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CONTENTS

M E D I C I N S K I  P R E G L E D
ČASOPIS DRUŠTVA LEKARA VOJVODINE SRPSKOG LEKARSKOG DRUŠTVA
Novi Sad Vase Stajića 9 Srbija

Med Pregl 2019; LXXII (11-12): 335-410. Novi Sad: novembar-decembar.

## SADRŽAJ

# ORIGINAL STUDIES
# *ORIGINALNI NAUČNI RADOVI*

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## THE EFFICACY AND TOLERABILITY OF AVARICON® HEMOR MEDICAL PREPARATION IN THE TREATMENT OF HEMORRHOIDS COMPARED TO PLACEBO – A PROSPECTIVE DOUBLE BLIND RANDOMIZED CLINICAL STUDY

## *EFIKASNOST I PODNOŠLJIVOST AVARICON® HEMOR MEDICINSKOG SREDSTVA U LEČENJU HEMOROIDNE BOLESTI U POREĐENJU SA PLACEBOM – DVOSTRUKO SLEPA RANDOMIZIRANA PROSPEKTIVNA KLINIČKA STUDIJA*

**Nataša ZDRAVKOVIĆ[1], Jovana BRADIĆ[2], Jelena ŽIVIĆ[1], Berislav VEKIĆ[3, 4], Sergey BOLEVICH[5] and Vladimir JAKOVLJEVIĆ[5, 6]**

**Summary**

**Introduction.** Despite the high incidence of hemorrhoidal disease and the widespread use of numerous topical preparations, there is still a lack of information regarding their efficacy. Therefore, the aim of this study was to assess the efficacy and tolerability of a new topical medical preparation containing sodium hyaluronate, calendula extract, hamamelis extract and mentha piperita essential oil as major components. **Material and Methods.** This prospective double-blind randomized clinical study included 49 patients with a diagnosis of hemorrhoidal disease. The patients were randomly assigned to two groups: Avaricon® group that included patients who applied 0.20% Avaricon® Hemor and a placebo group who applied placebo during 2 weeks. The effects of Avaricon® Hemor on the symptoms of hemorrhoidal disease, its safety, tolerability as well as compliance and adherence of study patients were analyzed. **Results.** Our results showed that Avaricon® Hemor was significantly superior to placebo in controlling most symptoms of hemorrhoidal disease. **Conclusion.** The tested medical agent showed to be effective with good tolerability and safety profile indicating its possible use in various therapeutic protocols in the management of hemorrhoidal disease.

**Key words**: Hemorrhoids; Treatment Outcome; Administration, Topical; Hyaluronic Acid; Drug Tolerance; Patient Compliance; Medication Adherence

**Sažetak**

**Uvod.** Uprkos velikoj učestalosti hemoroidne bolesti i širokoj upotrebi brojnih aktuelnih preparata, još uvek nedostaju informacije o njihovoj efikasnosti. Stoga je cilj naše studije bio da se proceni efikasnost i podnošljivost novog medicinskog proizvoda koji sadrži natrijum-hijaluronat, ekstrakt nevena, ekstrakt hamamelisa i esencijalno ulje nane kao glavne komponente. **Materijal i metode.** Ovo je bila dvostruko slepa randomizirana prospektivna klinička studija koja je obuhvatila 49 pacijenata sa dijagnostikovanom hemoroidnom bolešću. Pacijenti su nasumično svrstani u dve grupe: *Avaricon*® grupa koja je uključivala pacijente koji su primenjivali 0,20% *Avaricon*® *Hemor* medicinski uređaj i placebo grupu koja je uključivala bolesnike koji su primenjivali placebo dve nedelje. Zabeleženi su efekti medicinskog sredstva *Avaricon*® *Hemor* na simptome hemoroidne bolesti, bezbednost, podnošljivost i komplijansu i adherenciju ispitanika. **Rezultati.** Naši rezultati su pokazali da je medicinski uređaj *Avaricon*® *Hemor* bio značajno superiorniji od placeba u kontroli većine simptoma hemoroidne bolesti. **Zaključak.** Ispitivani medicinski uređaj je efikasan sa dobrom podnošljivošću i bezbednosnim profilom što ukazuje na njegovu moguću primenu u različitim terapijskim protokolima za lečenje hemoroidne bolesti.

**Ključne reči:** hemoroidi; ishod lečenja; topikalna primena; hijaluronska kiselina; tolerancija na lekove; komplijansa; adherenca

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***Abbreviations***

HA – hyaluronic acid
SH – sodium hyaluronate
CE – Calendula extract
HE – Hamamelis extract
MPO – essential oil from Mentha piperita
VAS – visual analogue scale
MMAS – Morisky medication adherence scale
SD – standard deviation
SEM – standard error of the mean

## Introduction

Hemorrhoidal disease is one of the most common anorectal conditions which affects up to one quarter of adults worldwide [1, 2]. It is characterized by clusters of vascular tissues, smooth muscles and connective tissues which occur due to abnormal dilatation of the vascular channel and pathological changes within the anal cushions [3, 4]. Although the etiology underlying the development of hemorrhoids has not been completely elucidated, increased intra-abdominal pressure during pregnancy or straining may be the cause venous engorgement of the hemorrhoidal plexus [5, 6]. Patients who suffer from hemorrhoids experience bleeding, fecal soiling, pruritus etc., which strongly affects their quality of life [7]. While internal hemorrhoids are often painless, external hemorrhoids are associated with significant pain and discomfort due to activation of perianal innervations associated with thrombosis [3, 5].

Numerous surgical and nonsurgical strategies have been proposed for the management of hemorrhoidal disease with the aim to relieve the symptoms of hemorrhoids rather than to cure them [3, 4]. Additionally, medical approach depends on the type and severity of hemorrhoids; topical and systemic drugs can be applied in grade 1 and grade 2 hemorrhoids, while surgical procedures are required in more severe cases [4, 8]. Topical preparations involve creams, gels and suppositories usually containing local anesthetics, corticosteroids, lubricants and or anti-inflammatory agents which provide temporary symptomatic relief [1, 9–11]. Nevertheless, true benefits of these preparations are still lacking, indicating a need to test the effectiveness of numerous available, as well as novel formulations, in randomized clinical trials [4, 8]. Few papers have addressed the encouraging potentials of hyaluronic acid (HA) that promotes extracellular matrix remodeling [1, 12]. Protective effects of topical formulations used for hemorrhoids may be achieved by adding components of plant origin together with HA. However, results in this field still need to be examined [12].

Regarding all the above mentioned, the aim of our study was to assess the efficacy and tolerability of a new topical medical preparation containing sodium hyaluronate (SH), calendula extract (CE), hamamelis extract (HE) and mentha piperita essential oil (MPO) as major ingredients.

## Material and Methods

This study was designed as a randomized, double-blind, parallel group, placebo-controlled mono-center trial. It was conducted at the Clinic of Gastroenterology, Clinical Center of Kragujevac, Serbia, from January to April 2019.

The study protocol was approved by the Ethics Committee of the Clinical Center of Kragujevac and it was carried out according to the Declaration of Helsinki, Good Clinical Practice and International Conference on Harmonization guidelines. All the participants were informed about the research protocol before giving their written consent to participate in the study.

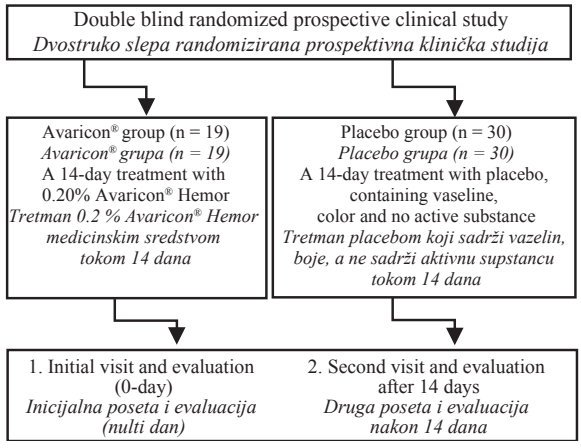
The study included 49 patients with a diagnosis of hemorrhoidal disease that were randomly assigned to two groups:

– Avaricon® group - patients (n = 19) who applied 0.20% Avaricon® Hemor at the affected site according to the manufacturer's instructions, 2 or 3 times daily for 14 days

– Placebo group - patients (n = 30) who applied placebo in the same manner.

– Avaricon® Hemor and placebo were applied at a dose of 0.5 to 1 g corresponding to a quantity of 1 to 2 cm of cream [12].

The inclusion criteria were: both genders, informed written consent from each participant, age over 18 years, and diagnosis of grades I - III hemorrhoids according to the international classification. The exclusion criteria were: suspected hypersensitivity and/or contraindications to active components or excipients of the examined product, breast feeding, oocyte donation or implantation during the study period, patients who cannot follow the investigation due to language comprehension problems, speech impediment, medical intervention or state that may interfere with the study protocol, patients who were included in another clinical trial at least 30 days before the beginning of the current study, patients subjected to surgical interventions of the anal region in the last 30 days, presence of inflammatory disease or other diseases of the gastrointestinal system [12]. Each participant could leave the clinical study at any time for any reason without consequences. All patients who met the inclusion criteria were enrolled in the investigations. Participants were subjected to clinical evaluation and examination by a gastroenterologist one day before the beginning of the study (baseline) and after completion of the 14-day treatment (14th day) **(Scheme 1).**




**Scheme 1.** Study flow diagram
***Shema 1.*** *Dijagram toka studije*

*Assessment of the Avaricon® Hemor efficacy*

In order to determine the efficiency of Avaricon® Hemor, the following parameters were recorded at the beginning (baseline) and at the end of the treatment (14th day): anal pain intensity, pain during defecation, bleeding self-evaluation, and assessment of the grade of hemorrhoidal disease.

*Visual analog scale for the efficacy assessment of Avaricon® Hemor*

The visual analogue scales (VAS) is a numerical a self-evaluation scale (0 – 100) where zero means absence of symptoms, and 100 mm corresponds to severe symptoms [12]. The following parameters were assessed by VAS: intensity of anal pain, intensity of anal pain during defecation, and intensity of anal bleeding.

*Assessment of the safety and tolerability of Avaricon® Hemor*

The safety and tolerability of the examined medical preparation were evaluated by a physician and by self-reporting unspecific and specific adverse effects. Tolerability was measured on a 4-point scale: 1 ("very good"), 2 ("good"), 3 ("poor") and 4 ("very poor") after 14th day of treatment and 7 days after treatment cessation. Additionally, at the same time, a 3-point descriptive scale: 1 ("better"), 2 ("no change") and 3 ("worse") was used for global assessment of improvement of the disease [12].

*Compliance of the study population assessment*

Direct and indirect methods were used for the assessment of compliance after 7 and 14 days of treatment. These procedures included self-reports about the amount of residual test substance, direct medical checking of the residual test substance, patients' statements regarding the applied dose, as well as the time and route of administration during the study protocol [13].

*Assessment of the study population adherence*

Adherence was assessed via medication event monitoring system (MEMS) and Morisky medication adherence scale (MMAS) after the treatment. The MMAS consists of four yes/no questions "Do you ever forget to take your medication?"; "Do you take care about the time when you should take the medication?"; "If you feel better, do you sometimes skip taking your medication?", and "Do you stop taking your medication if you feel worse?" The total score varies from 0 to 4; 0 corresponds to high degree of adherence, 1 – 2 to moderate adherence, and 3 – 4 to low degree of adherence [14].

*Used medications*

Avaricon® Hemor cream (20 g tube with applicator) is a certified product of Pharmanova (Belgrade, Serbia) pharmaceutical company that contains purified water, poloxamer 407, macrogol 400, liquid paraffin, Calendula officinalis flower liquid extract, ethanol, dexpanthenol, Hamamelis virginiana leaf dry extract, disodium phosphate dodecahydrate, mentha piperita essential oil, allantoin, sodium hyaluronate, sodium dihydrogen phosphate dihydrate, and chlorhexidine digluconate. The placebo contained Vaseline and color which made the formulation indistinguishable from Avaricon® Hemor cream.

Statistical analysis was performed using Statistical Package for the Social Sciences, 20.0 version for Windows. Descriptive statistics were used to calculate arithmetic mean with dispersion measures – standard deviation (SD) and standard error of the mean (SEM). Values were expressed as mean ± SEM. Distribution of data was checked by Shapiro–Wilk test and Mann–Whitney test; Chi squared test and Pearson coefficient were used for data analysis. Values of $p < 0.05$ were considered to be statistically significant, while $p < 0.01$ were highly statistically significant.

## Results

*General characteristics of the study population*

Baseline clinical and demographic characteristics of the patients enrolled in the research are presented in **Table 1.** There was no difference between groups, except in the frequency of diagnosis ($p < 0.05$).

*The effects of Avaricon® Hemor on the hemorrhoid symptoms after a 14-day treatment compared with the placebo*

A significant drop in the number of patients with anal pain was found both after the application of Avaricon® Hemor cream and placebo ($p < 0.05$). At the beginning of the study and prior to treatment, the intensity of pain was statistically significantly higher in the Avaricon® group ($p < 0.05$). However, at the end of 14-day Avaricon® Hemor cream treatment, there was a decrease in anal pain intensity compared

**Table 1.** Patient baseline descriptive characteristics
***Tabela 1.*** *Deskriptivne karakteristike pacijenata*

| | Avaricon® group<br>*Avaricon® grupa* | Placebo group<br>*Placebo grupa* | p<br>*p* |
|---|---|---|---|
| Gender/*Pol* | | | |
| Male/*Muški, n (%)* | 10 (52.6) | 19 (63.3) | > 0.05 |
| Female/*Ženski, n (%)* | 9 (47.4) | 11 (36.7) | |
| Age (mean ± SEM)/*Starost (sredina ± SEM)* | 52.32 ± 2.5 | 45.87 ± 2.2 | > 0.05 |
| Diagnosis/*Dijagnoza* | | | |
| External hemorrhoids, n (%)/*Spoljašnji hemoroidi, n (%)* | 12 (63.16) | 7 (23.33) | < 0.05 |
| Internal hemorrhoids, n (%)/*Unutrašnji hemoroidi, n (%)* | 7 (36.84) | 23 (76.67) | |
| Duration of disease (mean ± SEM)/*Trajanje bolesti (sredina ± SEM)* | 39.68 ± 8.07 | 34.60 ± 6.99 | > 0.05 |

**Table 2.** Assessment of disease severity and incidence of hemorrhoid symptoms
***Tabela 2.*** *Skala za procenu težine bolesti i prisustva simptoma hemoroida*

| | Avaricon® group/*Avaricon® grupa* | | Placebo group/*Placebo grupa* | |
|---|---|---|---|---|
| | Baseline *Početne vrednosti* | After 14 day *Nakon 14 dana* | Baseline *Početne vrednosti* | After 14 day *Nakon 14 dana* |
| Anal pain/*Bol analne regije, n (%)* | 13 (68.42) | 11 (57.89)* | 23 (76.66) | 21 (70)* |
| No anal pain/*Odsustvo bola analne regije, n (%)* | 6 (31.58) | 8 (42.11)* | 7 (23.33) | 9 (30)* |
| Anal pain intensity 1-10 (mean ± SEM) *Intenzitet bola analne regije 1-10 (sredina ± SEM)* | 4.74 ± 0.63 | 2 ± 0.29** | 3.57 ± 0.29# | 2.57 ± 0.23# |
| Anal pain at defecation, n (%) *Bol analne regije tokom defekacije, n (%)* | 13 (68.42) | 12 (63.16) | 28 (93.33) | 27 (90) |
| No anal pain at defecation, n (%) *Odsustvo bola analne regije tokom defekacije, n (%)* | 6 (31.58) | 7 (36.84) | 2 (6.66) | 3 (10) |
| Visible anal bleeding/*Vidljivo analno krvarenje, n (%)* | 11 (57.89) | 12 (63.16) | 28 (93.33) | 25 (83.33) |
| No visible anal bleeding, n (%) *Odsustvo vidljivog analnog krvarenja, n (%)* | 8 (42.11) | 7 (36.84) | 2 (6.66) | 5 (16.67) |
| Intensity of anal bleeding 1-10 (mean ± SD) *Intenzitet analnog krvarenja 1-10 (sredina ± SD)* | 3.05 ± 0.46 | 1.63 ± 0.19** | 2.93 ± 0.15 | 2.13 ± 0.17 |
| Grade of hemorrhoids (mean ± SEM) *Stepen hemoroida (sredina ± SEM)* | 2.16 ± 0.68 | 1.68 ± 0.58* | 2.7 ± 0.54 | 2.73 ± 0.52 |

*Statistically significant difference at the level $p < 0.05$ at 14th day of treatment compared to baseline; #statistically significant difference at the level $p < 0.05$ at the same time compared to Avaricon® group
** Statistički značajna razlika na nivou p < 0,05 nakon 14. dana tretmana u poređenju sa početnim vrednostima; #statistički značajna razlika na nivou p < 0,05 u istom trenutku u poređenju sa Avaricon® grupom*

to baseline values ($p < 0.01$) and values in the placebo group ($p < 0.05$). There was no difference in distribution of patients with pain during defecation and visible bleeding after the treatment in both groups ($p > 0.05$). Additionally, application of Avaricon® Hemor cream induced a significant decline in the mean value of bleeding intensity ($p < 0.01$) and grade of hemorrhoids ($p < 0.05$), while the placebo cream did not affect these parameters ($p > 0.05$) **(Table 2).**

*The effects of Avaricon® Hemor on the hemorrhoid symptoms after a 14-day treatment compared with the placebo (VAS assessment)*

A significant decrease in anal pain intensity, intensity of pain during defecation and intensity of bleeding was observed after a 14-day treatment in the group using Avaricon® ($p < 0.01$) in comparison to baseline values, while there was no change in these parameters after the placebo treatment ($p > 0.05$) **(Table 3).**

*Safety and tolerability of Avaricon® Hemor during and after treatment compared with the placebo*

Regarding the assessment of safety and tolerability of applied treatment, only one patient in the Avaricon® group reported a side effect, while none of them experienced side effects in the placebo group, both measured at the end of a 14-day protocol and 7 days later. Furthermore, in regard to the tolerability, 50% of the subjects have answered "very good" and 50% "good" in the Avaricon® group, while in the placebo group one patient defined tolerability of treatment as "very good", 16 as "good", 7 patients as "poor" and 1 patient as "very poor". When assessing improvement of symptoms after the treatment, most of the patients (n = 14 after the treatment, n = 13 seven days after treatment cessation) in the Avaricon® group answered "better", while the rest answered "no change". Also, most of the patients (n = 14) treated with the placebo answered "no change", while 7 of them reported "better" and even 4 described it as "worse".

*Compliance and degree of adherence of the study population during Avaricon® Hemor treatment compared with the placebo*

Overall assessment of compliance, by analyzing the usage of Avaricon® Hemor/placebo, 7 and 14 days after the beginning of treatment, indicated favorable compliance in both groups. A total of 94.74% of patients applied Avaricon® Hemor cream anally 3 times a day and used the whole tube of the examined cream. Also, 96.67% patients used the placebo anally 3 times a day, while 93.34% of them used the whole tube. Patients using Avaricon® Hemor cream showed more adherent with significantly lower number of patients who stopped treatment when feeling either better or worse ($p < 0.05$).

## Discussion

The main goal of all medical strategies for the treatment of hemorrhoidal disease is reduction of perianal pain, itching, tenesmus, and bleeding, rather than improvement in anal canal [1, 15]. Literature data suggest that agents possessing analgesic, local anesthetic, anti-inflammatory and venotonic properties may play a role in the management of this common gastrointestinal disease [8, 16]. Despite the numerous available topical formulations, there is insufficient and poor quality evidence regarding their efficacy, indicating the necessity for further

**Table 3.** Visual analogue scale for the assessment of Avaricon® Hemor efficacy
***Tabela 3.*** *Vizuelna analogna skala za procenu efikasnosti Avaricon® Hemor medicinskog sredstva*

| Parameter/*Parametar* | Avaricon® group/*Avaricon® grupa* | | Placebo group/*Placebo grupa* | |
|---|---|---|---|---|
| | Baseline *Početne vrednosti* | After 14 day *Nakon 14 dana* | Baseline *Početne vrednosti* | After 14 day *Nakon 14 dana* |
| Anal pain intensity (mean ± SEM) *Intenzitet bola analne regije (sredina ± SEM)* | 27.47 ± 6.27 | 8.63 ± 2.25** | 18.33 ± 2.54 | 11.53 ± 1.79 |
| Intensity of pain at defecation (mean ± SEM)/*Intenzitet bola prilikom defekacije (sredina ± SEM)* | 28.89 ± 6.45 | 7.58 ± 2.03** | 16.63 ± 2.41 | 10.27 ± 1.44 |
| Intensity of bleeding (mean ± SEM) *Intenzitet krvarenja (sredina ± SEM)* | 16.95 ± 3.91 | 4.26 ± 0.74 | 11.6 ± 1.9 | 6.43 ± 1.14 |

*Statistically significant difference at the level $p < 0.05$ at 14th day of treatment compared to the baseline
**Statistički značajna razlika na nivou p < 0,05 nakon 14.dana tretmana u poređenju sa početnim vrednostima*

evaluation via randomized controlled trials [8]. Throughout the history, topical application of plant-derived products such as extracts, oils etc. has been recognized to heal hemorrhoids, while in recent years attention has been directed to HA [1, 16]. Therefore, we aimed to reveal if Avaricon® Hemor that contains SH, calendula flower extracts (CE), dry hamamelis extract (HE) and essential oil from mentha piperita (MPO) may be efficient in reducing symptoms and improving hemorrhoidal disease.

Our results showed that a 14-day application of both Avaricon® Hemor and the placebo per se, were efficient in reducing anal pain, thus suggesting that use of any type of cream may alleviate symptoms of hemorrhoids to a certain extent [1, 12]. Baseline higher pain intensity in the Avaricon® group was markedly decreased after the treatment, leading to more prominent pain alleviation in comparison to the placebo. Positive effects of Avaricon® Hemor were also evidenced by a decrease in bleeding intensity and grade of hemorrhoids in the Avaricon® group. Moreover, as estimated by VAS, Avaricon® Hemor provided a greater relief of anal pain and pain during defecation, as well as a decline in intensity of bleeding in comparison to the placebo cream. In summary, Avaricon® Hemor cream showed to be significantly superior to the placebo in most of the symptoms, thus improving discomfort and patients' quality of life.

Previous studies have pointed out that abnormalities in collagen composition, decrease in mechanical stability, and tensile strength in extracellular matrix may be associated with hemorrhoidal disease [18]. In this respect, as a natural remarkable extracellular matrix component, HA may help rebuilding the structures anchoring the hemorrhoids and explain our results [19, 12]. Anti-inflammatory and wound healing effects of HA may also be useful in the treatment of this medical condition [12, 20]. Apart from HA, presence of plant extracts and essential oils from plant species may also help in the reduction of symptoms related to hemorrhoidal disease via improvement in microcirculation, capillary flow and vascular tone [21]. Moreover, anti-inflammatory, antioxidant and astringent properties of CE, HE and MPO in our examined formulation significantly contribute to overall observed protective effects [16, 22–25]. The astringent potential of plant-derived constituents of Avaricon® Hemor thus heals mucous membranes due to the presence of tannins, which probably promotes vein elasticity and exerts vasoconstrictor activity in the perianal area [21]. Another advantage of CE, HE and MPO in our formulation is based on their capacity to prevent the breakdown of HA through both antioxidant activity and hyaluronidase inhibition [12, 26]. Furthermore, plant extracts and essential oils increase the potency of SH, since they are able to act against a number of pathogenic bacteria that produce hyaluronidase [12, 27–29].

Assuming that providing safety of the treatment strategy represents a major world health concern, we wanted to find out if our medical preparation is associated with the occurrence of adverse effects. In the current research, neither significant adverse effects related to treatment nor other safety related issues were observed. A difference in favor of Avaricon® Hemor was found when patients assessed the tolerability of treatment. In fact, all patients treated with Avaricon® Hemor scored the tolerability as "good" or "very good", while none of them scored it as "poor" or "very poor" as it was noticed in the placebo group. A notably better tolerability profile in the Avaricon® group, apart from a more prominent improvement of symptoms evaluated by patients, undoubtedly indicates the advantage of Avaricon® Hemor cream. It is well documented that extensive use of HA in numerous drug formulations is due to its biodegradability, biocompatibility, non-toxicity and non-immunogenicity [30]. Our results are in correlation with a previously conducted investigation which also proved the efficacy and safety of topical gel with SH in combination with tea tree oil and methylsulfonylmethane [12].

The second part of our research assessed the compliance and adherence of patients using the examined medication. It has been considered that treatment non-adherence might be a problem for patients with chronic diseases who have to use a time-consuming topical drug [31]. Data suggest that adherence rates to topical treatments are low, although gels and creams are considered more acceptable among topical formulations [32]. Moreover, we noticed that the majority of patients (94.74%) who applied Avaricon® Hemor cream were

fully adherent to the prescribed therapy and they did not stop using it when they felt better or worse. Treatment effectiveness of Avaricon® Hemor supports the data obtained for satisfactory adherence, indicating that patients are more likely to continue using the therapy if they believe it to be beneficial.

## Conclusion

Our results showed that treatment with Avaricon® Hemor was more effective and safe in comparison to the placebo, with a greater potential to reduce numerous symptoms of hemorrhoids. This study provides a rational basis for implementation of Avaricon® Hemor in the management of hemorrhoidal disease, either alone or as an additional therapy following invasive procedures. Nevertheless, a long-term follow-up study is certainly necessary with more participants to completely establish all the therapeutic possibilities of Avaricon® Hemor in this common anorectal condition.

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# A TEN-YEAR TREND OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN VOLUNTARY BLOOD DONORS IN THE SOUTH BAČKA DISTRICT OF VOJVODINA

## *DESETOGODIŠNJI TREND INFEKCIJE VIRUSOM HUMANE IMUNODEFICIJENCIJE KOD DOBROVOLJNIH DAVALACA KRVI U JUŽNOBAČKOM REGIONU VOJVODINE*

**Jasmina GRUJIĆ[1, 2], Nevenka BUJANDRIĆ[1, 2] and Zorana BUDAKOV OBRADOVIĆ[1, 2]**

**Summary**

**Introduction.** The first cases of human immunodeficiency virus infection and the first transmission of human immunodeficiency virus infection through blood transfusion in Vojvodina were registered in 1985. The aims of this study were to determine the ten-year trend of human immunodeficiency virus infection among blood donors in South Bačka District of Vojvodina, routes of transmission and risk factors. **Material and Methods.** A retrospective study was conducted at the Blood Transfusion Institute of Vojvodina during 2009 - 2018, and human immunodeficijenca antigens and antibodies were analyzed. A total of 300 936 blood donor samples were screened using combined antibody-antigen tests (fourth generation enzyme-linked immunosorbent assay and chemiluminescence). Further testing included confirmatory immunoblot assay and molecular assay. **Results.** The overall human immunodeficiency virus seroprevalence was 3.7 per 100 000 donations. The number of blood donors found to be human immunodeficiency virus positive has increased during the study period showing a positive trend. The highest number of human immunodeficiency virus positive results was reported among blood donors aged 26-35 (36%) and 46-55 years (27%). The infection was more frequent in males. The leading rout of transmission was through unprotected sexual intercourse. Higher human immunodeficiency virus prevalence was among regular blood donors (73%). **Conclusion.** This study points to the importance of careful selection of blood donors and their education about risk behavior in order to reduce the risk of human immunodeficiency virus transmission. Concomitant use of sensitive serological and molecular tests is crucial to increase the blood safety.

**Key words:** Blood Donors; HIV Seroprevalence; HIV Infections; Risk Factors; Blood Transfusion; Blood Safety; Donor Selection; Sexual Behavior

**Sažetak**

**Uvod.** Prvi slučajevi infekcije virusom humane imunodeficijencije u Vojvodini registrovani su 1985. godine kada je registrovan i prvi prenos infekcije virusom humane imunodeficijencije putem transfuzije krvi. Cilj studije bio je da se utvrdi desetogodišnji trend infekcije virusom humane imunodeficijencije kod dobrovoljnih davalaca krvi Južnobačkog okruga Vojvodine, način prenosa infekcije i faktori rizika. **Materijal i metode.** U retrospektivnoj studiji analizirani su rezultati kombinovanog testiranja antigena i antitela na virusu humane imunodeficijencije kod davalaca krvi u Zavodu za transfuziju krvi Vojvodine u periodu od 2009. do 2018. godine. Za testiranje 300.936 uzoraka krvi davalaca krvi korišćeni su testovi četvrte generacije imunoenzimskih testova i testovi hemiluminiscencije. Dodatno testiranje uključivalo je potvrdni imunoblot test i molekularno testiranje. **Rezultati.** Ukupna prevalencija infekcije virusom humane imunodeficijencije iznosila je 3,7 na 100.000 jedinica krvi. Broj davalaca krvi pozitivnih na virus humane imunodeficijencije povećavao se tokom perioda studije (pozitivan trend). Najveći broj davalaca krvi pozitivnih na virus humane imunodeficijencije registrovan je u uzrastima 26−35 (36%) i 46−55 godina (27%). Infekcija je češće registrovana kod muškaraca. Vodeći način prenosa infekcije bio je putem nezaštićenog seksualnog odnosa. Utvrđena je viša prevalencija infekcije kod višestrukih davalaca krvi (73%). **Zaključak.** Studija prikazuje značaj koji pravilna selekcija davalaca krvi i njihova edukacija o rizičnom ponašanju imaju na smanjenje rizika prenosa infekcije virusom humane imunodeficijencije. Istovremena upotreba osetljivih seroloških i molekularnih testova krucijalna je za povećanje bezbednosti transfuzije krvi.

**Ključne reči:** donori krvi; HIV seroprevalencija; HIV infekcije; faktori rizika; transfuzija krvi; sigurnost krvi; izbor donora; seksualna aktivnost

## Introduction

The first cases of human immunodeficiency virus (HIV) were reported in 1981 in the United States in apparently healthy men. The patients were diagnosed with Pneumocystis jiroveci pneumonia or Kaposi's sarcoma, which are commonly associated with weakened immune system. The unknown disease was called acquired immunodeficiency syndrome (AIDS), but very soon the cause was discovered and it was HIV [1]. Initially, it was thought that the virus affected only "risk groups" of population, such as men who have sex with men (MSM), intravenous drug users, patients on dialysis, as well as

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***Abbreviations***

HIV – human immunodeficiency virus
MSM – men who have sex with men
BD – blood donor
BTIV – Blood Transfusion Institute of Vojvodina
NAAT – nucleic acid-amplification testing
WHO – World Health Organization

patients receivng blood transfusions [2]. The first case of transfusion-transmitted HIV infection was reported in late 1982, in the United States, while the first HIV antibody test was introduced in March of 1985, as a part of a routine screening of the donated blood [3]. The first cases of HIV infection and transfusion-transmitted HIV infection in Vojvodina were registered in 1985 [4].

Although a great progress has been made in the treatment of persons with HIV infection and in the preventon of HIV transmission through blood, there still remains a residual risk [5, 6]. Continuous, efficient, timely, reliable, and safe blood supply of health facilities represents the basis of the strategy of developing a good health care system in every country. A well-organized transfusion service with high quality level of each process, starting with the selection of blood donors (BD), blood collection, processing, storage, testing and distribution are prerequisites to achieve this aim [7]. According to the Law of Transfusion Medicine in Serbia, screening of all blood donations to blood-transmissible diseases (HIV I/II, hepatitis B and C and syphilis) is mandatory [8].

The aims of this study were to determine the HIV infection ten-year trend in BDs in the South Bačka District of Vojvodina, demographic characteristics of HIV positive BDs, routes of transmission and risk factors in low-risk donors as factors that may affect the transmission of the virus through blood transfusion.

## Material and Methods

This retrospective observational study analyzed the results of HIV tests performed among BDs in the South Bačka District of Vojvodina from January 2009 to December 2018. Data were collected from the monthly reports and Donor Deferral Registry of the Blood Transfusion Institute of Vojvodina (BTIV).

A specially designed blood donor questionnaire, including questions about risky behaviors and the donors' health status, was used to collect data from BDs before donating blood. A BD was selected to donate blood after he or she passed eligibility criteria according to the Law of the Transfusion Medicine in Serbia (medical history, physical examination, pulse, blood pressure, hemoglobin level etc.).

A total of 300 936 blood samples of all donated blood were screened using the fourth generation of the enzyme-linked immunosorbent assay (ELISA) (BioRad Geenscreen Ultra HIV I/II Ag/Ab) and Chemiluminescent immunoassay (Abbott Architect HIV I/II Ag/Ab Combo; Siemens Advia Centaur HIV I/II Ag/Ab Combo Assay) for the detection of HIV antigens and HIV antibodies, which could detect HIV between 13 and 24 days after exposure (window period).

In case of repeatedly reactive results, the sample was tested by confirmatory immunoblot assay (Fujirebio Inno-Lia HIV I/II Score) at the BTIV. In confirmed HIV-positive BDs, their stored blood samples from the last donation were thawed and tested by immunoassay and nucleic acid-amplification testing (NAAT) for presence of HIV markers.

All BDs with positive results for HIV who confirmed positive using an immunoblot assay were invited to a confidential interview at the BTIV and their initial questionnaire was reviewed. After they were informed about their positive results and about possible ways of HIV transmission, they were encouraged to inform their partners who should also be tested, and were referred to the HIV Counseling Center of the Institute of Public Health of Vojvodina. They were also referred for further examination and additional molecular testing for HIV at the Clinic of Infectious Disease of the Clinical Center of Vojvodina.

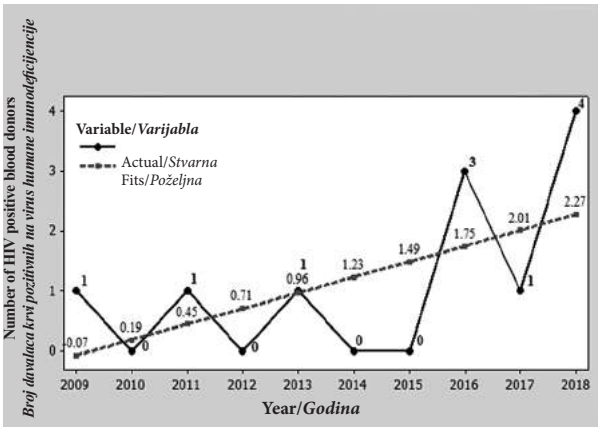
Test results were statistically analyzed by Minitab® 16 Statistical Software.

## Results

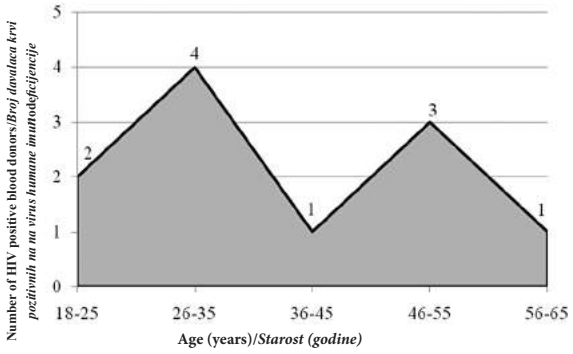
During the study period a total of 300 936 blood samples were tested, from 14 714 to 20 983 per year. Among these samples, 11 were found positive with prevalence of 3.7 HIV infections per 100.000 blood donations. Although there was a small number of positive cases, the trend of HIV infection among BDs increased between 2009 - 2018 **(Graph 1)**.

The highest number of HIV positive results was reported among BDs aged 26 - 35 (36%) and 46 - 55 years (27%) **(Graph 2)**. HIV infection was more frequently detected in males (91%).

Out of all HIV positive BDs, 3 (27%) were first time BDs, while 8 (73%) were regular BDs. In six




**Graph 1.** Trend of HIV infection in blood donors in the South Bačka District of Vojvodina, 2009 – 2018
***Grafikon 1.*** *Trend infekcije virusom humane imunodeficijencije kod davalaca krvi u Južnobačkom okrugu 2009–2018*




**Graph 2.** Age distribution of HIV positive blood donors in the South Bačka District of Vojvodina, 2009 – 2018
***Grafikon 2.*** *Starosna distribucija davalaca krvi pozitivnih na virus humane imunodeficijencije u Južnobačkom okrugu, 2009–2018*

regular BDs the time period between the last donation and the HIV positive donation was longer than one year, while two regular BDs gave blood every three months. For all of the regular BDs, the stored blood samples from the last donation screened negative, while one blood sample was NAAT positive. The BD with NAAT positive result at previous donation donated blood three months before and tansmitted HIV virus through blood transfusion, as the consequence of the "window period".

The majority of HIV positive BDs (81.8%) were from the city of Novi Sad.

The leading mode of transmission (91%) was through unprotected sexual intercourse: transmissions from MSM in 54.6%, and heterosexual mode in 36.4% of cases. In 9% of BDs, the mode of transmission could not be determined. The review of the BD questionnaire showed that all BDs denied risk behavior.

## Discussion

The study identified the highest number of HIV infections among male BDs aged 26-35 (36%) and 46-55 (27%), from urban environment, regular BDs with year or more since the last blood donation. The infection occurred through unprotected sexual intercourse in the majority of cases, mainly in MSM. All infected BDs denied any form of risk behaviors in the BD questionnaire.

In the latest report published by the European Centre for Disease Prevention and Control and the World Health Organization (WHO) Regional Office for Europe, HIV transmission remains a major public health concern. The HIV affects more than 2 million people in the WHO European Region, particularly in the eastern part of the Region. Nearly 160 000 people were diagnosed with HIV in the European Region in 2017. The increasing trend in newly diagnosed HIV infections continued for the Region overall [9]. Countries with the lowest rates were Slovenia (1.9) and Slovakia (1.3), and Lithuania (18.8) and Estonia (16.6) with the highest rates [10]. According to the report of the Institute of Public Health of Vojvodina, with an average of 41 newly identified cases in the total population per year, Vojvodina's population belongs to the group of low rate HIV infections with approximately two newly diagnosed HIV infections per 100 000 inhabitants. In the period from 1985 to 2017, the transmission of HIV infection through blood transfusion in Vojvodina presents 0.5% of all HIV transmission modes [11].

The prevalence of HIV positive BDs in Europe is 8.9 per 100 000 donations: 1.8 in Western Europe and 37.6 in Eastern Europe [12]. In our study, the prevalence of HIV was 3.7 per 100 000 donations in the territory of the South Bačka District of Vojvodina, which means it has a higher prevalence of HIV positive BDs than western European countries. This group also includes Italy (3.8), Spain (6.0) and countries of the Central Europe (3.8) [10, 12]. It is evident that there were no cases of HIV infected BDs in the four observed years. Also, it is important to emphasize that at the end of 2015, the prevalence of HIV positive BDs in the South Bačka District of Vojvodina was considerably lower than later. It can be concluded that blood transfusion still remains a potential risk for the transmission of viral infections, including HIV. The high cost of antiretroviral therapy and potential late diagnosis may potentially lead to increased morbidity and mortality among infected persons.

The study shows that there was an increase in newly diagnosed cases of HIV among BDs, and the highest number of HIV infected BDs was idetified in 2018. All infected BDs diagnosed during 2018 donated blood in the territory of the municipality of Novi Sad.

Since August 2019, the new Law on Transfusion Medicine has introduced NAAT as part of the mandatory screening of donated blood in Serbia [8]. Implementation of NAAT testing will reduce the risk of transfusion-transmitted viral infections, because it can detect viremia earlier (aproximately within 11 days) than current screening methods [13]. With reducing the "window period" by using NAAT, the safety of blood products will increase.

Although HIV infection is usually discovered in first-time donors, who are not fully aware of the risk factors that can lead to transmission of infection, this study found something different [14]. Namely, among the BDs with confirmed HIV infection, regular BD were more frequent than first-time BDs. During the post-testing interview with the doctor, not one of the blood donors suspected that he was infected, but all of them were aware of their risk behavior that could lead to the transmission of HIV infection. The dishonest or misleading answers in the donors questionnaire could have been conscious or unconscious, as a result of two reasons: they did not suspect that they were infected, or they wanted to be tested. Fearing stigma and discrimination, they donated blood and they were tested for sexually transmitted diseases in a way that allowed them to avoid people to know about their lifestyles and

risk behaviors. This is supported by the fact that the largest number of infected BDs was from the group of men who were infected through sexual relations with other men. In Serbia, BDs with such sexual orientation are permanently excluded from blood donation, which confirms the fact that they knowingly avoided to be honest and accurately fill out health questionnaires. Most European countries also permanently decline BDs who are MSM [15]; Hungary, Slovakia and United Kingdom defer them temporarily for three to six months [16], while Spain and Italy have no restrictions for MSM [17].

One of the main reasons why Vojvodina is a region with a low incidence of newly diagnosed HIV infections is the low rate (11.2) of HIV testing per 1,000 people in Vojvodina [18], compared to the countries of the European Union (e.g. France has a rate of testing 80.6.) [19]. On the other hand, a large number of the tested persons in Vojvodina belong to BDs. For example, during 2016, a total of 79 389 HIV tests were performed in Vojvodina, while 57 741 (72.7%) of them belonged to BDs [18].

## Conclusion

This study shows the importance of proper selection of blood donors, application of sensitive screening tests, and nucleic acid amplification testing for preparation of safe blood units and blood products. Providing more privacy for blood donors when filling out the questionnaire and education focused on all the possible consequences of failure to report "risk behavior", may contribute to lower prevalence of human immunodeficiency virus infection in the population of blood donors. Sufficient information about human immunodeficiency virus infection allows blood donors "self-exclusion" from the process of blood donation in case of risk factors and reduce the residual risk of transmitting the virus in the "window period". It is also important for potential blood donors to be informed about the possibility of confidential counseling and anonymous and free of charge human immunodeficiency virus testing daily at the Counceling Center of the Institute of Public Health of Vojvodina.

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# AGREEMENT BETWEEN PARENTS' PROXY REPORTS AND CHILDREN'S SELF-REPORTS OF PHYSICAL ACTIVITY AND SEDENTARY BEHAVIOR IN CHILDREN AGED 7 – 10 YEARS IN VOJVODINA

## *POVEZANOST PODATAKA O FIZIČKIM I SEDENTARNIM AKTIVNOSTIMA DECE DOBIJENIH OD DECE UZRASTA OD 7 DO 10 GODINA I NJIHOVIH RODITELJA U VOJVODINI*

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**Summary**

**Introduction.** The aim of this study was to determine the agreement between parents' and children's reports of children's physical activity and screen-based sedentary activities. **Material and Methods.** The sample included 7 - 10 year-old children (n = 94) and their parents (n = 94) in a local community in Vojvodina. Parents and children separately completed questionnaires about the types of physical and sedentary activities and the time children spent in different activities during one day. The agreement between children's and parents' responses was calculated using Cohen's kappa. The differences in parents' and children's responses in relation to gender and grade the students attended were analyzed using $\chi^2$ test. P-values less than 0.05 were considered statistically significant. **Results.** The highest level of agreement ($\kappa = 0.74$; $p = 0.00$) was found for the questions concerning physical activity in the morning before going to school. The lowest level of agreement was found for watching TV in the morning before going to school ($\kappa = 0.21$; $p = 0.04$). Children reported spending more time in screen-based sedentary activities than their parents. **Conclusion.** This research showed that there are differences in reports of children's physical activities and screen time obtained from children aged 7 to 10 years and their parents. The lowest level of agreement was found for watching television, indicating low level of awareness and control of this screen-based sedentary behavior.

**Key words:** Sedentary Behavior; Screen Time; Exercise; Self Reports; Parents; Child; Surveys and Questionnaires

**Sažetak**

**Uvod.** Cilj rada je da se utvrdi povezanost podataka o fizičkim i sedentarnim aktivnostima dece dobijenih od dece uzrasta od 7 do 10 godina i njihovih roditelja. **Materijal i metode**. Uzorak je uključivao decu uzrasta 7–10 godina (n = 94) i njihove roditelje (n = 94) iz jedne lokalne zajednice u Vojvodini. Deca i roditelji su odvojeno popunjavali upitnik o vrsti fizičkih i sedentarnih aktivnosti i vremenu koje su deca provela u navedenim aktivnostima tokom jednog dana. Slaganje odgovora dece i roditelja analizirano je primenom Koenove kapa metode. Razlike u slaganju odgovora roditelja i dece u odnosu na razred koji deca pohađaju utvrđene su primenom hi kvadrat testa. Vrednosti p manje od 0,05 smatrane su statistički značajnim vrednostima. **Rezultati.** Najviši nivo slaganja odgovora roditelja i dece ($\kappa = 0{,}74$; $p = 0{,}00$) utvrđen je za pitanja koja su se odnosila na upražnjavanje fizičkih aktivnosti tokom prepodneva pre odlaska u školu. Najniži nivo slaganja odgovora roditelja i dece ($\kappa = 0{,}21$; $p = 0{,}04$) utvrđen je za pitanja o gledanju televizijskog programa tokom prepodneva pre odlaska u školu. Deca su prijavila značajno duže vreme provedeno u sedentarnim aktivnostima nego roditelji. **Zaključak.** Istraživanje je pokazalo da postoje značajne razlike u podacima o fizičkim i sedentarnim aktivnostima dece dobijenim od dece i njihovih roditelja. Najniži nivo slaganja odgovora dece i roditelja, utvrđen za gledanje televizijskog programa, ukazuje na nizak nivo svesti i kontrole ovog oblika sedentarnog ponašanja dece.

**Ključne reči:** sedentarno ponašanje; vreme provedeno ispred ekrana; fizička aktivnost; odgovori; roditelji; deca; istraživanja i upitnici

## Introduction

Accurate assessment of children's physical activity and sedentary behavior is a challenging research topic. Numerous parameters and variability of physical activity in children make it difficult for monitoring [1]. Children are engaged in different activities during the day. Their activity pattern during unstructured spontaneous play is characterized by short intervals of intense physical activities combined with different intervals of low and moderate physical activities [1]. When children start going to school, their activity patterns change significantly [2].

Different methods and instruments have been developed for the assessment of physical activity and sedentary behavior in children. Considering strengths and limitations of each method, the choice of the most suitable method for a particular study depends on the research topic, target population, costs, etc [3].

Objective methods for the assessment of children's physical activity include accelerometry, pedometry, heart rate monitoring, doubly-labeled water, and direct

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***Abbreviations***
WHO – World Health Organization
METs – metabolic equivalents
BMI – body mass index

observation [3]. The most commonly used instruments for objective assessment of the amount of physical activity and sedentary time are accelerometers and pedometers [4]. Accelerometers are small instruments used to measure the intensity of physical activity or energy expenditure by capturing body's acceleration during movement [3]. Pedometers also provide valid measurement of children's physical activity by counting steps during a given period. Although they are suitable for estimating intensity and duration of physical activity, accelerometers and pedometers do not provide information about the type of children's physical activity [3, 5]. Practical aspects and cost-effectiveness make objective methods less appropriate for large population based studies or researches with limited resources [4, 5]. Therefore, population based studies often use self-report measures to asses children's physical activity and sedentary behavior [4–6]. Self-report measures are simple and quick to apply in large samples with relatively low costs [4–6]. Although self-report measures provide information about the type and context of physical activity and sedentary behaviors, in children they have limited validity and reliability in estimating their physical activity and sedentary behavior [4–6]. Children under the age of 10 years are prone to recall bias while reporting their physical and sedentary activities [4, 6, 7]. In order to overcome recall bias, proxy reports by parents are often used to estimate children's physical and sedentary activities [4, 8]. Some studies showed that parents are a more reliable source of information about their children's physical activity than children themselves [9, 10]. However, other studies show that validity of parents' proxy reports of their children's physical activities may vary across different age groups of children [5, 10, 11]. Parents are also prone to social desirability bias when reporting children's physical and sedentary activities [12].

The aim of this study was to determine the level of agreement between parents' reports of their children's physical and sedentary activities and children's self-reports of physical and sedentary activities.

## Material and Methods

The study included first to fourth grade students recruited from two elementary schools in a local community in Vojvodina and their parents. All students attending first to fourth grade from both schools were asked to participate in this study. Two classes from each grade agreed to participate in the study. In order to obtain written parental consents, parents were sent letters of consent and information about the research. A total of 94 parents provided written consent making a response rate of 48.9%. Sample characteristics are presented in **Table 1**.

The study was performed as a part of the doctoral dissertation which was approved by Ethics Committee of the Faculty of Medicine, University of Novi Sad.

According to World Health Organization (WHO), physical activity is defined as "any bodily movement produced by skeletal muscles that requires energy expenditure" [13]. Physical activity includes exercise, but also other bodily movements which involve playing, working, travelling etc. [13]. Children's physical activity measured in this study included physical activities performed as a part of spontaneous outdoor play and organized sports activities. Sedentary behavior included behaviors practiced while awake in sitting, reclining or lying posture with required energy expenditure less than 1.5 metabolic equivalents (METs) [14]. Screen time measured in this study referred to sedentary screen time which included time spent using screen-based devices while being sedentary [14].

Data about children's physical activity and screen time were collected using a questionnaire "My activities for one day" developed for this study. The questionnaire consisted of two sets of questions that were presented as pictures of different physical activities (playing outdoors, different sports activities, riding a bicycle) and screen-based devices (TV, computer, telephone). Children were asked to report activities they performed, devices they used and the time (only third and fourth grade children) spent in performing certain activities or using screen-based devices during one day. To boost children's recall questions were organized in parts of the day (in the morning and in the noon - before going to school;

**Table 1.** Sample characteristics
***Tabela 1.*** *Osobine uzorka*

| | n | % |
|---|---|---|
| Grade/*Razred* | | |
| First/*Prvi* | 13 | 13.8 |
| Second/*Drugi* | 20 | 21.3 |
| Third/*Treći* | 25 | 26.6 |
| Fourth/*Četvrti* | 36 | 38.3 |
| Gender/*Pol* | | |
| Boys/*Dečaci* | 47 | 50 |
| Girls/*Devojčice* | 47 | 50 |
| Total/*Ukupno* | 94 | 100 |

**Table 2.** Agreement between children's self-reports and parents' reports of children's physical activities and screen-based sedentary activities
***Tabela 2.*** *Slaganje odgovora dece i roditelja o fizičkim aktivnostima i sedentarnom ponašanju dece*

| Activities<br>*Aktivnosti* | Reported by parents<br>*Prijavili roditelji*<br>% (n) | Reported by children<br>*Prijavila deca*<br>% (n) | Responses agreed<br>*Odgovori se slažu*<br>% (n) | Responses disagreed<br>*Odgovori se ne slažu*<br>% (n) | Weighted kappa<br>*kapa* | p<br>*p* |
|---|---|---|---|---|---|---|
| Watching TV in the morning and noon<br>*Gledanje televizora pre podne* | 74.5 (70) | 77.7 (73) | 71.2 (67) | 28.7 (27) | 0.21 | 0.04 |
| Playing computer games in the morning and noon/*Igranje na kompjuteru pre podne* | 8.5 (8) | 10.6 (10) | 91.5 (86) | 8.5 (8) | 0.59 | 0.00 |
| Physical activities in the morning and noon<br>*Fizičke aktivnosti pre podne* | 44.7 (42) | 42.6 (40) | 87.2 (82) | 12.7 (12) | 0.74 | 0.00 |
| Watching TV in the afternoon and evening<br>*Gledanje televizora popodne i uveče* | 69.1 (65) | 62.8 (59) | 73.4 (69) | 26.6 (25) | 0.39 | 0.00 |
| Playing computer games in the afternoon and evening/*Igranje na kompjuteru popodne i uveče* | 11.7 (11) | 9.6 (9) | 93.6 (88) | 6.4 (6) | 0.66 | 0.00 |
| Physical activities in the afternoon and evening<br>*Fizičke aktivnosti popodne i uveče* | 64.9 (61) | 42.6 (40) | 72.3 (68) | 27.6 (26) | 0.47 | 0.00 |

in the afternoon and in the evening - after school). Data were collected during one school day which started at 2 p.m. and finished at 5:35 or 6:25 p.m. Physical activities performed at school were not analyzed in this study.

The questionnaire for parents included the same questions as the questionnaire for children, without pictures. Parents were asked to specify physical activities their children were involved in, screen-based devices they used and the time spent in different activities or using screen-based devices during one day. Parents and children completed questionnaires separately for the same day.

The body mass index (BMI) was calculated based on children's height and weight reported by parents. Instruction letters for measuring children's body height and weight were created according to the guide for anthropometric measurements of the United States Centers for Disease Control and Prevention and sent to parents prior to obtaining data [15]. The participants were categorized into three groups based on BMI and age: normal weight (n = 51; 53.2%), overweight and obese (n = 26; 26.6%), and underweight (n = 3; 3.2%) [16]. A total of 14 parents (13.8%) did not report their children's height and weight.

Statistical Package for the Social Sciences version 18.0 (SPSS Inc., New York) was used for statistical analysis of the data. Parents' and children's answers to certain questions were categorized into the following groups: physical activities before school, using screen-based devices before school, physical activities after school, using screen-based devices after school. Answers about the time spent in physical activities and screen-based time were also categorized in groups based on the part of the day. The level of agreement between parents' reports and children's self-reports was calculated with weighted kappa [16]. The difference in parents' reports and children's self-reports were analyzed using $\chi^2$ test in relation to children's gender and grade. P-values less than 0.05 were considered statistically significant.

## Results

Parents' and children's responses to certain questions, the agreement between children's self-reports and parents' reports of physical activities and using screen-based devices are presented in **Table 2.**

Most children and parents agreed that children were using computers before school (91.5%) and after school (93.6%). The lowest percentage of children and parents agreed that children were watching TV in the morning (71.2%) **(Table 3).**

The $\chi^2$ test revealed significant differences between parents' reports and children's self-reports of physical activities and screen-based sedentary behavior in regard to the grade children attended. Significantly more first grade children and their parents (69.2%) ($\chi^2 = 4.19$; $p = 0.00$) reported physical activity in the morning than children from third (24.0%) and fourth grades (19.4%). Watching TV in the morning was reported significantly more often by second grade children and their parents (80%) ($\chi^2 = 12.29$; $p = 0.01$) than children and parents from first (23.1%), third (56%) and fourth grades (69.4%). Children from the first grade (46.2%) were significantly more likely to disagree with parents about watching TV at noon ($\chi^2 = 16.28$; $p = 0.00$) than children from the second (15%), third (8%), and fourth grades (2.8%). Most first grade children and their parents disagreed about watching television (46.2%) ($\chi^2 = 19.32$; $p = 0.00$) in the afternoon and playing computer games at noon (15.4%), ($\chi^2 = 15.48$; $p = 0.02$).

Significantly more girls (60.9%) than boys (39.1%) agreed with their parents about the time spent playing computer games in the afternoon ($\chi^2 = 11.23$; $p = 0.01$). Girls (59.5%) were also more likely to agree with their parents about the time

**Table 3**. Agreement between children's self-reports and parents' reports of children's screen-based sedentary time during the day (n = 61)
***Tabela 3.*** *Slaganje odgovora dece i roditelja o vremenu provedenom u sedentarnim aktivnostima tokom dana (n = 61)*

| Activities/*Aktivnosti* | Responses agreed *Odgovori se slažu* % (n) | Responses disagreed *Odgovori se ne slažu* % (n) | Children reported more time *Deca prijavila duže vreme* % (n) | Children reported less time/*Deca prijavila kraće vreme* % (n) |
|---|---|---|---|---|
| Watching TV in the morning and noon *Gledanje televizora pre podne* | 73.8 (45) | 21.3 (13) | 11.5 (7) | 9.8 (6) |
| Watching TV in the afternoon *Gledanje televizora popodne* | 63.9 (39) | 26.3 (16) | 21.4 (13) | 4.9 (3) |
| Watching TV in the evening *Gledanje televizora uveče* | 60.7 (37) | 36.0 (22) | 18.0 (11) | 18.0 (11) |
| Playing computer games in the morning and noon *Igranje na računaru pre podne* | 67.2 (41) | 26.3 (16) | 19.7 (12) | 6.6 (4) |
| Playing computer games in the afternoon and evening *Igranje na računaru popodne i uveče* | 68.9 (42) | 21.3 (13) | 11.5 (7) | 9.8 (6) |

spent playing computer games in the evening ($\chi^2$ = 8.32; p = 0.04) than boys (40.5%).

Overweight and obese children (85.7%) were more likely than normal weight children (14.3%) to report more time spent watching TV ($\chi^2$ = 26.38; p = 0.01) than their parents.

## Discussion

The results of this study show that there are differences in the perception of physical activities and screen-based sedentary behavior between 7 – 10-year-old children and their parents. The **Table 2** shows that the agreement between children's and parents' responses was significant for each question of the questionnaire, but the level of agreement varied on some issues. The highest level of agreement was found concerning physical activity before school. The analysis of agreement between responses of parents and children showed that most parents and children agreed that children were physically active before school. These results may indicate that children were able to recall physical activities during the morning because those physical activities (playing in the playground, training) were part of their daily routines and parents were aware of the same. Unlike physical activity in the morning, the level of agreement between responses of parents and children about physical activities after school was just below the moderate. Approximately one fourth of children and parents disagreed whether children were physically active after school. More parents than children reported that children were engaged in physical activity after school. The above result may indicate that it was more difficult for children to remember what they did in the afternoon than in the morning, especially if afternoon activities vary from day to day more than late morning activities. It can be assumed that in the period when this study was performed, children spent more time outdoors before school than after school. Several studies showed that parents tend to give socially desirable answers when it comes to physical activity of their children [8, 17]. All this may indicate that the disagreement between children's self-reports and parent's reports of physical activities after school may be a result of overestimation of physical activity of children by their parents or tendency of parents to give desirable answers.

A moderate level of agreement (kappa = 0.59; kappa = 0.66) between responses of parents and children was found regarding playing computer games before and after school. The analysis of agreement between individual responses of parents and children indicated that they generally agreed that children did not play computer games in the morning and in the afternoon. This result may indicate that playing computer games was to some extent controlled by parents and that the use of computers was not available as much as watching TV. The study by Sithole et al. also showed higher degree of agreement between responses of parents and children about playing computer games than watching TV [17].

The lowest level of agreement between the answers of parents and children was about watching TV before and after school (kappa = 0.21; kappa = 0.39). This result is consistent with other studies which showed that responses of parents and children matched the least when it comes to watching TV [8, 17]. This finding may indicate that watching TV was less controlled by parents than playing computer games and that television was more accessible to children than the computer. The analysis of individual responses revealed that the lowest percentage of children and parents agreed that children did not watch TV in the morning. This result indicates that watching TV was less under control of parents in the morning than in the afternoon, which was expected given that parents are usually at work in the morning.

The **Table 3** shows agreement between parents' proxy reports and children's self-reports of time spent watching TV and playing computer games. Approximately one fifth of parents and children did not agree on the amount of time children spend

watching TV in the morning or playing computer games in the evening. Around one fourth of children and parents disagreed on the time children spend watching TV in the afternoon or playing computer games in the morning. About one third of parents and children disagreed on the time children spend watching TV in the evening. An equal number of children reported spending more or less time watching TV in the evening compared to their parents. The obtained result is in line with previously presented results related to the agreement between parents' and children's responses about watching TV after school. Both results confirm that neither parents nor children have a clear awareness of how much time children spend watching TV. In accordance with findings of other studies, the results of this research indicate that both children and parents lack awareness of children's screen-based sedentary time [8, 17]. The children reported more screen-based sedentary time than their parents. Previous studies showed that parents as proxy reporters were prone to socially desirable responding and reporting less screen-based sedentary time [8, 17]. These results are important considering that home environment is strongly correlated with children's physical activity and sedentary behavior [18].

Analyzing the difference between reports of parents and children, we found that significantly more first grade children and their parents reported that children were engaged in physical activities before school. This result is expected given that different studies showed that younger children are more physically active than older children [19]. Among those who disagreed with their parents in terms of watching TV or playing computer games before and after school, most of them were children from the first grade. This finding is consistent with other researches confirming that 7- to 8-year-old children are not able to independently provide reliable data on food intake and physical activity [3, 20–22].

The finding that girls' responses agreed more with the responses of their parents regarding the time spent playing computer games can be explained by the fact that boys play computer games more often than girls and that computer use is less under control of parents in boys than in girls [23].

The only significant difference between parents' reports and children's self-reports in relation to BMS was concerning the time children spent watching TV before school. Overweight children were significantly more likely than normal weight children to report longer time spent watching TV compared to their parents. This result may be due to the fact that parents of overweight children tended to give socially desirable responses by reporting less screen-based sedentary time. Sithole et al. found that overweight and obese children reported less time watching TV than their parents [17]. The sample of this study included children from the fifth grade, so it can be assumed that older children are more prone to socially desirable responses than younger children.

This study was conducted on a small sample of 7- to 10-year-old children from two elementary schools and therefore the results may not be representative for the entire population of school aged children in Serbia. The study compared parents' reports and children's self-reports without comparisons with more objective methods for assessing physical activity and screen-based sedentary time in children (accelerometer, pedometer, observation). Thus, it is not possible to determine whether parents or children gave more reliable information. Data on height and weight were based on the information given by parents. However, Huybrechts et al. have shown that parents can give reliable information about height and weight of their children if they received instructions on how measurements should be performed [24, 25].

Future research should test differences in the assessment of physical activities and screen-based sedentary time on a representative sample of school-aged children and their parents.

## Conclusion

This research showed that there were differences in reports of physical activities and screen-based sedentary time between children aged 7 to 10 years and their parents, which must be taken into consideration when using questionnaires for self-assessment of physical and screen-based sedentary behavior in children. The greatest differences in the assessment of physical activity was found between 7-year-old children and their parents and the recommendation is that 7-year-old children should not fill out questionnaires about physical activities and screen-based sedentary behavior by themselves. Parents' and children's reports agreed the least about the time spent watching television indicating low level of awareness and control of this screen-based sedentary behavior.

## Implications for practice

Due to the inability to precisely estimate the time spent watching television, questionnaires for the assessment of screen-based sedentary behavior should use intervals of time within which it is easier for children and parents to estimate screen-based sedentary time.

The research using objective methods for the assessment of physical activities and screen-based sedentary behavior (observation) would show whether children or parents are more reliable in reporting physical activities and screen-based sedentary time in children.

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# MORPHOLOGICAL CHARACTERISTICS OF FORAMEN VESALIUS IN DRY ADULT HUMAN SKULLS

*MORFOLOŠKE KARAKTERISTIKE VEZALIJUSOVOG OTVORA SUVIH LOBANJA ODRASLIH OSOBA*

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**Summary**

**Introduction.** The foramen Vesalius is a variable foramen located at the skull base, anteromedial to the foramen ovale behind and lateral to the foramen rotundum. This foramen is also known as emissary sphenoidal foramen. The aim of the research was to determine the anatomical characteristics of the foramen Vesalius in adult human skulls and foramina classification according to their type, shape, and sex distribution. **Material and Methods.** The study included 26 dry adult human skulls of both sexes from the collection of the Department of Anatomy, Faculty of Medicine of the University of Novi Sad. The skulls were macroscopically analyzed according to the presence or absence of the foramen Vesalius. **Results.** The foramen Vesalius was found in 16 skulls (61.54%) and it was absent in 10 skulls (38.46%). The incidence of bilateral and unilateral foramen Vesalius was 87.5% (14 skulls) and 12.5% (2 skulls), respectively. The foramen Vesalius was found in 10 male skulls (62.5%) and in 6 female skulls (37.5%). **Conclusion.** Based on the morphological analysis of the skulls, the study showed that the foramen of Vesalius can be unilateral or bilateral. The bilateral foramen was more common and it was usually round and symmetrical. In regard to the sex prevalence, the foramen was more prevalent in male than in female skulls. The results of the study showed that foramen Vesalius is not an uncommon anatomical variation, and its presence and morphological appearance are important information for physicians in various fields.

**Key words:** Skull Base; Sphenoid Bone; Cranial Fossa, Middle; Anatomic Variation; Adult

**Sažetak**

**Uvod.** Vezalijusov otvor je varijabilan otvor koji se nalazi na bazi lobanje, ispred i unutra od ovalnog otvora, a spolja od okruglog otvora. Vezalijusov otvor je poznat još i po nazivu emisarni sfenoidalni otvor. Cilj ovog rada bila je morfološka analiza Vezalijusovog otvora na lobanji odraslog čoveka i klasifikacija otvora u odnosu na tip, oblik i polnu zastupljenost. **Materijal i metode.** U istraživanju je korišćeno 26 suvih ljudskih lobanja, koje pripadaju Zavodu za anatomiju Medicinskog fakulteta Novi Sad, Univerziteta u Novom Sadu. Lobanje su makroskopski analizirane na prisustvo ili odsustvo Vezalijusovog otvora. **Rezultati.** Vezalijusov otvor je bio prisutan na 16 (61,54%), a odsutan na 10 (38,46%) lobanja. Incidencija obostrano prisutnog otvora bila je 87,5% (14 lobanja), a jednostrano prisutnog 12,5% (dve lobanje). Vezalijusov otvor je bio prisutan na 10 lobanja muškog pola (62,5%) i šest lobanja ženskog pola (37,5%). **Zaključak.** Na osnovu morfološke analize lobanja, studija je pokazala da Vezalijusov otvor može biti jednostrano i/ili obostrano prisutan. Otvor je bio češće prisutan obostrano i obično je bio okrugao i simetričan. Otvor je bio zastupljeniji kod lobanja muškog nego kod lobanja ženskog pola. Iz rezultata ove studije, može se zaključiti da Vezalijusov otvor nije retka anatomska varijacija, a njegovo prisustvo i morfološki izgled predstavljaju važne informacije za lekare iz različitih oblasti.

**Ključne reči:** baza lobanje; sfenoidna kost; srednja kranijalna jama; anatomske varijacije; odrasla osoba

## Introduction

The greater wings of the sphenoid bone contain several openings that connect the middle cranial fossa to the pterygopalatine and infratemporal fossa. The permanent foramina of the sphenoid bone are foramen rotundum, foramen ovale and foramen spinosum, whereas the foramen of Vesalius (FV) and meningo-orbital foramen (Hyrtl's canal) are inconstant openings. All permanent and inconstant openings of this region contain veins that connect the extracranial and intracranial venous systems.

Andreas Vesalius [1] first described and drew this opening, which was named after him "foramen of Vesalius" (foramen venosum Vesalii, sphenoid emissary foramen, canaliculus sphenoidal). The FV is a small, non-permanent opening, with the upper end located on the cerebral surface of the greater wings of the sphenoidal bone, anterior and medial to the foramen ovale and lateral to foramen rotundum. Its lower end is located on the upper part of the lateral pterygoid plate, inferior and lateral to the scaphoid fossa.

The FV can be unilateral or bilateral. The mean diameter of the foramen is 1 mm in infants, while

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***Abbreviations***
FV – foramen of Vesalius

in adults it is 1.4 – 2 mm [2]. Anthropological studies have shown that there are numerous disagreements among authors about the presence of the FV, which range from 25 – 100% in the population [3].

According to Wood-Jones [4], the FV is a characteristic of the human race and it is an indicator of the complex cranial venous system in humans. The FV does not exist in any primate other than humans.

The emissary vein passes through the FV and it was named as the foramen itself, i.e. Vesalius vein, which establishes communication between the cavernous sinus and the pterygoid plexus. Along with the emissary vein, the accessory meningeal artery also passes through the foramen [5]. The FV is sometimes divided with a bony septum, reducing the space for the emissary veins and artery, resulting in vascular compression.

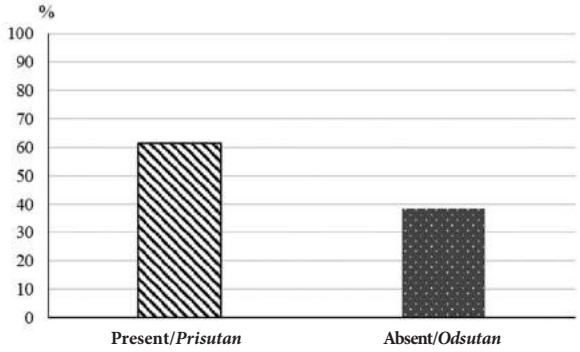
Since there is poor information on the presence and characteristics of the FV in our population, in comparison with the data in different populations, this study represents a morphological analysis of the adult skulls that show the FV. The aim of the research was to determine the anatomical characteristics of the FV in adult skulls and their classification according to the type, shape, and sex distribution.

## Material and Methods

The study included 26 dry adult skulls (13 male and 13 female) that belong to the Osteological Collection of the Department of Anatomy of the Faculty of Medicine, University of Novi Sad. Only skulls without visible deformities and damage were examined.

The skull sex determination was done using the sex-modified visual determination protocol modified by Ferembach [6] and Buikstra [7].

The examined skulls were photographed with an Olympus camera (18 x optical zoom) and the images were then transferred to the magnetic medium of the computer system.




**Graph 1.** Percentage of skulls with and without foramen of Vesalius
***Grafikon 1.*** *Procenat lobanja sa prisutnim i odsutnim Vezalijusovim otvorom*

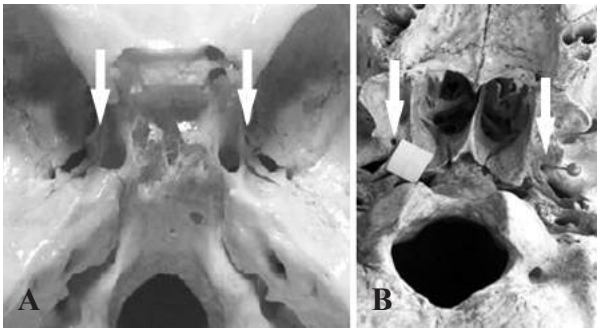



**Figure 1.** Bilateral foramen of Vesalius on the internal (A) and external surface (B) of the base of the skull (arrows)
***Slika 1.*** *Obostrano prisutan Vezalijusov otvor na unutrašnjoj (A) i spoljašnjoj (B) strani baze lobanje (strelica)*

Based on the macroscopic appearance and shape of the FV, the skulls were morphologically analyzed and divided into basic groups.

The incidence of bilateral and unilateral FV was calculated and presented in relation to the sex distribution. The obtained results were graphically presented.

## Results

In a sample of 26 dry adult skulls, presence of the FV, their shape on the internal and external surface of the base of the skull, as well as sex distribution, were analyzed. Based on the morphological characteristics, foramina were classified into two main groups: bilateral and unilateral.

The FV was found in 16 skulls (61.54%), while in 10 skulls (38.46%) it was absent **(Graph 1).**

Bilateral FV is located on the right and the left sides of the skull **(Figure 1)**, while unilateral foramen is located only on the right or left side **(Figure 2)**. In the sample of 16 skulls, bilateral FV was found in 14 skulls (87.5%) and unilateral in 2 skulls (12.5%).

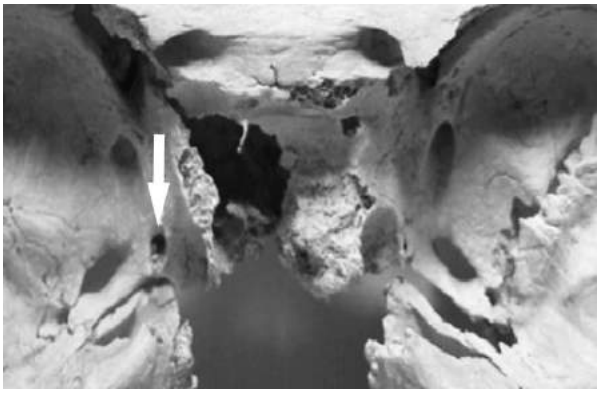


**Figure 2.** Unilateral foramen of Vesalius (arrow) on the internal surface of the base of the skull
***Slika 2****. Jednostrano prisutan Vezalijusov otvor (strelica) na unutrašnjoj strani baze lobanje*

**Table 1**. Incidence of the FV in different populations (%)
***Tabela 1.*** *Procenat prisustva Vezalijusovog otvora kod različitih populacija*

| Author and year<br>*Autor i godina* | Nation<br>*Narod* | Sample number<br>*Uzorak* | Incidence of the foramen Vesalius (%)<br>*Procenat prisustva Vezalijusovog otvora* |
|---|---|---|---|
| Boyd (1930) | England/*Engleska* | 1500 skulls/*lobanja* | 36.5% |
| Lanzieri (1988) | America/*Amerika* | 50 CT scans/*skenova* | 64% |
| Ginsberg (1994) | America/*Amerika* | 123 CT scans/*skenova* | 80% |
| Kodama (1997) | Japan/*Japan* | 400 skulls/*lobanja* | 21.75% |
| Gupta (2005) | India/*Indija* | 35 skulls/*lobanja* | 42.90% |
| Ramalho (2007) | Brazil/*Brazil* | 80 skulls/*lobanja* | 71.87% |
| Kaplan (2007) | Turkey/*Turska* | 10 skulls/*lobanja* | 100% |
| Shaik (2007) | India/*Indija* | 125 skulls/*lobanja* | 36% |
| Shinohara (2010) | Brazil/*Brazil* | 400 skulls/*lobanja* | 33.5% |
| Raval (2015) | India/*Indija* | 150 skulls/*lobanja* | 60% |
| Murlimanju (2015) | India/*Indija* | 78 skulls/*lobanja* | 37.2% |
| Bayrak (2018) | Turkey/*Turska* | 317 CBCT scans/*skenova* | 28.1% |
| Costa do Nascimento (2018) | Brazil/*Brazil* | 194 skulls/*lobanja* | 18.55% |
| Nayak (2018) | India/*Indija* | 30 skulls/*lobanja* | 30% |
| Leonel (2019) | Brazil/*Brazil* | 170 skulls/*lobanja* | 45.2% |
| Kaplan (2019) | Turkey/*Turska* | 350 CBCT scans/*skenova* | 41.1% |
| Our study (2020) | Serbia/*Srbija* | 26 skulls/*lobanja* | 61.54% |

Legend/*Legenda*: * CBCT - cone-beam computed tomography/*Kompjuterizovana tomografija na bazi koničnih zraka*

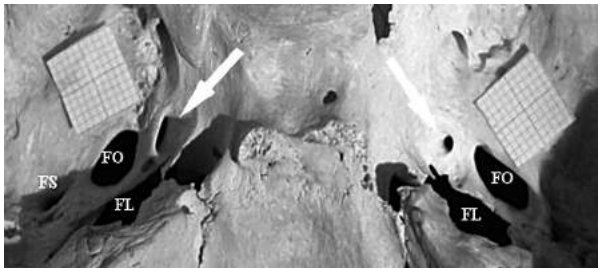
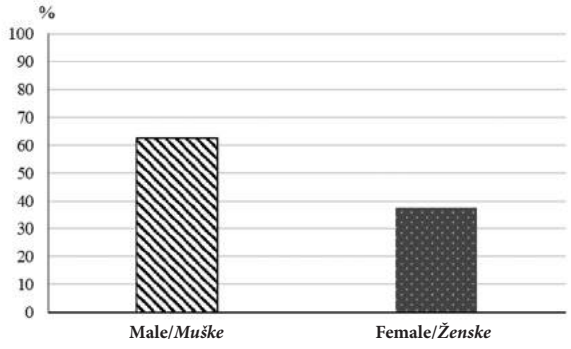



**Figure 3.** Bilateral asymmetric Vesalius foramina (arrows), foramen ovale (FO), foramen spinosum (FS), foramen lacerum (FL) on the internal surface of the base of the skull
***Slika 3.*** *Obostrano prisutan asimetričan Vezalijusov otvor (strelice), ovalni otvor (FO), otvor bodlje (FS), proderani otvor (FL) na unutrašnjoj strani baze lobanje*




**Graph 2.** Percentage of male and female skulls with Vesalius foramina
***Grafikon 2.*** *Procenat lobanja muškog i ženskog pola sa prisutnim Vezalijusovim otvorom*

All the examined cases presented with a foramen on the internal and external surface of the base of the skull, so it could be called the Vesalius canal.

In our sample, all the foramina were open. The upper end of the canal was behind and lateral to the foramen rotundum and in front and medial to foramen ovale. The lower end of the canal was located below and lateral to the scaphoid fossa. Closed canals at either end, or double foramina were not detected.

Asymmetric FV was found in 5 skulls. In 11 cases, foramina were small and round; in 5 cases on one side they were larger, oval and elongated **(Figure 3)**. The FV were present in 10 male skulls (62.5%) and 6 female skulls (37.5%) **(Graph 2).**

## Discussion

In our study, FV was observed in 61.54% of skulls **(Table 1)**, which corresponds to the findings of Lanzieri [8], Romalha [9], and Raval [10]. The most common foramen is bilateral, round and symmetrical, found in 87.5% of the skulls. Our results differ from the findings of other authors [5, 11–19], where the presence of foramina is lower **(Table 1).** According to most authors, the presence of the foramen is lower than ours, but when comparing the results of different authors of the same nationality, we may notice that their data also differ **(Table 1).** The FV in our sample was bilateral in 87.5% and unilateral in 12.5% of cases, which corresponds to the findings of Kodama [20], Nayak [17] and Kale [21]. Most authors reported approximately equal distribution of bilateral and unilateral foramina [5, 10–12], while some studies showed that unilateral openings were more frequent [14, 16, 18].

In our sample, the FV was more common in male subjects, unlike Gupta [11] and Rossi [22], who reported that it was more common in females. Kodama [20] did not detect a difference between sexes after examining 400 skulls.

The incidence of variable FV is probably due to evolutionary and adaptive skeletal responses to local biomechanical stimuli. The FV represents the communication between the extracranial and intracranial spaces of the skull. When present, it contains an emissary vein that connects the venous sinus of the skull and the venous vessels of the head, particularly the cavernous sinus with the pterygoid plexus. Through this canal, pathological processes and infections can pass from the extracranial into the cranial space (causing thrombosis of the cavernous sinus and intracranial infection). Given that the mandibular nerve and the corresponding blood vessels of the middle cranial fossa are nearby, pathological changes can affect the neurovascular elements and lead to certain symptoms [23–25]. The surgical significance of this foramen is that during percutaneous trigeminal rhizotomy, the needle may pass through this nonpermanent opening and lead to cavernous sinus puncture and intracranial hemorrhage, which can be a life-threatening condition [23, 24].

Asymmetry of the FV was observed in 5 cases and in most cases it was likely the result of a pathological process than a normal variant. The occurrence of asymmetry, i.e. an enlarged foramen on one side is most often the result of an acquired abnormality caused by an invasion of nasopharyngeal melanoma, angiofibroma, carotid-cavernous fistula, and neurofibromatosis [8].

Based on the results, it can be concluded that variations of FV are not rare anatomical phenomena as previously thought. The presence of several foramina, given that it is more frequent, should be suspected during the radiological diagnostic examination of the middle cranial fossa [23]. The finding of FV, its shape, symmetry, and asymmetry, provides important information for anatomists, radiologists, neurosurgeons, and maxillofacial surgeons.

## Conclusion

In this study, the macroscopic examination of skulls showed that foramen of Vesalius can be unilateral or bilateral. The incidence of bilateral foramen was much higher than unilateral, and it was mostly round and symmetrical. With regard to gender prevalence, the foramen was more frequent in male than in female skulls. From this, it can be concluded that foramen Vesalius is not an uncommon finding, and its presence and morphological appearance represent important information for physicians in various fields.

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Erratum

Hereby we offer our apologies for an unintentional error published in the paper entitled PROLIFERATION OF B-LYMPHOCYTES IN INFLAMMATORY AND HEMATOLOGICAL DISEASES published in the journal *Medical Review: 11 - 12/ 2018*, on pages 377 - 381 in the section Original Scientific Papers, by: Tamara Tešic, Dajana Lendak, Ivana Urošević, Igor Mitić and Vanja Andrić, where incorrect affiliations were attributed to Ivana Urošević and Igor Mitić. The correct affiliations and authors are as follows:

University of Novi Sad, Faculty of Medicine Novi Sad[1]
Clinical Center of Vojvodina, Novi Sad
Clinic of Otorhinolaryngology and Head and Neck Surgery[2]
Clinic of Infection Diseases[3]
Clinic of Hematology[4]
Clinic of Nephrology and Clinical Immunology[5]

Original study
*Originalni naučni rad*
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https://doi.org/10.2298/MPNS1812377T

## PROLIFERATION OF B-LYMPHOCYTES IN INFLAMMATORY AND HEMATOLOGICAL DISEASES

*PROLIFERACIJA B-LIMFOCITA U INFLAMATORNIM I HEMATOLOŠKIM BOLESTIMA*

**Tamara TEŠIĆ[1,2], Dajana LENDAK[1,3], Ivana UROŠEVIĆ[1,4], Igor MITIĆ[1,5] and Vanja ANDRIĆ[1]**

Editorial Board

Eratum

U radu pod naslovom PROLIFERATION OF B-LYMPHOCYTES IN INFLAMMATORY AND HEMATOLOGICAL DISEASES publikovanom u časopisu „Medicinski pregled“ u **dvobroju 11-12/2018**, na stranama 377-381 u rubrici Originalni naučni radovi, autora: Tamara Tešić, Dajana Lendak, Ivana Urošević, Igor Mitić and Vanja Andrić došlo je do nenamerne greške u navođenju afilijacija autora Ivane Urošević I Igora Mitića.

Izvinjavamo se zbog učinjenog propusta i navodimo ispravku načinjenog propusta:

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Original study
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**Tamara TEŠIĆ[1,2], Dajana LENDAK[1,3], Ivana UROŠEVIĆ[1,4], Igor MITIĆ[1,5] and Vanja ANDRIĆ[1]**

Uredništvo

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Professional article
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## THE ASSOCIATION BETWEEN PSYCHOACTIVE SUBSTANCE USE AND VIOLENCE

*POVEZANOST KORIŠĆENJA PSIHOAKTIVNIH SUPSTANCIJA I NASILJA*

**Vladimir A. KNEŽEVIĆ[1-3], Dragana RATKOVIĆ[1-3], Ana Marija VEJNOVIĆ[1-3], Svetlana IVANOVIĆ KOVAČEVIĆ[1-3], Jovan MILATOVIĆ[1-3] and Jelena KNEŽEVIĆ[4,5]**

**Summary**

**Introduction.** The association between substance use and aggression derives from the fact that among violent crime offenders there is a large number of people with alcohol and drug abuse. The objective of the study was to determine the incidence of psychoactive substance addicts in the population of violent crime offenders, as well as to consider possible measures to reduce the crime rates in this population. **Material and Methods.** Forensic psychiatric reports were used to assess 94 violent offenders from 2001 to 2018. The expert reports were obtained from the Psychiatry Clinic in Novi Sad, Serbia, including court case files, medical records, and psychiatric and psychological records of all offenders. **Results.** We have found that of the total of 94 violent crime offenders, as many as 25% suffered from some form of mental disorder. More than half of those with mental disorders from this group suffered from disorders induced by psychoactive substances, which means that as many as 15% of all offenders had some form of addiction. On the other hand, as much as 50% of all the offenders were under the influence of psychoactive substances at the time of the offense, predominantly by alcohol. **Conclusion.** Further research is needed in order to develop specific programs for the prevention of violence in the population using psychoactive substances.

**Key words:** Aggression; Violence; Crime; Psychotropic Drugs; Alcohol Drinking; Mental Disorders; Risk Factors; Forensic Psychiatry; Demography

**Sažetak**

**Uvod.** Povezanost korišćenja psihoaktivnih supstancija i agresije proizilazi iz činjenice da postoji veliki broj ljudi koji zloupotrebljavaju alkohol i droge u grupi učinilaca nasilnih krivičnih dela. Cilj ovog istraživanja bio je da se utvrdi učestalost zavisnika od psihoaktivnih supstancija u populaciji učinilaca nasilnih krivičnih dela, kao i da se razmotre moguće mere koje bi dovele do smanjenja kriminaliteta u ovoj populaciji. **Materijal i metode.** Razmatrana su sudsko-psihijatrijska veštačenja u kojima su procenjivani 94 učinilaca krivičnih dela od 2001. do 2018. godine. Ova veštačenja su sprovedena na Klinici za psihijatriju u Novom Sadu i u njima su razmatrane informacije iz sudskih spisa, medicinske dokumentacije, psihijatrijskog pregleda i psihološkog testiranja svih učinilaca nasilnih krivičnih dela. **Rezultati.** Utvrdili smo da je čak 25% od ukupno 94 učinilaca nasilnih krivičnih dela bolovalo od nekog oblika mentalnog poremećaja. Više od polovine osoba sa mentalnim poremećajima iz ove grupe je bolovalo od mentalnog poremećaja uslovljenog korišćenjem psihoaktivnih supstancija, što znači da je čak 15% svih učinilaca imalo neki oblik bolesti zavisnosti. S druge strane, čak 50% svih učinilaca je bilo intoksicirano psihoaktivnim supstancijama, dominantno alkoholom, u vreme izvršenja dela. **Zaključak.** Potrebno je sprovoditi dalja istraživanja u cilju razvijanja specifičnih programa prevencije nasilja u populaciji osoba koje zloupotrebljavaju psihoaktivne supstancije.

**Ključne reči:** agresija; nasilje; kriminal; psihoaktivne supstance; zloupotreba alkohola; mentalni poremećaji; faktori rizika; forenzička psihijatrija; demografija

## Introduction

The use of psychoactive substances is one of the most significant public health problems due to its high prevalence, as well as major medical and social consequences. It was estimated that 1 out of 20 people between the ages of 15 and 64 years, had used an illicit drug during one year. The magnitude of this problem becomes more apparent when considering that more than 10% of those people are suffering from mental disorders due to psychoactive substance use [1].

Human aggression is defined as a behaviour that is intended to hurt another person, resulting or likely

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to result in injury, death or psychological harm [2]. It is known that aggression is a result of a complex interaction of neurobiological, psychological and environmental factors [3]. Unfortunatelly, we must agree that aggressiveness of people with mental disorders, including people with substance use disorders, attracts little scientific attention despite its obvious importance [4]. Contrary to previous professional opinions, that the risk of aggressive behavior in the population of psychiatric patients was not higher than in the general population, today it is known that people with certain psychiatric diagnoses are at increased risk for committing violent acts [5]. In other words, a mental disorder is, independently from other factors, associated with increased aggression rate and the risk is estimated to be about 4% [6]. The risk of aggressive behavior is even greater when there is a comorbidity of mental disorder and substance abuse [7, 8]. This connection between aggression and psychoactive substance use in people with mental disorders can be explained by acute pharmacological effects of substances that can exacerbate psychiatric symptoms or lead to poor compliance [9].

The association between substance use and aggression derives from the fact that among violent crime offenders there is a large number of people with alcohol or drugs abuse. Substance use-related aggression results in considerable personal suffering and socioeconomic costs [10–12]. This is consistent with the fact that a large number of aggressive offenders are assessed as having been under the influence at the time of the offense [13]. According to the data of the World Health Organization, alcohol consumption is associated with violence more closely than the use of any other psychoactive substance [14]. Literature data show that alcohol intoxication plays an important role in about half of all violent crimes worldwide [13]. It is estimated that over 3 million violent crimes occur each year in the United States where the offenders were under the influence of alcohol [15]. Besides that, specific studies have shown a significant association between alcohol use and homicide all over the world [16]. Alcohol-related aggression is not associated only with acute intoxication, it is also associated with chronic alcohol consumption. When compared to healthy controls, the risk of aggression is five times higher in people with mental disorders due to alcohol use [17]. However, alcohol-related violence does not occur in the majority of all alcohol-dependent patients [18]. In contrast to the above, a recent meta-analysis of 32 studies, showed that the effect of alcohol on aggression was medium [19].

When considering psychiatric treatment of addiction in the context of reducing the risk of aggression in alcoholics, it is important to note that in some studies violent behavior decreased significantly in patients who were successfully treated and remained abstinent at least for one year [20]. On the other hand, noncompliance with psychiatric treatment is described as an important factor for the development of aggressive behavior in all people with mental disorders [21].

The aim of this study was to determine the incidence of people with substance use disorders in the population of violent crime offenders, as well as to consider possible measures to reduce crime rates in this population.

## Material and Methods

For study purposes, forensic psychiatric reports of 94 violent offenders from 2001 to 2018 were examined. The expert reports were obtained from the Psychiatry Clinic in Novi Sad, Serbia, including court case files, medical records, and psychiatric and psychological records of all offenders. All forensic records are the property of the Clinical Centre of Vojvodina and their use was authorized by the Ethics Committee in 2017. We emphasize that the Local Court rules require psychiatric evaluation of all the individuals charged with violent crimes during the trial to assess their mental capacity and criminal liability.

Sample baseline characteristics were summarized using means or frequencies, as appropriate. Data processing included methods of descriptive statistics, numerical features were presented through measures of central tendency (arithmetic mean) and measure of variability (range of values), and attributive features using frequencies and percentages.

## Results

The sample included 94 violent offenders, who were mainly male (85%) and young people (median age $37 \pm 4.1$ years). Forensic examination and court case files revealed that more than a quarter (27%) of them had first degree relatives who suffered from psychiatric disorders. The most prevalent mental disorder among relatives of violent offenders was alcoholism (68%).

As for the mental state of crime offenders, the data showed that almost one third of all subjects (32%) had a psychiatric diagnosis before they committed the current crime. Substance use disorder was the most frequent diagnosis, accounting for 27.9%. Forensic psychiatric evaluation of the sample showed that one quarter (25%) of all offenders had a major mental disorder at the time when the crime was committed. A "major mental disorder" was defined earlier for research purposes and included mood disorders, psychotic disorders and substance use disorders. More than half (60%) of individuals with major mental disorders received a diagnosis from the group of substance use disorders (F1 according to the International Classification of Diseases – 10th revision). This means that 15% of all offenders from our sample suffered from psychoactive substance use disorder at the time of the offense, while the most common diagnosis was alcohol use disorder (9%) followed by opioid use disorder (5%).

In addition to the above mentioned, it is important to emphasize that exactly half of all the violent crimes were committed under the influence of psychoactive substances. This means that 50% of violent offenders were intoxicated at the time of the crime and the vast majority of them were under the influence of alcohol (96%). Data from the court case files, mainly laboratory findings, enabeled the reconstruction of the level of acute alcohol intoxication among violent offenders at the time of the offense. The most frequent level of alcohol intoxication was mild (64%), while moderate and severe degree of intoxication was present in 36%. The severity of intoxication is important for further assessment of the accountability of offenders at the time of committing the offense, because alcohol, among other factors, can negatively impact cognitive and volitional mental functions.

## Discussion

The results of our research are in line with literature data with respect to the demographic characteristics of the sample. Our study included 85% of males, confirming the known predominance of males in the population of violent offenders [22]. The average age of the offenders was 37 years, which is consistent with literature data describing the offenders as young adults and middle-aged persons [23].

In view of results showing a high prevalence of mental disorders, mostly alcoholism, in close relatives of violent offenders from our sample, we emphasize the known fact that a family history of mental disorders is considered a major independent risk factor for violence [24].

Almost one third of the sample (32%) was diagnosed and treated by psychiatrists before the offense. This is important in the context of prevention, because adequate treatment lowers and poor compliance increases the risk of violent behavior in the population of people with mental disorders.

In general, high incidence of people with mental disorders (25%) and substance use disorders (15%) in our study sample is consistent with literature data and points out the responsibility of psychiatry as a clinical discipline to at least try to partially reduce the risk of violence [25, 26]. This could be done by timely and adequate treatment of people with mental disorders.

Exactly half of the offenders from the sample were acutely intoxicated at the time of the offense and alcohol was the most frequently used substance (96%). This result is consistent with the results of similar studies reporting that 34–59% of men commit crimes under the influence of psychoactive substances, and that most of the violent offenders were under the influence of alcohol [27].

## Conclusion

A large number of people with mental disorders in the group of violent offenders demonstrate that improving prevention, recognition and treatment of mental disorders may contribute to reducing the crime rates. Knowledge about the etiology and treatment of psychoactive substance induced aggression is insufficient, despite the high prevalence of such behavior. Further research is needed to clarify why some people who use psychoactive substances exhibit violence, while others do not. In order to develop specific programs for the prevention of violence in the population using psychoactive substances, controlled studies comparing the specific forms of intervention with the standard treatment of addictive disorders are necessary.

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# REVIEW ARTICLES
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## PHYSICIAN HEALTH CHALLENGES AND RETURN TO WORK - INSIGHTS FROM A RESEARCH FOR PHYSICIANS BY PHYSICIANS

## *POREMEĆAJI ZDRAVLJA I POVRATAK NA POSAO OBOLELIH LEKARA – UVID IZ ISTRAŽIVANJA LEKARA ZA LEKARE*

**Karen BELKIĆ[1-3] and Olesja NEDIĆ[4]**

**Summary**

**Introduction.** High rates of burnout, suicide and hypertension complications among physicians suggest an occupational etiology. Generic assessments of the work environment are insufficient. We examine how physicians' "participatory action research" with appropriate theoretical underpinnings provides insights. **Work Stressor Models with Instruments Created "for Physicians by Physicians".** Specific instruments based on the Job Demands-Resources model were developed by radiologists and psychosocial oncologists, aimed at ameliorating burnout. Increasing perceived value of work, frank discussions and communication between senior and junior colleagues were key The physician-specific Occupational Stressor Index based on an additive burden model informed by cognitive ergonomics was also developed by physicians. Total occupational stressor burden was high among physicians with cardiovascular disease. Long workhours, speed-up and job loss threat were associated with case status. Anesthesiologists and surgeons had the highest stressor burden, with nightshift work targeted for lowering risk. Associations between job stressors and cardiovascular risk were strongest among female physicians. **Return to Healthier Work.** Intervention studies on return-to-work regarding burnout, cardiovascular disease or malignancy are sparse among physicians. Reduced workhours and paid "protected" time to discuss shared experience may be helpful. Clinical experience suggests that the physician-specific Occupational Stressor Index facilitates return to healthier work. **Conclusions.** Occupation-specific instruments developed "for physicians by physicians" based on work stressor models can improve physicians' work conditions and health. Finding the best strategies for return-to-work among physicians with stress-related disorders remains a challenge. Modified work conditions can yield positive results. Return to *healthier* work enhances physician empowerment and often improves general work climate.

**Key words:** Return to Work; Physicians; Occupational Stress; Burnout, Professional; Workload; Health Behavior; Stress, Psychological; Cardiovascular Diseases; Neoplasms

**Sažetak**

**Uvod.** Visoke stope obolevanja do sindroma izgaranja, komplikacija arterijske hipertenzije i samoubistava među lekarima ukazuju na značaj profesionalne etiologije. Uopštena procena uslova rada nije dovoljna. Zato ukazujemo na to kako sami lekari, teorijski opravdanim postupcima, doprinose rasvetljavanju ove problematike. **Modeli procene profesionalnih stresora metodama koje su kreirali lekari za lekare.** Instrumenti bazirani na modelu *Zahtevi i resursi posla* (engl. *Job Demands-Resources model)* razvili su radiolozi i onkolozi, kako bi smanjili sindrom izgaranja. Ključni faktori su uvažavanje uloženog rada i iskrena, dobronamerna komunikacija starijih kolega sa mlađim. Indeks profesionalnih stresora specifičan za lekare, zasnovan na modelu ukupnog opterećenja, procenjenog kognitivnom ergonomijom, takođe su razvili lekari. Ukupan indeks opterećenja stresorima radnog mesta visok je kod lekara sa kardiovaskularnim oboljenjima. Dugo radno vreme, ubrzavanje rada i pretnja gubitkom posla su prisutniji kod obolelih. Anesteziolozi i hirurzi imaju najveće opterećenje, uz potrebu za intervencijom na smanjenju noćnog rada radi snižavanja rizika do obolevanja. Povezanost profesionalnih stresora i kardiovaskularnog rizika je značajnija kod žena lekara. **Povratak na zdravije radno mesto.** Vrlo retke su interventne studije povratka na radno mesto obolelih lekara od sindroma izgaranja, kardiovaskularnih ili malignih oboljenja. Skraćivanje radnog vremena i diskusija o problemima na radu mogu biti korisni. Klinička iskustva ukazuju da *Indeks profesionalnih stresora* za lekare pomaže povratku na zdravije radno mesto. **Zaključak.** Specifični instrumenti koje su razvili lekari za lekare, zasnovani na modelima profesionalnih stresora, mogu poboljšati uslove rada i zdravlje lekara. Nalaženje najbolje strategije povratka na posao za obolele lekare ostaje i dalje izazov. Poboljšanje uslova rada daje pozitivne rezultate. Povratak na zdravije radno mesto osnažuje lekare i često popravlja radnu klimu.

**Ključne reči:** povratak na posao; lekari; okupacioni stresori; profesionalno izgaranje; radno opterećenje; zdravlje; psihički stres; kardiovaskularna oboljenja; maligne bolesti

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***Abbreviations***

| | |
|---|---|
| BMI | – body mass index |
| OSI | – occupational stress index |
| CVD | – cardiovascular disease |
| RTW | – return to work |
| IHD | – ischemic heart disease |
| TAV | – threat avoidance vigilance |
| JD-R | – job demands-resources |

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## Introduction

Completing long, difficult training prior to entering paid working life entails a rigorous selection process for physicians. This reflects a super "healthy worker effect" [1]. It is anticipated that disease incidence and prevalence will be lower among physicians compared to other working populations. In other words, to effectively handle difficulties, physicians are selected to be physically and mentally healthy. Moreover, physicians are cognizant of behavioral and other factors that affect health. The consistent findings of high rates of burnout, depression and suicide among physicians [2, 3] suggest that harmful working conditions are causative. In physician-specific publications, various work stressors have been implicated [4-6]. Job stressors among physicians are also implicated in the progression from hypertension to ischemic heart disease (IHD). In our 7-year longitudinal study [7], once physicians developed hypertension, their risk of cerebrovascular or cardiovascular complications was significantly greater than among employees not directly providing patient care. However, generic work stressor models have often failed to explain why physicians are at-risk for mental health disorders and progression of hypertension to IHD. The Job Strain Model, e.g., categorizes physicians' work as "active" (high demands and high decision-making latitude) and thus expected to engender salutogenic ways of coping and mental health, as well as lower IHD risk [8].

Clarification requires appropriate methodologies for assessing work stressors, especially to identify potentially modifiable factors in the physician's work environment. Occupation-specific instruments are needed. When persons from the occupation are involved, this can become "participatory action research" [9], with deeper insights gleaned about the actual work situation and motivation strongest to improve it. A proactive role is thereby suggested for physicians regarding our own work environment [10]. As we now review, physician-specific instruments based on two work stressor models have been developed "for physicians by physicians".

### Work Stressor Models with Instruments Created "for Physicians by Physicians"

*The Job Demands-Resources Model tailored to Physician-Specialist Groups*

Burnout is defined as a syndrome of chronic exhaustion, with a negative attitude and diminished efficacy at work [11]. Burnout reportedly occurs when job demands surpass job resources [12]. According to the Job Demands-Resources (JD-R) model, role conflict and ambiguity, job insecurity and work overload are demands; resources include social support, feedback, coaching and autonomy. The JD-R model has been applied to many work endeavors, including the health care sector, and can be tailored to specific occupations [13].

Radiologists developed a specific JD-R based instrument for colleagues in training, with 19 items related to demands and resources in the radiology residency environment [14]. Emphasis was placed on personal accomplishment which was poor among these specialists-in-training. Based on their findings, the following recommendations were made as to how to ameliorate burnout among radiology residents:

(a) Provide feedback mechanisms to foster resident personal growth and development;
(b) Facilitate educational and social forums for residents themselves, aimed at promoting rapport with frank discussions of problems;
(c) Promote interdisciplinary learning opportunities, encouraging senior colleagues to share their clinical and research experience, and emphasizing the importance of radiology residents' work.

A study applying the JD-R among psychosocial oncologists [15] included an additional dimension of emotional demands [16] that were considered especially relevant to this problem area. Perceived value and work engagement were found to be protective against burnout, while high job demands plus overcommitment were associated with increased burnout risk among this profile of health-care providers.

*The Physician-Specific Occupational Stressor Index, OSI*

The Occupational Stressor Index (OSI) [17-19], an additive burden model informed by cognitive ergonomics and brain research, assesses mobilization and allocation of mental resources, and how these are controlled by the individual. The total job stressor load is gauged by the OSI, which also analyzes the nature of that burden, considering task and organizational level factors, physical/chemical exposures, and work scheduling, *inter alia*. The OSI considers the work environment in its entirety, akin to taking a full oc-

cupational history. It also provides quantitative/normative data. Less apparent stressors are identified, including threat avoidant vigilance (TAV). Facing potentially harmful consequences contributes greatly to the stressor burden [20] with the nervous system selectively attending to threatening stimuli [21]. Having to follow such information, responding quickly, with errors/delays having serious, even fatal consequences: this is TAV [22, 23]. The OSI includes TAV, but most other job stressor models do not and thus underestimate the stressor burden of physicians as well as nurses, airline pilots, professional drivers, police and firefighters, *inter alia* [18]. The OSI is a two-dimensional matrix: the rows are levels of information transmission, as per Welford [24]. The 1st two levels (sensory input and decision-making) are frequently "invisible" and often missed, whereas reports of "working fast" mainly reflect the task performance level. A 4th general level includes elements related to the overall work environment and is critical for identifying modifiable stressors. The columns are 7 stressor aspects (underload, high demand, strictness, external time pressure, aversive physical exposures, TAV and conflict/uncertainty). Each element is weighted equally, scored from 0 (not present) to 2 (strongly present). The total OSI score is the summation of these factors . [10, 19]. Several occupation-specific OSI instruments have been developed, all compatible within the same theoretical and numerical framework [1]. Questions about the fixed characteristics of a given line of work are omitted, to focus on variable features, especially modifiable stressors.

The physician-specific OSI was developed by us physicians, motivated to improve our work environment and health [18, 25]. The first larger-scale study using the physician-specific OSI focused on cardiovascular disease (CVD) risk [26] and was carried out in Novi Sad, Serbia, an area known for a heavy burden of CVD and its risk factors [27]. Notably, lifestyle-related risk factors were highly prevalent among the Novi Sad physicians. Over 25% had a body mass index (BMI) > 28, over 30% were current smokers, almost 20% consumed alcohol daily, and fewer than 30% regularly engaged in recreational physical activity [26]. This case-control study included 101 physicians with one or more acquired, i.e. stress-related, cardiovascular disorders: myocardial infarction, angina pectoris, arterial hypertension, and certain arrhythmias [28]. The 107 referent physicians had not been diagnosed with any of these disorders. The total OSI scores were significantly greater among cases, with high demands and TAV most notably higher. Three modifiable stressors: long work hours, speed-up and threat of job loss were significantly associated with case status [26]. An OSI-based study among Novi Sad physicians examined the occupational concomitants of a favorable lifestyle-related profile (normal BMI, non-smoker, regular recreational physical activity and minimal alcohol consumption) [29], with stratified analysis for two groups. One included anesthesiologists and surgeons, reportedly at particularly elevated risk for burnout and suicide, with long work hours and frequent night call implicated [30, 31]. Compared to other physicians profiles, surgeons and anesthesiologists had significantly higher total OSI scores and fewer modifiable stressors. Nightshift work was significantly associated with life-style related profile: anesthesiologists and surgeons with lower nightshift work scores were more likely to have normal BMI, be non-smokers, not daily alcohol consumers with regular recreational physical activity. Thus, nightshift work was suggested for interventions aimed at lowering risk among anesthesiologists and surgeons [29]. For other physicians, the favorable profile was associated with a lower total OSI score. Thus, broader intervention strategies were possible, targeting the work environment together with risk behaviors.

The relation between job stressors evaluated by the OSI and stress-related CVD was most evident among female physicians [29, 32-34]. An OSI high demand score above the mean was associated with over 3-times greater hypertension risk, accounting for BMI as a covariate [34]. The female physicians with a favorable risk profile, defined in that study [34] as not a current smoker and no diagnosed hypertension, had significantly lower total OSI scores. Fewer interruptions from people, less often listening to emotionally-disturbing occurrences, and lower TAV scores were significantly more often found among those who were non-smokers and normotensive. The association between work conditions and lifestyle-related profile was most apparent among female physicians. Significant multivariate relations with these lifestyle factors were reported for total OSI scores, several OSI aspects, especially TAV and some OSI elements (insufficient help with clinical difficulties, supervisory duties, technical problems hampering patient care and long work hours) [29, 32].

Given the increased risk of stress-related disorders among physicians, issues of sick leave and return to work (RTW) become salient. We now examine how improvements in work conditions could impact on RTW among our colleagues, and more broadly among working populations.

### Return To Healthier Work for Those Afflicted With Stress-Related and Other Disorders: Sparse Data among Physicians

Return to work, RTW, is vital to quality of life after illness, often critical for maintaining

[1] Use of the OSI instruments is provided free of charge with permission from the first author, the originator of the OSI [17].

economic and emotional stability. The question is: to what type of work is the patient returning—to a healthy job, or to one that adds yet another burden?

*Burnout and Related Mental Health Disorders*

Mental health disorders are among the leading causes of long-term disability [35, 36] with RTW very challenging for psychiatric disorders. Employees who develop mental health problems are likely to conceal these from employers. Physicians appear to be especially reluctant to seek care for mental health conditions due to fear of revocation of their medical license [37]. Notwithstanding many programs for RTW among those with mental health disorders, the evidence concerning their effectiveness is limited [38]. For physicians in training, concerns about confidentiality, overburdening colleagues and career trajectory impact on RTW. It is noted that more research is needed regarding RTW for physicians with mental health disorders [39]. Despite their high risk, there are few intervention studies regarding RTW for physicians with burnout. A Norwegian study indicates that professional counseling plus reduced work hours helped ameliorate burnout [40]. It was suggested that recognizing their heavy cognitive load would help design strategies to reduce physician burnout [3]. A randomized clinical trial over 19 weeks was implemented among U.S. physicians offering paid "protected" time for discussion of shared experience, and small-group learning, reflection and mindfulness. Rates of depersonalization, emotional exhaustion and overall burnout decreased in the trial intervention arm and were sustained 1 year after the study [41]. A subsequent meta-analysis of interventions aimed at physician burnout indicates:

"both individual-focused and structural or organisational strategies can result in clinically meaningful reductions in burnout among physicians. Further research is needed to establish which interventions are most effective…how individual and organisational solutions might be combined to deliver even greater improvements in physician well-being than those achieved with individual solutions" (p. 2272) [42].

*The Acquired Cardiovascular Disorders*

Clinical guidelines concerning the workplace and CVD have mainly focused on physical exertion [43]. Thus, when evaluating work capacity limitation, these are based on non-invasive evaluation via exercise testing and echocardiography [44]. Although cardiac rehabilitation improves physical capacity and survival in patients with IHD, this does not consistently promote RTW [44]. Many work exposures are associated with elevated risk of IHD or hypertension, unrelated to physical exertion. This is true for physicians with IHD or hypertension. We suggested that myocardial infarction among physicians should be considered potentially work-related [45]. As articulated over 3 decades ago, the challenge remains to offer the patient with CVD a style of work and life which protects health and the right to be productive [46]. These issues are salient vis-à-vis psychosocial job stressors, since such exposures are associated with increased risk of recurrent cardiac events after myocardial infarction [47-49]. In light of the importance of psychosocial and other workplace factors in the etiology and progression of IHD and hypertension, we suggested an integrated, graded approach to occupational cardiologic work-up, based upon degree of disease severity [50]. These guidelines are especially relevant for physicians, among whom, as noted [7], there is heightened risk of cardiovascular and cerebrovascular complications once hypertension develops. Blood pressure and electrocardiographic monitoring during work are crucial [18, 44, 50].

*Malignancies*

A Norwegian study examining RTW among patients with breast, prostate or testicular cancer confirmed that holding a job is vital to healthy survivorship [51]. A Swedish study of women with lymphedema after breast cancer surgery found that many participants increased their percentage of work time after a multi-faceted rehabilitation program [52]. Based on evidence that tumor growth is accelerated by suppression of melatonin secretion, women with previous or current breast cancer are advised against working night shifts [53]. Recently, physicians were found to have the highest breast cancer risk of all occupational groups [54], with shift work and ionizing radiation exposure implicated. There are very limited, mainly anecdotal data, concerning RTW among physicians after treatment of breast cancer or other malignancies. Dr. Carolyn Kaelin, oncologic surgeon and Director of the Comprehensive Breast Health Center at Brigham & Women's Hospital, described her experiences "living through breast cancer" [55, 56]. Of her many insights, the most salient to RTW for physicians are the need to take full account of the extreme fatigue which accompanies chemotherapy, issues of disclosure and the importance of social support from colleagues, staff as well as patients.

*Clinical Experience Applying the OSI for Return to Work among Physicians Afflicted with Stress-Related and Other Health Disorders*

In various settings, the OSI has aided RTW by helping improve work conditions for physicians on sick leave due to breast cancer, other malignancies, burnout, depression, hypertension, IHD and/or rheumatologic disorders [18, 19, 26, 57]. The OSI also aided physicians who sought to avoid taking sick leave, by guiding

improvements in work conditions. The process begins by assessing the baseline job situation. Elements of work that bolster self-confidence and sense of achievement are preserved. Job stressors are identified, focusing on those likely to impact on clinical status and overall well-being. Feasible ways are sought to implement needed changes, aimed at lowering the total OSI score. Numerical guidelines and norms are given in reference 19, whereby a total OSI above 88 indicates the need for urgent intervention and above 100 as the acute danger level.

This comprehensive approach via the OSI, viewed together with clinical status, avoids over-sheltering physicians, who are encouraged to perform challenging, but not over-taxing tasks. Thereby, overload and underload are averted. Underload reinforces the debilitating view that mental health disorder, cancer or other major illness will deprive a physician from successfully engaging in work activity. We consistently observed that this RTW strategy promotes a more positive outlook, resulting in feelings of empowerment and a sense of competence and dignity. When workplace modifications are successfully implemented, physicians flourish on an individual level, and often contribute to improving the work climate, e.g. promoting social support among colleagues [19]. It is critical to place an upper limit on work hours for physicians, taking into account clinical work plus pedagogical, research and administrative duties, and work-related activity performed at home. Especially for the initial RTW phase, nightshift work should usually be avoided. If the clinical situation is severe, part-time work is recommended. Regular, uninterrupted rest breaks with needed time for meals are essential.

The TAV and conflict/uncertainty scores are often very high among physicians on or potential candidates for sick leave. Some of these TAV stressors are fixed or relatively fixed: frequently encountering visually-disturbing scenes and emotionally-disturbing occurrences, potentially fatal consequences of a wrong decision, risk of infection due to close contact with blood and other body fluids. Caring for severely ill patients, threat of violence from patients' families, accidents, being faced with formal complaints or work-related litigation, and patient suicide further contribute to TAV burden. Among the relatively fixed stressors from the conflict/uncertainty aspect for physicians are the contradictory/conflicting nature of information and decision-making in medicine. Yet, much of the conflict/uncertainty burden could be ameliorated: frequent interruptions and other external factors that hamper patient care, emotionally-charged work atmosphere, blockage of career advancement and other violations of norms of behavior, performing multiple clinical and other tasks that objectively require separate time allocation. Further distress occurs if obliged to perform tasks that appear to be unnecessary [58].

*Published Case Studies* Several clinical cases among physicians and other health professionals relevant to RTW were published in Refs. [18, 19, 26, 59]. The most detailed is that of a psychiatrist with burnout and suicidal ideation, with baseline total OSI score of 106 [19]. She had:

"become burned-out, in large measure, due to her work. Yet, return to work is pivotal for her recovery. We present a realistic plan for modifying the conditions under which she works, tailored to her clinical status, capacities and affinities. This is an iterative process; we start with the steps that can be immediately implemented, in order for [her] to get back to work with reasonable safety" [19].

These initial steps included elimination of nightshift work, limitation of total work hours (40 hours/week maximum) and scheduled genuine rest breaks. She was temporarily freed from emergency room duty, since it was a traumatic event therein, patient suicide, which triggered her clinical deterioration. With these and other measures, the total OSI was reduced to 88.9. These measures provided the precondition for addressing the more complex, psychosocial job stressors, some of which will be discussed here (see reference 19, for the full presentation, including stressors faced by academic physicians). Counter-measures for underload included recognition of good work, especially the many patients helped by her expertise and efforts. Efforts were made to repair breakdowns in communication between senior and junior physicians, which often resulted in clinical misjudgments, including the patient suicide which triggered our colleague's crisis. The emotional toll of caring for patients with severe psychiatric illness was candidly confronted. As her self-confidence was bolstered, she initiated a positive process encouraging colleagues to help each other with clinical difficulties, striving to nurture a "culture of validation and respect". With these and other measures, our colleague's total OSI score was reduced to 84.9, still high, but below the level at which urgent intervention is needed. Notably, the conditions under which she was working prior to her illness were not uncommon. The total OSI scores of other colleagues in her unit ranged from 95 to 108. Her case served as an occupational sentinel health event [60] whereby patterns of ill-health events caused by occupational factors go beyond a purely individual case approach to identify others at the workplace, who have also been affected. This is pivotal for intervening at the workplace to prevent outbreaks of occupational illness.

## Conclusions

Participatory action research among physicians is a promising strategy for improving the work conditions and health of our profession. Occupation-spe-

cific instruments developed "for physicians by physicians" based on work stressor models play a key role, as seen in a substantial body of empirical literature. Finding the best strategies to aid return to work for physicians with stress-related or other disorders remains a challenge, although clinical experience suggests that modified work conditions can yield positive results. Namely, return to *healthier* work enhances the physician's empowerment and often contributes to improving the general work climate.

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# CASE REPORTS
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Case report
*Prikaz slučaja*
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# CEREBRAL AUTOSOMAL DOMINANT ARTERIOPATHY WITH SUBCORTICAL INFARCTS AND LEUKOENCEPHALOPATHY: DIFFERENT CLINICAL FEATURES IN A FAMILY – A CASE REPORT

## *CEREBRALNA AUTOZOMNO DOMINANTNA ARTERIOPATIJA SA SUPKORTIKALNIM INFARKTIMA I LEUKOENCEFALOPATIJOM: RAZLIČITA KLINIČKA EKSPRESIJA UNUTAR JEDNE PORODICE − PRIKAZ SLUČAJA*

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**Summary**

**Introduction.** Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy is the most common monogenic disease of small blood vessels. It commonly presents with repeated episodes of brain ischemia leading to progressive subcortical vascular dementia, migraine attacks and mood disorders. **Case Report.** A 46-year-old male patient was admitted with clinical presentation of stroke. The neurological examination revealed mild divergent strabismus and a left homonymous hemianopia. Brain magnetic resonance imaging showed subacute infarction in the region of the posterior cerebral artery to the right, as well as similar lesions in the splenium of the corpus callosum, numerous mostly confluent and some discrete T2-weighted/fluid attenuated inversion recovery hyperintense lesions of the centrum semiovale, corona radiata, frontoparietal subcortex, capsula externa, periventricularly at the level of occipital and temporal horns of lateral chambers bilaterally, and small punctiform lesions in the region of the corpus callosum. The magnetic resonance angiography findings were normal. The patient's brother underwent neurological examination at the age of 42 due to severe headaches, double vision, confusion, and numbness in the right arm. The magnetic resonance imaging of the endocranium showed multifocal confluent ischemic lesions predominantly in the frontal and temporal lobes, as well as focal microangiopathic changes in the gangliocapsular regions bilaterally in the brainstem and cerebellum. In agreement with the patient and his brother, genetic analyses were performed in both of them, and a mutation in exon 3 of the neurogenic locus notch homolog protein 3 gene was confirmed (c.505C > t, p.R169C). **Conclusion.** Although there is no causal therapy, it is very important to diagnose cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy in order to implement measures to prevent cerebrovascular diseases in both patients and their family members.

**Key words:** CADASIL; Cerebral Small Vessel Diseases; Cerebrovascular Disorders; Signs and Symptoms; Magnetic Resonance Imaging; Genetic Testing; Stroke

**Sažetak**

**Uvod.** Cerebralna autozomno dominantna arteriopatija sa supkortikalnim infarktima i leukoencefalopatijom najčešća je monogenska bolest malih krvnih sudova koja se često prezentuje ponovljenim ishemijskim moždanim udarima koji vode u supkortikalnu vaskularnu demenciju, atacima migrene i promenama raspoloženja. **Prikaz slučaja.** Muškarac star 46 godina primljen je pod kliničkom slikom moždanog udara. U neurološkom nalazu blag divergentni strabizam, homonimna hemianopsija levo. Magnetnorezonantni imidžing mozga pokazao je subakutni infarkt u regiji zadnje moždane arterije desno, kao i promene sličnih karakteristika u splenijumu korpusa kalozuma, brojne slivene, delimično i pojedinačne *T2-weighted/fluid attenuated inversion recovery* hiperintenzne lezije centruma semiovale, korone radijata, frontoparijetalnog supkorteksa, kapsule eksterna, periventrikularno u nivou okcipitalnih i temporalnih rogova lateralnih komora obostrano, manje punktiformne leyije u regiji tela korpusa kalozum. Magnetnorezonantna angiografija uredna. Pacijent ima brata koji je u 42. godini života neurološki ispitivan zbog intenzivne glavobolje, duplih slika, zbunjenosti, trnjenja desne ruke. Magnetnorezonantnim imidžingom endokranijuma registrovane su multifokalne konfluentne ishemijske lezije predominantno u frontalnim i temporalnim režnjevima kao i fokalne mikroangiopatske promene u ganglio-kapsularnim regijama obostrano u stablu i u malom mozgu. U dogovoru sa pacijentom i njegovim bratom, obojici je načinjena genetska analiza i potvrđeno je postojanje mutacije u egzonu 3 *neurogenic locus notch homolog protein 3* gena (c.505C > t, p.R169C). **Zaključak.** Iako kauzalna terapija ne postoji, veoma je važno postaviti dijgnozu cerebralne autozomno dominantne arteriopatije sa supkortikalnim infarktima i leukoencefalopatijom u cilju sprovođenja mera prevencije cerebrovaskularnih oboljenja kako kod pacijenta, tako i kod članova njegove porodice.

**Ključne reči:** CADASIL; cerebralna autozomno dominantna arteriopatija; cerebrovaskularne bolesti; znaci i simptomi; magnetna rezonanca; genetsko testiranje; moždani udar

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***Abbreviations***

CADASIL – Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
PCA – posterior cerebral artery
FLAIR – fluid attenuated inversion recovery
NOTCH3 – neurogenic locus notch homolog protein 3
CVD – cerebrovascular disease
MRI – magnetic resonance imaging

## Introduction

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common hereditary cause of cerebral angiopathy and vascular dementia in adults. Clinically, CADASIL is associated with repeated episodes of brain ischemia leading to progressive subcortical vascular dementia, migraine attacks and mood disorders. Brain magnetic resonance imaging (MRI) shows diffuse, often confluent and symmetric white matter lesions, multiple lacunar infarctions, and cerebral micro hemorrhages. The CADASIL diagnosis is confirmed by identification of the neurogenic locus notch homolog protein 3 (NOTCH3) gene mutation located on the 19p13 chromosome [1–3]. The criteria for diagnosis include: 1. Onset of illness before the age of 50 years; 2. At least two of the following clinical symptoms attack with persistent neurological signs, migraines, mood disorders, subcortical dementia; 3. Absence of vascular risk factors; 4. Autosomal dominant inheritance, and 5. White mass lesions without cortical infarction [1–3]. There is no causal treatment. We present a case with different clinical expressions of the same gene mutation within one family.

## Case Report

A 46-year-old male patient was admitted due to sudden visual disturbances, confusion, disorientation in time and space, and occipital headaches. The complaints started a few days before the admission. The patient was a smoker, moderate alcohol consumer, and had no other previously registered risk factors for cerebrovascular disease (CVD). On admission, he presented with bradypsychia and disorientation in time and space. His eyes were slightly divergent, and he reported diplopia in all directions of gaze. Right-sided homonymous hemianopia (National Institutes of Health Stroke Scale - NIHSS 4, modified Rankin Scale - mRS III) was evident. Brain computed tomography (CT) showed a subacute ischemic lesion in the right posterior cerebral artery (PCA) circulation, chronic ischemia in the pons, hypodensity in the supratentorial white matter, mostly subcortically. Ultrasonographic findings of the head and neck blood vessels were all normal. Contrast transcranial Doppler (TCD) was normal, and electrocardiography (ECG) did not show heart rhythm disturbances. Brain MRI examination confirmed an extensive subacute PCA lesion on the right, similar changes in the splenium of the corpus callosum, numerous mostly confluent and some discrete T2W/fluid attenuated inversion recovery (FLAIR) hyperintense lesions of the centrum semiovale, corona radiata, frontoparietal subcortex, capsula externa, periventricularly at the level of occipital and temporal horns of lateral chambers bilaterally, and smaller punctiform lesions in the corpus callosum region. Similar bilateral changes were found in the temporal subcortical region, slightly more pronounced on the left, all corresponding to CADASIL or vasculitis. The magnetic resonance angiography was normal **(Figure 1)**. Immunological tests were within standard ranges, and routine hemostasis parameters and thrombophilia markers were normal. The patient was methylenetetrahydrofolate reductase (MTHFR) A1298C heterozygous. The results of psychological testing indicated global mild to moderate cognitive impairment, resulting from dysfunction of prefrontal-subcortical circuits. Most pronounced were deficits in executive functions, and there were also disturbances in performing movements. Medical history data were obtained. The patient had a twin brother who had no complaints at the time, and a year elder brother who was neurologically examined at the age of 42 due to severe headache, double vision, confusion, and numbness in the right arm. Brain MRI recorded multifocal confluent ischemic lesions predominantly in the frontal and temporal lobes as well as focal microangiopathic bilateral changes in gangliocapsular regions in the stem and cerebellum **(Figure 2)**.

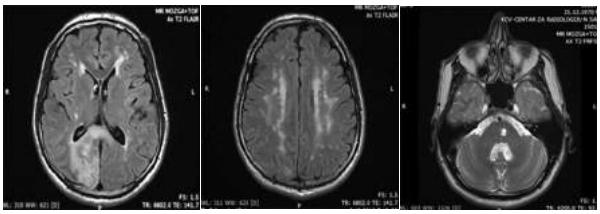

**Figure 1.** Subacute extensive lesion located occipitally on the right and in the corpus callosum splenium; confluent, partly discrete hyperintense lesions on T2 FLAIR in frontoparietal subcortex and periventricularly; chronic lacunar ischemia of the pons

***Slika 1.*** *Subakutna ekstenzivna lezija okcipitalno desno i u splenijumu korpusa kalozuma. Slivene, delom i pojedinačne hiperintenzne lezije T2/fluid attenuated inversion recovery sekvence frontoparijetalnog supkorteksa i preriventrikularno. Hronična lakunarna ishemija ponsa*

The recommended genetic testing for CADASIL was not performed. Thereafter, the patient was taking aspirin and had no new complaints. Neurological examination registered only divergent strabismus of the left eye. He was depressed because his daughter had migraine headaches and epileptic seizures. Neuroimaging was not done. Their mother had similar problems. Our patient and his brother agreed to undergo genetic testing and it confirmed a mutation in the exon 3 of the NOTCH3 gene (c.505C > t, p.R169C). Genetic counseling and testing of other family members was recommended, but it has not been accepted by the time of writing this report.

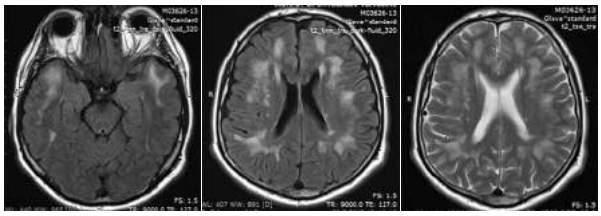

**Figure 2.** Multiple confluent changes of increased intensity on T2 and FLAIR, localized temporopolarly, periventricularly and in the deep subcortical white matter, with several lacunar lesions of the same MRI characteristics
***Slika 2.*** *Višestruke i slivene promene povišenog intenziteta signala u T2 i FLAIR, lokalizovane temporopolarno, periventrikularno i u dubokoj supkortikalnoj beloj masi, sa nekoliko lakunarnih lezija istih karakteristika na snimcima magnetne rezonancije*

## Discussion

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy is the most common and most recognized monogenic disease of small blood vessels. Several smaller and national registers estimate that its prevalence reaches 2 – 5 cases per 100,000 people [4]. Morphologically, CADASIL represents non-atherosclerotic, non-amyloid arteriopathy which most commonly affects the penetrating and leptomeningeal arteries. The pathohistological basis involves the accumulation of granular osmiophilic material around the smooth muscle cells and their subsequent degeneration. The changes are systemic and may also be detected in blood vessels of various organs and the skin. The disease develops due to mutations in the NOTCH 3 gene on the chromosome 19p13 [2]. This gene encodes a large transmembrane protein receptor on smooth muscle cells of the arteries and plays an important role in organogenesis and vasculogenesis. The penetrance of the disease is probably 100%. However, the expression varies in the age of onset, severity of the clinical picture and disease progression within one family [4]. The diagnosis of CADASIL is established based on the typical clinical picture, characteristic brain MRI findings, biopsy of the nerves, muscles and skin, and genetic analysis. The disease manifests clinically between 30 and 50 years of age. The most frequent clinical manifestation is recurrence of lacunar ischemic strokes in the absence of conventional risk factors for CVD, usually between the ages of 35 and 45, although there are huge discrepancies in the literature (from 20 to 70 years) [5]. Recent studies have shown that smoking in these patients doubles the risk of stroke and increases the risk of dementia by three times [6]. Development of territorial infarcts in the vascularization areas of large arteries is rare and probably accidental. In patients of East Asian origin, a higher prevalence of intracranial stenosis was reported, which suggested in several studies that in the absence of vascular risk factors the involvement of large blood vessels was associated with CADASIL [7, 8]. Approximately 20 – 40% of patients have migraine with aura and up to 60% of patients have migraine headache without aura. Migraine may precede stroke even by several years [5]. Impairment of the executive functions and working memory may be present even before the occurrence of transient ischemic attack (TIA) and stroke. Episodic memory may be preserved until the advanced stage of the disease. However, repeated lacunar strokes lead to pseudobulbar palsy and pronounced subcortical vascular dementia before the age of 65. Psychiatric disorders may vary from mild personality disorders to severe depression and mania. Migraine and development of psychiatric disorders are seen in the earlier stage of the disease and in some families represent the dominant clinical finding. Approximately 10% of patients with CADASIL have epileptic seizures [3]. Neuroradiological findings obtained by MRI are specific. Prior to the first clinical manifestations, white matter hyperintensity on the T2W and FLAIR sequences may be registered, which tend to be symmetrical, bilateral, distributed periventricularly, in the deep white matter, with predilection sites in the parietal and frontal lobes, temporopolar white matter and in the capsula externa [1]. Changes in the frontal temporal lobe have high sensitivity and specificity (90%) and are useful in establishing the diagnosis. Changes in the capsula externa have high sensitivity (90%) but lower specificity (50%) [1]. Abnormalities in the corpus callosum are rarely present in small vessel disease; they have been described in CADASIL and are common in multiple sclerosis, which is one of the reasons why CADASIL may be misdiagnosed as multiple sclerosis. Lacunar infarcts are most frequently localized in the centrum semiovale, thalamus, basal ganglia and pons. This is the most important MRI parameter that correlates with the degree of cognitive impairment. Cerebral microbleedings are registered on T2 sequences and T2* in 30 – 70% of patients, most commonly in the thalamus but also in the cortex, subcortically, in the white matter and brainstem, usually outside ischemic lesions [9]. CADASIL is primarily a subcortical disease, and recent studies using 7T MRI recorded changes in the cortex in the form of microinfarctions and initial diffuse cortical changes [4]. The diagnosis can also be established by skin biopsy; it is a standard procedure, however, the results of almost one half of the studies were false negative. The gold standard for establishing the diagnosis is the genetic confirmation of point mutations of the NOTCH gene, most frequently in exon 4 in the European population, followed by exons 8 and 3. In Serbia, three families (now four) with the diagnosis of CADASIL and point mutations in the exon 3 of this gene have been described so far [10, 11]. To date, over 150 mutations in exons 2 – 24 in over 500 families have been reported worldwide. Although these mutations have been registered in patients with a family history of CADASIL, recently de novo mutations have also been confirmed [12, 13]. That is why genetic testing should be carried out if there is clinical and morphological suspicion of CADASIL, regardless of the absence of family history of the disease, and it is recom-

mended in cases with positive family history even in the absence of clinical manifestations or with atypical clinical picture. Differential diagnosis includes multiple sclerosis, acute disseminated encephalomyelitis, hypertensive arteriosclerotic encephalopathy, cerebral angiitis, amyloid angiopathy, fabric disease, and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy.

## Conclusion

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy is the most common monogenetic disease of the small blood vessels that should be suspected in a younger population with registered signs of small blood vessels disease, especially if they are without detected conventional risk factors for cerebrovascular diseases and there is a positive family history for cerebrovascular disease. The penetrance of the disease is high, but the clinical expression varies widely in terms of age of onset and presentation, as well as the severity of the clinical symptoms. Although causal therapy does not exist, it is very important to diagnose cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy in order to implement prevention measures for both the patients and their family members.

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Case report
*Prikaz slučaja*
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# HYPOTHYROIDISM AS THE CAUSE OF REVERSIBLE DILATED CARDIOMYOPATHY – A CASE REPORT

## *HIPOTIROIDIZAM KAO UZROK REVERZIBILNE DILATATIVNE KARDIOMIOPATIJE – PRIKAZ SLUČAJA*

**Vanja DRLJEVIĆ TODIĆ[1], Božidar DEJANOVIĆ[2], Iva POPOV[1], Miloš VUKOVIĆ[3,4], Aleksandra VULIN[1,3] and Mirko TODIĆ[5]**

**Summary**

**Introduction.** The causes of dilated cardiomyopathy can be divided into ischemic and nonischemic. Although the importance of thyroid hormones for proper functioning of the cardiovascular system is well known, dilated cardiomyopathy is a rare presentation of hypothyroidism. **Case Report.** This is a case report of a 38-year-old man admitted to the Intensive Cardiac Care Unit with signs and symptoms of advanced heart failure. Dilated cardiomyopathy was established by echocardiography, while blood test showed highly elevated levels of thyroid stimulating hormone and decreased levels of free triiodothyronine and free thyroxine. The ejection fraction was significantly improved with levothyroxine replacement therapy. **Conclusion.** Hypothyroidism should always be considered as the cause of dilated cardiomyopathy. Thyroid hormone tests should be performed in all patients with dilated cardiomyopathy.

**Key words:** Hypothyroidism; Cardiomyopathy, Dilated; Thyroid Hormones; Diagnosis; Signs and Symptoms; Heart Failure; Echocardiography; Electrocardiography; Hormone Replacement Therapy; Treatment Outcome

**Sažetak**

**Uvod.** Etiološki činioci za nastanak dilatativne kardiomiopatije najčešće se dele na ishemijske i neishemijske. Iako je važnost tiroidnih hormona za adekvatno funkcionisanje kardiovaskularnog sistema dobro poznata, dilatativna kardiomiopatija je retka prezentacija hipotiroidizma. **Prikaz slučaja.** Prikazali smo slučaj 38-godišnjeg muškarca primljenog u jedinicu intenzivne kardiološke nege sa simptomima i znacima uznapredovale srčane slabosti. Ehokardiografski registrovana je dilatativna kardiomiopatija, dok su laboratorijskim testovima utvrđene visoke vrednosti tirostimulišućeg hormona i niske vrednosti slobodnog trijodtironina i slobodnog tiroksina. Ejekciona frakcija značajno je poboljšana nakon uvođenja terapije levotiroksinom. **Zaključak**. Hipotiroidizam uvek mora bit razmotren kao uzrok dilatativne kardiomiopatije. Ispitivanje tiroidnih hormona treba uraditi kod svih pacijenata sa dilatativnom kardiomiopatijom.

**Ključne reči:** hipotireoidizam; dilatativna kardiomiopatija; tireoidni hormoni; dijagnoza; znaci i simptomi; srčana slabost; ehokardiografija; elektrokardiografija; hormonska supstituciona terapija; ishod lečenja

## Introduction

Cardiomyopathy is defined as a myocardial disease which includes both morphological and functional heart disorders. Although there are different classification criteria, the most common is by morphological characteristics including four subtypes - hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy, and left ventricular (LV) non/compaction cardiomyopathy [1].

In DCM, the left ventricle is enlarged with poor contractility. Usually, the causes of DCM are divided in two groups, of ischemic and nonischemic origin.

Impaired thyroid hormone production and blood concentration, higher or lower than normal, affect the cardiovascular system causing changes in blood pressure, systemic vascular resistance, heart rhythm and rate and change in myocardial gene expression [2]. Even though structural myocardial changes can be explained by affected protein synthesis in myocytes, DCM is not a common presentation of hypothyroidism [3].

This case report is about a young male with hypothyroidism and DCM, which was improved after hormone replacement therapy with levothyroxine.

## Case Report

A young 38-year-old male was admitted to the Intensive Care Unit of the Institute of Cardiovascular Diseases of Vojvodina with symptoms and signs

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***Abbreviations***

| | |
|---|---|
| ECG | – electrocardiography |
| DCM | – dilated cardiomyopathy |
| LV | – left ventricle |
| TSH | – thyroid stimulating hormone |
| EF | – ejection fraction |
| FT4 | – free thyroxine |

of advanced congestive heart failure. He reported progressive dyspnea and stomachache that started 12 hours before admission. There was no history of chest pain. The previously diagnosed hypertension was treated by recommended medication.

On examination, the patient presented with bradypsychia, hypertension (165/115 mmHg), heart rate of 100 per minute, and bilateral chest crepitations, pretibial edema, without heart murmur and hepatomegaly.

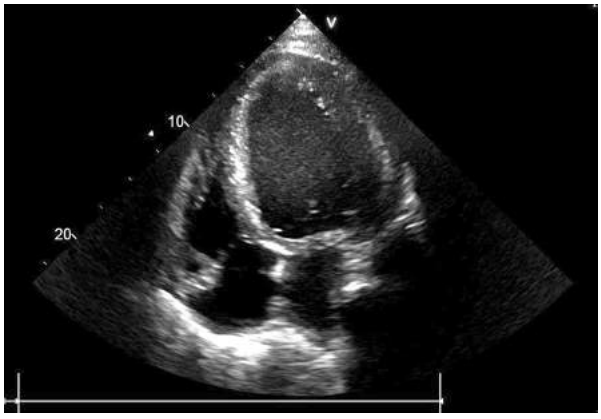

**Figure 1.** Echocardiography on admission
***Slika 1.*** *Ehokardiografski nalaz pri prijemu*

Blood test showed increased creatinine 180 μmol/l and N-terminal precursor of brain natriuretic peptide (NT-pro-BNP) 16241 [0.0-125.0] pg/ml. Liver function tests were also slightly increased: aspartate aminotransferase (AST) 67 [0.0-31.0] U/l, alanine aminotransferase (ALT) 85 [0.0-34.0] U/l, creatin kinase (CK) 395 [0.0-150.0] U/l, creatine kinase

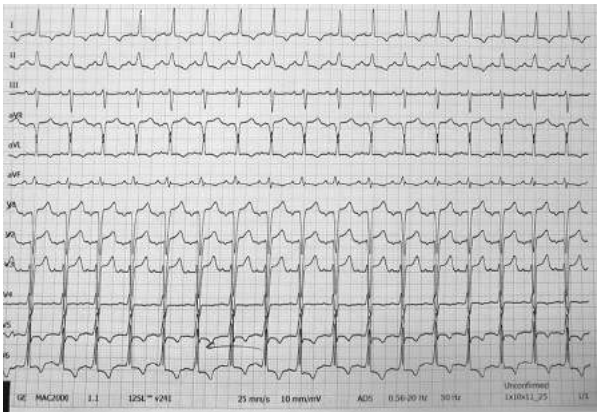

**Figure 2.** Electrocardiogram
***Slika 2.*** *Elektrokardiogram*

muscle-brain fraction (CK-MB) 52 [0.0-25.0] U/l. Serum protein levels, high-sensitive troponin (HsTni) levels and electrolytes were within reference values.

Echocardiography (ECG) showed a dilated left ventricle with severe systolic dysfunction, with ejection fraction (EF) of 14%, moderate mitral regurgitation and diastolic dysfunction with impaired relaxation **(Figure 1).** Ultrasound revealed no intra-abdominal pathology. The ECG showed negative T wave in the inferior-lateral leads (DI, DII, DIII, aVL, aVF, V4 - V6) **(Figure 2)**. The chest X-ray showed a dilated cardiac silhouette and bilateral pleural effusions, while computerized tomography (CT) coronary angiogram found no coronary vessel pathology. Serologic tests for herpes simplex virus type 1, cytomegalovirus, influenza type 1, coxsackievirus type A and B, Epstein-Barr virus, hepatitis type B and C, human immunodeficiency virus (HIV) were negative.

Following the European Society of Cardiology heart failure guidelines [4], thyroid hormone levels were examined showing highly elevated serum levels of thyroid stimulating hormone (TSH) 49.99 [0.25-5.0] μIU/ml, and decreased level of free triiodothyronine (FT3) 0,70 [4.0 - 8.3] pmol/l and free thyroxine (FT4) 1,00 [9.0-20.0] pmol/l. Levothyroxine therapy with 25

**Table 1.** Echocardiographic parameters and TSH values
***Tabela 1.*** *Ehokardiografski parametri i vrednosti TSH*

| | Follow up period (date)/*Period praćenja (datum)* | | |
|---|---|---|---|
| | August/*Avgust* | January/*Januar* | August/*Avgust* |
| | 2018 | 2019 | 2019 |
| TSH (μIU/l) | 49,99 | 16,34 | 4,2 |
| EF (%) | 14 | 41 | 55 |
| LVIDd (mm) | 80 | 54 | 50 |
| LVIDs (mm) | 75 | 34 | 30 |

Legend**:** TSH - thyroid stimulating hormone; EF - ejection fraction; LVIDd - left ventricle internal dimensions in diastole; LVIDs - left ventricle internal dimensions in systole

*Legenda**:** TSH – tiroidni stimulišući hormon; EF – ejekciona frakcija; LVIDd –* u*nutrašnje dimenzije leve komore u dijastoli; LVIDs –* u*nutrašnje dimenzije leve komore u sistoli*

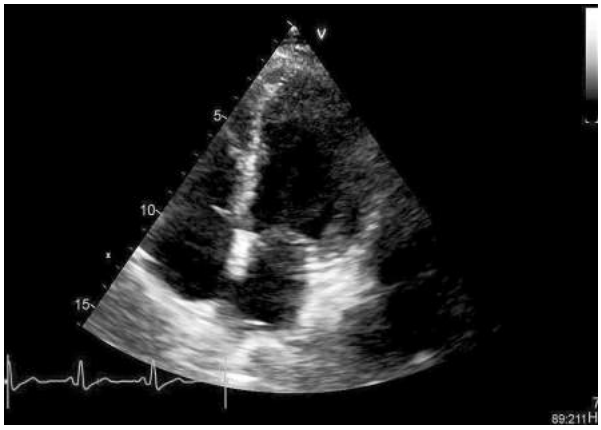


**Figure 3.** Echocardiography one year after discharge
***Slika 3.*** *Ehokardiografski nalaz godinu dana posle otpusta*

mcg was promptly initiated and the dosage was gradually increased to 50 mcg daily, in addition to the therapy he was receiving for acute heart failure. The patient was discharged after 10 days with improvement in symptoms and clinical status.

The ECG examinations were performed 5 and 12 months after discharge, showing increased EF (41%, 55%, respectively) and decreased left ventricular chamber dimensions **(Table 1**, **Figure 3)**. The TSH and FT4 values were normalized by hormone replacement therapy.

The patient is now taking medications for hypertension and hypothyroidism only. The control examination showed normalized ECG after 3 months, while liver function tests and myocardial enzyme values were normalized after one month.

## Discussion

It is well known that thyroid hormones affect almost every cell in the body causing structural and functional changes when it comes to thyroid function impairment.

Effects of thyroid hormones on cardiovascular system can be divided into two main groups - genomic and non-genomic, both affecting the heart muscle and blood vessels [5, 6]. There are many studies which have shown that heart function disturbances are mostly due to impaired protein synthesis [5]. The assessment of TSH is a diagnostic test recommended by the European Society of Cardiology Guidelines for diagnosis and treatment of heart failure (class I - stage C) [4].

It has also been shown that expression of some potassium gated ion channels, sodium/potassium (Na-K) pump functioning is also thyroid hormone dependent [7].

Even subclinical thyroid disorders are associated with changes in ECG parameters, particularly affecting the heart rate and QTc interval which was not registered in our patient [8].

The most consistent effect of hypothyroidism is diastolic function impairment, due to elevated pressure in the early filling phase and changes in the ventricular filling velocity. Bradycardia and hypertension are also common findings, while systolic function is usually slightly affected.

However, there are few case studies in the literature, reporting about reversible DCM caused by hypothyroidism [9, 10], suggesting that in patients with DCM, hypothyroidism should always be excluded as a potential cause.

In this case report, we have presented a case of a male patient with DCM and symptoms of acute heart failure. With introduction of hormone replacement therapy, LV dimension and EF normalized showing that severe hypothyroidism was the cause of DCM.

## Conclusion

Dilated cardiomyopathy is usually an idiopathic disease with uncertain prognosis, but it rarely develops due to other conditions (pregnancy, alcohol abuse, hyper and hypothyroidism etc.).

Although its exact pathogenesis remains unknown and could be a topic for future investigations, hypothyroidism should always be considered as the cause of dilated cardiomyopathy, because this life threatening disease is potentially reversible with adequate hormone replacement therapy.

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## POST-DURAL PUNCTURE HEADACHE: EPIDEMIOLOGY, ONSET MECHANISMS, CLINICAL SYMPTOMS, DIAGNOSIS AND THERAPY

*GLAVOBOLJA NASTALA POSLE DURALNE PUNKCIJE – EPIDEMIOLOGIJA, MEHANIZAM NASTANKA, KLINIČKA SLIKA, DIJAGNOZA I TERAPIJA*

**Svetlana S. SIMIĆ[1, 2], Aleksandar Š. KOPITOVIĆ[1, 2], Tamara RABI ŽIKIĆ[1, 2], Jelena KNEŽEVIĆ[1], Ljiljana RADMILO[3] and Dragan S. SIMIĆ[4]**

**Summary**

**Introduction.** Post-dural puncture headache is classified as a secondary headache attributable to non-vascular intracranial disorders and belongs to the group of headaches caused by low cerebrospinal fluid pressure. **Etiopathogenesis.** The pathogenesis is not completely clear, but it is thought to be caused by the cerebrospinal fluid leak through the duct opening. Cerebrospinal fluid efflux leads to a decrease in intracranial pressure and stretching of the pain sensitive intracranial structures. A drop in intracranial pressure can cause compensatory cerebrovascular vasodilation, contributing to the onset of a headache. **Diagnosis and Therapy.** Post-dural puncture headache clinically presents as an orthostatic headache. In most cases, the diagnosis is made based on a typical clinical picture and it can be confirmed by magnetic resonance imaging and measurement of cerebrospinal fluid pressure. The condition is usually benign, most often with spontaneous recovery. The therapy involves conservative treatment, medications, as well as some invasive methods: epidural blood patches, blockage of the greater occipital nerve, and in most severe cases, epidural injection of fibrin sealant or surgical dural repair. **Conclusion.** Post-dural puncture headache is a common complaint in the clinical practice of neurologists and anesthesiologists. The prognosis is usually favourable, while the therapy may include conservative or invasive treatment procedures.

**Key words:** Post-Dural Puncture Headache; Headache Disorders, Secondary; Cerebrospinal Fluid Leak; Intracranial Pressure; Blood Patch, Epidural; Intracranial Hypotension; Hypotension, Orthostatic; Spinal Puncture; Headache

**Sažetak**

**Uvod.** Glavobolja nakon punkcije dure je svrstana u sekundarnu glavobolju koja se pripisuje nevaskularnim intrakranijalnim poremećajima i nalazi se u grupi glavobolja koje se pripisuje niskom pritisku likvora. **Etiopatogeneza.** Patogeneza nije u potpunosti jasna, a smatra se da je uzrokovana odlivanjem cerebrospinalne tečnosti kroz otvor na duri. Odlivanje likvora dovodi do smanjenja intrakranijalnog pritiska i istezanja na bol osetljivih intrakranijalnih struktura. Pad intrakranijalnog pritiska može izazvati kompenzatornu cerebrovaskularnu vazodilataciju, što doprinosi razvoju glavobolje. **Dijagnoza i terapija.** Klinički se prezentuje kao ortostatska glavobolja. Dijagnoza se u većini slučajeva postavlja na osnovu tipične kliničke slike, a može se potvrditi magnetnom rezonancijom i merenjem pritiska likvora. Tok je obično benigan sa najčešće spontanim oporavkom. U terapiji se mogu primeniti konzervativne mere lečenja, medikamenti i invazivne metode u vidu epiduralne krvne zakrpe, blokade velikog okcipitalnog nerva, a u najtežim slučajevima primenjuje se epiduralna injekcija fibrinskog lepka ili hirurška reparacija dure. **Zaključak.** Glavobolja nakon punkcije dure je česta žalba sa kojom se susreću neurolozi i anesteziolozi u kliničkoj praksi. Prognoza je obično povoljna. U terapiji se mogu primeniti konzervativne i invazivne mere lečenja.

**Ključne reči:** glavobolja nakon duralne punkcije; sekundarne glavobolje; curenje cerebrospinalne tečnosti; intrakranijalni pritisak; epiduralne krvne zakrpe; intrakranijalna hipotenzija; ortostatska hipotenzija; spinalna punkcija; glavobolja

## Introduction

Dural or lumbar puncture (LP) involves passing of a needle through the dura mater into the subarachnoid space filled with cerebrospinal fluid (CSF). Headache is sometimes an uncomfortable complication of LP. It may occur after a planned LP performed for diagnostic or therapeutic purposes, after spinal anesthesia, but also after accidental dural puncture during epidural anesthesia [1, 2]. The resulting headache is called post-dural puncture headache (PDPH). In the International Classification of Headache Disorders, this headache is classified as a secondary headache attributed to non-vascular

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***Abbreviations***

LP – lumbar puncture
CSF – cerebrospinal fluid
PDPH – post-dural puncture headache
G – gauge
SSRIs – serotonin reuptake inhibitors
MRI – magnetic resonance imaging
EBP – epidural blood patches

intracranial disorders and belongs to the group of headaches caused by low CSF pressure [3, 4].

**Pathophysiology**

It is not entirely clear how post-dural puncture causes headache. There are two main hypotheses. The first is that the headache is caused by leaking of the CSF through the duct opening, and the consequent decrease in the CSF volume and development of cerebrospinal hypotension. The consequence is sinking of the brain, loss of brain buoyancy, and stretching of pain sensitive structures [5]. The second hypothesis is related to the compensatory vasodilatation resulting from the activation of adenosine receptors due to reduced CSF volume. Most likely both mechanisms play a role [3, 6]. In general, pathophysiological mechanisms do not differ significantly from those described for spontaneous intracranial hypotension [7].

**Epidemiology and risk factors**

After back pain, post-dural puncture is the second most common complication of the lumbar puncture [1]. The incidence of PDPH varies highly in different studies, from 2.6% to 30% [8]. A number of factors affect the incidence of PDPH. The large variation in the incidence of PDPH indicates a possible inaccuracy in defining this type of headache. This is supported by the fact that PDPH is rare in childhood, but one of the reasons for this is a more difficult diagnosis at an early age [9]. The PDPH is 2 - 3 times more frequent after diagnostic LP, than after spinal anesthesia [10]. This may be due to the use of thinner needles for spinal anesthesia and smaller volume of CSF loss [11]. Although headache is found to be more common after general than after spinal anesthesia, the high incidence of non-specific postoperative headaches after spinal anesthesia requires special attention [12]. The incidence of PDPH after lumbar puncture varies depending on individual patient characteristics (invariable factors), needle type and technique used (variable factors), as well as the diagnostic criteria and monitoring methods applied [8]. The shape and the size of perforation of the dura mater depends on the diameter of the needle, the thickness of the brain membrane at the injection site, and the direction of the injection with respect to the longitudinal axis of the fibers in the dura mater. Of the variable factors that may affect the appearance of PDPH, the most important is the thickness, that is, the diameter of the used needle. Numerous studies have confirmed that the incidence of PDPH is less frequent if thinner needles are used [13]. A smaller diameter needle makes a hole in the dura smaller, which causes both CSF leakage and the frequency of PDPH to be lower. When using 16 - 19 gauge (G) needles, the incidence of headache is 70%. When using 20 - 22 G needles, the incidence of headaches is 40%. Given that these are needles that are commonly used in diagnostic LP, this is probably one of the reasons for the more frequent occurrence of PDPH in these cases. As thinner needles are used in epidural and spinal anesthesia, the incidence of PDPH is less frequent in these cases. Specifically, when using 24 - 27 G needles, the incidence of headache is 12% [5]. Regarding the type of needles used for diagnostic punctures, some reports indicate that the size of the needle, not the shape, is important [14]. Other studies show that the incidence of headache is significantly lower when using atraumatic needles [15]. Davis et al. showed that when using atraumatic needles, the incidence of headache is 27.1% compared to the use of traumatic needles when the incidence is 60.4%. This difference is explained by the fact that tips of atraumatic needles, with smaller surface and rounded edges, like a tip of a pencil, separate the longitudinal fibers of the dura mater, while obliquely incised tips of traumatic needles cut them while passing through [6]. The incidence of PDPH is also affected by the direction of the needle during the procedure. If the needle is inserted perpendicular to the direction of the fibers of the dura mater, it cuts through the fibers and makes a larger hole in it. The insertion of the needle tip along the longitudinal axis of the dura fibers can potentially reduce CSF swelling and consequent headache. However, only 30% of practitioners were found to orient the needle tip along the longitudinal axis of the dura fibers [16]. When performing spinal anesthesia, the paramedial approach is less likely to lead to PDPH than the medial approach, but this has not been confirmed by studies. The medial approach involves the needle passing through the supraspinous and interspinous ligaments and the ligamentum flavum, and the paramedial approach avoids the supraspinous and interspinous ligaments and accesses the ligamentum flavum directly after passing through the paraspinal muscles. The paramedian approach is easier to perform, especially in the elderly who have sclerosis of ligaments, degenerative spinal changes, and who have more difficulties to take the proper position [17]. Furthermore, puncture of the dura mater and the arachnoid at different angles creates a valvular mechanism that reduces the possibility of spinal fluid efflux into the epidural space [18]. Variable factors that can affect the incidence of PDPH include the skill, level of training and experience of the LP practitioner. The PDPH occurs less frequently with more experienced practitioners [19]. Multiple attempts with more holes in the dura lead to a higher incidence of PDPH. If a patient moves during the procedure it will cause a larger slit in the dura than if the patient is still during the procedure [7, 20].

Age is a well-documented risk factor for PDPH. Adolescents have lower incidence than adults, it is rare in children and very rare in young children [21]. Although there are differences in relation to the age group with the highest risk for PDPH, these differences are not significant. Research studies report different age categories and that is why groups with the highest risk are aged 20 - 30 years, 20 - 40 years, and 31 - 50 years [8, 22, 23]. The incidence of PDPH drops after 40 years of age, and PDPH is rare after the age of 60 years. The causes of different incidence of PDPH across different age categories are numerous. Changes in hormonal status, especially in women, in various age categories can contribute to the onset of PDPH. The elasticity of the dura decreases with age, making it difficult for the liquor to swell. Cerebral blood vessels are less responsive to CSF. Reduction of the vertebral extra-dural space allows accumulation of a small amount of CSF and thus reduces its runoff from the subarachnoid space [22]. Gender is an independent risk factor for the development of PDPH [24]. The development of PDPH is twice as common in women as in men [25]. Physiological and psychosocial gender characteristics and different processing of painful impulses in women contribute to higher incidence of painful conditions in women [26, 27].

A lower body mass index (BMI) has been found to be associated with a higher incidence of PDPH [28, 29]. On the other hand, obese patients are less likely to have PDPH. Most likely, the excess abdominal panniculus acts as a binder and increases the intra-abdominal pressure, which reduces the efflux of the CSF [22].

The presence of comorbidity has been poorly understood. Patients who suffer from other headaches are more likely to develop PDPH. It is similar with the previous episodes of chronic pain. Also, patients who have had a headache with a previous LP are more likely to have it at repeated punctures, regardless of the time period elapsed from the previous puncture [30]. Some studies have linked epilepsy and PDPH [31]. The most prevalent symptoms of somatization in the general population in Serbia are fatigue, back pain and headaches [32]. Patients always fear from adverse effects of LP and even associate problems resulting from the illness itself with the diagnostic LP. The emotional status is a controversial item in PDPH understanding, but administration of anxiolytics does not reduce the risk of PDPH [28, 33]. Flying may contribute to the onset or relapse of PDPH [34].

## Clinical signs and symptoms

Orthostatic headache is a pathognomonic feature of PDPH [1]. The pain is usually located in the trigeminal or/and occipital region, whereas the temporal region, vertex, and nuchal regions are less commonly affected. In 90% of cases, the headache occurs within the first three days, and in 66% it begins within the first 48 hours of the procedure. According to the diagnostic criteria, the headache develops within five days of the LP [3, 4]. Rarely, the headache develops between 5 - 14 days after the procedure, as well as shortly after the dura puncture [35]. A headache occurring within 20 min of spinal anesthesia has also been described [36]. In most cases, the headache resolves spontaneously within a week and in 95% of cases within six weeks [37]. Cases of chronic headache have also been described [38]. The proliferation of fibroblasts begins after 48 hours of perforation of the dura and continues for seven days, contributing to the formation of collagen and closing of the duct opening. If this process does not occur, PDPH may persist for months and years. A case of a woman who has been rehospitalized for PDPH one month after spinal anesthesia has been described. She was treated with selective serotonin reuptake inhibitors (SSRIs) due to panic attacks. Clinically relevant doses of SSRI have been experimentally proven to reduce CSF production, and their intake may be associated with prolonged PDPH [39]. Fast development of a headache probably reflects faster efflux of the CSF and is more often accompanied by a stronger headache [40]. Any movement that increases intracranial pressure (such as coughing, sneezing, straining, or ocular compression) can cause exacerbation of symptoms. Complementary symptoms such as nausea, vomiting, pain, and tightness in the neck may occur [20].

Tinnitus is more common in patients with longer headache duration and in tall patients. Etiologically, except for hearing impairment, it is related to the efflux of perilymphatic fluid from the cochlea to the cerebrospinal space, via the cochlear aqueduct, which is functionally open in about 50% of adults [41].

## Diagnosis

Based on the clinical picture and course of the condition, it is usually easy to make the diagnosis. An easy to perform clinical test is a manoeuvre where one hand is placed on the back and the other is pressing the abdomen for half an hour. This increases the intra-abdominal pressure and the pain will cease with the pressure, but it will reoccur after the release.

The CSF may present with mild proteinorachia and increased lymphocyte count. Measuring the CSF pressure may reveal CSF hypotension. Measuring the blood flow in the upper ophthalmic vein provides a simple, rapid and non-invasive diagnostic method for suspected intracranial hypotension [42]. On magnetic resonance imaging (MRI), the ventricles appear small, the brain looks shrunken and descends into the posterior pit, yielding a Chiari type 1 malformation. Fluid accumulation in the subdural space can be observed – swelling of dura mater. Dilation of the cerebral venous sinuses and flattening or displacement of the optic chiasm and enlargement and prominence of the pituitary can also be seen. Enlarged epidural venous plexuses and extra-arachnoid fluid collections can be observed on spinal MRI. Unfortunately, MRI cannot show

the leak site because the fluid expands in many different directions [43].

## Therapy

The goals of the therapy are to achieve vasoconstriction, recovery of the CSF, and sealing the leakage site using methylxanthine derivatives such as caffeine, aminophylline, theophylline, triptans, anticonvulsant gabapentin and pregabalin, and steroids. The traditional approach with intravenous hydration, horizontal bed rest, and use of standard analgesic and symptomatic therapy has not been proven to be efficient, but since it has no side effects it is usually recommended. The application of abdominal clamp with the idea that abdominal pressure will lead to an increase in CSF pressure is rarely applied due to lack of evidence of efficacy and discomfort [44, 45]. Methylxanthine derivatives, primarily caffeine, are administered orally or intravenously. Caffeine blocks adenosine receptors and thus leads to vasoconstriction on one hand, and on the other, it can lead to PDPH improvement by stimulating the sodium pump [46]. Triptans are selective 5-hydroxytryptamine receptor agonists with consequent vasoconstriction, inhibition of neurogenic inflammation, and inhibition at the level of the caudal trigeminal nucleus. There are individual case reports, and case series about the use of sumatriptan in PDPH therapy with a favourable therapeutic response, but there is no sufficient evidence to suggest that its use can be recommended as standard therapy. Frovatriptan has been found effective in the prevention of PDPH, but these results also need to be confirmed by well-designed studies [47–49].

Antiepileptics, structural analogues of gamma amino butyric acid pregabalin and gabapentin (with better efficacy of pregabalin) have shown positive effects in some cases of PDPH [50].

Although the exact mechanism of action of steroids in PDPH has yet to be determined, the clinical efficacy has been confirmed in some cases. Their efficacy can be partly explained by the improvement of re-absorption of liquor from the extradural space, as well as by anti-inflammatory action at the site of dura puncture and reduced production of algogenic substances [51].

Invasive procedures that may be applied are epidural blood patches (EBP) and blockage of the greater occipital nerve. The EBP is an effective method in treating PDPH and it is the gold standard in the treatment of PDPH. It causes a prolonged increase in subarachnoid and epidural pressure, and it is possible that the coagulum itself makes a "patch" at the opening of the dura and prevents further leakage of the CSF. Complications such as acute and chronic low back pain, radiculopathy, infection, arachnoiditis, spinal subdural hematoma, spinal epiarachnoid hematoma, intrathecal hemorrhage are less frequent [52]. In the most severe cases, epidural injection of fibrin glue or surgical repair of the dura is used [53]. Greater occipital nerve blockage is a very effective method that is technically easy to perform, it is minimally invasive, and with a low rate of neurological complications [54].

## Conclusion

Post-dural puncture headache is an unpleasant complication that occurs after diagnostic or therapeutic lumbar puncture or incidentally with epidural and spinal anesthesia. It is clinically manifested as an orthostatic headache. The factors that contribute to the development of this headache can be divided into independent and dependent risk factors. The incidence of post-dural puncture headache can be reduced by the size and the direction of the needle. The prognosis is usually favourable. Conservative and invasive treatment measures can be applied in therapy.

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# THE USE OF ELECTRONIC HEALTH TOOLS FROM THE VERY BEGINNING OF LIFE - TIME-LAPSE EMBRYO MONITORING

## *PRIMENA ELEKTRONSKOG ZDRAVSTVA OD SAMOG POČETKA ŽIVOTA – KONTINUIRANI VIDEO MONITORING EMBRIONA*

**Artur BJELICA[1, 2], Maja ŠOĆ[3] and Marijana DESPOTOVIĆ ZRAKIĆ[4]**

**Summary**

**Introduction.** In addition to the already widespread use of electronic health services in monitoring the health status of patients, one should also refer to the possibility of using electronic health tools from the very beginning of life by monitoring growth and development of the embryo. In this way, the concept of electronic health would expand to cover the whole human life, from its very beginning to the end. **Monitoring the embryo development.** The efforts to improve in vitro fertilization success rate have been accompanied by the introduction of various procedures, including the evaluation of the embryo quality. A detailed monitoring of the kinetics of embryo development is achieved by laboratory techniques that provide continuous insight into the embryo development through applying time-lapse monitoring system. **Embryo quality evaluation programs based on time-lapse monitoring.** Special bioinformatic programs have been developed for automatic analysis of images obtained by time-lapse monitoring which allow a quantitative evaluation of the key moments in the development of the embryo and its morphology. **Implementation and limitations of time-lapse embryo monitoring.** The majority of authorities in the field of in vitro fertilization consider the use of time-lapse monitoring as a great advancement in the in vitro fertilization technology, obviously leading to a higher success rate. **Conclusion.** The systems for time-lapse monitoring of embryos represent powerful tools which help clinicians involved in in vitro fertilization and embryologists to select the best embryos, with the aim of improving the in vitro fertilization success rate. Despite all the advantages, these systems also have some shortcomings and limitations.

**Key words:** Time-Lapse Imaging; Fertilization in Vitro; Reproductive Techniques, Assisted; Embryonic Development; Embryo Transfer; Patient Safety

**Sažetak**

**Uvod.** Pored već široke upotrebe elektronskih zdravstvenih usluga za praćenje zdravstvenog stanja pacijenata, treba pomenuti i mogućnost upotrebe alata za elektronsko zdravstvo od samog početka života odnosno praćenja formiranja i razvoja embriona. Na ovaj se način pojam elektronskog zdravstva proširio na čitav ljudski život, od njegovog početka do kraja. **Nadzor razvoja embriona.** Napor da se poveća stopa uspeha oplodnje *in vitro* praćen je uvođenjem različitih postupaka, među kojima je i procena kvaliteta embriona. Detaljan nadzor kinetike razvoja embriona postiže se laboratorijskim tehnikama kojima se omogućava kontinuirani uvid u razvoj embriona primenom sistema kontinuiranog video-monitoringa. **Programi za evaluaciju kvaliteta embriona zasnovani na kontinuiranom video-nadzoru.** Kreirani su posebni bioinformatički programi za automatsku analizu slika dobijenih kontinuiranim video-nadzorom koji omogućavaju kvantitativnu procenu ključnih trenutaka u razvoju embriona i njegove morfologije. **Implementacija i ograničenja kontinuiranog video monitoringa embriona**. Većina eksperata u oblasti *in vitro* oplodnje smatra da je primena sistema kontinuiranog video-monitoringa veliki napredak tehnologije u ovoj oblasti koja dovodi do većeg stepena uspeha postupka. **Zaključak.** Sistemi za video-monitoring embriona predstavljaju moćne alate koji pomažu kliničarima koji sprovode vantelesnu oplodnju i embriolozima da odaberu najbolje embrione, sa ciljem da se poveća stopa uspeha vantelesne oplodnje. Uprkos svim prednostima, ovi sistemi imaju i neke nedostatke i ograničenja.

**Ključne reči:** time-lapse imidžing; in vitro fertilizacija; asistirane reproduktivne tehnike; embrionalni razvoj; embriotransfer; bezbednost pacijenta

## Introduction

Electronic health (e-health) is an "emerging field in the intersection of medical informatics, public health and business, referring to health services and information delivered or enhanced through the Internet and related technologies" [1]. In 2015, the European Commission proposed a definition of e-health as the application of information-communication technologies (ICTs) to meet the needs of citizens, patients, health service providers, and creators of health policy [2].

The use of ICTs in e-health is aimed at:

– Advancement and improvement of prevention, diagnostics, treatment, and disease management;

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***Abbreviations***

E-health – electronic health
ICTs – information-communication technologies
IVF – in vitro fertilization
ICSI – intracytoplasmic sperm injection

– Improvement of the accessibility to health services and enhancement of their quality by improving the efficiency of the health sector;
– Exchange of information between patients and health service providers, hospitals, health workers;
– Use of telemedicine and portable equipment for monitoring health conditions and other kinds of information.

The aim of this article is to give a short review of the use of e-health in monitoring the growth and development of the embryo with the emphasis on the time-lapse method, as a comprehensive tool of e-health.

Infertility is a serious health problem that affects a constantly increasing number of people all over the world [3]. After the birth of the first "test-tube" baby in 1978, in vitro fertilization (IVF) has become a widely practiced technique, appearing as a crucial solution to all those couples who went through all the procedures of infertility treatment [4]. It is estimated that globally more than 8.5 million children were born through IVF [5]. This procedure has become increasingly widespread in solving the infertility problem of many couples. Although a constant increase in its success rate is evident, it still remains relatively low. A strategy to increase its efficiency, which is still present in some countries and centers, is transfer of a larger number of embryos. However, this strategy increases the risk of multiple pregnancy, which may cause a number of harmful effects on both mother and child(ren). That is why, elective transfer of a single embryo is an imperative, which cannot be realized without proper selection and evaluation of its quality. Embryos can be selected by applying different methods – non-invasive or invasive. In non-invasive methods the emryos are selected based on their morphology or using techniques based on the analysis of their molecular components – the levels of proteomes and metabolomes. Detailed monitoring of the kinetics of embryo development is achieved by using techniques of different time-lapse monitoring systems [6].

## Monitoring the embryo development

The development of human embryo is a dynamic process which begins after the union of the sperm and the ovum, forming a zygote. The zygote continues its further development through a series of mitotic cleavage divisions, forming blastomeres every 12 to 24 hours. Thus, on day 3, the embryo has 8 cells, on day 4 a morula is formed, and on day 5 the embryo forms a blastocyst [7]. Classical monitoring of this dynamic embryo development means static observations performed from time to time by an embryologist. Such an approach has two major shortcomings [8]:

1. Static observations are performed only in certain time moments during the day, which means an inevitable loss of valuable information and disturbance of optimal conditions for embryo cultivation;
2. This approach allows only individual, sequential interpretations based on subjective evaluations, and as such it shows large inter-observer errors and moderate intra-observer errors.

Standard microscopic observation of the embryo mans its evaluation once a day. According to the consensus given by the Special Interest Group of the European Association for Human Reproduction and Embryology and Alpha Scientists in Reproductive Medicine, the checking is carried out in the following time intervals [9]:
– Fertilization is checked 17 hours post insemination and syngamy after 23 hours
– Stage of early cleavage is checked 26 hours post intracytoplasmic sperm injection (ICSI) and 28 hours after classical IVF procedure
– On day 2, embryo is assessed 44 hours post insemination
– On day 3, embryo is assessed 68 hours post insemination
– On day 4, embryo is assessed 92 hours post insemination
– On day 5, embryo is assessed 116 hours post insemination.

More frequent controls are not acceptable, due to the disturbance of conditions in the cultivation medium, such as the stability of the pH, temperature, and humidity, which are of paramount importance for good embryo development.

The use of a system consisting of a camera, microscope, incubator, and a computer provides a situation in which embryos can be continuously monitored during their cultivation without disturbing the optimal conditions. Depending on the system used, embryos are photographed every 5 to 20 minutes. Such an approach represents the basis for a continuous monitoring of embryos, the so-called time-lapse monitoring.

Time-lapse monitoring system provides digital images of the embryo in the given time intervals. The obtained digital images are processed using a special software, resulting in time-lapse sequences of embryo development. Besides, time-lapse systems can also provide computer-assisted evaluation of morphokinetic parameters, thus enabling a semi-quantitative evaluation of embryo quality [10]. In this way, by applying appropriate software algorithms, it is possible to achieve theoretically most precise embryo selection, whose most important advantages are that they are non-invasive and non-subjective [11].

The time-lapse monitoring shows the time dimension changes as a variable, which is very important for assessment of the embryo quality and selection of the best ones for transfer. In the classical approach, the time dimension is considered as a discretionary variable, whereas in the time-lapse monitoring time dimension is transformed into a continuous variable, which corresponds more to the reality.

The time-lapse monitoring system provides about 1000 images of the embryo during the five-day development in the incubator, whereas classical monitoring gives evaluation of the embryo development only in 2 to 4 static moments. The use of time-lapse monitoring allows a detailed insight into division of the embryo cells and other morphological embryo events, such as the beginning of compaction and formation of the blastocyst cavity, along with some other phenomena, like disappearance of multinuclearity and fragmentations [12].

The above facts clearly show the advantages of the time-lapse monitoring over the conventional method of embryo observation. Comparative characteristics of the conventional and time-lapse monitoring are summarized in **Table 1**.

Generally, there are two types of systems for continuous embryo monitoring: 1. the existing incubator is additionally equipped with a microscope and a camera (Eeva system; PrimoVision system), and 2. an integrated incubator system with microscope + camera (EmbryoScope). Both system types use digital inverted microscope which takes images of the embryos in predefined time intervals, which allows creation of a video record of the events in the period of 72 or 120 hours. PrimoVision and Eeva systems are incorporated into the existing incubators, while EmbryoScope is a compact incubator with the built-in microscope/camera system. All three systems use different light sources. Also, they differ in the way the embryos enter the imaging field. EmbryoScope and PrimoVision use bright field technology, whereas Eeva system uses dark field technology. Bright field technology allows evaluation of the kinetic parameters of the embryo growth and development, whereas dark field provides an excellent evaluation of the kinetics and a weaker evaluation of the embryo morphology. The systems also differ in the way of embryo cultivation. PrimoVision and Eeva culture dishes are suited for group cultivation, whereas EmbryoScope uses individual cultivation (EmbryoSlide). Group cultivation in the Eeva system uses special dishes for 12 embryos and in the PrimoVision system the dishes have space for 9 to 16 embryos sharing 50 - 120 μl of the medium. According to some authors, group cultivation is favorable, since it has demonstrated that it improves embryo development. Individual cultivation in the EmbryoScope system is performed in dishes with a space for 12 embryos, each of which contains 20 - 25 μl of medium.

Technical and clinical characteristics of the particular time-lapse monitoring systems are presented in **Table 2** and **Table 3**.

## Evaluation programs based on time-lapse monitoring

The main point in using time-lapse monitoring in IVF is objective evaluation of embryo quality. The limiting factors of automatic embryo development monitoring are associated with image quality, morphological differences in different stages of embryonic development, position and transparency of the embryo. Special bioinformatic programs have been created for automatic analysis of images obtained by time-lapse monitoring which allow quantitative evaluation of the key moments in the development of the embryo and its morphology. Initially, it was recommended that each IVF institution should design its own algorithm based on the locally obtained data by time-lapse monitoring. Still, such an approach did not appear acceptable, since the number of institutions had a small number of patients to provide valid evaluation algorithms [13].

PrimoVision software has been developed specifically for PrimoVision time-lapse monitoring. It

**Table 1.** Comparison of conventional and time-lapse monitoring of embryo development in IVF
***Tabela 1.*** *Poređenje konvencionalnog monitoringa i kontinuiranog video-monitoringa embriona tokom postupka vantelesne oplodnje*

| Conventional monitoring/*Konvenconalni monitoring* | Time-lapse monitoring/*Kontinuirani video-monitoring* |
|---|---|
| The embryologist removes the embryos from the incubator at defined time intervals and examines them under a micrpscope/*Embriolog u određenim vremenskim intervalima vadi embrione iz inkubatora i stavlja ih pod mikroskop radi procene* | The microscope/camera system takes images of embryos continuously at predefined time intervals *Sistem mikroskop-kamera kontinuirano snima embrione* |
| After completing the observation the embryos are returned to the incubator *Nakon završene procene vraća ih u inkubator* | The microscope/camera system is placed in the incubator or the integrated incubator/microscope/camera system is used/*Sistem mikroskop–kamera se postavlja u inkubator ili se koristi jedinstveni sistem inkubator–mikroskop–kamera* |
| Every manipulation changes parameters in the incubator *Manipulacija inkubatorom menja parametre u inkubatoru* | Predefined parameters are constantly maintained in the incubator *Konstantno održavanje zadatih parametara u inkubatoru* |
| Disturbance of the optimal environment for embryo development *Narušavanje optimalnog okruženja za razvoj embriona* | Stable environment for embryo development *Stabilno okruženje za razvoj embriona* |
| Subjective evaluation of embryo development *Subjektivna procena razvoja embriona* | Objective evaluation of embryo development *Objektivnija procena razvoja embriona* |

**Table 2.** Technical characteristics of the particular time-lapse monitoring systems
***Tabela 2.*** *Tehničke karakteristike pojedinih sistema kontinuiranog video-monitoringa embriona*

| Technical characteristic<br>*Tehničke karakteristike* | EmbryoScope<br>*EmbryoScope* | PrimoVision<br>*PrimoVision* | Eeva<br>*Eeva* |
|---|---|---|---|
| Integrated incubator/*Integrisani inkubator* | Yes/*Da* | No/*Ne* | No/*Ne* |
| Optics/*Optika* | Bright field/*Svetlo polje* | Bright field/*Svetlo polje* | Dark field/*Tamno polje* |
| Image frequency/*Frekvencija slika* | 10 min/*10 minuta* | 10 min/*10 minuta* | 5 min/*5 minuta* |
| Focal plane/*Žižna ravan* | 7 | 1 | 1 |
| Patient capacity/*Kapacitet pacijenata* | 6/system/*6/sistem* | 1/camera/*1/kamera* | 1/camera/*1/kamera* |
| Number of embryos/patients<br>*Broj embriona/pacijenata* | 12 individual cultures<br>*12 individualnih kultura* | 16 group cultures<br>*16 grupnih kultura* | 12 group cultures<br>*12 grupnih kultura* |

gives insight into embryonic events and enables distant monitoring and consultations among fertility specialists and embryologists. Smart tool helps to measure blastomere fragmentation and symmetry. It possesses predefined work profiles which allow easy and fast acquisition of data about the kinetics of the embryo development and its morphology, offering different ways of comparison of particular embryos, which facilitates taking the final decision regarding their future – transfer, cryopreservation or rejection [14].

The Eeva system developed the EevaTest software which, based on embryo development, categorizes embryos into two categories of their potential: high and low.

EmbryoScope, using the EmbryoViewer software, enables formation of one or more models in concordance with specific clinical criteria that compare the monitored embryos using the Compare & Select program.

The software used in the EmbryoScope system gives the so-called KIDScore D3 for embryos on day 3 and KIDScore D5 for embryos on day 5. This is a morphokinetic score for each embryo, from 1 to 5, and in this way it measures the relative evaluation potential for implantation of each particular embryo. The tested model was validated through clinical practice [15].

## Implementation and limitations of time-lapse embryo monitoring

The majority of authorities in the field of IVF consider time-lapse monitoring as a great advancement in the IVF technology, leading obviously to a higher success rate. Despite of this, there are opponents to this method [16]. Still, most of the arguments are on the side of those who support the use of time-lapse monitoring, since this technology offers more than the mere algorithms for kinetics of embryo development. The application of time-lapse monitoring can also detect numerous morphological events which cannot be noticed by conventional evaluation. Besides, it allows obtaining valuable data without taking embryos out of the stable environment of incubator and does not disturb their physiological development. This technology offers the possibility of selection of the best embryo for transfer since it gives an insight into its morphological and morpho-kinetic parameters.

The application of time-lapse monitoring results in:

1. Lower rate of early pregnancy losses
2. Higher implantation rate
3. Shorter time leading to IVF success, measured by live births.

Despite all of the advantages offered by time-lapse monitoring, it also has some shortcomings, such as the impossibility of embryo rotation. This prevents the possibility of observing all the em-

**Table 3.** Clinical characteristics of the particular time-lapse monitoring systems
***Tabela 3.*** *Kliničke karakteristike pojedinih sistema kontinuiranog video-monitoringa embriona*

| Clinical characteristic<br>*Kliničke karakteristike* | EmbryoScope<br>*EmbryoScope* | PrimoVision<br>*PrimoVision* | Eeva<br>*Eeva* |
|---|---|---|---|
| Automatic diagnostics/*Automatska dijagnostika* | No/*Ne* | No/*Ne* | Yes/*Da* |
| Need for operator/*Potreba za operaterom* | Yes/*Da* | Yes/*Da* | No/*Ne* |
| Time needed for analysis<br>*Vreme potrebno za analizu* | Yes/*Da* | Yes/*Da* | Automatic analysis<br>*Automatska analiza* |
| Algorithm selection<br>*Algoritam selekcije* | Defined by user<br>*Korisnik definiše* | Defined by user<br>*Korisnik definiše* | Yes/*Da* |
| Prediction of obtaining blastocyte on day 3<br>*Predikcija dobijanja blastociste na nivou 3. dana* | No/*Ne* | No/*Ne* | Yes/*Da* |
| Prediction of blastocyte implementation on day 3<br>*Predikcija implantacije blastociste na nivou 3. dana* | Yes/*Da* | No/*Ne* | No/*Ne* |

bryo's tridimensional aspects, as they are visible only in one plane. Besides, the exposure of the embryos to continuous microscopic observations is also undesirable. Namely, depending on the type of time-lapse monitoring system, the embryos are (by mechanical movement) placed under the microscope at a predefined frequency (EmbryoScope system) or are cultivated in the dishes with common medium, so that rotational movements of the embryo dishes are avoided (systems PrimoVision and Eeva). Also, one should not forget the possible negative effects of light during the monitoring [17–19].

## Conclusion

The time-lapse embryo monitoring systems represent powerful tools which help clinicians involved in "in vitro" fertilization and embryologists to select the best embryos, with the aim of enhancing the success rate of in vitro fertilization. Despite of all the advantages, these systems also have some shortcomings and limitations. It is expected that further technological development will certainly contribute to a more direct communication between the human life in its very conception and clinicians engaged in its coming into being.

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# LETTERS TO THE EDITORIAL BOARD
## *PISMA UREDNIŠTVU*

**Nevenka Rončević.** Remembering Prof. Dr. Dimitrije R. Miletić On The 30Th Anniversary of Death (*Sećanje na prof. dr Dimitrija R. Miletića povodom 30. godišnjice njegove smrti*)

In April of 2019, it has been thirty years since Prof. Dr. Dimitrije R. Miletić, an eminent pediatrician and outstanding figure in the Serbian and Yugoslav pediatrics, passed away. In recognition for all that he has taught us pediatricians, we would like to pay our tribute and respect once again and show our young colleagues the personality and accomplishments of Prof. Dimitrije R. Miletić, founder of the Institute of Child and Youth Health Care of Vojvodina.

Prof. Dr. Dimitrije R. Miletić, a great and unforgettable name in the Serbian and Yugoslav pediatrics, has not been with us for thirty years. On the occasion of the 30th anniversary of his death, we would like to introduce this prominent Serbian and Yugoslav pediatrician to younger generations of doctors and those of us who were fortunate enough to have him as a teacher and a role model, as well as to remember and thank him again for everything he tought us.

Prof. Dr. Dimitrije Miletić was born on March 7, 1920 in Sarajevo, to father Risto, a doctor at the General Hospital in Sarajevo and mother Kornelija Ninković, opera singer and prima donna of the Belgrade Opera between the two wars. His father Risto was from a prominent merchant family Miletić-Šajin from Mostar. Kornelija`s father, Dušan Ninković, a civil engineer educated in Switzerland, constructed the first Belgrade sewage system.

Prof. Miletić completed a primary school in Mostar and grammar school in Novi Sad, where his father was a manager of the Provincial Hospital until the beginning of the Second World War. Dimitrije started his medical studies in 1939 in Belgrade, but his studies were interrupted by the outbreak of the war.

As a member of the National Liberation Movement, he took an active part in the liberation of Serbia and Belgrade and fought at the Srem Front. When the war ended, he continued his medical studies in Belgrade and graduated from medicine in 1949. As a student, he was a demonstrator with Prof. Aleksandar Kostić in the subject of histology and embryology for three years.

He started his pediatric medical specialization at the Pediatric Clinic of the School of Medicine in Belgrade with Prof. Dr. Matija Ambrozić and Prof. Dr. Smilja Kostić Joksić.

By the order of authorities, he was transferred to the Pediatric Clinic of the Faculty of Medicine in Sarajevo and started working with Professor Dr. Milivoje Sarvan. At the end of his specialization, in 1952, he was elected a teaching assistant. He passed

his specialization exam at the Pediatric Clinic in Belgrade with the flying colours in 1953.

He was elected Assistant Prof. in 1959 and Associate Prof. of the Faculty of Medicine in Sarajevo in 1963. Prof. Miletić was transferred to the Faculty of Medicine in Novi Sad in 1963, where he was elected Associate Prof. of pediatrics. At the same time, he was appointed manager of the Clinic of Children`s Diseases. He was elected a Full Prof. in 1978.

From December 1969 to October 1971, through international technical assistance, he attended the famous Makarere College, Kampala University, Uganda. During his stay in Kampala, he broadened and enriched his great clinical knowledge and experience in the field of tropical medicine.

Upon his return to the country, Prof. Miletić initially worked as assistant director of the Institute of Mother and Child Health Care in Novi Sad, and in 1972 he was elected director of the Institute. From 1976 to 1978, he was the Director of the Department Child Health at the Clinical Hospital Center Dedinje. He left for Banja Luka in 1978, where he was one of the founders of the Faculty of Medicine and its first dean. He spent two terms in this capacity, being both the founder and the first head of the Department of Pediatrics in Banja Luka.

Prof. Miletić expanded his knowledge of pediatrics in numerous foreign institutions with the most respected pediatricians of the time. In 1954, he spent

three months at the International Children's Center in Paris, where he attended a course in social pediatrics and in 1957 he worked with Prof. E. Debre and Prof. Dr. J. Marija for seven months. In the afternoons he worked with Prof. Dr. B. Lastradet in the laboratory for metabolic deseases at the Children's Clinic in Paris. He also spent two months at the Faculty of Medicine in Paris, attending a course in endocrinology.

In 1959, he spent some time in Birmingham with Prof. Dr. Mary Crosse, an expert of the World Health Organization on problems with preterm children.

He retired in 1982 and passed away on April 22, 1990 in Belgrade, where he was buried.

One could talk about his professional, educational, scientific, organizational, ethical and human virtues for hours, unfortunately the time is limited.

His knowledge of pediatrics was extraordinary. He followed all the contemporary and new findings in this field and immediately applied them in practice. He was also an impressive practicioner. In addition to the habilitation work entitled "Neurotoxic Infant Syndrome" and doctoral dissertation "Protein Malnutrition in the Light of Hematological and Serum-Protein Events" he published numerous professional and scientific studies in the country and abroad.

As an educator, he was original, clear, convincing, confident, practical, considerate, and to cut the long story short, he had all the qualities of an outstanding lecturer.

When he made rounds in the hospital, it was a real treat for all his coworkers, as they represented an original and effective teaching practice by setting a good example. After every round, his associates were richer and more confident and they would get a new incentive to improve further on. He was exceptional in organizing all postgraduate and other forms of teaching.

While teaching as a Full Prof., Miletić used both hospital and extrahospital capacities, presenting physiological and pathological processes with healthy and sick children, focusing attention of medical students to comprehensive observation of a human being in the period of growth and development.

He mentored many specialists, masters and doctors of science. He was an initiator, mentor and associate in numerous studies and projects of doctors, professors and students. He introduced teamwork in clinical and scientific research at the institutions he managed. Rapidly, the General Children`s Hospital developed into a modern Europen Children`s Clinic with numerous subspecialities.

Pediatric school of Novi Sad created by Prof. Miletić as its director gained international recognition.

In addition to his work at the Clinic, he organized a course in social pediatrics in Vojvodina and courses for multi-function nurses.

The Pediatric School in Banja Luka, founded by Prof. Miletić, significantly improved professional work and stimulated further scientific research in Bosnia and Herzegovina over a very short course of time.

All the institutions he managed had established professional and scientific cooperation with many other medical institutions worldwide.

He was a member of the Serbian Medical Society, of the Pediatric Section, the head of the Pediatric Section of Vojvodina, a member of Medical Society of Bosnia and Herzegovina and a member of East African Association of Physicians; he was the president of the Coordinating Commitee for Scientific Research of the Yugoslav Association of Pediatrics, president of the Association of Teachers of the Faculty of Medicine in Novi Sad, and a member of the editorial boards of journals *Medical Review* and *Yugoslav Pediatrics*. He was awarded a Medal for Merit, the Order of Work of the Third Order and the Order of Work with the Gold Wreath.

His attitude towards his young patients and their parents was always extremely warm, friendly and cordial. He respected his young patients and their personalities and he advised his younger colleagues to keep in mind that the patient is always right no matter what problems or misuderstandings they might encounter during their work.

He was highly moral both in life and profession, in relation to people and work.

Being highly educated, he had a broad general knowledge that included literature, classical and modern music, knowledge of foreign languages (English and French) which offered him the opportunity to communicate easily and made him an interesting and charming communicator.

He made an impression of a great intellectual, with broad general education and vast life experience. He was an exceptional authority and respected Professor, a benevolent teacher and a colleague, a reliable friend and associate, favourite doctor to children, a strict and just director. He was extremely attentive and kind to his coworkers, taking great care to encourage young colleagues and support them to overcome everyday problems.

He believed that the young are encouraged best by setting a personal example. His personal character traits and values were reflected in his simple and unassuming behaviour.

His family members (wife and two sons) will always remember him as a man with the best human qualities. Apart from his two sons, he left many spiritual sons and daughters who will forever be grateful, and feel admiration and respect towards him. One of his students, Dr. Lolić from Banja Luka probably put it the best way: "The central part of my doctor's office belongs to the photograph of the respected Prof. Miletić and on Sundays when I light candles in church for all my fellow men, one of them is for the soul of late Prof. Dimitrije Miletić for whom the words of Njegoš, the famous writer, stand true: "A good reason had he to be alive!".

# REGISTAR ZA 2019. GODINU

## INDEKS AUTORA

## INDEX KEY WORDS

## INDEKS KLJUČNIH REČI

# UPUTSTVO ZA AUTORE

Časopis ***Medicinski pregled*** objavljuje radove koji prethodno nisu objavljeni niti poslati u drugi časopis. U Časopisu mogu biti objavljeni radovi iz različitih oblasti biomedicine, koji su namenjeni lekarima različitih specijalnosti.

Od 1. januara 2013. godine *Medicinski pregled* je počeo da koristi usluge *e-Ur* − Elektronskog uređivanja časopisa. Svi korisnici sistema − autori, recenzenti i urednici, moraju biti registrovani korisnici sa jednom elektronskom adresom.

Korisnici časopisa treba da se registruju na adresi:
http://aseestant.ceon.rs/index.php/medpreg/user/register

Prijava rada treba da se učini na adresi:
http://aseestant.ceon.rs/index.php/medpreg/

U postupku prijave neophodno je da se pošalje saglasnost i izjava autora i svih koautora da rad nije delimično ili u celini objavljen ili prihvaćen za štampu u drugom časopisu.

Elektronsko uređivanje časopisa obezbeđuje korišćenje sistema *CrossCheck*, koji prijavljene radove automatski proverava na plagijarizam i autoplagijarizam. Autori ne bi smeli da pošalju isti rad u više časopisa istovremeno. Ukoliko se to desi, glavni urednik časopisa *Medicinski pregled* ima pravo da rad vrati autorima bez prethodnog slanja rada na recenziju; da odbije štampanje rada; da se obrati urednicima drugih časopisa u koje je rad poslat ili da se obrati direktoru ustanove u kojoj su autori rada zaposleni.

Primaju se samo radovi koji su napisani na engleskom jeziku, uz sažetak rada i naslov rada koji treba da budu napisani na engleskom i srpskom jeziku.

Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

U časopisu *Medicinski pregled* objavljuju se: uvodnici, originalni članci, prethodna ili kratka saopštenja, pregledni članci, stručni članci, prikazi slučajeva, članci iz istorije medicine i drugi članci.

**1. Uvodnici** – do 5 strana. Sadrže mišljenja ili diskusiju o posebno značajnoj temi za Časopis, kao i o podacima koji su štampani u ovom ili nekom drugom časopisu. Obično ih piše jedan autor po pozivu.

**2. Originalni članci** – do 12 strana. Predstavljaju rezultate istraživanja autora rada i njihovo tumačenje. Istraživanje treba da bude obrađeno i izloženo na način da se može ponoviti, a analiza rezultata i zaključci jasni da bi se mogli proveriti.

**3. Pregledni članci** – do 10 strana. Predstavljaju sistematsko, sveobuhvatno i kritičko izlaganje problema na osnovu analiziranih i diskutovanih podataka iz literature, a koji oslikavaju postojeću situaciju u određenom području istraživanja. Literatura koja se koristi u radu mora da sadrži najmanje 5 radova autora članka iz uže naučne oblasti koja je opisana u radu.

**4. Prethodna ili kratka saopštenja** – do 4 strane. Sadrže izuzetno važne naučne rezultate koje bi trebalo objaviti u što kraćem vremenu. Ne moraju da sadrže detaljan opis metodologije rada i rezultata, ali moraju da imaju sva poglavlja kao originalni članci u sažetoj formi.

**5. Stručni članci** – do 10 strana. Odnose se na proveru ili prikaz prethodnog istraživanja i predstavljaju koristan izvor za širenje znanja i prilagođavanja originalnog istraživanja potrebama postojeće nauke i prakse.

**6. Prikazi slučajeva** – do 6 strana. Opisuju retke slučajeve iz prakse. Slični su stručnim člancima. U ovim radovima prikazuju se neuobičajeni oblici i tokovi oboljenja, neočekivane reakcije na primenjenu terapiju, primene novih dijagnostičkih procedura ili retke i nove bolesti.

**7. Članci iz istorije medicine** – do 10 strana. Ovi članci opisuju događaje iz prošlosti sa ciljem da omoguće očuvanje medicinske i zdravstvene kulture. Imaju karakter stručnih članaka.

**8. Ostali članci** – U časopisu Medicinski pregled objavljuju se feljtoni, prikazi knjiga, izvodi iz strane literature, izveštaji sa kongresa i stručnih sastanaka, saopštenja o radu pojedinih zdravstvenih organizacija, podružnica i sekcija, saopštenja Uredništva, pisma Uredništvu, novosti u medicini, pitanja i odgovori, stručne i staleške vesti i članci napisani u znak sećanja (*In memoriam).*

**Priprema rukopisa**

Kompletan rukopis, uključujući tekst rada, sve priloge i propratno pismo, treba poslati na elektronsku adresu koja je prethodno navedena.

Propratno pismo:

– mora da sadrži izjavu svih autora da se radi o originalnom radu koji prethodno nije objavljen niti prihvaćen za štampu u drugim časopisima;

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

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

**Rukopis**

**Opšta uputstva**

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

Rukopis treba da sadrži sledeće elemente:

**1. Naslovna strana**

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

**2. Sažetak**

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

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

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

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

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

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

**3. Tekst članka**

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

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

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

**Uvod**

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

**Materijal i metode**

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

**Rezultati**

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

**Diskusija**

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

**Zaključak**

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

**4. Literatura**

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

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

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

∗ Organizacija kao autor

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

∗ Bez autora

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

∗ Volumen sa suplementom

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

∗ Sveska sa suplementom

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

∗ Sažetak u časopisu

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

Knjige i druge monografije

∗ Jedan ili više autora

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

∗ Urednik (urednici) kao autor (autori)

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

∗ Poglavlje u knjizi

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

∗ Zbornik radova sa kongresa

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

∗ Disertacija

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

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

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

∗ Monografija u elektronskom formatu

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

∗ Kompjuterska datoteka

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

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

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

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

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

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

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

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

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

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

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

**6. Dodatne obaveze**

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

## *INFORMATION FOR AUTHORS*

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

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

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

Manuscript submission should be made on the web address:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Preparation of the manuscript**

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

**The covering letter:**

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

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

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

**The manuscript:**

**General instructions.**

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

The manuscript should contain the following elements:

**1. The title page.**

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

**2. Summary.**

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

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

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

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

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

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

**3. The text of the paper.**

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

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

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

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

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

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

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

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

Articles in journals

∗ *A standard article*

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

∗ *An organization as the author*

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

∗ *No author given*

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

∗ *A volume with supplement*

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

∗ *An issue with supplement*

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

∗ *A summary in a journal*

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

Books and other monographs

∗ *One or more authors*

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

∗ *Editor(s) as author(s)*

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

∗ *A chapter in a book*

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

∗ *A conference paper*

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

∗ *A dissertation and theses*

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

Electronic material

∗ *A journal article in electronic format*

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

∗ *Monographs in electronic format*

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

∗ *A computer file*

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

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

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

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

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

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

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

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