurological signs which were also observed, and with anamnestic data about recent return from Africa, where he had a brief episode of high fever, all pointed to a possible infectious etiology.

After a detailed laboratory analysis of blood and cerebrospinal fluid, when infectious disease of CNS was excluded, and when neuroradiological imaging demonstrated the presence of metal deposits in brain structures, several differential diagnosis resolution were discussed: panthotenate kinase-associated neurodegeneration (PKAN) formerly called Hallevorden Spatz syndrome, Wilson's disease and aceruloplasminemia. Neuroimaging indicated the existence of metal deposits in specific CNS structures which was not enough to resolve the differential diagnosis between these diseases. Similarities in clinical presentation – the presence of neurological and psychiatric symptoms, characteristic for all these disorders did not contribute to further precise diagnosis. More detailed diagnostic determination was achieved with laboratory analysis of blood and urine: normal values o f laboratory analysis of iron excluded diagnosis of PKAN. The absence of signs of diabetes mellitus, anemia, and retinal degeneration, with normal iron and ferritin concentrations excluded the diagnosis of aceruloplasminemia [20]. At the same time, significantly lower levels of ceruloplasmin and copper in serum indicated the presence of Wilson's disease, despite the absence of pathognomonic signs: Kayser-Fleischer rings, metal deposits in liver and decreased values of copper in urine [12].

After clinical decision to initiate penicillamine therapy, which led to the improvement of mental and general health of the patient, the diagnosis of Wilson's disease was obtained

*ex-iuvantibus* [21]. No sooner than 2 months after the beggining of treatment, ophthalmological examination showed Kayser-Fleisher rings, and 3 months later, the results of molecular genetic analysis confirmed the diagnosis of Wilson's disease [22].

**Conclusion**

Atypical form of Wilson's disease can remain undetected for a long time if it is masked by dominant or exclusive psychiatric symptoms. If clear clinical signs and symptoms (neurological, ophthalmological) are not fully present, and unambiguous laboratory findings (decreased concentrations of copper and ceruloplasmin in serum and increased concentration of copper in urine), it is necessary to perform molecular genetic analysis in order to confirm the definitive diagnosis.

Early diagnosis and effective treatment improve the outlook. The prognosis of Wilson's disease is excellent provided that the treatment starts before irreversible damage.

R E F E R E N C E S


1. *Wilson SAK*. Progressive lenticular degeneration: a familial nervous disease associated with cirrhosis of the liver. Brain 1912; 34: 395–509.
2. *Tomić A, Dobricić V, Novaković I, Svetel M, Pekmezović T, Kresojević N*, et al. Mutational analysis of ATP7B gene and the genotype-phenotype correlation in patients with wilson's disease in serbia. Vojnosanit Pregl 2013; 70(5): 457−62.
3. *Ferenci P*. Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. Hum Genet 2006; 120(2): 151−9.
4. *Houwen RH, van Hattum J, Hoogenraad TU*. Wilson disease. Neth J Med 1993; 43(1−2): 26-37.
5. *Scheinberg IH, Sternlieb I*. Wilson's disease. In: *Smith LH*, editor. Major problems in internal medicine. Philadelphia, PA: WB Saunders; 1984. p. 25–35.
6. *Ferenci P*. Wilson's disease. Ital J Gastroenterol Hepatol 1999; 31(5): 416−25.
7. *Schmidt HH*. Role of genotyping in Wilson's disease. J Hepatol 2009; 50(3): 449−52.
8. *Reilly M, Daly L, Hutchinson M*. An epidemiological study of Wilson's disease in the Republic of Ireland. J Neurol Neurosurg Psychiatr 1993; 56(3): 298−300.
9. *Loudianos G, Kostic VS, Solinas P, Lovicu M, Dessi V, Svetel MV*, et al. Characterization of the molecular defect in the ATP7B gene in Wilson disease patients from Yugoslavia. Genet Test 2003; 7(2): 107−12.
10. *Ferenci P*. Review article: diagnosis and current therapy of Wilson's disease. Aliment Pharmacol Ther 2004; 19(2): 157−65.
11. *Dong Q, Wu Z*. Advance in the pathogenesis and treatment of Wilson disease. Transl Neurodegener 2012; 1(1): 23.
12. *Youn J, Kim JS, Kim H, Lee J, Lee PH, Ki C*, et al. Characteristics of neurological Wilson's disease without Kayser-Fleischer ring. J Neurol Sci 2012; 323(1−2): 183−6.
13. *Benhamla T, Tirouche YD, Abaoub-Germain A, Theodore F*. The onset of psychiatric disorders and Wilson's disease. Encephale 2007; 33(6): 924−32. (French)
14. *Srinivas K, Sinha S, Taly AB, Prashanth LK, Arunodaya GR, Janardhana RY*, et al. Dominant psychiatric manifestations in Wilson's disease: a diagnostic and therapeutic challenge. J Neurol Sci 2008; 266(1−2): 104−8.
15. *Akil M, Schwartz JA, Dutchak D, Yuzbasiyan-Gurkan V, Brewer GJ*. The psychiatric presentations of Wilson's disease. J Neuropsychiatry Clin Neurosci 1991; 3(4): 377−82.
16. *Mak CM, Lam C*. Diagnosis of Wilson's disease: a comprehensive review. Crit Rev Clin Lab Sci 2008; 45(3): 263−90.
17. *Roberts EA, Schilsky ML; American Association for Study of Liver Diseases (AASLD)*. Diagnosis and treatment of Wilson disease: an update. Hepatology 2008; 47(6): 2089−111.
18. *Jukić I, Titlić M, Tonkić A, Dodig G, Rogosić V*. Psychosis and Wilson's disease: a case report. Psychiatr Danub 2006; 18(1−2): 105−7.
19. *Bidaki R, Zarei M, Mirhosseini SM, Moghadami S, Hejrati M, Kohnavard M,* et al. Mismanagement of Wilson's disease as psychotic disorder. Adv Biomed Res 2012; 1: 61.
20. *Miyajima H*. Aceruloplasminemia, an iron metabolic disorder. Neuropathology 2003; 23(4): 345−50.
21. *Walshe JM*. Penicillamine: the treatment of first choice for patients with Wilson's disease. Mov Disord 1999; 14(4): 545−50.
22. *Ferenci P, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I,* et al. Diagnosis and phenotypic classification of Wilson disease. Liver Int 2003; 23(3): 139−42.


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CASE REPORT

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# Li-Fraumeni syndrome: A case report

## Li-Fraumenijev sindrom

**Miroslav P. Ilić*‡, Kiralj Aleksandar*‡, Borislav Markov*, Ivana Mijatov*‡, Saša Mijatov*‡, Nada Vučković†‡**

***Clinic for Maxillofacial and Oral Surgery, †Centre for Pathology and Histology, Clinical Center of Vojvodina, Novi Sad, Serbia; ‡Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia**

**Abstract**

**Introduction.** Li-Fraumeni syndrome (LFS) is a very rare familial disease with the predisposition to the development of malignant tumors, such as osteosarcoma, breast cancer, brain neoplasm, leukemia, and adrenal tumors. Inheritance is autosomal dominant and is caused by heterozygous mutations in the p53 gene. The diagnosis is based on clinical criteria: a person under the age of 45 years suffering from sarcoma, the closest relative younger than 45 years diagnosed with cancer and a relative of the first or second degree, which is up to 45 years, was diagnosed with cancer and was diagnosed with sarcoma at any age. **Case report.** The presented family with three members diagnosed with malignant disease typical for LFS suggests the need to carefully follow those diagnosed with LFS related tumor. A 24-year-old man diagnosed and treated for osteosarcoma of the maxilla died in the first year. His younger brother was submitted to surgery due to osteosarcoma of the mandible three years later, and a year later in his 24 year he had no signs of locoregional recurrence. Their mother was operated in 1996 for glioblastoma multiform brain cancer and ductal carcinoma, and died two years later at the age of 33. **Conclusion.** The presented family highlights the need for careful examination, inspection and notification of the risks of family members diagnosed with LFS related tumors.

**Key words:**
**li-fraumeni syndrome; diagnosis; treatment outcome; prognosis.**

**Apstrakt**

**Uvod.** Li-Fraumenijev sindrom (LFS) predstavlja veoma retko familijarno obolenje sa predispozicijom za razvoj pojedinih malignih tumora, kao što su: osteosarkom, karcinom dojke, neoplazma mozga, leukemija i adrenalni tumori Nasleđuje se autozomno dominantno, a nastaje usled heterozigotne mutacije na genu p53. Dijagnoza se postavlja na osnovu kliničkih kriterijuma: osoba mlađa od 45 godina obolela od sarkoma, najbliži srodnik mlađi od 45 godina oboleo od karcinoma i srodnik prvog ili drugog kolena koji je do 45. godine oboleo od karcinoma ili oboleo od sarkoma u bilo kom uzrastu. **Prikaz bolesnika.** U radu je prikazana porodica u kojoj su kod tri člana dijagnostikovana maligna obolenja karakteristična za LFS. Muškarac star 24 godine oboleo i lečen od osteosarkoma gornje vilice preminuo je u toku prve godine. Njegov mlađi brat je 3 godine kasnije operisan od osteosarkoma donje vilice, a godinu dana kasnije u svojoj 24 godini bio je bez znakova lokoregionalnog recidiva. Njihova majka je 1996. godine operisana od multiformnog glioblastoma mozga i duktalnog karcinoma dojke. Umrla je dve godine kasnije u 33. godini života. **Zaključak.** Ovaj prikaz ukazuje na potrebu pažljivog pregleda, kontrolisanja i obaveštavanja o rizicima članova porodice obolelih od tumora koji su u vezi sa LFS.

**Ključne reči:**
**li-fraumeni sindrom; diagnosis; lečenje, ishod; prognoza.**

### Introduction

Li-Fraumeni syndrome (LFS) is a hereditary cancer predisposition syndrome caused by heterozygous mutation in the p53. This syndrome is very rare with ~ 400 families reported in the literature. This syndrome is characterized by autosomal dominant inheritance and early appearance of tumors, multiple tumors within a person, and multiple affected family members [1]. Osteosarcoma, soft tissue sarcoma, leukemia, brain cancer, breast cancer, and adrenal cortical tumors are the most common types of cancer found in LFS families. This syndrome is also known as sarcoma, breast, leukaemia and adrenal gland (SBLA) syndrome [2].

Classic LFS syndrome is defined as proband with a sarcoma under the age of 45 and the first-degree relative (sibling, parent, or child) with any cancer diagnosed before the

**Correspondence to:** Miroslav P. Ilić, Clinic for Maxillofacial and Oral for Maxillofacial and Oral Surgery, Clinic Centre of Vojvodina, 21 000 Novi Sad, Serbia. E-mail: mikimfh@gmail.com

age of 45, and the first- or second-degree relative (grandparent, uncle, aunt, nephew, niece, or grandchild) diagnosed with any cancer before the age of 45 or with sarcoma diagnosed at any age [3].

There is also a hereditary condition of cancer predisposition that has been called Li-Fraumeni-like syndrome (LFL) which is defined as proband with any childhood cancer, or a sarcoma, adrenocortical tumor, or brain tumor before the age of 45, the first- or second-degree relative in the same lineage with LFS tumor at any age, and the first- or second-degree relative in the same lineage with any cancer before the age of 60 [4].

LFS and LFL are genetic conditions. Approximately 70% of LFS cases and 40% of LFL cases contain germline mutations in the p53 gene on chromosome 17p13.1 [5]. TP53 mutations have been primarily implicated in Li–Fraumeni syndrome [6]. Mutations in another gene, called CHEK2, have been found in another form of Li-Fraumeni syndrome (LFS2). Women with CHECK2 mutations could be in increased risk for breast cancer [6]. A third locus has been mapped on the long arm of chromosome 1 (1q23) but the gene has not been identified.

**Case report**

We presented a family with three members diagnosed with a malignant disease typical for LFS, so that the clinical criteria for LFS were met.

*A proband with sarcoma before the age of 45-elder son with osteosarcoma died at the age of 24*

A 24-year-old man with swelling in the region of upper incisors observed in January 2008, visited maxillofacial surgeon in May 2008. Local extraoral finding showed swelling of premaxilla with a lobular tumor dimension 30 × 20 × 10 mm in the oral premaxilla region which partially covered the cheek surface of the upper central incisors, but the mucosa over the tumor was preserved and seemed unchanged (Figure 1). Left

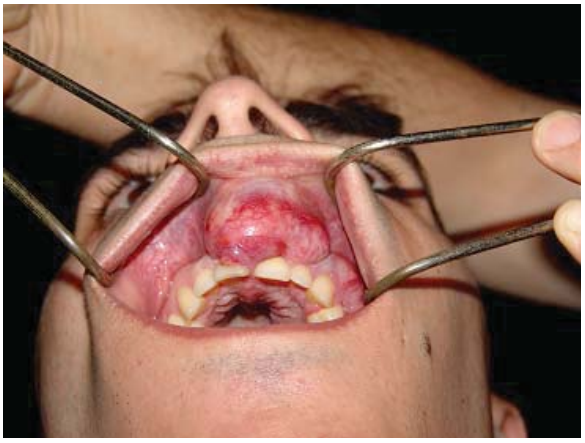

**Fig. 1 – Tumor mass in the oral premaxilla region which partially covered the cheek surface of the upper central incisors in the elder son-proband.**

maxillary alveolar process was completely ballooned. Pathology of biopsy indicated a chondrosarcoma. CT scan showed the sclerotic, osteolytic areas with inhomogeneous and prominent periosteal reaction in the alveolar process of the left maxilla and the lower part of the left maxillary sinus was filled with tumor mass that infiltrated the lower nasal concha. The neck had no of enlarged lymph nodes.

In early June 2008 the patient underwent total maxillectomy which included a resection of the premaxilla until the contralateral (right) first premolar and resection of the lower nasal concha. Histological findings showed an osteosarcoma with small cells with some fibroblastic and chondroblastic foci (Figure 2).

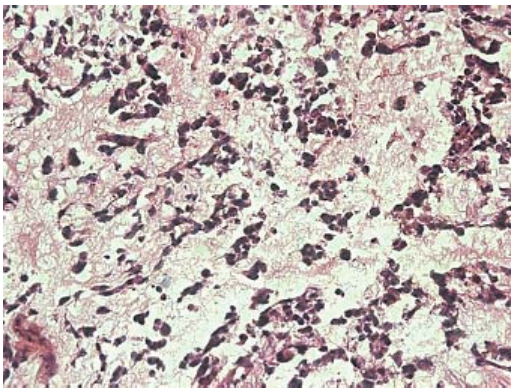

**Fig. 2 – Maxillary tumor osteosarcoma with small cells in the myxoid stroma in the elder son-proband (HE, ×200).**

The radiotherapy started in the optimal period, but only a month after maxillectomy a recurrence in the midline of the nasal cavity was diagnosed. A resection of the tumor was followed, and the finishing of previously established radiotherapy. In the coming months there was a locoregional recurrence – sphenoid bone infiltration and left orbital tumor progression leading to blindness. Distant metastases in the lungs and multiple secondary cutaneous deposits occurred. The death was due to cardiopulmonary failure in December 2008. Subsequent histological analysis indicated high grade conventional osteosarcoma chondro and osteoblastic types.

*A first-degree relative diagnosed with any cancer before the age of 45 – mother with breast and brain cancer died at the age of 33*

From the data of the previous two patients found out that their mother was born in 1965 underwent separately brain and breast surgery in 1996. Pathohystological findings showed invasive ductal carcinoma of breast and brain multiform glioblastoma (Figure 3). She died in 1998. She had no siblings.

*A first-degree or second-degree relative diagnosed with any cancer before the age of 45 or diagnosed with sarcoma at any age – younger son with osteosarcoma diagnosed at age of 23*

A 23-year male observed a sweling and deformity of the left mandible in April 2012 (Figure 4). Two months before he noticed a numbness of the lower lip. Maxillofacial surgeon examined him and endosseous biopsy was performed a few days later. Pathology findings indicated a ma-

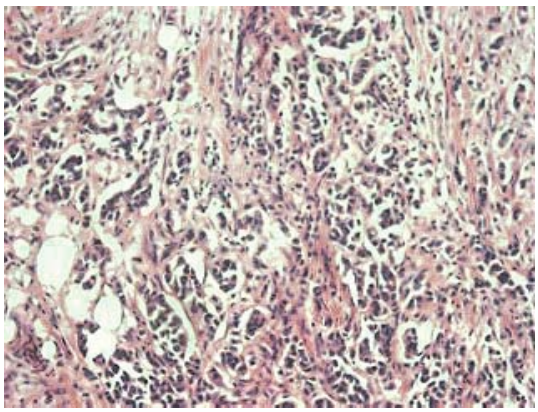

**Fig. 3 – Ductal infiltrative breast carcinoma in the mother (HE, ×200).**

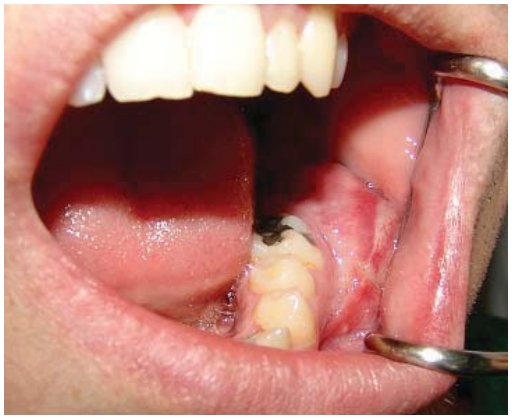

**Fig. 4 – Deformity of the left mandible in the younger son.**

lignant mesenchymal tumor, probably chondrosarcoma. The radiologist on computed tomography (CT) scanning described extended body of the mandible from the mental foramen to the left lower jaw angle. The medulla was modified by a bone mass characterized with inhomogeneous higher attenuation and cortex showed individual partial erosion. In the neck region II were present lymph nodes greatest diameter 17 mm, oval.

In the beginning of June, he underwent hemimandibulectomy with supraomohyoid neck dissection. Histological findings were: osteosarcoma with fibroblastic and chondroblastic foci (Figure 5). Immunohistochemical characteristics

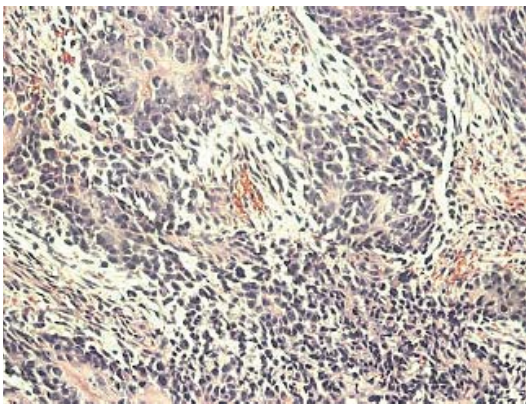

**Fig. 5 – Mandibular osteosarcoma with the small round cells surrounding homogenous eosinophylic osteoid deposits with some fibroblastic cells in the younger son (HE, ×200).**

of tumor cells were: vimentin and S-100 and CD99 positive, and negative on CD79a, CD3, HMB45, desmin, MyoD1, EMA, CK18, CK5/6. The proliferative index (Ki67) was rather uneven and very high, on the average 30% of cells. The neck was tumorfree.

A combined chemo- and radiotherapy followed. The patient was regularly controlled clinically and by imaging magnetic resonance imaging (MRI) of the head, chest CT, abdominal ultrasound (US). Locoregional recurrence and distant metastases were not registered. He regularly performed his profesional and daily activities.

## Discussion

Li–Fraumeni syndrome is named in honor of Frederick Pei Li and Joseph F. Fraumeni, Jr. [7], the American physicians who first recognized and described the syndrome. In the study on 648 childhood rhabdomyosarcoma patients, they identified 4 families in which siblings or cousins had a childhood sarcoma. These 4 families also had distinct histories of breast cancer and other neoplasm, assuming a new familial cancer syndrome of different tumors. The following prospective study confirmed the high risk of different tumors in family members [8]. In contrast to other hereditary cancer syndromes, which are mainly characterized by site-specific cancers, LFS presents a diverse of tumor types. The most common types are soft tissue sarcomas and osteosarcomas, breast cancer, leukemia, and adrenocortical carcinoma.

Pearson et al. [9] reported 2 families with LFS. In the first, the mother had breast cancer and 3 of her 4 children had adrenocortical carcinoma, rhabdomyosarcoma, and medulloblastoma; in the other, the mother had breast cancer and 2 of her 3 children had rhabdomyosarcoma and adrenocortical carcinoma. An increased risk for melanoma [10], Wilms' tumor, and cancers of gaster, esophagus, colon, pancreas, gonadal germ cells and lung, have also been reported [11–13]. Someone who has LFS may be at risk for neoplasm of almost every part of the body.

The syndrome is characterised by the appearance of tumors associated to LFS at an early age [14] and the occurrence of multiple primary malignant tumors [1, 14]. Tumors in the case of family were diagnosed in 30, 24 and 23 years of age. The mother was diagnosed by multiple primary malignance – of the brain and the breast, during one calendar year. The median age of breast cancer diagnosis in LFS woman is 33 years average and 16 years of brain tumor onset [15]. Determination of TP53 gene mutation is not as high-sensitivity method; it is positive in 50–70% of cases. LFS is a very rare disease, so we suspected on this one when the younger brother was diseased. The same surgeon operated both of brothers and set of the clinical diagnosis of LFS.

The early diagnosis of LFS raises the issue of preventive screening for carriers of mutations that can have long-term risk of development of malignancy, which is estimated at 73% for men and 100% for women carriers [16]. Malignant brain tumors and breast cancer are particularly common in older TP 53 mutation carriers, requiring full annual clinical examination and regular screening for breast cancer for females. A high de-

gree of suspicion should be maintained for LFS carriers who complain of unexplained persisting symptoms.

## Conclusion

The case of families with Li-Fraumeni Syndrome indicates the need of getting a detailed family history in patients with tumors associated with this syndrome. Mutations in the TP53 gene do not prove a specific finding for Li-Fraumeni Syndrome which is the reason for the annual clinical review and full physial examination with regular screening for breast cancer for females. Informing family members about the possible risks related to Li-Fraumeni Syndrome could lead to the early detection of Li-Fraumeni Syndrome related tumors.

## REFERENCES


1. *Hisada M, Garber JE, Fung CY, Fraumeni JF, Li FP.* Multiple primary cancers in families with Li-Fraumeni syndrome. J Natl Cancer Inst 1998; 90(8): 606−11.
2. *Lynch HT, Guirgis HA.* Childhood cancer and the SBLA syndrome. Med Hypotheses 1979; 5(1): 15−22.
3. *Li FP, Fraumeni JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA,* et al. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988; 48(18): 5358−62.
4. *Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM,* et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. Cancer Res 1994; 54(5): 1298−304.
5. *Bachinski LL, Olufemi S, Zhou X, Wu C, Yip L, Shete S,* et al. Genetic mapping of a third Li-Fraumeni syndrome predisposition locus to human chromosome 1q23. Cancer Res 2005; 65(2): 427−31.
6. *Malkin D.* Li-Fraumeni syndrome. Genes Cancer 2011; 2(4): 475−84.
7. *Li FP, Fraumeni JF.* Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome. Ann Intern Med 1969; 71(4): 747−52.
8. *Li FP, Fraumeni JF.* Prospective study of a family cancer syndrome. JAMA 1982; 247(19): 2692−4.
9. *Pearson AD, Craft AW, Ratcliffe JM, Birch JM, Morris-Jones P, Roberts DF.* Two families with the Li-Fraumeni cancer family syndrome. J Med Genet 1982; 19(5): 362−5.
10. *Hartley AL, Birch JM, Marsden HB, Harris M.* Malignant melanoma in families of children with osteosarcoma, chondrosarcoma, and adrenal cortical carcinoma. J Med Genet 1987; 24(11): 664−8.
11. *Strong LC, Stine M, Norsted TL.* Cancer in survivors of childhood soft tissue sarcoma and their relatives. J Natl Cancer Inst 1987; 79(6): 1213−20.
12. *Li FP, Fraumeni JF, Mulvihill JJ, Blattner WA, Dreyfus MG, Tucker MA,* et al. A cancer family syndrome in twenty-four kindreds. Cancer Res 1988; 48(18): 5358−62.
13. *Masciari S, Dewanwala A, Stoffel EM, Lauwers GY, Zheng H, Achatz MI,* et al. Gastric cancer in individuals with Li-Fraumeni syndrome. Genet Med 2011; 13(7): 651−7.
14. *Gonzalez KD, Noltner KA, Buzin CH, Gu D, Wen-Fong CY, Nguyen VQ,* et al. Beyond Li-Fraumeni syndrome: Clinical characteristics of families with p53 germline mutations. J Clin Oncol 2009; 27(8): 1250−6.
15. *Olivier M, Goldgar DE, Sodha N, Ohgaki H, Kleihues P, Hainaut P,* et al. Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. Cancer Res 2003; 63(20): 6643−50.
16. *Chompret A, Brugières L, Ronsin M, Gardes M, Dessarps-Freichey F, Abel A,* et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br J Cancer 2000; 82(12): 1932−7.


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# Obstetric complications of placenta previa percreta

## Akušerske komplikacije placente previje perkrete

**Radmila Sparić*, Ljiljana Mirković*†, Uroš Ravilić*, Tijana Janjić***

***Clinic of Gynecology and Obstetrics, Clinical Center of Serbia, Belgrade, Serbia;**
**†Faculty of Medicine, University of Belgrade, Belgrade, Serbia**

**Abstract**

**Introduction**. Placenta previa is related to severe maternal and fetal morbidity. The increasing incidence of cesarean delivery rate causes a marked increase in abnormally invasive placenta over the past decades. The abnormally invasive placenta is becoming the foremost cause of obstetric hemorrhage and postpartum hysterectomy, causing a significant maternal and fetal morbidity and even mortality. Maternal morbidity in such cases also comprise politransfusion, development of disseminated intravascular coagulation, uterine rupture, cystostomy, fistula formation, ureteral stricture, intensive care unit admission, infection, and prolonged hospitalization, adult respiratory distress syndrome, renal failure, septicemia and even death. **Case report.** A 38-year-old gravida 3, para 2, was admitted to our hospital at 27 weeks of gestation as an emergency due to vaginal bleeding, previously diagnosed with an anterior placenta previa. Following tocolytic therapy, bleeding stopped. The patient was informed on the diagnosis and the possibility of life-threatening hemorrhage necessitating preterm delivery. She was given corticosteroids to enhance fetal lung maturity. At 28 weeks of gestation, she experienced massive vaginal bleeding, and a decision was made to perform emergency cesarean section. We made a corporeal transverse uterine incision well above the uterovesical fold and tortuous vessels, at the same time avoiding the superior edge of the placenta. The placenta was found to be densely adherent to the lower uterine segment, penetrating through it and infiltrating the posterior wall of the urinary bladder. An attempt to remove the placenta resulted in injury to the bladder wall and the uterine rupture at a previous cesarean scar. The decision was made to perform total abdominal hysterectomy with placenta left *in situ*. At present, both mother and the baby are well. **Conclusion.** Anticipation and the surgeon's judgment are leading factors for surgery, from the choice of uterine incision type to the decision to proceeding to hysterectomy in order to reduce maternal morbidity.

**Key words:**
**placenta previa; risk assessment; cesarean section; hysterectomy; treatment outcome.**

**Apstrakt**

**Uvod**. Placenta previja je stanje koje je povezano sa teškim maternalnim i fetalnim morbiditetom. Povećanje incidencije carskih rezova dovelo je do značajnog povećanja abnormalno invazivne placente u prošloj deceniji. Abnormalno invazivna placenta je jedan od vodećih uzroka postpartalnih histerektomija, krvarenja kao i značajnog broja maternalnog i fetalnog morbiditeta i mortaliteta. Maternalni morbiditet u ovakvim slučajevima kompromituje transfuziju, dovodi do razvoja diseminovane intravaskularne koagulacije, do rupture uterusa, cistostomije, formiranja fistule, uretralnih striktura, dovodi do povećanja broja prijema u jedinice intenzivne nege, do infekcija, produženja hospitalizacije, razvoja adultnog respiratornog distres sindroma, otkazivanja bubrega, septikemije, čak i do smrti. **Prikaz bolesnika.** Trudnica, stara 38 godina, u trećoj trudnoći, primljena je u našu kliniku u 27 nedelji gestacije kao hitan slučaj zbog vaginalnog krvarenja. Prethodno kod trudnice dijagnostikovano je postojanje placente previje. Nakon primenjene tokolitičke terapije krvarenje je prestalo. Trudnica je informisana o dijagnozi i o mogućem, po život ugrožavajućem krvarenju, koje može prouzrokovati prevremeni porođaj. Primila je kortikosteroidnu terapiju u cilju maturacije fetalnih pluća. U 28 nedelji gestacije trudnica je obilno vaginalno prokrvarila, te je doneta odluka da se uradi hitan carski rez. Učinjen je transverzalni rez na telu uterusa iznad plike vezikouterine i proširenih krvnih sudova, izbegavajući gornji vrh placente. Placenta je probila ožiljak na donjem segmentu uterusa i infiltrirala se u zadnji zid mokraćne bešike, pa bi došlo do povrede mokraćne bešike i rupture uterusa ukoliko bi se pokušalo manuelno odvajanje i to na mestu ožiljka od prethodnog carskog reza. Totalna abdominalna histerektomija urađena je sa placentom na mestu upravo zbog mogućeg krvarenja. Majka i beba sada su dobro. **Zaključak**. Predviđanje i odluka hirurga odlučujući su faktori za operaciju, od izbora uterusne incizije pa do odluke da se nastavi ka histerektomiji, sve u cilju sniženja maternalnog morbiditeta.

**Ključne reči:**
**placenta previja; rizik, procena; carski rez; histerektomija; lečenje, ishod.**

**Correspondence to:** Ljiljana Mirković, Clinic for Gynecology and Obstetrics, Clinical Center of Serbia, Višegradska 26, 11 000 Belgrade, Serbia. Phone: +381 11 366 3600. E-mail: drljiljamirkovic@gmail.com

## Introduction

Placenta previa is related to severe maternal and fetal morbidity. Its incidence is growing due to the rising rate of cesarean sections and advanced maternal age on delivery [1]. Increasing incidence of cesarean delivery rate is also causing a marked increase of abnormally invasive placenta over the past decades [2, 3]. It is 1 in 533 deliveries and the occurrence has risen 10-fold over the last five decades, which can mainly be attributed to the increased number of cesarean deliveries [3, 4].

Therefore, abnormally invasive placenta is becoming the foremost cause of obstetric hemorrhage and postpartum hysterectomy, because it usually results in significant maternal and fetal morbidity and even mortality [3]. Most of the women with abnormally invasive placenta present with known predisposing factors [3]. Both placenta previa and abnormally invasive placenta have the same major risk factors, namely tissue insult and scaring. The usual causes are previous cesarean section, myomectomy, histeroscopic surgery, suture of uterine perforation, infection and dilatation and curettage [3, 5]. The risk of abnormally invasive placenta increases proportionately with the number of previous cesarean deliveries [6]. Advanced maternal age is an independent risk factor for both conditions [3]. In the subgroup of women with placenta previa the risk of abnormally invasive placenta is predominantly increased. In the presence of placenta previa, the risk of having placenta accreta rises from 24% in women with one cesarean section to 67% for women with three previous cesarean sections [6].

Abnormally invasive placenta is categorized into accreta/increta/percreta in the order of increasing severity and invasion of placental villi through the myometrium. The most severe form is placenta percreta, which involves placental penetration through the full thickness of the uterine wall and in some cases into adjacent organs such as urinary bladder, broad ligament, cervix, uterine artery and bowel [5]. Separation of such placenta from the uterine wall can lead to extensive hemorrhage. Placenta percreta with urinary bladder invasion is a principally uncommon, life-threatening complication of pregnancy [6]. It poses a significant risk of hemorrhage and carries a very high morbidity and mortality risk for both mother and the fetus.

The majority of women with abnormally invasive placenta require a hysterectomy. Although successful conservative management has been described, there are currently inadequate data to endorse this approach to management routinely [6, 7]. Maternal morbidity in cases of placenta percreta also comprises politransfusion, development of disseminated intravascular coagulation, uterine rupture, cystostomy, fistula formation, ureteral stricture, intensive care unit admission, infection, and prolonged hospitalization, adult respiratory distress syndrome, renal failure, septicemia and even death [6–8].

## Case report

A 38-year-old woman gravida 3, para 2, was admitted to our hospital at 27 weeks of gestation as an emergency due to vaginal bleeding. The patient had a history of prior two cesarean sections, the first 9 years before and the second 4 years prior to the present pregnancy. She had been previously diagnosed with anterior placenta previa. Apart from several episodes of mild vaginal bleeding, her antenatal course was uneventful.

Her vital signs on admission were stable and hemoglobin was 102 g/L. Coagulation profile was normal. An ultrasound examination showed normal fetal anatomy and growth and confirmed the diagnosis of placenta previa. Following tocolytic therapy, bleeding stopped. The patient was informed of the diagnosis and the possibility of life-threatening hemorrhage necessitating preterm delivery. She was given corticosteroids to enhance fetal lung maturity and antibiotic prophylaxis. The patient was followed conservatively without further complications for 6 days.

At 28 weeks of gestation, she experienced massive vaginal bleeding, and a decision was made to perform emergency cesarean section under general anesthesia. Both the patient and her family were informed on the possible complications and a prior informed written consent to the probability of emergency cesarean hysterectomy and its associated morbidity was obtained.

On opening the abdominal wall by making lower median laparotomy incision omentum was found densely adherent to the anterior uterine wall, limiting its visualization and necessitating omental resection. We found the engorgement of the vessels around the vesicouterine peritoneal fold and the bladder outer surface, because of which we made a corporeal transverse uterine incision well above the uterovesical fold and tortuous vessels, at the same time avoiding the superior edge of the placenta. A male fetus with Apgar scores of 2 and 3 at 1 and 5 min, respectively, weighing 1,240 g was delivered. The neonate was transferred into neonatal intensive care unit. The placenta was found to be densely adherent to the lower uterine segment, penetrating through it and infiltrating the posterior wall of the urinary bladder. An attempt to remove the placenta resulted in injury to the bladder wall and the uterine rupture at a previous cesarean scar.

The patient became hypotensive, with blood systolic pressure 60 mm Hg and a heart rate 150 beat per min. The decision was made to perform a total abdominal hysterectomy with placenta left *in situ*. Intraoperative urologic consultation was obtained, which confirmed ureteric patency and trigon uninjured. The bladder wall was repaired in 2 layers.

Intraoperative coagulation studies were: platelets 80 000/μL, prothrombin time 26.6 s (reference values 10–13), international normalized ratio 2.48 (reference values 0.8–1.2), partial thromboplastin time (PTT) 112 s (normal range 60–70 s), fibrinogen 0.59 g/L (normal range 1.5–4.5 g/L), antithrombin III 14.6 (reference values 80-120), D dimmer 3.12 (< 0.5).

Over the course of 210-min surgical procedure massive blood transfusion was necessary: 11 units of packed red blood cells and 1,200 mL of autologous blood in order to replace blood loss. The patient was also given 10 units of fresh frozen plasma, 12 units of platelets, 10 units of cryoprecipi-

tate, 1,500 mL of colloid and 3,800 mL of crystalloid. She also received a course of prophylactic intravenous antibiotics. She was transferred to the intensive care unit where she received an additional of 3 units of packed red blood cells. The patient was monitored in the intensive care unit for 5 days.

The postoperative course was uneventful. The urinary catheter was left in place for 13 days. The patient was discharged within 2 weeks after the surgery voiding well. During follow up in the outpatient department there were no complications, including urological ones. The baby spent 26 days in the neonatal intensive care unit, and was discharged 69 days after the delivery without early neonatal sequelae. At present, both the mother and the baby are well.

The pathology report showed the placental villi extending through the myometrium of the lower uterine segment thus confirming the diagnosis of placenta previa percreta causing uterine rupture (Figure 1).

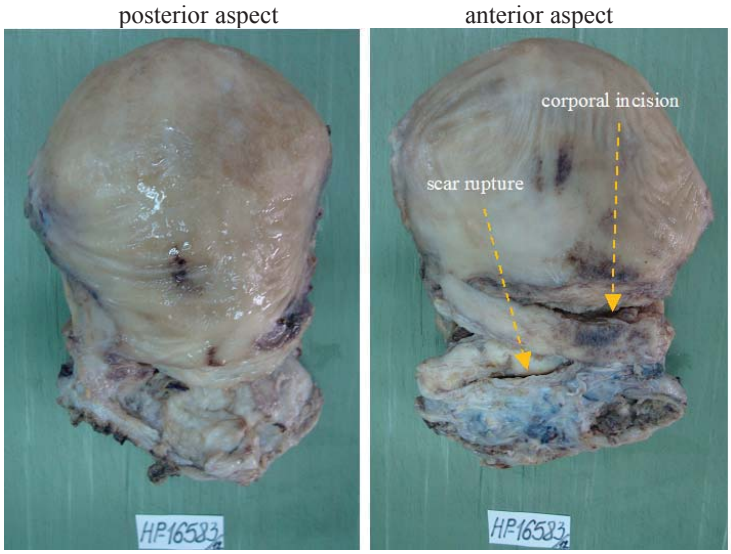


**Fig. 1 − Macroscopic appearance of the uterus**

## Discussion

The incidence of abnormally invasive placenta has been reported to be increasing, and a persistent rise in cesarean delivery rates will cause its further rise [9, 10]. This condition has been present worldwide as the primary indication for emergency postpartum hysterectomy, accounting for up to 50% of all emergency peripartum hysterectomies nowadays [1, 2, 5]. According to the results of our previous research, based on histological diagnosis, prevalence of abnormally invasive placenta in our institution is 0.19 *per* 1,000 deliveries (0.06% of cesarean sections) [1].

The presented 38-year-old patient had most of the significant risk factors for placenta percreta, namely 2 prior cesarean sections, anterior placenta previa. When a multipara with previous cesarean sections is found to have placenta previa, as in the presented case, the possibility of abnormally invasive placenta should be born in mind. Gray scale, color and power Doppler, as well as three-dimensional ultrasonography and magnetic resonance imaging have been shown to be useful in antenatal diagnosis [3, 7]. In the presented case, the diagnosis was not recognized until emergency delivery. Prediction of abnormally invasive placenta relies on adequate screening with ultrasound, which was not performed in the presented case. Taking into account the rise in cesarean delivery rate, it would be recommendable to perform a routine screening for abnormally invasive placenta in cases with the presence of risk factors, such was the presented one. It is also advisable to use magnetic resonance imaging in cases with doubtful bladder and parametrial invasion [3].

This case report demonstrates that, even in the absence of sophisticated diagnostic techniques, such as 3-dimensional ultrasound and magnetic resonance imaging, a high index of suspicion and an experienced surgeon can provide favorable results. Necessity to bear in mind possibility of abnormally invasive placenta in women with major risk factors is essential for good outcomes in such cases.

The maternal morbidity in women with abnormally invasive placenta is high, especially in cases of placenta percreta [8]. The maternal mortality ranges from 7% to 22% in the literature [6]. In a meta-analysis of placenta percreta with bladder invasion published by Washecka and Behling [8] there were three maternal deaths out of 54 cases. Maternal outcome can be significantly improved with the prenatal diagnosis [3]. Lethal outcome is more frequent in patients like ours, with antenataly unrecognized abnormally invasive placenta. The main cause of death in such cases is hemorrhage and disseminated intravascular coagulation [6]. The use of cell salvage has been advocated in the literature in woman with anticipated massive hemorrhage [3]. In the presented case, autologous blood salvage was used without any complications registered. The literature data suggests that mean blood loss at cesarean delivery in such cases is over 2.5 L, which explicates the routine use of intraoperative autologous blood salvage [3]. As presented in this case, the use of autologous blood transfusion can contribute favorable maternal outcome.

Extensive hemorrhage and urinary complications can be caused by manual removal of the placenta previa percreta, as in the presented case. The prenatal diagnosis of the condition in risk cases is important as scheduled cesarean hysterectomy can reduce maternal morbidity and mortality. With this attitude, there is concern for risk of neonatal morbidity because of premature birth as well. More data are necessary before making recommendations about the optimal timing of delivery in such women.

Treatment options vary from conservative approaches to obstetric hysterectomy [3]. Hysterectomy is considered to be the gold standard in management, particularly for women who do not wish to continue their fertility [5]. At the moment, this procedure seems to be the only appropriate treatment available but fertility-sparing options need to go into further research [5]. Having in mind uterine rupture, bladder involvement, age and patient's obstetric history, as well as intractable hemorrhage we undoubtedly performed emergency cesarean hysterectomy as a lifesaving procedure in the presented case, without considering conservative management. The conservative options include leaving the placenta *in situ*, awaiting either spontaneous resorption or expulsion. It is also manageable with prophylactic or therapeutic uterine artery embolization, internal artery ligation and methotrexate, uterine compression sutures and/or over sewing of the placental vascular bed. Because of collateral vasculature in the pelvis, hypogastric artery occlusion does not completely stop uterine blood flow and success rate in arresting hemorrhage with this technique [6]. The outcome of leaving the placenta in place varies extensively, and includes complete remission, an intrauterine infection requiring prolonged administration of antibiotics, delayed vaginal bleeding and hysterectomy [3]. There are cases reported with placenta percreta that was left *in situ* with good outcome with adjuvant therapy given, including methotrexate, transarterial embolization of the uterine arteries or both [3]. The potential benefits of fertility sparing management must be weighed against the associated risks and the possibility of recurrent abnormally invasive placenta in these women. Surgery in cases of abnormally invasive placenta can cause injury to adjacent organs thus increasing maternal morbidity and mortality. Cystostomy is usually necessary, to facilitate dissection of the bladder [8]. Urological complications of placenta percreta with bladder involvement include massive hematuria, bladder laceration, urinary tract fistula, ureteric transection, cystectomy and small capacity bladder [8]. Preoperative ureteric stent placement may help reduce the risk of ureteric injury [3]. Recognition of bladder injuries at operation is paramount to prevent urinary fistula formation and further operation [3].

## Conclusion

Anticipation and the surgeon's judgment must be leading factors for surgery, from the choice of uterine incision type to the decision to proceeding to hysterectomy in order to reduce maternal morbidity. In addition, we suggest informed consent for cesarean delivery to include data about increased risk of abnormally invasive placenta in future pregnancies.

## REFERENCES


1. *Mirkovic Lj, Janjic T, Sparic R, Ravlic U, Raslic Z.* Placenta accreta: incidence and risk factors. J Perinat Med 2013; 41(Suppl 1): 1196.
2. *Sparić R, Dokić M, Argirović R, Kadija S, Bogdanović Z, Milenković V.* Incidence of postpartum post-cesarean hysterectomy at the Institute of gynecology and obstetrics, Clinical center of Serbia, Belgrade. Srp Arh Celok Lek 2007; 135(3−4): 160−2. (Serbian)
3. *Doumouchtsis SK, Arulkumaran S.* The morbidly adherent placenta: an overview of management options. Acta Obstet Gynecol 2010; 89(9): 1126−33.
4. *Wu S, Kocherginsky M, Hibbard JU.* Abnormal placentation: twenty-year analysis. Am J Obstet Gynecol 2005; 192(5): 1458−61.
5. *Comstock CH.* The antenatal diagnosis of placental attachment disorders. Curr Opin Obstet Gynecol 2011; 23(2): 117−22.
6. *Guleria K, Gupta B, Agarwal S, Suneja A, Vaid N, Jain S.* Abnormally invasive placenta: changing trends in diagnosis and management. Acta Obstet Gynecol Scand 2013; 92(4): 461−4.
7. *Allahdin S, Voigt S, Htwe TT.* Management of placenta previa and accreta. J Obstet Gynaecol 2011; 31(1): 1−6.
8. *Washecka R, Behling A.* Urologic complications of placenta percreta invading the urinary bladder: a case report and review of the literature. Hawaii Med J 2002; 61(4): 66−9.
9. *Zizza A, Tinelli A, Malvasi A, Barbone E, Stark M, de Donno A,* et al. Caesarean section in the world: a new ecological approach. J Prev Med Hyg 2011; 52(4): 161−73.
10. *Silver LE, Hobel CJ, Lagasse L, Luttrull JW, Platt LD.* Placenta previa percreta with bladder involvement: new considerations and review of the literature. Ultrasound Obstet Gynecol 1997; 9(2): 131−8.


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# ERRATUM

The article “Understanding sensitivity, specificity and predictive values”. Vojnosanit Pregl 2014; 71(11): 1062–65 (DOI:10.2298/VSP1411062S)

Listed the authors as: Miodrag Stojanović, Marija Apostolović, Dijana Stojanović, Zoran Milošević, Aleksandra Toplaović, Vesna Mitić Lakušić, Mlađan Golubović

The list of authors should read as: Miodrag Stojanović, Marija Andjelković-Apostolović, Dijana Stojanović, Zoran Milošević, Aleksandra Ignjatović, Vesna Mitić Lakušić, Mladjan Golubović

**VOJNOSANITETSKI PREGLED**
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd, Srbija**
Tel/faks: +381 11 2669689
vsp@vma.mod.gov.rs

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

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

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

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

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**Od 1. januara 2012. godine Vojnosanitetski pregled prešao je na e-Ur: Elektronsko uređivanje časopisa.**

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

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

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*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. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

**Ilustracije**

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

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

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Koristiti samo standardne skraćenice, izuzev u naslovu i apstraktu. Pun naziv sa skraćenicom u zagradi treba dati kod prvog pominjanja u tekstu.

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