YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2021; May Vol. 78 (No. 5): pp. 483–584.

Vojnosanitetski Pregled 2021 May Vol. 78 (No. 5): pp. 483–584.

vol. 78, No 5, 2021

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of Vojno-sanitetski glasnik which was published between 1930 and 1941

PUBLISHER
University of Defence, Ministry of Defence of the Republic of Serbia, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Editorial Office: University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Serbian Citation Index (SCIndex), DOAJ. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

vol. 78, br. 5, 2021

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Ministarstvo odbrane Republike Srbije, Univerzitet odbrane, Beograd, Srbija

ISSN 0042-8450
eISSN 2406-0720

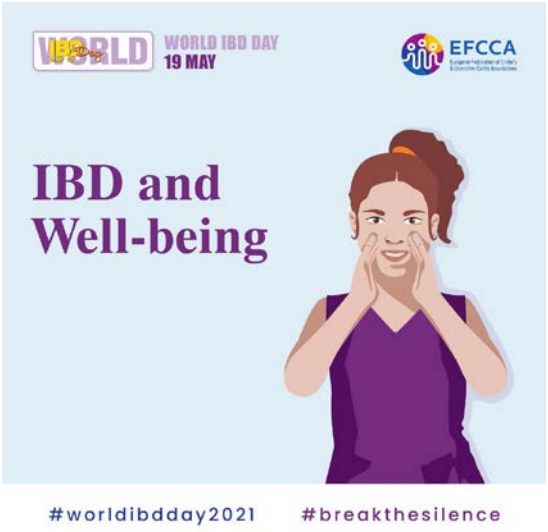
Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu" indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Srpski citatni indeks (SCIndeks), DOAJ. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ



World Inflammatory Bowel Diseases (IBD) Day takes place on May 19 each year with the aim to unite people worldwide in their fight against Crohn's disease and ulcerative colitis, known as inflammatory bowel diseases. Throughout the month of May, the European Federation of Crohn's & Ulcerative Colitis Associations (EFCCA) is working to increase awareness of IBD, educate the public about these diseases, and raise funds to support efforts in finding better IBD cures and improving the patients quality of life.

Svetski dan inflamatornih bolesti creva (*inflamatory bowel diseases* – IBD) održava se 19. maja svake godine sa ciljem da ujedini ljude širom sveta u borbi protiv Kronove bolesti i ulceroznog kolitisa, poznatih kao inflamatorne bolesti creva. Tokom meseca maja, Evropska federacija asocijacija za Kronovu bolest i ulcerozni kolitis (EFCCA) nastoji da poveća svest o IBD, da obrazuje javnost u vezi sa ovim bolestima, kao i da prikupi sredstva koja su neophodna za pronalaženje boljih lekova za IBD i poboljšanje kvaliteta života obolelih.

ORIGINAL ARTICLE

UDC: 616.43:618.11-006
DOI: https://doi.org/10.2298/VSP190715099S

Is there a correlation between blood glucose curve and the insulin resistance during oral glucose tolerance test in females suffering from polycystic ovary syndrome?

Da li postoji korelacija oblika glikemijske krive i rezistencije na insulin u toku testa oralnog opterećenja glukozom kod žena obolelih od sindroma policističnih jajnika?

Svetlana Spremović Radjenović*†, Miljan Pupovac*

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

Abstract

Background/Aim. Insulin resistance occurs in approximately 60–80% of women with polycystic ovary syndrome (PCOS) and in 95% of obese women with PCOS. Previous studies have shown that the shape of the glucose curve obtained during an oral glucose tolerance test (OGTT) may be useful as a metabolic screening parameter and could give insight into the future risk of diabetes mellitus type II. The aim of this study was to determine the frequency of insulin resistance according to the Homeostasis model assessment of insulin resistance (HOMA-IR) and indirect assessment method of insulin resistance based on insulinemias during the oral glucose tolerance test (OGTT) (here in after "insulinemias during the OGTT"), as well as, frequency of glucose curve shapes (monophasic, biphasic, triphasic) in patients with PCOS. Also, the aim of research was testing the correlation between glucose curve shape and peak time of glucose during the OGTT with presence of insulin resistance in patients with PCOS. **Methods.** Patients were observed according to the following parameters: presence of insulin resistance, glucose curve shape and peak time of glucose and insulin during the OGTT. **Results.** The observed prevalence of insulin resistance in the PCOS group according to the HOMA-IR > 2.5 was 66.19% and according to insulinemias during the OGTT was 78.42%. The shape of the glucose curve was monophasic in 293 (70.26%), biphasic in 56 (13.43%) and triphasic in 68 (16.31%) of patients. There was statistically significant difference in the frequency of insulin resistance, according to glucose curve shape, only when it was defined by insulinemias during the OGTT ($p = 0.005$). **Conclusion.** According to results of the study, the most patients with PCOS have a monophasic shape of glucose curve. When we take frequency of insulin resistance in account, we notice approximately the same frequency in all types of curves, when it is defined by the HOMA-IR. On the other hand, when insulin resistance is defined by insulinemias during the OGTT, resistant patients with PCOS mostly have triphasic glucose curve shape.

Key words:
polycystic ovary syndrome; glucose tolerance test; blood glucose, insulin resistance.

Apstrakt

Uvod/Cilj. Učestalost insulinske rezistencije u grupi bolesnica sa sindromom policističnih jajnika (PCOS) kreće se od 60% do 80%, dok u populaciji gojaznih žena sa PCOS iznosi čak 95%. Ranija istraživanja su pokazala da oblik glikemijske krive u toku testa oralnog opterećenja glukozom (OGTT) može biti koristan metabolički skrining parametar za rizik od nastanka dijabetesa melitusa tip II. Cilj rada bio je odrediti učestalost insulinske rezistencije prema homeostaznom modelu procene insulinske rezistencije (HOMA-IR) i metodom indirektne procene insulinske rezistencije na osnovu insulinemija u toku OGTT (u daljem tekstu "vrednosti insulinemija u toku OGTT"), kao i učestalost oblika glikemijske krive (monofazne, bifazne, trifazne) u populaciji bolesnica sa PCOS. Takođe, cilj studije je bio ispitivanje povezanosti oblika glikemijske krive, kao i vremena glikemijskog pika u toku OGTT sa insulinemijama, sa prisustvom insulinske rezistencije u populaciji žena sa PCOS. **Metode.** Bolesnice smo posmatrali u odnosu na prisustvo insulinske rezistencije, oblik glikemijske krive i u odnosu na vreme glikemijskog i insulinskog pika u toku OGTT. **Rezultati.** Učestalost insulinske rezistencije na osnovu HOMA-IR > 2,5 iznosila je 66,19%, dok je na osnovu vrednosti insulinemija u toku OGTT iznosila 78,42%. Monofazni oblik glikemijske krive

Correspondence to: Miljan Pupovac, University of Belgrade, Faculty of Medicine, Dr Subotića Street 8, 11 000 Belgrade, Serbia. E-mail: miljanpupovac@gmail.com

u toku OGTT je imalo 293 (70,26%), bifazni 56 (13,43%), a trifazni oblik krive 68 (16,31%) bolesnica. Statistički značajna razlika u pogledu učestalosti insulinske rezistencije u odnosu na oblik glikemijske krive registrovana je kada je insulinska rezistencija bila definisana na osnovu vrednosti insulinemija u toku OGTT (p = 0,005). **Zaključak.** Prema rezultatima studije, većina bolesnica sa PCOS ima monofazni oblik glikemijske krive. Kada govorimo o učestalosti insulinske rezistencije u odnosu na oblik krive, uočava se približno ista učestalost kada insulinsku rezistenciju definišemo po HOMA-IR. S druge strane, kada insulinsku rezistenciju definišemo na osnovu vrednosti insulinemija u toku OGTT, bolesnice sa insulinskom rezistencijom većinom imaju trifazni oblik glikemijske krive.

Ključne reči:
jajnik, policistični, sindrom; glukoza, test tolerancije; glikemija; insulin, rezistencija.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrinopathy affecting 6–10% of reproductive-aged women [1–3]. Beside the reproductive dysfunction, androgenic excess and polycystical structure of ovaries, which stands for the basic parameters that define this syndrome, the special attention should be paid to metabolic aspect of this complex disorder. PCOS is a significant risk factor in the occurrence of glucose intolerance (IGT), metabolic syndrome and diabetes mellitus type II in females [4, 5]. The frequency of insulin resistance in a group of patients with PCOS varies from 60–80%, while in a group of obese women with PCOS it goes up to 95% [6, 7].

The oral glucose tolerance test (OGTT) with determining the level of insulinemia is used as an accepted clinical method in assessing the occurrence of insulin resistance. It is most commonly used in patients suffering from PCOS. Earlier studies showed that the shape of glucose curve during the OGTT can be used as a useful metabolic screening parameter [8] for a risk of occurring diabetes mellitus type II [9]. Monophasic glucose curve shape goes in favour of the worsening of insulin sensitivity and the damage of beta cells [10], while the more complex shapes of glucose curve are correlated with the better glucose tolerance [11].

The aims of this study were: to determine the frequency of insulin resistance in a population of females, suffering from PCOS according to the Homeostasis model assessment of insulin resistance (HOMA-IR) and according to indirect assessment method of insulin resistance based on insulinemias during the OGTT (here in after "insulinemias during the OGTT"); to determine the frequency of the shape of the glucose curve (monophasic, biphasic and triphasic) in a population of females suffering from PCOS; to examine the presence of the correlation between the glucose curve shape and the insulin resistance during the OGTT with determining the level of insulinemias in patients suffering from PCOS; to examine the possible correlation between the time of the glycemic peak during the OGTT with determining the level of insulinemias and the presence of insulin resistance in population of females suffering from PCOS.

Methods

Totally 417 female patients were included in this retrospective study, that were hospitalized on the Department of Gynecological Endocrinology, Clinic for Gynecology and Obstretics, Clinical Center of Serbia, in a period from January 1st, 2017 until December 31st, 2018. Patients were hospitalized due to the plan, regarding the further examination of irregular menstrual cycles or due to the clinical signs of hyperandrogenism. During the hospitalization patients were completely examined, including physical examination, ultrasound examination and laboratory analysis. The blood was taken from each patient in order to determine the basic hormonal status (follicle stimulating hormone – FSH, luteinizing hormone – LH, prolactin, estradiol, progesterone and testosterone) and androgen status (androstendione, 17-OH-progesterone, dehydroepiandrosterone sulfate – DHEA-S, sex hormone binding globulin – SHBG). The OGTT with determining the level of insulinemias was carried out. Patients' data were collected from medical histories.

The diagnosis of PCOS was established based on the Rotterdam criteria [12]. For establishing the diagnosis two out of the following three criteria were necessary to be fulfilled: hyperandrogenism – clinical symptoms (hirsutismus and/or acne) and/or confirming biochemical analysis (increased levels of testosterone and/or androstenedione); chronic anovulation – oligomenorrhea/amenorrhea; polycystic morphology of ovaries – at least 12 follicles with diameter 2–9 mm and volume of at least one ovary should be greater than 10 mL.

Patients, diagnosed with some other endocrinological disease (hyperprolactinaemia, hyper/hypothireoidism, Cushing syndrome, nonclassic adrenal hyperplasia) and also the patients, who were using oral contraceptive therapy or drugs that could affect the metabolism of glucose and have an impact on insulin sensitivity were excluded from this study. All stated data was collected from the medical histories of patients.

The OGGT was performed as follows: first of all, the basic sample for glycemia and insulin level was taken from patients. Hereupon, patients drank a standard 75 g glucose solution, and samples were taken for determining glycemia and insulin at 0, 30, 60, 120 and 180 minutes.

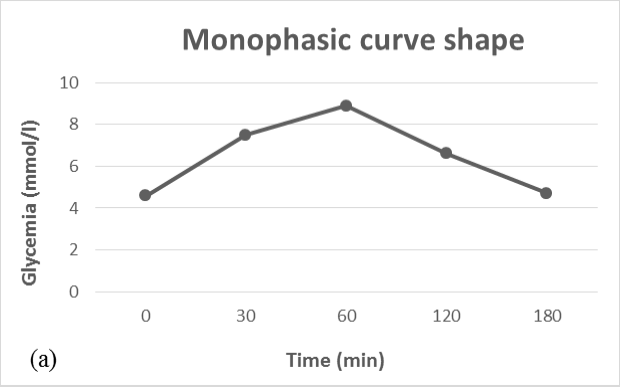
Insulin resistance was defined in 2 different ways. The first way of determining the insulin resistance was by using the HOMA-IR. It was calculated using the following formula: fasting glycemia (mmol/L) × fasting insulinemia (μIU/mL)/22.5 [13]. The borderline of the HOMA-IR was from 2.5 and it was taken from the basic research. Values above mentioned one were defined as insulin resistance.

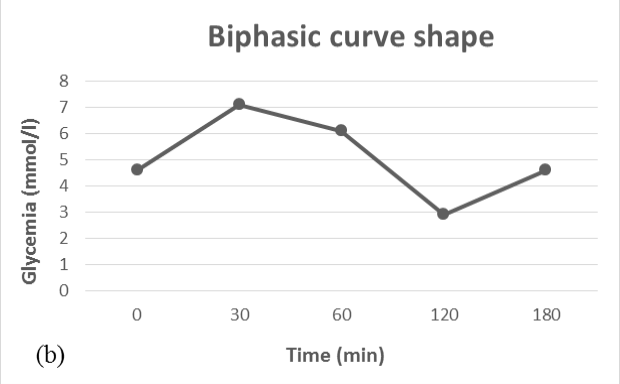
The second indicator of insulin resistance was defined based on borderline values for insulin in 0 minute

(I0 > 22.1 μIU/mL), 60 minute (I60 > 130 μIU/mL) and in 120 minute (I120 > 30 μIU/mL), that were given in Greenspan's Basic & Clinical Endocrinology [14]. We will refer to this method in the text as "indirect assessment method of insulin resistance based on insulinemias during OGTT" ("insulinemias during OGTT").

The glucose curve shape

Monophasic shape of the glucose curve is characterized by the glycemic peak between the 30th and the 120th minute, as well as the significant decrease in glycemia values from minimum 0.25 mmol/L between the 120th and the 180th minute upon starting the OGTT (G180–G120 < -0.25 mmol/L) [8] (Figure 1a).





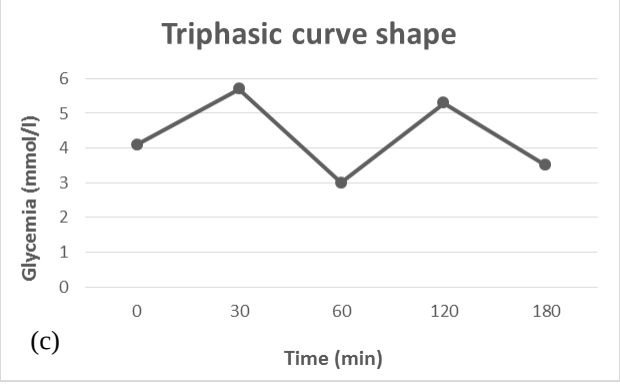


Fig. 1 – The glucose curve shape: (a) Monophasic curve shape; (b) Biphasic curve shape; (c) Triphasic curve shape.

Biphasic shape of glycemic curve is characterized by peak of glycemia in the 30th or the 60th minute, as well as the significant increase in glycemia values between the 120th and the 180th minute (G180–G120 > 0.25 mmol/L). If the peak is reached in the 30th minute, one should have in mind the significant increase of glycemia between 60th and 180th minute (G180–G60 > 0.25 mmol/L) [8, 15] (Figure 1b).

Triphasic shape curve is characterized by peaks in the 30th and 120th minute, as well as the negative peak in the 60th minute. The significant decrease in glycemia values between the 120th and the 180th minute criterion has to be fulfilled (G180–G120 < -0.25 mmol/L) [8, 15] (Figure 1c).

The shape of the curve that is described as unclassified occurs when insignificant changes of glycemia values between the 120th and the 180th minute (< 0.25 mmol/L) are noticed. The patients with this glucose curve shape were excluded from the study.

Statistical analysis

The database was created in Microsoft Office Excel. Statistical data analysis was done using SPSS software 20.0 (Statistical Package for the Social Sciences) for Windows (SPSS Inc., Chicago, IL, USA). For statistical data processing, methods of descriptive and analytical statistics were used. Relative numbers and arithmetic meanings were used from descriptive statistical methods. To test the significance of the difference in arithmetic meanings, the *t*-test and ANOVA were used, while the χ^2 test was used to test the significance of the frequency difference. In case the necessary condition for using the mentioned tests were not fulfilled, the appropriate nonparametric tests were applied: Kruskal-Wallis test and Mann-Whitney test.

Results

Study group consisted of 417 patients suffering from polycystic ovary syndrome. Monophasic shape of glucose curve obtained through the OGTT had 293 (70.26%), biphasic 56 (13.43%) and triphasic shape of glucose curve had 68 (16.31%) patients. By comparing the mean values of body mass index (BMI) in patients with monophasic (23.68 ± 5.09 kg/m^2), biphasic (22.12 ± 3.55 kg/m^2) and triphasic (22.41 ± 4.6 kg/m^2) shape of glucose curve we found the statistically significant difference between these groups ($p < 0.05$). However, these correlations showed no signs of clinical significance, due to the fact that the mean values of BMI in all three groups of patients belong to a group of normally nourished patients.

The frequency of insulin resistance based on the HOMA-IR > 2.5 amounts to 66.19% (276 out of total 417 patients). The majority of women with insulin resistance had a monophasic shape of glucose curve. Elaborately, 198 patients with insulin resistance had a monophasic glucose curve shape, which represents 67.58% of the total number of patients with monophasic shape curve. Additionally, a triphasic shape of glucose curve, that was noticed in 41 patients (60.29% from a total number of patients with triphasic glu-

cose curve shape) was on the second place. Finally, biphasic glucose curve shape was seen in 37 patients with insulin resistance (66.07% of patients with biphasic shape of glucose curve obtained during OGTT). There was no statistically significant difference in comparison between the frequency of insulin resistance and the shape of glucose curve ($p > 0.05$) (Figure 2a).

The frequency of insulin resistance according to insulinemias during the OGTT amounts to 78.42% (327 out of total of 417 patients). The majority of patients with insulin resistance had a monophasic shape of glucose curve. Elaborately, 231 patients had insulin resistance, which represented 78.84% from the total of patients with monophasic shape of glucose curve. Triphasic glucose curve shape, that was noticed in 60 patients (88.23% from a total number of patients with triphasic shape of glucose curve) was on the second place. Finally, biphasic shape of glucose curve was determined in 36 patients with insulin resistance (64.28% of patients with biphasic shape of glucose curve obtained during OGTT). There was a statistically significant difference noticed in comparison between the frequency of insulin resistance and the shape of glucose curve ($p = 0.005$) (Figure 2b).

Regarding the time of the glycemic peak, from the total number of 293 patients with monophasic curve, 146 (49.83%) had a peak time in the 30th minute, 139 (47.44%) in the 60th minute and 8 (2.73 %) in the 120th minute (Figure 3a). When talking about patients with a biphasic curve, from the total number of 56 patients, 44 (78.57%) had a peak time in the 30th minute, 11 (19.64%) had a peak in the 60th minute and 1 (1.79%) in the 180th minute (Figure 3b). As for patients with triphasic glycemic curve, from a total number of 68, 66 (97.06%) patients had a peak time of the 30th minute and 2 (2.94%) in the 120th minute (Figure 3c). There was no statistically significant difference in the frequency of insulin resistance compared to the glycemic time ($p > 0.05$). The same holds true for all criteria of insulin resistance (Figure 3).

Fig. 2 – The frequency of insulin resistance in the group of patients with monophasic, biphasic and triphasic glucose curve shape, when the insulin resistance is defined according to the homeostasis model assessment of insulin resistance (HOMA-IR) > 2.5 (a) and according to insulinemias during the oral glucose tolerance test (OGTT) (b). (-)IR patients without insulin resistance; (+)IR patients with insulin resistance.

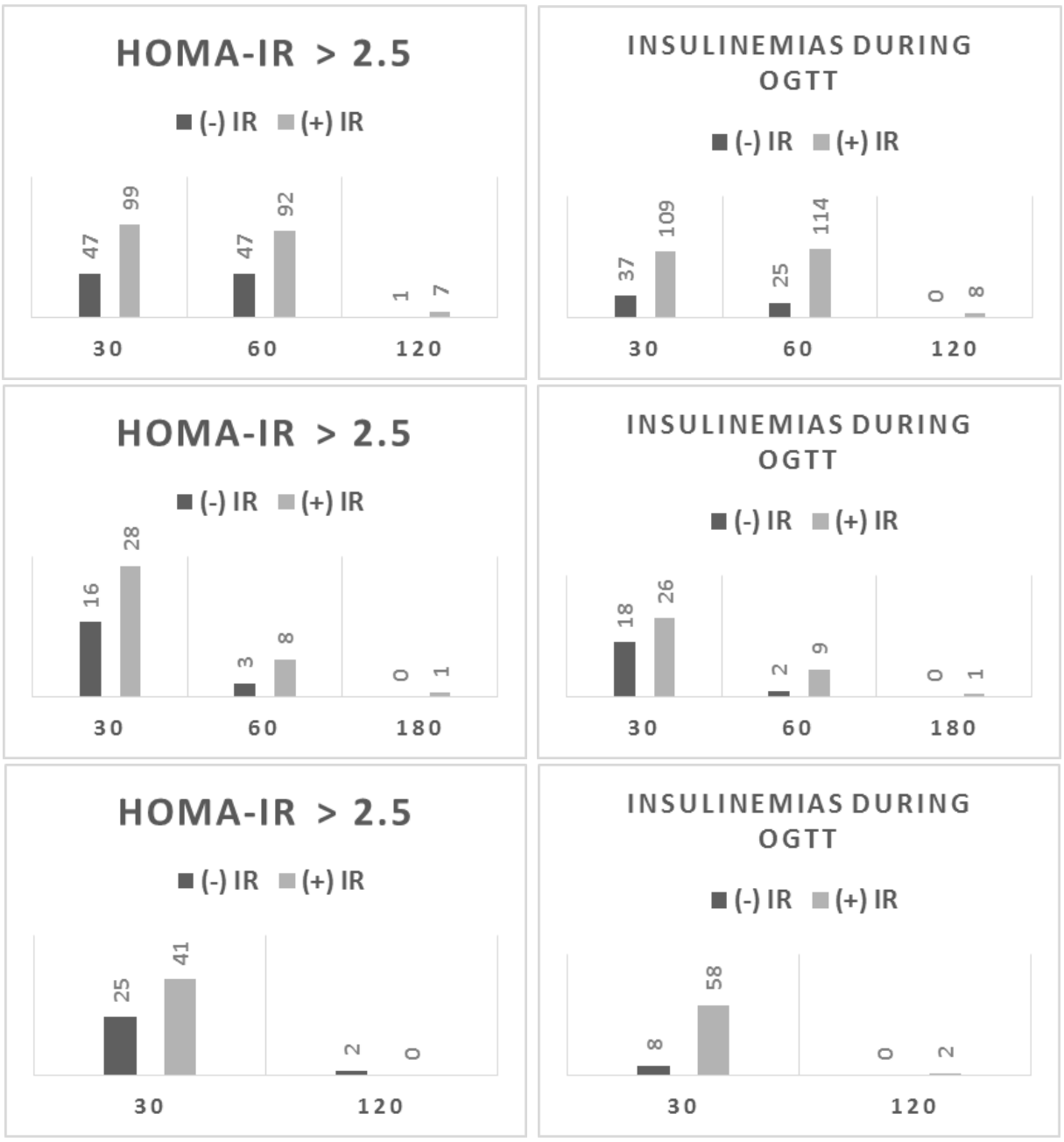


Fig. 3 – The frequency of insulin resistance (IR) in patients with glycemic peak in the 30th, 60th, 120th, 180th minute, when insulin resistance is defined according to the homeostasis model assessment of insulin resistance (HOMA-IR) > 2.5 and according to insulinemias during oral glucose tolerance test (OGTT).
(-)IR patients without insulin resistance; (+)IR patients with insulin resistance.

There was no statistically significant difference regarding the frequency of insulin resistance compared to the time of insulin peak, when it was defined according to the HOMA-IR > 2.5 ($p > 0.05$) (Figure 4a).

There was statistically significant difference regarding the frequency of insulin resistance compared to the time of insulin peak, when it was defined according to insulinemias during the OGTT ($p = 0.002$) (Figure 4b).

When comparing the areas underneath the monophasic, biphasic and triphasic glucose curves (AUCglu) no statistical significance was found ($p > 0.05$). The same holds true for comparing the areas underneath the AUCglu of patients that were suffering from insulin resistance and the ones who were not (especially for the HOMA-IR > 2.5 and according to insulinemias during the OGTT) ($p > 0.05$).

When comparing the areas underneath the insulinemia curve (AUCins) of patients with monophasic, biphasic and triphasic shapes of glucose curves no statistical significance were calculated ($p > 0.05$). When compared the surface underneath the AUCins of patients with insulin resistance and

Fig. 4 – The frequency of insulin resistance (IR) in patients with insulin peak in the 30th, 60th, 120th and 180th minute, when insulin resistance is defined according to the homeostasis model assessment of insulin resistance (HOMA-IR) > 2.5 (a) and according to insulinemias during the oral glucose tolerance test (OGTT) (b).
(-)IR patients without insulin resistance; (+)IR patients with insulin resistance.

the ones without it and when insulin resistance was defined according to the HOMA-IR > 2.5, no statistical significant difference was found ($p > 0.05$). There was a statistically significant difference in the AUCins in patients with insulin resistance and the ones without insulin resistance, when insulin resistance was defined according to insulinemias during the OGTT ($p = 0.003$).

Discussion

The OGTT with determining the level of insulinemias is widely used in clinical praxis, as the most useful method in assessing the presence of insulin resistance. It is mostly used, when patients are suffering from PCOS. Based on the previously acquired data, simple and more practical methods for measuring insulin resistance have been developed. Among them stands homeostasis model assessment of insulin resistance (HOMA-IR) that was used in our current study.

Beside this parameter, in our investigation we used consolidated reference values for insulin in the 0th, 60th, 120th minute of OGTT defined by Greenspan's Basic and Clinical Endocrinology, as indicator of insulin resistance [14].

Due to the fact that there is no consistent method of the OGTT interpretation and that there is no precise way of de-

fining insulin resistance, we wanted to see if the glucose curve shape and the time of glucose peak could be a useful tool in detecting insulin resistance in patients suffering from polycystic ovary syndrome. As far as it is known, based on studying available literature, correlation between glucose curve shape and insulin resistance in women with PCOS is an original idea of this study and it is published in this article for the first time.

Previous research that was performed on this topic, has shown that there is an increased risk of the occurrence of prediabetes in adults with the following morphological characteristics of the glucose curve: the time of glycemic peak after the 30th minute, the glucose concentration in the 60th minute ≥ 8.6 mmol/L and monophasic shape curves [9, 16–18].

It has been observed that most people with normal glucose values and normal insulin sensitivity have peak glycemia in the 30th minute or earlier [15, 19, 20], while on the other hand, the delayed glycemic time (≥ 60 minutes) is observed in adults who are suffering from type II diabetes mellitus [19].

When talking about the time of the glycemic peak of the monophasic curve, an equal number of patients with insulin resistance show an early peak in the 30th minute and a delayed peak at the 60th or the 120th minute.

Based on our results, total insulin response during the OGTT correlates with insulinemias during the test, whereas the HOMA-IR does not correlate. Clinical importance of these two data should be defined and requires further study.

In the study that was engaged in the reproducibility of morphological parameters of the glucose curve during the OGTT, such are the time of the insulin peak, the time of glycemia peak, the shape of the curve, the glucose concentration in 1h after testing, that the time of the glycemic peak proved to be the most reliable parameter [21].

In general, it has been shown that people with biphasic glucose curves have lower BMI, better glucose tolerance, insulin sensitivity, and beta cell function, compared to those patients that are characterized by the monophasic shape of the glucose curve [8, 10, 11].

A more complicated glycemic and insulin response during the 3h-OGTT (that involves a greater number of phases) is associated with better glucose tolerance, beta cell function and greater insulin sensitivity [11].

In our study, the majority of patients were characterized by the monophasic shape of the glucose curve (70.26%), while the frequency of biphasic (13.43%) and triphasic curves (16.31%) was almost identical. Having in mind the results of previous studies that dealt with the shapes of glucose curve in the population of patients with normal glucose tolerance, as well as in the population of patients with impaired glucose tolerance (IGT) and diabetes mellitus, it was expected that the incidence of insulin resistance was the highest in the group of patients with monophasic glucose curve shape, while the more complex shapes of the curve (biphasic and triphasic in our case) should act protective, in the sense of improving the insulin sensitivity. This was not the case in our research. As already mentioned, most patients with PCOS have a monophasic shape of glucose curve, but when the insulin resistance is defined by HOMA-IR, we notice approximately the same frequency. On the other hand, when the insulin resistance is defined by insulinemias during the OGTT, the frequency of insulin resistance in the population of patients with triphasic shape of the glucose curve is as high as 88.23%.

Conclusion

According to results of the study, the most patients with PCOS have a monophasic shape of glucose curve. When we take frequency of insulin resistance into account, we notice approximately the same frequency in all types of curves, when it is defined by the HOMA-IR. On the other hand, when insulin resistance is defined by insulinemias during the OGTT, resistant patients with PCOS mostly have triphasic glucose curve shape. The time of glycemic peak is not related to the frequency of insulin resistance in patients with PCOS, under no criteria of insulin resistance.

REFERENCES

1. *Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG,* et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab 1999; 84(11): 4006–11.
2. *Asunción M, CalvoRM, San Milla´n JL, Sancho J, Avila S, Escobar-Morreale HF.* A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Meta. 2000; 85(7): 2434–8.
3. *Azziz R, WoodsKS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO.* The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 2004; 89(6): 2745–9.
4. *Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS.* Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome. Endocr Rev 2015; 36(5): 487–525.
5. *Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W,* et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. J Clin Endocrinol Metab 2010; 95(5): 2038–49.
6. *Carmina E, Lobo RA.* Use of fasting blood to assess the prevalence of insulin resistance in women with polycystic ovary syndrome. Fertil Steril 2004; 82(3): 661–5.
7. *DeUgarte CM, Bartolucci AA, Azziz R.* Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. Fertil Steril 2005; 83(5): 1454–60.
8. *Tschritter O, Fritsche A, Shirkavand F, Machicao F, Häring H, Stumvoll M.* Assessing the shape of the glucose curve during an oral glucose tolerance test. Diabetes Care 2003; 26(4): 1026–33.
9. *Abdul-Ghani MA, Lyssenko V, Tuomi T, Defronzo RA, Groop L.* The shape of plasma glucose concentration curve during OGTT predicts future risk of type 2 diabetes. Diabetes Metab Res Rev 2010; 26(4): 280–6.
10. *Kanauchi M, Kimura K, Kanauchi K, Saito Y.* Beta-cell function and insulin sensitivity contribute to the shape of plasma glu-

cose curve during an oral glucose tolerance test in non diabetic individuals. Int J Clin Pract 2005; 59(4): 427–32.

11. *Tura A, Morbiducci U, Sbrignadello S, Winhofer Y, Pacini G, Kautzky-Willer A.* Shape of glucose, insulin, C-peptide curves during a 3-h oral glucose tolerance test: any relationship with the degree of glucose tolerance? Am J Physiol Regul Integr Comp Physiol 2011; 300(4): R941–8.
12. *The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group.* Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19(1): 41–7.
13. *Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.* Homeostasis model assessment: insulin resistance and beta-cell functionfrom fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28(7): 412–9.
14. Apendix: Normal Hormone Reference Ranges. In: *Gardner DG, Shoback D,* editors. Greenspan's Basic and Clinical Endocrinology, 10th ed. The International Edition: The McGraw-Hill Companies; 2018. p. 847–68.
15. *Nolfe G, Spreghini MR, Sforza RW, Morino G, Manco M.* Beyond the morphology of the glucose curve following an oral glucose tolerance test in obese youth. Eur J Endocrinol 2012; 166(1): 107–14.
16. *Bianchi C, Miccoli R, Trombetta M, Giorgino F, Frontoni S, Faloia E,* et al. Elevated 1-hour postload plasma glucose levels identify subjects with normal glucose tolerance but impaired beta-cell function, insulin resistance, and worse cardiovascular risk profile: the GENFIEV study. J Clin Endocrinol Metab 2013; 98(5): 2100–5.
17. *Chung ST, Kasturi K, Bingham BA, Onuzuruike A, Baker RL, Utumatwishima JN,* et al. Time to Glucose Peak During an Oral Glucose Tolerance Test Identifies High Risk for Prediabetes: Results from a Multiethnic Study. Endocr Soc 2017; 2017: OR14.
18. *Kim J, Michaliszyn S, Nasr A, Lee S, Tfayli H, Hannon T,* et al. The Shape of the Glucose Response Curve During an Oral Glucose Tolerance Test Heralds Biomarkers of Type 2 Diabetes Risk in Obese Youth. Diabetes Care 2016; 39(8): 1431–9.
19. *Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA.* Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes 2006; 55(5):1430–5.
20. *Yeckel CW, Taksali SE, Dziura J, Weiss R, Burgert TS, Sherwin RS,* et al. The normal glucose tolerance continuum in obese youth: evidence for impairment in beta-cell function independent of insulin resistance. J Clin Endocrinol Metab 2005; 90(2): 747–54.
21. *Kramer CK, Vuksan V, Choi H, Zinman B, Retnakaran R.* Emerging parameters of the insulin and glucose response on the oral glucose tolerance test: reproducibility and implications for glucose homeostasis in individuals with and without diabetes. Diabetes research and clinical practice. 2014; 105(1): 88–95.

Received on July 15, 2019.
Revised on August 18, 2019.
Accepted August 20, 2019.
Online First September, 2019.

ORIGINAL ARTICLE

UDC: 539.1:61]:617.581/.583-089.161-022.7-07
DOI: https://doi.org/10.2298/VSP190807101P

Rational and reliable diagnosis of periprosthetic hip and knee joint infection using two nuclear-medicine methods: ^{99m}Tc-ciprofloxacin and ^{99m}Tc-MDP scintigraphy

Racionalna i pouzdana dijagnoza periprotetske infekcije kuka i kolena korišćenjem dve nuklearno-medicinske metode: ^{99m}Tc-ciprofloksacin i ^{99m}Tc-MDP scintigrafije

Dragan Pucar*†, Zoran Janković*†, Srdjan Starčević†‡

Military Medical Academy, *Institute of Nuclear Medicine, ‡Clinic for Orthopedic Surgery and Traumatology, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. There is a constant and dramatic increase in hip and knee prosthetic implantations worldwide. However, a decrease in the percentage of failed (infected) prosthetic implantations is not significant (0.5% up to 2%). The real challenge is whether prosthetic joint loosening was caused by aseptic inflammation or by infection. The aim of the present study was to attempt to distinguish sterile inflammation from infection in patients with a painful hip or knee prosthetic joint. Another objective was to determine the accuracy of cumulative bone scintigraphy with methylenediphosphonate (MDP) and ciprofloxacin in diagnosis of periprosthetic joint infection (PJI). **Methods.** Three-phase bone scintigraphy with ^{99m}Tc-MDP and ^{99m}Tc-ciprofloxacin was used. The patient selection criterion for this study was a suspicion of PJI followed by painful and limited movement of the prosthetic joint, accompanied by elevated unspecific inflammatory factors. Forty five patients with 39 implanted hips and 24 knee prostheses were included and evaluated. All prosthetic joints were examined (although some of them were asymptomatic) and underwent plain radiography. An average time span between the two nuclear medicine imaging procedures was 3–5 days. Three-phase ^{99m}Tc-MDP bone scintigraphy was performed first. Scintigraphy with ^{99m}Tc-ciprofloxacin was necessarily involved calculation of the accumulation index. The obtained results were confirmed by microbiological findings as a gold standard. Statistical analysis of the results was performed using SPSS version 20 software (descriptive statistics, χ^2-test). Sensitivity, specificity, and predictive values were also calculated. **Results**. Microbiologically confirmed PJI was found in 16 out of 39 hip prostheses. Positive scintigraphy was obtained in 15 out of 39 prosthetic hip joints. PJI was found using scintigraphy of the knee, and microbiologically confirmed in all 13 out of 24 suspected joints. Estimated sensitivity/specificity of ^{99m}Tc-MDP bone scintigraphy alone (for both joints) was 90%/69%, for ^{99m}Tc-ciprofloxacin scintigraphy it was 93%/97%, and for cumulative results it was 96.5%/97%. **Conclusion.** Cumulative ^{99m}Tc-MDP scintigraphy with ^{99m}Tc-ciprofloxacin scintigraphy increases the ability of differentiation between aseptic loosening and PJI, with high accuracy of 97%.

Key words:
technetium tc 99m ciprofloxcin; technetium tc 99m medronate; radionuclide imaging; hip prosthesis; knee prosthesis; infection.

Apstrakt

Uvod/Cilj. Postoji dramatičan i konstantan porast ugradnje novih proteza kuka i kolena širom sveta. Međutim, procenat neuspelih (inficiranih) protetskih implantacija se ne smanjuje značajno (0,5% do 2%). Pravi izazov je pitanje da li je nastalo razlabavljenje protetskog zgloba uzrokovano aseptičnom upalom ili infekcijom. Cilj studije bio je pokušaj odvajanja sterilne upale od infekcije kod bolesnika sa bolnim protetskim zglobovima kuka ili kolena. Pored toga, naš cilj je bio da utvrdimo tačnost kombinovane scintigrafije kostiju metilendifosfonatom (MDP) i ciprofloksacinom u dijagnostici periprotetske infekcije zglobova (PJI). **Metode**. Korišćena je trofazna scintigrafija kostiju sa ^{99m}Tc-MDP i ^{99m}Tc-ciprofloksacin. Kriterijum za odabir bolesnika za ovo istraživanje bila je sumnja na PJI praćenu bolom i ograničenim pokretima protetskog zgloba uz povišenje nespecifičnih faktora upale. Uključeno je i procenjeno 45 bolesnika sa implantiranih 39 proteza kuka i 24 proteze kolena. Pregledani su svi protetski zglobovi (iako su neki zglobovi bili asimptomatski) i kod svih je bila urađena planarna radiografija. Vremenski razmak između izvedenih

Correspondence to: Dragan Pucar, Military Medical Academy, Institute of nuclear medicine, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: draganpucar@yahoo.com

nuklearno-medicinskih metoda iznosio je u proseku 3–5 dana. Prvo je izvođena trofazna ^{99m}Tc-MDP scintigrafija kostiju. Scintigrafiju ^{99m}Tc-ciprofloksacinom obavezno je pratilo izračunavanje indeksa akumulacije. Dobijeni rezultati su bili potvrđeni mikrobiološkim nalazima kao zlatnim standardom. Statistička analiza dobijenih rezultata urađena je pomoću softvera SPSS, verzija 20 (deskriptivna statistika, χ^2-test). Takođe su bili izračunati osetljivost, specifičnost i prediktivne vrednosti. **Rezultati**. Mikrobiološki potvrđene PJI bile su prisutne kod 16 od 39 proteza kuka. Pozitivna scintigrafija je dobijena kod 15 od 39 proteza kuka. Scintigrafija kolena je bila pozitivna kod svih 13 od 24 protetska zgloba kolena koji su imali mikrobiološku potvrdu PJI. Izračunata osetljivost/specifičnost za ^{99m}Tc-MDP scintigrafiju kosti (za oba zgloba) iznosila je 90%/69%, za ^{99m}Tc-ciprofloksacin scintigrafiju 93%/97%, a za kombinaciju obe scintigrafije 96,5%/97%. **Zaključak.** Kombinovana scintigrafija sa ^{99m}Tc-MDP i ^{99m}Tc-ciprofloksacinom povećava sposobnost razlikovanja aseptičnog labavljenja protetskog zgloba od PJI sa visokom tačnošću od 97%.

Ključne reči:
tehnecijum tc 99m ciprofloksacin; tehnecijum tc 99m medronat; radioizotopsko snimanje; kuk, proteza; koleno, proteza; infekcija.

Introduction

One of the most important advances in surgery in the last few decades was the joint replacement. This routine surgical procedure improves patients' quality of life by providing pain relief and regaining of joint function, but also patient mobility and independence from others persons [1]. Implantation of hip and knee prostheses makes up more than 95% of all joint replacements. Joint replacements of the hip and knee are highly successful surgical interventions. The most common complications of that surgical procedure are aseptic failure and periprosthetic joint infection (PJI). About 25% of all prostheses will demonstrate evidence of loosening, especially after revision arthroplasty [2]. But infection is one of the most unpleasant complications. Many studies suggest that in patients with primary joint replacement, the infection rate in the first 2 years is usually in the range of 0.5%–2% [3]. There is an increasing number of studies indicating that the reported infection rate is probably underestimated, since many cases regarded as aseptic failure may be due to unrecognized infection [4]. PJI after surgical revision is usually more frequent (even 25% to 40%) than after primary replacement [5]. Surgical site infections are the most important risk factor for infection, although surgical complexity, osseous tissue status surrounding the prosthesis, immune status of the patient, previous total hip or knee arthroplasty, older age, malnutrition, joint disease such as rheumatoid arthritis, obesity, diabetes mellitus, remote infection, are also important [4, 6, 7]. Commonly isolated bacteria were Gram-positive *cocci*: *Coagulase-negative staphylococcus* (CoNS), *Staphylococcus aureus* and *Enterococci* (65%). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels are neither sensitive nor specific for PJI. Differentiation of PJI from aseptic loosening is very important because treatments are completely different. Plain radiography is always performed but it is not specific enough [8]. Computed tomography (CT) provides a better contrast between normal and abnormal tissue, bone erosion, and it is useful in detecting sinus tracts [9]. Magnetic resonance imaging (MRI) can only be performed in patients with titanium or tantalum implants [9]. Optimal treatment of PJI represents a real challenge. It is difficult to distinguish superficial wound infections or cellulitis from true periprosthetic infections [10].

It is generally accepted that nuclear medicine is a modality of choice among imaging diagnostic methods for evaluation of suspected PJI. Bone scintigraphy with methylene diphosphonate (MDP) is extremely sensitive for detection of bone remodelling changes, but it has low specificity for infection. Labelled white blood cells (WBC) are generally preferred for osteomyelitis and PJI, but the labelling of leukocytes is complicated and does not have high specificity [11]. ^{67}Gallium (^{67}Ga) imaging is superior only in vertebral disc infections, but increased uptake of ^{67}Ga can occur in fractures and tumours (not specific for PJI) [11, 12]. Long ago, the labelling of antibiotics was considered a potentially good tracer. Ciprofloxacin labelled with ^{99m}Tc was introduced in the 1990s [13, 14]. This radiopharmaceutical was more specific in infection but not easy to interpret only by visual assessment [15, 16]. Calculation of the accumulation index is the only rational way of distinguishing between aseptic inflammation and infection, based on increased uptake on scintigrams [15–19]. Considering the most commonly used radiopharmaceuticals for the detection of PJI, Love et al. [20], in a review article, listed examples of almost all relevant nuclear medical methods with unfortunately unsatisfactory sensitivity and specificity. The authors only single out the successful combination of labelled leukocytes and bone marrow scintigraphy (sensitivity of 96%, specificity of 87% and accuracy of 91%). The use of fusion images obtained with single-photon emission computed tomography (SPECT/CT) gamma cameras when using ^{99m}Tc-cipofloxacin provides a better contrast and clearer differentiation between soft tissue structures and bone. The procedure (one-day protocol) [21] is also simpler.

The aim of this study was to confirm or reject good diagnostic accuracy in differentiation between aseptic loosening and PJI of the prosthetic hip and knee, obtained in a preliminary study [22], but now involving a larger number of patients. This study also combined bone scintigraphy with ^{99m}Tc-MDP and ^{99m}Tc-ciprofloxacin.

Methods

Patient selection criteria for this study were based on suspected PJI: a painful prosthetic joint, restricted joint movements, and increased values of ESR and CRP. The study included 45 patients (14 men and 29 women) with a to-

tal of 63 implanted joints (39 hip and 24 knee prostheses), median age 68.6 years, range 43–82 years (Figure 1).

All patients also underwent plain radiography. In all patients, three-phase ^{99m}Tc-MDP bone scintigraphy was performed. Scintigrams were analyzed visually without any quantification. The results obtained with ^{99m}Tc-MDP were interpreted visually as clearly positive/negative or as borderline positive or borderline negative (unconvincingly positive/negative). Three to five days after the bone scan, we performed scintigraphy using ^{99m}Tc-ciprofloxacin with necessary quantification and calculation of the accumulation index. The value of accumulation index above 1.5 is considered positive for infection, while the value below 1.5 indicates aseptic inflammation [17]. Accumulation index values from 1.41 to 1.50 were considered borderline negative, and those from 1.51 to 1.60 as borderline positive [17, 18]. PJI was confirmed on the basis of microbiological or histopathological findings.

We used the SPSS statistical software version 20 (descriptive statistics, χ^2 test). Sensitivity, specificity, and predictive values were also calculated.

Results

Microbiological analysis of PJI was confirmed in 29 out of 45 patients (Table 1). Regarding these microbiological results as the total number of evaluated hip and knee prosthetic joints (63), PJI was confirmed in 16 (41%) of 39 hip prostheses and in 13 (54.2%) of 24 knee prosthetic joints.

Diagnostic results of PJI obtained with bone scintigraphy with ^{99m}Tc-MDP are shown in Table 2, and diagnostic results of PJI obtained with bone scintigraphy with ^{99m}Tc-ciprofloxacin in Table 3. Both scintigraphic modalities

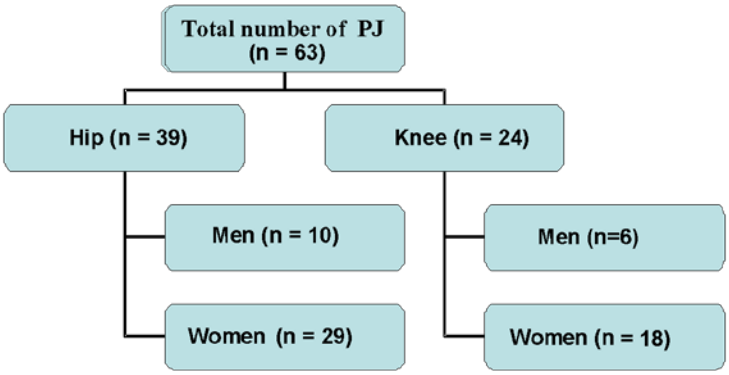


Fig. 1 – Total number of examined prosthetic joints with insight into the number of hip or knee prostheses, as well as their distribution by gender.

Table 1

Microbiologically confirmed periprosthetic joint infection (PJI) was found in 29 of 45 patients although unspecific parameters of inflammation were found in almost all patients

PJI findings	Patients, n (%)
Microbiological confirmation	
unconfirmed	16 (35.6)
confirmed	29 (64.4)
Biochemical indicators	
elevated CRP	43 (95.6)
elevated ESR	42 (93.3)

CRP – C-reactive protein; ESR – erythrocyte sedimentation rate.

Table 2

Results obtained with a ^{99m}Tc-MDP bone scan showed statistical significance

Finding	Infection, n (%)		Total
	no	yes	
Negative	9 (100)	0 (0)	9 (100)
Borderline negative	14 (82.4)	3 (17.6)	17 (100)
Borderline positive	5 (45.5)	6 (54.6)	11 (100)
Positive	6 (23.1)	20 (76.9)	26 (100)
Total	34 (54)	29 (46)	63 (100)
Significance assessment	$\chi^2 = 23.5$;	$p < 0.001$	

MDP – methylenediphosphonate.

(MDP and ciprofloxacin scintigraphy) were statistically processed using χ^2-test.

When the final decision on the presence of PJI was made on the basis of cumulative findings (^{99m}Tc-MDP scintigraphy and ^{99m}Tc-ciprofloxacin scintigraphy), the results of the diagnosis of PJI were more accurate (Table 4). These cumulative results are shown in Figure 2.

The graph shows that the detection of PJI was almost unmistakable if both methods were combined (^{99m}Tc-MDP and ^{99m}Tc-ciprofloxacin) in case when the results of both methods were positive or negative, but also in borderline cases, the results were reliable.

Different modalities of negative and positive findings of the presence of PJI or aseptic loosening were apparently useful.

Sensitivity, specificity, and accuracy of scintigraphy with ^{99m}Tc-MDP and with ^{99m}Tc labelled ciprofloxacin, as well as the cumulative findings of these two methods, are shown in Table 5.

Cumulative findings obtained by these two methods showed higher positive predictive values (PPV) and negative predictive values (NPV) of PJI than those of these methods alone. Positive findings of both scintigraphies with ^{99m}Tc-

Table 3

Results obtained with ^{99m}Tc-ciprofloxacin scintigraphy in detection of periprosthetic joint infection (PJI) were much better practically in all modalities of the findings

Finding	Infection, n (%)		Total
	no	yes	
Negative	29 (96.7)	1 (3.3)	30 (100)
Borderline negative	4 (80)	1 (20)	5 (100)
Borderline positive	1 (12.5)	7 (87.5)	8 (100)
Positive	0 (0)	20 (100)	20 (100)
Total	34 (54)	29 (46)	63 (100)
Significance assessment	$\chi^2 = 52.4$;	$p < 0.001$	

Table 4

Results obtained with combined scintigraphy that used both radiopharmaceuticals (^{99m}Tc-MDP and ^{99m}Tc-ciprofloxacin)

Cumulative findings of ^{99m}Tc-MDP/ ^{99m}Tc-ciprofloxacin	Infection, n (%)		Total
	no	yes	
Negative	22 (100)	0 (0)	22 (100)
Borderline negative	11(91.7)	1 (8.3)	12 (100)
Borderline positive	1 (8.3)	11 (91.7)	12 (100)
Positive findings	0 (0)	17 (100)	17 (100)
Total	34 (54)	29 (46)	63 (100)
Significance assessment	$\chi^2 = 55.6$;	$p < 0.001$	

MDP – methylenediphosphonate.

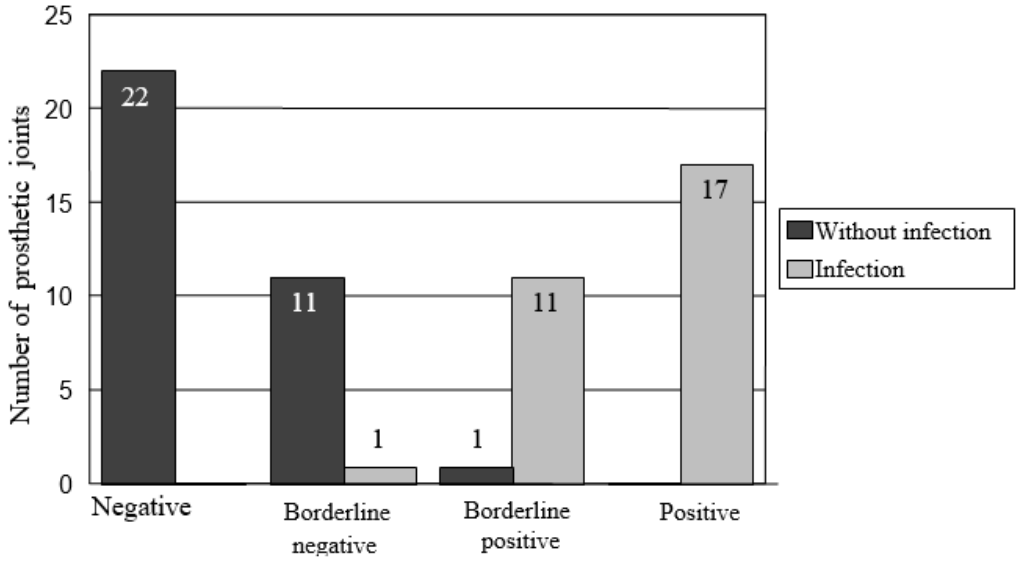


Fig. 2 – Cumulative results obtained by using both methods (^{99m}Tc-MDP and ^{99m}Tc-ciprofloxacin).
MDP – methylendiphosphonate.

MDP and ^{99m}Tc-ciprofloxacin that clearly indicated the presence of PJI are shown in Table 6.

Positive scintigraphy obtained by three-phase ^{99m}Tc-MDP is shown in Figure 3, and positive scintigraphy with ^{99m}Tc-ciprofloxacin is presented in Figure 4.

Positive scintigraphy with ^{99m}Tc-MDP but negative scintigraphy with ^{99m}Tc-ciprofloxacin indicate aseptic loosening (Figures 5 and 6).

High NPV of scintigraphy with ^{99m}Tc-MDP indicates that there is no need for ^{99m}Tc-ciprofloxacin scintigraphy. Increased accumulation of tracer in the left hip joint suggests left hip coxarthrosis (Figure 7).

Table 5

Cumulative findings (^{99m}Tc-MDP/^{99m}Tc-ciprofloxacin) gave the highest accuracy

Scintigraphy	Sensitivity %	Specificity %	Accuracy %
^{99m}Tc-MDP	90	69.7	79
^{99m}Tc-ciprofloxacin	93	97	95
Cumulative findings based on both scintigraphy	98.5	95.5	97

MDP – methylenediphosphonate.

Table 6

Positive and negative predictive values (PPV and NPV, respectively) for scintigraphy with ^{99m}Tc-MDP, ^{99m}Tc-ciprofloxacin, and their combination

Scintigraphy method	PPV (%)	NPV (%)
^{99m}Tc-MDP	70	90.5
^{99m}Tc-ciprofloxacin	96%	94
^{99m}Tc-MDP/^{99m}Tc-ciprofloxacin	96.5	97

MDP – methylendiphosphonate.

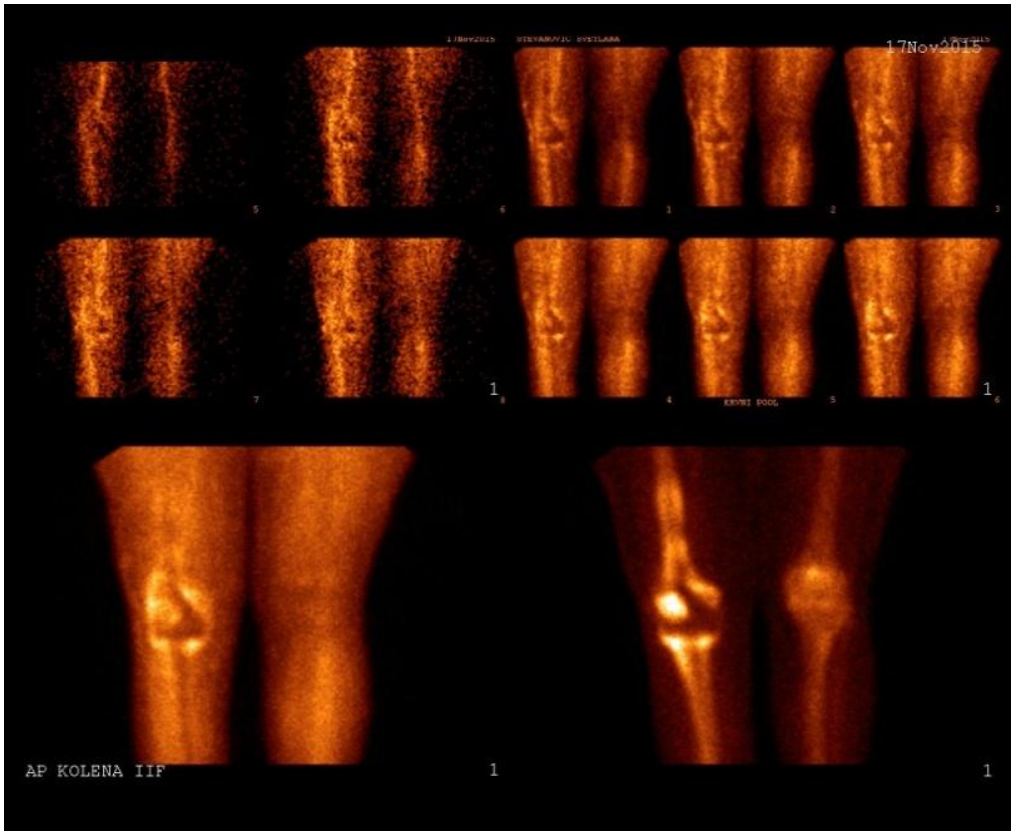


Fig. 3 – ^{99m}Tc-MDP scintigraphy of a 60-year-old female, with a right knee joint replacement. In the region of the right knee replacement we observed increased activity at a flow and pool pattern at the proximal tibia heel. On the last static scintigram we also observed increased activity indicating infection at the same place.
MDP – methylendiphosphonate.

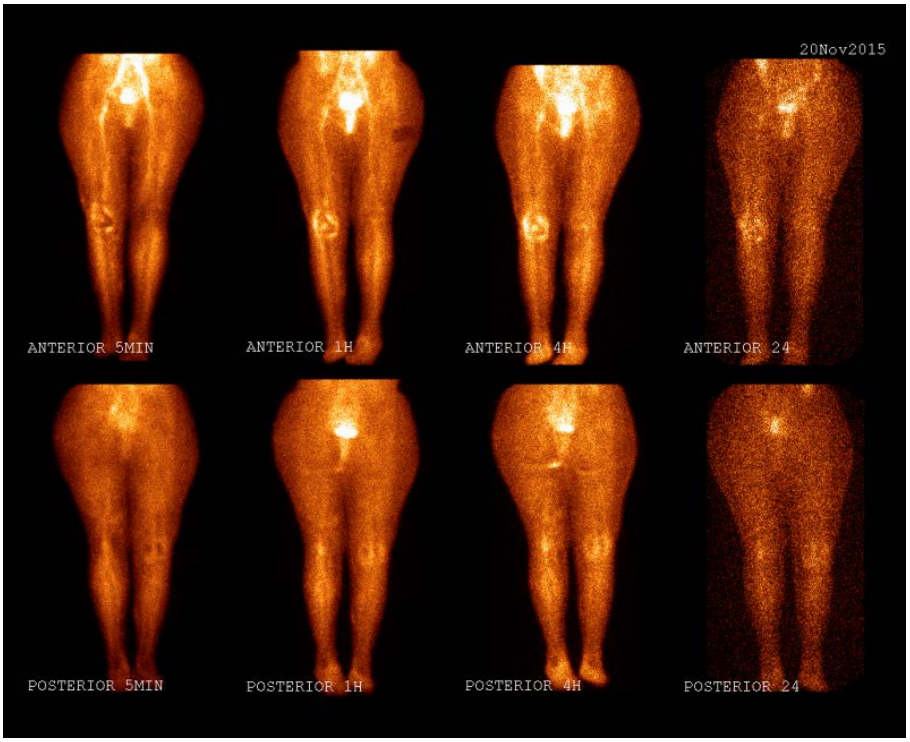


Fig. 4 – ^{99m}Tc-ciprofloxacin scintigraphy showed increased uptake in the region of the right knee prosthesis, while the accumulation index at all times of imaging was significantly higher than the "cut off" value of 1.5. Final microbiological confirmation was periprosthetic infection. The right knee prosthesis was removed and antibiotic therapy was applied.

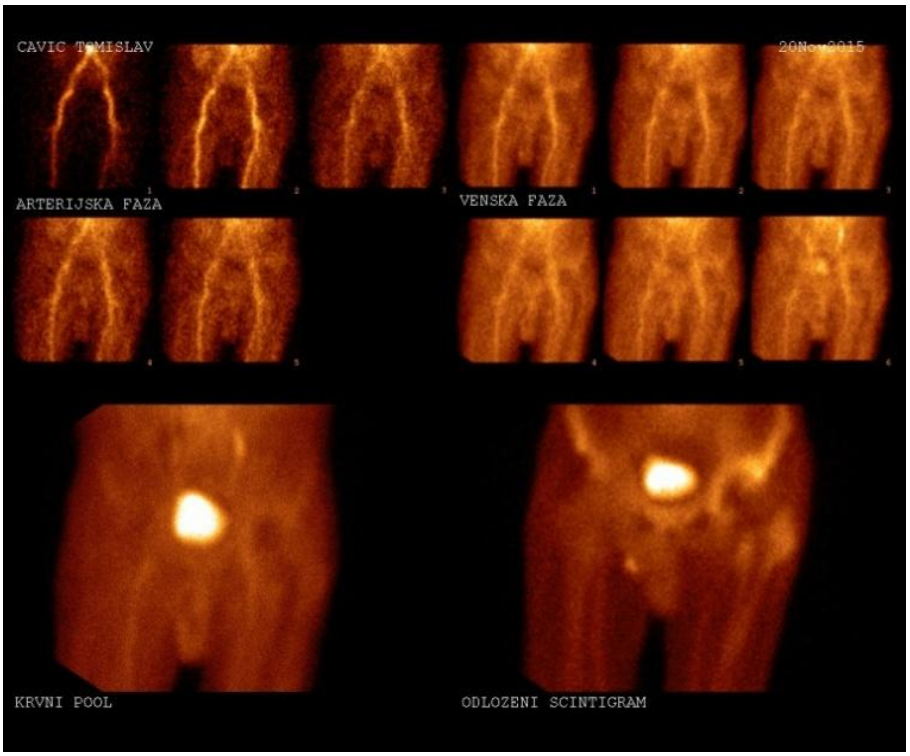


Fig. 5 – Scintigraphy with ^{99m}Tc-MDP of a 77- year-old male with a left hip joint replacement. In the region of the left hip joint replacement we observed increased activity on delayed scintigram, i.e. after 4 hours in the region of acetabulum and a great trochanter indicating possible infection.
MDP – methylendiphosphonate.

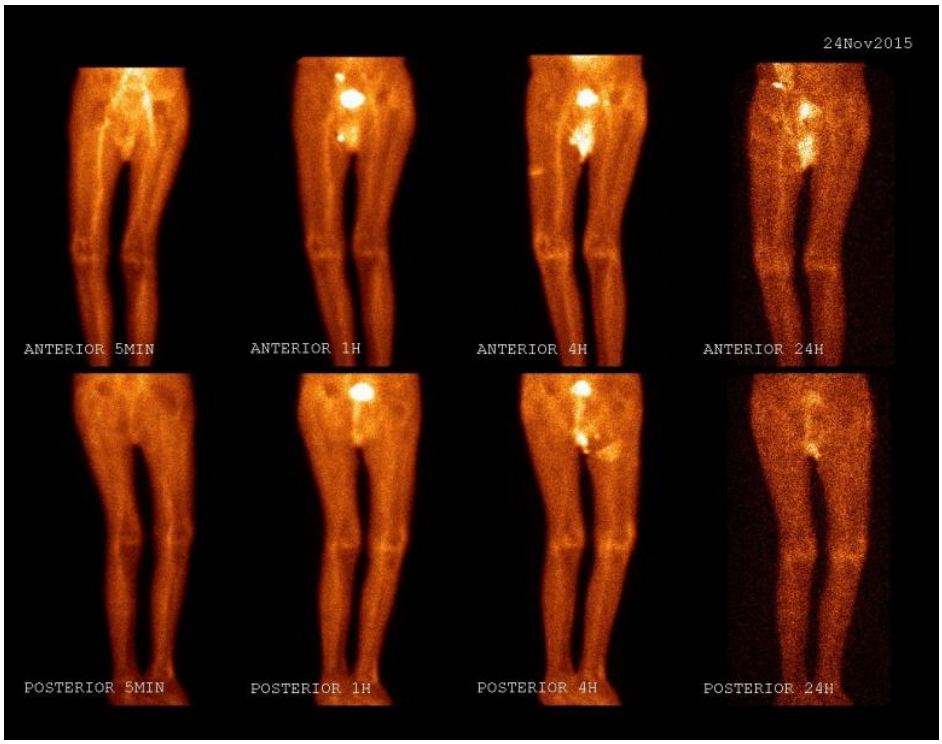


Fig. 6 – ^{99m}Tc-ciprofloxacin scintigraphy (the same patient as in Fig. 5) showed practically no increased uptake in the region of the left hip joint replacement at all times of imaging, indicating aseptic loosening despite positive scintigram with 99mTc-MDP. MDP – methylendiphosphonate.

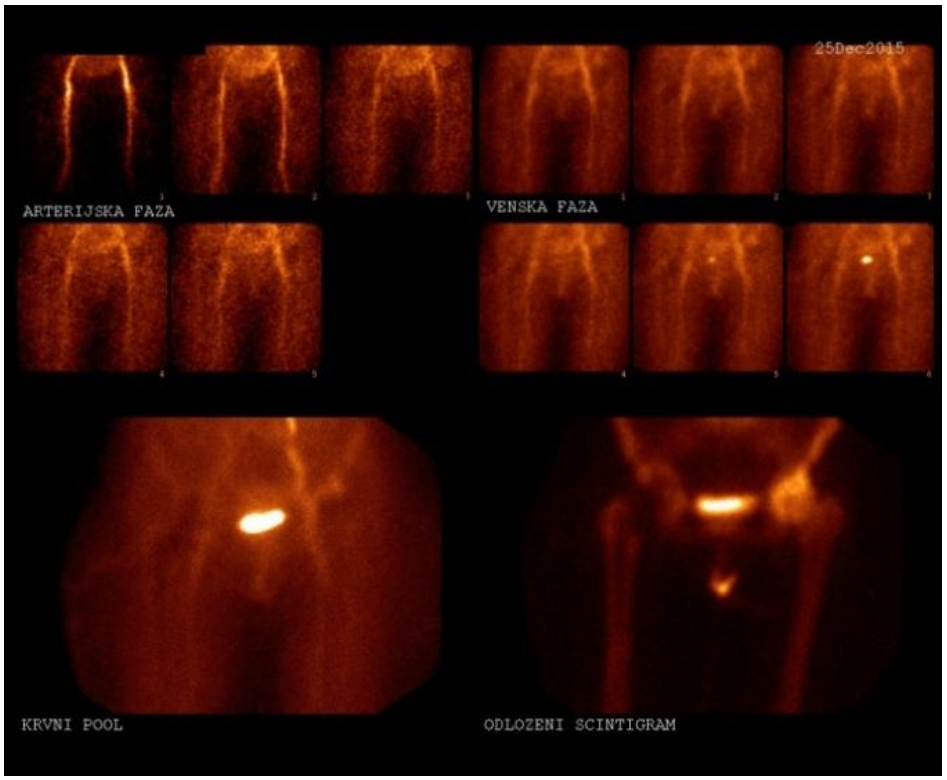


Fig. 7 – Scintigraphy with ^{99m}Tc-MDP of a 73-year-old female with a right hip joint replacement. In the region of the right hip joint replacement we did not observe significantly increased activity in any of the three phases. No need for ^{99m}Tc-ciprofloxacin scintigraphy. Diagnosis was *Coxarthrosis sin*. MDP – methylendiphosphonate.

Discussion

Combined assessment of painful prosthetic joints with limited motion by two scintigraphic methods (^{99m}Tc-MDP and ^{99m}Tc-ciprofloxacin), in the most part resulted in definitive findings: aseptic instability or PJI.

A normal bone scan with ^{99m}Tc-MDP is a scan in which periprosthetic activity of the investigated joint is practically adjacent to non-articular bone or the healthy contralateral joint. A three-phase bone study is usually performed because of its sensitivity for detection of bone remodelling changes around prosthetic joints, and when the orthopaedic surgeon suspects PJI. Three-phase MDP bone scintigraphy is also usually performed when there is suspicion of cellulitis, and it demonstrates enhanced perfusion of soft tissue in the region of interest. It is important to include both extremities (affected and unaffected) and to set them symmetrically [11]. Nagoya et al. [23] reported that the test was 88% sensitive and 90% specific for hip replacement infection. Many other authors, however, reported good sensitivity, unsatisfactory specificity, or both [4, 10, 24]. Since MDP bone scintigraphy has a high NPV, normal results of the study make it very unlikely that the patient's symptoms are related to PJI [10, 23, 24].

It should be noted that bone scintigraphy can remain positive even a year after an uncomplicated hip replacement, and even two years after insertion of a prosthesis without cement (that reflects increased bone mineral turnover). Those scintigrams cannot be used to distinguish infection from an aseptic loosening [10].

We overcame lack of specificity of ^{99m}Tc-MDP scintigraphy in the diagnosis of PJI with an additional, more specific diagnostic method for detection of infection. ^{99m}Tc-ciprofloxacin scintigraphy is a method of high specificity for detection of infection [13, 14, 17, 18]. The great advantage of ^{99m}Tc-ciprofloxacin is that it should not be accumulated in a healthy bone [13, 14]. Our preliminary research, concerning calculation of the accumulation index in ^{99m}Tc-ciprofloxacin scintigraphy, has established the importance of its use in increasing the specificity in the diagnosis of osteomyelitis [17, 18]. Only visual interpretation has low specificity. ^{99m}Tc-MDP bone scintigraphy combined with ^{99m}Tc-ciprofloxacin scintigraphy yielded good results in differentiation of aseptic instability of the prosthetic hip and knee joint from infection of periprosthetic tissue [19].

A limitation of this study is the relatively small number of patients for separate evaluations of the hip and knee joints, but sufficient for an overall evaluation. Consequently, further trials involving a larger number of patients are needed.

Conclusion

Confirmed low specificity but high NPV of the ^{99m}Tc-MDP bone scan in combination with high specificity of ^{99m}Tc-ciprofloxacin significantly facilitates differentiation between aseptic loosening and PJI as a cause of prosthetic failure. ^{99m}Tc-ciprofloxacin scintigraphy has high PPV for detecting periprosthetic infection only if combined with calculation of the accumulation index. Negative results of a MDP bone scan virtually exclude the periprosthetic infection. The study also suggests that in most cases ^{99m}Tc-chiprofloxacin scintigraphy should be performed first due to its high specificity.

REFERENCES

1. *Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM*, et al. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2013; 56(1): e1–e25.
2. *Kurtz* S, *Ong K, Lau E, Mowat F, Halpern M.* Projections of primary and revision hip and knee arthroplasty in the United States from 2005 to 2030. J Bone Joint Surg Am 2007; 89(4): 780–5.
3. *Lentino JR.* Prosthetic joint infections: bane of orthopaedists, challenge for infectious disease specialists. Clin Infect Dis 2003; 36(9): 1157–61.
4. *Palestro CJ.* Nuclear medicine and the failed joint replacement: Past, present, and future. World J Radiol 2014; 6(7): 446–58.
5. *Zimmerli W, Trampuz A, Ochsner PE.* Prosthetic-joint infections. N Engl J Med 2004; 351(16): 1645–54.
6. *Berbari EF, Hanssen AD, Duffy MC, Steckelberg JM, Ilstrup DM, Harmsen WS*, et al. Risk factors for prosthetic joint infection: case-control study. Clin Infect Dis 1998; 27(5): 1247–54.
7. *Bongartz T, Halligan CS, Osmon DR, Reinalda MS, Bamlet WR, Crowson CS,* et al. Incidence and risk factors of prosthetic joint infection after total hip or knee replacement in patients with rheumatoid arthritis. Arthritis Rheum 2008; 59(12): 1713–20.
8. *Tigges S, Stiles RG, Roberson JR.* Complications of hip arthroplasty causing periprosthetic radiolucency on plain radiographs. Am J Roentgenol 1994; 162(6): 1387–91.
9. *Cahir JG, Toms AP, Marshall TJ, Wimhurst J, Nolan J.* CT and MRI of hip arthroplasty. Clin Radiol 2007; 62(12): 1163–71; discussion 1172–3.
10. *Cobo J, Del Pozo JL.* Prosthetic joint infection: diagnosis and management. Expert Rev Anti Infect Ther 2011; 9(9): 787–802.
11. *Love C, Palestro CJ.* Radionuclide imaging of infection. J Nucl Med Technol 2004; 32(2): 47–57; quiz 58–9.
12. *Truluck CA.* Nuclear medicine technology: Inflammation and infection imaging. J Radiol Nurs 2007; 26(3): 77–85.
13. *Vinjamuri S, Hall AV, Solanki KK, Bomanji J, Siraj Q, O'Shaughnessy E,* et al. Comparison of 99mTc infection imaging with radiolabelled white-cell imaging in the evaluation of bacterial infection. Lancet 1996; 347(8996): 233–5.
14. *Britton KE, Wareham DW, Das SS, Solanki KK, Amaral H, BhatnagarA*, et al. Imaging bacterial infection with (99m)Tc-ciprofloxacin (Infecton). J Clin Pathol 2002; 55(11): 817–23.
15. *Dumarey N, Blocklet D, Appelboom T, Tant L, Schoutens A.* Infecton is not specific for bacterial osteo-articular infective pathology. Eur J Nucl Med Mol Imaging 2002; 29(4): 530–5.
16. *Sarda L, Saleh-Mghir A, Peker C, Meulemans A, Crémieux AC, Le Guludec D.* Evaluation of (99m)Tc-ciprofloxacin scintigraphy in a rabbit model of Staphylococcus aureus prosthetic joint infection. J Nucl Med 2002; 43(2): 239–45.
17. *Pucar D.* Validity of 99mTc-ciprofloxacin scintigraphy with estimation of accumulation index in diagnostic of bone infection [thesis]. Belgrade: Military Medical Academy; 2006. (Serbian)
18. *Pucar D, Jankovic Z, Dugonjic S, Popovic Z.* Estimation of 99mTc-ciprofloxacin accumulation indexes in bone and joint bacterial infections. Vojnosanit Pregl 2009; 66(5): 395–8.

19. *Gemmel F, Hans Van den Wyngaert, Love C, Welling MM, PP Gemmel Palestro CJ*. Prosthetic joint infections: radionuclide state-of-the-art imaging. Eur J Nucl Med Mol Imaging 2012; 39(5): 892–909.
20. *Love C, Tronco G, Yu A, Marwin S, Nichols K, Palestro C*. Diagnosing lower extremity (LE) prosthetic joint infection: Bone, gallium & labelled leukocyte imaging. J Nucl Med 2008; 49(Suppl 1): 133P.
21. *van der Bruggen W, Bleeker-Rovers CP, Boerman OC, Gotthardt M, Oyen WJ*. PET and SPECT in Osteomyelitis and Prosthetic Bone and Joint Infections: A Systematic Review. Semin Nucl Med 2010; 40(1): 3–15.
22. *Pucar D, Janković Z, Bascarevic Z, Starcevic S, Radulovic M, Sisic M,* et al. Combined 99mTc-MDP bone scintigraphy and 99mTc-Ciprofloxacin scintigraphy in differentiation of hip and knee prosthesis aseptic loosening and infection: Preliminary study. Vojnosanit Pregl 2017; 74(7): 666–71.
23. *Nagoya S, Kaya M, Sasaki M, Tateda K, Yamashita T*. Diagnosis of peri-prosthetic infection at the hip using triple-phase bone scintigraphy. J Bone Joint Surg Br 2008; 90(2): 140–4.
24. *Zajonz D, Wuthe L, Tiepolt S, Brandmeier P, Prietzel T, von Salis-Soglio GF*, et al. Diagnostic work-up strategy for periprosthetic joint infections after total hip and knee arthroplasty: A 12-year experience on 320 consecutive cases. Patient Saf Surg 2015; 9: 20.

Received on August 7, 2019
Revised on August 21, 2019
Accepted August 23, 2019
Online First September, 2019

ORIGINAL ARTICLE

UDC: 616.89-008.454::616-053.9
DOI: https://doi.org/10.2298/VSP190719098G

Prevalence of depression in elderly and relations to chronic diseases

Prevalencija depresije kod starih osoba i povezanost sa hroničnim bolestima

Gordana Gajović*, Sanja Kocić†‡, Snežana Radovanović†‡, Ivana Simić Vukomanović†‡, Katarina Janićijević†, Svetlana Radević†

***Health Center Arandjelovac, Arandjelovac, Serbia; †University of Kragujevac, Faculty of Medical Sciences, Department of Social Medicine, Kragujevac, Serbia; ‡Institute of Public Health, Kragujevac, Kragujevac, Serbia**

Abstract

Background/Aim. Depression is the most prevalent mental disorder which affects approximately 7% of the world's older population. This study aimed at examining the prevalence of depression among older adults and its relations to chronic illnesses. **Methods.** Study was conducted within the National Health Survey of the Serbian population in 2013. The questionnaires used as instruments in this study were created in accordance with the questionnaires of the European Health Interview Survey – Second Wave. The Patient Health Questionnaire-8 (PHQ-8) was used to evaluate the presence of depressive symptoms. The relations between depression symptoms (a dependent variable) and a set of independent variables were examined with univariate and multivariate logistic regression analyses. **Results.** The study showed that there was a 10.0% prevalence of depression within this population with statistically significant differences between the genders – 12.6% of women and 6.5% of men. The multivariate analysis revealed that multimorbidity [odds ratio (OR) = 1.89], chronic pain (OR = 2.35) and self-evaluations of poor health (OR = 8.37) were strongly associated to depression. In terms of individual chronic illnesses, the study showed that strokes double the odds of developing depression (OR = 1.82) while the deformities of lower spine increased this odds by 27%. **Conclusion.** Depression is very frequent in older persons who suffer from chronic diseases and medical conditions. It is crucial to enable adequate screening in primary healthcare institutions in order to diagnose depression in its early stages and start its treatment as soon as possible.

Key words:
depression; chronic disease; comorbidity; aged; prevalence; serbia.

Apstrakt

Uvod/Cilj. Depresija je najčešći mentalni poremećaj koji pogađa oko 7% starije svetske populacije. Cilj ovog istraživanja bio je utvrđivanje prevalencije depresije u starijoj populaciji i njene povezanosti sa hroničnim oboljenjima. **Metode.** Istraživanje je sprovedeno u okviru nacionalne studije "Istraživanje zdravlja stanovništva Srbije" 2013. godine. Kao istrument istraživanja korišćeni su upitnici kreirani u skladu sa upitnicima Evropskog istraživanja zdravlja – drugi talas. Za procenu prisustva simptoma depresije korišćen je *the Patient Health Questionnaire-8* (PHQ-8). Povezanost prisustva depresivnih simptoma (zavisna varijabla) i skupa nezavisnih varijabli ispitana je univarijantnom i multivarijantnom logističkom regresijom. **Rezultati.** Rezultati ukazuju na stopu prevalencije depresije od 10,0% sa statistički značajnim razlikama među polovima – 12,6% kod žena i 6,5% kod muškaraca. Multivarijantna analiza je pokazala da postoje jake veze između depresije i multimorbiditeta [*odds ratio* (OR) = 1.89], hroničnog bola (OR = 2,35) i samoprocena lošeg zdravlja (OR = 8,37). Što se tiče pojedinačnih oboljenja, studija je pokazala da moždani udari dvostruko povećavaju šanse za pojavu depresije (OR = 1,82), dok prisustvo deformiteta donje kičme te šanse povećava za 27%. **Zaključak.** Depresija je veoma česta kod starijih osoba koje pate od hroničnih bolesti i stanja. Ključno je omogućiti adekvatan skrining u ustanovama primarne zdravstvene zaštite kako bi se depresija dijagnostikovala u ranoj fazi i što pre započelo njeno lečenje.

Ključne reči:
depresija; hronična bolest; komorbiditet; stare osobe; prevalenca; srbija.

Correspondence to: Gordana Gajović, Health Center Aranđelovac, Department of Social Medicine, Kralja Petra I 62, 34 300 Aranđelovac, Serbia. E-mail: gordana.gajovic@gmail.com

Introduction

The world's population has been ageing rapidly [1]. Simultaneously, mental health problems in elderly populations have become an important public health issue [2, 3]. According to the World Health Organization (WHO) report, approximately 15% of adults aged 60 and over suffer from some mental disorder [4]. Depression is the most prevalent among them. It affects approximately 7% of the global older population and has severe physical, psychological and social impacts on individuals, their families and entire societies [5].

Depressive symptoms among the elderly are generally associated with several adverse health outcomes – they decrease functional ability and life quality, worsen the outcomes of other medical illnesses and increase the risks of mortality and suicide [6–10]. Depressive episodes had been present in about 80% of those aged 74 and over who committed suicide [11]. Furthermore, depression substantially increases health care costs due to higher health care needs and improper health behavior [3, 6, 12]. Depression with older adults requires special attention since it is commonly under-recognised and undertreated [7]. It is inadequately dealt with predominantly because it is generally considered as a natural phenomenon in aging [13, 14]. Previous studies have shown that a half of the depression cases commonly remains undiagnosed [2].

Depression in late life can result from a complex interaction of biological, physical, psychosocial and social factors [3, 15]. Studies have shown that chronic diseases are one of the leading factors for developing depression [16, 17]. Depression is two times more common in patients with chronic diseases than in general population, whereas risks of suffering from depression increase with the number of chronic diseases a patient has [5]. Even though any chronic medical condition can result in depressive symptoms, there are certain medical diagnoses that are more likely to lead to depression, e.g. coronary heart disease, diabetes, stroke, cancer, rheumatoid arthritis and hypertension [18, 19]. It is well known that depression which appears as comorbidity to chronic diseases can affect mortality rates, clinical outcomes, adherence to prescribed therapy and treatments and functional abilities to perform daily tasks [2, 14].

The relations between depression in the elderly population and their chronic diseases, on one hand, and their subjectively evaluated medical conditions, on the other hand, have not been fully explored in Serbia. Therefore, this study aimed at examining the depression prevalence in older adults and its potential links to chronic diseases on a large and representative sample of the Serbian old population.

Methods

Study population and sample

This epidemiological population-based study was conducted as a part of the 2013 National Health Survey initiated and carried out by the Ministry of Health of the Republic of Serbia. The survey was conducted from 7th October to 30th December, 2013. The ethics approvals were granted by the Institute of Public Health of Serbia "Dr Milan Jovanovic Batut". The written informed consents were obtained from all the individuals who participated in this study.

The study was conducted in accordance with the methodology and instruments of the European Health Interview Survey – Second Wave (EHIS wave 2). The target population of the National Health Survey were the individuals aged 15 and over who lived in private households. The survey excluded persons who lived in collective households or institutions (e.g. foster homes, social and gerontology institutions, prisons, psychiatric facilities, etc.). In order to obtain a reliable assessment of a large number of factors for the population health at the national level, as well as in four geographic areas (Vojvodina, Belgrade, Šumadija and Western Serbia, Southern and Eastern Serbia) and in different settlement types separately, the National Health Survey used a stratified two-stage sample. The units of the first sampling stage included 670 census enumeration areas defined in the 2011 Population Census. The units of the second sampling stage were randomly selected households. The study included 6,500 randomly selected households (3,909 from urban and 2,591 from other areas). The response rate was 94.1%. Finally, 13,756 respondents (aged 15 and over) successfully completed the survey.

The target population for this particular analysis were the individuals aged 65 and over who lived in private households in Serbia at the time of the data collection. The number of participants who fulfilled this age criterion was 3,540. The final sample of this study thus comprised of 3,540 elderly adults.

Instruments

The questionnaires used as instruments in this study were created in accordance with the questionnaires of the EHIS wave 2 which had been created based on internationally accepted and defined criteria. They are adapted here to the particularities of the Serbian context.

Depression was selected as a dependent variable. It was evaluated with the Patient Health Questionnaire-8 (PHQ-8) [20] that was incorporated in a „face-to-face" questionnaire for respondents aged 15 and over. The respondents were asked to evaluate how often they had been bothered by any of the given mental problems during the previous two weeks. Their responses were marked as 0 ("not at all"), 1 ("for a few days"), 2 ("more than seven days" and 3 ("almost every day"). After summing up the points for every answer, we obtained the scores ranging from 0 to 24 points. The values within the range 0–4 indicated that there were no symptoms of depression. The values from 5–9 were taken as a proof of mild depression (subsyndrome depression) and the range from 10 to 24 as a high probability of a depressive episode. Depressive episodes (i.e. depression) were further classified as: moderate (10–14 points), moderately severe (15–19 points) and severe (20–24 points).

The evaluations of the medical conditions included: 1) self-evaluations of medical states; 2) the presence of a long-term disease or a medical condition (where *a long-term disease* refers to an illness and a medical condition that had lasted or was expected to last for at least 6 months), and 3) the presence and intensity of bodily pain during the month preceding the time of the data collection and its influence on performing usual activities and tasks.

The study included 17 different chronic diseases or medical conditions reported by the participants in the last 12 months: 1) Asthma (J45), Status asthmaticus(J46); 2) Chronic bronchitis, chronic obstructive pulmonary disease, emphysema (J40-J44), and Chronic lower respiratory diseases excluding asthma but including chronic asthmatic bronchitis (J47); 3) Myocardial infarction (heart attack) or chronic consequences of myocardial infarction [I21 (Acute myocardial infarction – AMI)], I22 (Subsequent myocardial infarction), I23 (Certain current complications following AMI), (consequences of former MI included partly also under I25)); 4) Coronary heart disease or angina pectoris (I20-I25); 5) High blood pressure [I10-I13 and I15 (Hypertensive diseases)]; 6) Stroke [I60-I69 (Cerebrovascular diseases)]; 7) Arthrosis (M15-M19, arthritis excluded); 8) Low back disorder or other chronic back defect [No specific ICD-10 codes can be used but the condition is included under some M40-M54 (Dorsopathies) diagnosis (excluding M45-Ankylosing spondylitis and M50 – Cervical disc disorders)]; 9) Neck disorder or other chronic neck defect [No specific ICD-10 codes can be used but the condition is included under some M40-M54 (Dorsopathies) diagnosis (excluding M45 – Ankylosing spondylitis and M51 – Other intervertebral disc disorders)]; 10) Diabetes [E10-E14 (Diabetes mellitus)]; 11) Depression [F31-F39 (Mood (affective) disorders excluding F30 – Manic episode; F41.2 (Mixed anxiety and depressive disorder), F53.0 (Mild mental and behavioural disorders associated with the puerperium, not elsewhere classified)]; 12) Malignant diseases; 13) Increased fats in the blood serum; 14) Allergy, such as rhinitis, hay fever, eye inflammation, dermatitis, food allergy or other allergy (allergic asthma excluded) [J30 (Vasomotor and allergic rhinitis), L20-L30 (Dermatitis and eczema excluding L21 – Seborrhoeic dermatitis), and other allergies irrespective of the origin]; 15) Cirrhosis of the liver [K70 (Alcoholic liver disease), as secondary to other diseases (K71.7 – Toxic liver disease with fibrosis and cirrhosis of liver), part of K74 (Fibrosis and cirrhosis of liver); K76.1 (Chronic passive congestion of liver)]; 16) Urinary incontinence, problems in controlling the bladder [R32 (Unspecified urinary incontinence); N39.3 (Stress incontinence); N39.4 (Other specified urinary incontinence)]; 17) Kidney problems [Chronic conditions under N00- N08 (Glomerular diseases), N10-N16 (Renal tubulointerstitial diseases) and N17- N19 (Renal failure), N25-N29 (Other disorders of kidney and ureter)].

Multimorbidity, i.e. a simultaneous presence of two or more chronic diseases/conditions in one person, was also taken into consideration.

Statistical analysis

All the data of interest obtained through the above described methods were analyzed by adequate statistical tools. The proportions between different population groups were compared with the Chi-square (χ^2) test. The results with probabilities lower than 5% are considered as statistically significant. The relations between a dependent variable (depressive symptoms) and a set of independent variables were examined by univariate and multivariate logistic regressions. The unadjusted odds ratios (ORs) with their corresponding 95% confidence intervals (CIs) were also obtained. All statistical calculations were performed with a commercial, standard software package SPSS, version 18.0. [The Statistical Package for Social Sciences software (SPSS Inc, version 18.0, Chicago, IL)].

Results

The sample included 1,528 men and 2,012 women whose mean age was 73.9 years [standard deviation (SD) = 6.3 years]. Based on PHQ-8 scores, the depression prevalence was 10.0%. There were statistically significant differences between women (12.6%) and men (6.5%) (χ^2 = 95.534, p < 0.001). Mild depressive symptoms (sub-syndrome depression) were present in every fifth female (21.2%) and every eighth male subject (12.7%). More than a half of the depressive episodes were mild (57.9%). Moderately severe depression episodes were recorded in 26.3% and severe depressive episodes in 15.8% of the cases. There were no statistically significant gender differences with respect to the severity of depression (χ^2 = 0.293, p = 0.864) (Table 1).

Table 1

The gender prevalence of depression in the population aged 65 and over

PHQ-8 score (range)	Total		Males		Females		*p*
	n	%	n	%	n	%	
0–4 (non-depressive)	2,656	72.5	1,235	80.8	1,330	66.1	
5–9 (mildly depressive)	621	17.5	194	12.7	427	21.2	< 0.001
10–24 (depressive episodes)	354	10.0	99	6.5	255	12.7	
10–14 (moderate)	205	5.8	56	3.7	149	7.4	
15–19 (moderately severe)	93	2.6	28	1.8	65	3.2	0.864
20–24 (severe)	56	1.6	15	1.0	41	2.1	

The mean PHQ-8 depression score for the elderly in Serbia was estimated at 3.6 ± 4.6. The difference between women and men was statistically significant (t = -10.763, p < 0.001). The mean depression score for females amounted to 4.2 ± 4.9 and for males it was 2.6 ± 4.1 points.

88.9% of the participants claimed to suffer from the investigated chronic diseases and medical conditions. 19% of them reported just one diagnosis while 69.9% reported two or more chronic health issues (multimorbidity). The most prevalent diseases were: increased blood pressure (65.8%), lower back and neck deformities or other chronic back conditions (35.2%), coronary heart disease and angina pectoris (28.8%), arthrosis (24%) and increased blood fats (22%). Malignant diseases were the least frequent among those suffering from depression (2.7%).

The prevalence of depression was significantly higher in respondents with multimorbidity (13.4%) in comparison to individuals without chronic diseases and medical conditions (27.1%). There were statistically significant differences in the likelihood of depression co-occurring with the given diagnoses. Depressive episodes appeared with every third person who had had a stroke (30.1%), and almost every fifth individual who had suffered from urinary incontinence (21.4%), myocardial infarction (20.0%) or arthrosis (18.6%). The distributions of depression with respect to chronic diseases and medical conditions in the Serbian elderly population are given in Table 2.

There were wide discrepancies among the results obtained through PHQ-8 and patients' claims. Namely, only 33.0% of respondents suffering from mild depression (according to PHQ-8) reported depressive symptoms. In other words, among those who claimed not to suffer from depression, 211 (6.8%) were evaluated as depressed by PHQ-8 instrument.

The univariate logistic regression analysis showed that depression was more likely to occur with any analyzed chronic disease than without them. Those differences proved to be statistically significant in each particular case. The respondents with two or more chronic medical conditions were five times more likely to undergo depressive episodes (OR = 5.63). Depressive symptoms were most likely to develop in those who had suffered from: a stroke (OR = 3.64), arthrosis (OR = 3.01), lower back deformities (OR = 2.94) neck deformities (OR = 2.85) and urinary incontinence (OR = 2.51). Chronic bodily pain made it five times more likely for an individual to suffer a depressive episode (OR = 5.37). Self-evaluations of poor medical conditions proved to be a strong indicator of depression. Namely, individuals who evaluated their health status as bad were 17 times more likely to be depressed with respect to those who evaluated it as good (OR = 17.99).

The multivariate analysis showed that multimorbidity (OR = 1.89), chronic bodily pain (OR = 2.35) and negative health self-evaluations (OR = 8.37) were strongly associated with depression. When it comes to individual diseases, we must note that strokes doubled the chances for suffering from depression (OR = 1.82) while lower spine deformities increased those chances by 27% (Table 3).

Table 2

Depressive symptoms and chronic diseases/conditions (number of diseases)

Disease/condition	Non-depressive		Mildly depressive		Depressive episodes		*p*
	n	%	n	%	n	%	
No chronic diseases	390	93.3	20	4.8	8	1.9	
One chronic disease	601	89.7	51	7.6	18	2.7	< 0.001
Two or more chronic diseases	1574	64.2	550	22.4	328	13.4	
Asthma	145	60.2	55	22.8	41	17.0	< 0.001
Chronic bronchitis/chronic obstructive pulmonary disease	166	56.1	83	28.0	47	15.8	< 0.001
Myocardial infarction	140	55.1	63	24.8	51	20.0	< 0.001
Coronary disease or angina pectoris	614	60.7	249	24.6	149	14.8	< 0.001
Hypertension	1576	68.4	458	19.9	270	11.7	< 0.001
Stroke	85	44.0	50	25.9	58	30.1	< 0.001
Arthrosis	459	54.4	227	26.9	158	18.6	< 0.001
Lower back deformities	722	58.2	313	25.2	205	16.6	< 0.001
Neck deformities	428	54.8	212	27.1	141	18.0	< 0.001
Diabetes	418	65.4	146	22.8	75	11.8	< 0.001
Malignant diseases	59	60.8	25	25.8	13	13.4	< 0.001
Increased blood fats	491	65.4	172	22.9	88	11.8	< 0.001
Depression	115	30.3	125	33.0	139	36.7	< 0.001
Allergy	203	61.7	81	24.6	45	1	< 0.001
Cirrhosis of the liver	8	57.1	5	35.7	1	7.1	0.202
Urinary incontinence	244	56.2	101	22.4	100	21.4	< 0.001
Kidney problems	228	56.6	104	25.8	71	17.6	< 0.001

Table 3

Odds ratios (OR) and 95% confidence intervals (CI) for the depression depending health characteristics

Disease	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Asthma	1.83 (1.39–2.40)	< 0.001	0.85 (0.60–1.21)	0.368
Chronic bronchitis/chronic obstructive pulmonary disease	2.23 (1.75–2.85)	< 0.001	1.26 (0.92–1.72)	0.151
Myocardial infarction	2.29 (1.77–2.97)	< 0.001	1.02 (0.75–1.39)	0.908
Coronary disease or angina pectoris	2.22 (1.89–2.60)	< 0.001	1.07 (0.88–1.30)	0.497
Hypertension	1.92 (1.62–2.26)	< 0.001	1.06 (0.85–1.32)	0.600
Stroke	3.64 (2.72–4.89)	< 0.001	1.82 (1.29–2.58)	< 0.001
Arthrosis	3.01 (2.55–3.54)	< 0.001	1.28 (1.04–1.57)	0.190
Lower back deformities	2.94 (2.52–3.43)	< 0.001	1.27 (1.02–1.59)	0.034
Neck deformities	2.85 (2.42–3.38)	< 0.001	1.22 (0.97–1.55)	0.094
Diabetes	1.53 (1.27–1.83)	< 0.001	0.96 (0.77–1.20)	0.727
Malignant diseases	1.73 (1.15–2.62)	< 0.05	0.96 (0.60–1.52)	0.851
Allergy	1.73 (1.37–2.19)	< 0.001	1.08 (0.82–1.44)	0.585
Cirrhosis of the liver	1.98 (0.69–5.73)	0.206	1.37 (0.41–4.56)	0.605
Urinary incontinence	2.51 (2.05–3.08)	< 0.001	1.38 (1.08–1.77)	< 0.05
Kidney problems	2.26 (1.83–2.79)	< 0.001	1.15 (0.89–1.49)	0.294
Multimorbidity	5.63 (4.51–7.05)	< 0.001	1.89 (1.38–2.57)	< 0.001
The presence of moderate, severe or very severe bodily pain	5.37 (4.46–6.46)	< 0.001	2.35 (1.88–2.93)	< 0.001
Self-evaluation of poor medical condition	17.99 (13.01–24.89)	< 0.001	8.37 (5.79–12.08)	< 0.001

Discussion

The WHO has estimated that the total depression prevalence rate among older adults in the world varies from 10–20% [21]. The rate among the Serbian elderly population, as found in this study, was 10.0%. Similar values (10.3%) were reported in a meta-analysis of 84 independent studies which reported the rate variations ranging from 4.7–16.0% [22]. However, some countries documented considerably higher rates of depression prevalence among elderly population. The community-based studies conducted in India reported the variations within the 13–25% range [21]. The meta-analysis for the older Chinese calculated this rate as 23.6% [23]. In Brasil, nearly 30% of the elderly people suffered from depression [24]. The studies carried out in America recorded the depression prevalence rates ranging from 15% to 19% [25]. The findings of the studies indicated that mean depression prevalence rates in elderly population are similar in Asia, Europe and America, but significantly lower in Australia [22]. About 8% of older Australians are currently experiencing depressive symptoms [26]. These huge variations in depression prevalence among older adults may result from the differences in methodological approaches to data collection, from the use of different scales for geriatric depression, as well as from the socio-demographic and cultural variations [2]. They may also arise from the existent regional and racial differences between the countries [27].

It was shown in our study that older women were more likely to suffer from depression than men. This finding is in accordance with the results of the previous studies in the field [2, 28, 29]. Quite contrary, Baiyewu et al. [27] found no differences between the opposite sexes. Higher likelihood for developing depression in an older age in female population may be attributed to numerous factors (i.e. genetic, biological and psychological factors), as well as to different social roles of the two genders and the more unfavorable social positions of females [24]. These findings may also follow from the fact that, due to their longer life expectancy, women are generally more exposed to medical problems and undesirable events which all may contribute to depression [7].

Population studies have indicated that depression is a disorder of high comorbidity. They have also emphasized the strong links between chronic diseases and depressive symptoms in elderly population [5]. Chronic medical conditions may trigger depression or worsen its symptoms, but depression may also precede chronic diseases and deteriorate their outcomes [30]. This study showed that depression was higher in respondents with multimorbidity. Even though the studies have shown that the relations between chronic diseases and depressive symptoms vary among different chronic diseases, the number of chronic diseases is more strongly related to depression than any specific individual diagnosis [17]. The studies have shown that depression prevalence increases with the number of chronic diseases [31]. The WHO research carried out in 60 countries documented the depression prevalence of 23% in individuals with two or more chronic medical conditions and depression prevalence of 3.2% in healthy population [32].

The Netherlands Study of Depression in Old Persons (NESDO) found that the presence of cardiovascular, musculoskeletal and somatic diseases was strongly associated with depressive disorder during the two-year monitoring of the patients [33]. With each additional chronic

somatic disease, the chances of developing moderate and severe chronic depression increased by 92% [34].

The study conducted in the U.S. showed that people with chronic diseases were three times more likely to experience depressive episodes with respect to a control group. Three times higher depression rates were also recorded in patients with terminal-stage kidney insufficiency, chronic obstructive pulmonary disease and cerebrovascular diseases. Twice as high depression rates were detected in patients suffering from coronary diseases, hypertension and diabetes [35]. The evaluations of depression prevalence in patients with chronic diseases like coronary heart disease, diabetes and previous strokes ranged from 15% to 25%, depending on the screening method [36].

Cardiovascular diseases frequently coexist with psychiatric disorders, but they can also develop as a complication of psychiatric problems and *vice versa*. In almost a half of the patients with cardiovascular diseases, there were depressive episodes which were getting worse as the disease was progressing and which eventually increased the risk of deadly outcomes by two to three times [37].

Some studies have shown that depression co-occurring with coronary arterial disease is a factor of high risk for this disease; besides, numerous studies have determined the depression prevalence of 18% after an acute myocardial infarction [38]. The other study showed that about 25% of the individuals developed severe depression after myocardial infarction while an additional 25% of them ended up with mild depression. During 4-month period following an acute myocardial infarction, fatal outcomes were almost four times more likely in depressed than in non-depressed patients [39].

According to Jiang [40], almost 25% of the patients who had had a stroke developed clinical depression during the same year. Ozaki et al. [38] confirmed these results by proving that depression appeared in 19–23% stroke survivors and by concluding that depression increased the risk of strokes in the time interval ranging from 10 to 15 years ahead. The depression prevalence in patients suffering from diabetes ranged from 11% to 31% with diabetes doubling the risks for developing depression.

Finally, it must be noted that depressive symptoms may follow almost all chronic diseases. Their existence can contribute to poorer adherence to prescribed treatments, lifestyle deterioration and increased morbidity and mortality [38].

This study has certain limitations. The study had a cross-sectional design so causal relations between depression and chronic diseases are not strong and clear. Secondly, one may argue that PHQ-8 is not a clinical tool for diagnosing depression. Thus, depression may not be accepted as a definite diagnosis. However, this instrument is most commonly used in evaluating depression prevalence in general population and we decided to follow this widespread practice. We also relied on comorbidities that were reported by participants without being able to check the real morbidity prevalence from their medical records.

Conclusion

Depression is very frequent in older persons who suffer from chronic diseases and medical conditions. It is crucial to enable adequate screening in primary healthcare institutions in order to diagnose depression in its early stages and start its treatment as soon as possible. The key factors for reducing more severe outcomes of depressive disorders for individuals, their families and larger communities include: the proper identification of risk factors for developing depression in older population, an early diagnosis, a timely and efficient treatment and a proper depression management.

R E F E R E N C E S

1. *Mohebbi M, Agustini B, Woods RL, McNeil JJ, Nelson MR, Shah RC, et al.* Prevalence of depressive symptoms and its associated factors among healthy community-dwelling older adults living in Australia and the United States. Int J Geriatr Psychiatry 2019; 34(8): 1208–16.
2. *El-Gilany AH, Elkhawaga GO, Sarraf BB.* Depression and its associated factors among the elderly: A community-based study in Egypt. Arch Gerontol Geriatr 2018; 77: 103–7.
3. *Dao ATM, Nguyen VT, Nguyen HV, Nguyen LTK.* Factors Associated with Depression among the Elderly Living in Urban Vietnam. Biomed Res Int 2018; 2018: 2370284.
4. *WHO.* Mental health of older adults. 2017 December [cited 2019 Jun 12]. Available from: https://www.who.int/news-room/fact-sheets/detail/mental-health-of-older-adults
5. *Pruchno RA, Wilson-Genderson M, Heid AR.* Multiple Chronic Condition Combinations and Depression in Community-Dwelling Older Adults. J Gerontol A Biol Sci Med Sci 2016; 71(7): 910–5.
6. *Wright PP, Thorpe CW.* Triple Threat among the Elderly: Depression, Suicide Risk, and Handguns. J Emerg Nurs 2016; 42(1): 14–8.
7. *Aly HY, Hamed AF, Mohammed NA.* Depression among the elderly population in Sohag governorate. Saudi Med J 2018; 39(2): 185–90.
8. *Alamri SH, Bari AI, Ali AT.* Depression and associated factors in the hospitalized elderly: a cross-sectional study in a Saudi teaching hospital. Ann Saudi Med 2017; 37(2): 122–9.
9. *Li N, Chen G, Zeng P, Pang J, Gong H, Han Y,* et al. Prevalence of depression and its associated factors among Chinese elderly people: A comparison study between community-based population and hospitalized population. Psychiatry Res 2016; 243: 87–91.
10. *Ni Y, Tein JY, Zhang M, Yang Y, Wu G.* Changes in depression among older adults in China: A latent transition analysis. J Affect Disord 2017; 209: 3–9.
11. *Alexopoulos SG.* Depression in the elderly. Lancet 2005; 365(9475): 1961–70.
12. *Buvneshkumar M, John KR, Logaraj M.* A study on prevalence of depression and associated risk factors among the elderly in a rural block of Tamil Nadu. Indian J Public Health 2018; 62(2): 89–94.
13. *Laksham KB, Selvaraj R, Kameshvell C.* Depression and its determinants among the elderly in selected villages of Puducher-

ry - A community-based cross-sectional study. J Family Med Prim Care 2019; 8(1): 141–4.
14. *Patra P, Alikari V, Fradelos EC, Sachlas A, Kourakos M, Rojas Gil AP,* et al. Assessment of Depression in Elderly. Is Perceived Social Support Related? A Nursing Home Study: Depression and Social Support in Elderly. Adv Exp Med Biol 2017; 987: 139–50.
15. *Behera P, Sharan P, Mishra AK, Nongkynrih B, Kant S, Gupta SK.* Prevalence and determinants of depression among elderly persons in a rural community from northern India. Natl Med J India 2016; 29(3): 129–35.
16. *Zhang H, Gao T, Gao J, Kong Y, Hu Y, Wang R, Mei S*. A comparative study of negative life events and depressive symptoms among healthy older adults and older adults with chronic disease. Int J Soc Psychiatry 2017; 63(8): 699–707.
17. *Han J*. Chronic Illnesses and Depressive Symptoms among Older People: Functional Limitations as a Mediator and Self-Perceptions of Aging as a Moderator. J Aging Health 2018; 30(8): 1188–204.
18. *Jani BD, Purves D, Barry S, Cavanagh J, McLean G, Mair FS*. Challenges and implications of routine depression screening for depression in chronic disease and multimorbidity: a cross sectional study. PLoS One 2013; 8(9): e74610.
19. *Ahn S, Kim S, Zhang H*. Changes in Depressive Symptoms among Older Adults with Multiple Chronic Conditions: Role of Positive and Negative Social Support. Int J Environ Res Public Health 2017; 14(1): pii: E16.
20. *Kroenke K, Strine TW, Spritzer RL, Williams JB, Berry JT, Mokdad AH*. The PHQ-8 as a measure of current depression in the general population. J Affect Disord. 2009; 114(1–3): 163–73.
21. *Rangaswamy SM*. World Health Report: Mental health: New Understanding New Hope. Geneva, Switzerland: The World Health Organization; 2001.
22. *Barua A, Ghosh M.K, Kar N, Basilio MA.* Prevalence of depressive disorders in the elderly. Ann Saudi Med 2011; 31(6): 620–4.
23. *Li D, Zhang DJ, Shao JJ, Qi XD, Tian L*. A meta-analysis of the prevalence of depressive symptoms in Chinese older adults. Arch Gerontol Geriatr 2014; 58(1): 1–9.
24. *Nogueira EL, Rubin LL, Giacobbo Sde S, Gomes I, Cataldo Neto A.* Screening for depressive symptoms in older adults in the Family Health Strategy, Porto Alegre, Brazil. Rev Saude Publica 2014; 48(3): 368–77.
25. *Cahoon CG*. Depression in Older Adults. Am J Nurs 2012; 112(11): 22–30.
26. *Pirkis J, Pfaff J, Williamson M, Tyson O, Stocks N, Goldney R,* et al. The community prevalence of depression in older Australians. J Affect Disord 2009; 115(1–2): 54–61.
27. *Baiyewu O, Yusuf AJ, Ogundele A.* Depression in elderly people living in rural Nigeria and its association with perceived health, poverty, and social network. Int Psychogeriatr 2015; 27(12): 2009–15.
28. *He G, Xie JF, Zhou JD, Zhong ZQ, Qin CX, Ding SQ*. Depression in left-behind elderly in rural China: Prevalence and associated factors. Geriatr Gerontol Int 2016; 16(5): 638-43.
29. *Sendra-Gutiérrez JM, Asensio-Moreno I, Vargas-Aragón ML*. Characteristics and factors associated with depression in the elderly in Spain from a gender perspective. Actas Esp Psiquiatr 2017; 45(5): 185–200.
30. *National Collaborating Centre for Mental Health*. Depression in adults with a chronic physical health problem. Treatment and management. NICE Clinical Guidelines, No. 91. Leicester: British Psychological Society; 2010.
31. *Gunn JM, Ayton DR, Densley K, Pallant JF, Chondros P, Herrman HE,* et al. The association between chronic illness, multimorbidity and depressive symptoms in an Australian primary care cohort. Soc Psychiatry Psychiatr Epidemiol. 2012; 47(2): 175–84.
32. *Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B.* Depression, chronic diseases, and decrements in health: results from the World Health Surveys. Lancet 2007; 370(9590): 851–8.
33. *Comijs HC, Nieuwesteeg J, Kok R, van Marwijk HW, van der Mast RC, Naarding P,* et al. The two-year course of late-life depression; results from the Netherlands study of depression in older persons. BMC Psychiatry 2015; 15:20.
34. *Hegeman JM, van Fenema EM, Comijs HC, Kok RM, van der Mast RC, de Waal MWM*. Effect of chronic somatic diseases on the course of late-life depression. Int J Geriatr Psychiatry. 2017; 32(7): 779–87.
35. *Egede LE*. Major depression in individuals with chronic medical disorders. Prevalence, and correlates and association of health resource utilization, lost productivity and functional disability. Gen Hosp Psychiatry 2007; 29(5): 409–16.
36. *Jani BD, Purves D, Barry S, Cavanagh J, McLean G, Mair FS*. Challenges and implications of routine depression screening for depression in chronic disease and multimorbidity: a cross sectional study. PLoS One 2013; 8(9): e74610.
37. *Fan H, Yu W, Zhang Q, Cao H, Li J, Wang J,* et al. Depression after heart failure and risk of cardiovascular and all cause mortality: a meta-analysis. Prev Med 2014; 63: 36–42.
38. *Ozaki Y, Sposito AP, Bueno DR, Guariento ME*. Depression and chronic diseases in the elderly. Rev Soc Bras Clin Med 2015; 13(2): 149–53.
39. *Romanelli J, Fauerbach J, Buch D, Ziegelstein R*. The significance of depression in older patients after myocardial infarction. J Am Geriatr Soc 2002; 50(5): 817–22.
40. *Jiang W*. Depression and Cardiovascular Disorders in the Elderly. Psychiatr Clin North Am 2018; 41(1): 29–37.

Received on December 10, 2018
Revised on February 1, 2019
Accepted February 21, 2019
Online First March, 2019

ORIGINAL ARTICLE

UDC: 616.61-08
DOI: https://doi.org/10.2298/VSP190730097A

The influence of vitamin E-coated dialysis membrane on oxidative stress during a single session of on-line hemodiafiltration

Uticaj dijalizne membrane obložene vitaminom E na oksidacioni stres u toku pojedinačne seanse *on-line* hemodijafiltracije

Svetlana Antić*, Nevena Draginić†, Dejan Pilčević*, Vladimir Živković‡, Ivan Srejović‡, Nevena Jeremić†, Dejan Petrović§‖, Vladimir Jakovljević‡¶

***Military Medical Academy, Clinic for Nephrology, Belgrade, Serbia; University of Kragujevac, Faculty of Medical Sciencies, †Department of Pharmacy, ‡Department of Physiology, §Department of Internal Medicine, Kragujevac, Serbia; ‖Clinical Center Kragujevac, Clinic for Urology and Nephrology, Center for Nephrology and Dialysis, Kragujevac, Sebia; ¶1st Moscow State Medical University IM Sechenov, Department of Human Pathology, Moscow, Russia**

Abstract

Background/Aim. Oxidative stress is an important risk factor for the development of cardiovascular atherosclerotic diseases in the population of patients treated with regular hemodialysis. Bioincompatibility of the dialysis membrane and increased concentration of endotoxin in the hemodialysis solution are two main factors that can trigger oxidative stress. This paper was intended to examine the effect of a vitamin E-coated membrane on oxidative stress during a single session of on-line hemodiafiltration. **Methods.** Twenty-four patients undergoing hemodiafiltration with vitamin E-coated polysulfone dialysis membrane (Leoceed 21H) were examined, followed by a polysulfone dialysis membrane treatment without vitamin E (FX800). The following parameters of oxidative stress were measured: superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), thiobarbutyric acid reactive substances (TBARS), nitric oxide (NO_2^-), catalase (CAT), superoxide dizmutase (SOD), and reduced glutathione (GSH) activity. Statistical analysis included the Kolmogorov-Smirnov test, Student-*t* test and Wilcoxon test. **Results.** On-line hemodiafiltration using a high-flux polysulfone vitamin E-coated membrane led to significant reduction of TBARS concentration and SOD activity, while the on-line hemodiafiltration session using a high-flux polysulfone membrane that is not vitamin E-coated induced a significant increase in H_2O_2 concentration in the serum and a decrease in SOD activity. There was no statistical significance among the other parameters of oxidative stress. **Conclusion**. A single session of on-line hemodiafiltration using a vitamin E-coated polysulfone membrane significantly affects oxidative stress. After a single session of on-line hemodiafiltration using a vitamin E-coated membrane, the concentration of TBARS has significantly decreased. The decreased activity of superoxide dismutase could be a consequence of an increased loss of microelements during an on-line hemodiafiltration session using a high-flux polysulfone membrane. Patient selection, continuous on-line hemodiafiltration using a vitamin E-coated polysulfone membrane over a 3–6 month period and increased antioxidant protection capacity could possibly reduce the risk of cardiovascular morbidity and mortality in patients treated by hemodialysis.

Key words:
hemodiafiltration; membranes, artificial; oxidative stress; vitamin e.

Apstrakt

Uvod/Cilj. Oksidacioni stres je značajan faktor rizika od razvoja kardiovaskularnih aterosklerotskih bolesti u populaciji bolesnika koji se leče redovnom hemodijalizom. Bioinkompatibilnost dijalizne membrane i povećana koncentracija endotoksina u rastvoru za hemodijalizu su dva glavna faktora koja mogu da podstaknu oksidacioni stres. Rad je imao za cilj da ispita uticaj membrane obložene vitaminom E na oksidacioni stres u toku pojedinačne seanse *on-line* hemodijafiltracije. **Metode.** Ispitana su 24 bolesnika koja su lečena *on-line* hemodijafiltracijom sa polisulfonskom dijaliznom membranom obloženom vitaminom E (Leoceed 21H), a zatim i polisulfonskom dijaliznom membranom neobloženom vitaminom E (FX800). Glavni parametri oksidacionog stresa, koji su praćeni, bili su: superoksidni anjon (O_2^-), vodonik peroksid (H_2O_2), reaktivne supstancije vezane za tiobarbituričnu kiselinu

Correspondence to: Svetlana Antić, Military Medical Academy, Clinic for Nephrology, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: drsvetlana.antic@gmail.com

(TBARS), azotni oksid (NO_2^-), katalaza (CAT), superoksid dizmutaza (SOD) i aktivnost redukovanog glutationa (GSH). Za statističku analizu korišćeni su: Kolmogorov-Smirnov test, Student-ov *t*-test za vezane uzorke i Wilcoxon-ov test. **Rezultati.** *On-line* hemodijafiltracija sa *high-flux* polisulfonskom membranom obloženom vitaminom E statistički je značajno smanjivala koncentraciju TBARS i aktivnost SOD u serumu. Nakon seanse *on-line* hemodijafiltracije sa *high-flux* polisulfonskom membranom koja nije bila oboložena vitaminom E statistički se značajno povećala koncentracija H_2O_2 u serumu, dok se aktivnost SOD značajno smanjila. Kod ostalih ispitivanih parametara oksidacionog stresa nije utvrđena statistički značajna razlika između korišćenih membrane. **Zaključak.** Pojedinačna seansa *on-line* hemodijafiltracije sa polisulfonskom membranom obloženom vitaminom E statistički značajno utiče na oksidacioni stres. Koncentracija TBARS u serumu je statistički značajno niž posle pojedinačne seanse *on-line* hemodijafiltracije sa membranom obloženom vitaminom E. Smanjena aktivnost SOD mogla bi da bude posledica pojačanog gubitka mikroelemanata u toku seanase *on-line* hemodijafiltracije sa *high-flux* polisulfonskom membranom. Izbor bolesnika, *on-line* hemodijafiltracija sa polisulfonskom membranom obloženom vitaminom E kontinuirano u vremenskom periodu od 3–6 meseci i povećanje kapaciteta antioksidacione zaštite mogli bi da smanje rizik od kardiovaskularnog morbiditeta i mortaliteta kod bolesnika na hemodijalizi.

Ključne reči:
hemodijafiltracija; membrane, veštačke; stres, oksidativni; vitamin e.

Introduction

Oxidative stress is a significant risk factor for the development of cardiovascular diseases in the population of patients treated with regular hemodialysis [1]. In these patients, oxidative stress happens as a consequence of increased effect of prooxidative factors and reduced activity of antioxidant protection systems (non-enzyme and enzyme systems). The prooxidation factors include: age, diabetes mellitus, uremic background, chronic inflammatory status, bioincompatible dialysis membrane and presence of endotoxins in the hemodialysis solution. On the other hand, decreased activity of antioxidant non-enzyme mechanisms happens due to a lack of vitamin C and vitamin E, while decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx), is due to a lack of cofactors [increased selenium (Se) and zinc (Zn) loss through the dialysis membrane during hemodialysis] and reduced activity of glutathione (GSH) system [2, 3].

Hemodialysis is itself a trigger for the occurence of oxidative stress. The main pathophysiological mechanisms of the increased formation of reactive oxygen species (ROS) during the hemodialysis session are the bioincompatibility of the dialysis membrane, presence of endotoxins in the hemodialysis solution and increased loss of cofactor oligoelements that are necessary for the activity of antioxidant enzymes [2–6].

Dialysis membranes play a central role in the hemodialysis process (hemodialysis). They can be natural and synthetic. Natural membranes include cellulose derivatives with water permeability coefficient – Kuf < 10 mL/h × mmHg ("low-flux" membrane), low clearance of moderate molecular weight uremic toxins and a lower biocompatibility degree compared to synthetic membranes. Synthetic membranes are biocompatible membranes that have a high water permeability coefficient – Kuf > 20 mL/h × mmHg ("high-flux" membranes) and high clearance of medium molecular weight uremic toxins [3–6]. The evaluation of the dialysis membrane efficacy is based on the coefficient of mass transfer (KoA), which can be calculated by multiplying the coefficient of transmission (Ko) and the surface of the membrane (A). Highly effective dialysis membranes are those that have KoA > 600–700 [3–6]. Highly effective (KoA > 600–700) and highly permeable water membranes with ultrafiltration coefficient of Kuf ≥ 50 mL/h × mmHg are used for on-line hemodiafiltration [3–6].

During a hemodialysis session, there is a direct activation of the polymorphonuclear leukocytes due to direct contact of the blood and the surface of the dialysis membrane, which is the result of myeloperoxidase activity which increases the formation of free oxygen radicals. Measurement of serum myeloperoxidase concentrations released from polymorphonuclears during a hemodialysis session indicates the severity of oxidative stress induced by different bioincompatibility degrees of dialysis membranes [7].

During a single 4-hour session of hemodialysis, the patient's organism is exposed to approximately 120 liters of dialysis solution. Therefore, a high microbiological quality of the dialysis solution (ultra-pure dialysis solution) is required [8, 9]. According to the European Best Practice Guidelines/European Renal Best Practice, ANSI/AAMI RD52 (American National Standards Institute/Association for the Advancement of Medical Instrumentation RD 52) and ANSI/AAMI/ ISO 11663 for the Advancement of Medical Instrumentation ISO 11663, the ultra-pure dialysis solution is defined as a solution in which the number of bacterial colonies is < 0.1 (colony-forming units – CFU)/mL and the endotoxin concentration is E < 0.03 (endotoxin units – EU/mL). This solution is used for high-flux hemodialysis (HFHD) and hemodiafiltration (HDF), while a solution with endotoxin concentration ≤ 0.50 EU/mL (≤ 0.25 EU/mL) and the number of colonies ≤ 100 CFU/ml (≤ 50 CFU/mL) is used for low-flux hemodialysis (LFHD), according to current recommendations [8, 9]. Endotoxin and other bacterial products can pass from the dialysis solution through the dialysis membrane to the patient's

blood via backdiffusion/backfiltration processes and activate the mononuclear and polymorphonuclear cells to boost the formation and release of free oxygen and pro-cationic cytokine radicals interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα), all of this resulting in the development of oxidative stress, microinflammatory and accelerated atherosclerosis [8, 9].

In patients treated with regular hemodialysis, the activity of enzymatic and non-enzymatic antioxidant systems is reduced. Reduced concentration of trace elements, such as Se, Cu and Zn decreases the activity of antioxidant enzymes (SOD, Gpx). This is probably a result of insufficient intake, but also increased loss during HFHD/HDF [10]. Also, because of a lack of vitamins C and E, the capacity of non-enzymatic antioxidative protection systems is reduced [10].

The present investigation aimed to examine the effect of a vitamin E-coated membrane on oxidative stress during a single session of on-line HDF.

Methods

The study included 24 patients treated with on-line HDF at the Center for Nephrology and Dialysis of the Clinical Center Kragujevac. The research was conducted in accordance with the Helsinki Declaration on Medical Research, the approval from the Ethics Committee of the Clinical Center Kragujevac and the consent of the patients.

The patients examined in this study were regularly treated with on-line HDF, three times a week for 4 hours (12 h per week), over a period of more than three months with a convective volume of 17 liters per session, on machines with controlled ultrafiltration type Fresenius 5008S, Gambro AKA200US and Gambro Artis, with the blood flow strength - Qb = 250 mL/min and the dialysis flow strength-Qd = 500 mL/min were examined. A standard ultra-pure solution for on-line hemodiafiltration (endotoxin concentration – E < 0.03 EU/mL) was used. For on-line HDF, the high-flux dialysis membrane was coated with vitamin E-Leoceed 21H (polysulfonic membrane with the effective area of 2.1 m^2, KoA = 1351 mL/min, high-flux - Kuf = 88 mL/h × mmHg, sterilized by gamma rays, Asachi Kasei Medical Europe, Germany) and a high-flux dialysis membrane that is not coated with vitamin E – FX800 (polysulphonic membrane with the effective area of 2.0 m^2, KoA = 1365 mL/min, high-flux - Kuf = 62 mL/h × mmHg, sterilized by steam, Fresenius Medical Care, Germany). Exclusion criteria were as follows: patients with proven active bleeding, active systemic inflammation or infection, with uncontrolled malignancies, as well as patients treated with immunosuppressive and antioxidant drugs.

In order to evaluate the influence of the dialysis membrane coated with vitamin E on oxidative stress during an individual session of on-line HDF, several parameteres were followed: anemia (hemoglobin, hematocrit, erythrocyte indexes), iron status in the patient's organism (iron and ferritin serum concentration, iron saturation of transferin), microinflammation (C-reactive protein), nutritional status (prealbumin, transferin), secondary hyperparathyroidism (iPTH, vitamin D), oxidative stress: (superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), thiorbutyric acid reactive substances (TBARS), nitric oxide in the form of nitrites (NO_2^-), superoxide dizmutase (SOD), catalase (CAT), reduced glutathione activity (GSH)), carotid artery intima media thickness, blood flow through the vascular hemodialysis approach (Qavf) and the dialysis adequacy parameter (spKt/V).

Blood samples for laboratory analyses were taken prior to initiation and after completion of a single on-line HDF, before subcutaneous administration of heparin and before intravenous administration of iron and vitamin B complex at the end of the on-line HDF session. Blood samples were taken from the same group of patients before and at the end of an individual HDF session with a "high-flux" polysulfone membrane of type Leoceed 21H, as well as before and after type FX800 treatment. Routine laboratory analyses were performed using standard laboratory tests and calculated as an average value of three measurements over three consecutive months.

The serum ferritin concentration was determined by the turbidimetric method, on the Beckman Coulter AU680 apparatus. In patients treated with regular hemodialysis, the normal serum ferritin concentration is 100–500 ng/mL.

The serum C-reactive protein (CRP) concentration was determined by the turbidimetric method on the Olympus AU680 and calculated as the average value of two measurements over two consecutive months. The normal serum CRP concentration is ≤ 5 mg/L. Microinflammation is defined as the concentration of CRP in the serum of 5 mg/L.

The concentration of vitamin D in the serum was determined by electrochemiluminescence, on the Cobas e 411. The normal vitamin D concentration in the serum is 20–40 ng/mL. In patients treated with regular hemodialysis, the normal vitamin D concentration is ≥ 30 ng/mL (30–80 ng/mL). A severe deficit is defined as the concentration of vitamin D < 10 ng/mL. Vitamin D deficiency exists if the concentration is 10–20 ng/mL, and the insufficiency is defined as the concentration of vitamin D in the serum of 20–30 ng/mL.

The concentration of intact parathormone (PTH) in the serum was determined by the immunodiathymetric method (IRMA), on the gamma counter WALLAC WIZARD 1470. The normal concentration of intact PTH in the serum is 11.8–64.5 pg/mL. In patients with hemodialysis the upper normal limit is 300 pg/mL.

The principle of determining the concentration of superoxide anion ($O_2^{\cdot-}$) in blood plasma samples uses the O_2 reaction with nitro tetrazolium blue (Nitro Blue Tetrazolium – NBT) to nitroformase blue. Measurement takes place at a wavelength λ = 550 nm.

The method for determining the concentration of hydrogen peroxide (H_2O_2) is based on the oxidation of

phenol red by the hydrogen peroxide reaction, which catalyses Horse Radish Peroxidase (HRPO). The final result of this reaction is the formation of a compound with a maximum absorption λ_{max} = 610 nm.

The determination of the lipid peroxidation index was carried out indirectly through products of the lipid peroxidation reaction with thiobarbituric acid reactive substances (TBARS). The principle of this method is based on the determination of lipid peroxide levels based on the reaction of one of them, malonildialdehyde (MDA), with thiobarbutyric acid (TBA). Measurement takes place at a wavelength λ= 530 nm.

Determination of the concentration of nitrogen monoxide (NO_2^-) was carried out on the basis of the amount of released nitrites. The principle of this method involves the use of a Griess reagent, which builds a diazo complex with nitrites, which gives the purple color. Measurement takes place at a wavelength λ= 550nm.

An adrenaline method was used to determine the activity of SOD. The principle of this method, which normally belongs to the group of the "negative" type, is to monitor the reduction in the self-oxidation rate of adrenaline in the alkaline environment, which is dependent on $O_2^{-\cdot}$. Given that $O_2^{-\cdot}$ Is removed by the present SOD, the adrenaline authoxidation reaction is inhibited. The system monitors the rate of adrenaline autoxidation change through the change in absorbance at 480 nm, which is inversely proportional to SOD activity.

The Beutler method was used to determine the CAT activity. The principle is the spectrophotometric monitoring of the rate of decomposition of H_2O_2 in the presence of CAT at a wavelength of 230 nm, in which H_2O_2 absorbs light.

For the determination of reduced GSH activity, the Beutler spectrophotometric method was used. The principle of the method is based on the oxidation of GSH by 5,5-dithio-bis-6,2-nitrobenzoic acid (DTNB).

The hemodialysis adequacy was assessed on the basis of the single-pool Kt/Vsp index calculated according to Daugirdas second-generation formula: Kt/Vsp = -ln (C2/C1 - 0.008 x T) + (4 - 3.5 x C2/C1) x UF/W (mmol/L), T - hemodialysis duration (h), UF - interdialysis yield (L), W - body weight after the hemodialysis (kg). According to K/DOQI guidelines, hemodialysis is adequate if Kt/Vsp ≥ 1.2.

Urea reduction rate (URR) index was calculated using the following formula: URR = (1-R) × 100%, where R represents the ratio of urea concentration in the serum after and before the hemodialysis treatment. Hemodialysis is adequate if the URR index = 65–70%.

Blood flow through the vascular approach (Qavf) was determined by the Color Doppler ultrasound scan, on the Logic P5 apparatus, using a 7.5 MHz probe, wherein the blood flow is calculated from the formula: Qavf = $r^2\pi/4$ × Vmean × 60 (mL/min), where: r – radius of vascular access, and Vmean – mean blood flow velocity through vascular approach. The blood flow is calculated as the mean of three measurements, 2–4 cm on the vein vascular approach, proximal to the anastomosis site. Blood flow through a vascular approach that provides adequate hemodialysis is 500–1000 mL/min.

The thickness of carotid arterial intimal media (IMT) was determined by the Color Doppler ultrasonic examination, on the Logic P5 apparatus, using a 7.5 MHz probe, as the average value of three individual measurements on the right and left carotid arteries. Measurements were performed 1–2 cm below the bifurcation of carotid arteries by the same ultrasonograph apparatus. The normal thickness of the intima media is defined as a value of less than 0.9 mm.

The tests used for the statistical analyses of the obtained data were following: the Kolmogorov-Smirnov test, Student *t*-test and Wilcoxon test. Significance threshold was a probability of 0.05 and 0.01.

Results

In the Center for Nephrology and Dialysis of the Clinical Centre Kragujevac, a cross-sectional study was conducted, which included patients who have been treated with regular on-line HDF over a period of more than three months. Twenty four patients (19 men, 5 women) were examined (mean age: 60.92 ± 8.20 years, average dialysis length: 9.53 ± 5.45 years, and average spKtV dialysis index: 1.20 ± 0.18). General data on patients are shown in Table 1.

Anemia treatment that was used included short-acting and long-acting erythropoietin, i.v. administration of iron, i.v. preparation of vitamin B and folic acid perorally. The average monthly dose for short-acting erythropoietin was 18,000.00 ± 12,055.43 IU, and for long-acting erythropoietin it was 140.00 ± 36.33 μg. The average monthly intravenous iron dose was 256.25 ± 131.50 mg, the average monthly dose of vitamin B_{12} was 2,916.67 ± 1592.56 μg, and the average monthly folic acid dose was 181.25 ± 62.23 mg. Secondary hyperparathyroidism in the examined patients was treated with calcium-containing phosphate linkers, active metabolites of vitamin D and paricalcitol. The average monthly dose of rocaltrol was 3.08 ± 5.10 μg, and the average monthly dose of i.v. paracalcitol was 2.50 ± 12.25 μg.

The average values of anemia parameters, iron status, microinflammation, nutritional status, secondary hyperparathyroidism and ultrasound examination of carotid arteries are shown in Table 2.

In order to evaluate the influence of the dialysis membrane type on oxidative stress during a single session of on-line hemodiafiltration, the following parameters were examined: $sO_2^{-\cdot}$, H_2O_2, TBARS, NO_2^-, SOD, CAT, and reduced activity GSH. They are shown in Table 3.

The patients treated with HDF were shown a significantly ($p < 0.05$) lower concentration of TBARS and significantly ($p < 0.01$) lower SOD activity after the on-line HDF session with a vitamin E-coated membrane (Leoceed 21H), as shown in Table 3. When we treated the

same patients in a single on-line HDF session using a membrane without vitamin E-coating (FX800), t H_2O_2 values were significantly higher, while the SOD activity was significantly lower after the treatment ($p < 0.01$) than before the onset of HDF (Table 3). There was no significant difference in values of the rest of oxidative stress parameters: CAT, GSH, O_2^-, NO_2^- ($p > 0.05$), before and after the session of on-line HDF using a vitamin E-coated membrane (Leoceed 21H), as well as a a membrane without vitamin E-coating (FX800) (Table 3).

Table 1

General data on patients

Data	Values
Number of patients	24
Gender (M/F), n (%)	19/5 (79.17/20.83)
Age (years), mean ± SD	60.92 ± 8.20
Length of hemodyalisis treatment (years), mean ± SD	9.53 ± 5.45
Body mass index (kg/m^2), mean ± SD	25.63 ± 3.53
Systolic arterial blood pressure (mmHg), mean ± SD	131.67 ± 13.73
Diastolic arterial blood pressure (mmHg), mean ± SD	77.50 ± 6.76
Mean arterial blood pressure (mmHg), mean ± SD	95.56 ± 8.49
Body weight (kg), mean ± SD	74.21 ± 12.69
Interdialytic weight gain (kg), mean ± SD	2.33 ± 0.92
Interdialytic weight gain (%),mean ± SD	3.22 ± 1.32
Ultrafiltration rate (mL/kg/h), mean ± SD	8.04 ± 3.30
Ultrafiltration (mL/h), mean ± SD	583.33 ± 229.21
Residual diuresis (mL/24 h), mean ± SD	425.00 ± 591.98
Arteriovenous fistula flow (mL/min), mean ± SD	967.08 ± 415.62
Index of hemodyalisis adequacy (Kt/Vsp), mean ± SD	1.20 ± 0.18
Primary kidney disease, n (%)	
glomerulonephritis chronica	3 (12.5)
nephropathia hypertensive	10 (41.66)
nephropathia diabetica	1 (4.16)
nephropathia obstructiva	1 (4.16)
nephropathia chronica	4 (16.67)
renes polycystici	5 (20.83)
Comorbidity, n (%)	
hypertension	22 (91.66)
hypotension	1 (4.17)
diabetes mellitus	1 (4.17)

M – male; F – female; SD – standard deviation.

Table 2

Basic investigation parameters

Parameters	Values (mean ± SD)
Hemoglobine (g/L)	105.88 ± 14.56
Hematocrit (%)	32.09 ± 4.47
Mean corpuscular volume (fL)	93.74 ± 4.86
Mean corpuscular hemoglobin concentration (g/L)	329.50 ± 6.53
Vitamine B_{12} serum concentration (pg/mL)	962.33 ± 503.24
Folic acid serum concentration (ng/mL)	20.42 ± 13.16
Iron serum concentration (μmol/L)	9.62 ± 4.04
Transferrin saturation (%)	24.46 ± 9.77
Feritine serum concentration (ng/mL)	591.96 ± 318.31
C-reactive proteine serum concentration (mg/L)	6.08 ± 6.74
Albumine serum concentration (g/L)	37.96 ± 3.24
Prealbumine serum concentration (g/L)	0.29 ± 0.08
Transferine serum concentration (g/L)	1.57 ± 0.28
Vitamin D serum concentration (ng/mL)	20.16 ± 9.69
Intact parathormon serum concentration (pg/mL)	228.92 ± 287.42
Average right carotid artery intima-media thickness (mm)	1.21 ± 0.24
Average left carotid artery intima-media thickness (mm)	1.19 ± 0.26
Average intima-media thickness of both carotid arteries (mm)	1.20 ± 0.23

SD – standard deviation.

Table 3

The influence of the dialysis membrane type on oxidative stress parameters during a single session of on-line hemodiafiltration (HDF)

Parameters	On-line HDF membrane					
	Leoceed 21H (vitamin E-coated)		*p*	FX800 (without vitamin E-coating)		*p*
	before HDF	after HDF		before HDF	after HDF	
$O_2^{-\cdot}$	3.45 ± 3.51	1.87 ± 2.06	0.078	1.96 ± 1.23	1.83 ± 1.14	0.548
H_2O_2	4.82 ± 1.99	4.74 ± 1.56	0.878	7.14 ± 1.72	7.95 ± 1.54	0.003
TBARS	1.20 ± 0.26	1.07 ± 0.10	0.031	0.88 ± 0.15	0.90 ± 0.24	0.567
NO_2	3.65 ± 1.24	3.34 ± 0.80	0.223	7.79 ± 2.29	8.09 ± 2.00	0.174
SOD	37.99 ± 27.12	19.33 ± 11.46	0.003	27.13 ± 13.72	18.66 ± 11.38	0.010
CAT	2.38 ± 1.51	1.86 ± 1.28	0.107	1.79 ± 1.23	2.15 ± 1.52	0.626
GSH	110,615.52 ± 27,561.68	106,438.65 ± 32,204.63	0.992	90,988.86 ± 14,909.96	92,846.14 ± 12,470.16	0.364

***Note*: Results are given as mean ± standard deviation.**

$O_2^{-\cdot}$ – superoxide anion radical (nmol/mL); H_2O_2 – hydrogen peroxide (nmol/mL); TBARS – thiobarbituric acid reactive substances (μmol/L); NO_2^- nitric oxide in the form of nitrites (nmol/mL); SOD – superoxide dismutase (U/gHb × 10^3); CAT – catalase (U/gHb × 10^3); GSH – reduced glutathione (U/gHb × 10^3).

Discussion

Cardiovascular diseases have remained the leading cause of death in patients with a final stage of chronic kidney disease treated with regular dialysis. Oxidation stress is considered to be a non-traditional risk factor for the development of cardiovascular disease in this population of patients [11, 12]. It occurs as a disbalance between formation of free oxygen radicals and activity of antioxidant protection systems. In the population of patients treated with hemodialysis, four types of oxidative stress can be distinguished: standard oxidation stress, chlorinated stress, nitrosative stress and carbonyl stress. Antioxidant protection against oxidative stress includes enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic systems of protection which include hydrophilic vitamin C and lipophilic vitamin E [11–13]. The main clinical consequences of oxidative stress in patients with a terminal stage of chronic kidney disease treated with regular hemodialysis are: accelerated atherosclerosis, erythropoietin resistance (anemia) and amyloidosis caused by β_2-microglobulin [11–13].

Biocompatibility degree of the dialysis membrane significantly affects oxidative stress in patients with a terminal stage of chronic kidney disease treated with hemodialysis. The results of the conducted studies that compared the influence of two different dialysis membranes on oxidative stress indicate that the hemodialysis session with cuprophane membrane significantly increases the serum MDA concentration relative to the dialysate session with the polysulfone membrane [14]. After a hemodialysis session using a polysulfone and cuprophane membrane, the activity of antioxidant enzymes increases, but this increase is significant only for CAT in patients dialysed using a polysulphonic membranes [14]. However, comparing the effects of the hemodialysis session usingdifferent membranes, hemophane and polysulfone, it has been found that the polysulfone membrane significantly increases the concentration of MDA in the serum and significantly reduces selenium concentration and Gpx activity relative to the hemophane dialysis membrane [15]. The hemodialysis session using the "high-flux" polysulfone membrane significantly lowers the formation of free oxygen radicals during hemodialysis compared to the "low-flux" polysulfone membrane [16]. The hemodialysis session with the "high-flux" polysulfone membrane also provides better control of the neutrophil function compared to the "low-flux" polysulfone membrane [17].

On-line HDF, a biocompatible highly permeable dialysis membrane and an ultrapure solution for hemodialysis could ameliorate oxidative stress and slow the development of atherosclerosis [17, 18]. During a single session of on-line HDF, using membranes that have a large surface and high water permeability (Kuf > 50 mL/h × mmHg), there is an increased loss of trace oligoelements (selenium, zinc, copper), which are significant cofactor of enzymatic antioxidant enzymes (SOD). A significantly lower concentration of oxidative stress parameters is achieved on-line by HFD with a vitamin E-coated membrane in continuity over a 3–6 months period relative to standard hemodialysis using a “low-flux” membrane without vitamin E-coating. The results of clinical trials suggest that treatment with on-line HDF, with “high-flux” membranes over a period of 3–6 months, significantly reduces inflammation, oxidative stress and resistance to erythropoietin activity, compared to hemodialysis with “low-flux” membranes [17, 18]. Hemodialysis membranes (vitamin E-coated hemodialysis) have also shown the reduction of lipid peroxidation parameters values in the serum, such as: MDA, TBARS and oxidized low density lipoprotein (oxLDL) cholesterol [19–24]. Some studies highlight that these membranes also reduce the concentration of oxidative nucleic acid parameters such as 8-hydroxydeoxyguanosine (8-OHdG) and the concentration of microinflammatory parameters (CRP, interleukin-6) [19–24]. Treatment with “high-flux” hemodialysis using polysulphonic membrane-bound vitamin E over a period of three to six months

significantly reduces oxidative stress, microinflammation, and erythropoietin resistance index. It also corrects the treatment of anemia, reduces the amount of erythropoietin and the thickness of intima-media of carotid arteries in a population of patients treated with regular hemodialysis without affecting hemodialysis adequacy parameters (Kt/V index) [19–24]. The results of this study showed that after a single session of on-line HDF using a "high-flux" polysulfone membrane coated with vitamin E (Leocced 21H), the concentration of TBARS and the SOD activity are significantly reduced. This can be explained by the effect of vitamin E, but also by the loss of microelements, the cofactor of SOD during on-line HDF session [25, 26]. The results of the tests carried out so far show that a significant amount of microelements (selenium, zinc, copper) is lost during the on-line HDF and hemodialysis using a "high-flux" polysulfone membrane, which results in a decrease in the activity of antioxidant enzymes, such as SOD and Gpx [27–30]. Normal serum zinc concentration is 70–110 μg/dL, in erythrocytes 40–44 μg/g Hb, and normal activity of SOD in erythrocytes is 1.102–1.601 IU/g Hb. In patients treated with regular dialysis, zinc is administered at a dose of 100 mg/day for 8 weeks. The results of the study show that the zinc applied in this dose significantly increases the activity of SOD in erythrocytes, while the serum MDA concentration significantly decreases [31, 32]. In addition to the oligoelements, during the dialysis session, hydrosoluble vitamins that exhibit an antioxidant effect (vitamin C) are lost. Vitamin C clearance during the hemodialysis session is 30–50%, and during "high-flux" hemodialysis and hemodynamic filtration over 50%. Due to reduced antioxidant capacity and reduced GSH concentration during HDF using "high-flux" polysulphonic vitamin E-coated membranes, oligoelements, vitamin C and vitamin E should be used to prevent these events. Vitamin C should be administered at a dose of 300 mg i.v. after each individual hemodialysis session for 8–12 weeks (with monitoring of serum oxalate concentration), while vitamin E is administered perorally at a dose of 400–800 IU/day for 12–16 weeks for secondary prevention of cardiovascular events; a strong antioxidant effect is achieved at a dose of 1000 mg/day perorally for 8 weeks (alpha tocopherol: 1.0 mg = 1.5 IU) [28–30].

Conclusion

A single session of on-line HDF using a "high-flux" large-surface polysulfone vitamine E-coated membrane ($A \geq 2.0$ m^2) significantly affected parameters of oxidative stress relative to the individual session of the on-line HDF using the "high-flux" polysulfone membrane with surface ≥ 2.0 m^2, which is not coated with vitamin E. After a single session of on-line HDF using the "high-flux" polysulfone vitamine E-coated membrane with surface ≥ 2.0 m^2, the concentration of TBARS was significantly decreased, while the activity of SOD was decreased with both dialysis membranes, probably as a consequence of the increased loss of trace elements, which are the cofactors of enzymatic antioxidative system components (SOD). On-line HFD using "high-flux" vitamine E-coated polysulfone membrane should be applied in a 3–6 months period, with an appropriate assessment of the antioxidant protection capacity during treatment with this dialysis modality. In order to achieve the optimal efficiency of the treatment, individualization of HDF prescription is needed.

Acknowledgement

Authors would like to express their deepest gratitude to the Ministry of Education, Science and Technological Development of the Republic of Serbia for the Grant N^{0}175014, as well as to the Faculty of Medical Sciences, University of Kragujevac for their Junior Grant N^{0}02/19 from which the funds were used as one of the sources to financially support this paper.

R E F E R E N C E S

1. *Cozzolino M, Mangano M, Stucchi A, Ciceri P, Conte F, Galassi A.* Cardiovascular disease in dialysis patients. Nephrol Dial Transplant 2018; 33(1): 28–34.
2. *Descamps-Latscha B, Drüeke T, Witko-Sarsat V.* Dialysis-induced oxidative stress: biological aspects, clinical consequences, and therapy. Semin Dial 2001; 14(3): 193–9.
3. *Liakopoulos V, Roumeliotis S, Gorny X, Dounousi E, Mertens PR.* Oxidative Stress in Hemodialysis Patients: A Review of the Literature. Oxid Med Cell Longev 2017; 2017: 3081856.
4. *Liakopoulos V, Roumeliotis S, Zarogiannis S, Eleftheriadis T, Mertens PR.* Oxidative stress in hemodialysis: Causative mechanisms, clinical implications, and possible therapeutic interventions. Semin Dial 2019; 32(1): 58–71.
5. *Antić S, Draginić N, Nikolić T, Jeremić N, Petrović D.* Oxidative stress in hemodialysis patients: pathophysiological mechanisms, clinical consequences and basic principles of treatment. Ser J Exp Clin Res 2019; DOI: 10.2478/sjecr-2019-0008.
6. *Ronco C, Clark WR.* Hemodialysis membranes. Nat Rev Nephrol 2018; 14(6): 394–410.
7. *Wu CC, Chen JS, Wu WM, Liao TN, Chu P, Lin SH*, et al. Myeloperoxidase serves as a marker of oxidative stress during single hemodialysis session using two different biocompatible dialysis membranes. Nephrol Dial Transplant 2005, 20(6): 1134–9.
8. *Ward RA.* Ultrapure Dialysate. Semin Dial 2004; 17(6): 489–97.
9. *Glorieux G, Neirynck N, Veys N, Vanholder R.* Dialysis water and fluid purity: more than endotoxin. Nephrol Dial Transplant 2012; 27(11): 4010–21.
10. *Aziz MA, Majeed GH, Diab KS, Al-Tamimi RJ.* The association of oxidant-antioxidant status in patients with chronic renal failure. Ren Fail 2016; 38(1): 20–6.
11. *Ling XC, Kuo KL.* Oxidative stress in chronic kidney disease. Ren Replacement Ther 2018; 4: 53.
12. *Krata N, Zagozdzon R, Foroncewicz B, Mucha K.* Oxidative Stress in Kidney Disease: The Cause or the Consequence? Arch Immunol Ther Exp 2018; 66(3): 211–20.
13. *Liakopoulos V, Roumeliotis S, Bozikas A, Eleftheriadis T, Dounousi E.* Antioxidant Supplementationem in Renal Replacement

Therapy Patients: Is There Evidence? Oxidative Med Cell Long 2019; 2019: 9109473.
14. *Varan HI, Dursun B, Dursun E, Ozben T, Suleymanlar G.* Acute effects of hmodialysis on oxidative stress parameters in chronic uremic patients: Comparison of two dialysis membranes. Int J Nephrol Renovasc Dis 2010; 3: 39–45.
15. *Yavuz O, Bicik Z, Cinar Y, Guney Y, Guler S.* The effect of different dialysis membranes on oxidative stress and selenium status. Clin Chim Acta 2004; 346(2): 153–60.
16. *Ward RA, Ouseph R, McLeish KR.* Effects of high-flux hemodialysis on oxidant stress. Kidney Int 2003; 63(1): 353–9.
17. *den Hoedt CH, Bots ML, Grooteman MP, van der Weerd NC, Mazairac AH, Penne EL,* et al. CONTRAST Investigators. Online hemodiafiltration reduces systemic inflammation compared to low-flux hemodialysis. Kidney Int 2014; 86(2): 423–32.
18. *Panichi V, Scatena A, Rosati A, Giusti R, Ferro G, Malagnino E,* et al. High-volume online hemodiafiltration improves erythropoiesis-stimulating agents (ESA) resistance in comparison with low-flux bicarbonate dialysis: results of the REDERT study. Nephrol Dial Transplant 2015; 30(4): 682–9.
19. *Tarng DC, Huang TP, Liu TY, Chen HW, Sung YJ, Wei YH.* Effect of vitamin E-bonded membrane on the 8-hydroxy 2,-deoxyguanosine level in leukocyte DNA of hemodialysis patients. Kidney Int 2000; 58(2): 790–9.
20. *Panichi V, Rosati A, Paoletti S, Ferrandello P, Migliori M, Beati S,* et al. A vitamin E-coated polysulphone membrane reduces serum levels of inflammatory markers and resistance to erythropoietin-stimulating agents in hemodialysis patients: results of a randomized cross-over multicenter trial. Blood Purif 2011; 32(1): 7–14.
21. *Yang SK, Xiao L, Xu B, Xu XX, You F, Liu FY,* et al. Effects of vitamin E-coated dialyzer on oxidative stress and inflammation status in hemodialysis patients: a systematic review and meta-analysis. Ren Fail 2014; 36(5): 722–31.
22. *Yamadera S, Nakamura Y, Inagaki M, Ohsawa I, Gotoh H, Goto Y*, et al. Vitamin E-Coated Dialyzer Inhibits Oxidative Stress. Blood Purif 2017; 44(4): 288–93.
23. D'Arrigo G, Baggetta R, Tripepi G, Galli F, Bolignano D. Effects of vitamin E-Coated versus Conventiional Membranes in Chronic Hemodialysis Patients: A Systemic Review and Meta-Analysis. Blood Purif 2017; 43(1–3): 101–22.
24. *Locatelli F, Andrulli S, Vigano SM, Concetti M, Urbini S, Giacchino F,* et al. Evaluation of the Impact of a New Synthetic Vitamin E-Bonded Membrane on the Hypo-Responsiveness to the Erythropoietin Therapy in Hemodialysis Patients: A Multicenter Study. Blood Purif 2017; 43(4): 338–45.
25. *Akiyama S, Inagaki M, Tsuji M, Gotoh H, Gotoh T, Washio K,* et al. Comparison of effect of vitamin E-coated dialyzer and oral vitamin E on hemodialysis-induced Cu/Zn-superoxide dismutase. Am J Nephrol 2005; 25(5): 500–6.
26. *Nakamura Y, Inagaki M, Kenmotsu S, Yamadera S, Ohsawa I, Gotoh H,* et al. Significance of Cu/Zn-Superoxide Dismutase Levels in Hemodialysis Patients: A Mini Review. MRI 2017; 6(2): 9–13.
27. *Torregosa E, Jaras H, Sastre J, Pons R, Calvo HG, Gordo CC*, et al. Analysis of oxidative stress in patients on-line hemodiafiltration. Nefrologia 2007; 27(5): 612–8.
28. *Singer RF.* Vitamin C supplementation in kidney failure: effect on uraemic symtoms. Nephrol Dial Transplant 2011; 26(2): 614–20.
29. *Coombes JS, Fassett RG.* Antioxidant therapy in hemodialysis patients: a systematic review. Kidney Int 2012; 81(3): 233–46.
30. *Kosmadakis G, DaCosta Correia E, Odette Carceles O, Somda F, Aguilera D.* Vitamins in dialysis: who, when and how much? Ren Fail 2014; 36(4): 638–50.
31. *Wang LJ, Wang MQ, Hu R, Yang Y, Huang YS, Xian SX*, et al. Effect of Zinc Supplementation on Maintenance Hemodialysis Patients: A Systematic Review and Meta-Analysis of 15 Randomized Controlled Trials. Biomed Res Int 2017; 2017: 1024769.
32. *Mazani M, Argani H, Rashtchizadeh N, Ghorbanihaghjo A, Hamdi A, Estiar MA,* et al. Effect of zinc supplementation on antioxidant status and lipid peroxidation in hemodijalysis patients. J Ren Nutr 2013; 23(3): 180–4.

Received on July 30, 2019.
Revised on August 21, 2019.
Accepted on August 21, 2019.
Online First September, 2019.

ORIGINAL ARTICLE

UDC: 617-089.161:616.893
DOI: https://doi.org/10.2298/VSP190303088L

Preoperative alcohol consumption, intraoperative bleeding and postsurgical pain may increase the risk of postoperative delirium in patients undergoing radical retropubic prostatectomy

Preoperativno konzumiranje alkohola, intraoperativno krvarenje i postoperativni bol mogu povisiti rizik od nastanka postoperativnog delirijuma kod bolesnika nakon radikalne retropubične prostatektomije

Nikola Ladjević*, Nebojša Nick Knežević†‡, Andjela Magdelinić*, Ivana Likić Ladjević*, Otaš Durutović*, Dušica Stamenković§‖, Vesna Jovanović*¶, Nebojša Ladjević*¶

***University of Belgrade, Faculty of Medicine, Belgrade, Serbia; †Advocate Illinois Masonic Medical Center, Department of Anesthesiology, Chicago, IL, USA; ‡University of Illinois, Department of Anesthesiology, Chicago, IL, USA; §Military Medical Academy, Department of Anesthesiology and Intensive Therapy, Belgrade, Serbia; ‖Universit of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ¶Clinical Center of Serbia, Center for Anesthesiology and Reanimatology, Belgrade, Serbia**

Abstract

Background/Aim. The incidence of postoperative delirium (POD) after non-cardiac surgery is a problem not often recognized by many anesthesiologists. The objective of our study was to detect POD and its possible cause, in patients undergoing radical retropubic prostatectomy (RRP) under general anesthesia. **Methods.** After Ethical Committee approval, we enrolled 80 patients, ASA (the American Society of Anestesiology) status II, scheduled to undergo RRP under general anesthesia, in a prospective study. All patients completed MMSE tests (the Folstein Mini Mental State Exam) the evening before, and 48 hours after the surgery. Assessment for the presence and severity of delirium was performed using CAM (the Confusion Assessment Method), and an assessment of the degree of agitation and sedation using RASS (the Richmond Agitation and Sedation Scale). **Results.** The average preoperative MMSE score (28.59 ± 1.04) significantly decreased following the surgery (27.74 ± 1.52) ($p < 0.0001$). The average postoperative MMSE score trend descended in correlation to intraoperative bleeding ($p = 0.036$). The patients with higher pain scores had significant decline in MMSE after the surgery (28.75 vs. 26.25; $p < 0.001$). Five patients were considered positive for delirium, and four of them reported regular alcoholic drinks intake (> 1 drink *per* day) preoperatively ($p < 0.0001$). Based on RASS score, 13 patients (16.3%) were agitated or sedated, and they had statistically significantly higher intraoperative bleeding ($p < 0.001$). **Conclusion.** Results of this study emphasize the importance of proper preoperative evaluation; especially regarding the alcohol consumption since all the patients that developed POD reported moderate alcohol consumption. Furthermore, greater intraoperative bleeding and postoperative pain scores did not influence the occurrence of delirium, but resulted in lower postoperative MMSE scores, which highlights the importance of adequate intraoperative treatment of patients during surgery and anesthesia in order to reduce the risk of developing POD.

Key words:
delirium; postoperative complications; alcohol consumption; bleeding; postoperative pain; risk factors; prostatectomy.

Apstrakt

Uvod/Cilj. Postoperativni delirijum (POD) kod bolesnika nakon nekardiohirurških procedura je često neprepoznat od strane anesteziologa. Cilj naše studije bio je procena učestalosti postoperativnog delirijuma (POD) i mogućih faktora rizika od njegovog nastanka kod bolesnika koji su bili u opštoj anesteziji usled hirurškog zahvata kod radikalne retropubične prostatektomije (RRP). **Metode.** Nakon dobijanja dozvole Etičkog komiteta, prospektivna studija

Correspondence to: Nebojša Ladjević, Clinical Center of Serbia, Center for Anesthesiology and Reanimatology, Resavska 51/IV floor, 11 000 Belgrade, Serbia. E-mail: nladjevic@yahoo.com

obuhvatila je 80 bolesnika, ASA (*the American Society of Anestesiology*) skor II, koji su planirani za RRP u opštoj anesteziji. Bolesnici su ispunili MMSE test (*the Folstein Mini Mental State Exam*) preoperativno (veče pred operaciju) i postoperativno (48 sati nakon operacije). U studiji smo ipitivali prisutsvo i težinu delirijuma upotrebom CAM (*the Confusion Assessment Method*), a stepen agitacije i sedacije primenom RASS (*the Richmond Agitation and Sedation Scale*). **Rezultati.** Prosečni preoperativni MMSE skor (28,59 ± 1,04) bio je značajno snižen u postoperativnom periodu (27,74 ± 1,52; $p < 0{,}0001$). Sniženje prosečnog postoperativnog MMSE skora bilo je u korelaciji sa intraoperativnim krvarenjem ($p = 0.036$). Bolesnici sa višim intenzitetom bola imali su značajno snižen postoperativni MMSE skor (28,75 vs. 26,25; $p < 0{,}001$). Kod četiri od pet bolesnika koji su imale delirijum, zabeležen je redovni unos alkohola (> 1 pića dnevno) u preoperativnom periodu ($p < 0{,}0001$). Na osnovu RASS skora, agitacija je registrovana kod 13 (16,3%) bolesnika, i kod svih je zabeleženo značajno veće intraoperativno krvarenje u odnosu na ostatak ispitanika ($p < 0.001$). **Zaključak.** Rezultati naše studije pokazuju da je u preoperativnoj evaluaciji značajno registrovati preoperativnu konzumaciju alkohola, uzevši u obzir da su svi bolesnici koji su u postoperativnom period razvili POD, preoperativno konzumirali alkohol u većoj količini. Iako veće intraoperativno krvarenje i postoperativni bol višeg intenziteta nisu uticali na učestalost pojave delirijuma, snižavali su MMSE skor, što ukazuje na značaj adekvatnog intraoperativnog tretmana bolesnika u toku hirurgije i anestezije u cilju smanjenja rizika od razvoja POD.

Ključne reči:
delirijum; postoperativne komplikacije; alkohol, pijenje; krvarenje; bol, postoperativni; faktori rizika; prostatektomija.

Introduction

The incidence of postoperative delirium after non-cardiac surgery in patients older than 18 years of age could range between 19% and 44.5% [1, 2]. This problem is often underestimated and not recognized by many anesthesiologists. Postoperative delirium is more frequent in the elderly but is also perceived in younger patients as well. Since the world population over the age of 65 is increasing, this would be a more commonly observed problem in the post-anesthesia care unit (PACU) and intensive care unit (ICU) in the upcoming years [3, 4]. Postoperative delirium in patients undergoing surgical procedures under general anesthesia is very important because it is associated with poor outcomes, increased mortality rate, increased length of stay in the PACU and overall hospital stay as well [5, 6].

Pathogenesis of delirium is poorly understood. In several attempts, researchers tried to develop predictor model to identify postoperative risk for delirium by looking at severe illness, visual impairment, cognitive impairment, nitrogen/creatinine ratio, neurological impairments, and social habits (smoking, ethanol abuse) [7–9]. However, none of these parameters direct significant sensitivity toward delirium determination. On the other hand, delirium could have iatrogenic etiology triggered by anesthetic medications. Sieber et al. [10] in a randomized study showed that the use of light propofol vs. deep sedation could reduce the prevalence of postoperative delirium by 50% in patients undergoing hip fracture repair under spinal anesthesia.

Different screening tools have been used in hospitalized patients for the screening of delirium [11]. The Mini Mental State Exam (MMSE), initially described by Folstein et al. [12] in 1975 is recommended as a simple tool in the early detection of cognitive impairment and state of delirium. Even though it cannot have a final diagnostic accountability, it can serve in screening for mental state function validation. Sensitivity and specificity for delirium/dementia are 87% and 82%, respectively, calculated when 24 out of 30 were used as cut-off score [13]. The Confusion Assessment Method (CAM) test was designed to be used by clinicians that are not mental health professionals. In a systematic review of 9 different studies, Orman et al. [14] showed very high sensitivity and specificity of this test in several studies (80% and 95.9%, respectively). Furthermore, CAM scale has the highest level of compatibility with the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders) classification, which is now considered to be the gold standard in the diagnosis of delirium [15]. The Richmond Agitation and Sedation Scale (RASS) is a 10-point scale that was developed in collaboration with critical care physicians, nurses, and pharmacists [16]. It was initially developed to assess the level of agitation or sedation in order to ensure precise medication titration. This scale has been frequently used in the research and clinical practice settings for delirium assessment. In a prospective cohort study on 510 ICU patients, Vasilevskis et al. [17] showed that RASS in combination with CAM is a sustainable and reliable measure of delirium and sedation along a bedside.

The objective of our study was to detect postoperative delirium using pre- and postoperative MMSE, postoperative CAM and RASS, as well as possible risk factors in male patients undergoing radical retropubic prostatectomy (RRP) under general anesthesia.

Methods

This prospective observational study was conducted after receiving approval from Ethical Committee of the Clinical Center of Serbia in Belgrade, Serbia. We consented and enrolled 80 male patients who were scheduled for radical retropubic prostatectomy at the Clinic of Urology, Clinical Center of Serbia. All the patients who had clinically significant cardiovascular, respiratory, hepatic, renal, neurological diseases or psychiatric disorders, those who had history of benzodiazepine abuse or those who had undergone a general anesthesia 30 days before screening were excluded from the study.

All 80 patients underwent radical retropubic prostatectomy under general anesthesia. Half an hour prior to the induction of anesthesia, the patients were pre-medicated with midazolam 5 mg *im* and atropin 0.5 mg *im*. Common methods of balanced general anesthesia were applied. All patients received 1.5 μg/kg *iv* of fentanyl and 2 mg/kg *iv* of propofol for induction of anesthesia, and 0.6 mg/kg *iv* of rocuronium bromide muscle relaxant to facilitate tracheal intubation. General anesthesia was maintained by a mixture of sevoflurane (Fex = 0.8%), nitrous oxide and oxygen (FiO_2 = 40). Neuromuscular antagonism maintenance dose 0.15 mg/kg of rocuronium bromide was administered when 2 responses to TOF ("Train of Four") stimulation were present. Analgesia was maintained by intravenous injection of opioids that included 0.5–1.0 μg/kg *iv* fentanyl bolus injection. Intraoperative monitoring for all patients included continuous recording of five-lead electrocardiogram (ECG) with special attention to ST segment, oxygen saturation by pulse oximetry, and noninvasive blood pressure, airway gas analysis, capnography and TOF stimulation. At the end of the surgery, residual neuromuscular blockade was reversed by mixture of atropine 0.75 mg *iv* and neostigmine 1.5 mg *iv*.

Upon admission to the ICU, patients received continuous *iv* infusion of tramadol 400 mg/day and diclofenac-Na^+ 75 mg *im* every 12 hours if the pain scores were more than 3 out of 10 on the Numeric Rating Scale (NRS).

We collected the following variables: demographic information (age, height, weight, education level), comorbidity (detailed medical history with emphasis on neuropsychiatric disorders), as well as alcohol consumption (number of drinks *per* day), the American Society of Anestesiology (ASA) status, duration of anesthesia, duration of surgery, total blood loss, length of stay in the ICU and total length of stay in the hospital. Furthermore, we collected the MMSE scores preoperatively and postoperatively, and postoperatively CAM, RASS and NRS scores.

Twelve hours before the surgery patients were interviewed, and the Folstein MMSE questionnaire, written in Serbian, language were completed. The MMSE is an 11-question assessment tool that can be completed within 5–10 minutes, with the maximum test score of 30. This test is a global assessment of many domains including: orientation of time and place, registration of 3 words, attention and calculation (recall of 3 words, language and visual construction), which allows detection of mood changes, abnormal mental experiences and thought process impairment [12]. Reassessment of cognitive status using MMSE score was performed 48 hours after the surgery.

The CAM test was used to evaluate the presence and severity of delirium and agitation. This test is easy to perform for the short period of time (5 minutes). The RASS was used to assess the level of sedation. This 10-point scale has one level to denote a calm/alert state (0), five levels of sedation (-1 to -5) and four levels to detect anxiety or agitation (+1 to +4). These two scales, CAM and RASS, were collected 48 hours after the surgery. One person interviewed patients and collected all MMSE, CAM and RASS scores to prevent any inconsistency.

Pain scores were recorded on an 11-point NRS (0–10), every 6 hours postoperatively in the first 48 hours after the surgery.

Statistical analysis

The sample size estimated for this study was 78, based on the difference in pain scores at $\alpha = 0.05$, power = 0.95, and effect size of 0.36. We considered the difference of 3 in MMSE pain scores to be a clinically significant improvement. Statistical analysis included measures of central tendency (the statistical variability of the series, the interval of variation, mean with standard deviation and weighted average). The Student's *t*-test and Pearson's Chi-square (χ^2) test were applied for testing differences between variables; as for testing the correlation between variables, we used the Pearson's correlation coefficient. A *p*-value less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 20.0 software (IBM Corporation, Armonk, NY).

Results

The study included 80 hospitalized patients who underwent RRP. The enrolled patient age range was between 44 and 74 years (the average age was 65 ± 6 years). All the patients were assigned ASA II status. The majority of the patients, 48 of them (60%), had a normal body mass index (BMI). Regarding the level of education, most of them, 46 (57.5%), had high level of education (college degree or graduate degree). Only 4 patients (5%) reported regular consumption of more than one drink *per* day.

The average preoperative MMSE score of 28.59 ± 1.04 was within normal score range, in accordance to patient's age and level of education, whereas score measured 48h after the surgery was 27.74 ± 1.52. When MMSE values were compared with the preoperative baseline, the mean MMSE scores decreased significantly following the surgery (*t*-test = 4.602, $p < 0.0001$). The older patients had lower postoperative MMSE scores, but without any statistical significance (Figure 1). The patients with lower level of education showed higher cognitive deterioration postoperatively according to MMSE scores, however, the difference was not statistically significant ($p > 0.05$) (Figure 2).

The surgery duration was between 97 and 145 minutes (average 125 ± 11 minutes). The average duration of anesthesia was 151 ± 13 minutes (ranging from 121 to 171 minutes). There was no correlation between postoperative delirium and duration of surgery or anesthesia ($p > 0.05$).

On average, the blood loss during the surgery was 1,058 ± 278 mL. Throughout the entire surgery, hematocrit values were checked regularly, and blood transfusion was initiated if the hematocrit levels were below 0.33. The patients received 1–3 units (equivalent to 300–900 mL) of packed red blood cells (pRBCs). Compared to intraoperative bleeding, there was the mean postoperative MMSE scores decline. Specifically, less intraoperative bleeding was in correlation with the highest postoperative MMSE score ($\rho = 0.5397$), which was expressed as statistically significant $p = 0.036$ (Figure 3).

Postoperatively, patients reported pain scores between 0

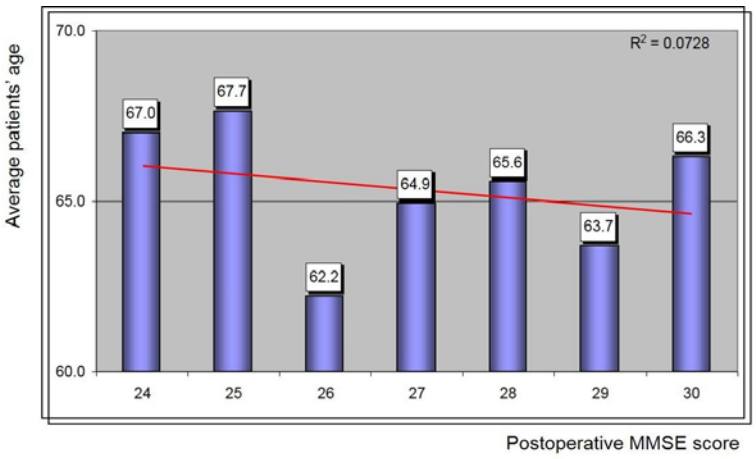


Fig. 1 –Postoperative Mini Mental State Exam (MMSE) scores and average patients' age.

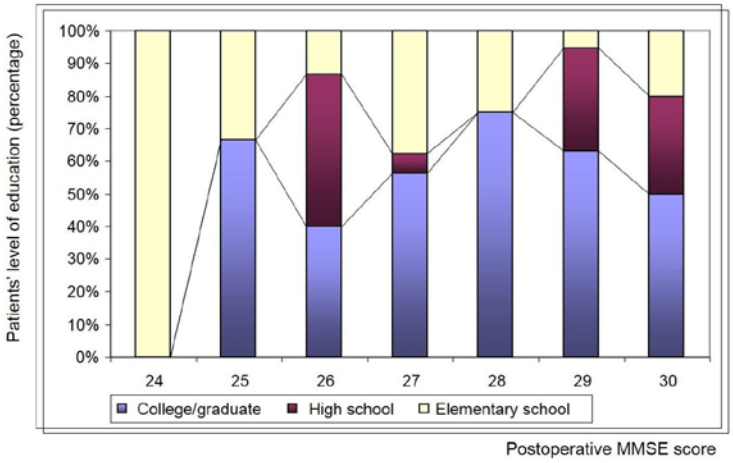


Fig. 2 – Postoperative Mini Mental State Exam (MMSE) scores and patients' level of education.

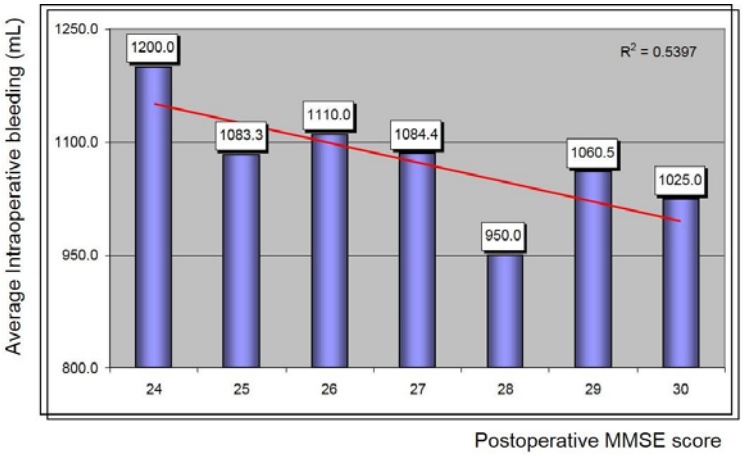


Fig. 3 – Postoperative Mini Mental State Exam (MMSE) scores and average intraoperative bleeding.

and 4 on an 11-point NRS scale (0–10), with an average of 2.33 ± 1.11. The majority of patients had pain scores 3/10 (57.5%), and only 4% of them had 4/10. Patients with higher pain scores had significant MMSE scores decline after the surgery (28.75 vs. 26.25; $p < 0.001$). Correlation between postoperative pain scores and MMSE scores decline was statistically significant ($p = 0.002$).

The CAM diagnostic algorithm was utilized for all the patients. According to the CAM scale, five patients were considered positive for delirium. Four out of 5 patients were classified as moderate alcohol consumers because they were consuming up to 2 drinks *per* day preoperatively ($\chi^2 = 63.16$; $p < 0.0001$). Patients that developed delirium were a few months older (65.75 years) when compared to those that did not develop delirium (64.40 years), which had no statistical significance. The patients that developed delirium had lower MMSE scores preoperatively (27.80), compared to those that did not develop delirium (28.64), and that was without significant difference. Additionally, these patients also had greater blood loss compared to the others (1,100 mL and 1,053.7 mL respectively), without statistical significance as well.

The RASS score (score of agitation and sedation) ranged from -2 to +4 for all patients. Most of the patients, 67 (83.7%), were awake, alert and demanding with the RASS score = 0 and 13 patients (16.3%) were agitated or sedated. Three out of 5 patients with delirium had mixed delirium, 1 patient had hypoactive and 1 patient had hyperactive delirium. The patients with the RASS score = 0 had less intraoperative bleeding (average 1,004.03 ± 211.03 mL) then patients that were agitated or sedated (average, 1,244.44 ± 391.41 mL) ($F = 11.91$; $p < 0.001$).

Furthermore, there was a statistically significant correlation between preoperative MMSE scores and postoperative RASS ($R = 0.552$; $p = 0.018$). RASS scores increased postoperatively for most of the patients with low preoperative MMSE. However, postoperative MMSE descending score was related to lower RASS, but without statistical significance ($R = 0.044$; $p = 0.881$).

Patients that had POD stayed in the ICU longer (average 95 ± 19 hours) than patients without POD (average 49 ± 11 hours) and this difference was statistically significant ($p = 0.0411$). Furthermore, patients with POD stayed in the hospital slightly longer (10 ± 3 days) than patients without POD (8 ± 2 days); however, this difference was not statistically significant ($p > 0.05$).

Discussion

Our results revealed that only 6.25% of patients developed delirium after RRP under general anesthesia, which is significantly lower incidence than observed (21.23%) in a study by Tai et al. [18]; however, our patients were, on average, six years younger than patients in their study. Studies that followed incidence of delirium for patients after other (non-urological) types of surgeries showed the incidence ranging from 0.84% up to 51% [19–21].

Results of our study pointed out that the risk factors for developing delirium in our patient population were moderate alcohol consumption, intraoperative bleeding and postoperative pain. Based on CAM scale, four out of 5 patients that developed delirium reported the use of more than one drink *per* day which fits into criteria for moderate alcohol consumption *per* Dietary Guidelines for Americans 2015–2020. [22]. The other authors found that alcohol abuse was one of the predictors for the development of delirium as well [19, 20, 23].

In a retrospective database analysis, Fineberg et al. [19] showed the incidence of postoperative delirium of 0.84% in patients undergoing spine surgical procedures. They found that patients who developed delirium were elderly (≥ 65 years) and with the history of alcohol and/or drug abuse, having depression, some neurological or psychiatric disorders, electrolyte, pulmonary or renal abnormalities, anemia or congestive heart failure. They also found that delirium was associated with 7.6 times increased mortality rate [19].

Shah et al. [20] showed that 11.5% of 774 study patients undergoing major resection of head and neck squamous carcinoma developed delirium. They showed that older age (≥ 69 years), preexisting cognitive impairment, surgery duration (longer than 6 hours) and alcohol consumption are predictors for developing delirium [20]. It was found that asking the patients whether they have ever been advised on cutting back on drinking alcohol or abstained for at least a week in the past year could help in postoperative delirium risk identification [20].

Hudetz et al. [23] conducted a prospective study with 28 patients over the age of 55 with self-reported alcohol abuse, and the same number of matched non-consuming alcohol controls, undergoing elective surgery under general anesthesia. Even though experimental patients' group did not consume alcohol for 5 weeks prior to the surgery, they had a higher incidence of postoperative delirium due to impaired executive (frontal lobe) functions even without neurological defects [23]. The results of our study, as well as other studies, [19–21] confirmed that physician should emphasize the question regarding alcohol consumption prior to the surgery.

We excluded patients with clinically significant cardiovascular, respiratory, hepatic, renal, neurological and psychiatric diseases, yet confirmed that cognitive impairment is an important predictor for post-operative delirium, as shown by many other authors [9, 24, 25]. The etiology of cognitive impairment observed in elderly patients is multifactorial. When dealing with elderly patients in the preoperative anesthesia clinic, anesthesiologists should assess the cognitive function and identify all risk factors that might be associated with cognitive dysfunction [24].

Several already existing models are able to identify patients with predisposing factors for developing postoperative delirium [9, 25]. Marcantonio et al. [25] developed a set of scores for patients undergoing elective non-cardiac surgery including factors such as: age, poor cognitive and functional status, significantly abnormal preoperative glucose, sodium and potassium levels, as well as self-reported alcohol abuse.

It is important to recognize that even intraoperative management may play a role in the development of POD. Results of our study showed that patients that had more intraoperative bleeding had lower postoperative MMSE scores than the RASS scores, which revealed either agitation or sedation. Olin et al. [21] observed 51 patients (average 75.1 years of age) after major abdominal surgeries and showed that 26 of them (51%) developed delirium, and where delirium lasted for more than 3 days there was significantly greater blood loss.

The results of our study showed that patients experiencing more pain had significant MMSE decline after surgery. Leung et al. [26] also found that patients with higher postoperative pain, having received higher doses of opioids, had 3.6 times greater risk for developing POD.

Our patients who developed POD stayed longer in the ICU. Observing 48 patients, Ely et al. [27] studied the relationships between delirium in the ICU and outcomes including length of hospitalization. Multivariate analysis showed that POD was the most important independent factor for the length of hospitalization [27]. When compared to the other patients that have not developed delirium, our patients who developed POD did not stay in the hospital much longer. However, it is well known that these patients usually have prolonged hospitalization, which is related to increased morbidity and mortality [5, 19, 28].

Veiga at al. [29] evaluated the incidence and determinants for delirium development during the immediate postoperative period in 680 adult PACU patients. The patients that developed delirium (18.8%) were elderly (average 71 years of age), had higher ASA physical status, were more likely to have emergency surgery, and were more severely ill (hypertension, hyperlipidemia, ischemic heart disease, congestive heart disease, cerebrovascular disease, or previous renal insufficiency). They also stayed in the PACU and hospital longer, and also received higher volume of intraoperative fluids. They showed that POD was an independent determinant for hospital mortality and post 6-month follow-up mortality [29].

Witlox et al. [28] conducted a meta-analysis of 42 studies that investigated delirium in elderly patients and showed that it is associated with poor outcomes, increased risk of death, institutionalization, and dementia. However, they also showed that delirium was independent of other confounders such as age, sex, comorbid illness or illness severity, or the presence of dementia at baseline. Delirium can be prevented in some cases; nevertheless, once present, management of delirium has very limited results in improving long-term mortality [30]. The most important is to identify the patients at high risk for developing delirium and apply different strategies to prevent delirium occurrence.

In a meta-analysis of 29 randomized controlled studies that reported perioperative interventions and postoperative delirium after non-cardiac surgeries, Moyce et al. [31] showed that perioperative geriatric consultation and lighter anesthesia were associated with the reduced risk of POD.

The limitations of our study are that it was done in a single center, patients were younger than 65 years of age, and certain patients had some form of psychiatric impairment, which could be the reason for relatively low incidence of postoperative delirium.

Conclusion

The results of this study emphasize the importance of proper preoperative evaluation, encouraging physicians to spend more time interviewing patients and getting details from their medical and social history, especially regarding the alcohol consumption, since all the patients that developed POD reported moderate alcohol consumption. Furthermore, greater intraoperative bleeding and postoperative pain scores did not influence the occurrence of delirium, but rather resulted in lower postoperative MMSE scores, which highlights the importance of adequate intraoperative treatment of patients during surgery and anesthesia in order to reduce the risk of developing postoperative delirium.

Conflict of interest

Nothing to declare.

R E F E R E N C E S

1. *Gani H, Domi R, Kodra N, Prifti P, Naco M, Beqiri V,* et al. The incidence of postoperative delirium in elderly patients after urologic surgery. Med Arch 2013; 67(1): 45–7.
2. *Shi CM, Wang DX, Chen KS, Gu XE.* Incidence and risk factors of delirium in critically ill patients after non-cardiac surgery. Chin Med J (Engl) 2010; 123(8): 993–9.
3. *Noimark D.* Predicting the onset of delirium in the post-operative patient. Age Ageing 2009; 38(4): 368–73.
4. *Deiner S, Silverstein JH.* Long-term outcomes in elderly surgical patients. Mt Sinai J Med 2012; 79(1): 95–106.
5. *Bhattacharya B, Maung A, Barre K, Maerz L, Rodriguez-Davalos MI, Schilsky M,* et al. Postoperative delirium is associated with increased intensive care unit and hospital length of stays after liver transplantation. J Surg Res 2017; 207: 223–8.
6. *Steinmetz J, Christensen KB, Lund T, Lohse N, Rasmussen LS.* ISPOCD Group. Long-term consequences of postoperative cognitive dysfunction. Anesthesiology 2009; 110(3): 548–55.
7. *Kalisvaart KJ, Vreeswijk R, de Jonghe JF, van der Ploeg T, van Gool WA, Eikelenboom P.* Risk factors and prediction of postoperative delirium in elderly hip-surgery patients: implementation and validation of a medical risk factor model. J Am Geriatr Soc 2006; 54(5): 817–22.
8. *Lindroth H, Bratzke L, Purvis S, Brown R, Coburn M, Mrkobrada M,* et al. Systematic review of prediction models for delirium in the older adult inpatient. BMJ Open 2018; 8(4): e019223.
9. *Freter SH, Dunbar MJ, MacLeod H, Morrison M, MacKnight C, Rockwood K.* Predicting post-operative delirium in elective orthopaedic patients: The Delirium Elderly At-Risk (DEAR) in

strument. Age Ageing 2005; 34(2): 169–71.
10. *Sieber FE, Zakriya KJ, Gottschalk A, Blute MR, Lee HB, Rosenberg PB,* et al. Sedation depth during spinal anesthesia and the development of postoperative delirium in elderly patients undergoing hip fracture repair. Mayo Clin Proc 2010; 85(1): 18–26.
11. *De J, Wand AP.* Delirium Screening: A Systematic Review of Delirium Screening Tools in Hospitalized Patients. Gerontologist 2015; 55(6): 1079–99.
12. *Folstein MF, Folstein SE, McHugh PR.* "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12(3): 189–98.
13. *Kukull WA, Larson EB, Teri L, Bowen J, McCormick W, Pfanschmidt ML.* The Mini-Mental State Examination score and the clinical diagnosis of dementia. J Clin Epidemiol 1994; 47(9): 1061–7.
14. *Orman ES, Perkins A, Ghabril M, Khan BA, Chalasani N, Boustani MA.* The confusion assessment method for the intensive care unit in patients with cirrhosis. Metab Brain Dis 2015; 30(4): 1063–71.
15. *Grover S, Kate N.* Assessment scales for delirium: A review. World J Psychiatry 2012; 2(4): 58–70.
16. *Sessler CN, Gosnell MS, Grap MJ, Brophy GM, O'Neal PV, Keane KA,* et al. The Richmond Agitation-Sedation Scale: validity and reliability in adult intensive care unit patients. Am J Respir Crit Care Med 2002; 166(10): 1338–44.
17. *Vasilevskis EE, Morandi A, Boehm L, Pandharipande PP, Girard TD, Jackson JC,* et al. Delirium and sedation recognition using validated instruments: reliability of bedside intensive care unit nursing assessments from 2007 to 2010. J Am Geriatr Soc 2011; 59 Suppl 2: S249–55.
18. *Tai S, Xu L, Zhang L, Fan S, Liang C.* Preoperative risk factors of postoperative delirium after transurethral prostatectomy for benign prostatic hyperplasia. Int J Clin Exp Med 2015; 8(3): 4569–74.
19. *Fineberg SJ, Nandyala SV, Marquez-Lara A, Oglesby M, Patel AA, Singh K.* Incidence and risk factors for postoperative delirium after lumbar spine surgery. Spine (Phila Pa 1976) 2013; 38(20): 1790–6.
20. *Shah S, Weed HG, He X, Agrawal A, Ozer E, Schuller DE.* Alcohol-related predictors of delirium after major head and neck cancer surgery. Arch Otolaryngol Head Neck Surg 2012; 138(3): 266–71.
21. *Olin K, Eriksdotter-Jonhagen M, Jansson A, Herrington MK, Kristiansson M, Permert J.* Postoperative delirium in elderly patients after major abdominal surgery. Br J Surg 2005; 92(12): 1559–64.
22. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015 – 2020 Dietary Guidelines for Americans. 8th ed. Available from: https://health.gov/dietaryguidelines/2015/guidelines/.
23. *Hudetz JA, Iqbal Z, Gandhi SD, Patterson KM, Hyde TF, Reddy DM,* et al. Postoperative cognitive dysfunction in older patients with a history of alcohol abuse. Anesthesiology 2007; 106(3): 423–30.
24. *Coburn M, Fahlenkamp A, Zoremba N, Schaelte G.* Postoperative cognitive dysfunction: Incidence and prophylaxis. Anaesthesist 2010; 59(2): 177-84; quiz 185.
25. *Marcantonio ER, Goldman L, Mangione CM, Ludwig LE, Muraca B, Haslauer CM,* et al. A clinical prediction rule for delirium after elective noncardiac surgery. JAMA 1994; 271(2): 134–9.
26. *Leung JM, Sands LP, Lim E, Tsai TL, Kinjo S.* Does preoperative risk for delirium moderate the effects of postoperative pain and opiate use on postoperative delirium? Am J Geriatr Psychiatry 2013; 21(10): 946–56.
27. *Ely EW, Gautam S, Margolin R, Francis J, May L, Speroff T, et al.* The impact of delirium in the intensive care unit on hospital length of stay. Intensive Care Med. 2001; 27(12): 1892–900.
28. *Witlox J, Eurelings LS, de Jonghe JF, Kalisvaart KJ, Eikelenboom P, van Gool WA.* Delirium in elderly patients and the risk of postdischarge mortality, institutionalization, and dementia: a meta-analysis. JAMA 2010; 304(4): 443–51.
29. *Veiga D, Luis C, Parente D, Fernandes V, Botelho M, Santos P,* et al. Postoperative delirium in intensive care patients: risk factors and outcome. Rev Bras Anestesiol. 2012; 62(4): 469-83.
30. *Pitkala KH, Laurila JV, Strandberg TE, Tilvis RS.* Multicomponent geriatric intervention for elderly inpatients with delirium: a randomized, controlled trial. J Gerontol A Biol Sci Med Sci 2006; 61(2): 176–81.
31. *Moyce Z, Rodseth RN, Biccard BM.* The efficacy of peri-operative interventions to decrease postoperative delirium in non-cardiac surgery: a systematic review and meta-analysis. Anaesthesia 2014; 69(3): 259–69.

Received on March 3, 2019
Revised on June 26, 2019
Accepted on August 23, 2019
Online First September, 2019

ORIGINAL ARTICLE

UDC: 616.155.392-08
DOI: https://doi.org/10.2298/VSP190620095U

The efficacy of generic imatinib in patients with chronic myeloid leukemia – a single center experience

Efikasnost generičkog imatiniba u lečenju bolesnika sa hroničnom mijeloidnom leukemijom – rezultati jednog centra

Ivana Urošević*†, Ivanka Perčić*†, Marina Dragičević Jojkić*†, Marina Dokić*†, Amir El Farra*†, Aleksandar Savić*†, Ivana Milošević*†, Nada Vlaisavljević*†, Borivoj Sekulić*†, Bela Balint‡§

***Clinical Center Vojvodina, Clinic of Hemathology, Novi Sad, Serbia; †University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; ‡Serbian Academy of Sciences and Arts, Department of Medical Sciences, Belgrade, Serbia; §Institute of Cardiovascular Diseases "Dedinje", Department of Transfusiology, Belgrade, Serbia**

Abstract

Background/Aim. The treatment of chronic myeloid leukemia (CML) has changed dramatically with the advent of targeted therapies. This study aimed to assess the efficacy of generic imatinib in CML patients treated in our center. **Methods.** The study was retrospective. It included 101 patients diagnosed with CML – chronic phase (CP). The patients were divided into two groups. Group 1 included 55 patients initially treated with branded imatinib and then switched to generic imatinib. Group 2 consisted of 46 newly diagnosed patients who received only generic imatinib from the beginning of therapy. **Results.** The patients were treated with branded imatinib for the mean of 42 months (range 6–132 months) before switching to generic imatinib. Treatment with generic imatinib lasted for 25 months on average (range 3–66 months). A quarter of the patients from the group 1 lost their cytogenetic response after being switched to generic imatinib, but without signs of transformation to acute leukemia. The patients treated with branded imatinib had a significantly longer event-free survival (EFS) and failure-free survival (FFS) (log-rank $p = 0.01$ and $p = 0.03$, respectively). These results could have been influenced by frequent changes of the brand and dosage formulation of generic imatinib. **Conclusions.** Our study showed a significantly longer EFS and FFS in the patients who were initially treated with branded imatinib, compared to those treated with generic imatinib only. These results provide useful information, but have to be interpreted within the context of the crossover study.

Key words:
leukemia, myeloid, chronic-phase; imatinib mesylate; drugs, generic; survival.

Apstrakt

Uvod/Cilj. Ciljna terapija je značajno izmenila uspeh lečenja bolesnika sa hroničnom mijeloidnom leukemijom (CML). Cilj rada je bio da se proceni efikasnost lečenja obolelih od CML generičkim imatinibom u našem centru. **Metode.** Istraživanje je bilo retrospektivno. Obuhvatilo je 101 obolelog od CML u hroničnj fazi. Bolesnici su bili podeljeni u dve grupe. Prvu grupu je činilo 55 bolesnika koji su inicijalno lečeni originalnim imatinibom i koji su kasnije tokom lečenja prevedeni na terapiju gneričkim imatinibom. Drugu grupu činilo je 46 novodijagnostikovanih bolesnika koji su od početka lečeni generičkim imatinibom. **Rezultati.** Bolesnici su originalnim imatinibom bili lečeni u proseku 42 meseca (od 6 do 132 meseca) nakon čega su prevedeni na generički imatinib. Lečenje generičkim imatinibom je u proseku trajalo 25 meseci (od 3 do 66 meseci). Četvrtina bolesnika prve grupe izgubila je citogenetski odgovor nakon prevođenja na generički imatinib. Nije bilo znakova za transformaciju u akutnu leukemiju. Bolesnici lečeni originalnim imatinibom imali su statistički značajno duže preživljavanje bez događaja koje podrazumeva smrtni ishod (*event-free survival* – EFS) i preživljavanje bez neuspeha terapije (failure-free survival – FFS) (log-rank $p = 0{,}01$ i $p = 0{,}03$, redom). Na ovakve rezultate mogla je da utiče učestala promena dozne formulacije i proizvođača generičkog imatiniba. **Zaključak.** Naše istraživanje ukazalo je na značajno duže EFS i FFS kod bolesnika koji su lečenje započeli originalnim imatinibom u odnosu na bolesnike koji su sve vreme lečeni generičkim imatinibom. Navedeni rezultati pružaju korisnu informaciju, ali se moraju tumačiti u kontekstu studije po tipu unakrsnog izajna.

Ključne reči:
leukemija, mijeloidna, hronična; imatinib mesilat; lekovi, generički; preživljavanje.

Correspondence to: Ivanka Perčić, Clinical Center Vojvodina, Clinic of Hematology, Novi Sad, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 1-3, 21 000 Novi Sad, Serbia. Email: IVANKA.PERCIC@mf.uns.ac.rs

Introduction

The treatment of chronic myeloid leukemia (CML) has changed dramatically with the advent of targeted therapies [1]. Imatinib mesylate is a highly selective inhibitor of tyrosine kinase used in the treatment of CML. It has shown long-term efficacy and safety in published randomized clinical trials [2]. Results from the landmark International Randomized Study of Interferon and STI571 (IRIS) comparing interferon alpha (IFNα) plus low-dose cytarabine (LDAC) with imatinib for the clinical management of CML patients led to the adoption of the first targeted therapy (ie. imatinib) as standard first-line treatment [3]. Overall survival at eight years was 85%; for only CML-related deaths and those before stem cell transplantation, the survival was 93% [4]. The IRIS trial also showed that imatinib provided significant advantages regarding health-related quality of life (HRQOL) over IFNα + LDAC [4].

On the other hand, the high cost of tyrosine kinase inhibitors (TKI) developed for CML is a significant concern for health care payers in countries with restricted resources. It is true that generics lead to considerable cost savings, but also give rise to questions associated with their efficacy, safety, and quality [5].

In Serbia, as in most countries, for the registration of generics, official regulations only require evidence of pharmaceutical and biological equivalence, but no evidence of efficacy and safety. Generic imatinib was approved by the Medicines and Medical Devices Agency of Serbia in January 2012. In July 2012, The National Health Insurance Fund of Serbia introduced this drug in the positive list.

This study aimed to assess the efficacy of generic imatinib in CML patients treated in our center.

Methods

Since the introduction of TKI in Serbia, all CML patients on TKI from the region of Vojvodina, Serbia, have been treated at the Clinic of Hematology, Clinical Center of Vojvodina, Novi Sad, Serbia. During August and September 2012, all CML patients treated at the Clinic of Hematology, Clinical Center of Vojvodina, Novi Sad, Serbia, were switched from branded imatinib to generic imatinib. All newly diagnosed CML patients' treatment started with generic imatinib.

Subjects

This retrospective study was performed at the Clinic of Hematology, Clinical Center of Vojvodina, Novi Sad. It included 101 patients diagnosed with CML – chronic phase (CP). All patients included in the study received treatment with TKI, and they represented all CML patients on TKI in the region of Vojvodina, Serbia, in the period from June 2006 to August 2017. The patients were divided into two groups. The group 1 included 55 patients initially treated with branded imatinib and then switched to generic imatinib. The group 2 consisted of 46 newly diagnosed patients who received only generic imatinib from the start of therapy. Four commercial generics of imatinib have been available in Serbia since 2012. The dose formulation and type of generic drug were changed frequently, depending on the availability, without the influence of the treating physician.

CML patients that were not treated with TKI were not included in the study.

Methods

All data [patient's age and sex, whole blood count, cytogenetic results, results of polymerase chain reaction (PCR) for bcr/abl testing, date of the diagnosis, date of TKI treatment initiation, duration of treatment with branded imatinib, duration of treatment with generic imatinib, date of the loss of response to treatment, event-free survival (EFS – intolerance and death) and failure-free survival (FFS – failure to treatment) were collected from the medical documentation]. Hematologic and cytogenetic responses were monitored according to the recommendations of the European Leukemia Network (ELN) [6]. Molecular monitoring was performed by GeneXpert® from 2013.

Statistical analysis

Descriptive statistical analysis was performed determining patients' demographic characteristics, scores for calculating the relative risk concerning CML patients (Sokal, Hasford and EUTOS score — European Treatment and Outcome Study), mean values for treatment duration with generic and branded imatinib, rate of hematological, cytogenetic and molecular response, and rate of treatment failure in patients treated with generic imatinib. The efficacy of TKI treatment was compared in the two groups using a log-rank test by StatSoft, Inc. STATISTICA, version 10.0. Statistical significance was set at $p < 0.05$.

Results

Patients' characteristics

Medical records for a total of 101 CML-CP patients treated with imatinib were reviewed. The group 1 consisted of 55 patients (30 male and 25 female). Characteristics of the patients in the group 1 are summed up in Table 1. Upon diagnosis, most patients in the group 1 were stratified as high risk according to Sokal score (low 23.1%, intermediate 30.7%, high 46.2%) and low risk according to EUTOS score (low 61.5%, high 38.5%). The patients were treated with branded imatinib for the mean of 42 months (range 6–132 months) before switching to generic imatinib. Treatment with generic imatinib lasted for 25 months on average (range 3–66 months) until the end of the follow-up period. A quarter of the patients from the group 1 lost their cytogenetic response after being switched to generic imatinib but without signs of transformation to acute leukemia. Distribution of patients who lost cytogenetic response according to Sokal and EUTOS scores is shown in Table 2.

Table 1

Characteristics of patients treated with branded imatinib

Parameters	Mean	Min	Max
Age (years)	28.5	21	77
WBC (10^9/L)	107	12	411
Hemoglobin (g/L)	112	64	139
Platelets (10^9/L)	429.5	131	3795
Blasts (%)	2.5	1	8
Promyelocytes (%)	4	2	9
Eosinophls (%)	3	1	6
Basophils (%)	2	1	8

WBC – white blood cells.

Table 2

Distribution of patients who lost their complete cytogentc response (CCgR) after being switched to generic imatinib according to Sokal and Eutos scores

Group	Patients (%)	
	Sokal score	Eutos score
Low	23.1	53.8
Intermediate	23.1	
High	53.8	46.2

The group 2 consisted of 46 patients (26 male and 20 female) with newly diagnosed CML, treated only with generic imatinib. Characteristics of the patients in the group 2 are summed up in Table 3. Unlike the group 1, most of the patients were classified as low and intermediate risk according to Sokal score (low 41.3%, intermediate 43.5%, high 15.2%). As in the group 1, patients in the group 2 were also mostly low risk according to EUTOS (low 71.7%, high 28.3%).

Table 3

Characteristics of newly diagnosed patients treated with generic imatinib (n = 46)

Parameters	Mean	Min	Max
Age (years)	56.5	20	83
WBC (10^9/L)	127.7	16.2	367.8
Hemoglobin (g/L)	118	75	157
Platelets (10^9/L)	444.5	123	1280
Blasts (%)	4	1	7
Promyelocyte (%)	5	2	8
Eosinophyls (%)	2.5	1	4
Basophils (%)	3	1	4

WBC – white blood cells.

Ninety percent of patients treated only with generic imatinib achieved complete hematologic response (CHR) after a mean of 3 months. As shown in Figure 1, half of the patients (50%) in the group 2 had a complete cytogenetic response (CCgR) at six months after the beginning of treatment. At twelve months, 66.6% of patients were in CCgR. A molecular response with less than 1% of bcr/abl transcript was achieved by 28.6% of patients in the group 2 after six months (Figure 2). By 12 months, 28.6% of patients achieved a major molecular response (MMR) defined as less than 0.1% bcr/abl transcript (Figure 3). The patients who had treatment failure after 12 months of treatment with generic imatinib (28.6% of patients) were switched to the second generation of TKI (nilotinib).

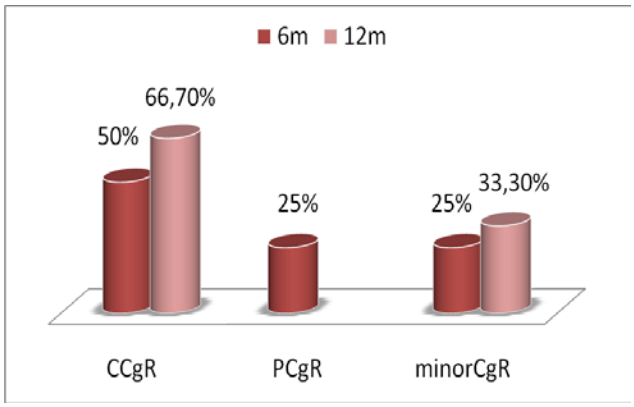


Fig. 1 – Cytogenetic response of patients treated with generic imatinib. CCgR – complete cytogentic response; PCgR – partial cytogenetic response.

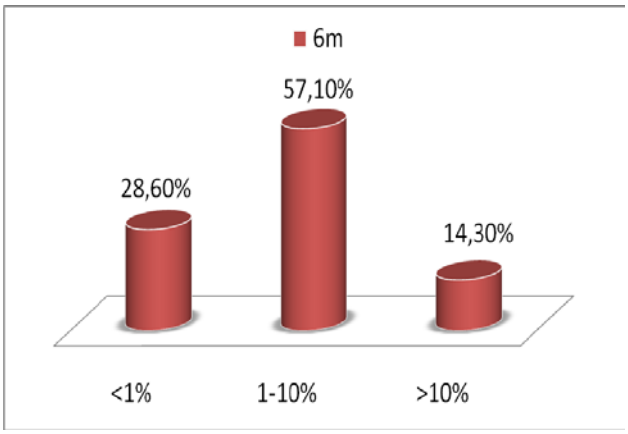


Fig. 2 – Molecular response at 6 months for patients treated with generic imatinib.

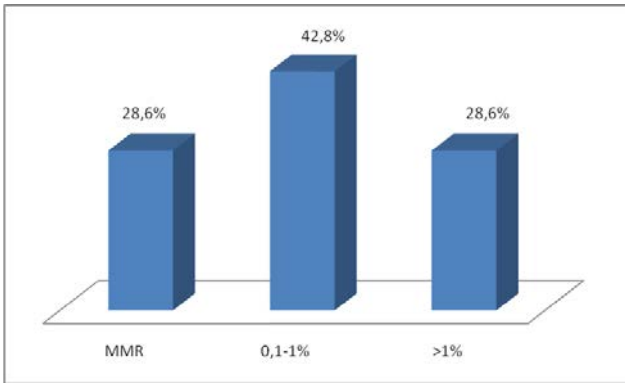


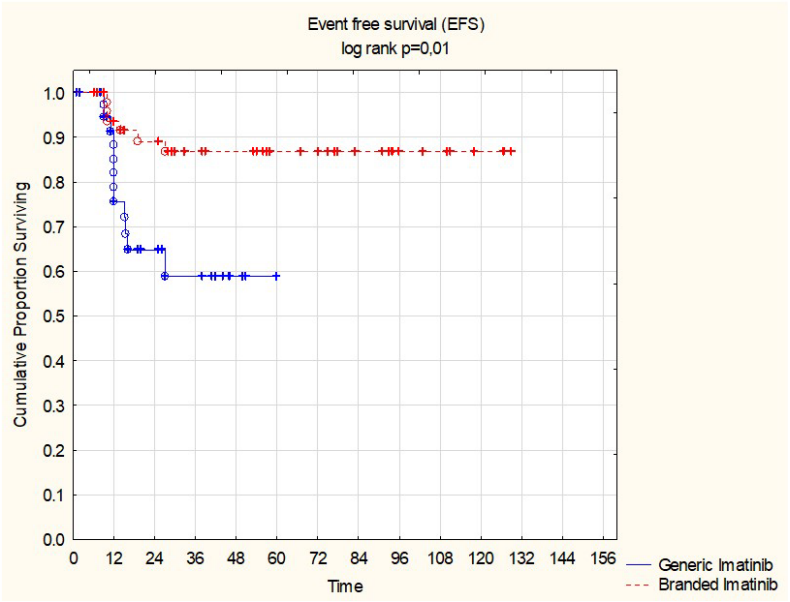
Fig. 3 – Molecular response at 12 months for patients treated with generic imatinib.

Comparisson of event-free survival (EFS) and failure-free survival (FFS)

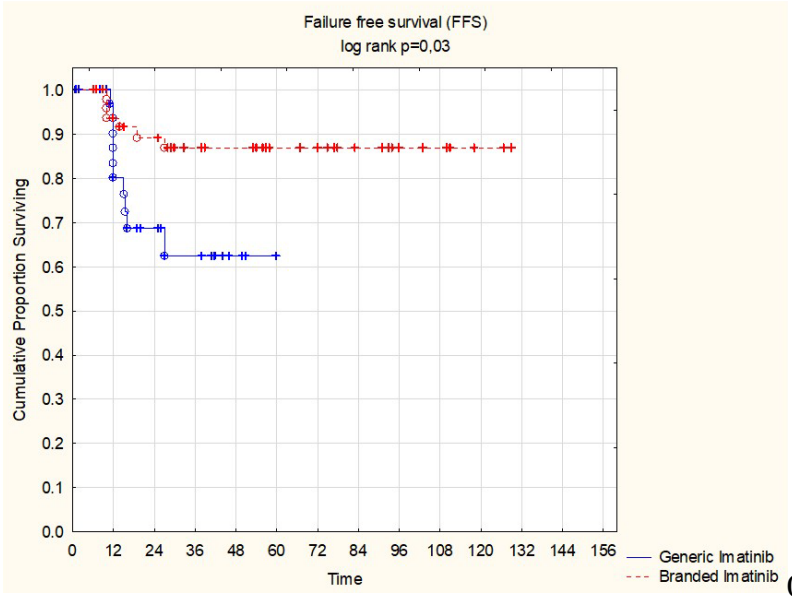
Five-year EFS and FFS were significantly different between the two groups of patients (87% vs. 59%, and 87% vs. 62%, respectively), meaning that the patients started on a therapy with branded imatinib had a significantly better EFS

(p = 0.01) and FFS (p = 0.03) compared with the patients treated with the generic drug (Figures 4a and 4b). It is important to note, as pointed out above, that it was a crossover study design.

Our results showed that 25% of patients initially treated with branded imatinib lost their cytogenetic response when switched to generic imatinib. Around half of the patients were classified as high risk according to Sokal and EUTOS



(a)



(b)

Fig. 4 – Kaplan Meier curve of event-free (a) and failure-free (b) survival.

Discussion

In published randomized clinical trials, imatinib has shown long-term efficacy and safety improving the 10-year survival rate from 20% to 85% [1]. However, the high cost of treatment led to the approval of generic formulations of imatinib.

Generally, generics are approved after a bioequivalent trial without long-term safety and efficacy data [5]. There has been confusion and uncertainty concerning the safe administration of patented drugs, quality-controlled generics, and copies of patented drugs and medicines of substandard quality [5].

scores (53.8% and 46.2%, respectively). In concordance with our study, four case reports showed that switching to a generic imatinib product was associated with a loss of CHR achieved while on branded imatinib [6–10].

In our study, 90% of patients treated initially with generic imatinib achieved CHR at three months. In international studies involving generic imatinib, rates of CHR were 96%–100% from 3 to 28 months [10–12]. On the other hand, a larger prospective study conducted by Alwan et al. [13] has shown that 33% of patients lost a hematologic response in 6 months.

Patients treated with generic imatinib reported by Eddou et al. [14] achieved 77% of major cytogenetic response (MCgR). In our group of patients, 50% achieved CCgR at six months, and 66.6% achieved CCgR at 12 months, which is slightly less than Eddou et al [14] reported.

Rates of MMR from 8–47% in 6 to 18 months have been reported in patients treated with generic imatinib [11, 12, 14]. The other two observational studies have shown interesting results. One of them is a study conducted by colleagues from Turkey involving 145 patients with CML [15]. There were two groups: one included patients receiving branded imatinib (Glivec®), and the other comprised patients who initially received Glivec® and then switched to the generic drug. MMR rate was quite similar between these two groups [15]. The other study from India included 213 patients with CML: 64% of patients were on Gippar Glivec®, while 36% were on generic imatinib [16]. It may be noted that cytogenetic and molecular responses were better in the group of patients treated with generic imatinib. These results could be explained by the fact that molecular responses were not documented in 42% of patients in the Glivec® group because of economic reasons [16].

In the ENESTnd study of 283 patients receiving branded imatinib, 22% reached MMR after 12 months of therapy and 53% after 36 months [17, 18]. In our study, molecular response with the level of bcr/abl transcript less than 1% at six months in patients treated only with generic imatinib was 28.6%. The same percentage of these patients achieved MMR at 12 months.

Five-year EFS and FFS were significantly better in patients treated with branded imatinib as shown in Figures 4a and 4b (log-rank $p = 0.01$ and $p = 0.03$, respectively). These results could have been influenced by frequent changes in the brand and dosage formulation of generic imatinib.

There are reliable data on CML patients in Europe using generic imatinib forms. Results from Bosnia and Herzegovina show that generic imatinib as first-line treatment was less efficient when compared to branded imatinib, but it did not influence the outcome of treatment when used as second-line treatment [19]. Another study involving 24 patients from Croatia shows adequate efficacy of generic imatinib alongside a decreased cost of treatment [20].

However, our results cannot be compared to the results achieved in patients taking the original imatinib due to the crossover study design, but they provide an informative view of patients initially treated with branded imatinib compared to those initially treated with the generic drug.

Conclusion

Our study showed a significantly longer EFS and FFS in the patients who were initially treated with branded imatinib, compared to those treated with generic imatinib only. These results provide useful information, but have to be interpreted within the context of the crossover study.

REFERENCES

1. *Kantarjian HM, Fojo T, Mathisen M, Zwelling LA.* Cancer drugs in the United States: Justum Pretium - The just price. J Clin Oncol 2103; 31(28): 3600–4.
2. *Cortes J, Hochhaus A, Hughes T, Kantarjian H.* Front-line and salvage therapies with tyrosine kinase inhibitors and other treatments in chronic myeloid leukemia. J Clin Oncol 2011; 29(5): 524–31.
3. *O'Brien SG, Guilhot F, Larson RA, Gathmman I, Baccarani M, Cervantes F*, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2003; 348(11): 994–1004.
4. *Deininger M, O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP*, et al. International randomized study of interferon vs STI571 (IRIS) 8-year follow up: sustained survival and low risk for progression or events in patients with newly diagnosed chronic myeloid leukemia in chronic phase (CML-CP) treated with imatinib [abstract]. Blood 2009; 114(22): 462. Abstract 1126.
5. *Geissler J, Sharf G, Cugurovic J, Padua R, Narbutas S, Remic M,* et al. Chronic myeloid leukemia patients call for quality and consistency when generics are introduced to treat their cancer. Leukemia 2016; 30(12): 2396–7.
6. *Baccarani M, Deininger M, Rosti G, Hochhaus A, Soverini S, Apperley JF.* European Leukemia Net recommendations for the management of chronic myeloid leukemia: 2013. Blood 2013; 122(6): 872–84.
7. *Goubran HA.* Failure of a non-authorized copy product to maintain response achieved with imatinib in a patient with chronic phase chronic myeloid leukemia: a case report. J Med Case Rep 2009; 3: 7112.
8. *Asfour IA, Elshazly SA.* Changing therapy from Glivec to a "copy" imatinib results in a worsening of chronic myeloid leukemia disease status: two case reports. Cases J 2009; 2: 9342.
9. *Chouffai Z.* Hematologic relapse after 2 years on a non-authorized copy version of imatinib in a patient with chronic myeloid leukemia in chronic phase: a case report. Case Rep Oncol 2010; 3(2): 272–6.
10. *Mattar M.* Failure of copy Imatib (CIPLA, India) to maintain hematologic and cytogenetic responses in chronic myeloid leukemia in chronic phase. Int J Hematol 2010; 91(1): 104–6.
11. *Razmkhah F, Razavi M, Zaker F, Kazemi A, Negari S, Rasighaemi P*, et al. Hematologic and molecular responses to generic imatinib in patients with chronic myeloid leukemia. Lab Med 2010; 41(9): 547–50.
12. *Nair V, Sharma A, Kotwal J, Bhishapathy M, Mishra DK, Das S*, et al. Monitoring of responce to therapy with Imatinib mesylate in chronic myeloid leukemia in chronic phase. Med J Armed Forces India 2014; 70(40): 315–20**.**
13. *Alwan AF, Matti BF, Naji AS, Muhammed AH, Abdulsahib MA.* Prospective single center study of chronic myeloid leukemia in chronic phase: switching from branded Imatinib to a copy drug and back. Leuk Lymphoma 2014; 55(12): 2830–4.
14. *Eddou H, Astati S, Mahtat EM.* The treatment of chronic myeloid leukemia by Imatinib Mesylate generic: About 26 cases. 16th Congress of the European Hematology Association; London, UK; 2011 June 9–12. Abstract Book (Abstract 1234.) Hematologica 2011; 96(Suppl 2): 1–678.
15. *Eskazan AE, Elverdi T, Yalniz FF, Salihoglu A, Ar MC, Ongoren Aydin S,* et al. The efficacy of generic formulations of Imatinib

Mesylate in the treatment of chronic myeloid leukemia. Leuk Lymphoma 2014; 55(12): 2935–7.
16. *Malhotra H, Sharma R, Singh Y, Chaturvedi H*. Report of chronic myeloid leukemia SMS Medical College Hospital, Jaipur. Indian J Med Paediatr Oncol 2013; 34(3): 177–9.
17. *Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C,* et al. Nilotinib versus Imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med 2010; 362(24): 2251–9.
18. *Larson RA, Hochhaus A, Hughes TP, Clark RE, Etienne G, Kim DW*, et al. Nilotinib vs Imatinib in patients with newly diagnosed Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase. ENESTnd 3 year follow up. Leukemia 2012; 26(10): 2197–203.
19. *Islamagic E, Hasic A, Kurtovic S, Suljovic Hadzimesic E, Mehinovic L, Kozaric M*, et al. The efficacy of generic imatinib as first- and second-line therapy: 3-year follow-up of patients with chronic myeloid leukemia. Clin Lymphoma Myeloma Leuk 2017; 17: 238–40.
20. *Ostojic A, Sertic D, Roncevic P, Peric Z, Granic P, Matic N*, et al. Comparison of branded and generic imatinib plasma concentrations in patients with chronic myelogenous leukemia: unicentric study. Clin Lymphoma Myeloma Leuk 2016; 16(8): 472–6.

Received on June 20, 2019.
Revised on August 24, 2019.
Accepted on August 26, 2019.
Online First September, 2019.

ORIGINAL ARTICLE

UDC: 581.6::615.451.2
DOI: https://doi.org/10.2298/VSP190703096K

Chemical and pharmacological characterization of aqueous and ethanolic extracts of *Cyclamen hederifolium* Ait. (Primulaceae) tuber

Hemijska i farmakološka karakterizacija vodenog i etanolnog ekstrakta lukovica *Cyclamen hederifolium* Ait. (Primulaceae)

Ksenija Kojičić*, Aleksandar Arsenijević†, Marija Marković‡, Vesna Stankov Jovanović§, Zoran Simić‖, Vanja Tadić¶, Snežana Cupara*

University of Kragujevac, Faculty of Medical Sciences, *Department of Pharmacy, †Department of Microbiology and Immunology, Faculty of Science, ‖Institute of Chemistry, Kragujevac, Serbia; University of Niš, Faculty of Science, ‡Department of Biology, §Institute of Chemistry, Niš, Serbia; ¶Institute for Medical Plant Research "Dr. Josif Pančić", Belgrade, Serbia

Abstract

Background/Aim. *Cyclamen hederifolium* (*C. hederifolium*) Ait. belongs to the family Primulaceae, which includes 23 species of cyclamen, naturally distributed in the Central and Southern Europe, Western Asia and some parts of North Africa. This plant is considered highly poisonous and not suitable for human use. However, tuber extracts have been used in traditional medicine and homeopathy. The aim of this study was to investigate *C. hederifolium* growing naturally in Serbia for its metal content and biological activities (antioxidant, antibacterial, antifungal and cytotoxic activity). **Methods.** Content of metals was determined by atomic absorption spectrophotometric method. We used several different assays for assessment of antioxidant activity of both aqueous and ethanol extracts of *C. hederifolium:* 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay, ferric-reducing antioxidant power (FRAP) assay, total reducing power assay (TRP) and cupric reducing antioxidant capacity (CUPRAC) assay. The disk diffusion assay was used to investigate sensitivity of laboratory bacterial and fungal control strains against investigated extracts. Aqueous and ethanol extracts of *C. hederifolium* were examined on 4 different tumoral cell lines by *in vitro* MTT bioassay. **Results.** The presence of Mn, Ca, Mg, Fe, Zn, K, Cu was confirmed in aqueous and ethanol extract, as well as in whole tubers and soil, while Cr, Ni, Pb and Cd were not detected. Both aqueous and ethanol extract of *C. hederifolium* tubers showed antioxidant activity, that positively correlated to content of phenols and flavonoids in it. Aqueous extract was slightly superior in these terms than ethanol one. None of the tested extracts showed antimicrobial activity. Both investigated extracts showed cytotoxicity against four cancer cell lines 4T1, HCT116, CT26, LLC1, in the concentration range 15,625–2,000 μg/mL. Ethanol extract showed stronger cytotoxicity than aqueous extract. **Conclusion.** Seven metals were identified in the *C. hederifolium* tubers, extracts and soil. Both extracts exhibited antioxidant and cytotoxic activity.

Key words:
antioxidants; cyclamen; flower essences; metals; phytotherapy; spectrophotometry, atomic.

Apstrakt

Uvod/Cilj. *Cyclamen hederifolium* (*C. hederifolium*) Ait. pripada familiji Primulaceae koja obuhvata 23 vrste ciklame, prirodno rasprostranjene u Centralnoj i Južnoj Evropi, Zapadnoj Aziji i nekim delovima Severne Afrike. Ta biljka se smatra otrovnom i nije pogodna za ljudsku upotrebu. Međutim, ekstrakti lukovica se koriste u tradicionalnoj medicini i homeopatiji. Cilj istraživanja je bio ispitivanje sadržaja metala, fenola, flavonoida i bioloških aktivnosti (antioksidativna, antibakterijska, antifungalna i citotoksična) *C. hederifolium* koji prirodno raste u Srbiji. **Metode.** Sadržaj metala određen je metodom atomske apsorpcione spektofotometrije. Za procenu antioksidativne aktivnosti i vodenog i etanolnog ekstrakta *C. hederifolium* korišćeno je više različitih testova: 2.2-*diphenyl-1-picrylhydrazyl* (DPPH) *radical scavenging assay*, 2,20-azino-bis (3-*ethylbenzothiazoline-6-sulfonic acid*) (ABTS) *radical scavenging assay*, *ferric-reducing antioxidant power* (FRAP) *assay*, *total reducing power* (TRP) *assay* i *cupric reducing antioxidant capacity* (CUPRAC) *assay*. Test disk difuzije je korišćen za ispitivanje osetljivosti laboratorijskih bakterijskih i gljivičnih sojeva na ispitivane ekstrakte. Vodeni i etanolni ekstrakti *C. hederifolium* ispitivani su na 4 različite tumorske ćelijske linije pomoću *in vitro* MTT testa. **Rezultati.** Prisustvo Mn, Ca,

Correspondence to: Ksenija Kojičić and Snežana Cupara, University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Svetozara Markovića 69, 34 000 Kragujevac, Serbia. E-mail: ksenija.kojicic@gmail.com, snezanacupara@yahoo.com

Mg, Fe, Zn, K, Cu je potvrđeno u vodenom i etanolnom ekstraktu, kao i u lukovicama i zemljištu, dok Cr, Ni, Pb i Cd nisu detektovani. I vodeni i etanolni ekstrakt lukovica *C. hederifolium* pokazali su antioksidativnu aktivnost, koja je bila u pozitivnoj korelaciji sa sadržajem fenola i flavonoida u njemu, pri čemu je vodeni ekstrakt bio superiorniji od etanolnog ekstrakta. Niti jedan od testiranih ekstrakata nije imao antimikrobnu aktivnost. Oba ekstrakta su ispoljila citotoksičnost protiv četiri tumorske ćelijske linije 4T1, HCT116, CT26, LLC1 u koncentracionom opsegu 15 625–2 000 μg/mL. Etanolni ekstrakt je pokazao snažniju citotoksičnost od vodenog ekstrakta. **Zaključak.** U zemlji, lukovicama i ekstraktima *C. Hederofolium* detektovano je sedam metala. Oba ekstrakta su pokazala antioksidativno i citotoksično dejstvo.

Ključne reči:
antioksidansi; ciklama; ekstrakti, biljni; metali; fitoterapija; spektrofotometrija, atomska apsorpciona.

Introduction

The number of newly diagnosed cancer cases as well as the percentage of deaths caused by cancer has been increasing in the world. As a result, many researchers focused attention on traditional herbal treatments, since it is an area from which a possible new antitumoral compound could arise. Investigators often search in local folk medicinal plant treatments of different regions, aiming to spot the possible anticancer activity of plants, since the current conventional (chemical) anticancer treatment has been accompanied by strong side effects [1, 2]. The studies on herbal material may reveal more than the desideratum. Various biological effects, such as antimicrobial, cytotoxic or antioxidant activity, have been often detected along the main investigation aim. This process that usually starts from *in vitro* or/and *in vivo* studies on herbal extracts, latter may or may not be rewarded by formulation of an efficient antitumor medicinal product. Nevertheless, it is a long, tedious, but often scientifically fruitful process. Therefore, we think that performing laboratory investigations as well as *in vitro* studies on plant material, especially on the species that are scarcely documented, is a first and prerequisite step on this path, regardless of current limitations in obtaining the final anticancer product [1, 2].

Cyclamen hederifolium (*C. hederifolium*) Ait. belongs to the family Primulaceae, which includes 23 species of cyclamen, naturally distributed in the Central and Southern Europe, Western Asia and some parts of North Africa. It is perennial, flowering plant, inhabiting partially shady woodlands and mountain meadows. There are several cultivated varieties and they differ in shape and color of flowers [3, 4]. Plant tubers of *C. hederifolium* are rich in toxic compounds which cause nausea, vomiting, diarrhea, abdominal pain, bloody stool, hemolysis, convulsions, skin irritation and blistering, and may even cause death due to the asphyxiation [3]. Though, the plant is considered highly poisonous and not suitable for human use, tubers have been used in homeopathy as well as in traditional medicine of different regions. *C. hederifolium* has been sporadically used in folk medicine in some parts of Serbia in the treatment of rheumatism, menstrual problems, migraine, and skin conditions, as a purgative and antitumor agent. Italian traditional medicine employs fresh tubers in the treatment of hemorrhoids and frostbites [3–7].

Several different biological activities have been noted for *Cyclamen* spp., due to the presence of secondary metabolites produced by plant as key elements of its defense mechanism. There are also some reports on bioactivity of isolated compounds from the tubers of *Cyclamen* spp. [8, 9].

However, biological activity of *C. hederifolium* has not been abundantly documented. Among available data for *Cyclamen* spp. there are some results of *in vitro* studies on *C. hederifolium* that suggest cytotoxic, antioxidant, antiinflammatory and antimicrobial activity [6, 7, 10–14]. The molecular structure of compounds responsible for these effects have not been elucidated, but authors attributed some of biological activities of *C. hederifolium* to its content of phenols and triterpenic saponosides [8, 15, 16]. There are saponins in *C. hederifolium* that have been identified as cyclamin, deglucocyclamin, cyclaminorin, hederifoliosides A–E and ardisicrenoside D. After isolation they were investigated for inducing apoptosis, cytotoxic and hemolytic effects by the change of the cell membrane permeability and intracellular signaling pathways [8, 9, 14–16].

Therefore, the aim of this study was to complement available knowledge on *C. hederifolium*, by augmenting data on species from natural habitats in Serbia. The starting point was folk medicine of eastern parts of the country that utilizes this plant as an antitumor agent. We evaluated the metal content and biological activities that have not been previously reported for this species from natural habitats in Serbia – antioxidant, antimicrobial and cytotoxic activity of *C. hederifolium* tubers. Our intention was to clarify or justify the use of this plant by traditional medicine, which offers no documented data for antitumor use.

Methods

Plant material

Tubers of *C. hederifolium* were collected in September 2018 on the slopes of the mountain Suva Planina, belonging to the Eastern region of Serbia. The plant material was identified by standard botanical keys for plant determination (Flora of Republic of Serbia and European Flora) at Department of Biology at the Faculty of Science, University of Niš, Serbia [17, 18]. A voucher specimen no. 13556 was deposited in the "Herbarium Moesiacum Niš", University of Niš. Fresh *C. hederifolium* tubers were cleaned from the soil, separated from all other plant parts and dried in the shadow, at room temperature. Fresh tubers were used for extract preparation as well as for metal quantity analysis. Surrounding soil was sampled by collecting it from 10–50 cm area near tubers,

cleaned from stones and prepared for analysis in the shade at room temperature (20–25 ºC).

Extract preparation

The plant material was ground and mixed thoroughly with solvents. Two extracts were prepared by maceration of powdered plant material in two different solvents – distilled water and 70% v/v ethanol. Each extract was allowed to stand for five days at room temperature, in a closed glass container, protected from the light, and subject to frequent agitation. Excess solvent was evaporated at a rotary vacuum evaporator at 40 ºC. The dry obtained extracts were stored in a desiccator until the experiment.

Chemicals and instruments

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), iron (III) hloride hexahydrate, Folin–Ciocalteu reagent, gallic acid (3,4,5-trihydroxybenzoic acid), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid, methanol, nitric acid, perchloric acid and all reagents used for cytotoxic assay were purchased from Sigma Chemicals Co. St. Louis, Missouri, USA. The chemical substances: neocuproine (2,9- dimethyl-1,10-phenanthroline), copper (II) chloride dihydrate, NaCO3, HCl, 2,4,2-tri(2-pyridyl)-s-triazine (TPTZ), K3[Fe(CN)6], phosphate buffer (NaH2PO4-Na2HPO4), ammonium acetate buffer, CCl3COOH, K2S2O8, FeSO4x7H2O, and DMSO (dimethyl sulphoxide) were purchased from Merck, Darmstadt, Germany. All chemicals were of analytical reagent grade. Absorbance measurement were performed on a double beam UV-Vis spectrophotometer Perkin Elmer lambda 15 (Massachusetts, USA) and microplate Zenyth 3100 Multimode detector (Anthos Labtec Instruments GmbH, Austria). Heavy metals were analysed by atomic absorption spectrophotometer (Perkin Elmer Company Model 3300/96 with MHS-10 hydride system).

Metal content determination

Comparative analysis of metal content was conducted in four different samples of *C. hederifolium tubers* – ethanol and aqueous extracts, fresh tubers and soil sample. Content of the following metals was determined by atomic absorption spectrophotometric method: Mn, Ca, Mg, Fe, Zn, K, Cu, Cr, Ni, Pb, Cd 19, 20. Determination of metal content in plant material was performed dissolving 1 g of sample by mixture of conc. HNO_3 and conc. H_2O_2. Mixture was heated. After cooling, 12 mL of distilled water was added and the mixture was filtrated. Determination of metal quantity in soil sample was performed after 1 g of sample was dissolved by 20 mL conc. HNO_3. The mixture was heated to 110ºC. After cooling, 2 mL conc. $HClO_4$ was added and the mixture was reheated to 130ºC. The heating was stopped when solution was evaporated to one third of the volume and clearing up of the solution was reached. After cooling, 12 mL of distilled water was added and the mixture was filtrated. Each measurement was performed in triplicate and results were expressed as mean (mg/kg) ± standard deviation.

Total phenolic and flavonoid content

Total phenolic content (TPC) was expressed as µg of gallic acid equivalents per mg of dry weight (µg GAE/mg dry extract). It was determined by Folin-Ciocalteu reagent, according to Singleton et al. [21]. Method is based on oxidation/reduction reaction. Briefly, 0.02 mL of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent, 5.03 mL diluted water and 2 mL sodium carbonate (20% Na_2CO_3). The mixture was allowed to stand in the dark for 30 min and absorbance was measured at 750 nm. Gallic acid (0.5 mg/mL) was used as standard. The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation.

The total flavonoid content (TFC) was determined by the colorimetric method based on formation of complex between flavonoid and aluminium chloride, described by Baba and Malik [22]. Briefly, 0.05 mL of extract was mixed with 0.15 mL of 5% $NaNO_2$. After 5 min of incubation, 0.75 mL of 2% $AlCl_3$ was added, and the mixture was allowed to stand for 5 min. Then, 1 mL of NaOH and 2.05 mL of distilled water were added. The mixture was allowed to stand for 15 min. After expiration of incubation time, absorbance was measured at 520 nm. Rutin was used as standard. The total flavonoid content was calculated from a calibration curve, and the result was expressed as µg of rutin equivalents per mg dry weight (µg RE/mg dry extract). The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation.

Antioxidant activity assays

We used several different assays for assessment of antioxidant activity of both aqueous and ethanol extracts of *C. hederifolium*: DPPH radical scavenging assay, ABTS radical scavenging assay, ferric-reducing antioxidant power (FRAP) assay, total reducing power assay (TRP) and cupric reducing antioxidant capacity (CUPRAC) assay.

DPPH radical scavenging capacity

The total antioxidant activity of the extracts was assessed by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay with slight modifications 23, 24. Method is based on the DPPH radical acceptation of H atom or electron from antioxidant molecules. DPPH radical changes the color from violet to yellow, when it is scavenged by reducing agents from extract and transformed to stable hydrazine. Absorbance was measured with spectrophotometer. Briefly, 1.5 mL of methanol solution of the DPPH radical (concentration 90 µmol/L), 0.002 mL of extract and 2.5 mL of methanol were placed in a test tube. Test tubes were allowed to stand at room temperature for 60 min in a dark place. The absorbance was measured at 515 nm against a

blank (methanol). All tests were carried out in triplicate and results were obtained from the calibration curve. Trolox in concentration 0.025 mg/mL was used as standard. Results are expressed as μg of Trolox equivalents per mg dry extract weight (μg TE/mg dry extract) and by EC-50 value which represents amount of extract necessary to scavenge the initial DPPH concentration by 50%.

ABTS radical scavenging activity

ABTS radical "scavenging" activity was performed followed the method of Dimitrijevic et al. [23], with some modifications. The working solution was prepared by mixing two stock solutions in equal quantities, 7 mM ABTS with 2.4 mM potassium persulfate. Blue/green ABTS radical was produced by the reaction of those solutions. Mixture was allowed to stand at room temperature in the dark for 12–16 h before use. The solution was then diluted by mixing 14.8 mL working solution with 240 mL of methanol. Absorbance was measured at 734 nm.

0.002 mL of plant extracts were mixed with 1.8 mL of diluted ABTS solution and then diluted with 2.198 mL of methanol. Mixture was allowed to stand for 6 min at room temperature. Antioxidants from extract cause discoloration of solution, proportional to their amount. The absorbance was measured at 734 nm. Trolox was used as standard and results are expressed as μg of TE per mg dry extract weight (μg TE/mg dry extract). The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation.

Ferric-reducing antioxidant power (FRAP) assay

Antioxidant activity was also determined by the assay based on reduction of Fe^{3+}–TPTZ (tripyridyltriazine) complex to the intensive blue Fe^{2+}–TPTZ form at acidic pH 2.5. FRAP reagent was prepared by mixing 200 mL of $CH_3COONa \times 3H_2O$, 20 mL of TPTZ and 20 mL of $FeCl_3$ in ratio 10 : 1 : 1. After that, 0.01 mL of extract was mixed with 1 mL of prepared FRAP reagent and 2.99 mL of distilled water. Mixture was allowed to stand for 5 min at 37ºC. Absorbance was measured at 595 nm. The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation. Results are expressed as μg Fe/mg dry extract.

Total reducing power (TRP) assay

The reducing power of water and ethanol *C. hederifolium* extracts was determined by the method of Oyaizu [26]. 0.01 mL of extract was mixed with 1 mL of 1% solution K3[Fe(CN)6], 1 mL phosphate buffer (pH 6.6) and 1.69 mL of distilled water. The mixture was allowed to stand at 50 ºC for 30 min. After expiration of the incubation time, 1 mL of 10% solution of CCl_3COOH and 0.6 mL of $FeCl_3$ were added. The absorbance was measured at 700 nm. Ascorbic acid was used as standard. The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation. Results were expressed as μg ascorbic acid equivalents per mg of dry extract weight (μg AAE/mg dry extract). Higher absorbance indicates higher reducing power.

Cupric reducing antioxidant capacity (CUPRAC) assay

The CUPRAC assay was performed using the method based on electron-transfer mechanism described by Özyürek et al. [27], with some modifications. The method is based on the reduction of a cupric neocuproine complex (Cu(II)–Nc) by antioxidants to the cuprous form (Cu(I)–Nc). The CUPRAC method is applicable to a wide range of hydrophilic and lipophilic antioxidant molecules, such as polyphenol acids, flavonoids, carotenoids, anthocyanins, synthetic antioxidants and vitamin C and E 23, 28. Briefly, 0.01 mL of extract was mixed with 1 mL of neocuproine, 1 mL of phosphate buffer (pH 7.0) and 1 mL of copper (II) chloride. 1.09 mL of distilled water was added to the mixture. The mixture was allowed to stand for 30 min at room temperature. The absorbance was measured at 700 nm and Trolox (410 μg/mL) was used as standard. The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation. Results are expressed as μg Trolox equivalents per mg of dry weight (μg TE/mg dry extract).

Antimicrobial activity

Disk diffusion assay

Both extracts of *C. hederifolium* (aqueous and ethanol) were examined for *in vitro* antimicrobial activity. The disk diffusion assay was used to investigate sensitivity of laboratory bacterial and fungal control strains (American Type Culture Collection – Maryland, USA) against investigated extracts [29, 30]. Initial concentration of extracts was 50 mg/mL. Bacterial strains were: Gram-positive bacteria *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538 and Gram-negative bacteria *Escherichia coli* ATCC 8739 and *Salmonella abony* NCTC 6017. *Candida albicans* ATCC 10231 was used as test strain for investigation of antifungal activity. Antibiotics streptomycin (10 μg/disk), chloramphenicol (30 μg/disk) and antimycotic nystatin (100 U/disk) were used as control substances. The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation.

Evaluation of cytotoxicity

Cell culture

Both extracts (aqueous and ethanol) of *C. hederifolium* were examined on 4 different cell cultures (3 mouse and 1 human cancer cell lines) by *in vitro* 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) bioassay. Mouse lung cancer cells (LLC1), breast cancer cells (4T1), colon cancer cells (CT26) and human colon cancer cells (HCT116) were obtained from the American Type Culture Collection (ATCC). The cells were maintained in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma, Germany), at 37 ºC in an atmosphere of 95% O2 and 5% CO2.

MTT bioassay

The investigated extracts of *C. hederifolium* were tested for *in vitro* cytotoxicity by standard colorimetric assay for measuring the cell viability (MTT test) [31]. Cancer cells were plated by adding 100 μL of media into each of the 96-well plates (Nunc A/S, Rockilde, Denmark). Plates were incubated at 37 ºC and 5% CO_2 overnight for adherence. After 24h, when the cells were attached to the surface of the plate, the medium was taken out and replaced with 100 μL of extracts, which had been serially diluted 2 fold in the medium to concentrations ranging 2,000 μg/mL, 1,000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL and 15.625 μg/mL. Both extract was tested in triplicate, repeated in three independent series, and cisplatin was used as positive control. Plates were incubated at 37ºC in 5% CO_2 for 24 h, 48 h and 72 h. Cells were periodically viewed under fluorescence microscope Olympus BX51. After incubation periods the supernatant was removed, 100 μL MTT solution (5 mg/mL in PBS) in DMEM (Dulbecco's modified Eagle's medium) (10 μL MTT and 90 μL, per well) was added to each well. In this reaction, purple crystals of formazan were formed due to the reduction of yellow MTT by mitochondrial dehydrogenase of viable cells. After 4 h incubation under aforementioned conditions, the medium with MTT was removed. Then, DMSO (150 μL) with glycine buffer (20 μL) was added to each well in order to dissolve formazan crystals. The absorbance was measured at 595 nm using microplate Zenyth 3100 Multimode detector (Anthos Labtec Instruments GmbH, Austria). Results are presented as percentage of dead cells.

Data analysis

The experiments were carried as three independent measurements and data were expressed as mean ± standard deviation (SD). EC-50 values were calculated by using Microsoft Office Excel 2007.

Results

Metals found in all four investigated samples are presented in Table 1 (ethanol and aqueous *C. hederifolium* extracts, fresh plant tuber and soil). Cr, Ni, Pb and Cd were not detected in all samples, due to the concentrations that were below the detection limit.

Total phenolic content (TPC) and total flavonoid content (TFC)

Amount of phenols and flavonoids found in aqueous and ethanol extracts of *C. hederifolium* is presented in Table 2.

Antioxidant activity

The results of antioxidant activity of aqueous and ethanol *C. hederifolium* extracts, estimated by five different methods are shown in Table 3.

The comparison of the radical scavenging activity of investigated extracts measured by DPPH and ABTS assays expressed by EC-50 is presented in Figure 1.

Table 1

Metal content found in different *Cyclamen hederifolium* samples

Metal	Aqueous extract (mg/kg)	Ethanol extract (mg/kg)	Fresh tuber (mg/kg)	Soil (mg/kg)
Mn	157.32 ± 1.60	17.62 ± 0.41	47.42 ± 0.52	1141.56 ± 38.61
Ca	4018.8 ± 11.09	2263.86 ± 38.43	9053.4 ± 38.98	16748.98 ± 92.24
Mg	12052.36 ± 44.5	6770.78 ± 39.42	7256.06 ± 43.57	3162.62 ± 35.46
Fe	1250.2 ± 22.64	462.3 ± 11.85	375.06 ± 6.82	6448.66 ± 37.57
Zn	62.02 ± 1.07	23.96 ± 0.37	10.72 ± 0.40	37.44 ± 0.65
K	6826.04 ± 30.57	5224.8 ± 27.71	7070.12 ± 27.81	3265.34 ± 38.55
Cu	5.454 ± 0.06	5.542 ± 0.04	9.502 ± 0.05	9.904 ± 0.06

Table 2

Total content of phenols and flavonoids in *Cyclamen hederifolium* extracts

*E*xtract	TPC (μg GAE/mg dw)	TFC (μg RE/ mg dw)
Aqueous	31.76 ± 2.92	28.17 ± 10.06
Ethanol	26.03 ± 1.32	23.2 ± 7.37

TPC – total phenolic content; TFC – total flavonoid content; GAE – gallic acid equivalents; RU – rutin equivalents.

Table 3

Antioxidant activity of *Cyclamen hederifolium* extracts (mean ± standard deviation)

Extract	DPPH	FRAP	ABTS	TRP	CUPRAC
	μg TE/mg dw	μg Fe/ mg dw	μg TE/mg dw	μg AAE/ mg dw	μg TE/ mg dw
Aqueous	8.153 ± 1.881	15.62 ± 0.576	6.21 ± 0.562	0.067 ± 0.008	5.893 ± 1.853
Ethanol	8.293 ± 1.167	12.57 ± 1.822	6.97 ± 0.534	0.05 ± 0.00	1.358 ± 1.160

DPPH – 2,2-diphenyl-1-picrylhydrazyl assay; FRAP – ferric-reducing antioxidant power assay; ABTS – 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay; TRP – total reducing power assay; CUPRAC – cupric reducing antioxidant capacity assay; TE – trolox equivalents; AAE – ascorbic acid equivalents.

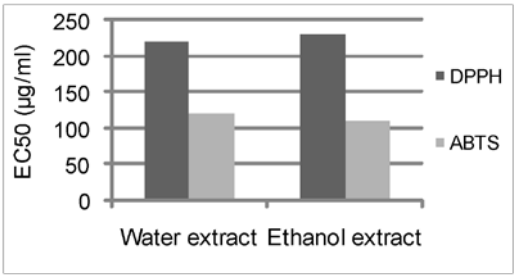


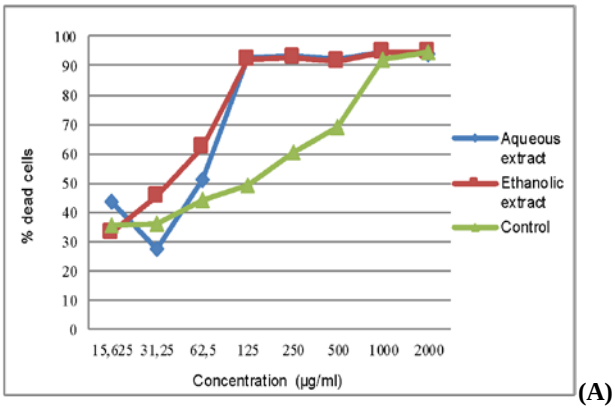
Fig. 1 – Free radical-scavenging of *Cyclamen hederifolium* extracts – DPPH vs. ABTS assay.
DPPH – 2,2-diphenyl-1-picrylhydrazyl assay; ABTS – 2,20-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) assay.

Antimicrobial activity

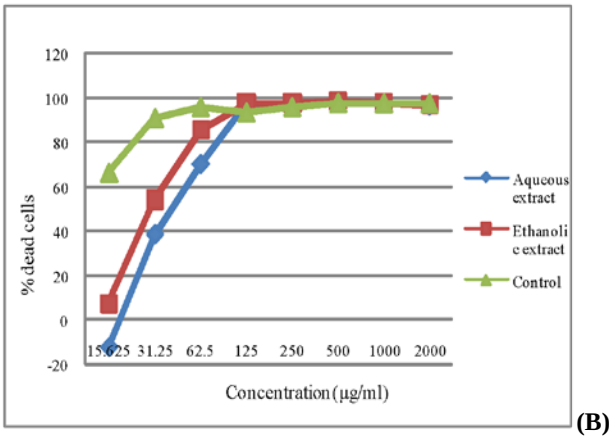
Antimicrobial activity of both investigated extracts of *C. hederifolium* against four bacterial and one fungal strain was negative.

Cytotoxic activity

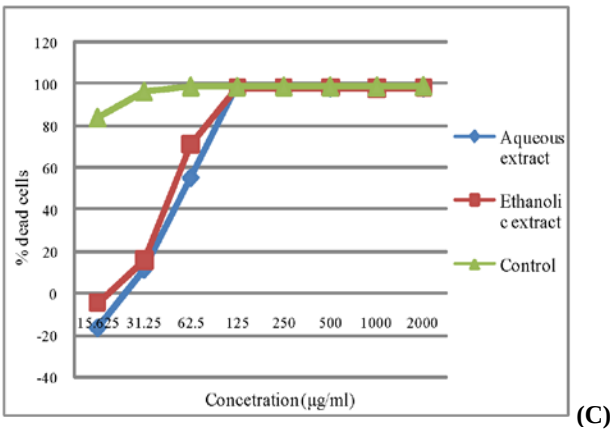
Results of the MTT assay show that both *C. hederifolium* extracts exhibit significant reduction of cell viability in all investigated carcinoma cells (Figures 2–5). Control was cisplatin.



(A)

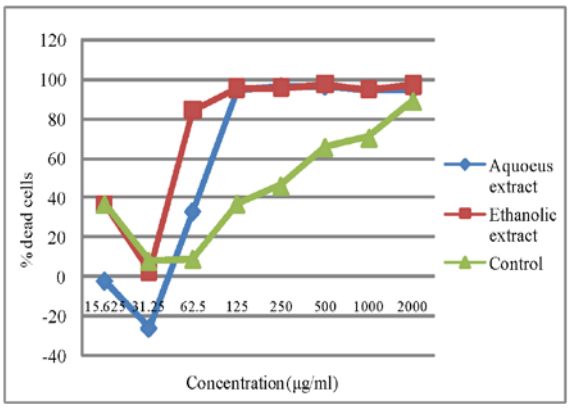


(B)

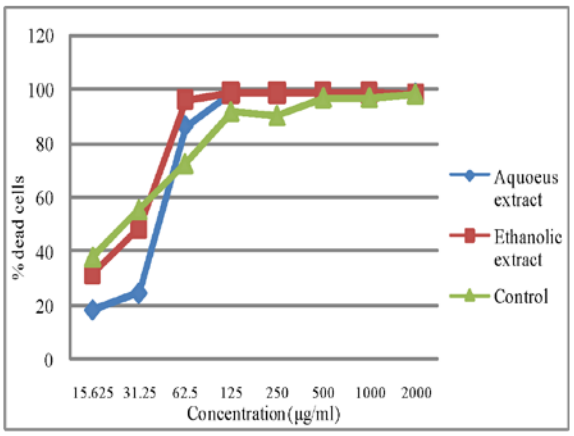


(C)

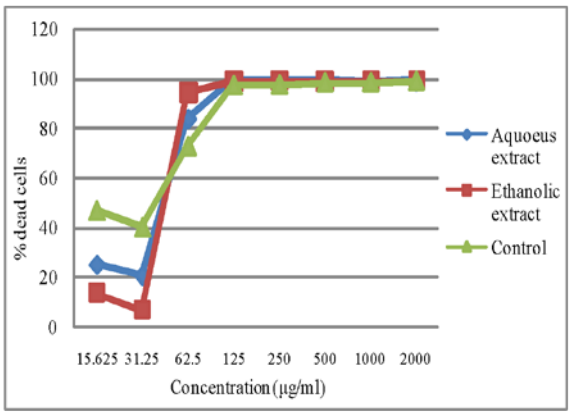
Fig. 2 – Mortality rate of 4T1 cell line exposed to aqueous and ethanolic extracts of *Cyclamen hederifolium* tuber during 24 h (A), 48 h (B) and 72 h (C).



(A)



(B)



(C)

Fig. 3 – Mortality rate of CT26 cell line exposed to aqueous and ethanolic extracts of *Cyclamen hederifolium* tuber during 24 h (A), 48 h (B) and 72 h (C).

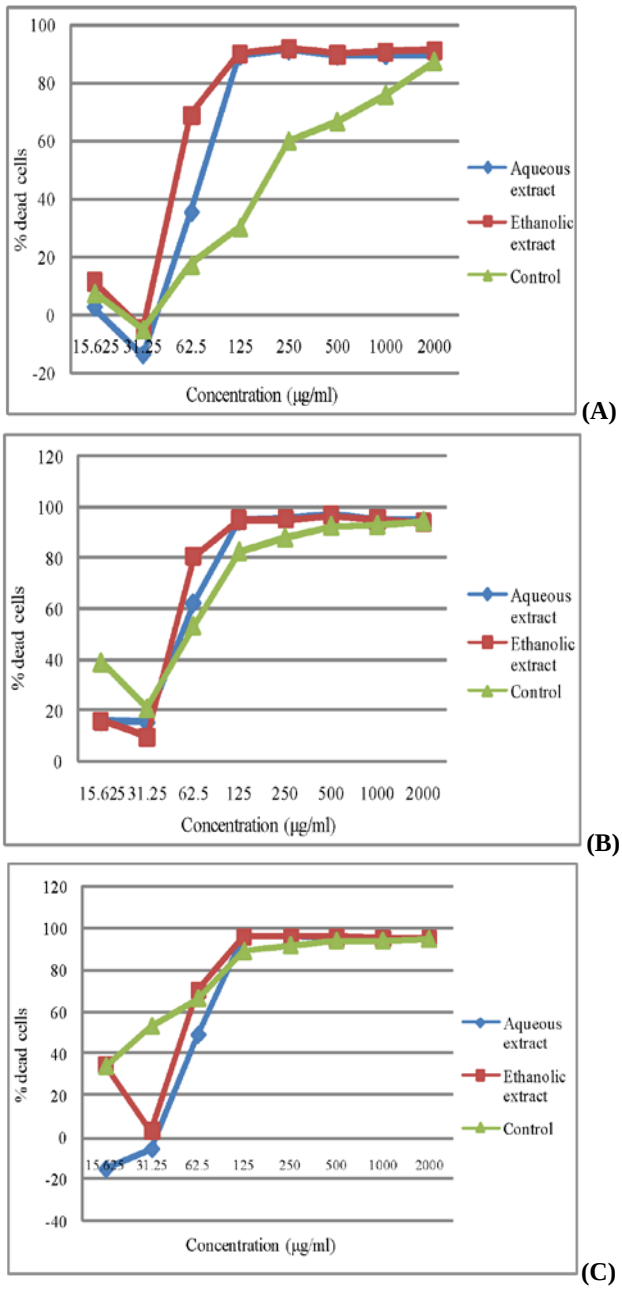


Fig. 4 – Mortality rate of HCT116 cell line exposed to aqueous and ethanolic extracts of *Cyclamen hederifolium* tuber during 24 h (A), 48 h (B) and 72 h (C).

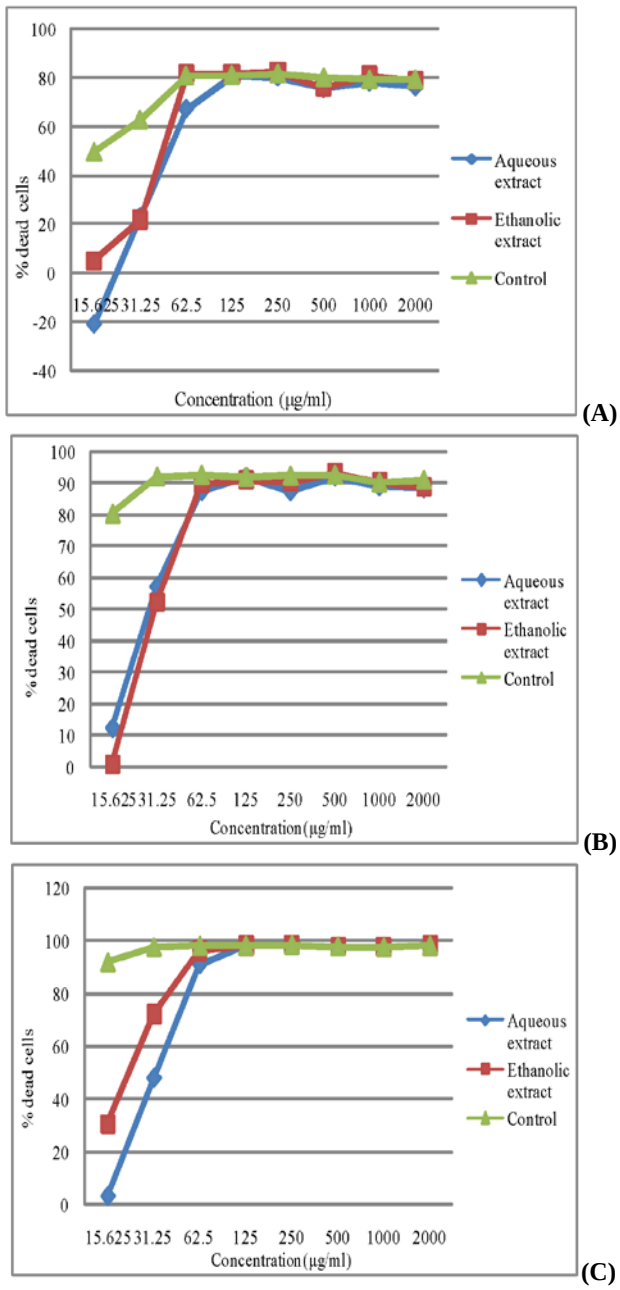


Fig. 5 – Mortality rate of LLC1 cell line exposed to aqueous and ethanolic extracts of *Cyclamen hederifolium* tuber during 24 h (A), 48 h (B) and 72 h (C).

Discussion

The tubers of *C. hederifolium* have been sporadically used in Serbian folk medicine in the treatment of different tumors. The absence of scientific evidence that supports use of *C. hederifolium* in this indication was a driving force to perform this study. Taking into account that most plants have been processed in folk medicine by water or alcohol (maceration, decoction, tinctures, etc.) and that have been no data regarding the bioactivity of *C. hederifolium* growing naturally in Eastern parts of Serbia, we investigated aqueous and 70% ethanol extracts for antioxidant, antibacterial, antifungal and cytotoxic properties, in order to evaluate the traditional application of this plant as anticancer agent.

Many plants have various uses due to their specific content of elements and secondary metabolites. Plants absorb water and minerals from soil where they are grown and store (accumulate) them in different parts. Many of micro and macro elements have an important role in human health, making monitoring of metal content plant material essential [32, 33]. The composition of minerals found in the plant material depends on species, parts of the plant, tendencies to take over and accumulate metals from soil, type of the growing environment (urban, industrial, rural, wilderness, etc.) and chemical composition of the soil [33, 34].

We followed metal content in four investigated samples in order to determine total quantity of metals present in *C.*

hederifolium fresh tubers and aqueous and ethanol extracts. Soil sample was monitored in order to determine if the plant is the accumulator of a specific mineral. The presence of Mn, Ca, Mg, Fe, Zn, K, Cu was confirmed in all four samples. Cr, Ni, Pb and Cd were not detected. Their below the limit concentrations are most likely related to unpolluted rural area where the tubers were collected from. Following metals were found in aqueous extract in the concentrations of the descending order: Mg > K > Ca > Fe > Mn > Zn > Cu, while ethanol extract contained: Mg > K > Ca > Fe > Zn > Mn > Cu. Aqueous extract had higher content of all observed metals than ethanol extract, except content of Cu which was similar in both extracts. Results of metal content analysis in relation to tuber, showed that aqueous extract had higher amount of Mn, Mg, Fe and Zn, while ethanol extract had more Fe and Zn. These findings indicate the difference in transfer of metals during extraction procedure. In this case, water has proven to be extraction solvent with higher potential for metal transfer than ethanol. Compared to soil, aqueous extract contained more Mg, Zn and K, while ethanol extract contained more Mg and K. The results demonstrated that the tuber samples contained higher quantity of Mg and K, in comparison to soil samples, which suggest potential phytoaccumulation of these metals [20].

The aqueous and ethanol extracts of *C. hederifolium* tubers were screened for composition of phenols and flavonoids, since these compounds have been mainly responsible for antioxidant activity of plants [23]. Aqueous extract contained slightly higher concentration of both phenols and flavonoids than ethanol extract. Several standard methods which were used to estimate antioxidant activity of investigated extracts (DPPH, FRAP, ABTS, CUPRAC and TRP assay) revealed that the aqueous extract of *C. hederifolium* exhibited stronger antioxidant activity than ethanol extract (FRAP and CUPRAC method). Therefore, we observe a positive correlation between TPC and TFC and antioxidant activity. Antioxidant activity of aqueous and ethanol extracts determined by DPPH, TRP and ABTS methods, however, did not confirm this difference. This absence of major differences between extracts in regards to the antioxidant activity as well as total phenol and flavonoid content could be attributed to predictable and small difference in polarity of solvents, which evidently exerted similar extraction potential.

There is generally a lack of the data in the literature on antioxidative activity of *C. hederifolium*. The only available information as regards antioxidative activity of *C. hederifolium* originated from Turkey – antioxidative activity of ethanol extracts of tubers was dose-dependent (by DPPH, ABTS and NO assays) [11]. We accentuate the results obtained in this study of *C. hederifolium* as the first information of phenol and flavonoid content and antioxidant activity of the plant material autochthon to this region.

Different *Cyclamen* species exhibited antimicrobial properties in varying degrees, such as *C. hederifolium* growing in Syria [31, 35, 36]. However, we may not compare our results to it, since extracts used in that research were made by different solvents that have no application in traditional medicine (methanol, petroleum ether, ethyl acetate and chloroform). The methanol extract showed better antimicrobial activities than the other extracts [6, 10]. This could be due to the differences in solvents polarity and their stronger ability to extract secondary metabolites responsible for antimicrobial activity. Aqueous and ethanol extracts of *C. hederifolium* tubers in our study showed absence of antimicrobial activity. We did not expected these findings, because some of secondary metabolites of *C. hederifolium* (e.g. saponins) in tubers may interfere with sterols in bacterial membrane and chaenge its integrity [9, 15, 16]. Since varying degrees of antimicrobial activity were detected for different types of *Cyclamen*, we consider that the limitation of this study was of number of strains that were tested as well as the selection of solvents used for extraction.

Some of previous studies documented that *Cyclamen* species may induce cell death in different tumor cell lines, although cytotoxic mechanisms have not been proposed [8, 13, 17]. Moderate cytotoxicity of *C. coum* was noted against cervical and lung cancer cells (HeLa and H1299, respectively) [35]. Moderate cytotoxic activity was reported for *C. trochopteranthum* against hepatocelular (HepG2) and colorectal adenocarcinoma (Caco-2) cell lines, while *C. pseudibericum* exhibited activity against nonsmall cell lung carcinoma cells (A549) [38, 39]. Although strong cytotoxic effect was observed in investigation of *C. libanoticum* and *C. persicum* against breast adenocarcinoma (SK-BR-3), colon adenocarcinoma (HT-29), hepatocelular (HepG2/3A), lung (NCI-H1299), pancreatic (BXPC-3), and prostate (22RV1) carcinoma cell lines, these effects could not be attributed to the extracts of these plants, since the experiments were performed with isolated and purified secondary metabolites (saponins) [8]. *C. hederifolum* was not tested on any of the cell lines we used in our investigation.

The effect of aqueous and ethanol extracts of *C. hederifolium* tubers on viability of several different cancer cell lines (4T1, HCT116, CT26, LLC1) were examined by MTT assay. Cell viability was tested after cell treatment with increasing concentrations of extracts, at different time of incubation (after 24 h, 48 h and 72 h). The results obtained in this study showed both aqueous and ethanol extracts of *C. hederifolium* exhibit cytotoxic activity in all investigated cell lines. Ethanol extract was superior in cytotoxic effect to aqueous extract, in all cell lines. Stronger cytotoxicity of both investigated extracts in comparison to control was observed in all carcinoma cell lines, in the first 24 h, with exception of LLC1 cell line. Concentrations lower than 125 μg/mL of both investigated extracts induced cell death up to 90%. However, higher concentrations of 125 μg/mL of both extracts showed an almost equal cytotoxic effect as control in all investigated cell lines, after 48 h and 72 h of incubation (90%–100%). The dose dependent manner of cytotoxcity was observed for different concentration in different cell line. Concentrations lower than 62.5 μg/mL for cell lines CT26 and LLC1 and concentrations lower than 125 μg/mL for 4T1 and HCT116, exerted dose dependent action. Higher concentration of extracts the for above mentioned cell lines reached a plateau in effectiveness (Figures 2–5). Unusual effect was observed for aqueous extract in concentrations up to 15.625 μg/mL, which produced no cytotoxic activity in some cell lines at different times of incubation – HCT116 and 4T1 cell line (24 h and 72 h of incubation) and CT26 (at 48 h and

72 h) and LLC1 (24 h). Based on these results, we might assume that ethanol as a solvent was more suitable for extraction of antitumor plant compounds than water.

To our knowledge, this is the first report for cytotoxic activity of endemic species *C. hederifolium* from natural habitats in Serbia. Although there were some previously reported data on cytotoxicity of *C. hederifolium*, there are major differences in methodology used in previous research and ours such as type of cytotoxic assay as well as the origin and preparation of the plant material (solvents used for the plant extraction and harvesting time of the plant material)[9, 12]. Both previous studies were done on plant material from Turkey. One study utilized plant material collected in autumn and evaluation of cytotoxicity by brine shrimp assay, while the other study investigated isolated compounds from *C. hederifolium* and reported no significant cytotoxicity. Therefore, we may not compare our results with aforementioned literature data which found low cytotoxic activity or absence of it in *C. hederifolium* from Turkey[9, 12]. Our results are however in accordance with other studies that point out the significant antitumor effects of *Cyclamen* spp. found on different cell lines[8, 34, 38, 39].

Since both investigated extracts showed cytotoxicity on four cancer cell lines (4T1, HCT116, CT26, LLC1) we consider this work as a starting point for further research, leading to elucidation of cell death mechanisms on proposed cell lines.

Limitations

The study was performed on 4 different tumor cell lines, but not on any control cell line. This is a potential limitation because it omitted the possibility of testing selectivity in cytotoxic activity of the examined extracts.

Conclusion

Seven metals have been identified in the *C. hederifolium* tubers, extracts and soil: Mn, Ca, Mg, Fe, Zn, K, Cu. Both aqueous and ethanol extract of *C. hederifolium* tubers exhibited antioxidant activity, which was positively correlated with content of phenols and flavonoids. Aqueous extract was slightly superior in these terms than ethanol extract. None of the tested extracts showed antimicrobial activity. Investigated extracts showed significant cytotoxicity in the concentration range 15.625–2,000 μg/mL. Ethanol extract showed stronger cytotoxic potential than aqueous extract. The reported data are the first data of this type for the species of *C. hederifolium*, with natural habitats in Serbia.

Acknowledgments

This work was supported by the projects No. OI 172051, No. OI 172047, and No. OI 175014 of the Ministry of Education, Science and Technological Development of the Republic of Serbia, as well as bilateral project with PR China (06/2018).

Conflict of interest

Authors declare that no conflict of interest exists.

R E F E R E N C E S

1. *De Vos P.* European material medica in historical texts: longevity of a tradition and implications for future use. J Ethnopharmacol 2010; 132(1): 28–47.
2. *Fürst R, Zündorf I.* Evidence-based phytotherapy in Europe: Where do we stand? Planta Med 2015; 81(12–13): 962–7.
3. *Grey-Wilson C.* Cyclamen: a guide for gardeners, horticulturalists and botanists. New edition. London: Batsford Ltd; 2003.
4. *Anderberg AA.* Phylogeny and subgeneric classification of Cyclamen L. (Primulaceae). Kew Bull 1994; 49(3): 455–67.
5. *Guarrera PM.* Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). Fitoterapia 2005; 76(1): 1–25.
6. *Mansour O, Rim S, Abbas L.* Screening of Antibacterial activity in vitro of Cyclamen hederifolium tubers extracts. Res J Pharm Tech 2016; 9(11): 1451–3.
7. *Mazouz W, Djeddi S.* A biological overview on the Genus Cyclamen. Eur J Sci Res 2013; 110(1): 7–22.
8. *El Hosry L, Di Giorgio C, Birer C, Habib J, Tueni M, Bun SS,* et al. In vitro cytotoxic and anticlastogenic activities of saxifragifolin B and cyclamin isolated from Cyclamen persicum and Cyclamen libanoticum. Pharm Biol 2014; 52(9): 1134–40.
9. *Altunkeyik H, Gulcemal D, Masullo M, Alankus-Caliskan O, Piacente S Karayildirim T.* Triterpene saponins from Cyclamen hederifolium. Phytochemistry 2012; 73(1): 127–33.
10. *Boissa NH, Yaziji M, Salame R.* Screening of antifungal activity in vitro of Cyclamen hederifolum tubers extracts. Res J Pharm Tech 2016; 9(10): 1677–80.
11. *Özay C, Uluköy G, Mammadov R, Sayin Z.* Radical scavenging activity and antibacterial effect of three Cyclamen L. tuber extracts on some fish pathogens. SDU J Nat App Sci 2018; 22(2): 562–8.
12. *Düşen S, Aydın C, Gul HY, Ozay C, Dusen O, Mammadov R.* In vitro cytotoxic activities of Cyclamen L. (Primulaceae) ethanol extracts from Turkey. Fresenius Envir Bull 2016; 25(12A): 6224–8.
13. *Yildiz M, Bozcu H, Tokgun O, Karagur ER, Akyurt O, Akca H.* Cyclamen exerts cytotoxicity in solid tumor cell lines: a step toward new anticancer agents? Asian Pac J Cancer Prev 2013; 14(10): 5911–3.
14. *Mohammed GJ, Hameed IH, Kamal SA.* Anti-inflammatory effects and other uses of Cyclamen species: A review. Ind J Pub Health Res Dev 2018; 9(3): 205–11.
15. *Koczurkiewicz P, Czyż J, Podolak I, Wójcik K, Galanty A, Janeczko Z,* et al. Multidirectional effects of triterpene saponins on cancer cells - mini-review of in vitro studies. Acta Biochim Pol 2015; 62(3): 383–93.
16. *Chudzik M, Korzonek-Szlacheta I, Król W.* Triterpenes as potentially cytotoxic compounds. Molecules 2015; 20(1): 1610–25.
17. *Nikolić V.* Genus Cyclamen L In: *Josifović M,* editor. Flora of SR Serbia III. Belgrade: Serbian Academy of Sciences and Arts; 1972. p. 500–2. (Serbian)
18. *Meikle DR, Sinnott HN.* Cyclamen L. In: *Tutin TG, Heywood WH, Burges NA, Moore DM, Valentine H, Walters SM,* et al, editors. Flora Europaea III. London, UK: Cambrige University Press; 1972. p. 25–6.
19. *Zlatić NM, Stanković MS, Simić ZS.* Secondary metabolites and metal content dynamics in Teucrium montanum L. and Teucrium chamaedrys L. from habitats with serpentine and calcareous substrate. Environ Monit Assess 2017; 189(3): 110.

20. *Brankovic S, Cupara S, Glisic R, Djelic G, Grbovic F, Kojicic K,* et al. Phytoaccumulation in plants of mountain GOC in Serbia. Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii 2017; 27(3): 196–201
21. *Singleton VL, Orthofer R, Lamuela-Raventos RM*. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999; 299(1): 152–78.
22. *Baba S, Malik S*. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. J Taibah Univ Sci 2015; 9(4): 449–54.
23. *Dimitrijevic M, Stankov Jovanovic V, Cvetkovic J, Mihajilov Krstev T, Stojanovic G, Mitic V*. Screening of antioxidant, antimicrobial and antiradical activities of twelve selected Serbian wild mushrooms. Anal Methods 2015; 7(10): 1–15.
24. *Tadić VM, Dobrić S, Marković GM, Dordević SM, Arsić IA, Menković NR,* et al. Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of hawthorn berries ethanol extract. J Agric Food Chem 2008; 56(17): 7700–9.
25. *Benzie IF, Strain JJ*. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996; 239(1): 70–6.
26. *Oyaizu M*. Studies on Products of Browning Reactions: Antioxidative Activities of Product of Browning Reaction Prepared from Glucosamine. Jpn J Nutr 1986; 44(6): 307–15.
27. *Özyürek M, Güçlü K, Apak R*. The main and modified CUPRAC methods of antioxidant measurement. Trends Anal Chem 2011; 30(4): 652–64.
28. *Apak R, Güçlü K, Demirata B, Ozyürek M, Celik SE, Bektaşoğlu B,* et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 2007; 12(7): 1496–547.
29. *Faleiro L, Miguel G, Gomes S, Costa L, Venâncio F, Teixeira A,* et al. Antibacterial and antioxidant activities of essential oils isolated from Thymbra capitata L. (Cav.) andOriganum vulgare L. J Agric Food Chem 2005; 53(21): 8162–8.
30. *Tomovic D, Bukonjic A, Jevtic V, Ratkovic ZR, Bogojeski JV, Dekovic A,* et al. DNA binding, antibacterial and antifungal activities of copper(II) complexes with some S-alkenyl derivatives of thiosalicylic acid. Transit Met Chem 2018; 43(2): 137–48.
31. *Tomovic DLJ, Bukonjic AM, Kocovic A, Nikolic MV, Mijajlovic MZ, Jevtic VV,* et al. Synthesis, characterization, and cytotoxicity of binuclear copper(II)-complexes with some S-alkenyl derivatives of thiosalicylic acid. Ser J Exp Clin Res 2017; 18(1): 13–8.
32. *Randjelovic S, Kostic D, Mitic S, Zarubica A, Mitic C, Velickovic J*. The correlation of metal content in medicinal plants and water their extract. Hem Ind 2013; 67(4): 585–91.
33. *Shah A, Niaz A, Ullah N, Rehman A, Akhlaq M, Zakir M,* et al. Comparative Study of Heavy Metals in Soil and Selected Medicinal Plants. J Chem 2013; ID 621265. doi: 10.1155/2013/621265.
34. *Grbovic F, Stankovic M, Vukajlovic F, Brankovic S, Simic Z, Topuzovic M*. Comparative study of the accumulation of metals in the plant Polygonum aviculare L. from different sites in the city of Kragujevac. Biologia Serbica 2016; 38(1): 3–11.
35. *Sajjadi ST, Saboora A, Mohammadi P*. Comparison of aglycon and glycosidic saponin extracts of Cyclamen coum tuber against Candida spp. Curr Med Mycol 2016; 2(2): 40–4.
36. *Turan M, Mammadov R*. Antioxidant, antimicrobial, cytotoxic, larvicidal and anthelmintic activities and phenolic contents of *Cyclamen alpinum*. J Pharm Pharmacol 2018; 9(4): 100–16.
37. *Alkowni R, Jodeh S, Hussein F, Jaradat N*. Phytochemical screening and antibacterial activity of *Cyclamen persicum* Mill tuber extracts. Pak J Pharm Sci 2018; 31(1): 187–92.
38. *Arslan S, Ozgun O*. Cyclamen Trochopteranthum: Cytotoxic activity and possible adverse interactions including drugs and carcinogens. Chin J Integr Med 2012; 1–8. Doi: 10.1007/s11655-012-1253-1.
39. *Karagur ER, Ozay C, Mammadov R, Akca H*. Antiinvasive effect of *Cyclamen pseudibericum* extract on A549 non-small cell lung carcinoma cells via inhibition of ZEB1 mediated by miR 200c. J Nat Med 2018; 72(3): 686–93.

Received on July 3, 2019.
Revised on September 5, 2019.
Accepted September 6, 2019.
Online First September, 2019.

ORIGINAL ARTICLE

UDC: 616.24-005-037
DOI: https://doi.org/10.2298/VSP190226103J

Different predictive value for short-term all-cause mortality with commonly used biomarkers regarding the cause of pulmonary embolism

Različite prediktivne vrednosti rutinskih biomarkera u proceni smrtnosti obolelih od plućne embolije u odnosu na njen uzrok

Ljiljana Jovanović*, Vesna Subota*, Milena Stavrić*, Boris Džudović†, Bojana Subotić†, Nataša Novčić†, Milica Mirić‡, Jovan Matijašević‡, Maja Nikolić§, Vladimir Miloradović§, Sonja Salinger‖, Nataša Marković Nikolić¶, Ljiljana Kos, Tamara Preradović Kovačević**, Slobodan Obradović†††**

Military Medical Academy, *Institute of Medical Biochemistry, †Clinic for Cardiology and Emergency Internal Medicine, Belgrade, Serbia; ‡University of Novi Sad, Faculty of Medicine, Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia; §University of Kragujevac, Faculty of Medicine, Clinical Center Kragujevac, Clinic for Cardiology, Kragujevac, Serbia; ‖University of Niš, Clinical Center Niš, Clinic for Cardiology, Niš, Serbia; ¶University of Belgrade, Faculty of Medicine, University Clinical Center Zvezdara, Belgrade, Serbia; **University of Banja Luka, Faculty of Medicine, Clinical Center Banja Luka, Clinic for Cardiology, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; †††University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. The evaluation of blood levels of cardiac troponin I (cTnI), D-dimer, B-type natriuretic peptide (BNP), and C-reactive protein (CRP) on admission and during the treatment of pulmonary embolism (PE) are the part of routine diagnostic process and estimation of mortality risk. The aim of this study was to evaluate the predictive value of these biomarkers on admission for all-cause 30-day mortality in consecutive PE patients regarding whether they classified as spontaneous, transiently provoked, or permanently provoked PE. **Methods.** This retrospective analysis was gained from the data of 590 PE patients from the Serbian University Multicenter Pulmonary Embolism Registry (SUPER). Patients had at least one of these biomarkers (BNP, CRP, cTnI, and D-dimer) measured during the first 24 hours upon admission. **Results.** Receiver operating characteristic (ROC) curve analyses demonstrated that BNP had the highest prognostic accuracy for 30-day mortality in patients (n = 219) who had data for all examined biomarkers. BNP provided an AUC of 0.785 ($p < 0.001$). Separately, BNP had the highest c-statistic for all three groups of patients. CRP had a modest predictive value for the 30-day all-cause mortality in the group with transient provoked PE. Troponin I had a very modest predictive value for the 30-day all-cause mortality only in patients with spontaneous PE, and D-dimer was a very weak predictor of this end-point only in patients with persistent provoked PE. **Conclusion.** Patients with spontaneous, transient provoked, and persistent provoked PE have a significantly different profile of blood biomarkers level with different prognostic significance for early all-cause mortality.

Key words:
pulmonary embolism; biological factors; mortality; risk assessment; natriuretic peptides; troponin I; c-reactive protein.

Apstrakt

Uvod/Cilj. Rutinski dijagnostički proces i procena rizika od smrtnosti tokom prijema i lečenja plućne embolije (PE) obuhvata i analizu nivoa kardijalnog troponina I (cTnI), D-dimera, natriuretičnog peptida B-tipa (BNP) i C-reaktivnog proteina (CRP). Cilj ove studije bio je utvrđivanje prediktivne moći ovih biomarkera kako bi se odredio rizik od 30-to dnevne smrtnosti kod bolesnika obolelih od PE i to u odnosu na različite uzroke bolesti koji mogu biti spontani ili kratkotrajno i dugotrajno provocirani. **Metode.** Ova retrospektivna studija obuhvatila je 590 bolesnika obolelih od PE, iz multicentričnog Registra za plućnu emboliju. Tokom prvih 24 časa od prijema, bolesnicima je vršeno merenje bar

Correspondence to: Slobodan Obradović, Military Medical Academy, Clinic for Cardiology and Emergency Internal Medicine, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: sloba.d.obradovic@gmail.com

jednog od analiziranih biomarkera (BNP, CRP, cTnI i D-dimer). **Rezultati.** *Receiver operating characteristic* (ROC) analiza je pokazala da BNP ima najvišu prognostičku vrednost u proceni 30-dnevne smrtnosti kod bolesnika (n = 219) kod kojih su bile poznate vrednosti svih analiziranih biomarkera. BNP je imao AUC od 0,785 ($p < 0,001$). Pojedinačno posmatrano, BNP je imao najvišu c-statistiku (*concordance*) za sve tri grupe bolesnika. CRP je pokazao skromnu prediktivnu moć za 30-dnevnu smrtnost u grupi bolesnika kod kojih je PE bila izazvana prolaznim faktorom. Troponin I je imao malu prediktivnu vrednost za 30-dnevnu smrtnost samo kod bolesnika sa spontanom PE, dok je D-dimer bio veoma slab prediktor i to kod bolesnika kod kojih je PE bila rezultat stalnih provocirajućih faktora. **Zaključak.** U odnosu na spontane, prolazne ili stalne provocirajuće uzroke nastanka PE, biohemijski profil ovih grupa bolesnika značajno je različit, kao i prognostički značaj biomarkera u proceni rane smrtnosti.

Ključne reči:
plućna embolija; biološki faktori; mortalitet; rizik, procena; natriuretski peptid; troponin I; c-reaktivni protein.

Introduction

Pulmonary embolism (PE) is a huge global health problem and life-threatening condition associated with significant morbidity and all-cause mortality [1–5]. PE is considered to be provoked in the presence of permanent or temporary risk factors or unprovoked in the absence thereof [6, 7]. Different states and diseases, sometimes quite clearly and sometimes completely hidden, often underlie PE. Their presence is often crucial for the management and prognosis of patients with PE. The determination of some blood biomarkers on admission and during the treatment of PE is a part of the routine diagnostic process and estimation of mortality risk [8–10]. The blood levels of different biomarkers used in PE patients for different purposes, such as brain natriuretic peptides (BNP), cardiac troponins (cTn), D-dimer, and C-reactive protein (CRP), depend on different but somehow connected pathophysiological mechanisms, and many of the underlying PE diseases and states can have a strong influence on their levels. Since that, we speculate that patients with spontaneous PE, transient provoked PE, and persistent provoked PE must have a significantly different profile of blood biomarkers level with different prognostic significance regarding the main grouping causes of PE.

The aim of this study was to evaluate the association of blood levels of commonly used biomarkers in PE patients: cardiac troponin I (cTnI), D-dimer, BNP, and CRP early in the course of admission with all-cause 30-day mortality in consecutive PE patients admitted to intensive care unit for at least one day, regarding the main causality grouping method to spontaneous, transiently provoked, and permanently provoked PE. This retrospective analysis was gained from the data of the Serbian University Multicenter Pulmonary Embolism Registry (SUPER), founded in 2015.

Methods

This retrospective study was performed after the approval of the local Ethics Committee. Patients over 18 years old of both genders, with confirmed acute PE diagnosis by multidetector computed tomography pulmonary angiography (MDCT-PA), were included in this study. During the study period from January 2015 to June 2018, 607 cases were enrolled from five university cardiology or pulmonology clinics (Military Medical Academy in Belgrade, Institute for Pulmonary Diseases of Vojvodina in Sremska Kamenica, Clinical Center in Niš, University Clinic Zvezdara in Belgrade, and Clinical Center in Kragujevac).

Participants were divided into three groups. Patients with idiopathic or unprovoked PE were included in the first group. Provoked PE groups were identified according to different risk factors associated with PE. In the second group, PE is considered a consequence of the setting-related reversible factors, such as surgical procedure, trauma, immobilization, oral contraceptive use, pregnancy, and infections. PE patients with underlying serious chronic medical illness (malignancies, chronic inflammatory disorders, such as systemic connective tissue diseases or inflammatory bowel disease, prolonged immobilization due to irreversible neurologic deficit) were taken in the third group. The endpoint of the study was the all-cause short-term (30-day) mortality defined as death due to any cause after the diagnosis of PE.

Biochemical analysis

Peripheral venous blood specimens from the antecubital vein were collected, centrifuged, and immediately analyzed using standard laboratory techniques. cTnI and D-dimer were measured upon admission, BNP and CRP were analyzed within 24 hours of the hospital admission (which minimizes the influence of administrated therapy). Serum was utilized for cTnI and CRP assays. Citrated plasma was utilized for D-dimer assay and EDTA-plasma for BNP measurements. cTnI was determined *via* the fully-automated electrochemiluminescent assay using the conventional ADVIA Centaur ultra-cTnI assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). D-dimer was measured by an immunoturbidimetric assay using the Innovance D-dimer assay (BCS, Siemens, Marburg, Germany). BNP was studied in Siemens ADVIA Centaur System (ADVIA Centaur BNP assay, Bulletin 10629823-EN Rev.U, 2017-07, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). CRP measurements were performed with an ADVIA 1800 analyzer (Siemens Healthcare Diagnostic, Tarrytown, NY, USA). The reference values in the healthy population were as follows: 0.01–0.04 μg/L for cTnI, 0–0.5 mg/L FEU for D-dimer, 0–4 mg/L for CRP, and BNP upper reference level was 100 ng/L.

Statistical analysis

The continuous variables were expressed as the median with a 25th–75th percentile range since all investigated variables did not have a normal distribution. Mann-Whitney U-tests were performed for the comparison of biomarker blood concentrations in patients who survived versus patients who died within 30 days in all groups and according to the presumed cause of PE across the three groups. *P*-values being < 0.05 denoted statistical significance. The area under the receiver operating characteristics (ROC) curve was used to determine the diagnostic and prognostic value of biomarkers and select optimal cutoffs. SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used for data processing and statistical analysis.

Results

The aim of the study was to investigate the effectiveness of the panel of biomarkers to predict adverse events in different PE subgroups. The baseline clinical characteristics of the study patients are presented in Table 1. Out of 607 patients, 301 (49.6%) were men, 306 (13.7%)

Table 1

Baseline characteristic pulmonary embolism (PE) subgroups

Variables	30-day mortality										
	all patients			unprovoked PE			transient provoked PE			persistent provoked PE		
	no n = 524	yes n = 83	*p*	no n = 271	yes n = 32	*p*	no n = 144	yes n = 18	*p*	no n = 109	yes n = 33	*p*
Age (years), mean (SD)	61 (16)	68 (16)	< 0.001	62 (15)	69 (17)	0.03	57 (18)	73 (10)	< 0.001	65 (14)	65 (16)	0.956
Gender, n (%)												
male	267 (51.0)	34 (41.0)	0.099	153 (56.5)	15 (46.9)	0.349	58 (40.3)	5 (27.8)	0.443	56 (51.4)	14 (42.4)	0.429
female	257 (49.0)	49 (59.0)		118 (43.5)	17 (53.1)		86 (59.7)	13 (72.2)		53 (48.6)	19 (57.6)	
Comorbidities, n (%)												
COPD	51 (9.7)	14 (16.9)	0.057	24 (8.9)	5 (15.6)	0.210	11 (7.6)	0 (0)	0.613	16 (14.7)	9 (27.3)	0.118
CHD	66 (12.6)	18 (21.7)	0.038	33 (12.2)	5 (15.6)	0.573	12 (8.3)	4 (22.2)	0.083	21 (19.3)	9 (27.3)	0.337
CAD	50 (9.8)	15 (19.5)	0.018	22 (8.3)	2 (7.4)	1.000	15 (11.0)	6 (35.3)	0.252	13 (12.0)	7 (21.2)	0.015
history of DVT/PE	67 (12.9)	6 (7.2)	0.203	54 (20.3)	4 (12.5)	0.353	6 (4.2)	0 (0)	1.000	7 (6.4)	2 (6.1)	1.000
hypertension	277 (53)	50 (60.2)	0.237	149 (55.2)	17 (53.1)	0.853	66 (45.8)	14 (77.8)	0.012	62 (56.9)	19 (57.6)	1.000
MI+stroke+ PAD	61 (11.7)	20 (24.1)	0.005	33 (12.2)	3 (9.4)	0.780	15 (10.5)	9 (50)	<0.001	13 (11.9)	8 (24.2)	0.096
DM	83 (15.9)	19 (22.9)	0.116	51 (18.9)	5 (15.6)	0.812	16 (11.1)	5 (27.8)	0.062	16 (14.7)	9 (27.3)	0.118
history of stroke	31 (5.9)	13 (15.7)	0.005	12 (4.4)	4 (12.5)	0.075	11 (7.6)	4 (22.2)	0.067	8 (7.3)	5 (15.2)	0.180
malignancy	59 (11.3)	15 (18.3)	0.100	4 (1.5)	4 (12.5)	0.005	9 (6.2)	2 (11.8)	0.327	46 (42.2)	9 (27.3)	0.155
creatinine clearance < 30 mL/min	28 (5.8)	20 (27.4)	< 0.001	17 (6.7)	7 (28.0)	0.003	0 (0.0)	3 (20.0)	0.001	11 (10.7)	10 (30.3)	0.011
Risk factors, n (%)												
PESI												
> 0	339 (67.3)	73 (94.8)	< 0.001	171 (65.0)	26 (92.9)	0.002	85 (63.4)	15 (93.8)	0.022	83 (77.6)	32 (97.0)	0.009
= 0	165 (32.7)	4 (5.2)		92 (35.0)	2 (7.1)		49 (36.6)	1 (6.2)		24 (22.4)	1 (3.0)	
Risk of PE, n (%)												
low	147 (28.4)	6 (7.3)	< 0.001	77 (28.8)	4 (12.9)	< 0.001	48 (33.6)	1 (5.6)	0.016	22 (20.4)	1 (3.0)	< 0.001
intermediate-low	137 (26.4)	8 (9.8)		72 (27.0)	1 (3.2)		27 (18.9)	2 (11.1)		38 (35.2)	5 (15.2)	
intermediate-high	184 (35.5)	32 (39.0)		99 (37.1)	13 (41.9)		50 (35.0)	9 (50.0)		35 (32.4)	10 (30.3)	
high	50 (9.7)	36 (43.9)		19 (7.1)	13 (41.9)		18 (12.6)	6 (33.3)		13 (12.0)	17 (51.5)	

COPD – chronic obstructive pulmonary disease; CHD – coronary heart disease; DVT – deep vein thrombosis; CAD – coronary artery disease; MI – myocardial infarction; PAD – pulmonary artery disease; DM – diabetes mellitus; PESI – pulmonary embolism severity index; SD – standard deviation.

Data were missing for CAD (3.5%), DVT/PE (1.0%), hypertension (0.2%), MI+stroke+PAD (0.3%), DM (0.2%), malignancy (0.2%), PESI (4.3%) and risk of PE (1.2%).

were women, with a mean age of 62 ± 16. Among all patients, 83 died within 30 days; of those, 50 patients died as a result of PE, which is 60.2% of all-cause deaths (50.4% vs. 27.5% vs. 22%, in spontaneous, transient, and persistent provoked PE group, respectively). Venous thromboembolism (VTE) is the second leading cause of death in cancer patients [11]. Therefore, in the persistent provoked group, a significant number of patients with malignancy died (27.3%), unlike 12.5% vs. 11.8% in the spontaneous and transient provoked PE group, respectively.

If we analyze the differences between the characteristics of patients across these three groups, we can see that in patients with spontaneous PE and PE caused by the transient provoked factor, patients who died at 30 days were older and there was no difference in age in comparison with the rate of death and survival in the group with persistent provoked factor (Table 1). The distribution of gender was similar regarding the 30-day mortality across these three groups (Table 1). History of arterial symptomatic disease was more prevalent in patients who died from spontaneous PE (Table 1). Both simplified Pulmonary Embolism Severity Index (sPESI) score and mortality score stratified patients as it was expected, and 30-day mortality rate were much higher in patients with sPESI > 0 comparing to sPESI 0, and increased significantly from the low, intermediate-low, intermediate-high to high-risk PE patients in all three groups (Table 1).

BNP and cTnI represent a part of risk stratification algorithm for intermediate-high risk or intermediate-low risk patients and indicate treatment strategies. Comparison of blood levels of biomarker between the deceased from any cause and patients who survived the 30-day period showed that CRP and BNP levels were significantly higher in deceased patients in all three groups and cTnI level only in spontaneous PE patients (Table 2). D-dimer levels were not different between the deceased and survivors in neither group (Table 2).

ROC curve analyses demonstrated that BNP had the highest prognostic accuracy for 30-day mortality in patients (n = 219) who had all examined biomarkers. BNP provided an AUC of 0.785 ($p < 0.001$). Besides BNP, D-Dimer had modest predictive power with an AUC=0.703 and *p*-value of 0.002. CRP showed an AUC of 0.672 ($p = 0.008$). The AUC for cTnI was 0.631, but it was not statistically significant ($p = 0.063$) (Figure 1).

In general, BNP separately as a continuous variable had the highest c-statistic for all three groups of patients, but with a modest predictive value for 30-day all-cause mortality in patients with persistent provoked PE with an AUC of 0.699 ($p = 0.025$). C-reactive protein had solid predictive value for the 30-day all-cause mortality in the group with transient provoked PE, and it was less appreciated in spontaneous and persistent provoked PE. cTnI had a very modest predictive value for the 30-day all-cause mortality only in patients with spontaneous PE, and D-dimer was a very weak predictor of this endpoint only in patients with persistent provoked PE.

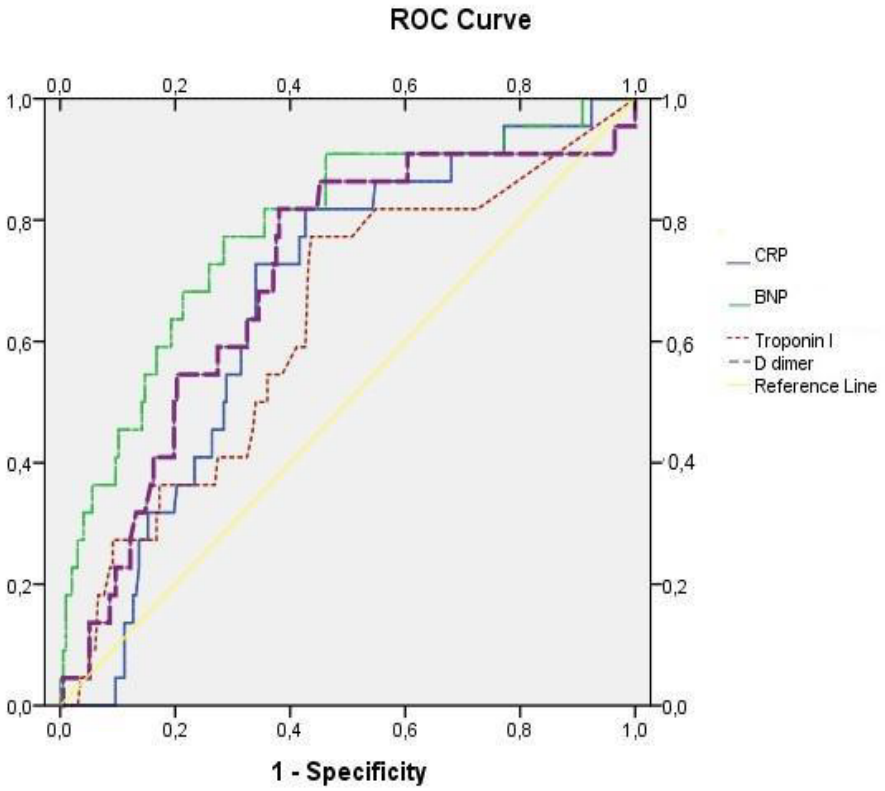


Fig. 1 – Receiver operating characteristic (ROC) curve analysis for assessing the capacity of biomarkers to predict 30-day mortality.
CRP – C-reactive protein; BNP – B-type natriuretic peptide.

Table 2

Association of biomarkers with outcomes. Statistical comparison of study parameters in pulmonary embolism (PE) subgroups with (P) vs without (N) adverse outcome

Parameters	All patients			Unprovoked PE			Transient provoked PE			Persistent provoked PE		
	yes	no	*p*	yes	no	*p*	yes	no	*p*	yes	no	*p*
CRP (mg/L)	n = 67 93.9 (57.0–164.3)	n = 506 43.5 (15.8–100.0)	< 0.001	n = 27 78.1 (50.5–145.8)	n = 261 28.0 (12.2-76.8)	0.001	n = 15 111.0 (77.0-240.0)	n = 138 60.6 (24.7-133.8)	0.004	n = 25 99.0 (37.5-175.6)	n = 107 52.0 (20.1-100.0)	0.009
BNP (ng/L)	n = 31 422.0 (161.6–970.0)	n = 284 117.0 (44.0–315.0)	< 0.001	n = 10 696,0 (129.4-1424.5)	n = 157 130,0 (45.6-323.5)	0.011	n = 7 413.6 (267.6-512.0)	n = 80 108.8 (40.5-303.5)	0.008	n = 14 426.5 (147.2-935.2)	n = 47 107.0 (49.2-351.0)	0.025
cTnI (µg/L)	n = 49 0.13 (0.04–0.88)	n = 323 0.05 (0.01–0.29)	0.001	n = 22 0.15 (0.08–0.72)	n = 195 0.05 (0.01–0.30)	0.021	n = 12 0.09 (0.02-0.81)	n = 78 0.04 (0.00-0.28)	0.168	n = 15 0.18 (0.04-1.10)	n = 50 0.05 (0.00-0.16)	0.092
D-dimer (mg/lFEU)	n = 73 6.9 (3.5–16.1)	n = 473 5.2 (2.5–10.0)	0.032	n = 29 5.4 (3.5-9.7)	n = 248 4.5 (2.3-9.5)	0.298	n = 16 7.4 (2.4-19.0)	n = 124 5.9 (3.3-10.6)	0.500	n = 28 8.8 (3.5-22.3)	n = 101 5.1 (2.2-9.9)	0.050

Note: Data are presented as a median, 25th – 75th percentile.

CRP – C-reactive protein; BNP – B-type natriuretic peptide; cTnI – cardiac troponin1.

Table 3

Receiver operating characteristic (ROC) Analysis for biomarkers predicting adverse outcomes in pulmonary embolism (PE) subgroups

Parameters	Unprovoked PE				Transient provoked PE				Persistent provoked PE			
	n	AUC	95% CI	*p*	n	AUC	95% CI	*p*	n	AUC	95% CI	*p*
CRP (mg/L)	288	0.695	0.589–0.801	0.001	153	0.728	0.620–0.835	0.004	132	0.669	0.552–0.785	0.009
BNP (ng/L)	167	0.741	0.553–0.929	0.011	87	0.804	0.696–0.911	0.008	61	0.699	0.544–0.854	0.025
Troponin-I (µg/L)	217	0.650	0.533–0.768	0.021	90	0.623	0.450–0.795	0.172	65	0.643	0.487–0.800	0.094
D- dimer (mg/L FEU)	277	0.559	0.458–0.660	0.298	140	0.552	0.381–0.723	0.500	129	0.621	0.493–0.749	0.050
CRP (mg/L)*	122	0.750	0.564–0.936	0.018	61	0.584	0.424–0.745	0.535	35	0.564	0.337–0.791	0.571
BNP (ng/L)*		0.701	0.477–0.924	0.058		0.835	0.734–0.936	0.014		0.799	0.622–0.977	0.008
Troponin-I (µg/L)*		0.578	0.354–0.803	0.460		0.635	0.386–0.884	0.320		0.705	0.513–0.897	0.070
D-dimer (mg/L FEU)*		0.672	0.480–0.865	0.104		0.626	0.325–0.927	0.352		0.761	0.593–0.928	0.021

***Patients who had all biomarkers.**

AUC – area under the curve; CI – confidence interval.

For other abbreviations see under Table 2.

Since not all patients had measured values of all markers, we performed a c-statistic analysis marker by marker (Table 3) and a combination of each marker with BNP (Table 4).

In the c-statistic analysis with a combination of markers and BNP, the CRP had a comparable, high c-statistic as BNP in spontaneous and transient provoked PE. However, both markers were not doing well for the prediction of 30-day all-cause mortality in patients with persistent provoked PE with C-indices below 0.700. On the other hand, cTnI in combination with BNP was significantly weaker from BNP for the prediction of 30-day all-cause mortality in patients with spontaneous and transient provoked PE, but much better, yet significantly less respectable as BNP for persistent provoked PE with an AUC of 0.712. D-dimer in combination with BNP also had a very low predictive value for 30-day all-cause mortality, but slightly better but not comparable with BNP in patients with transient and provoked PE.

In the previous study [12], it was demonstrated that none of these markers (CRP, cTnI, D-Dimer) added to BNP (adjusted to gender, age, and glomerular filtration rate calculated with Cockcroft-Gault formula) improved Cox regression prediction models for 30-day PE-related mortality.

BNP had a high predictive value for 30-day all-cause mortality in all three groups of patients, which means that heart failure with its surrogate BNP was an important predictive factor for death regardless of the cause of PE. There are a lot of studies that showed that BNP is a good predictor for short-term mortality in patients with PE regardless of the presence of left ventricle performance. However, our study showed that BNP is a good predictor for short-term mortality, especially in patients with spontaneous PE and PE with transient provoked factor, but less good in patients with provoked PE who had a persistent factor. The possible reason for that is that the cause of death in this group was more often the consequence of other reasons and not PE itself.

Table 4

Comparison of B-type natriuretic peptide (BNP) and its combination with other biomarkers

Parameters	Unprovoked PE				Transient provoked PE				Persistent provoked PE			
	n	AUC	95% CI	*p*	n	AUC	95% CI	*p*	n	AUC	95% CI	*p*
BNP												
BNP (ng/L)	167	0.741	0.553–0.929	0.011	87	0.804	0.696–0.911	0.008	61	0.699	0.544–0.854	0.025
30-day mortality (n, %)			10 (6.0)				7 (8.0)				14 (23.0)	
BNP + CRP												
BNP (ng/L)	162	0.744	0.558–0.930	0.010	86	0.801	0.693–0.910	0.009	60	0.693	0.535–0.850	0.030
CRP (mg/L)		0.772	0.621–0.923	0.004		0.728	0.573–0.883	0.047		0.596	0.435–0.758	0.297
30-day mortality (n, %)			10 (6.2)				7 (8.1)				14 (23.3)	
BNP + cTnI												
BNP (ng/L)	128	0.692	0.464–0.919	0.070	63	0.834	0.732–0.937	0.014	36	0.790	0.612–0.968	0.010
Troponin-I (μg/L)		0.578	0.352–0.804	0.463		0.631	0.380–0.882	0.334		0.712	0.522–0.902	0.060
30-day mortality (n, %)			8 (6.3)				5 (7.9)				9 (25.0)	
BNP + D-dimer												
BNP (ng/L)	164	0.743	0.555–0.930	0.010	83	0.805	0.700–0.909	0.008	57	0.722	0.560–0.884	0.016
D-dimer (mg/L FEU)		0.595	0.401–0.789	0.316		0.706	0.468–0.943	0.073		0.688	0.524–0.852	0.041
30-day mortality (n, %)			10 (6.1)				7 (8.4)				13 (22.8)	

For abbreviations see under Tables 2 and 3.

Discussion

Since PE includes various diseases and conditions behind heterogeneous pathophysiology, it is logically that different biochemical markers have different prognostic values for mortality depending on that. Besides that, the cause of PE-related mortality can be divided into at least three major groups, mortality caused by PE itself, mortality due to comorbidities, and hemorrhage complications. This study showed that several biomarkers had very different values for the prediction of 30-day all-cause death regarding the main cause of PE. In general, BNP had a solid predictive value for 30-day all-cause mortality regardless of the cause of PE. The serum concentration of CRP had a good predictive value for 30-day all-cause mortality in patients with spontaneous and transient provoked PE. Cardiac troponin I and D-dimer levels had only modest but significantly lower predictive value for 30-day all-cause mortality compared to BNP in both provoked groups of PE. Thus, the value of biomarkers for the prediction of 30-day all-cause mortality very much depends on whether the patient had spontaneous, provoked PE with transient or persistent factors.

CRP level also had a good predictive value for early all-cause mortality, especially in patients with spontaneous PE. Several inflammatory markers are elevated in patients with acute PE even when febrile state or pulmonary consolidation on computed tomography (CT) is not present. CRP, as a well-known marker of inflammation, has a good predictive value for mortality and bleeding complications which can also contribute to death. Since causes of death, other than PE, were presented more often in patients with provoked PE, CRP was not such a good predictor for early mortality in those patients.

According to previous reports, D-dimer was not a good indicator of PE prognosis and severity [13, 14], although the results of D-dimer have had general application in excluding PE due to its high negative predictive value [15]. In the present study, plasma D-dimer levels were increased in all subgroups of patients regardless of the causality. However, a significant difference was observed only between survivors and 30-day mortality for all cohorts of PE patients. In our study, D-dimer had a good predictive value for 30-day all-cause mortality only in the subgroup of patients with persistent provoked PE.

This result might be partly explained as active malignant disease, autoimmune diseases, and other severe comorbidities predominate in this subgroup of patients. Moreover, it is well-known that in these cohorts of patients, D-dimer *per* se is the predictive factor for mortality [16–18].

There are a lot of assays for cardiac troponins with various sensitivity and likelihood ratios making usefulness for cardiac troponins in the prediction of outcome complicated for clinical practice [19]. In our study, the cTnI assay had a relatively weak prognostic value for 30-day all-cause mortality only in the subgroup of patients with spontaneous PE when it was used as a solo marker. As we mentioned before, in this group of patients, PE-related death was the most prominent cause of death, and the myocardial necrosis during the stretching of the right ventricle (RV) was the mechanism of raised troponin in this subgroup. Groups with provoked PE had predominantly other pathological mechanisms of death with a lower role of RV failure and a smaller amount of release of cardiac troponin.

The main limitation of this study was that a considerable number of patients did not have all tested biomarkers. However, according to the European Society of Cardiology (ESC) guidelines, patients with low and intermediate-low risk of PE do not require results of all these biomarkers.

Conclusion

Overall, this study showed that the cause of PE very much influences the predictive power of biomarkers for early PE-related mortality. Brain natriuretic peptide is the probable biomarker that is the most resistant to different influences because heart failure, surreally presented through BNP blood level, is the final process firmly associated with death outcome. C-reactive protein and cardiac troponin I blood concentrations were better markers for the prediction of early death in patients with spontaneous PE than in provoked PE. Although D-dimer had predictive value for early mortality in the whole group of PE patients, it had poor predictive value in patients with spontaneous PE and PE provoked with transient factor. However, it also had a modest predictive value for PE patients with persistent provoked PE.

REFERENCES

1. *Konstantinides SV, Barco S, Lankeit M, Meyer G.* Management of Pulmonary Embolism: An Update. J Am Coll Cardiol 2016; 67(8): 976–90.
2. *Pernod G, Caterino J, Maignan M, Tissier C, Kassis J, Lazarchick J.* DIET study group. D-Dimer Use and Pulmonary Embolism Diagnosis in Emergency Units: Why Is There Such a Difference in Pulmonary Embolism Prevalence between the United States of America and Countries Outside USA? PLoS One 2017; 12(1): e0169268.
3. *Dudzinski DM, Giri J, Rosenfield K.* Interventional Treatment of Pulmonary Embolism.Circ Cardiovasc Interv 2017; 10(2): pii: e004345.
4. *Hendriksen JM, Lucassen WA, Erkens PM, Stoffers HE, van Weert HC, Büller HR*, et al. Ruling Out Pulmonary Embolism in Primary Care: Comparison of the Diagnostic Performance of "Gestalt" and the Wells Rule. Ann Fam Med 2016; 14(3): 227–34.
5. *Jaber WA, Fong PP, Weisz G, Lattouf O, Jenkins J, Rosenfield K*, et al. Acute Pulmonary Embolism: With an Emphasis on an Interventional Approach. J Am Coll Cardiol 2016; 67(8): 991–1002.
6. *Konstantinides SV, Torbicki A, Agnelli G, Danchin N, Fitzmaurice D, Galiè N*, et al. 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism.Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). Eur Heart J 2014; 35(43): 3033–69, 3069a–69k.
7. *Kunutsor SK, Seidu S, Blom AW, Khunti K, Laukkanen JA.* Serum C-reactive protein increases the risk of venous thromboembolism: a prospective study and meta-analysis of published prospective evidence. Eur J Epidemiol 2017; 32(8): 657–67.
8. *Meyer G, Planquette B, Sanchez O.* Risk stratification of pulmonary embolism: clinical evaluation, biomarkers or both? Eur Respir J 2015; 46(6): 1551–3.
9. *Wang Q, Ma J, Jiang Z, Ming L.* Prognostic value of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in acutepulmonary embolism: a systematic review and meta-analysis. Int Angiol 2018; 37(1): 4–11.
10. *Gjonbrataj E, Kim JN, Gjonbrataj J, Jung HI, Kim HJ, Choi WI.* Risk factors associated with provoked pulmonary embolism. Korean J Intern Med 2017; 32(1): 95–101.
11. *Donnellan E, Khorana AA.* Cancer and Venous Thromboembolic Disease: A Review. Oncologist 2017; 22(2): 199–207.
12. *Jovanovic L, Subota V, Stavric M, Subotic B, Dzudovic B, Novicic N*, et al. Biomarkers for the prediction of early pulmonary embolism related mortality in spontaneous and provoked thrombotic disease. Clin Chim Acta 2019; 492: 78–83.
13. *Tanabe Y, Obayashi T, Yamamoto T, Takayama M, Nagao K.* Predictive value of biomarkers for the prognosis of acute pulmonary embolism in Japanese patients: Results of the Tokyo CCU Network registry. J Cardiol 2015; 66(6): 460–5.
14. *Ay MO, Kozaci N, Avci M, Cekic B, Cerit N, Keskin O*, et al. Utility of biochemical markers and RVD/LVD ratio in acute pulmonary embolism risk classification in Emergency Department. Eur Rev Med Pharmacol Sci 2017; 21(19): 4391–7.
15. *Expert Group on Biomarkers.* Biomarkers in Cardiology - Part 2: In Coronary Heart Disease, Valve Disease and Special Situations. Arq Bras Cardiol 2015; 104(5): 337–46.
16. *Li W, Tang Y, Song Y, Chen SH, Sisliyan N, Ni M*, et al. Prognostic Role of Pretreatment Plasma D-Dimer in Patients with Solid Tumors: a Systematic Review and Meta-Analysis. Cell Physiol Biochem 2018; 45(4): 1663–76.
17. *Zhang J, Guo Z, Yang W, Zhu Z, Kong W, Zheng S*, et al. D-dimer levels are correlated with disease activity in Chron's patients. Oncotarget 2017; 8(38): 63971–7.
18. *Liang Y, Xie SB, Wu CH, Hu Y, Zhang Q, Li S*, et al. Coagulation cascade and complement system in systemic lupus erythematosus. Oncotarget 2018; 9(19): 14862–81.
19. *Bajaj A, Rathor P, Sehgal V, Kabak B, Shetty A, Al Masalmeh O*, et al. Prognostic value of biomarkers in acute non-massive pulmonary embolism: a systemic review and meta-analysis. Lung 2015; 193(5): 639–51.

Received on Fabruary 26, 2019.
Revised on July 29, 2019.
Accepted on September 12, 2019.
Online First October, 2019.

ORIGINAL ARTICLE

UDC: 615.464::616.31
DOI: https://doi.org/10.2298/VSP161208034Z

Survival rate of ceramic inlay and onlay restorations in posterior teeth with one-surface or multi-surface after a 10-year follow-up: A systematic review and meta-analysis

Stopa trajanja keramičkih inlej i onlej restaurativnih nadoknada sa jednom ili više površina u bočnim zubima posle 10-godišnjeg praćenja: sistematski pregled i meta analiza

Yun Zou, Jing Bai, Jing Zhou Xiang

Ansteel Group Hospital, Department of Stomatology, Anshan, China

Abstract

Background/Aim. A large number of studies have been conducted on the survival rate of ceramic single tooth restorations, but few studies have discussed the influence of the number of restoration surfaces on their survival rate. This study aimed to evaluate the survival rate of ceramic inlay and onlay restorations in posterior teeth with one-surface or multi-surface after a 10-year follow-up. **Methods**. PubMed, Web of Science, Cochrane Library, Embase, and Wanfang databases were searched for articles published by July 31, 2016. Randomized controlled trials and non-randomized trials were collected and patients with posterior teeth defect were included. Publication bias and sensitivity analysis were also assessed. **Results**. Five studies comprising 6,720 cases were included in this meta-analysis. The results indicated that the survival rate of ceramic inlay and onlay restorations with two-surface was significantly higher than that of one-surface restorations (within 10 years) [*hazard ratio* (HR) = 2.11; 95% *confidence interval* (CI) = 1.33–3.36, $p = 0.002$], and the survival rate of three-surface restorations was higher than that of two-surface ones (HR = 2.50; 95% CI = 1.36–4.59, $p = 0.003$). **Conclusion.** The current meta-analysis shows that the increase in the ceramic inlay and onlay restoration surfaces increases their survival rate within a 10-year period.

Key words:
denture, partial, fixed; meta- analysis as topic; survival rate

Apstrakt

Uvod/Cilj. Velik broj studija bavio se ispitivanjem veka trajanja keramačkih restaurativnih nadoknada na pojedinačnim zubima, dok je manji broj njih proučavao uticaj broja površina tih nadoknada na njihov vek trajanja. Cilj ove studije bio je da proceni stopu trajanja keramičkih inlej i onlej restaurativnih nadoknada sa jednom ili više površina u bočnim zubima posle 10-godišnjeg praćenja. **Metode.** Pretražene su baze PubMed, Web of Science, Cochrane Library, Embase i Wanfang radi pronalaženja radova objavljenih do 31. jula 2016. godine. Prikupljeni su radovi o randomizovanim kontrolisanim kliničkim ispitivanjima i nerandomizovanim kliničkim ispitivanjima, u koja su bili uključeni pacijenti sa oštećenjem bočnih zuba. Takođe, bila je procenjena pristrasnost i izvršena analiza osetljivosti u ovim publikacijama. **Rezultati.** U ovu meta-analizu bilo je uključeno pet studija sa 6 720 pacijenata. Rezultati su pokazali da je tokom desetogodišnjeg praćenja vek trajanja inlej i onlej keramičkih restaurativnih nadoknada sa dvostrukom površinom bio znatno duži od onih sa jednom površinom [*hazard ratio* (HR) = 2,11; 95% *confidence interval* (CI): 1,33–3,36, $p = 0,002$], kao i da je vek trajanja nadoknada sa tri površine bio duži od onih sa dve površine (HR = 2,50, 95% CI: 1,36–4,59, $p = 0,003$). **Zaključak.** Ova meta-analiza pokazuje da povećanje površina keramičkih inlej i onlej restaurativnih nadoknada produžava njihov vek trajanja tokom perioda od 10 godina.

Ključne reči:
zub, trajne nadoknade; meta analiza; preživljavanje, stepen

Introduction

As a result of patients' increasing demand for highly esthetic restorations, issues concerning the use of composite resins for large restorations in posterior teeth, and discussions regarding possible side effects of dental amalgam have increased indications for tooth-color partial-coverage restorations to restore posterior teeth [1–7]. As an

Correspondence to: Jing Zhou Xiang, Ansteel Group Hospital, Department of Stomatology, 3 JiangShen Street, Tiedong District, Anshan 114000, China. E-mail: xiangjingzhou11@163.com

alternative to direct partial restorations, indirect restorations were more widely used because they could provide more control over shape and function, particularly in larger defects in posterior teeth. Numerous materials are currently available for making indirect partial restorations [8–9], but the mechanical strength must be taken into account in posterior applications. With the development of adhesive technologies and escalation in aesthetic demands, it is likely that most indirect restorations are currently made from ceramic materials.

Indirect ceramic restorations can be made either by a dental technician in the laboratory or by using computer aided design/computer aided manufacturing (CAD/CAM) systems to make chairside restorations in a single session. The procedure of placing indirect inlay restorations includes many steps and a wide variation of ceramic materials and luting cements that can be used. Clinical studies on the success of stress-bearing all-ceramic inlays in permanent posterior teeth have already identified that the longevity of dental restorations is dependent on many different factors, including material-, patient- and dentist-related factors. Amounts of factors related to the materials, such as ceramic properties or characteristics of the adhesive luting technique, have been investigated extensively *in vitro* [5, 10–13]. Clinical studies with limited sample size also have shown the influence of factors related to patients and operators on the clinical outcome of ceramic inlays [13–16]. However, there is a lack of clinical studies analyzing the role of surfaces risk factors on restoration longevity and performance.

This systematic review and meta-analysis aimed to evaluate the difference in longevity of ceramic inlay restorations with one-surface or multi-surface after 10-year follow-up associated with the main clinical outcomes reported in randomized controlled trials (RCTs) and retrospective studies.

Methods

Information sources

We searched the following databases for articles published between 1983 and 2016 that reported on survival of ceramic inlay restorations: PubMed, Web of Science, Cochrane Library, Embase, and Wanfang (by July 31, 2016). References of the included articles were further checked manually. We selected the year 1983 as the starting point because adhesive procedures for ceramics with the use of hydrofluoric acid and silanization were first introduced in that year [17].

Search strategy

PubMed, Web of Science, Cochrane Library, Embase, and Wanfang databases were searched for articles with broad key terms, such as “ceramic,” “Cerec”, “inlay,” “onlay”, “survival,” and “long-term”. The search strategy was carried out using the retrieval type as following: (“ceramic” OR “Cerec”) AND (“survival” OR “long-term”) AND (“inlay” OR “onlay”). In addition, we searched all these databases to avoid missing relevant studies published before July 31, 2014. Only studies published in English and Chinese were included. Manual search of reference lists of retrieved articles was also performed.

Study selection and eligibility criteria

All titles and abstracts of the selected studies were first assessed for the following inclusion criteria: clinical studies related only to all-ceramic inlays in human posterior teeth and those with clinical follow-up (prospective studies, retrospective studies, or RCTs). The full text was evaluated for articles without abstracts or for abstracts with an insufficient description. After evaluating the full text of the articles according to the previously defined exclusion criteria, articles with the following features, without language restrictions, were considered ineligible: 1) articles without a description of the procedure or those in which uncommon preparations had been performed (e.g, bridge abutments, splinting, uncommon bonding procedures, occlusal coverage of posterior teeth without preparation, or implant abutments or restorations including metal); 2) case reports; 3) literature or systematic reviews, protocols, interviews, and *in vitro* studies; 4) studies conducted in isolated groups (bruxism, hypoplasia, others); 5) studies with the same sample (the most recent and/or most complete was considered); 6) studies without a survival analysis or those with incomplete data for the analysis; 7) studies with a dropout rate higher than 30%; and 8) studies with a follow-up shorter than 10 years.

Data extraction

We extracted information from the studies (that had been collected based on the aforementioned criteria), such as author names, publication year, volume and issue; article design; number of cases and placebos, efficacy and safety assessment. Yun Zou and Jing Bai independently checked the data from all the included studies. Subsequently, a third reviewer (Jingzhou Xiang) discussed inconsistent evaluations and thereby helped to reach a final agreement.

Quality assessment of the included studies

The quality of all the included studies was assessed according to the Newcastle-Ottawa quality Assessment Scale [18] independently by 2 reviewers (Yun Zou, Jing Bai). Disagreements were resolved by another reviewer (JingZhou Xiang). The Newcastle-Ottawa Quality Assessment Scale falls into three categories, including Selection, Comparability, and Outcome. The categories Selection and Outcome have four and three items, respectively. The category Comparability has only one item. When a study is assessed item by item, it is awarded a maximum of one star (★) for each item within the Selection and Outcome categories. A maximum of two stars can be awarded for the

Comparability category. Generally, the study which was awarded more than five stars in total was considered to be included in this meta-analysis.

Statistical analysis

The strength of association between one-surface and multi-surface was estimated by hazard ratio (HR) value and 95% confidence interval (CI). A meta-analysis of surfaces risk factors on restoration longevity and performance, were performed using Review Manager Version 5.3 software (provided by the Cochrane Collaboration) to obtain a HR. *Z*-test determined the significance of the pooled HR and $p < 0.05$ was considered as statistically significant. Heterogeneity of the studies was assessed using the Cochrane Q and I^2 statistic [19], which represents the percentage of total variation among studies that is attributed to heterogeneity rather than chance [20]. Both fixed-effects and random-effects models were used: if the I^2 test < 50% or $p \geq 0.05$ (Q-test), we used the fixed-effects model; if there was significant heterogeneity among the included studies (I^2 test > 50%), the random-effects model was employed.

Egger rank correlation tests were used to assess the extent of publication bias. In addition, sensitivity analysis was also performed. Those two procedures were conducted using STATA 11 (Stata, College Station, TX, USA).

Results

Study selection

The search strategies employed yielded 569 studies (Figure 1). The titles and abstracts were screened and 21 studies were excluded at this step. Then, full-text articles were screened against the inclusion criteria. Thus, 5 studies [15, 21–24] comprising 6,720 ceramic inlay restorations were included in our study. We followed the PRISMA guidelines and illustrated the study selection by the PRISMA flow diagram (Figure 1).

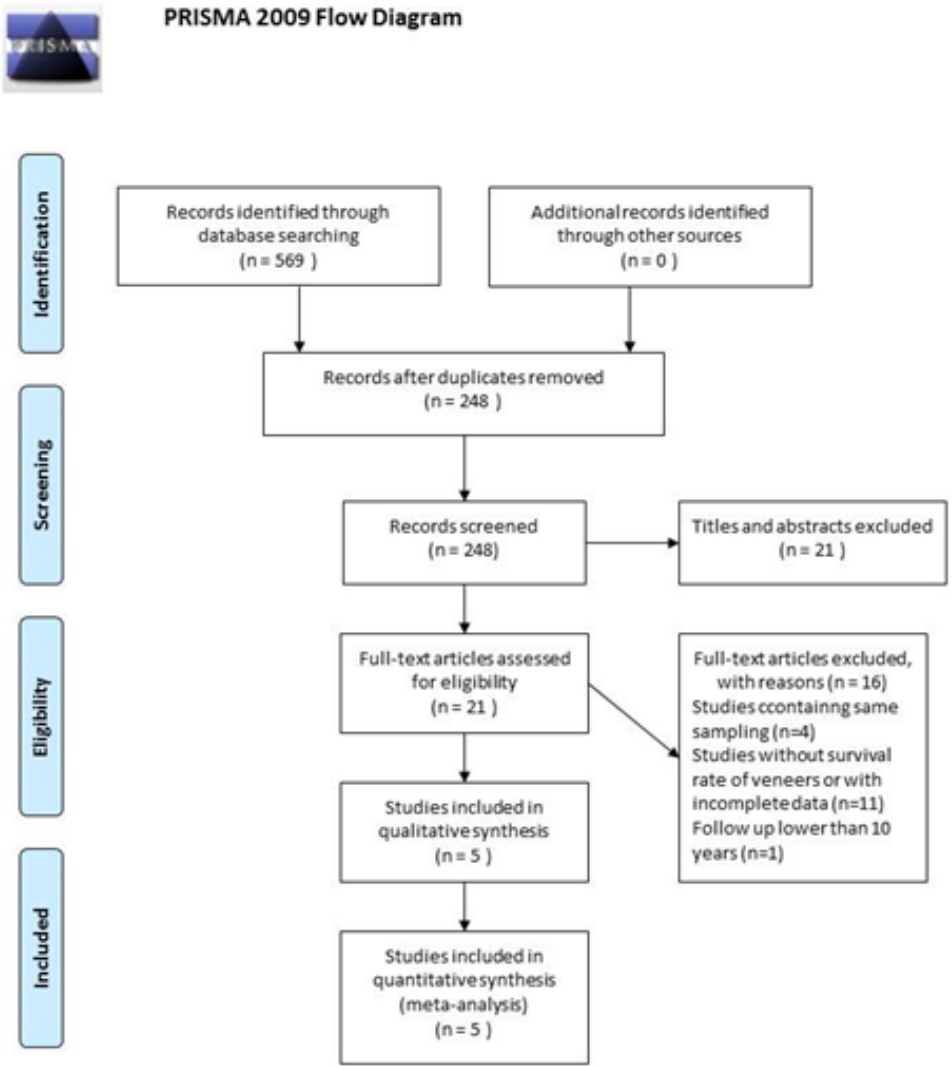


Fig. 1 – PRISMA flow diagram for the study selection process. In this meta-analysis, 5 studies were selected for qualitative analysis.

Table 1

The characteristics of the studies [15, 21–24] included in this meta-analysis

First author	Year of publication	Country	Type of study	Follow-up (years)	Material	Number.of restored surfaces	Evaluation criteria
Reiss [22]	2000	Germany	Retrospective cohort	10	Feldspathic porcelain	32 one-surface 344 two-surface 519 three-surface	USPHS
Otto [24]	2002	Switzerland	Retrospective cohort	10	Feldspathic porcelain	23 one-surface 67 two-surface 85 three-surface	USPHS
Stoll [15]	2007	Germany	Retrospective cohort	10	Glass-ceramic	304 one-surface 754 two-surface 438 three-surface	NS
Beier [21]	2012	Austria	Retrospective cohort	20	Glass-ceramic	38 one-surface 141 two-surface 155 three-surface	CDA
Collares [23]	2016	Brazil	randomized controlled trial	10	Ceramic	205 one-surface 1359 two-surface 2256 three-surface	NS

All studies were published in English.
CDA – California Dental Association; USPHS – United States Public Health Service; NS – not specified.

Table 2

Results of literature [15, 21–24] quality assessment according to the Newcastle-Ottawa quality Assessment Scale

First author, year of publication	Selection	Comparability	Outcome
Reiss, 2000 [22]	★★★★	★★	★★
Otto, 2002 [24]	★★★★	★★	★★
Stoll, 2007 [15]	★★★★	★★	★★
Beier, 2012 [21]	★★★★	★★	★★
Collares, 2016 [23]	★★★	★★	★★★

Study characteristics

The characteristics of the included studies are presented in Table 1. The selected articles were published from 2000 to 2016. The 5 aforementioned studies included 6,720 ceramic inlay restorations characterized by one-surface, two-surface and three-surface. The experiment group included ceramic inlays with one-surface, and the control group included ceramic inlays with multi-surface.

All the included studies were marked by more than five stars on quality assessment (Table 2). These studies all illustrated explicit diagnostic criteria, good comparability between subgroups, and clear results.

Meta-analysis

The substantial heterogeneity was described with an I^2 value of 0%; thus, fixed effects models were used, showing that the survival of one-surface was significantly different from that of two-surface (95%CI:1.33,3.36 $p = 0.002$) (Figure 2). Because the I^2 value was 0% ($I^2 = 0\%$; $p = 0.41$), the data extracted were those obtained by the fixed effects

model showing a significant difference between one-surface and three-surface (95%CI: 1.36, 4.59 $p = 0.003$) (Figure 3).

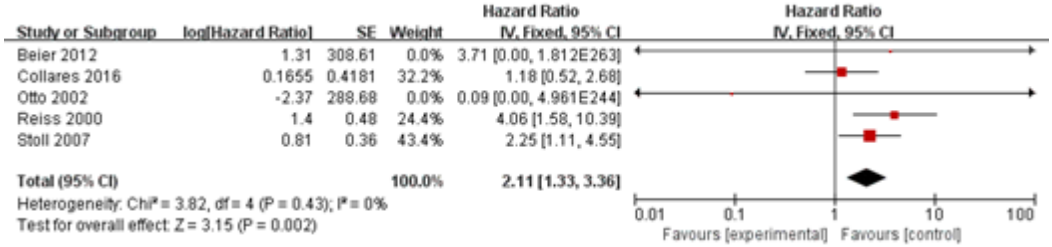


Fig. 2 – Forest plot of inlays, one-surface vs. two-surface.
CI – confidence interval; SE – standard error.

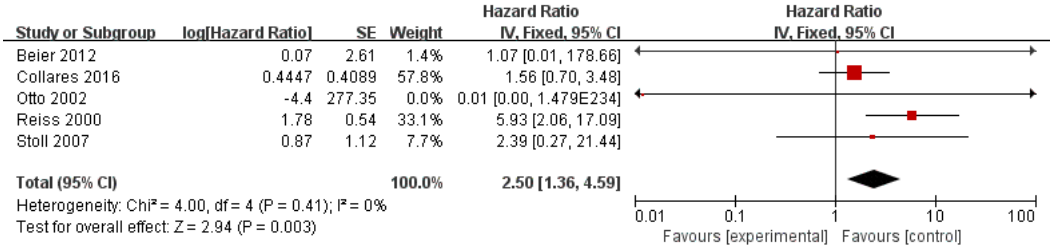


Fig. 3 – Forest plot of inlays, one-surface vs. three-surface.
For abbreviations see under Fig. 2.

Publication

The Egger rank correlation tests showed that there was no publication bias in these two meta-analyses ($p = 0.937$, $p = 0.968$).

Discussion

According to the present systematic review and meta-analysis, and concerning the outcome, the survival rate of ceramic inlays and onlays increases with the increase in the number of inlay and onlay surfaces. This conclusion was different from the conclusions in the studies included. In general, several factors may be associated with the survival rate of ceramic inlays: the design of the inlays, fabrication methods, bonding procedures, use of composite resin cements, vital or non-vital teeth habit of the participants, and the evaluation criterion of the study.

The descriptions of all-ceramic inlays preparation were presented only in one study [21], which emphasizes the shoulder preparation and occlusal reduction of at least 1.5 mm from the deepest pit in the fossae, because this could improve the fracture resistance strength of all-ceramic restoration. The other four studies did not describe the preparation of inlays, and the influence of the surface on the survival of ceramic inlays could therefore not be evaluated.

Three studies [15, 21, 22] divided the restorations into two groups: vital teeth and non-vital teeth. Restorations on vital teeth showed significantly fewer failures compared to restorations on non-vital teeth during the 10-year follow-up in all these studies. However, the samples of vital teeth were obviously much larger than those of non-vital teeth, and items were not subgrouped by surface. The evidence was therefore insufficient to indicate whether vital teeth may or may not be the factors in the survival rate of ceramic inlays with one-surface or multi-surface.

The restorations were polished in one follow-up study [15], while an *in-vitro* study showed that the ceramic polishing with rotating instruments may creating microcracks in the marginal zone. Hence, whether or not to apply polishing is still debatable. Besides, none of the studies involved proved that polishing during the treatment has an effect on the survival rate of the ceramic inlays surface. Each restoration received dual-cured resin composite at the time of treatment. A 10-year prospective study compared the performance of inlays cemented with a chemically cured and dual-cured resin composite. After 10 years of clinical service, the inlays luted with chemically cured resin composite had a higher survival rate (89%) compared to dual-cured resin composite (77%). Three studies [21–23] mentioned using dentin bonding, and one of the studies [21] showed that although more than half of the failures occurred in restorations with no dentin bonding, the differences were not significant. The surface survival rate of multi-surface was lower than that of one-surface. Reiss and Walther [22] suggested that the risk of failure was significantly reduced when a dentin adhesive was applied. It was also confirmed that the survival rate of multi-surface was higher than that of one-surface. Besides, Hass et al. [25] reported that a survival rate of 95% after 7 years means the dentin adhesive used had no significant influence on the results either. In contract, Posselt and Kerschabaum [26] found significantly higher survival probabilities for inlays incorporated with a dentin

adhesive, but there was no significant evidence to arrive at the conclusion above.

Clelland et al. [27] suggested that application of silanation had a greater effect on improving the strength of the ceramic restoration, particularly if the surface was rough. Two of the studies included in this meta-analysis refer to silanation. In one of the studies [22], all the ceramic inlays were silanized directly after drying before seating, and the outcome indicated that the survival rate of multi-surface was higher than one-surface. In the other studies, 86% of inlays were silanized before placement and the results showed that survival rate of one-surface was higher than multi-surface. However, the current evidence was not sufficient to draw the conclusion on whether silanation has an effect on the survival rate of the surface of inlays. One study [15] showed that the number of surfaces did not influence their longevity.

Restorations in two studies [15, 21] were fabricated at the Department of Operative Dentistry, School of Dental Medicine. All of the patients in the other three studies [22–24] had been recruited from a single private dental office. Trials undertaken in a university hospital environment are normally conducted in accordance with fixed placement and evaluation protocols, in idealized conditions without the restraints of time and available materials. The data obtained in this manner should show the optimum performance of the restorative system. University studies are normally fixed-term studies with a defined placement and evaluation time-scale. The trials undertaken in a dental practice environment tend to be influenced by reduced clinical working-time, variation in the use of luting materials, and usually the inability to adhere to a strict case selection protocol [28]. Therefore, different locations of research could influence the survival rate of the surface of inlays.

Two studies [21, 24] pointed out that the patients who participated in the research were diagnosed with bruxism. Otto and De Nisco [24] confirmed the fact that during the follow-up, two to three patients with multiple failures were diagnosed with bruxism. This may mean that this particular group of patients should be considered a risk group with regard to Cerec restorations. In the clinical study by Beier et al. [21], 33% of all fractured inlays occurred in patients with signs of bruxism, but no significant differences were reported. Therefore, the conclusion regarding whether or not bruxism has an impact on the survival of the surface of inlays could not be drawn.

A large number of studies have been conducted on the survival rate of ceramic single tooth restorations. Different factors were held responsible for the survival of the restorations. Only a few studies discussed whether the number of surfaces has an influence on the survival rate. The meta-analysis consisted of three studies [21, 23, 24] confirmed that the survival rate was decreasing with the surface increased. However, one study [22] showed that the survival rate was increasing with the surface increased. The present study showed that the survival risk was decreasing with the surface increased. The current evidence indicates that the survival rate increased with the increase of the number of inlay surfaces. The clinical dentists should take account of the number of surfaces during treatment.

Several limitations and sources of bias should be considered in this meta-analysis. First, only studies published in English and Chinese were searched in the process of study selection. No evidence of significant publication bias in this study was reflected by the test. Second, few studies reported the survival rate of ceramic inlay surfaces, and the sample used in this meta-analysis is not large enough to perform a subgroup analysis. Therefore, more original studies are needed. Furthermore, the included studies were mostly from Europe. The absence of representative data from other parts of the world may have made the results more prone to potential selection bias.

Conclusion

In conclusion, this meta-analysis indicates that the survival rate increased with the increase in the number of surfaces of inlays and onlays. We suggest the clinical dentists should take into account the influence of the number of surfaces during treatment, and improve the survival rate of the ceramic inlays.

Acknowledgement

This study was approved by the China Medical University Ethical Review Board. We had no source of funding for the study and have no conflicts of interest.

R E F E R E N C E S

1. *Boushell LW, Ritter AV*. Ceramic inlays: A case presentation and lessons learned from the literature. J Esthet Restor Dent 2009; 21(2): 77–87.
2. *Bergman MA*. The clinical performance of ceramic inlays: A review. Aust Dent J 1999; 44(3): 157–68.
3. *Tyas MJ*. Dental amalgam – What are the alternatives? Int Dent J 1994); 44(3): 303–8.
4. *Roulet JF*. Benefits and disadvantages of tooth-coloured alternatives to amalgam. J Dent 1997; 25(6): 459–73.
5. *Manhart J, Chen H, Hamm G, Hickel R*. Buonocore Memorial Lecture. Review of the clinical survival of direct and indirect restorations in posterior teeth of the permanent dentition. Oper Dent 2004; 29(5): 481–508.
6. *El-Mowafy O, Brochu JF*. Longevity and clinical performance of IPS-Empress ceramic restorations – a literature review. J Can Dent Assoc 2002; 68(4): 233–7.
7. *Sjögren G, Lantto R, Granberg A, Sundström BO, Tillberg A*. Clinical examination of leucite-reinforced glass-ceramic crowns (Empress) in general practice: A retrospective study. Int J Prosthodont 1999; 12(2): 122–8.
8. *Thordrup M, Isidor F, Horsted-Bindslev P*. A prospective clinical study of indirect and direct composite and ceramic inlays: ten-year results. Quintessence Int 2006; 37(2): 139–44.
9. *Pol CW, Kalk W*. A systematic review of ceramic inlays in posterior teeth: an update. Int J Prosthodont 2011; 24(6): 566–75.

10. *Frankenberger R, Krämer N, Appelt A, Lohbauer U, Naumann M, Roggendorf MJ*. Chairside vs. labside ceramic inlays: effect of temporary restoration and adhesive luting on enamel cracks and marginal integrity. Dent Mater 2011; 27(9): 892–8.
11. *Magne P, Paranhos MP, Schlichting LH*. Influence of material selection on the risk of inlay fracture during pre-cementation functional occlusal tapping. Dent Mater 2011; 27: 109–13.
12. *Magne P, Knezevic A*. Thickness of CAD-CAM composite resin overlays influences fatigue resistance of endodontically treated premolars. Dent Mater 2009; 25(10): 1264–8.
13. *Frankenberger R, Reinelt C, Petschelt A, Kramer N*. Operator vs. material influence on clinical outcome of bonded ceramic inlays. Dent Mater 2009; 25(8): 960–8.
14. *Schulz P, Johansson A, Arvidson K*. A retrospective study of Mirage ceramic inlays over up to 9 years. Int J Prosthodont 2003; 16(5): 510–4.
15. *Stoll R, Cappel I, Jablonski-Momeni A, Pieper K, Stachniss V*. Survival of inlays and partial crowns made of IPS empress after a 10-year observation period and in relation to various treatment parameters. Oper Dent 2007; 32(6): 556–63.
16. *van Dijken JW, Hasselrot L*. A prospective 15-year evaluation of extensive dentin-enamel-bonded pressed ceramic coverages. Dent Mater 2010; 26(9): 929–39.
17. *Horn HR*. Porcelain laminate veneers bonded to etched enamel. Dent Clin North Am 1983; 27(4): 671–84.
18. *Stang A*. Critical evaluation of the Newcastle-Ottawa scale for assessing the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25(9): 603–5.
19. *Colditz GA, Burdick E, Mosteller F*. Heterogeneity in meta-analysis of data from epidemiologicstudies: a commentary. Am J Epidemiol.1995; 142: 371–82.
20. *Higgins JP, Thompson SG*. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21(11): 1539–58.
21. *Beier US, Kapferer I, Burtscher D, Giesinger JM, Dumfahrt H*. Clinical performance of all-ceramic inlay and onlay restorations in posterior teeth. Int J Prothodont 2012; 25(4): 395–402
22. *Reiss B, Walther W*. Clinical long-term results and 10-year Kaplan-Meier analysis of Cerec restorations. Int J Comput Dent 2000; 3(1): 9–23. (English, German)
23. *Collares K, Corrêa MB, Laske M, Kramer E, Reiss B, Moraes RR*, et al. A practice-based research network on the survival of ceramic inlay/onlay restorations. Dent Mater 2016; 32(5): 687–94.
24. *Otto T, De Nisco S*. Computer-aided direct ceramic restorations: a 10-year prospective clinical study of Cerec CAD/CAM inlays and onlays. Int J Prosthodont 2002; 15(2): 122–8.
25. *Haas M, Arnetzl G, Pertl R, Polansky R, Smetan M*. Cerec vs laboratory Inlays. In: *Mormann W*, editor. CAD/CIM in Aesthetic Dentistry. Chicago: Quintessence; 1996. p. 299–312.
26. *Posselt A1, Kerschbaum T*. Longevity of 2328 chairside Cerec inlays and onlays. Int J Comput Dent 2003; 6(3): 231–48.
27. *Clelland NL, Warchol N, Kerby RE, Katsube N, Seghi RR*. Influence of interface surface conditions on indentation failure of simulated bonded ceramic onlays. Dent Mater 2006; 22(2): 99–106.
28. *Martin N, Jedynakiewicz NM*. Clinical performance of CEREC ceramic inlays: a systematic review. Dent Mater 1999; 15(1): 54–61.

Received on December 8, 2016.
Accepted on March 26, 2021.
Online First March, 2021.

SHORT COMMUNICATION

UDC: 616.831-089
DOI: https://doi.org/10.2298/VSP190611093L

Sitting position awake craniostomy with drainage for chronic subdural hematoma: a viable alternative?

Kraniostomija sa drenažom u lokalnoj anesteziji i sedećem položaju za lečenje hroničnog subduralnog hematoma

Milan Lepić*†, Stefan Mandić-Rajčević‡, Goran Pavlićević†§, Andrija Savić*‖, Aleksandra Lokaj§, Lukas Rasulić*‖

University of Belgrade, *Faculty of Medicine, ‡Innovation Centre of Faculty of Technology and Metallurgy, Belgrade, Serbia; †Military Medical Academy, Clinic for Neurosurgery, Belgrade, Serbia; §University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ‖Clinical Center of Serbia, Clinic for Neurosurgery, Belgrade, Serbia

Abstract

Background/Aim. Chronic subdural hematoma (CSDH) is one of the most frequent neurosurgical conditions with an overall incidence ranging from 1.72 to 20.6 per 100,000 persons per year. The surgical procedure for CSDH is relatively simple and usually performed in the supine position. Reported reoccurrence rates range from 11.7% to 28%. Postperative pneumocephalus was previously identified as a sole predictor of reoccurrence. The aim of this study was to assess the advantage of the procedure in the sitting position in patients with CSDH and a possible impact on the reoccurrence rate. **Methods.** The study included 31 patients who underwent awake craniostomy with closed system drainage for CSDH (16 in supine and 15 in sitting position) in our department from December 2016 to March 2018. **Results.** A total of 22 males and 9 females were included in the study. The overall reoccurrence rate was 19% (22% and 18% in females and males, respectively). The reoccurrence was noted in 5 patients who had undergone surgery in the supine position, and in one case in the sitting position. Our results revealed a lower reoccurrence rate in patients undergoing surgery in the sitting position, although not reaching statistical significance [odds ratio (OR): 0.18, 95% confidence interval: 0.01–1.42, $p = 0.172$]. **Conclusion.** Craniostomy in the sitting position under local anesthesia is a safe, simple, and reliable procedure for CSDH treatment. Besides being very comfortable for the patient, according to our initial results, it might also lead to a lower reoccurrence rate, probably due to the better management of the air inflow, and consequent pneumocephalus.

Key words:
hematoma, subdural; neurosurgical procedures; posture; recurrence; sitting position; treatment outcome.

Apstrakt

Uvod/Cilj. Hronični subduralni hematom (HSH) je jedan od najčešćih neurohirurških entiteta sa ukupnom incidencom javljanja od 1,72 do 20,6 na 100 000 osoba godišnje. Hirurška procedura za lečenje HSH je relativno jednostavna i obično se izvodi u ležećem položaju. Stopa recidiva iznosi od 11,7% do 28%. Postoperativni pneumocefalus se smatra nezavisnim pojedinačnim prediktorom recidiva. Cilj rada bio je da se proceni prednost procedure u sedećem položaju u lečenju bolesnika sa HSH, kao i njen eventualnim uticaj na stopu recidiva. **Metode.** Studija je obuhvatila 31 bolesnika kojima je urađena kraniostomija sa zatvorenim sistemom za drenažu HSH pod lokalnom anestezijom (16 u ležećem položaju na leđima i 15 u sedećem položaju), u periodu od decembra 2016. do maja 2018. godine. **Rezultati.** Studijom su obuhvaćena 22 muškarca i 9 žena. Ukupna stopa recidiva iznosila je 19% (22% kod žena i 18% kod muškaraca). Recidiv se javio kod 5 operisanih u ležećem položaju i kod samo jedne bolesnice operisane u sedećem položaju. Naši rezultati su pokazali trend ređe pojave recidiva kod bolesnika operisanih u sedećem položaju, iako razlika nije bila statistički značajna [*odds ratio* (OR): 0.18; 95% interval poverenja: 0.01–1.42, $p = 0.172$]. **Zaključak.** Kraniostomija u sedećem položaju pod lokalnom anestezijom je sigurna, jednostavna i pouzdana procedura za lečenje HSH. Pored toga što je veoma komforna za bolesnika, prema našim inicijalnim rezultatima, mogla bi da vodi i sniženju stope recidiva, verovatno zahvaljujući boljoj kontroli ulaska vazduha i pojave posledičnog pneumocefalusa.

Ključne reči:
hematom, subduralni; neurohirurške procedure; položaj tela; recidiv; položaj, sedeći; lečenje, ishod.

Correspondence to: Milan Lepić, Military Medical Academy, Clinic for Neurosurgery, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: milanlepic@gmail.com

Introduction

A subdural hematoma is a blood collection between the dura and arachnoid layers of meninges, which is considered chronic when developed within 21 days or more [1]. Chronic subdural hematoma (CSDH) is one of the most common neurosurgical occurrences, with the overall incidence reported to range from 1.72 to 20.6 per 100,000 persons per year [2]. The reported reoccurrence rate, after the standard surgical procedure, ranges from 11.7% to 28% [3]. Most likely, CSDH develops due to traumatic head injury, which is often minor and not always evident, especially in light of antiplatelet or anticoagulation therapy in older patients, although it may develop through the lysis of an acute hematoma of any origin [4, 5]. The incidence of CSDH may double in the next ten years due to the growth of the aging population [6]. The pathophysiology of the disease is controversial, being a combination of multiple interrelated mechanisms following trauma, including inflammation, membrane formation, angiogenesis and fibrinolysis, that propagate an increase in CSDH volume [4].

Craniostomy is the gold standard for CSDH treatment, although, there are many different "styles" of performing this procedure [7]. The simple procedure of craniostomy still brings many dilemmas: a twist-drill or a burr hole, single or double craniostomy, or an enlarged single burr hole [8]. There is no consensus, but some evidence-based recommendations are available, suggesting to irrigate the CSDH and to place postoperative closed-system drainage to prevent reoccurrence [7, 9, 10]. Pneumocephalus is a common surgical complication, representing a significant independent predictor of CSDH treatment failure, which almost doubles the reoccurrence rate [11, 12]. If the postoperative computed tomography (CT) images reveal significant pneumocephalus, a simple re-operation is proposed, to refill the hematoma cavity with saline [12]. Craniostomy is usually performed in the semilateral position or in the supine position, with the head in the neutral position or rotated opposite the lesion site [8]. The burr hole should be kept at the vertex of the head during this procedure. Improper head posture can result in a large quantity of subdural air, no matter how many holes are drilled [8, 12].

When performing the procedure on an awake patient, the sitting position is the most comfortable for the patient, while also being very convenient for the surgeon. Due to head elevation and positioning, the burr hole, which is easily made at the vertex of the patient's head (and the vertex of the CSDH), becomes a natural barrier, preventing gas from entering the subdural cavity. Nevertheless, in neurosurgery, the sitting position is usually linked with complications (venous air embolism in particular), although recent studies demonstrate decreased rates of complications in patients undergoing surgeries in the sitting position [13, 14]. Hovewer, anesthesiologists and surgeons continue to avoid this position [15]. Up to date, no reports of patients undergoing craniostomy in the sitting position for CSDH have been published.

Methods

A small prospective cohort study in our patients undergoing awake craniostomy for CSDH was performed. The patients were selected for either the supine or sitting position according to the treating physician's preference.

The aim of the study was to recommend the introduction of the sitting position, as at least noninferior to the standard supine position, as well as to evaluate the possible impact of positioning (sitting vs. supine) on the outcome, with a possible influence on complications, namely the most frequent one – CSDH reoccurrence.

All patients who underwent craniostomy with drainage for primary CSDH in both supine and sitting positions at our department from December 21st 2016 to March 31st 2018 were included.

Inclusion criteria were: patients who underwent craniostomy with drainage for CSDH under local anesthesia from December 21st, 2016 to March 31st, 2018; either the sitting or supine position; CSDH verified by CT; either bilateral or unilateral hematoma/s.

Exclusion criteria were: the prone position; iatrogenic CSDH (related to previous ventriculoperitoneal shunt or surgery); CSDH with significant neomembranes or acute blood clot formation, according to CT; reocurring CSDH.

Patients were divided into two groups, sitting and supine, according to their intraoperative positioning.

Surgical procedure

After CSDH was confirmed by CT, if excessive neomembranes formation was detected, the patient was excluded from this study, and referred for either craniostomy and drainage followed by postoperative corticosteroid treatment, or craniotomy and evacuation of hematoma by way of neomembranes resection. The patients with a significant acute blood clot underwent craniotomy and evacuation of hematoma.

After the patient had been checked for bleeding, the procedure under local anesthesia was indicated. In cases where coagulation status was altered due to anticoagulation drugs, an antidote therapy was prescribed to reduce the international normalized ratio (INR) to less than 1.5, and activated partial thromboplastin time (APTT) to 30–40 s. Patients taking antiaggregating drugs were not operated on the day of admission if the medication had been taken on that day. The first dose of prophylactic 2nd generation cephalosporin antibiotic was given at the ward or in the emergency room (ER), before the procedure. The operating field was shaved in a radius of approximately 3 cm around the incision.

The patient was informed regarding the surgical procedure, the type of anesthesia, as well as potential risks and expected outcomes. A standardized informed consent form was signed by each patient or their legal guardian.

The patient was placed on the operating table in the supine position. To achieve this position, the head was slightly flexed, and rotated to the side opposite the lesion.

The sitting position was reached by manipulating the operating table. The head was flexed towards the torso and secured using a pillow (see Figure 1). While the patient is comfortably seated armrests may be helpful to further improve the patient's comfort [13].

Fig. 1 – Illustration of a patient in the sitting position prepared for an awake craniostomy for a chronic subdural hematoma.

Initial 5 mL lidocaine was administered locally to impregnate the area of incision and drain placement. The drainage system was prepared in the meantime.

The incision was made over the hematomas highest thickness aligned with the vertex of the head, after which a burr hole was made and filled around with surgical wax. The dura was exposed, and the drain was pulled through the skin, approximately 1 cm behind the incision. A T-shaped dural incision was made, and a 6 cm perforated part of the drain was pushed through the capsule into the subdural space. The burr hole was filled with an oxidized cellulose hemostatic agent, and sutures were placed. The same sutures were used to fix the cotton gauze. After initial hematoma release and pressure relief, 20 mL of 0.9% saline solution was then injected through the proximal part of the drain for irrigation, 3–5 times. Afterwards, the drain was connected through the distal part to the drainage bottle placed below the patient, to proceed with controlled closed system drainage.

The patient was placed in a bed, in a comfortable supine position and transferred to the ward. Solutions infusion and paracetamol were administered, and the drainage was continued. Antibiotics were continued until the drain was removed. A head CT was performed on the second morning after the procedure, and the drain was removed.

A control head CT was performed one month after the surgery, together with the follow-up examination. At that time, in cases where significant residual hematoma was detected, a re-operation was indicated. Where a head CT was normal, or with an insignificant subdural collection, only clinical follow-up was advised.

Statistical analysis

Categorical data were presented as the number of patients in each category and the corresponding percentages. The χ^2 test and the Fisher exact test (in case the number of patients in a group was below 5) were performed to verify the statistical significance between the various groups. Numerical data were first analyzed graphically, and the normality of their distribution was checked by performing the Shapiro-Wilk test. Where the Shapiro-Wilk test confirmed the normal distribution, numerical data were presented as means and standard deviation, and the Student's *t*-test or ANOVA were applied to compare two or more groups, respectively. Otherwise, numerical data were presented as median values with minimum and maximum values. The Kruskal-Wallis test was performed to compare numerical variables between different groups.

The statistical analysis was performed in the R Language and Environment for Statistical Computing (v. 3.4.2 – "Short Summer") [16]. The data were imported from Excel using the *openxlsx* package, processed using the *Hmisc*, *dplyr*, *stringr*, and *tidyr* packages, and presented using the *compareGroups* and *ggplot2* packages [17, 18].

Results

Out of 36 patients who underwent 42 surgeries in the observed period, this study included a total of 31 patients, each with a single operation (31 in sum) for primary CSDH. Five patients were excluded due to the CSDH related to a previous surgery (4), and due to significant acute clot formation (1). The remaining 6 surgeries were reoperations (two of these in a single patient), while one of the male patients was referred to another institution for reoperation after reoccurrence.

Nine patients (29%) were female, and 22 (29%) were male (71%). Table 1 shows general information about the patients' characteristics, as well as the characteristics of the surgery and the outcomes. In the majority of cases, the patients were operated immediately after the diagnosis of CSDH or on the following day, although in some cases the procedure was delayed for up to 10 days. Sixteen (52%) of the patients were operated in the supine position, while 15 patients (48%) were operated in the sitting position.

Most of the patients (81%) had no complications during the follow-up period, while reoperation for recurrent CSDH was necessary for 6 patients (19%). One patient, operated on in the sitting position developed a headache that lasted for 2 weeks postoperatively and resolved with the use of paracetamol. No other complications occurred in the group of patients undergoing primary craniostomy and drainage for CSDH.

Pneumocephalus was less frequent in the group of patients undergoing surgery in the sitting position, and the two cases of massive pneumocephalus were both in the group of supine-positioned patients. However, there was no statistically significant correlation between pneumocephalus formation and hematoma reoccurrence; neither was there any statistically significant correlation between positioning and pneumocephalus formation.

Table 1

General characteristics of the patients and surgical procedure by gender

Parameter	All patients (n = 31)	Female (n = 9)	Male (n = 22)	*p* (overall)
Age (years)	72 (49–89)	70 (59–88)	74 (49–89)	0.433
Location of CSDH:				0.670
bilateral	6 (19)	1 (11)	5 (23)	
right	15 (48)	4 (44)	11 (50)	
left	10 (32)	4 (44)	6 (27)	
Position, n				0.252
supine	16 (52)	3 (33)	13 (59)	
sitting	15 (48)	6 (67)	9 (41)	
Outcome				1.000
no complications	25 (81)	7 (78)	18 (82)	
recurrent hematoma	6 (19)	2 (22)	4 (18)	

All values are expressed as median (minimum–maximum) or number (percentage).

Table 2

General characteristics of the patients with chronic subdural hematoma (CSDH) and surgical procedure by the outcome

Parameter	All patients (n = 31)	No complications (n = 25)	Recurrent hematoma (n = 6)	*p* (overall)
Age, years	72 (49–89)	70 (49–89)	76 (63–78)	0.920
Gender:				1.000
female	9 (29)	7 (28)	2 (33)	
male	22 (71)	18 (72)	4 (67)	
Location of CSDH:				0.833
bilateral	6 (19)	5 (20)	1 (17)	
right	15 (48)	11 (44)	4 (67)	
left	10 (32)	9 (36)	1 (17)	
Position:				0.172
supine	16 (52)	11 (44)	5 (83)	
sitting	15 (48)	14 (56)	1 (17)	

All values are expressed as median (minimum–maximum) or number (percentage).

Table 2 shows the characteristics of the patients and the surgery by the outcome. Reoperation was necessary for a total of 6 patients, of which two were female, and 4 were male. There were no statistically significant differences between male and female patients regarding their basic characteristics and the outcome of the surgery. The reoperated patients had a median age of 76 years (63–78), and those with a positive outcome a median age of 70 years (49–89). Still, there was no statistically significant difference in the age of the patients with or without a complication. None of the previously recognized factors such as age, gender, time to surgery, or position, had a significant influence on the reoccurrence of CSDH during the follow-up period. As for the position of the patients, 5 out of 6 of the patients with reoccurring CSDH were initially operated on in the supine position, while only one patient was operated on in the sitting position (see Figure 2). As for other characteristics of the patients and the surgical procedure, there were no statically significant differences between the patients with a positive outcome and those with reoccurring CSDH.

The influence of the patients' characteristics and surgical procedure on the outcome was quantified by calculating odds ratios (OR) with 95% confidence interval (CI), as reported in Table 3. The patients' characteristics, such as age and gender, were not significant predictors of the reoccurrence. As far as the surgical procedure is concerned, the location of CSDH, time to surgery, as well as the position (supine or sitting), in our study neither of these had any influence on the outcome.

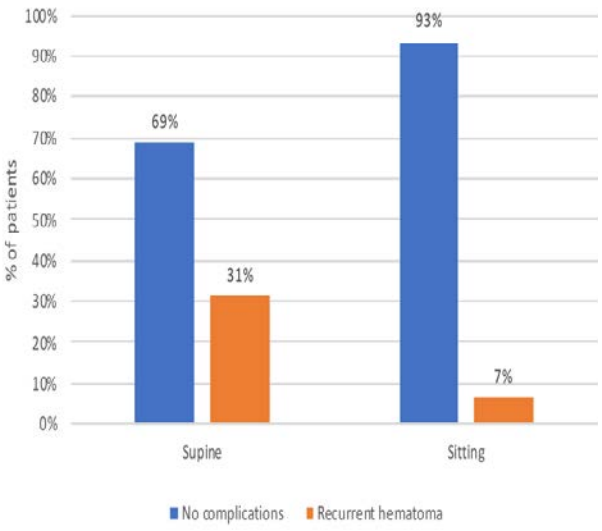


Fig. 2 – The number of reoperations according to the intraoperative position.

Table 3

Factors influencing the outcome of the surgery in patients with chronic subdural hematoma (CSDH)

Parameter	All patients (n = 31)	No complications (n = 25)	Recurrent hematoma (n = 6)	OR	*p* (ratio)	*p* (overall)
Age, years	72 (49–89)	70 (49–89)	76 (63–78)	1.00 (0.91; 1.09)	0.973	0.920
Gender:						1.000
female	9 (29)	7 (28)	2 (33)	Ref.	Ref.	
male	22 (71)	18 (72)	4 (67)	0.77 (0.11;7.31)	0.796	
Location of CSDH:						0.833
bilateral	6 (19)	5 (20)	1 (17)	Ref.	Ref.	
right	15 (48)	11 (44)	4 (67)	1.65 (0.17;53.5)	0.698	
left	10 (32)	9 (36)	1 (17)	0.58 (0.01;25.8)	0.750	
Position:						0.172
supine	16 (52)	11 (44)	5 (83)	Ref.	Ref.	
sitting	15 (48)	14 (56)	1 (17)	0.18 (0.01;1.42)	0.111	

All values are expressed as median (minimum–maximum) or number (percentage); OR – odds ratio (95% confidence interval).

Discussion

CSDH drainage is one of the simplest neurosurgical procedures, sometimes even performed by general surgeons [19]. Apart from infection, a reoccurrence rate of up to 28% after surgical treatment is one of the most common and the most significant complications [3]. We performed awake craniostomy and drainage for CSDH in 31 patients, 9 females, and 22 males. The median age of patients was 72 years, and the gender distribution was in accordance with previously published studies [2]. Our study groups, based on the position during surgery, were homogenous according to age and gender distribution. Sixteen patients underwent the surgical procedure in the supine position, while 15 patients underwent surgery in the sitting position. The overall reoccurrence rate was 19% (22% and 18% in females and males respectively), 76 years being the median age of patients with the reoccurrence, similar to previously published studies [20–22]. There was no statistically significant difference in the hematoma reoccurrence based on the patients' general characteristics. We noted hematoma reoccurrence in 5 patients operated on in the supine position, and only in one patient operated on in the sitting position. The intraoperative sitting position was related to the reduced rate of reoccurrence, although this difference in our group of patients was not statistically significant (see Table 3).

The most common patient-related risk factors for an undesirable outcome of this procedure are chronic alcoholism, seizure disorders, and history of a ventriculoperitoneal shunt [20, 23]. The impact of diabetes mellitus remains controversial [23, 24]. Most studies suggest that age, sex, hypertension, cardiac disease, and use of anticoagulants or antiplatelets do not affect the reoccurrence rate [23, 25], but do influence the overall outcome [26]. Considering the age of the patients suffering from CSDH, it can be expected that some patients undergoing surgery for CSDH may not be able to undergo awake surgery under local anesthesia in the supine or prone positions due to age-related pathology (e.g. severe cardiac or pulmonary disease). In this specific group of patients, the sitting position preserves the benefits of the procedure under local anesthesia regardless of the underlying condition, therefore avoiding general anesthesia and related complications.

Radiologic risk factors for CSDH reoccurrence include a preoperative appearance of heterogeneous hematoma or higher-density hematoma, greater midline shift, bilateral hematomas, or postoperative appearance of poor brain reexpansion or greater subdural air accumulation [11]. Only one of these risk factors, subdural air collection (pneumocephalus) developed in the course of burr-hole craniostomy, which is considered a sole predictor of hematoma reoccurrence, can be prevented during the surgical procedure [12]. Controlled blood evacuation when the dural incision is made at the vertex of CSDH, precludes the air ingress into the subdural space. The CSDH vertex exposure and incision are performed simply and reliably in the sitting position through the burr hole positioned at the vertex preventing uncontrolled blood spill and air ingress (Figure 3). Although head tilting also prevents air ingress, it is much less comfortable for the patient, and is therefore difficult to maintain in an awake patient. Our results suggest that significant pneumocephalus formation is less likely to occur in the sitting position with the head flexed. However, no statistically significant correlation was found.

Surgical risk factors for CSDH reoccurrence include the use of twist-drill as opposed to burr hole craniostomy or craniotomy, although craniotomies predispose patients to higher morbidity rates [27]. Burr hole craniostomy is considered to be related to shorter hospital stay, as well as to lower reoccurrence rate [9]. Other surgical risk factors include lack of or poor postoperative closed-system drainage. The impact of intraoperative irrigation and postoperative patient position (flat vs. upright) is controversial, although some studies found no significant influence of postoperative patient posture on the hematoma reoccurrence [20, 22, 28, 29]. Our surgical technique was uniform in both groups of patients, and included burr hole craniostomy and closed system drainage and irrigation with saline solution to achieve the best possible outcome in both groups of patients [27].

Complications are the main reason for avoidance of wider use of the sitting position in surgery in general, especially in neurosurgery [13]. There are even guidelines and protocols for anesthesiologists to reduce the complication

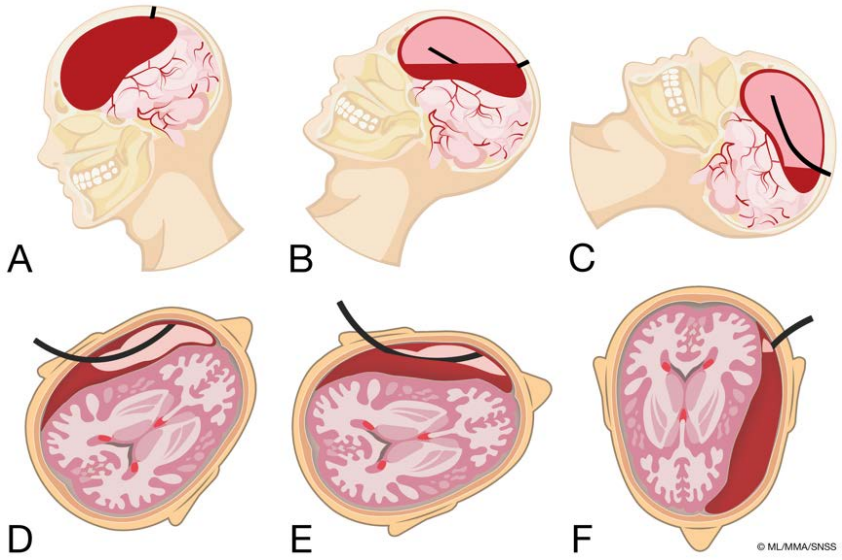


Fig. 3 – Patient's head positioning and potential air ingress during craniostomy. The impact of flexion: A) Head in the sitting position with craniostomy positioned at the vertex (prevents air ingress); B) Head in the supine position and posteriorly positioned craniostomy (significant air ingress may occur); C) Head in the supine position without flexion (significant air ingress may occur). The impact of rotation: D) Head tilted towards the contralateral side (air ingress may be prevented); E) Head tilted towards the contralateral side, nearly parallel to the table (air ingress may be prevented); F) Prone position head appearance (prevents air ingress).

rates in patients positioned this way during surgery [14, 15]. Previous studies have analyzed the sitting position within general anesthesia; therefore, many of the complications may be related to the duration of the procedure and prolonged sitting [14, 15]. On the other hand, specific complications such as tension pneumocephalus occur in the sitting position only during the posterior *fossa surgeries*, when cerebrospinal fluid (CSF) leak is not maintained under control [13, 30].

Our study is the first to assess the sitting position when craniostomy for CSDH is performed. No particular complication which could be attributed to the sitting position was noted or revealed, probably due to the short duration of the procedure (less than half an hour for single-sided, or less than 45 min for bilateral CSDH), the awake state of the patient (able to move the extremities when feeling the need), and the burr hole placed at the vertex (Figure 4). For the same reasons and because of uniqueness of our study, the complications related to the sitting position in awake craniostomy for CSDH cannot be compared to other studies on procedures usually performed in the similar positions until other studies on this procedure become available.

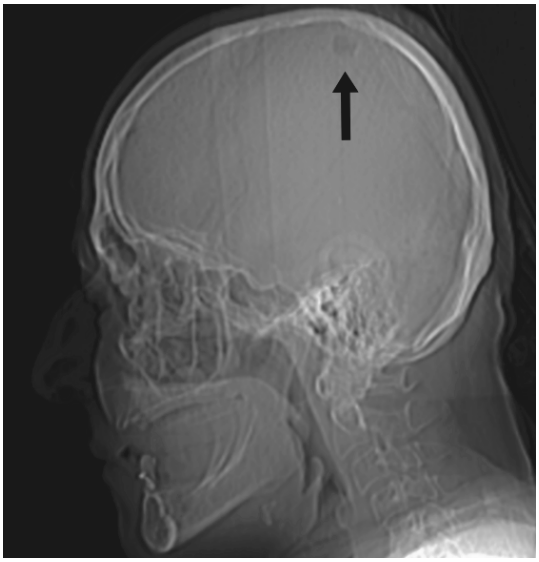


Fig. 4 – Cranial roentgenogram after craniostomy in the sitting position (the arrow points at the burr hole).

Study limitations

Regarding the limitations of the study, the most notable is the small size of the group of patients. Being an initial group undergoing CSDH treatment in the sitting position, the results are encouraging but not statistically significant, and should be taken with caution. Some bias avoidance was achieved through the exclusion of patients with any competing factors, considered to influence the outcome. The impact of bilateral hematomas was considered in the analysis, but these patients were considered as a single patient, rather than two independent hematomas.

Future studies, preferably multicentered, randomized, and performed on a larger number of patients, could verify these findings. Multivariate analysis with the inclusion of more factors affecting outcomes may provide additional strength to the conclusions of the studies and should be performed. This would allow for wider implementation and use of the sitting position in CSDH treatment.

Conclusion

The sitting position is comfortable and easily accepted by the patient, leading to better patient-surgeon compliance and cooperation, even if the patient is disoriented, which is common in elderly neurosurgical patients. This study showed that this positioning of patients during craniostomy may reduce CSDH reoccurrence, possibly owing to the prevention of subdural air formation. Therefore, the sitting position for craniostomy in CSDH could represent a viable alternative to the more commonly used supine position, not resulting in a higher percentage of complications.

Acknowledgement

We acknowledge the support of the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project TR 37001.

R E F E R E N C E S

1. *Sahyouni R, Goshtasbi K, Mahmoodi A, Tran DK, Chen JW*. Chronic Subdural Hematoma: A Historical and Clinical Perspective. World Neurosurg 2017; 108: 948–53.
2. *Yang W, Huang J*. Chronic Subdural Hematoma: Epidemiology and Natural History. Neurosurg Clin N Am 2017; 28(2): 205–10.
3. *Montano N, Stifano V, Skrap B, Mazzucchi E*. Management of residual subdural hematoma after burr-hole evacuation. The role of fluid therapy and review of the literature. J Clin Neurosci 2017; 46: 26–9.
4. *Edlmann E, Giorgi-Coll S, Whitfield PC, Carpenter KLH, Hutchinson PJ*. Pathophysiology of chronic subdural haematoma: inflammation, angiogenesis and implications for pharmacotherapy. J Neuroinflammation 2017; 14(1): 108.
5. *Novaković N, Lepić M, Minić L, Radenović K, Rotim A, Rasulić L*. Combined Treatment of Ruptured Middle Cerebral Artery Aneurysm Followed by Subarachnoid Hemorrhage and Acute Subdural Hematoma in Multiple Aneurysm Disease of Cerebral Blood Vessels: Case Report. Acta Clin Croat 2016; 55(4): 659–62.
6. *Welling LC, Welling MS, Teixeira MJ, Figueiredo EG*. Chronic Subdural Hematoma: So Common and So Neglected. World Neurosurg 2018; 111: 393–4.
7. *Liu W, Bakker NA, Groen RJ*. Chronic subdural hematoma: a systematic review and meta-analysis of surgical procedures. J Neurosurg 2014; 121(3): 665–73.
8. *Winn HR*. Youmans and Winn Neurological Surgery E-Book. 7th ed. Philadelphia, PA: Elsevier; 2016.
9. *Buchanan IA, Mack WJ*. Minimally Invasive Surgical Approaches for Chronic Subdural Hematomas. Neurosurg Clin N Am 2017; 28(2): 219–27.
10. *Rasulić L, Lepić M*. Neurosurgical clinical practice guidelines and recommendations: Experience, Evidence and Enrichment. World Neurosurg 2019; 26: 76–8.
11. *Mori K, Maeda M*. Surgical treatment of chronic subdural hematoma in 500 consecutive cases: clinical characteristics, surgical outcome, complications, and recurrence rate. Neurol Med Chir (Tokyo) 2001; 41(8): 371–81.
12. *You C G, Zheng XS*. Postoperative pneumocephalus increases the recurrence rate of chronic subdural hematoma. Clin Neurol Neurosurg 2018; 166: 56–60.
13. *Himes BT, Mallory GW, Abcejo AS, Pasternak J, Atkinson JLD, Meyer FB,* et al. Contemporary analysis of the intraoperative and perioperative complications of neurosurgical procedures performed in the sitting position. J Neurosurg 2017; 127(1): 182–8.
14. *Ammirati M, Lamki TT, Shaw AB, Forde B, Nakano I, Mani M*. A streamlined protocol for the use of the semi-sitting position in neurosurgery: a report on 48 consecutive procedures. J Clin Neurosci 2013; 20(1): 32–4.
15. *Jadik S, Wissing H, Friedrich K, Beck J, Seifert V, Raabe A*. A standardized protocol for the prevention of clinically relevant venous air embolism during neurosurgical interventions in the semisitting position. Neurosurgery 2009; 64(3): 533–8; discussion 8–9.
16. *R Core Team* (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available online at https://www.R-project.org/.
17. *Subirana I, Sanz H, Vila J*. Building Bivariate Tables: The compareGroups Package for R. J Statistical Software 2014; 57(12): 1–16.
18. *Wickham H*. Ggplot2 Elegant Graphics for Data Analysis. 2nd ed. New York: Springer; 2009.
19. *Kanyi JK, Ogada TV, Oloo MJ, Parker RK*. Burr-Hole Craniostomy for Chronic Subdural Hematomas by General Surgeons in Rural Kenya. World J Surg 2018; 42(1): 40–5.
20. *Desai VR, Scranton RA, Britz GW*. Management of Recurrent Subdural Hematomas. Neurosurg Clin N Am 2017; 28(2): 279–86.
21. *Torihashi K, Sadamasa N, Yoshida K, Narumi O, Chin M, Yamagata S*. Independent predictors for recurrence of chronic subdural hematoma: a review of 343 consecutive surgical cases. Neurosurgery 2008; 63(6): 1125–9; discussion 1129.
22. *Abouzari M, Rashidi A, Rezaii J, Esfandiari K, Asadollahi M, Aleali H, et al.* The role of postoperative patient posture in the recurrence of traumatic chronic subdural hematoma after burr-hole surgery. Neurosurgery 2007; 61(4): 794-7; discussion 797.
23. *Chon KH, Lee JM, Koh EJ, Choi HY*. Independent predictors for recurrence of chronic subdural hematoma. Acta Neurochir (Wien) 2012; 154(9): 1541–8.
24. *Yamamoto H, Hirashima Y, Hamada H, Hayashi N, Origasa H, Endo S*. Independent predictors of recurrence of chronic subdural hematoma: results of multivariate analysis performed using a logistic regression model. J Neurosurg 2003; 98(6): 1217–21.
25. *Kamenova M, Lutz K, Schaedelin S, Fandino J, Mariani L, Soleman J*. Does Early Resumption of Low-Dose Aspirin After Evacuation of Chronic Subdural Hematoma With Burr-Hole Drainage Lead to Higher Recurrence Rates? Neurosurgery 2016; 79(5): 715–21.
26. *Kwon CS, Al-Awar O, Richards O, Izu A, Lengvenis G*. Predicting Prognosis of Patients with Chronic Subdural Hematoma: A New Scoring System. World Neurosurg 2018; 109: e707–e14.
27. *Stanisić M, Pripp H*. A Reliable Grading System for Prediction of Chronic Subdural Hematoma Recurrence Requiring Reoperation After Initial Burr-Hole Surgery. Neurosurgery 2017; 81(5): 752–60.
28. *Wang QP, Yuan Y, Guan JW, Jiang XB*. A comparative study of irrigation versus no irrigation during burr hole craniostomy to treat chronic subdural hematoma. BMC Surg 2017; 17(1): 99.
29. *Wang W, Liu H, Yang J*. Burr hole craniostomy irrigation with and without drainage during surgical treatment of chronic subdural hematoma: A retrospective study of 87 cases. Turk Neurosurg 2017; doi: 10.5137/1019-5149.JTN.19747-16.2.
30. *Ganslandt O, Merkel A, Schmitt H, Tzabazis A, Buchfelder M, Eyupoglu I,* et al. The sitting position in neurosurgery: indications, complications and results. a single institution experience of 600 cases. Acta Neurochir (Wien) 2013; 155(10): 1887–93.

Received on June 11, 2019.
Revised on August 21, 2019.
Accepted on August 28, 2019.
Online First September, 2019.

CASE REPORTS

UDC: 340.6:615.099
DOI: https://doi.org/10.2298/VSP170508090K

Fatal poisoning case involving drug interaction due to the inhibition of cytochrome P450

Slučaj trovanja sa fatalnim ishodom koji uključuje interakciju lekova zbog inhibicije citohroma P450

Hiroshi Kinoshita, Naoko Tanaka, Mitsuru Kumihashi, Mostofa Jamal, Asuka Ito, Tadayoshi Yamashita, Kiyoshi Ameno

Kagawa University, Faculty of Medicine, Department of Forensic Medicine, Kagawa, Japan

Abstract

Introduction. Cytochrome P450 (CYP) enzymes are responsible for the metabolism of various drugs and chemicals. The forensic pathologist should consider not only pharmacodynamic interactions by multiple drugs but also the pharmacokinetic drug interactions of each drug in the case of multiple drug ingestion. **Case report.** A female in her forties, receiving therapy for alcohol dependence, was found dead in her house. The medicolegal autopsy revealed no findings suggestive of natural cause of death. Quantitative toxicological analysis showed that levomepromazine, promethazine, dextromethorphan, estazolam, clomipramine, risperidone, and flunitrazepam concentrations in femoral blood were 0.750 μg/mL, 0.701 μg/mL, 0.332 μg/mL, 0.390 μg/mL, 0.216 μg/mL, 0.031 μg/mL, and 0.002 μg/mL, respectively, along with a blood ethanol level of 377 mg/dL. **Conclusion.** We concluded that the cause of death was the interaction between ethanol and multiple psychotropic drugs. Pharmacokinetic drug interactions due to the CYPs inhibition should be considered in the evaluation of toxicity in poisoning cases involving multiple drugs.

Key words:
autopsy; cytochrome p-450 enzyme system; drug interactions; ethanol; forensic pathology; phenothiazines; poisoning; psychotropic drugs.

Apstrakt

Uvod. Enzimi citohroma P450 (CYP) su odgovorni za metabolizam različitih lekova i hemikalija. Forenzički patolog treba da razmotri ne samo farmakodinamske interakcije između više lekova već i farmakokinetičke interakcije svakog leka u slučaju multiple ingestije lekova. **Prikaz bolesnika.** Žena u 40-im godinama, na lečenju od zavisnosti od alkohola, nađena je mrtva u svojoj kući. Medikolegalnom autopsijom nisu nađeni znaci koji bi upućivali na prirodni uzrok smrti. Kvantitativnom toksikološkom analizom nađeno je da su koncentracije levomepromazina, prometazina, dekstrometorfana, estazolama, klomipramina, risperidona i flunitrazepama u femoralnoj krvi bile: 0.750 μg/mL, 0.701 μg/mL, 0.332 μg/mL, 0.390 μg/mL, 0.216 μg/mL, 0.031 μg/mL, i 0.002 μg/mL, redom, uz etil alkohol u krvi u koncentraciji od 377 mg/dL. **Zaključak.** Zaključili smo da je uzrok smrti bila interakcija između etil alkohola i više psihotropnih lekova. Farmakokinetičke interakcije lekova zbog inhibicije CYP trebalo bi uzeti u obzir kod procene toksičnosti u slučajevima trovanja koji uključuju ingestiju više lekova.

Ključne reči:
autopsija; citohrom p-450; lekovi, interakcija; alkohol, etil; patologija, sudska; fenotiazini; trovanje; psihotropni lekovi.

Introduction

Fatal cases following multiple drug ingestion are sometimes observed in forensic casework. In such cases, the forensic pathologist should consider not only pharmacodynamic interactions between multiple drugs but also the pharmacokinetic drug interactions of each drug [1]. Cytochrome P450 (CYP) enzymes are responsible for the metabolism of various drugs and chemicals, including psychotropic drugs [2]. CYP3A4 and CYP2D6 are two major enzymes involved in drug metabolism [2, 3]. These two enzymes metabolize approximately 50% of drugs [2]. CYP2D6 is involved in the metabolism of many psychotropic drugs, such as antipsychotic agents, tricyclic antidepressants, opioids, and antiarrhythmic agents [4, 5]. Hence, inhibition of these enzymes results in significant pharmacokinetic drug interactions, which can cause

Correspondence to: Hiroshi Kinoshita, Kagawa University, Faculty of Medicine, Department of Forensic Medicine. 1750-1 Miki, Kita, 761-0793 Kagawa, Japan. E-mail: kinochin@med.kagawa-u.ac.jp

adverse reactions. Here, we report a case of death due to the combined use of ethanol with multiple psychotropic drugs and discuss the pharmacokinetic interactions among them.

Case report

A female in her forties was found dead in her house. Subsequent police investigations revealed that the deceased was receiving therapy for alcohol dependence. Although she had been prescribed psychotropic drugs, such as estazolam, flunitrazepam, levomepromazine, promethazine, and risperidone, she could not stop drinking. The medicolegal autopsy revealed no injury except an old scar on her forehand. No findings suggestive of natural disease and, consequently, natural cause of death was not observed.

The deceased was 161 cm tall and had 84.5 kg. Her heart weighed 386 g and contained 200 mL of blood without coagulum. Her brain weighed 1,470 g and was slightly edematous. The left and right lung weighed 475 g and 642 g, respectively, and were congested. The stomach contained foodstuff in greyish fluid (100 mL). There were no notable changes in other organs other than congestion. A drug screening test using a Triage™ (Biosite Diagnostic Inc., San Diego, CA, USA) panel was positive for benzodiazepines. *Postmortem* blood, urine, bile, and stomach content samples were collected for toxicological investigation.

Toxicological analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed using a slightly modified method from that previously reported [6]. In brief, the liquid chromatography separations were carried out using Ekspert™ UltraLC 100-XL (Eksigent part of AB Sciex, Framingham, MA, USA). An L-column2 ODS (1.5 mm × 150 mm, 5.0 μm particle size, Chemicals Evaluation and Research Institute, Tokyo, Japan) was used with a mobile phase of solvent A (5% methanol containing 10 mM ammonium formate) and solvent B (95% methanol containing 10 mM ammonium formate) with a flow rate of 0.1 mL/min. A QTrap® 4500 tandem mass spectrometer (AB Sciex) was used to obtain the mass spectra. Quantitation of ethanol was performed using headspace gas chromatography.

The toxicological analysis identified levomepromazine, promethazine, dextromethorphan, estazolam, clomipramine (and its metabolite desmethylclomipramine), risperidone (and its metabolite paliperidone), flunitrazepam (and its metabolite 7-aminoflunitrazepam), and acetaminophen in the subject's body fluid samples. Ethanol concentrations in blood and urine were 377 mg/dL and 406 mg/dL, respectively. Table 1 shows the quantification of each drug in the victim's blood, along with the currently established fatal and therapeutic levels [7–9].

Table 2 shows the metabolic enzymes for each drug and the enzymes inhibited by each drug found in the patient's blood.

Table 1

Concentrations of each drug and metabolite in the *postmortem* samples (μg/mL)

Drug	Blood	Urine	Bile	Stomach contents	Therapeutic range *	Toxic range *	Lethal range *
Levomepromazine	0.750	0.056	2.206	41.493	0.005–0.2	0.4	0.5
Promethazine	0.701	0.062	1.501	15.847	0.05–0.4	1–2	1.8–5.4
Dextromethorphan	0.332	0.333	1.097	2.266	0.01–0.04	0.1	3
Estazolam	0.390	0.100	2.457	2.354	0.055–0.2	–	0.48
Clomipramine	0.216	0.023	1.912	1.928	0.02–0.4	0.4–0.6	1–2
Desmethylclomipramine	0.005	0.001	0.076	0.063	–	–	–
Risperidone	0.031	0.058	0.206	0.780	0.002–0.02	0.12	1.8
Paliperidone	0.015	0.057	0.470	0.149	0.02–0.06	0.12	–
Flunitrazepam	0.002	0.001	B.D.L	0.291	0.005–0.015	0.05	> 0.16 **
7–aminoflunitrazepam	0.011	0.002	0.541	0.131	–	–	
Acetaminophen	0.065	7.491	12.203	B.D.L	5–25	100–150	200–300

*** Therapeutic, toxic, and lethal ranges are cited [7, 8]; ** Lethal range includes the total of flunitrazepam and 7-aminoflunitrazepam [9]; B.D.L – below the detection limit.**

Table 2

Enzymes involved in the metabolism of each drug and enzyme inhibition by each drug

Drug	Metabolic enzyme	Enzyme inhibition
Levomepromazine	CYP3A4 [12]	CYP1A2, CYP2D6, CYP3A4 [5, 12]
Promethazine	CYP2D6 [5]	CYP2D6 [5, 13]
Dextromethorphan	CYP2D6, CYP3A4 [4, 15]	
Estazolam	CYP3A4 [16]	
Clomipramine	CYP1A2, CYP2C19, CYP2D6 [4, 5, 13]	CYP2D6 [4, 5, 13]
Risperidone	CYP2D6, CYP3A4 [2, 5]	
Flunitrazepam	CYP2C19, CYP3A4 [17]	
Acetaminophen	CYP1A2, CYP2E1 [5]	

Discussion

Analytical results indicated a fatal level of levomepromazine and toxic levels of promethazine, dextromethorphan, and estazolam in blood, while risperidone (and its metabolite paliperidone) and clomipramine (and its metabolite desmethylclomipramine) were in their therapeutic ranges, and flunitrazepam (and its metabolite 7-

aminoflunitrazepam) and acetaminophen were below their therapeutic ranges.

Levomepromazine is a phenothiazine derivative used as an antipsychotic drug and has pronounced sedative effects. Its overdose results in nausea, vomiting, coma, and convulsions [8]. Promethazine is also a phenothiazine derivative that is used as an antihistaminic, antiemetic, and sedative agent, and can cause coma, respiratory depression, and circulatory failure in overdose [8]. These drugs, along with ethanol, exert additive or synergistic effects, resulting in suppression of central nervous system function [10]. Based on autopsy findings and the results of the toxicological examination, we concluded that the combined toxicity of ethanol and multiple psychotropic drugs led to death in this case.

Most of the drugs are metabolized by CYPs [2, 4, 5, 10–17]. However, many of these drugs also inhibit CYPs. Among them, levomepromazine, promethazine, and clomipramine have potent inhibitory effects on CYP2D6, and levomepromazine also inhibits CYP3A4 [5, 11–13].

CYP2D6 is part of a group of enzymes responsible for the metabolism of many drugs. Since more than 65 commonly used drugs are metabolized by this enzyme [4], considering pharmacokinetic drug interactions in cases of multiple drug ingestion is important. The subfamily of CYP3A (including CYP3A4) is probably the most important of all drug-metabolizing enzymes [4].

Individual differences in the activity of CYP2D6 due to genetic polymorphism have been reported, based on which individuals are categorized as the following four phenotypes: poor, intermediate, extensive, and ultrarapid metabolizers [2, 4, 15]. Dextromethorphan is a synthetic analogue of codeine, which is prescribed as an antitussive agent. It is also used as a clinical probe for CYP2D6 phenotyping, as its major metabolic pathway is mediated by both CYP2D6 and CYP3A4 [8, 15]. The postmortem blood concentration of dextromethorphan in the present case was within the toxic range. Life-threatening intoxication with dextromethorphan resulting from inhibition of CYP2D6 as a result of interactions of other drugs has been clinically reported [18, 19]. Based on previous evidence, it is speculated that the drug levels in the present case might have been elevated due to the inhibition of CYP2D6 by drugs such as levomepromazine, promethazine, and clomipramine, and of CYP3A4 and CYP1A2 by levomepromazine. The inhibitory effects of other drugs depend on their dose and the ability of the inhibitor to bind to the enzyme, which usually occurs immediately after drug ingestion [3]. Estazolam is a triazolobenzodiazepine derivative metabolized by CYP3A4, and is prescribed for the short-term management of insomnia; somnolence, respiratory depression, and coma are observed with its overdose [8, 16]. In the current case, blood estazolam level might also have been elevated due to the inhibition of CYP3A4.

We speculate that the accumulation of dextromethorphan and estazolam in the patient's blood was possibly due to an inhibitory pharmacokinetic drug interaction. Thus, these two drugs might also have contributed to the patient's death.

The present case showed that more attention should be paid to the interactions between multiple psychotropic drugs.

Conclusion

We concluded that the cause of the patient's death was the interaction between ethanol and multiple psychotropic drugs. Pharmacokinetic drug interactions due to the inhibition of CYPs should be considered in the evaluation of toxicity.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. *Kinoshita H, Tanaka N, Takakura A, Kumihashi M, Jamal M, Ito A, et al.* Multiple drug poisoning case caused by a pharmacokinetic interaction involving paroxetine. Rom J Leg Med 2015; 23(3): 208–10.
2. *Zanger UM, Schwab M.* Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013; 138(1): 103–41.
3. *Lynch T, Price A.* The effects of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician 2007; 76(3): 391–6.
4. *Wilkinson GR.* Drug metabolism and variability among patients in drug response. N Engl J Med 2005; 352(21): 2211–21.
5. *Flockhart DA.* Drug Interactions: Cytochrome P450 Drug Interaction Table. Indiana University School of Medicine. (2007). Available from: https://drug-interactions.medicine.iu.edu/Home.aspx [accessed 2019 April 3].
6. *Kinoshita H, Tanaka N, Takakura A, Kumihashi M, Jamal M, Ito A, et al.* Flunitrazepam in stomach contents may be a good indicator of its massive ingestion. Rom J Leg Med 2017; 25(2): 193–5.
7. *Schulz M, Iwersen-Bergmann S, Andresen H, Schmoldt A.* Therapeutic and toxic blood concentrations of nearly 1000 drugs and other xenobiotics. Crit Care 2012; 16(4): R136.
8. *Baselt RC.* Disposition of toxic drugs and chemicals in man. 8th ed. Foster City, CA: Biomedical Publications; 2008.
9. *Drummer OH, Odell M.* The forensic pharmacology of drug of abuse. London: Arnold; 2001.
10. *Koski A, Ojanperä I, Vuori E.* Interaction of alcohol and drugs in fatal poisonings. Hum Exp Toxicol 2003; 22(5): 281–7.
11. *Gervasini G, Caballero MJ, Carrillo JA, Benitez J.* Comparative cytochrome p450 in vitro inhibition by atypical antipsychotic drugs. ISRN Pharmacol 2013; 2013: 792456.
12. *Basińska-Ziobroń A, Daniel WA, Wǒjcikowski J.* Inhibition of human cytochrome P450 isoenzymes by a phenothiazine neuroleptic levomepromazine: An in vitro study. Phramacol Rep 2015; 67(6): 1178–82.
13. *Vandel P, Haffen E, Nezelof S, Broly F, Kantelip JP, Sechter D.* Clomipramine, fluoxetine and CYP2D6 metabolic capacity in depressed patients. Hum Psychopharmacol 2004; 19(5): 293–8.
14. *Suzuki A, Yasui-Furukori N, Mihara K, Kondo T, Furukori H, Inoue Y,* et al. Histamine H_1-receptor antagonists, promethazine

and homochlorcyclizine, increase the steady-state plasma concentrations of haloperidol and reduced haloperidol. Ther Drug Monit 2003; 25(2): 192–6.

15. *Frank D, Jaehde U, Fuhr U*. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. Eur J Clin Pharmacol 2007; 63(4): 321–33.
16. *Miura M, Otani K, Ohkubo T*. Identification of human cytochrome P450 enzymes involved in the formation of 4-hydroxyestazolam from estazolam. Xenobiotica 2005; 35(5): 455–65.
17. *Kilicarslan T, Haining RL, Rettie AE, Busto U, Tyndale RF, Sellers EM*. Flunitrazepam metabolism by cytochrome P450s 2C19 and 3A4. Drug Metab Dispos 2001; 29(4 Pt 1): 460–5.
18. *Forget P, le Polain de Waroux B, Wallenmacq P, Gala JL*. Life-threatening dextromethorphan intoxication associated with interaction with amitriptyline in a poor CYP2D6 metabolizer: a single case re-exposure study. J Pain Symptom Manage 2008; 36(1): 92–6.
19. *Adachi Y, Uchisaki S, Itagaki T, Suzuki K, Obata Y, Doi M,* et al. Serotonin syndrome caused by an overdose of dextromethorphan, Medicon®. Masui 2009; 58(12): 1531–3. (Japanese)

Received on May 8, 2017.
Revised on June 22, 2019.
Accepted July 17, 2019.
Online First September, 2019.

CASE REPORT

UDC: 616-056.7::616-008.9
DOI: https://doi.org/10.2298/VSP190613094V

Acute intermittent porphyria – A case report

Akutna intermitentna porfirija

Tatjana Vulović*, Aleksandra Ristanović†, Rade Vuković†, Milić Veljović†‡

***University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia; †Military Medical Academy, Clinic for Anesthesiology and Intensive Care, Belgrade, Serbia; ‡University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia**

Abstract

Introduction. Acute intermittent porphyria is a rare inherited metabolic disorder caused by a decreased level of porphobilinogen deaminase. Subsequent accumulation of by-products in neural elements causes a classic triad of abdominal pain, neurological dysfunction, and psychiatric disturbances. **Case report.** A 22-year-old female patient with convulsions, episodes of blindness and progressive development of quadriparesis, bulbar paralysis, and respiratory failure was admitted to our intensive care unit twelve days after undergoing colon resection at the local hospital. The diagnosis was confirmed by a high level of porphobilinogen in urine. Previous use of oral contraceptives, antidepressants, and thiopental as induction agents for general anesthesia could represent precipitating factors. The patient was treated conservatively with high carbohydrate intake and human hemin. Six months after admission, the patient was transferred to the Department of Physical Medicine and Rehabilitation. **Conclusion.** Early diagnosis of acute intermittent porphyria is the cornerstone for successful treatment. The next step includes adequate therapy followed by the prevention of attacks.

Key words:
diagnosis, differential; neurologic manifestation; porphyrias; porphyria, acute intermittent; porphobilinogen; risk factors; treatment outcome.

Apstrakt

Uvod. Akutna intermitentna porfirija je redak nasledni metabolički poremećaj uzrokovan nedostatkom porfobilinogen deaminaze. Posledično nakupljanje međuproizvoda u nervnim strukturama dovodi do trijasa kliničkih simptoma: bol u trbuhu, neurološki ispadi i psihijatrijski poremećaji. **Prikaz bolesnika.** Dvanaest dana nakon resekcije debelog creva u lokalnoj bolnici, 22-godišnja bolesnica primljena je u jednicu intenzivnog lečenja zbog konvulzija, epizoda slepila i progresivne razvojne kvadripareze, bulbarne paralize i respiratorne insuficijencije. Dijagnoza je postavljena na osnovu visokog nivoa porfobilinogena u mokraći. Prethodna upotreba oralnih kontraceptiva, antidepresiva i tiopentala kao indukcionog agensa u opštoj anesteziji mogli su biti precipitirajući faktori. Bolesnica je lečena konzervativno visokim unosom ugljenih hidrata i humanim heminom. Šest meseci posle prijema prebačena je na Odeljenje fizikalne medicine i rehabilitacije. **Zaključak.** Postavljanje rane dijagnoze je osnovni korak uspešnog lečenja napada akutne intermitentne porfirije. Sledeći korak podrazumeva adekvatnu terapiju, praćenje i prevenciju nastanka ponovnih napada.

Ključne reči:
dijagnoza, diferencijalna; neurološke manifestacije; porfirija; porfirija, akutna, intermitentna; porfobilnogen; faktori rizika; lečenje, ishod.

Introduction

Porphyrias are rare inherited or acquired disorders caused by partial deficiency of enzymes in the biosynthesis of heme. There are cutaneous and neurological forms of the disease. However, generally looking, the disease is a result of enzyme dysfunction of the same biosynthetic pathway, but differences in organ affection are from mistakes in different isoenzymes of one enzyme, different processing of mRNA, and different microenvironment in cell matrix [1]. Acute intermittent porphyria (AIP) is a rare autosomal dominant disorder. Prevalence in Europe is 1–2 *per* 100,000 (highest is in Scandinavia with 1 *per* 1,500) [2–4].

Case report

A 22-year-old female patient was admitted from the local hospital under suspicion of developing sepsis. After

Correspondence to: Milić Veljović, Military Medical Academy, Clinical for Anesthesiology and Intensive Care, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: milic1210@yahoo.com

interviewing her parents, we found out that, at the beginning of the year, she had laparoscopic surgery of ovarian cysts and subsequently received advice to take oral contraceptives (one of the main provoking factors). After just a few weeks, she stopped the suggested therapy because of nausea, vomiting, apathy, and sleepiness. Three months later, she was hospitalized in a local hospital with significant epigastric pain and vomiting, back pain, and constipation that lasted for four days. During her hospital stay, she had also suffered from insomnia and hallucinations. The ward doctor consulted a psychiatrist who established the diagnosis of the somatoform disorder and prescribed her sulpiride (Eglonyl®, Alkaloid AD, N. Macedonia), one of the porphyrinogenic drugs. After series of medical tests and examinations, the patient was discharged with the diagnosis of biliary gastritis. The next day, at the request of her parents, she was admitted to a higher-level hospital in a town nearby due to further worsening of the abdominal pain and the appearance of a new symptom – inability to climb the stairs (due to proximal neuropathy). Computed tomography (CT) scan of the abdomen revealed widening of the transverse colon, which was interpreted as toxic megacolon, and the patient underwent a transversal colon resection. Twelve days later, she was transferred to our hospital.

Upon admission, the patient was awake, had brief periods of confusion, dyspneic with SpO_2 85% on room air, blood pressure 160/100 mmHg, heart rate 140/min, body temperature 37.7°C, central venous pressure (CVP) 6 cmH_2O, abdominal pain, active peristalsis, intestinal contents visible on stoma, and light yellow urine in urine bag. Laboratory values were: erytrocyte sedimentation rate (ESR) 47 mm/h, C-reactive protein (CRP) 119 mg/L (normal range less than 5 mg/L), leukocytes 17.4×10^9/L (normal range 3–12 $\times 10^9$/L), pH = 7.38 (normal range 7.35–7.45), $PaCO_2 = 52$ mmHg (normal range 38–42 mmHg), $PaO_2 = 119$ mmHg (normal range 75–100 mmHg), $HCO_3^- = 30.8$ mmol/L (normal range 23–29 mmol/L).

After admission, the patient had episodes of epileptiform seizures that lasted for a few minutes, and they were accompanied by apnea and complete loss of responsiveness. CT of thorax, abdomen, and pelvis revealed bilateral pleural effusions and pneumothorax on the right side, which was drained with a chest tube. After consulting with a neurologist, a CT scan of her head, electroencephalography (EEG) and electromyoneurography (EMNG) were performed. On the next day, further deterioration with more episodes of convulsions, quadriplegia, bulbar paralysis, and respiratory failure occurred, and eventually, intubation and mechanical ventilation were applied. We took blood cultures, smears, and 24 h collection of urine for porphobilinogen.

Brain CT scan revealed signs of suprasellar subarachnoid recession in the sella turcica and parenchymal cortical reduction frontally, bilaterally. There were no areas of demyelination.

EEG finding was well expressed with minor diffuse dysfunction and elevated irritability of frontal regions bilaterally. EMNG findings showed a loss of conduction in the proximal group of muscles with the existence of spontaneous muscle activity and preserved sensory fibres. Finally, when the results of porphobilinogen in urine arrived (904 μg/24 h, and normal values are less than 150 μg/24 h) diagnosis of acute porphyria was made.

Hyponatremia, present from the beginning, was treated with 3% saline infusions and specific therapy with 10% and 50% dextrose i.v. along with enteral feeding and administration of human hemin (Normosang® 25 mg/mL, Orphan Europe SARL, Puteaux, France) in the dose of 4 mg/kg/day, four days in a row. Therapy with Normosang® was given on two occasions, after 30 days and after three months. The first dose led to a minimal improvement in clinical symptoms, while the second dose had a better effect.

The patient was tracheotomized, and a percutaneous endoscopic gastrostomy was done. After 160 days of intensive care, she was transferred to the Department of Physical Medicine and Rehabilitation. The patient was discharged from the Intensive Care Unit with spontaneous breathing, an act of swallowing, and movements in all extremities (Figures 1 and 2).

Fig. 1 – Patient on discharge from the Intensive Care Unit.

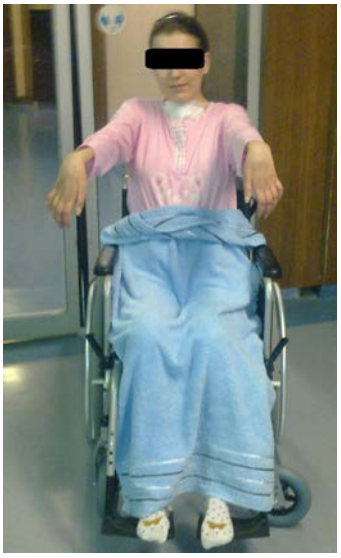


Fig. 2 – Patient with renewed movements in the extremities.

Discussion

AIP symptomatic is mainly heterozygotes and rarely homozygotes. It is characterized by a long latent period, and symptoms become manifested after puberty in the third and fourth decade of life after exposure to some provoking factors [5]. There are no cutaneous manifestations. AIP is a metabolic disorder caused by a deficiency of porphobilinogen deaminase (PBGD). Patients with AIP have acute attacks of neurovisceral symptoms (affection of the autonomic nervous system) followed by a high level of porphyrin precursors in urine. The first presentation of the disorder is in 85%–95% abdominal pain and in 45% peripheral neuropathy with motor weakness. The exact mechanism of neuronal damage is unknown; one of the proposed theories is the crystallization of byproducts in neural structures [5, 6].

A variety of clinical presentation is considerable: abdominal pain, nausea, vomiting, paralytic ileus, urinary retention or incontinence, tachycardia, arterial hypertension, sweating, tremor, postural hypotension, peripheral neuropathy (proximal because of axonal degeneration and demyelination), sensory neuropathy (paresthesias and dysesthesias), cranial neuropathy (VII and X), periodical cortical blindness (caused by vasospasm), epileptiform seizures and rarely bulbar paralysis, and death [7, 8].

Deficiency of PBGD is not enough for clinical manifestation; there must be a presence of provoking factors: medications (inductors of P450 cytochrome oxidase), fasting, hormones (progesterone), smoking, alcohol, or metabolic stress (infection, surgery, psychological stress) [1].

Diagnosis is based on clinical presentation and a high level of urine porphobilinogen. The most relevant thing is the high level of clinical suspicion [9, 10].

Treatment consists of the following steps: review all medications and discontinue any that can exacerbate acute porphyria, restore energy balance using an enteral route if possible, if not, apply dextrose in the dose of 300–400 g/day i.v., hemin 3–4 mg/kg i.v., given once a day for four days in order to prevent future attacks (treat intercurrent infections and other diseases promptly) [11, 12].

Chronic complications are hepatocellular carcinoma and renal failure [13, 14].

Conclusion

AIP is capable of mimicking a wide variety of medical conditions. It is important to have a high index of clinical suspicion and then, with a relatively simple biochemical test, confirm the diagnosis of AIP. Specialized care and prevention of future attacks are cornerstones of favorable outcomes. Nevertheless, we need more research in the future in order to obtain new therapeutic opportunities and detect parameters for monitoring the effects of therapy and prediction of outcome.

R E F E R E N C E S

1. *Bissell DM, Anderson KE, Bonkovsky HL*. Porphyria. N Engl J Med 2017; 377(9): 862–72.
2. *Nordmann Y, Puy H, Da Silva V, Simonin S, Robreau AM, Bonaiti C,* et al. Acute intermittent porphyria: prevalence of mutations in the porphobilinogen deaminase gene in blood donors in France. J Intern Med 1997; 242(3): 213–7.
3. *Elder G, Harper P, Badminton M, Sandberg S, Deybach JC*. The incidence of inherited porphyrias in Europe. J Inherit Metab Dis 2013; 36(5): 849–57.
4. *Chen B, Solis-Villa C, Hakenberg J, Qiao W, Srinivasan RR, Yasuda M*, et al. Acute Intermittent Porphyria: Predicted Pathogenicity of HMBS Variants Indicates Extremely Low Penetrance of the Autosomal Dominant Disease. Hum Mutat 2016; 37(11): 1215–22.
5. *Naik H, Stoecker M, Sanderson SC, Balwani M, Desnick RJ*. Experiences and concerns of patients with recurrent attacks of acute hepatic porphyria: A qualitative study. Mol Genet Metab 2016; 119(3): 278–83.
6. *Andersson C, Floderus Y, Wikberg A, Lithner F*. The W198X and R173W mutations in the porphobilinogen deaminase gene in acute intermittent porphyria have higher clinical penetrance than R167W. A population-based study. Scand J Clin Lab Invest 2000; 60(7): 643–8.
7. *Liu YP, Lien WC, Fang CC, Lai TI, Chen WJ, Wang HP*. ED presentation of acute porphyria. Am J Emerg Med 2005; 23(2): 164–7.
8. *Kumar S, Sharma N, Modi M, Sharma A, Mahi S, Varma S*. Spectrum of emergency department presentation in patients of acute intermittent porphyria: experience from a North Indian tertiary care center. Neurol India 2010; 58(1): 95–8.
9. *Anderson KE, Bloomer JR, Bonkovsky HL, Kushner JP, Pierach CA, Pimstone NR,* et al. Recommendations for the diagnosis and treatment of the acute porphyrias. Ann Intern Med 2005; 142(6): 439–50.
10. *Deacon AC, Peters TJ*. Identification of acute porphyria: Evaluation of a commercial screening test for urinary porphobilinogen. Ann Clin Biochem 1998; 35 (Pt 6):726.
11. *Harper P, Wahlin S*. Treatment options in acute porphyria, porphyria cutanea tarda, and erythropoietic protoporphyria. Curr Treat Options Gastroenterol 2007; 10(6): 444–55.
12. *Anderson KE, Bonkovsky HL, Bloomer JR, Shedlofsky SI*. Reconstitution of hematin for intravenous infusion. Ann Intern Med 2006; 144(7): 537–8.
13. *Stewart MF*. Review of hepatocellular cancer, hypertension and renal impairment as late complications of acute porphyria and recommendations for patient follow-up. J Clin Pathol 2012; 65(11): 976–80.
14. *Innala E, Andersson C*. Screening for hepatocellular carcinoma in acute intermittent porphyria: a 15-year follow-up in northern Sweden. J Intern Med 2011; 269(5): 538–45.

Received on June 13, 2019.
Revised on July 23, 2019.
Accepted on August 9, 2019.
Online First September, 2019.

CASE REPORT

UDC: 616.24-006-053.2-07/-08
DOI: https://doi.org/10.2298/VSP190530092V

A rare case of inflammatory myofibroblastic tumor presenting with pneumothorax

Redak slučaj inflamatornog miofibroblastnog tumora udruženog sa pneumotoraksom

Radomir Vešović*, Dragan Radovanović*†, Jelena Stojšić‡, Marko Popović*, Marina Moromila*

Clinical Center of Serbia, *Clinic for Thoracic Surgery, ‡Service of Histopathology, Belgrade, Serbia; †University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Abstract

Introduction. An inflammatory myofibroblastic tumor (IMT) is a rare mesenchymal tumor of unclear etiology, which demonstrates myofibroblastic differentiation accompanied by inflammatory cells. IMT is a frequent primary lung tumor in children and is of nonspecific symptomatology and imaging methods. Its definitive diagnosis requires histopathology and immunohistochemistry of the tissue sample obtained after a rigid bronchoscopy or after complete surgical resection. **Case report.** A 16-year-old male patient was admitted to our clinic for further treatment of IMT verified by rigid bronchoscopy. He had previously been treated at another institution for left-sided pneumothorax with thoracic drainage. Since it had not resulted in lung reexpansion, a chest computed tomography was performed followed by rigid bronchoscopy that eventually established IMT diagnosis in the distal part of the left main bronchus. Since the tumor surrounded the left lobar carina and infiltrated the pulmonary artery, pneumonectomy was undertaken. Its morphology and immunoprofile determined the IMT diagnosis. Four years after surgical resection, the patient showed no recidivism of the illness. **Conclusion.** IMT is one of the most frequent primary lung tumors in children and needs to always be suspected upon. Pneumothorax can appear as an IMT manifestation. Its occurrence could be the consequence of either a visceral pleura lesion in case of peripheral tumors or a ball valve mechanism in case of endobronchial tumors. Definitive diagnosis of IMT requires not only histopathology but also immunohistochemical analysis. Complete surgical resection results in the best survival rates. Further monitoring of patients is necessary due to the risk of recurrence.

Key words:
adolescent; diagnosis; lung neoplasms; pneumothorax; pneumonectomy; treatment outcome.

Apstrakt

Uvod. Inflamatorni miofibroblastni tumor (IMT) je redak mezenhimalni tumor, nejasne etiologije, koji pokazuje miofibroblastnu diferencijaciju udruženu sa inflamatornim ćelijama. IMT je čest primarni tumor pluća kod dece i nespecifične je simptomatologije kao i radiološkog nalaza. Za definitivnu dijagnozu potrebna je histopatološka i imunohistohemijska obrada materijala dobijenog nakon rigidne bronhoskopije ili nakon kompletne resekcije tumora. **Prikaz bolesnika.** Bolesnik, star 16 godina, primljen je u našu ustanovu radi nastavka lečenja IMT dokazanog rigidnom bronhoskopijom. Prethodno je bio lečen torakalnom drenažom zbog levostranog pneumotoraksa u drugoj ustanovi. Pošto reekspanzija pluća nije bila ostvarena, učinjena je kompjuterizovana tomografija grudnog koša, a potom i rigidna bronhoskopija kojom je postavljena dijagnoza IMT u distalnom delu levog glavnog bronha. Zbog zahvatanja leve lobarne karine i plućne arterije, učinjena je leva pneumonektomija. Morfološkom i imunohistohemijskom analizom dokazan je IMT. Bolesnik je bio bez recidiva četiri godine nakon operacije. **Zaključak.** Na IMT treba uvek posumnjati u dečijem dobu jer je jedan od najčešćih primarnih tumora pluća kod dece. Pneumotoraks se može javiti kao manifestacija IMT. Njegova pojava mogla bi biti posledica lezije visceralne pleure u slučaju perifernih tumora ili posledica valvularnog mehanizma kod endobronhijalnih tumora. U cilju postavljanja definitivne dijagnoze, osim histopatološke, neophodna je i imunohistohemijska analiza. Kompletna hirurška resekcija daje najbolju mogućnost za preživljavanje. Zbog mogućnosti recidiva neophodne su dalje kontrole ovih bolesnika.

Ključne reči:
adolescenti; dijagnoza; pluća, neoplazme; pneumotoraks; pneumonektomija; lečenje, ishod.

Correspondence to: Radomir Vešović, Clinical Centre of Serbia, Clinic for Thoracic Surgery, Koste Todorovića 26, 11 000 Belgrade, Serbia. E-mail: rvesovic@gmail.com

Introduction

An inflammatory myofibroblastic tumor (IMT) is a rare tumor. Its occurrence is more frequently documented in children than in adults [1, 2]. It usually manifests as peripheral nodes, less so endobronchially [3–5]. The etiology of IMT is uncertain. According to the classification of the World Health Organization (WHO), IMT is defined as an intermediate soft tissue tumor that demonstrates myofibroblastic differentiation accompanied by inflammatory cells, plasma cells, and lymphocytes [6]. It presents with nonspecific symptomatology, including cough, high fever, dyspnea, pneumonia, and chest pain [3–5]. Chest X-ray and computed tomography (CT) scan are not sufficient to determine it. Definitive diagnosis requires histopathology and immunochemical analysis of the adequate tissue obtained after rigid bronchoscopy and surgical procedure. Surgical resection is the method of choice in IMT treatment [2, 4]. We report a 16-year-old male patient with IMT in the lung manifested as a left-sided pneumothorax.

Case report

A 16-year-old male patient was admitted to our clinic for further diagnostics and treatment of IMT previously verified by rigid bronchoscopy.

The patient was originally hospitalized at another institution because of the left-sided spontaneous tension pneumothorax accompanied by pain, weakness, and cough. Thoracic drainage was performed, and because it did not result in lung reexpansion within a day, further tests were undertaken (Figure 1). Prior to hospitalization, the patient was experiencing weakness, chest pain, and cough to a smaller degree for a month. He had no history of lung diseases, thoracic trauma, or medical intervention that could have caused pneumothorax.

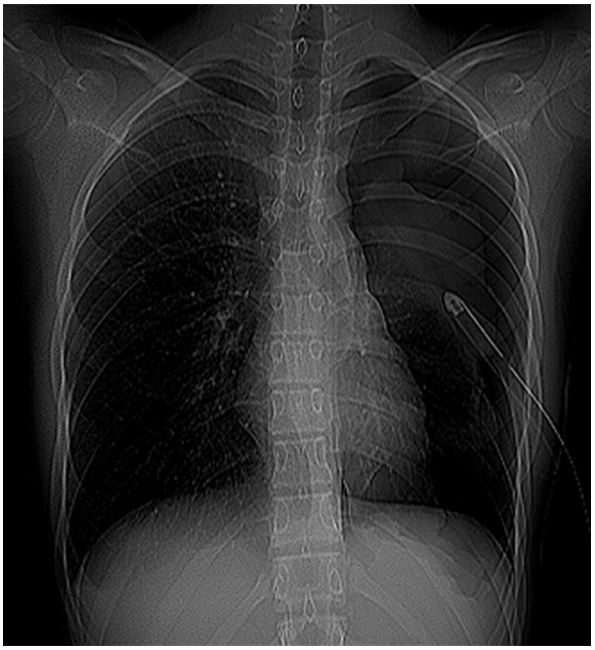


Fig. 1 – Chest X-ray showing left-sided pneumothorax and thoracic drain.

Thorax CT scan showed almost complete obstruction of the left main bronchus in its distal part by a hypodense soft-tissue tumor with dimensions 28 × 25 × 22 mm. On the neoplasm periphery, three amorphous calcified formations were detected, with the largest being 8 mm in diameter. The upper left lobe was atelectatic, while the lower was compressed with present pneumothorax (Figure 2).

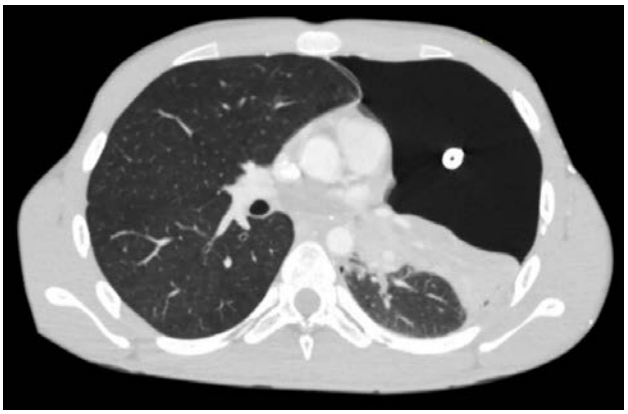


Fig. 2 – Computed tomography showing tumor almost completely obstructing left main bronchus and pneumothorax with atelectasis of the left upper lobe.

During rigid bronchoscopy, the tumor was seen as an off-white endobronchial mass in the left main bronchus located 4 cm from the carina of the trachea. Microscopically, according to its morphology and immunoprofile, the detected tumor was consistent with IMT with the understanding that its biological characteristics will be determined by surgical resection.

Laboratory analysis values fell within their respective reference intervals.

The patient was then referred to our clinic, where left pneumonectomy was performed by posterolateral thoracotomy after a completed preoperative procedure. Macroscopically, we noticed atelectasis of the upper left lobe that was unexpandable under positive airway pressure while the lower lobe was expandable. Visceral and parietal pleura were with no significant changes. A small amount of serous pleural effusion was present. Tumor location indicated pneumonectomy because it surrounded the left lobar carina and infiltrated the pulmonary artery. Neoplasm characteristics were consistent with endoluminal and intrapulmonary growth patterns. A solid off-white tumor was well separated from the surrounding lung tissue and was of dimensions 28 × 20 × 20 mm. The tumor did not spread to the visceral pleura. Microscopically, the tumor nodule was covered by regular respiratory epithelial cells (Figure 3a). The unencapsulated tumor consisted of a mixture of spindle cells in fascicles and those in storiform patterns. As expected, tumor cells had abundant light eosinophilic cytoplasm, oval nuclei, and inconspicuous nucleoli. Mitosis and cytologic atypia were not noticed. An inflammatory infiltrate containing numerous lymphocytes and a prominent number of plasma cells was between spindle mesenchymal cells. Histiocytes were also obtained, including some Touton-type giant cells (Figure 3b). Immunohisto-

chemically, all tumor cells expressed vimentin (Figure 3c), and most of them smooth-muscle-actin (SMA) (Figure 3d). Anaplastic lymphoma kinase (ALK) expression was detected in some tumor cells (Figure 3e). Ki67 was expressed in less than 10% nuclei of spindle tumor cells (Figure 3f).

The postoperative course of a surgical treatment proceeded with no complications. The patient was released from the hospital on the eighth postoperative day. In regular follow-up visits, four years after the procedure, the patient showed no recidivism of the illness.

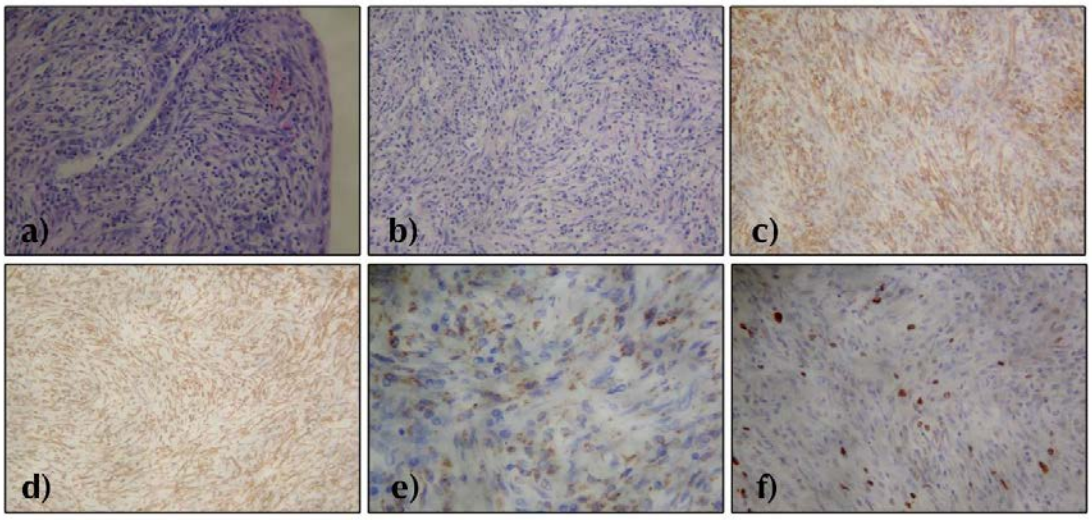


Fig. 3 – a) Endoluminal growth pattern of tumor, covered by respiratory epithelia [hematoxylin & eosin (HE), x10]; b) Mixed cellularity of pseudotumor, spindle, and inflammatory cells (HE, x40); c) The mesenchymal origin of the tumor was confirmed by vimentin (x20); d) Tumor cells originate from myofibroblasts [smooth-muscle-actin (SMA), x20]; e) Some tumor cells express anaplastic lymphoma kinase (ALK, x40); f) Ki67 is expressed in less than 10% nuclei of tumor cells (x40).

Discussion

IMT is a rare tumor of the mesenchymal original that more frequently occurs in children than in adults [1]. Of all reported IMTs, 35% were recorded in children under the age of 15 [2]. It is most commonly manifested as peripheral nodes in the lungs while much less so endobronchially [3–5]. In reported child cases, IMT accounts for around 20% of all primary lung tumors [7]. There are no gender differences in its incidence [7]. It gets detected most typically in the second decade of life [3].

The etiology of IMT is uncertain. According to the WHO classification from 2013, IMT is defined as an intermediate soft tissue tumor that demonstrates myofibroblastic differentiation accompanied by inflammatory cells, plasma cells, and lymphocytes [6].

It is considered that several genes and chromosomal abnormalities are linked to IMT occurrence. It is shown that chromosome translocation of ALK genes is present in around 50% of the reported IMT cases [1].

Recent studies have described fusions involving the ROS1 and PDGFRβ genes in a subset of ALK-negative cases [1]. Change in HMGIK (HMGA2) gene is also documented [8]. Aneuploidy is present in around half of the IMT patients and points to a tumor with aggressive behavior [9].

Various autoimmune diseases, trauma, as well as infections caused by *Mycobacterium tuberculosis* and other mycobacteraa, *Mycoplasma* spp., *Pseudomonas aeruginosa*, *Actinomycetes*, *Nocardia* spp., Epstein-Barr virus, and human herpesvirus are also believed to be linked to IMT occurrence [2, 3].

The clinical report is nonspecific. Around 40–50% of IMT patients are asymptomatic [2, 5] when it is accidentally discovered, while in other patients, it is manifested through cough, pain, hemoptysis, weakness, difficulty breathing, pneumonia, weight loss, and arthralgia [3–5]. Endobronchial tumor location is associated with the earlier manifestation of mentioned symptoms and a smaller percentage of asymptomatic cases, only around 21%, as opposed to the peripheral one [5].

In the literature, we were able to find only two cases of reported IMT associated with pneumothorax. In the first case, regarding peripheral tumor location, persistent pneumothorax existed, and its occurrence was explained by chronic visceral pleura lesion that was the result of repeated lung reexpansion and collapse accompanied by inflammation [10]. The significance of IMT on that lesion and pneumothorax development is unclear. We think that the peripheral IMT location could bring about chronic visceral pleura lesion and consequent pneumothorax. In the other reported case, the tumor was developed endobronchially in the presence of subcutaneous emphysema. It was hypothesized that, in this case, pneumothorax is a consequence of ball valve mechanism with subsequent air obstruction and alveolar rupture [5]. We consider this to be a pneumothorax development mechanism in the case of our patient, as well as subsequent complete upper lobe bronchus obstruction that could explain lung reexpansion inability after thoracic drainage and applied positive airway pressure during surgery. The existence of subcutaneous emphysema in the mentioned case could be explained by peribronchial air expansion further across medias-

tinum towards the neck and subcutaneous tissue, which did not happen in the case of our patient.

Chest radiographs are nonspecific as well. IMT is usually seen as a peripheral, solitary, lobulated, sharply circumscribed mass predisposed towards lower lobes [3].

Thorax CT scan is significant in determining the precise location of the lesion and its scope. The tumor manifests itself as a nonspecific heterogeneous mass with different contrast enhancement. Notable calcifications are infrequent, reported in only 15% of the cases [11], and more common in children than adults [3]. Lymphadenopathy is rarely documented [3, 11]. Multiple lesions are observed in 5% of the reported cases, and endobronchial involvement exists in 10% of the noted cases [3]. In the case of our patient, both endobronchial and intrapulmonary components of the tumor with peripheral calcifications were discerned.

Due to its nonspecific symptomatology and thus late diagnosis and treatment, its spreading towards mediastinum [3] is possible as was observed in our patient.

Radiographs, CT scans, fiberoptic bronchoscopy, and fine needle aspiration biopsy are usually not sufficient for definitive diagnosis. However, rigid bronchoscopy is advised when dealing with endobronchial tumors. It produces adequate tissue samples and facilitates definitive IMT diagnosis in over 80% of those cases [4]. In the case of our patient, rigid bronchoscopy was undertaken at the institution he was treated at before transferring to ours, and the obtained results supported IMT diagnosis, which was confirmed after surgical resection.

The established diagnosis of IMT was concluded upon surgical tumor specimen according to its growth pattern, morphology, and immunoprofile.

The characteristic morphology of this mostly unencapsulated tumor is a mixture of spindle cells with fascicular and storiform patterns and collagen, accompanied by lymphocytes, plasma cells, and histiocytes. We confirmed mesenchymal proliferation by vimentin expression and its myofibroblastic origin by focal SMA expression [12]. ALK was expressed in some tumor cells. It was reported that ALK was expressed in some tumor cells in 40% of the IMT cases [13]. No expression of S-100 protein excluded its neuroectodermal origin. The absence of cytokeratin-AE1/AE3 excluded lung spindle cell carcinoma, while its focal expression could be found in some cases of IMT. Its focal immunopositivity is potentially explained by alveolar entrapment. Ki67 was expressed in less than 10% of tumor cells, and that was a reason for 3–6-month follow-up visits due to the risk of IMT recurrence. The guidelines for IMT diagnosis followed the 2004 and 2015 WHO classification of lung tumors [12, 14].

The method of choice in IMT treatment is complete surgical resection. The 5-year postoperative survival rate is 91.3%, while recidivism rates are between 4% and 13% and are associated with incomplete surgical resection [4, 15].

When the complete resection is not doable or in the case of tumor recurrence or comorbidities, medical therapy alone or in combination with radiation needs to be considered.

Effects of nonsurgical IMT treatments are based upon single case reports and a small number of patients. Therefore, it is hard to talk about their contribution to future recommendations.

Carboplatin and paclitaxel chemotherapy could be used in certain cases, but generally, it did not provide satisfactory results. It is implemented in the treatment of recidivism and unresectable cases [16]. The application of vinorelbine and methotrexate has also shown success in certain IMT cases [17].

The use of corticosteroids is controversial because it resulted in complete tumor regression in certain cases [18] while, in other cases, it resulted in further tumor progression [19].

Nonsteroidal anti-inflammatory drugs (NSAID), such as celecoxib, due to its antiangiogenic effects, were implemented in some ALK and ROS-1 negative patients [20]. In ALK and ROS-1 positive patients, the use of tyrosine kinase inhibitor crizotinib produces certain positive effects [1].

Radiotherapy is used in patients when surgical treatment is not applicable, when recidivism is detected, or in the case of metastatic tumor [4].

IMT could be of different malignant potential, but it could generally be said that it has a benign course. Rare cases of distant metastases and spontaneous remission have been documented [3, 4]. The possibility of recidivism calls for further monitoring of these patients.

Conclusion

The inflammatory myofibroblastic tumor is one of the most frequent primary lung tumors in children, and it always needs to be suspected upon. Patients could be asymptomatic or manifest nonspecific symptomatology, such as cough, dyspnea, chest pain, exertion, and respiratory infections. Pneumothorax could be one of the IMT manifestations. Its occurrence could be the consequence of either a visceral pleura lesion in the case of peripheral tumors or a ball valve mechanism in the case of endobronchial tumors. Definitive diagnosis of IMT and treatment methods require not only histopathology but also immunohistochemical analysis. Complete surgical resection as a treatment of choice has, as a result, the best survival rates. Medical and radiation therapy are documented to be less effective and are used in selective cases. Further monitoring of patients is necessary due to the risk of recurrence.

REFERENCES

1. *Lovly CM, Gupta A, Lipson D, Otto G, Brennan T, Chung CT,* et al. Inflammatory myofibroblastic tumors harbor multiple potentially actionable kinase fusions. Cancer Discov 2014; 4(8): 889–95.
2. *Ochs K, Hoksch B, Frey U, Schmid RA.* Inflammatory myofibroblastic tumour of the lung in a five-year-old girl. Interact Cardiovasc Thorac Surg 2010; 10(5): 805–6.
3. *Narla LD, Newman B, Spottswood SS, Narla S, Kolli R.* Inflammatory pseudotumor. Radiographics 2003; 23(3): 719–29.
4. *Thistlethwaite PA, Renner J, Duhamel D, Makani S, Lin GY, Jamieson SW,* et al. Surgical management of endobronchial inflammatory myofibroblastic tumors. Ann Thorac Surg2011; 91(2): 367–72.

5. *El-Desoky T, Nasef N, Osman E, Osman A, Zaki A, Zalata K.* Endobronchial inflammatory pseudotumor: a rare cause of a pneumothorax in children. J Bronchology Interv Pulmonol 2013; 20(3): 256–60.
6. *Coffin CM, Fletcher JA.* Inflammatory myofibroblastictumour. In: *Fletcher CD, Bridge JA, Hogendoorn PC, Mertens F,* editors. World Health Organization classification of tumours. WHO classification of soft tissue and bone. 4th ed. Lyon: IARC Press; 2013. pp. 83–84.
7. *Hartman GE, Shochat SJ.* Primary pulmonary neoplasms of childhood: a review. Ann Thorac Surg 1983; 36(1): 108–19.
8. *Kazmierczak B, Dal Cin P, Sciot R, Van den Berghe H, Bullerdiek J.* Inflammatory myofibroblastic tumor with HMGIC rearrangement. Cancer Genet Cytogenet 1999; 112(2): 156–60.
9. *Biselli R, Ferlini C, Fattorossi A, Boldrini R, Bosman C.* Inflammatory myofibroblastic tumor (inflammatory pseudotumor): DNA flow cytometric analysis of nine pediatric cases. Cancer 1996; 77(4): 778–84.
10. *Mizuno Y, Iwata H, Shirahashi K, Matsui M, Takemura H.* Persistent spontaneous pneumothorax for four years: a case report. Prague Med Rep 2012; 113(4): 303–8.
11. *van den Heuvel DA, Keijsers RG, van Es HW, Bootsma GP, de Bruin PC, Schramel FM,* et al. Invasive inflammatory myofibroblastic tumor of the lung. J Thorac Oncol 2009; 4(7): 923–6.
12. *Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC.* World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press; 2004.
13. *Cessna MH, Zhou H, Sanger WG, Perkins SL, Tripp S, Pickering D,* et al. Expression of ALK1 and p80 in inflammatory myofibroblastic tumor and its mesenchymal mimics: a study of 135 cases. Mod Pathol 2002; 15(9): 931–8.
14. *Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG.* WHO Classification of Tumours of the Lung, Pleura. Lyon: Thymus and Heart; 2015.
15. *Hussain SF, Salahuddin N, Khan A, Memon SS, Fatimi SH, Ahmed R.* The insidious onset of dyspnea and right lung collapse in a 35-year-old man. Chest 2005; 127(5): 1844–7.
16. *Kubo N, Harada T, Anai S, Otsubo K, Yoneshima Y, Ijichi K,* et al. Carboplatin plus paclitaxel in the successful treatment of advanced inflammatory myofibroblastic tumor. Intern Med 2012; 51(17): 2399–401.
17. *Favini F, Resti AG, Collini P, Casanova M, Meazza C, Trecate G,* et al. Inflammatory myofibroblastic tumor of the conjunctiva: response to chemotherapy with low-dose methotrexate and vinorelbine. Pediatr Blood Cancer 2010; 54(3): 483–5.
18. *Bando T, Fujimura M, Noda Y, Hirose J, Ohta G, Matsuda T.* Pulmonary plasma cell granuloma improves with corticosteroid therapy. Chest 1994; 105(5): 1574–5.
19. *Panigada S, Sacco O, Girosi D, Magnano GM, Tuo P, Tarantino V,* et al. Corticosteroids may favor proliferation of thoracic inflammatory myofibroblastic tumors. Pediatr Pulmonol 2014; 49(3): E109–11.
20. *Ghani S, Desai A, Pokharel S, Demmy T, Dy GK.* Pneumonectomy-Sparing NSAID Therapy for Pulmonary Inflammatory Myofibroblastic Tumor. J Thorac Oncol 2015; 10(9): e89–e90.

Received on May 30, 2019.
Revised on July 21, 2019.
Accepted August 9, 2019.
Online First September, 2019.

CASE REPORT

UDC: 618.177-089.888.11-06:616.25-003.219
DOI: https://doi.org/10.2298/VSP190806100M

Catamenial pneumothorax after multiple failed *in vitro* fertilization cycles

Katamenijalni pneumotoraks nakon višestrukih neuspešnih ciklusa vantelesne oplodnje

Bojan Milačić*†, Obrad Vujadinović*, Batrić Vukčević†, Petar Milić‡

***Clinical Center of Montenegro, Center for Thoracic Surgery, Podgorica, Montenegro; †University of Montenegro, Faculty of Medicine, Podgorica, Montenegro; ‡General Hospital Kotor, General Surgery Department, Kotor, Montenegro**

Abstract

Introduction. Catamenial pneumothorax is spontaneous pneumothorax occurring within 72–96 h before and after menstrual bleeding. It is frequently associated with thoracic endometriosis. However, certain cases are not associated with any identifiable thoracic pathology. **Case report.** A 42-year-old woman with a history of pelvic endometriosis presented with sudden cough and shortness of breath on the first day of menstrual bleeding. Chest radiography revealed a complete right pneumothorax. The patient had previously undergone 7 failed *in vitro* fertilization cycles. Video-assisted thoracoscopic surgery showed pulmonary bullous lesions and a diaphragmatic fenestration. Atypical resection of the pulmonary apex was performed by an endostapler. Diaphragm plication was performed using Ethibond sutures. Definitive histopathological examination of the pulmonary tissue was negative for endometriosis. A postoperative course of gonadotropin-releasing hormone (GnRH) agonist triptorelin was administered during a period of 6 months. The patient's postoperative recovery was uneventful, without recurrence of pneumothorax to this day. **Conclusion.** There is a possibility that ovarian hyperstimulation caused the rupture of the pulmonary bullae. The patient may have developed endometriotic diaphragmatic fenestrations, activated by ovarian hyperstimulation, leading to pneumothorax. Early diagnosis and timely surgical treatment dealing with all thoracic pathology, as well as adjuvant hormonal treatment, may reduce the recurrence rate of catamenial pneumothorax.

Key words:
catamenial pneumothorax; fertilization in vitro; endometriosis; thoracic surgery, video-assisted.

Apstrakt

Uvod. Katamenijalni pneumotoraks je spontani pneumotoraks nastao u periodu od 72–96 h pre ili nakon menstrualnog krvarenja. Često je povezan sa torakalnom endometriozom. Međutim, u pojedinim slučajevima nije identifikovana torakalna patologija. **Prikaz bolesnika.** Bolesnica, stara 42 godine, sa prethodnom pelvičnom endometriozom javila se zbog naglog kašlja i osećaja nedostatka vazduha prvog dana menstrualnog ciklusa. Rendgenski snimak grudnog koša prikazao je kompletni desnostrani pneumotoraks. Pre ovog događaja, bolesnica je prošla 7 neuspešnih ciklusa vantelesne oplodnje. Video-asistirana torakoskopija pokazala je bulozne lezije pluća i fenestraciju dijafragme. Učinjena je atipična resekcija plućnog vrha endostaplerom, kao i plikacija dijafragme Ethibond šavovima. Definitivni histopatološki pregled tkiva nije dokazao endometriozu. Postoperativno, u toku 6 meseci, bio je primenjen triptorelin, agonist gonadotropin-*releasing* (oslobađajućeg) hormona (GnRH). Postoperativni tok je bio uredan, bez recidiva pneumotoraksa. **Zaključak.** Postoji mogućnost da je ovarijalna hiperstimulacija izazvala rupturu plućnih bula. Takođe, moguće je da je bolesnica imala endometriozne fenestracije dijafragme aktivirane ovarijalnom hiperstimulacijom, što je dovelo do pneumotoraksa. Rana dijagnoza i pravovremeni hirurški tretman, kao i adjuvantna hormonska terapija, mogu smanjiti stopu recidiva katamenijalnog pneumotoraksa.

Ključne reči:
katamenijalni pneumotoraks; oplođenje in vitro; endometrioza; hirurgija, torakalna, video-asistirana.

Correspondence to: Batrić Vukčević, University of Montenegro, Faculty of Medicine, Kruševac bb, 81 000 Podgorica, Montenegro. E-mail: batricvukcevic@gmail.com

Introduction

Catamenial pneumothorax (CP) is a term used to describe the occurrence of spontaneous pneumothorax during the period of 72–96 h before and after menstrual bleeding [1]. Traditionally, CP was considered a rare entity (not exceeding 3–6% of all spontaneous pneumothorax cases) [2], probably owing to a decreased disease awareness, as well as underdiagnosis [3]. CP is usually seen in women aged 32–35 [1], most commonly affecting the right lung [4, 5]. Haga et al. [6] suggested 4 criteria for distinguishing CP from spontaneous pneumothorax: right-sided pneumothorax, history of pelvic endometriosis, 31 years of age or older, and no history of smoking. CP is often associated with thoracic endometriosis (TE) [7]. TE is explained through several plausible mechanisms: migration of endometrial cells (through the fallopian tubes and the diaphragmatic fenestrations caused by the cyclical proliferation and necrosis of the diaphragmatic endometriotic foci), hematogenous microembolization, and lymphatic metastasis [1]. CP is the most common clinical manifestation of TE [8–11]. Pelvic endometriosis is described in 20–70% of CP patients – similar to TE, which is not always confirmed in cases of CP (either macroscopically or histologically) [3]. There are opinions that the histopathological diagnosis of TE can be made only if both endometrial stroma and glands are present in the examined tissue, while the presence of stroma alone yields only a "probable" diagnosis [12].

Certain cases of CP may present with no identifiable thoracic pathology [13]. Theoretical explanation of CP occurring independently of TE takes into account the physiological effect of prostaglandin E2 secreted during menses (causing bronchoconstriction and subsequent alveolar damage, especially in blebs) [14], as well as possible passage of air from the fallopian tubes through congenital fenestrations in the diaphragm [1]. A case of pneumothorax occuring after multiple in vitro fertilization (IVF) attempts is described herein, with a discussion of potential pathophysiological mechanisms.

Case report

In January 2018, a 42-year-old woman presented with sudden cough and shortness of breath on the first day of menstrual cycle. A chest radiography revealed a complete right pneumothorax (Figure 1). The patient's history was positive for pelvic endometriosis [presenting as bilateral ovarian cysts which were extirpated laparoscopically in 2010, followed by treatment with a gonadotropin-releasing hormone (GnRH) agonist goserelin]. The patient denied having previous pneumothoraces. During the period between 2010 and 2018, she underwent 7 failed IVF cycles.

On admission, she was treated primarily with a chest tube (Figure 2), followed by video-assisted thoracoscopic surgery (VATS) to explore the right hemithorax. The apex of the right lung showed bullous lesions (Figure 3), and an area of fenestration was noticed in the right tendinous portion of the diaphragm (Figure 4). An atypical resection of the pulmonary apex was performed with an endostapler. Also, diaphragm plication was performed with Ethibond sutures. The lung tissue was examined histologically *ex tempore*, with several tissue fragments showing the structure suggestive of endometriosis on hematoxylin and eosin stain. However, the definitive histopathological examination of the resected tissue showed bullous changes without definitive signs of endometriosis (lack of endometrial glands or stroma, as well as the negative immunohistochemical test for estrogen receptors). A postoperative course of GnRH agonist triptorelin was administered during the period of 6 months. The patient's postoperative recovery was uneventful, without a recurrence of pneumothorax to this day.

Fig. 1 – Chest radiography showing a complete right pneumothorax.

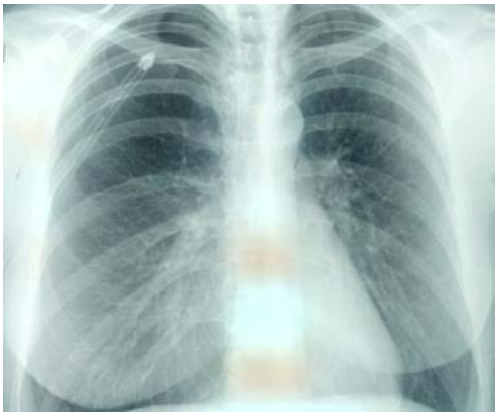


Fig. 2 – Chest radiography showing the resolution of pneumothorax after chest tube placement.

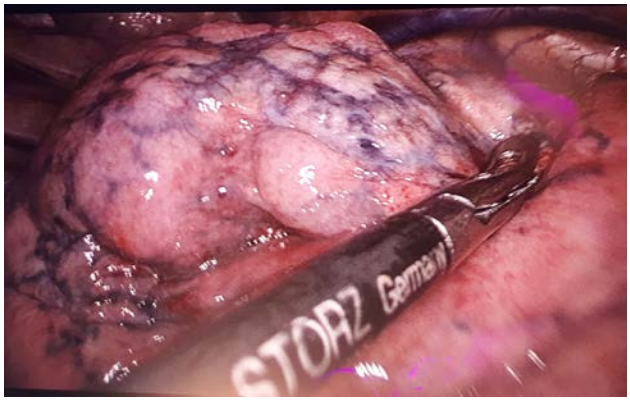


Fig. 3 – The right pulmonary apex seen during video-assisted thoracoscopic surgery, showing bullous lesions.

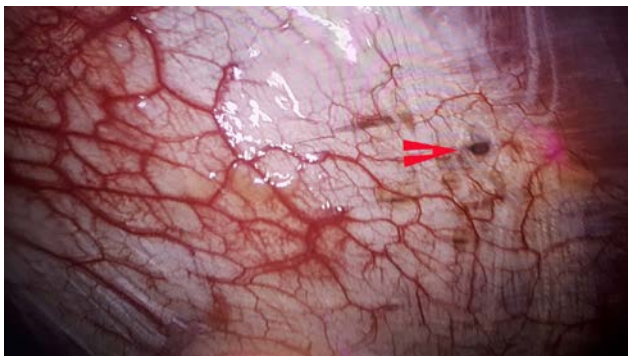


Fig. 4 – The right portion of the central tendon of the diaphragm showing a fenestration (red arrowhead).

Discussion

To the best of our knowledge, this is the fourth report of CP occurring after IVF attempts. Garg and McKenzie Gray [15] reported a case of a 26-year-old woman with a history of pelvic endometriosis, presenting with two episodes of CP which occurred three years after IVF. She underwent VATS pleurodesis and pleural abrasion, with several diaphragmatic endometriotic foci identified. Afterwards, she had two recurrences of CP, which ceased after the initiation of treatment with GnRH agonists [15]. Baisi et al. [16] reported a case of right-sided CP occurring after an unsuccessful IVF attempt with a GnRH agonist as a suppressant of pituitary activity, follitropin alpha [recombinant follicle-stimulating hormone (FSH)] for controlled ovarian hyperstimulation, and finally, human chorionic gonadotropin (hCG) for ovulation induction. During surgery, endometriotic foci of the parietal pleura and the tendinous portion of the diaphragm were observed, but without diaphragmatic rupture. Ruptured blebs were seen at the apex of the inferior lobe, and were removed by stapler. The endometrial foci were coagulated by argon plasma and removed, after which a partial pleurectomy (from the first to the sixth rib) and pleural abrasion were performed. Histopathology did not show any endometrial implants in the resected pulmonary tissue. During a 22-month follow up, the patient underwent a successful IVF-embryo transfer (ET) cycle, without a pneumothorax recurrence. The authors concluded that CP was caused by the rupture of the pulmonary blebs, which were likely caused by ovarian hyperstimulation [16]. Halvorson et al. [17] described a case of a woman with a history of endometriosis undergoing IVF with FSH, human menopausal gonadotropin and hCG. She presented with shortness of breath and right-sided pleuritic pain. Computed tomography (CT) revealed a large left pleural effusion and a right hydropneumothorax, treated with bilateral thoracentesis (serosanguinous fluid was extracted). Several months afterwards, the patient was diagnosed with a diaphragmatic hernia which was treated surgically. The intraoperative findings were suggestive of congenital diaphragmatic agenesis. Pleural biopsy showed endometrial tissue, and the patient presented with two additional episodes of CP, after which she was treated with pleurectomy and pleurodesis. The authors suggested that the congenital diaphragmatic defect allowed the endometrial cells to pass inside the thorax, creating endometriotic pleural foci which were inflamed by ovarian hyperstimulation, and presented with hemorrhagic pleural effusions [17]. The hypotheses made in these case reports suggest that ovarian hyperstimulation may facilitate the rupture of pulmonary blebs, as well as activate the endometrial foci in the thorax. There is a possibility that the ovarian hyperstimulation caused the rupture of the pulmonary bullae in the patient presented herein – similar to the case reported by Baisi et al. [16]. It is also plausible that this patient had endometriotic fenestrations in the diaphragm which were activated by ovarian hyperstimulation, leading to their rupture and subsequent pneumothorax.

Despite the fact that the diaphragmatic lesions were not examined histologically, their gross appearance leads to a suspicion that they are endometriotic in origin. The uncertainty of histological diagnosis of TE in CP cases is noted in the relevant literature. In a review of 9 CP cases, Furuta et al. [18] described 5 patients with circular red spots and/or perforations in the diaphragm, with a histopathologic verification of endometriosis in three patients.

Subotić et al. [2] presented 4 cases of CP owing to multiple small diaphragmatic lesions typical for CP (1–3 mm in size), associated with pulmonary bullae in two patients and a large diaphragm defect in one patient. Endometriosis was confirmed histologically in two patients [2]. Radiological criteria for CP are not defined. Several findings may lead to a clinical suspicion of CP: diaphragmatic hernia, defects or perforations [19], as well as pneumoperitoneum associated with right-sided pneumothorax [20]. CT may reveal the endometriotic foci viewed as ground-glass opacities or nodules. Magnetic resonance imaging (MRI) is superior to CT in displaying diaphragmatic lesions (especially hemorrhagic ones), and small pleural foci [11].

Video-assisted thoracoscopic surgery (VATS) is the approach of choice for the treatment of CP [1, 21], with all suspected endometriotic foci removed and all diaphragmatic fenestrations excised or closed. Korom et al. [9] proposed that thoracic exploration should be timed near the beginning of the menses in order to increase the visibility of the endometrial implants. Alifano et al. [12] suggested that pleurodesis plays a major role in the prevention of CP recurrence (from microscopic or newly implanted TE foci). Mechanical pleurodesis may fail to address the diaphragmatic surface. Therefore, parietal pleurectomy and selective use of talc on the diaphragmatic surface should be considered [14].

Hormonal therapy aims at suppression of the ectopic endometrium by oral contraceptive pills, danazol, progestins, and GnRH agonists [22]. GnRH agonists induce hypogonadotropic hypogonadism, which is believed to be useful in the prevention of CP recurrence during the period of 6–12 months after surgery [2], until effective pleurodesis is accomplished [3].

Immediate surgery followed by adjuvant hormonal suppression therapy is considered to be the optimal therapeutic approach [3, 23]. The positive patient outcome described in this paper serves as an example of a successful

combination of surgery and hormonal therapy. A possible see-saw effect of the IVF procedures on the diaphragmatic and pulmonary endometriotic foci may be considered: GnRH agonists may protect the patient from CP, while ovarian hyperstimulation has the potential to stimulate the ectopic endometrium, leading to CP recurrence.

Conclusion

CP should be suspected in young women with pneumothorax occuring during the perimenstrual period. Characteristic findings (mainly diaphragmatic holes or macroscopic endometriotic foci) are highly suggestive and – in our opinion – sufficient for the diagnosis, given the fact that the histopathologic findings of endometrial glands or stroma are rarely identified. The pattern of pneumothoraces may not always be catamenial (particularly when occurring in the premenstrual period). The surgical treatment should be early, with the aim to address all lesions (if feasible), as well as the presence of chronic disease (ie. endometriosis). Diaphragm reconstruction is required each time when fenestrations are found. Hormonal treatment aims at achieving amenorrhea immediately after surgery, and it should be administered in all cases of proven CP, unless there are significant contraindications. Early diagnosis and timely treatment dealing with all thoracic pathology (including diaphragmatic repair), together with multidisciplinary approach and hormonal treatment dealing with the main chronic disease, may reduce the recurrence rate of CP.

Conflict of interest

None declared.

REFERENCES

1. *Marjański T, Sowa K, Czapla A, Rzyman W*. Catamenial pneumothorax - a review of the literature. Kardiochir Torakochirurgia Pol 2016; 13(2): 117–21.
2. *Subotic D, Mikovic Z, Atanasijadis N, Savic M, Moskovljevic D, Subotic D*. Hormonal therapy after the operation for catamenial pneumothorax - is it always necessary? J Cardiothorac Surg 2016; 11(1): 66.
3. *Visouli AN, Zarogoulidis K, Kougioumtzi I, Huang H, Li Q, Dryllis G,* et al. Catamenial pneumothorax. J Thorac Dis 2014; 6(Suppl 4): S448–60.
4. *Rousset-Jablonski C, Alifano M, Plu-Bureau G, Camilleri-Broet S, Rousset P, Regnard JF,* et al. Catamenial pneumothorax endometriosis-related pneumothorax: clinical features and risk factors. Hum Repord 2011; 26(9): 2322–9.
5. *Fukuda S, Hirata T, Neriishi K, Nakazawa A, Takamura M, Izumi G,* et al. Thoracic endometriosis syndrome: Comparison between catamenial pneumothorax or endometriosis-related pneumothorax and catamenial hemoptysis. Eur J Obstet Gynecol Reprod Biol 2018; 225: 118–23.
6. *Haga T, Kataoka H, Ebana H, Otsuji M, Seyama K, Tatsumi K,* et al. Thoracic endometriosis-related pneumothorax distinguished from primary spontaneous pneumothorax in females. Lung 2014; 192(4): 583–7.
7. *Peikert T, Gillespie DJ, Cassivi SD*. Catamenial pneumothorax. Mayo Clin Proc 2005; 80(5): 677–80.
8. *Channabasavaiah AD, Joseph JV*. Thoracic endometriosis: revisiting the association between clinical presentation and thoracic pathology based on thoracoscopic fndings in 110 patients. Medicine (Baltimore) 2010; 89: 183–8.
9. *Korom S, Canyurt H, Missbach A, Schneiter D, Kurrer MO, Haller U*, et al. Catamenial pneumothorax revisited: clinical approach and systematic review of the literature. J Thorac Cardiovasc Surg 2004; 128(4): 502–8.
10. *Larraín D, Suárez F, Braun H, Chapochnick J, Diaz L, Rojas I*. Thoracic and diaphragmatic endometriosis: Single-institution experience using novel, broadened diagnostic criteria. J Turk Ger Gynecol Assoc 2018; 19(3): 116–21.
11. *Maniglio P, Ricciardi E, Meli F, Vitale SG, Noventa M, Vitagliano A*, et al. Catamenial pneumothorax caused by thoracic endometriosis. Radiol Case Rep 2017; 13(1): 81–5.
12. *Alifano M, Jablonski C, Kadiri H, Falcoz P, Gompel A, Camilleri-Broet S*, et al. Catamenial and noncatamenial, endometriosis-related or nonendometriosis-related pneumothorax referred for surgery. Am J Respir Crit Care Med 2007; 176(10): 1048–53.
13. *Visouli AN, Darwiche K, Mpakas A, Zarogoulidis P, Papagiannis A, Tsakiridis K*, et al. Catamenial pneumothorax: a rare entity? Report of 5 cases and review of the literature. J Thorac Dis 2012; 4(Suppl 1): 17–31.
14. *Marshall MB, Ahmed Z, Kucharczuk JC, Kaiser LR, Shrager JB*. Catamenial pneumothorax: optimal hormonal and surgical management. Eur J Cardiothorac Surg 2005; 27(4): 662–6.
15. *Garg V, McKenzie Gray B*. An unusual case of catamenial pneumothorax. J Obstet Gynaecol 2008; 28(3): 354–5.
16. *Baisi A, Raveglia F, De Simone M, Calati AM, Leporati A, Cioffi U*. Endometriosis-related pneumothorax after in vitro fertilization embryo transfer procedure: a case report. J Thorac Cardiovasc Surg 2010; 139(4): e88–9.
17. *Halvorson SA, Ricker MA, Barker AF, Patton PE, Harrison RA, Hunter AJ*. Thoracic endometriosis unmasked by ovarian hyperstimulation for in vitro fertilization. J Gen Intern Med 2012; 27(5): 603–7.
18. *Furuta C, Yano M, Numanami H, Yamaji M, Taguchi R, Haniuda M*. Nine cases of catamenial pneumothorax: a report of a single-center experience. J Thorac Dis 2018; 10(8): 4801–5.
19. *Roth T, Alifano M, Schussler O, Magdaleinat P, Regnard JF*. Catamenial pneumothorax: chest X-ray sign and thoracoscopic treatment. Ann Thorac Surg 2002; 74(2): 563–5.
20. *Downey DB, Towers MJ, Poon Py, Thomas P*. Pneumoperitoneum with catamenial pneumothorax. AJR Am J Roentgenol 1990; 155(1): 29–30.
21. *Junejo SZ, Singh Lubana S, Shina SS, Tuli SS*. A Case of Thoracic Endometriosis Syndrome Presenting with Recurrent Catamenial Pneumothorax. Am J Case Rep 2018; 19: 573–6.
22. *Augoulea A, Lambrinoudaki I, Christodoulakos G*. Thoracic endometriosis syndrome. Respiration 2008; 75(1):113–9.
23. *Hwang SM, Lee CW, Lee BS, Park JH*. Clinical features of thoracic endometriosis: A single center analysis. Obstet Gynecol Sci 2015; 58(3): 223–31.

Received on August 6, 2019.
Accepted on September 5, 2019.
Online First September, 2019.

CASE REPORT

UDC: 616.61-006
DOI: https://doi.org/10.2298/VSP190523108L

A case report of an unusual kidney tumor: Mucinous tubular and spindle cell carcinoma

Prikaz bolesnika sa retkim tumorom bubrega – mucinozni tubularni vretenastoćelijski karcinom

Tanja Lakić*†, Aleksandra Ilić*†, Mirjana Zivojinov*†, Jelena Ilić Sabo*†, Dimitrije Jeremić*‡, Dragan Grbić‡

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

Abstract

Introduction. Mucinous tubular and spindle cell carcinoma of the kidney (MTSCC) is a rare and recently described subtype of renal cell carcinoma (RCC). As its name indicates, the tumor is composed of three morphological structures: tubules consisted of cuboidal cells, spindle cells, and extracellular mucus. **Case report.** A 59-year-old female patient was admitted to the Emergency Center of the Clinical Center of Vojvodina due to injuries sustained in a traffic accident. After diagnostic procedures, computer tomography (CT) revealed a large asymptomatic cyst of the upper pole of the left kidney as an incidental finding. Four months later, after recovering from traumatic injuries, a control CT confirmed a well-circumscribed tumor, 90 mm in diameter, confined to a kidney, and a radical nephrectomy was performed. Histopathological evaluation showed that the necrotic tumor mass consisted of tubules made of cuboidal cells and cords made of spindle cells separated by pale mucinous material in some areas, while other tumor parts were of dense cellularity without mucin. No atypia was found. **Conclusion.** MTSCC is a variant of papillary RCC, thus, it is usually mistaken with papillary RCC with sarcomatoid differentiation. Because of the same immunoprofile as papillary RCC, histomorphology is imposed as the gold standard for making the diagnosis. MTSCC is a tumor with a generally favorable prognosis, and complete surgical excision appears to be adequate treatment, but single cases with metastatic disease have been reported. In this case, there were no signs of the disease one year after surgery.

Key words:
kidney neoplasms; carcinoma, renal cell; adenocarcinoma mucinous; diagnosis; nephrectomy; treatment outcome.

Apstrakt

Uvod. Mucinozni tubularni vretenastoćelijski karcinom bubrega (MTSCC) je redak i nedavno opisan podtip karcinoma bubrežnih ćelija (RCC). Kao što mu ime kaže, tumor se sastoji od tri morfološki različite strukture: tubuli sačinjeni od kubičnih ćelija, zatim vretenaste ćelije i ekstracelularna sluz. **Prikaz bolesnika.** U radu je prikazana 59-godišnja bolesnica koja je primljena u Urgentni centar Kliničkog centra Vojvodine zbog povreda nastalih u saobraćajnoj nesreći. Nakon dijagnostičkih procedura, kompjuterskom tomografijom (CT) otkrivena je asimptomatska velika cista gornjeg pola levog bubrega kao uzgredni nalaz. Četiri meseca kasnije, nakon oporavka od traumatskih povreda, kontrolnim CT pregledom potvrđen je dobro ograničen tumor, promera 90 mm, ograničen na bubreg, zbog čega je izvršena radikalna nefrektomija. Histopatološkom analizom ustanovljeno je da se radi o nekrotičnoj tumorskoj masi sastavljenoj od tubula sagrađenih od kubičnih ćelija i snopova vretenastih ćelija, razdvojenih bledim mucinoznim materijalom u nekim područjima, dok su ostali delovi tumora bili bez produkcije mucina i prisustva atipije. **Zaključak.** MTSCC je varijanta papilarnog RCC, pa se obično pogrešno dijagnostikuje kao papilarni RCC sa sarkomatoidnom diferencijacijom. Zbog istog imunoprofila sa papilarnim RCC, histomorfologija predstavlja zlatni standard za postavljanje dijagnoze. MTSCC je tumor sa, generalno povoljnom prognozom i kompletna hirurška ekscizija je adekvatan tretman lečenja, iako su opisani pojedinačni slučajevi metastatske bolesti. Kod ove bolesnice nije bilo znakova bolesti godinu dana nakon operacije.

Ključne reči:
bubreg, neoplazme; karcinom bubrežnih ćelija; adenokarcinom, mucinozni; dijagnoza; nefrektomija; lečenje, ishod.

Correspondence to: Tanja Lakić, Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. E-mail: tanja.lakic@mf.uns.ac.rs

Introduction

Mucinous tubular and spindle cell carcinoma of the kidney (MTSCC) is a rare and recently described subtype of renal cell carcinoma (RCC), which has been recognized as a specific entity in the 2004 World Health Organization (WHO) classification of renal cell carcinoma [1]. To this date, there are approximately 100 cases described in the literature [2]. The tumor mostly affects adults with a mean age of 53 years (range 18–82) with marked female predominance (4:1) as in our case [1], in contrast to RCC. MTSCC is a cytologically low-grade neoplasm, but both high-grade MTSCC and MTSCC with sarcomatoid differentiation are described [3, 4].

Clinical symptoms such as flank pain, abdominal mass, and hematuria are rare but possible [5]; most MTSCC tumors are solitary and were incidentally discovered by ultrasound or computer tomography examinations.

As its name indicates, the tumor is composed of three morphologically different structures: tubules consisted of cuboidal cells, spindle cells, and extracellular mucus and it mostly resembles type 1 papillary RCC but without true papilla formation [6]. In both cuboidal and spindle cells, nuclear atypia and mitoses are rare. The proportion of those morphological parts is different in different cases, and hemorrhage and/or necrosis, minor areas with clear cell and oncocytic change, are possible to find as well [7, 8]. Focal papillations may be found and are usually mistaken with papillary RCC with sarcomatoid differentiation.

Although the literature shows contradictory reports regarding its histogenesis [9, 10], the morphological, immunohistochemical, and genetic features suggest differentiation from collecting duct epithelium. Moreover, immunohistochemical analyses show expression of epithelial membrane antigen (EMA), alpha-methylacyl-coenzyme A racemase (AMACR), cytokeratin 7 (CK7), PAX-8, and vimentin in 80–100% of the cases, which confirms a possible origin from distal convoluted epithelial cells [11]. AMACR expression can also be seen in proximal tubule cells. Therefore, there is a belief that it could be a variant of papillary RCC [10]. CD-10 and RCC are often negative.

MTSCC is a renal tumor with an excellent prognosis, but it is not exclusive because there are single cases with the metastatic disease described.

Case report

In December 2017, a 59-year-old female patient was admitted to the Emergency Center of the Clinical Center of Vojvodina due to injuries sustained in a traffic accident. After diagnostic procedures, a serial fracture of ribs was registered, some subcutaneous hematomas, and no signs of internal bleeding. Moreover, computer tomography (CT) revealed a large cyst on the left kidney as an incidental finding without symptoms (Figure 1A). After recovering from traumatic injuries, the patient came to the Urology Clinic of the Clinical Center of Vojvodina because of hematuria. In April 2018, a control CT was made, and it confirmed the existence of a round, clearly demarcated, inhomogeneous mass, 85 millimeters in size, which changed the kidney contour and made pressure on calyces of the medium part and the upper pole of the kidney (Figure 1B). The lumen of the cyst was filled with debris-like material, and surgical treatment was indicated. A left radical nephrectomy was performed in June 2018, and the specimen was sent to the Pathology Department. Grossly, it was a well-circumscribed tumor, 90 mm in diameter, confined to the kidney with the necrotic content inside. A detailed histopathological examination verified the tumor mass characterized by a mixture of compressed anastomosing and tightly packed and parallelly arranged tubular structures consisted of cuboidal cells with round, pale nuclei (Figure 2A). Atypia was minimal. Tubular structures were separated by thin bundles of spindle cells and variable amounts of extra-

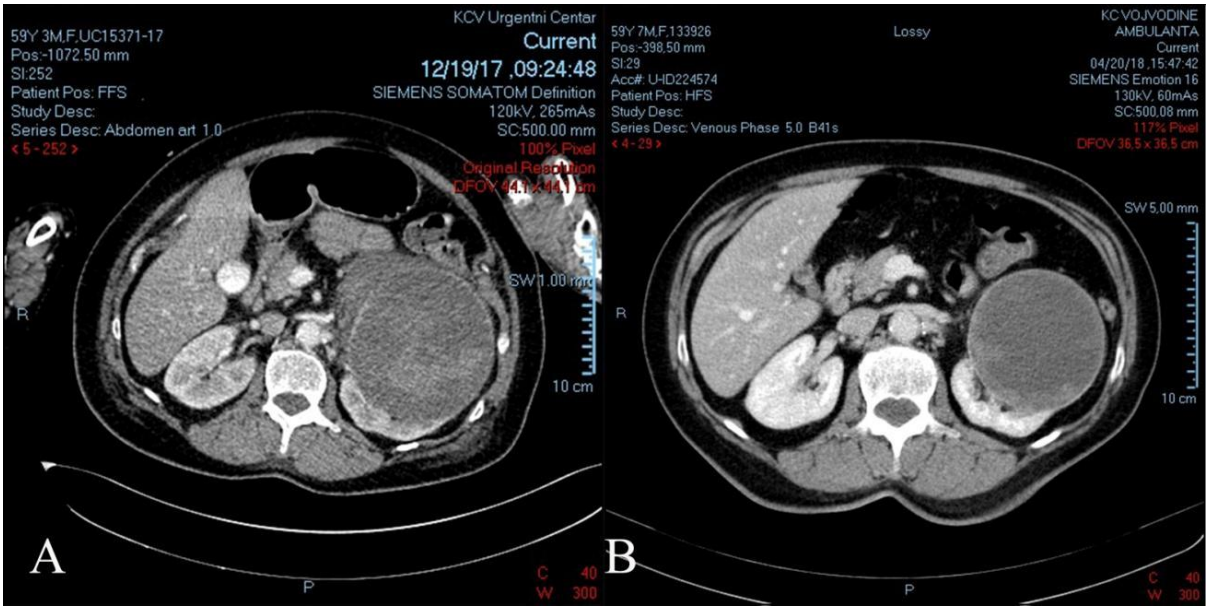


Fig. 1 – A) The first computer tomography (CT) scan showed a big cyst of the left kidney; B) Control CT with the same kidney cyst.

cellular blue-gray Alcian-blue-periodic-acid-Schiff positive mucinous matrix (Figure 2B). Extensive areas of necrosis were present without lymphovascular invasion. Special immunohistochemical stainings showed positivity for CK7 (Figure 2C), AMACR (Figure 2D), Vimentin (Figure 2E), as well as EMA and E-cadherin. RCC and CD-10 were negative. Based on the histological and immunohistochemical description of the tumor, the diagnosis of mucinous tubular and spindle cell renal cell carcinoma was made.

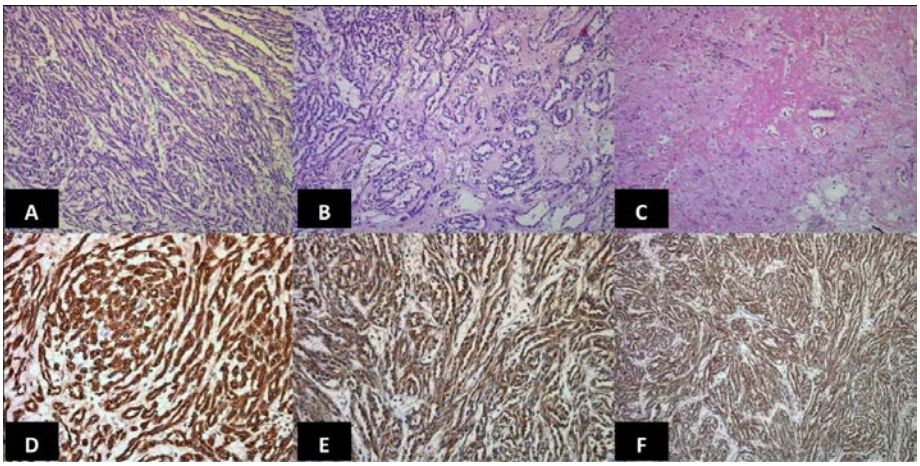


Fig. 2 – A) Spindle cell tumor component [hematohylin and eosin (HE), 10×]; B) Tubular structures of cuboidal cells (HE, 10×); C) Extracellular mucinous matrix (HE, 10×); D) Antibody cytokeratin 7 (CK7) (IHC, 20×); E) Antibody alpha-methylacyl-coenzyme (AMACR) (IHC, 20×); F) Antibody Vimentin (IHC, 10×). IHC – immunohistochemistry.

Discussion

Mucinous tubular and spindle cell renal cell carcinoma is a rare renal tumor. The histogenesis of this tumor is controversial. It has been shown differentiation toward the distal nephron, which led to different tumors, including low-grade tubular mucinous renal neoplasms and low-grade myxoid tumor. Recently, the literature showed immunohistochemical overlap with papillary RCC pointing to proximal tubular differentiation. In fact, MTSCC is a variant of papillary RCC. Although a close relationship to papillary RCC has been suggested, clinically, morphologically, and genetically, it is a distinct renal neoplastic entity. Histopathological examination is imperative for making the correct diagnosis. Immunohistochemistry is not a very useful method for discriminating between papillary RCC and MTSCC, thus histomorphology appears to be the gold standard for making the diagnosis. It is a tumor with a generally favorable prognosis, and complete surgical excision appears to be adequate treatment, but cases with metastatic disease have been reported [12]. Generally, it is estimated that 20–40% of patients with RCC develop metastases after surgery [13]. An unfavorable course of the disease like recurrence, regional lymph nodes, and distant sites metastases, or even death, are associated with atypical histological features such as high nuclear grade and sarcomatoid transformation [14, 15]. Although an innocent outcome is likely, a close follow-up is recommended. In our patient, there were no signs of the disease one year after surgery.

Conclusion

MTSCC is a variant of papillary RCC, hence it is usually mistaken with papillary RCC with sarcomatoid differentiation. Because of the same immunoprofile as papillary RCC, histomorphology is imposed as the gold standard for making the diagnosis. MTSCC is a tumor with a generally favorable prognosis, and complete surgical excision appears to be adequate treatment, but cases with metastatic disease have been reported. In this patient, there were no signs of the disease one year after surgery.

REFERENCES

1. *Eble JN, Sauter G, Epstein JI, Sesterhenn IA.* World Health Organization Classification of Tumours Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon: IARC Press; 2004.
2. *Chander B, Bharti R, Preet K, Deb P.* Mucinous tubular and spindle cell carcinoma of kidney: A case report of an unusual tumor. J Can Res Ther 2015; 11(3): 659.
3. *Dhillon J, Amin MB, Selbs E, Turi GK, Paner GP, Reuter VE.* Mucinous tubular and spindle cell carcinoma of the kidney with sarcomatoid change. Am J Surg Pathol 2009; 33(1): 44–9.
4. *Kuroda N, Hes O, Michal M, Nemcova J, Gal V, Yamaguchi T,* et al. Mucinous tubular and spindle cell carcinoma with Fuhrman nuclear grade 3: a histological, immunohistochemical, ultrastructural and FISH study. Histol Histopathol 2008; 23(12): 1517–235.
5. *Zhao M, He X, Teng X.* Mucinous tubular and spindle cell renal cell carcinoma: a review of clinicopathologic aspects. Diagn Pathol 2015; 1: 168.
6. *Lima MS, Barros-Silva GE, Pereira RA, Ravinal RC, Tucci S Jr, Costa RS, et al.* The imaging and pathological features of a mu-

cinous tubular and spindle cell carcinoma of the kidney: A case report. World J Surg Oncol 2013; 11: 34.

7. *Fine SW, Argani P, DeMarzo AM, Delahunt B, Sebo TJ, Reuter VE*, et al. Expanding the histologic spectrum of mucinous tubular and spindle cell carcinoma of the kidney. Am J Surg Pathol 2006; 30(12): 1554–60.
8. *Kuroda N, Tamura M, Hes O, Michal M, Kawada C, Shuin T*, et al. Renal cell carcinoma with extensive clear cell change sharing characteristics of mucinous tubular and spindle cell carcinoma and papillary renal cell carcinoma. Pathol Int 2009; 59(9): 687–8.
9. *Shen SS, Ro JY, Tamboli P, Truong LD, Zhai Q, Jung SJ*, et al. Mucinous tubular and spindle cell carcinoma of kidney is probably a variant of papillary renal cell carcinoma with spindle cell features. Ann Diagn Pathol 2007; 11(1): 13–21.
10. *Paner GP, Srigley JR, Radhakrishnan A, Cohen C, Skinnider BF, Tickoo SK*, et al. Immunohistochemical analysis of mucinous tubular and spindle cell carcinoma and papillary renal cell carcinoma of the kidney-significant immunophenotypic overlap warrants diagnostic caution. Am J Surg Pathol 2006; 30(1): 13–9.
11. *Ferlicot S, Allory Y, Compérat E, Mege-Lechevalier F, Dimet S, Sibony M,* et al. Mucinous tubular and spindle cell carcinoma: A report of 15 cases and a review of the literature. Virchows Arch 2005; 447(6): 978–83.
12. *Kenney PA, Vikram R, Prasad SR, Tamboli P, Matin SF, Wood CG*, et al. Mucinous tubular and spindle cell carcinoma (MTSCC) of the kidney: a detailed study of radiological, pathological and clinical outcomes. BJU Int 2015; 116(1): 85–92.
13. *Levakov I, Vojinov S, Marusic G, Popov M, Levakov O, Popov M,* et al. Safety profile of temsirolimus in patients with metastatic renal cell carcinoma. J BUON 2016; 21(6): 1442–8.
14. *Ursani NA, Robertson AR, Schieman SM, Bainbridge T, Srigley JR.* Mucinous tubular and spindle cell carcinoma of kidney without sarcomatoid change showing metastases to liver and retroperitoneal lymph node. Hum Pathol 2011; 42(3): 444–8.
15. *Thway K, du Parcq J, Larkin JM, Fisher C, Livni N.* Metastatic renal mucinous tubular and spindle cell carcinoma. Atypical behavior of a rare, morphologically bland tumor. Ann Diagn Pathol 2012; 16(5): 407–10.

Received on May 23, 2019.
Accepted September 30, 2019.
Online First October, 2019.

INSTRUCTIONS TO THE AUTHORS

The VSP publishes only papers not published before, nor submitted to any other journals, in the order determined by the Editorial Board. Any attempted plagiarism or self-plagiarism will be punished. When submitting a paper to the VSP electronic editing system **(http://aseestant.ceon.rs/index.php)**, the following should be enclosed: a statement on meeting any technical requirements, a statement signed by all the authors that the paper on the whole and/or partly has not been submitted nor accepted for publication elsewhere, a statement specifying the actual contribution of each author, no conflict of interest statement that make them responsible for meeting any requirements set. What follows subsequently is the acceptance of a paper for further editing procedure. The manuscripts submitted to the VSP pass in-house and external peer review. All authors pay "Article Processing Charge" for coverage all editing and publishing expenses. Domestic authors pay 5,000 RSD, and those from aboard 150 euros. The editing and publishing fee is required for substantive editing, facts and references validations, copy editing, and publishing online and in print by editorial staff of the Journal. No additional fees, other than stated above, are required even if an author who already paid the fee would have more articles accepted for publishing in the year when fee was paid. All authors who pay this fee may, if want, receive printed version of the Journal in year when fee is payed. Please note that the payment of this charge does not guarantee acceptance of the manuscript for publication and does not influence the outcome of the review procedure. The requirement about paying "Article Processing Charge" does not apply to reviewers, members of the Editorial Board and the Publisher's Council of the Journal, young researchers and students, as well as any of the subscribers of the Journal.

The VSP publishes: **editorials, original articles**, **short communications**, **reviews**/**meta-analyses, case reports**, **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, and other. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mmHg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: ***, †, ‡, §, ‖, ¶, **, ††, ...** .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

2. ***Abstract and key words***

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

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

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

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

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

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

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

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

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

Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

Illustrations

Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

Legends for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs.

Abbreviations and acronyms

Authors are encouraged to use abbreviations and acronyms in the manuscript in the following manner: abbreviations and acronyms must be defined the first time they are used in the text consistently throughout the whole manuscript, tables, and graphics; abbreviations should be used only for terms that appear more than three times in text; abbreviations should be sparingly used.

An alphabetical list of all abbreviations used in the paper, followed by their full definitions, should be provided on submission.

Detailed Instructions are available at the web site:

www.vma.mod.gov.rs/vsp

UPUTSTVO AUTORIMA

VSP objavljuje radove koji nisu ranije nigde objavljivani, niti predati za objavljivanje redosledom koji određuje uređivački odbor. Svaki pokušaj plagijarizma ili autoplagijarizma kažnjava se. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“**(http://aseestant.ceon.rs/index.php)** neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu koju potpisuju svi autori da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjavu o pojedinačnom doprinosu svakog od autora rada potpisanu od svih autora, treba skenirati i poslati uz rad kao dopunsku datoteku. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa čime postaju odgovorni za ispunjavanje svih postavljenih uslova. Ovome sledi odluka o prihvatanju za dalji uređivački postupak. Rukopisi pristigli u Redakciju časopisa podležu internoj i eksternoj recenziji. Svi autori dužni su da plate “Article Processing Charge” za pokriće troškova jezičke, stručne i tehničke obrade rukopisa, kao i njegovog objavljivanja. Domaći autori plaćaju iznos od 5 000 dinara, a inostrani 150 eura. Dodatna plaćanja nisu predviđena čak i u slučaju da autor koji je već prethodno platio traženi iznos, ima više prihvaćenih radova za objavljivanje u godini u kojoj je izvršio uplatu. Svi autori koji su platili “Article Processing Charge” mogu, ukoliko žele, dobijati štampanu verziju časopisa tokom godine u kojoj je izvršena uplata. Plaćanje ovog iznosa ne garantuje prihvatanje rukopisa za objavljivanje i ne utiče na ishod recenzije. Od obaveze plaćanja pokrića navedenih troškova oslobođeni su recenzenti, članovi Uređivačkog odbora i Izdavačkog saveta VSP, studenti i mladi istraživači, kao i pretplatnici časopisa.

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize**, **kazuistika, seminar praktičnog lekara,** članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespodenciju na konačnu saglasnost.

Priprema rada

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

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

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

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

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

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

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

Literatura

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

Primeri referenci:

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

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

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

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

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

Tabele

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

Ilustracije

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

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

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp