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Joshua Lederberg (1925-2008)

https://en.wikipedia.org/wiki/Joshua_Lederberg

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https://en.wikipedia.org/wiki/Joshua_Lederberg

In May of this year, we celebrate the 100th anniversary of the birth of Joshua Lederberg (May 23, 1925, Montclair, NJ, USA – February 2, 2008, New York, NY), a world-renowned molecular biologist and geneticist, whose discoveries opened the way to understanding the adaptation of microorganisms, including the development of resistance to antimicrobial drugs. While researching the genetics of bacteria, Dr. Lederberg showed that two bacteria can "exchange" genetic material through a bridge-like connection. He also proved a phenomenon known as transduction, in which DNA is transferred between bacteria by means of bacteriophages. He was only 33 years old when, for his discoveries concerning genetic recombination and the organization of the genetic material of bacteria, he shared the Nobel Prize in Physiology or Medicine (1958) with George W. Beadle and Edward L. Tatum.

U maju ove godine obeležavamo 100 godina od rođenja Džošue Lederberga (23. maj 1925, Montkler, Nju Džerzi, SAD – 02. februar 2008, Njujork, Njujork), svetski poznatog molekularnog biologa i genetičara, čija su otkrića otvorila put ka razumevanju adaptacije mikroorganizama, uključujući razvoj otpornosti na antimikrobne lekove. Istražujući genetiku bakterija, dr Lederberg je pokazao da dve bakterije mogu da "razmene" genetski material, preko veze koja podseća na most. Takođe, dokazao je fenomen poznat kao transdukcija, u kojem se DNK prenosi između bakterija putem bakteriofaga. Imao je samo 33 godine kada je, za svoja otkrića u vezi sa genetskom rekombinacijom i organizacijom genetskog materijala bakterija, podelio Nobelovu nagradu za fiziologiju ili medicinu (1958) sa Džordžom V. Bidlom i Edvardom L. Tejtumom.

ORIGINAL ARTICLES

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Compliance of extended infusion of piperacillin/tazobactam with the desired pharmacokinetic/pharmacodynamic index in septic patients

Usklađenost produžene primene infuzije piperacilina/tazobaktama sa željenim farmakokinetičkim/farmakodinamskim indeksom kod septičnih bolesnika

Nataša Tomić*, Saša Vukmirović†, Stanislav Sabo‡, Arsen Uvelin*, Radmila Popović*, Sanja Vicković*, Ljiljana Tomić§, Zdenko Tomić†

***University Clinical Center of Vojvodina, Clinic for Anesthesia, Intensive Care and Pain Therapy, Novi Sad, Serbia; †University of Novi Sad, Faculty of Medicine, Department of Pharmacology and Toxicology, Novi Sad, Serbia; ‡University of Health and Social Work of St. Elizabeth, Bratislava, Slovak Republic; §University of Bijeljina, Bijeljina, Bosnia and Herzegovina**

Abstract

Background/Aim. Piperacillin (PIP)/tazobactam (TAZ) – PIP/TAZ is a beta-lactam antibiotic used to treat Gram-negative sepsis. The portion of the dosing interval during which antibiotic concentrations are above the minimal inhibitory concentration – MIC (fT>MIC) or, even more, four times higher than the MIC (fT>4xMIC), represents the pharmacokinetic indices that best correlate with the clinical outcome. In light of the increasing resistance of bacteria in intensive care units (ICUs), it is important to examine the pharmacokinetic/pharmacodynamic (PK/PD) indices of different PIP/TAZ dosing regimens to determine whether this condition is met. The aim of this study was to analyze the efficacy of prolonged intermittent infusion of PIP/TAZ in patients with sepsis in the ICU to achieve the desired PK/PD index. **Methods.** A prospective, controlled, non-interventional study included patients with abdominal post-operative sepsis. Patients received PIP/TAZ in a dose of 4.5 g every 6 hrs in an extended (60-min) intermittent infusion in a daily therapeutic dose of 18 g as part of the prescribed therapy. Blood samples were collected during the first 36 hrs, and antibiotic concentrations were determined using high-performance liquid chromatography. The analysis included the most common isolates from the blood culture in the ICU that were sensitive to PIP/TAZ, and the MICs were also taken from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) database. The primary objective of the PK/PD study was to determine the indices fT>MIC and fT>4xMIC as the best indicators of therapeutic efficacy. For the pharmacodynamic target, this period was determined to be ≥ 50% of the time of the dosing interval. **Results.** The maximum achieved PIP concentrations in examined patients were 130.03 ± 32.43 μg/mL. Based on PK/PD data, the applied PIP/TAZ dosing regimen was effective against sensitive strains whose MIC did not exceed 16 μg/mL (fT>MIC = 56%). If we take fT>4xMIC≥50% as a target value, that percentage was significantly below the set goal (27%). For strains that include strains with a phenotypic resistance mechanism, PK/PD values for fT>4xMIC≥50% were far below the set goal (2–11%), except for *Escherichia coli* (79%). **Conclusion.** An intermittent 60-min infusion of PIP/TAZ meets the required pharmacodynamic target fT>MIC≥50% for sensitive strains of bacteria with an interruption point from 16 μg/mL. The indicated dosing regimen did not meet the target PK/PD values in the case of resistant strains.

Key words:
beta lactam, antibiotics; chromatography, high pressure liquid; drug resistance, microbial; infusion, intravenous; pharmacokinetics; pharmacology; sepsis.

Apstrakt

Uvod/Cilj. Piperacilin (PIP)/tazobaktam (TAZ) – PIP/TAZ je beta laktamski antibiotik koji se koristi u lečenju sepse uzrokovane Gram negativnim bakterijama. Deo intervala doziranja tokom kojeg su koncentracije antibiotika iznad minimalne inhibitorne koncentracije (*minimal inhibitory concentration* – MIC) (fT>MIC) ili značajnije, četiri puta veće od MIC-a (fT>4xMIC), predstavljaju farmakokinetske indekse koji najbolje koreliraju sa kliničkim ishodom. U svetlu sve veće rezistencije bakterija u jedinicama intenzivne nege (JIN), važno je ispitati farmakokinetske/farmakodinamske (*pharmacokinetic/pharmacodynamic* – PK/PD) indekse kod različitih režima doziranja PIP/TAZ, da bi se utvrdilo da li će taj uslov biti ispunjen. Cilj rada bio je da se analizira

Correspondence to: Zdenko Tomić, University of Novi Sad, Faculty of Medicine Hajduk Veljkova 3, 21 000 Novi Sad, Serbia. E-mail: zdenko.tomic@mf.uns.ac.rs

efikasnost produžene intermitentne infuzije PIP/TAZ kod bolesnika sa sepsom u JIN, kako bi se postigao željeni PK/PD indeks. **Metode**. Prospektivnom, kontrolisanom, neintervencijskom studijom obuhvaćeni su bolesnici sa abdominalnom postoperativnom sepsom. Bolesnici su primali PIP/TAZ u dozi od 4,5 g na 6 sati u produženoj (60-min) intermitentnoj infuziji u dnevnoj terapijskoj dozi od 18 g, kao propisanu terapiju. Uzorci krvi uzimani su tokom prvih 36 sati, a koncentracije antibiotika određene su primenom tečne hromatografije visokih performansi. U analizu su uključeni najčešći izolati iz hemokulture u JIN koji su bili osetljivi na PIP/TAZ, a MIC-e su preuzete iz baze *European Committee on Antimicrobial Susceptibility Testing* (EUCAST). Primarni cilj PK/PD studije bio je da se odrede indeksi fT>MIC i fT>4xMIC kao najbolji pokazatelji terapijske efikasnosti. Za farmakodinamski cilj određeno je da taj period bude ≥ 50% vremena intervala doziranja. **Rezultati**. Maksimalna koncentracija PIP kod ispitivanih bolesnika iznosila je 130,03 ± 32,43 μg/mL. Na osnovu PK/PD podataka, primenjeni režim doziranja PIP/TAZ bio je efikasan protiv osetljivih sojeva čija je MIC bila ispod 16 μg/mL (fT>MIC = 56%). Uzimanjem fT>4xMIC≥50% kao ciljne vrednosti, taj procenat bio je značajno ispod cilja (27%). Kod sojeva koji uključuju sojeve sa mehanizmom fenotipske rezistencije, vrednosti PK/PD za fT>4xMIC≥50% bile su značajno ispod postavljenog cilja (2–11%), osim za *Escherichia coli* (79%). **Zaključak**. Intermitentna 60-min infuzija PIP/TAZ ispunjava zahtevani farmakodinamski cilj fT>MIC≥50% za osetljive sojeve bakterija, sa tačkom prekida od 16 μg/mL. Navedeni režim doziranja nije ispunio ciljne PK/PD vrednosti u slučaju rezistentnih sojeva.

Ključne reči:
antibiotici, beta laktamski; hromatografija, tečna, pod vp; lekovi, rezistencija mikroorganizama; infuzije, intravenske; farmakokinetika; farmakologija; sepsa.

Introduction

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [1–3]. The alterations in organ and system functioning during sepsis can significantly affect the pharmacokinetics (PK) and pharmacodynamics (PD) of the administered antibiotic. Fluid extravasation into the interstitial space due to capillary damage and vascular dysfunction, hypoalbuminemia as a consequence of liver function disorders, kidney function disorders in the sense of increased renal clearance or acute renal damage, cardiovascular system dysfunction with great inter-individual variability are only part of the pathophysiological changes that significantly complicate adequate dosing medicines and often require therapeutic monitoring [4–5]. Due to these changes, therapeutic doses of antibiotics may be insufficient to meet the PK/PD target necessary to inhibit the growth of or kill the pathogen, which results in poor therapeutic outcomes [3–5]. Optimizing antibiotic therapy according to PK/PD indices and providing an individualized approach for the treatment of a septic patient is essential for a successful treatment outcome and prevention of antibiotic resistance [6]. The leading PK/PD indices are categorized as follows: the ratio of maximum drug concentration (Cmax) to minimal inhibitory concentration (MIC) (Cmax/MIC); the duration of time that the unbound drug concentration remains above the MIC during a dosing interval (fT>MIC); the area under inhibitory curve (AUIC) – the ratio of the area under the concentration-time curve during 24 hrs to MIC (area under the curve – $AUC_{0–24}$/MIC) [5]. However, the optimal PK/PD target of beta-lactams to achieve clinical cure in microbiological eradication in critically ill patients remains undefined, including decisions for different infusion durations [7].

Achieving PK/PD targets depends on the dose and the duration of administration. Unlike conventional intermittent infusions (infusion ≤ 30 min), administration of extended intravenous (i.v.) infusion, either as an extended infusion (antibiotic infused for at least half of the dosing interval) or as a continuous infusion, results in sustained beta-lactam concentrations consistent with these drugs' PD. The meta-analyses reported similar results, confirming reduced short-term mortality [relative risk (RR), 0.70; 95% confidence interval (CI), 0.57–0.87] with prolonged beta-lactam infusion [8–10]. A prolonged infusion is a feasible intervention if there is an appropriate i.v. access and available resources to ensure that the beta-lactam antibiotic is infused for the required time, which can become a significant problem where resources are limited, particularly in developing countries. However, the randomized international clinical trial BLING III conducted on 7,202 critically ill patients with sepsis who received piperacillin/tazobactam (PIP/TAZ) as intermittent or continuous infusion together with meropenem showed that there was no significant difference in all-cause mortality at day 90: 24.9% (continuous) vs. 26.8% (intermittent), absolute difference -1.9% (95% CI -4.9 to 1.1), odds ratio (OR)- 0.91 (95% CI 0.81 to 1.01), $p = 0.08$. There was no significant difference in all-cause ICU mortality, but clinical cure was significantly better in the continuous infusion group ($p = 0.001$) [11].

One of the most commonly used antibiotics in ICUs is a combination of PIP/TAZ, indicated for the treatment of severe intraabdominal infections mainly caused by *Enterobacterales* [12, 13]. To ensure a good clinical outcome, the leading PK/PD index, the duration of time (T) that the free antibiotic concentration remains above the MIC during a dosing interval (fT>MIC) must be more than 50%; however, expert opinion recommends drug levels of even 4–5xMIC for 100% of the dosing interval for critically ill patients with variable PK/PD parameters [14–16]. The recommended dose of PIP/TAZ (according to the summary of product characteristics – SmPC) for the treatment of severe infections is 4.5 g, which is administered every 6 hrs by intermittent i.v. infusion over 30 min [electronic medicines compendium (EMC), 2023] [17]. In recent documents, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [18] and the Food and Drug Administration (FDA) [12] recommend administering a 30-min or extended 3-hr infusion. However, recently pub-

lished research mentions the administration of PIP/TAZ in continuous infusion, which, according to the authors, achieves the longest time when the values are above the MIC [19]. These differences in recommendations often cause clinicians difficulty when deciding on the dosing regimens.

Rates of resistant pathogens are generally higher in the ICU compared to general hospital wards due to the use of broad-spectrum antibiotics, transmission within the ICU, and patients requiring invasive procedures [20]. Resistant pathogens present a challenge to PD, with elevated MICs requiring higher antibiotic concentrations to achieve an equivalent PK/PD target. Therefore, we analyzed the cut-off points of both sensitive strains of the most common causal bacteria of sepsis in the ICU and cut-off points that include 90% of all strains, both sensitive and those with some phenotypic resistance mechanisms, for the most common sepsis-causing bacteria (MIC_{90}) from the database EUCAST [18].

An official recommendation is the use of PIP/TAZ in an intermittent infusion over 30 min, and the existing studies favor the use in the form of prolonged or continuous infusion. In many clinical centers in developing countries, there is no possibility of administering antibiotics by the pump as a continuous or prolonged infusion, and a 60-min infusion is used in seriously ill patients. Therefore, this research aimed to study the effect of a 60-min infusion of a high-dose regimen of PIP/TAZ in patients with sepsis and septic shock in the ICU in the context of achieving the desired PK/PD parameters.

Methods

Setting

This prospective, controlled, non-interventional study was conducted in the University Clinical Center of Vojvodina ICU in Novi Sad, Serbia. PD measurements were conducted at the Faculty of Medicine in Novi Sad, Department for Pharmacology and Clinical Pharmacology. The approval for conducting the study was obtained from the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-20/610), with written informed consent from either the patient or the patient's nominated substitute decision-maker.

Patients

The study was performed on patients who met the inclusion criteria. The sufficient number of subjects was 13 to assess the relationship between fT>MIC and fT>4xMIC for the required $r > 0.7$, the statistical power of 80%, and a $p \leq 0.05$. Patients were eligible for enrolment if they were between 18 and 80 years of age, had *post*-surgical abdominal sepsis (AS) [Sequential Organ Failure Assessment (SOFA), Acute Physiology and Chronic Health Evaluation (APACHE II)], were hospitalized in the ICU, and were receiving prescribed PIP/TAZ as a part of the protocol for the treatment of sepsis. Exclusion criteria were as follows: patients who did not have an intraarterial line inserted as part of routine management or if the blood could not be obtained for another reason (to allow repeated plasma sampling without additional venipuncture); patients who had renal impairment (defined by a plasma creatinine concentration greater than 171 μmol/L) or who required renal replacement therapy; patients with a history of allergy to PIP; patients who decided not to participate in the study. The patient flow chart is shown in Figure 1.

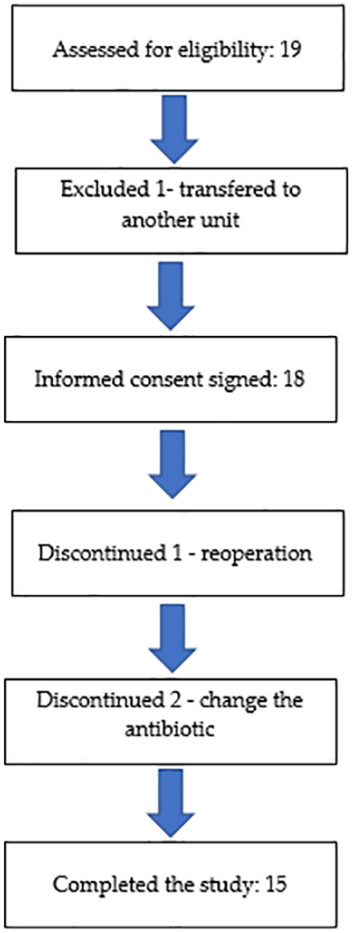

Fig. 1 – Consolidated Standards of Reporting Trials (CONSORT) flow chart of the patients.

Duration of the study

The inclusion period lasted 12 months (January 1 to December 31, 2019), which was necessary to collect sufficient participants. After inclusion in the study, we took the first sample, and after the first administration of PIP/TAZ, blood samples were taken for 36 hrs for PD analysis. In our sample of patients, we monitored mortality over 28 days from the start of the study.

Drugs

PIP/TAZ 4 g/0.5 g powder for solution for infusion was used. Each vial contains 4 g PIP (as sodium salt) and 0.5 g TAZ (as sodium salt).

PIP/TAZ was administered immediately after the diagnosis of sepsis as an intermittent 60-min i.v. infusion at a daily dose of 18 g, 4.5 g every 6 hrs.

Measurement of the concentration of piperacillin

A blood sample was obtained *via* venipuncture from the patients during the first 36 hrs of therapy. Samples were taken on the first day eight times after the first dose (15 min, 30 min, 60 min, and 1, 2, 3, 4, and 6 hrs). Afterward, blood was sampled before each subsequent dose and 10 min after the end of infusion (dose) until 36 hrs. The samples were obtained for 36 hrs, as we wanted to cover the concentrations after the first dose and at a steady state.

The amount of blood taken during individual sampling without coagulation was 1 mL. We stored serum samples at -20 °C until analysis.

The concentration of total PIP was determined using high-pressure liquid chromatography (HPLC) (Dionex, USA) with ultraviolet detector, according to the method of Rama Krishna et al. [21]. In the case of PIP, the free fraction is 70% of the total concentration, and based on that, we calculated the concentration of free PIP in the blood [22].

From the PK parameters, we calculate the Cmax, Cmin, and AUC_{0-24} μg/mL × hr and AUC_{24-36} μg/mL × hr using the PK Solver program (add-in program for Microsoft Excel, Microsoft Corporation, USA) [23].

Microbiological assay

The most frequently isolated bacteria from ICU patients with AS were used to calculate PK/PD parameters. The MIC value was determined using the EUCAST epidemiological cut-off values (ECOFF) breakpoint for the most susceptible strains of pathogens [18].

We used the EUCAST ECOFF databases for sensitive strains [18] as MIC breakpoints for the tested strains of bacteria. We also determined the MIC_{90}, which includes 90% of all reported strains, both those with and without phenotypically detectable acquired resistance mechanisms (non-wild type). We took all strains into account due to the high level of resistance of pathogens present in the ICU units.

Calculation of pharmacokinetic/pharmacodynamic indices

The primary endpoint investigated in this study was to calculate the PK/PD indices. We calculated two PK/PD indices as the best indicators of the effectiveness of therapy: %fT>MIC and AUIC.

The percentage of time during which the unbound concentration of PIP remains above the MIC for the analyzed bacterial strain (%fT>MIC) represents the PK/PD index associated with optimal PIP/TAZ activity. This index was defined as fT>MIC≥50%, which is also considered the PD target (PDT) [24]. However, data from critically ill patients suggest that patients may benefit from longer contact (e.g., 100% fT>MIC) [5, 25] with higher concentrations (e.g., 2–5 x MIC) [26] of beta-lactam antibiotics than those previously described. Therefore, when calculating the PK/PD index, we also calculated the ratio of the dosing interval during which the unbound PIP was higher than 4 x MIC (fT>4xMIC).

AUIC is a measure of the area under the concentration-time curve for 24-hr dosing/MIC (AUC_{0-24}/MIC). As previously described, target values for AUIC are 125–500, as evaluated for beta-lactam, with a target breakpoint stated as the value of 125. However, even higher values of 250 are needed for optimal treatment [27].

Statistical analysis

The data were presented in tables and graphs and evaluated using descriptive statistics (mean values and standard deviations).

The Mann-Whitney *U* test (with a threshold of $p = 0.05$) was used to determine a statistically significant difference between the mean PIP concentrations and the measured values fT>MIC and fT>4xMIC.

Results

Study sample

A total of 19 patients were assessed for eligibility. One was excluded due to a transfer to another unit. Eighteen patients signed informed consent. Two were excluded due to a change of antibiotics, and one was excluded due to reoperation. Finally, a total of 15 patients were included, hence total number of patients who completed the study was 15 (9 men and 6 women) (Figure 1). The average age of the respondents was 68.90 years, and the average body weight was 85.43 kg (Table 1). We used the SOFA and APACHE II scores as a criterion for sepsis diagnosis (Table 2). The 28-day mortality was 47.62%.

The most common bacterial isolates

The most common bacterial isolates from the patients with postoperative AS sensitive to PIP/TAZ at the ICU department were *Klebsiella pneumoniae*, *Escherichia coli (E. coli)*, *Pseu-*

Table 1

Demographic characteristics of the 15 patients with sepsis who received prolonged intermittent infusion of PIP/TAZ in the ICU.

Sex	n (%)	Age, years	Weight, kg
Men	9 (60)	66.41 ± 12.89	92.44 ± 18.02
Women	6 (40)	72.54 ± 11.50	72.80 ± 17.06
Total	15 (100)	68.90 ± 14.04	85.43 ± 19.90

PIP/TAZ – piperacillin/tazobactam; ICU – intensive care unit.
Values are given as numbers (percentages) or mean ± standard deviation.

domonas aeruginosa, and *Enterobacter spp.* (Table 3). Likewise, sporadically isolated but resistant to PIP/TAZ were *Clostridium tetani, Enterococcus faecium, Acinetobacter spp.*, and coagulase-negative *Staphylococcus.*

Pharmacokinetic calculation of piperacillin

We determined the AUC values of PIP for the first 24 hrs ($AUC_{0–24}$ = 1,141.12 μg/mL x hr) and the period 24–36 hrs ($AUC_{24–36}$ = 1,568.96 μg/mL x hr). The mean value of the Cmax for the applied dosing regimen was 130.03 μg/mL (Table 4). The highest concentrations ranged between 125 μg/mL and 132 μg/mL, while the lowest concentrations, obtained immediately before administering the next dose, were as low as 2.4 μg/mL and 2.8 μg/mL.

The calculated ratio of the free fraction of PIP concentrations/time curves and the MIC for the most common bacterial pathogens isolated from patients with sepsis is shown in Figure 2. The chart represents the basis for determining the PK/PD parameters.

Table 3 shows the MIC and MIC_{90} values [18] for the most common isolates sensitive to PIP. For each strain, PK/PD indices (fT>MIC and fT>4xMIC) based on PK data of PIP and PD values (MIC) of the causative agent are presented. We calculated the AUIC ($AUC_{0–24}$/MIC) ratio for the analyzed strains. Table 3 also shows the breakpoint of sensi-

Table 2

The values of APACHE II and SOFA scores in septic patients at the beginning of the study

Scores	Value
SOFA	6.93 ± 4.11
APACHE II	22.43 ± 19.65

SOFA – Sequential Organ Failure Assessment; APACHE – Acute Physiology and Chronic Health Evaluation.
Values are given as mean ± standard deviation.

Table 3

Calculation of PK/PD indices the most common sensitive isolates from blood culture in the ICU from the MIC and concentration/time curve of unbound piperacillin in the blood

Bacteria (% prevalence)	MIC (μg/mL) (EUCAST)	fT>MIC	fT>4xMIC	$AUC_{0–24}$/MIC (AUIC)	Breakpoint for PK/PD (fT>MIC≥ 50%)#
Klebsiella pneumoniae	8*	79 (67–81)	27 (22–31)	143	
(33.3)	64**	11 (8–14)	0	18	
Escherichia coli	8*	79 (67–81)	27 (22–31)	143	
(16.6)	8**	79 (67–81)	27 (22–31)	143	16 μg/mL
Pseudomonas aeruginosa	16*	56 (43–67)	11 (8–14)	71	
(5.6)	128**	2 (0–5)	0	9	
Enterobacter spp.	8*	79 (67–81)	27 (22–31)	143	
(5.6)	128**	2 (0–5)	0	9	

PK/PD – pharmacokinetic/pharmacodynamic; ICU – intensive care unit; MIC – minimal inhibitory concentration; EUCAST – European Committee on Antimicrobial Susceptibility Testing; fT>MIC – time period during which unbound drug concentration remains above the MIC; fT>4xMIC – time period during which unbound drug concentration remains above 4 times the MIC; AUC – area under the curve; AUIC – area under the inhibitory curve.
The fT>MIC and fT>4xMIC values are presented as mean (minimum-maximum) in percentages.
sensitive strains (without acquired resistance – EUCAST [18]); *MIC_{90} covering 90% of strains without and with a phenotypically detectable mechanism of resistance, calculated from EUCAST bases [18]; # – [13, 18, 23].**

Table 4

Pharmacokinetic parameters (AUC, Cmax, Cmin) of the free fraction of piperacillin in blood samples after 4 g/6 hr intravenously administration for 60 min, for 36 hrs

Pharmacokinetic parameter	Values
AUC_{0-24}, μg/mL × hr	1,141.12 ± 61.87
AUC_{24-36}, μg/mL × hr	1,568.96 ± 398.48
$AUC_{0-\infty}$, μg/mL × hr	3,062.25 ± 622.37
Cmax, μg/mL	130.03 ± 32.43
Cmin, μg/mL	2.60 ± 0.17

AUC – area under the curve; Cmax – maximum drug concentration; Cmin – minimum drug concentration.
Values are given as mean ± standard deviation.

tivity for the PK/PD indices based on literature data. The breakpoint for the PD target is defined based on literature data as 16 μg/mL (Figure 3). While for the sensitive strains of bacteria, the time above the MIC was 79%, concentrations of PIP 4xMIC were present for only 27% of the dosing interval. The PIP concentrations were lower than the 4xMIC for all dosing intervals for strains with a phenotypic resistance mechanism. We got the same results when calculating AUIC ($AUC_{0–24}$/MIC). For sensitive strains of bacteria, the $AUC/MIC_{0–24}$ ratio was higher than 100. It was as low as 9–18 for resistant bacteria, except for *E. coli* (143).

We found a statistically significant correlation between the mean PIP concentrations and the measured values of fT>MIC and fT>4xMIC ($r = 0.97$; $p = 0.001$).

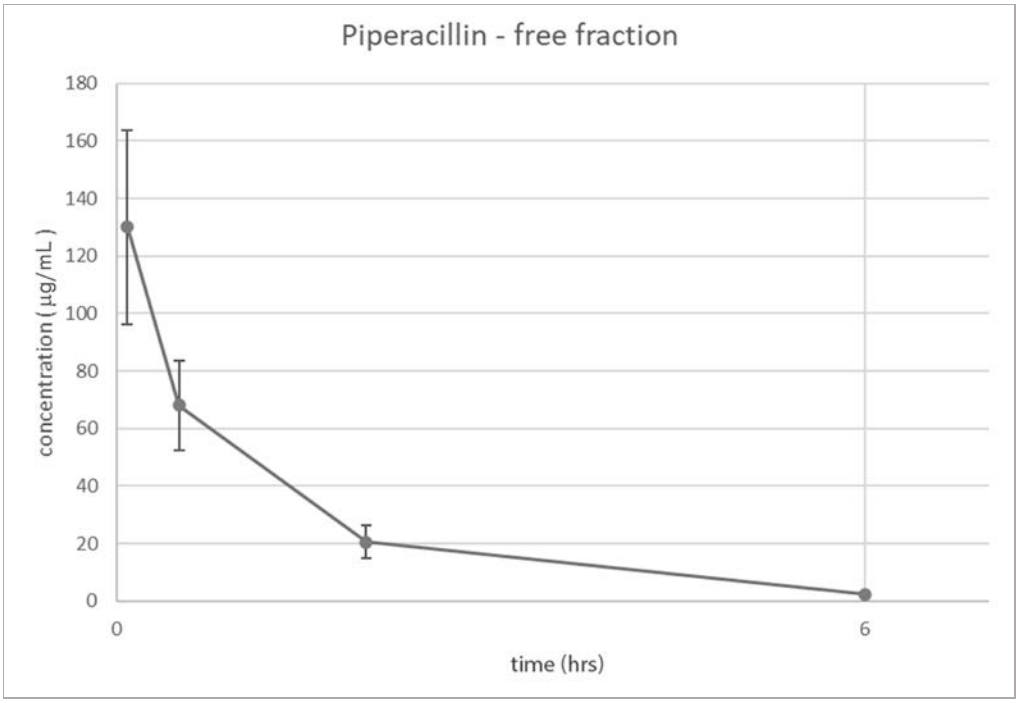

Fig. 2 – The ratio of the free fraction of piperacillin concentrations (μg/mL)/time curves for the first 6 hrs after administration of 4.5 g intravenously for 6 hrs. Values are given as mean ± standard deviation.

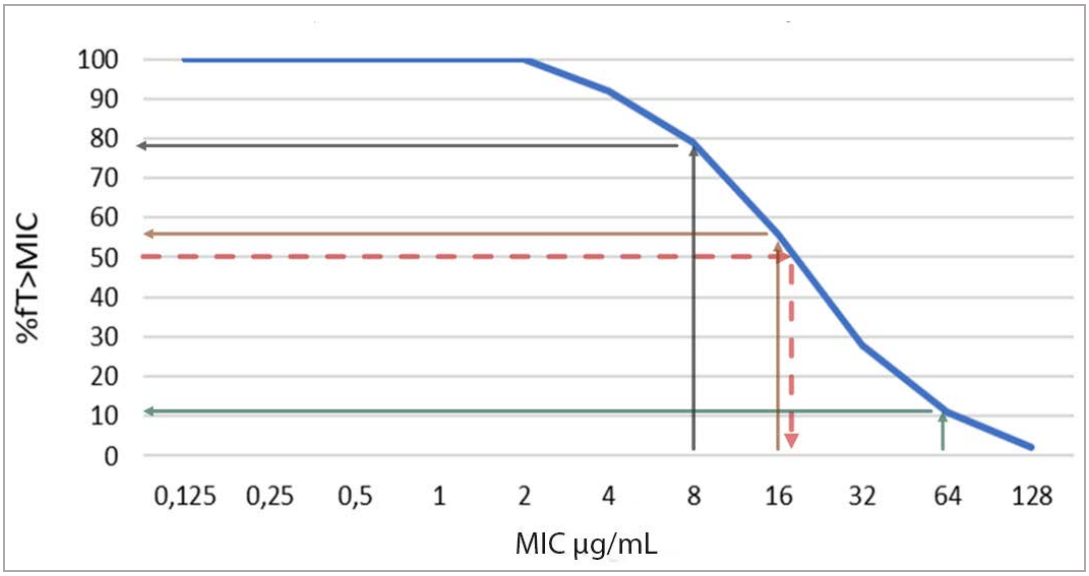

Fig. 3 – Relationship between fT>MIC and MIC of piperacillin/tazobactam, 4.5 g administered intravenously for 60 min. Assuming the pharmacodynamic target of fT>MIC≥50%, the cut-off point is 16 μg/mL (dashed arrows). The fT>MIC values are also marked, which are shown by solid arrows for MIC = 8 μg/mL (79%), MIC = 16 μg/mL (56%), and MIC = 64 μg/mL (11%). More detailed data are presented in Table 4.

MIC – minimal inhibitory concentration; fT>MIC – time period during which unbound drug concentration remains above the MIC; %fT>MIC – percentage of time period during which unbound drug concentration remains above the MIC.

Discussion

In everyday practice, real problems often require a different approach to assessing the PK/PD profile of antibiotics in septic patients, which can significantly reduce the accuracy of calculations. Given that in the first 24–48 hrs of sepsis treatment, which are considered crucial for the treatment outcome, it is not realistic to obtain microbiological analyses that confirm the identity and sensitivity of the causative agent (MIC), we are forced to use EUCAST [18] data or our own database. In this sense, this work is an example of the only possible PK/PD approach in many environments if this type of therapy evaluation is to be done.

In our study, a high-dose regimen of PIP/TAZ administered by partially extended intermittent administration (60 min) enabled the achievement of an average fT>MIC value of 79% for sensitive strains of three out of four most common pathogens (*Klebsiella*, *E. coli*, and *Enterobacter*), which suggests the possibility of successful clinical outcome. For *Pseudomonas*, the higher MIC breakpoint value of 16 μg/mL for susceptible strains shows fT>MIC of 56% within the set PDT. However, when considering fT>4xMIC, the period during which the concentrations were four times above the MIC value, even in susceptible strains with lower MIC values (8 μg/mL), was only 27%, which is significantly less than the target values (50%). AUIC values were correlated with fT>MIC values for susceptible strains and reached a value of 143, which is above the limit of 125.

In strains that include microorganisms with a phenotypic resistance mechanism, only in *E. coli*, the MIC_{90} value of 8 μg achieves PDT (fT>MIC≥50%) due to the large number of strains with low MIC. In other pathogens, their high MIC_{90} values (64–128 μg) resulted in low fT>MIC (2–11%) and AUIC (9–18) values, far below what is needed to achieve the desired goal.

PK/PD calculation suggests that intermittent dosing can achieve target exposures comparable to continuous infusion when pathogen MICs are low; however, in the presence of less susceptible pathogens, intermittent dosing is associated with a higher risk of treatment failure [28]. Intermittent dosing produces PIP concentrations below the MIC for most dosing intervals when pathogens with phenotypic resistance mechanisms are involved.

According to our results, the applied intermittent dosing, although lasting 60 min (16.6% of the dosing interval), was not sufficient to provide optimal PK/PD parameters (fT>4xMIC), which some authors state as minimum targets when it comes to severe infections [14, 15].

Following these results, using the PIP/TAZ combination in severe infections is accompanied by controversial conclusions related to the clinical outcome of therapy. According to one group of authors, there were no statistical differences in cure rates between the two treatment arms (continuous or conventional intermittent dosing) and no adverse events [29, 30]. Furthermore, the meta-analysis showed that therapeutic drug monitoring (TDM)-guided dosing of beta-lactam antibiotics improved clinical and microbiological cure but did not reduce mortality or length of stay [31]. According to recent studies, PIP/TAZ given *via* continuous/prolonged infusion [10, 24] improved clinical outcomes in critically ill patients. However, in our trial, it is evident that the investigated dosing regimen of PIP/TAZ is insufficient to achieve the target exposure of unbound PIP plasma concentration above the MIC for most of the dosing interval (100% fT>MIC) to ensure 40% to 70% of fT>4xMIC, as suggested by some authors for severe infections [32]. Although the investigated dose regimen failed to achieve PDT (50% fT>4xMIC) for most bacteria, including those with some phenotypic resistance mechanism, the predictive significance of the PK/PD index on the final treatment outcome of patients with sepsis remains an open question, in the context of recently published studies [31].

The next issue involves the guidelines for the use of antibiotics. When writing the SmPC as an official document for the administration of antibiotics, manufacturers follow the results of the tests carried out when they are introduced and placed on the market, which can, over time, result in decreased clinical outcomes [33]. However, individual patient differences cannot be considered when writing the guidelines, especially in sepsis. Antibiotic concentrations vary multiple times due to pathophysiological changes. Constant changes in MICs for pathogens are another factor that must be constantly considered [34]. Therefore, the application method should be strictly individualized (TDM, MIC, individual calculation of PK/PD parameters). However, especially in areas that do not have optimal conditions for this, this happens very rarely.

In most cases, doctors prescribe antibiotics according to the dosage instructions, not having enough time and opportunity to devote to each patient. This leads to unsatisfactory results in patients with a low PK profile (lower than average concentrations of PIP). This may be one of the main reasons the applied dosing regimens in critical patients failed to achieve the desired therapeutic outcome. Therefore, the assessment of the PK profile of each patient and the adjustment of the administration regimen could be associated with a different, optimal dosing regimen for such a subgroup of patients, ultimately leading to a better therapeutic outcome.

Limitations of the study

The study had several limitations. First, the research subjects were selected from a single center, which limited the size of the sample. Second, the negative microbiological samples in a number of participants, as well as the period for waiting for the microbiological samples, 3–5 days, which forced us to use the most common pathogens isolated from patients with postoperative AS in the ICU rather than individual microbiological results, are also limiting factors for the study. Future studies with larger sample sizes across all cohorts are needed to confirm these findings.

Conclusion

The obtained PK/PD parameters support the fact that with intermittent infusion, it is possible to achieve the PK/PD

goal (fT>MIC≥50%) for susceptible pathogens (breakpoint MIC ≤ 16 μg/mL). When including causative agents with phenotypic resistance mechanisms, the tested PIP/TAZ dosing regimen does not meet the PK/PD parameters, except in the case of *Escherichia coli*. Achieving the PK/PD target in treating severe infections (fT>4xMIC>50%), such as sepsis, with a given PIP/TAZ dosing regimen for the analyzed agents remains elusive. For this reason, administration of PIP/TAZ as a high-dose continuous infusion should be considered.

Conflict of interest

The authors declare no conflict of interest.

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Factors affecting the effectiveness/productivity of therapeutic plasma exchange in the treatment of immune-mediated neurologic disorders – a pilot study

Faktori koji utiču na efikasnost/produktivnost terapijske izmene plazme u lečenju imunski posredovanih neuroloških poremećaja – pilot studija

Marija Vraneš*, Aleksandar Lazović†, Bela Balint‡§, Vladimir Jakovljević‖¶, Milena Todorović Balint††‡‡,Tatjana Bošković Matić§§**

University Clinical Center Kragujevac, *Blood Bank Department, †Department of Surgery, Kragujevac, Serbia; ‡Serbian Academy of Sciences and Arts, Department of Medical Sciences, Belgrade, Serbia; §University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Kragujevac, Faculty of Medical Sciences, ‖Department of Physiology, §§Department of Neurology, Kragujevac, Serbia; ¶Center of Excellence for Redox Balance Research in Cardiovascular and Metabolic Disorders, Kragujevac, Serbia; **University IM Sechenov, 1st Moscow State Medical, Department of Human Pathology, Moscow, Russia; ††University Clinical Center of Serbia, Clinic for Hematology, Belgrade, Serbia; ‡‡University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Abstract

Background/Aim. Standard treatment for immune-mediated neurologic disorders (IMNDs) involves the use of immunosuppressive drugs and other therapies. Therapeutic plasma exchange (TPE) is an effective supplementary immunomodulatory approach. Its main goal is to decrease patients' load of pathogenic factors (including autoantibodies) to the levels that will allow clinical improvement. Immunosuppressive medications used simultaneously with TPE reduce the "rebound rise" of autoantibody synthesis. The aim of the study was to evaluate the effectiveness of our own standardized TPE protocol and determine the correlation of TPE treatment outcomes with paraclinical (laboratory) and apheresis parameters/data. **Methods.** The study included 32 patients with myasthenia gravis, Guillain-Barré syndrome or acute polyradiculoneuropathy, and multiple sclerosis. TPEs were carried out using Spectra-Optia® (Terumo-BCT, USA). Properties of our apheresis protocol used for IMND patients were as follows: a) total treatment – five single TPE procedures; b) TPE frequency – every other day; c) removed plasma – one patient's circulating plasma volume (range 2,800–3,100 mL). TPE effectiveness was determined based on recovery of neurologic deficiency and peripheral nerve conduction, positive findings in some paraclinical (laboratory) tests, and apheresis data monitoring. **Results.** Using the described apheresis protocol, a clear positive therapeutic effect was observed in patients treated by TPE procedures with no interruption. TPEs were advantageous in 84.4% of patients (effectiveness rate 89.3%; non-response rate 10.7%), while in 15.6% of cases, treatment was not completed due to patients' severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (followed by coronavirus disease 19 – COVID-19). Patients who completed five single TPEs had evident clinical improvement in terms of disability scale, muscle weakness, or neural conduction deficit. In the follow-up period, no relapses were observed. Significantly reduced values of erythrocyte parameters (especially hematocrit levels) were correlated with higher TPE effectiveness, due to increased plasma/blood cell volume ratio, followed by higher plasma-collection/removal efficacy. Other examined laboratory findings did not show a positive correlation with TPE effectiveness/productivity. Severe adverse events did not occur. There were no relapses in the following 6 months. **Conclusion.** In this study, the reduced levels of erythrocyte parameters (particularly hematocrit levels) were associated with an increased TPE effectiveness. For definitive conclusions, further randomized and larger clinical investigations are needed.

Key words:
blood component removal; guillain-barre syndrome; myasthenia gravis; multiple sclerosis; plasmapheresis; treatment outcome.

Correspondence to: Aleksandar Lazović, University Clinical Center Kragujevac, Department of Surgery, Zmaj Jovina 30, 34 000 Kragujevac, Serbia. E-mail: dr.lazovic.kg@gmail.com

Apstrakt

Uvod/Cilj. Standardno lečenje za imunski posredovane neurološke poremećaje (IPNP) uključuje upotrebu imunosupresivnih lekova i drugih terapija. Terapijska izmena plazme (TIP) je efikasan dopunski imunomodulacijski pristup. Njen osnovni cilj je smanjenje „opterećenja" bolesnika patogenim faktorima (uključujući autoantitela) na nivoe koji će omogućiti kliničko poboljšanje. Imunosupresivni lekovi, korišćeni istovremeno sa TIP, smanjuju „povratni porast" sinteze autoantitela. Cilj rada bio je da se proceni efikasnost protokola naše standardizovane TIP i utvrdi korelacija ishoda TIP tretmana sa parakliničkim (laboratorijskim) i afereznim parametrima/podacima. **Metode.** Istraživanjem su obuhvaćena 32 bolesnika sa miastenijom gravis, sindromom Guillain-Barré ili akutnom poliradikuloneuropatijom i multiplom sklerozom. TIP je izvođena korišćenjem aparata Spectra-Optia® (Terumo-BCT, SAD). Svojstva našeg afereznog protokola primenjenog kod bolesnika sa IPNP bila su: a) sveukupni tretman – pet pojedinačnih TIP procedura; b) učestalost TIP – svaki drugi dan; c) uklonjena plazma – jedan volumen cirkulišuće plazme bolesnika (opseg 2 800–3 100 mL). Efikasnost TIP procenjena je na osnovu oporavka neurološkog deficita i provodljivosti perifernih nerava, pozitivnih nalaza u para-kliničkim (laboratorijskim) testovima i praćenja afereznih parametara. **Rezultati.** Korišćenjem opisanog protokola afereze primećen je jasan pozitivan terapijski efekat kod bolesnika koji su bili podvrgnuti TIP procedurama bez prekida. Procedure TIP bile su korisne kod 84,4% bolesnika (stopa efektivnosti 89,3%; stopa izostanka odgovora 10,7%), dok kod 15,6% slučajeva tretman nije završen zbog infekcije bolesnika koronavirusom 2 izazivačem teškog akutnog respiratornog sindroma (*severe acute respiratory syndrome coronavirus 2* – SARS-CoV-2) (praćene koronavirusnom bolešću 19 – COVID-19). Bolesnici kod kojih je sprovedeno pet pojedinačnih TIP imali su evidentno kliničko poboljšanje u smislu skale invaliditeta, slabosti mišića ili deficita neuronske provodljivosti. U periodu praćenja nisu zabeleženi recidivi. Značajno smanjene vrednosti eritrocitnih parametara (posebno nivoa hematokrita) bili su u korelaciji sa većom efikasnošću TIP, zahvaljujući povišenom odnosu volumena plazme/krvnih ćelija, što je bilo praćeno većom efikasnošću prikupljanja/uklanjanja plazme. Ostali praćeni laboratorijski nalazi nisu pokazali pozitivnu korelaciju sa efikasnošću/produktivnošću TIP. Nije bilo teških neželjenih događaja. Nije bilo realpsa u narednih 6 meseci. **Zaključak.** U ovom istraživanju značajno smanjene vrednosti eritrocitnih parametara (posebno nivoa hematokrita) bili su u korelaciji sa većom efikasnošću TIP. Za definitivne zaključke potrebne su buduće randomizovane i veće kliničke studije.

Ključne reči:
afereza; poliradikuloneuritis; miastenija gravis; multipla skleroza; plazmafereza; lečenje, ishod.

Introduction

Therapeutic plasma exchange (TPE) involves removing a portion of the patient's plasma and replacing it with an appropriate fluid—such as plasma, colloids, or crystalloids—and autologous cells. Beneficial effects of TPE can be obtained by changing the "antigen-to-antibody" ratio, reduction of the concentration of immune complexes, and/or modifying the activities of immuno-inflammatory mediators, and sometimes even through a placebo effect [1–4]. During a single TPE procedure, the volume of removed plasma should be 35–55 mL/kg of the patient's body mass – this practically corresponds to one volume of circulating plasma [1, 5]. The applied TPE protocols vary depending on the type of devices (equipment characteristics) used, the category of the disease, and the patient's general condition. More than 150 disorders have previously been documented in which TPEs have been used, although no uniform therapeutic effects have been achieved [2, 5–9]. Nowadays, that number has been reduced to a few dozen indications where the use of TPEs is really justified and undoubtedly effective. For the currently accepted indications given according to the classification of the American Society for Apheresis (ASFA), specific categories of diseases/disorders and the degree of success of TPE are shown [10]. Immune-mediated neurological disorders (IMNDs) are among them. Therefore, TPE should be considered a treatment option for IMNDs, provided that clearly defined clinical criteria are met. Generally, the use of TPE is associated with improving patients' clinical status – recovery of neurologic deficiencies and reducing disease-related complications [1–4]. Although numerous authors consider that the precise determination of exact parameters of the TPE efficacy in the treatment of IMND is still not completely resolved [4, 10–13].

Finally, it should be emphasized that the use of TPE, in combination with immunosuppressive drugs and other medications, should never represent the last approach or option in the treatment of IMNDs.

The aim of this study was to evaluate the effectiveness of our standardized TPE treatment and the correlation of this protocol with paraclinical (laboratory) findings and specific apheresis parameters/data.

Methods

Study design and population

This clinical investigation was designed as a retrospective unicentric pilot study performed in treating patients with IMND at a single center, the University Clinical Center Kragujevac – UCCK, Serbia. The study was conducted on 32 patients (male to female ratio 1 : 1.29) with IMND or autoimmune neurologic diseases: 9 patients had myasthenia gravis (MG), 12 patients had Guillain-Barré syndrome or acute polyradiculoneuropathy (APRN), and 11 patients had multiple sclerosis (MS). The patients were all hospitalized at the Department of Neurology of UCCK. Investigations were carried out from December 2019 to July 2024. The study was approved by the Ethics Committee of the UCCK (approval No. 01/23-130, from

April 10, 2023). The treatment of the subjects was carried out in accordance with the principles of ethics (Declaration of Helsinki) and good clinical practice. Written consent for inclusion in the research was obtained from each respondent.

Standardized TPE protocol

TPEs were performed by Spectra-Optia® (Terumo-BCT, USA), an automated blood cell separator of the last generation. Simply put, this *ex vivo* system separated whole blood (WB) into components – plasma and blood cells. Then, plasma [standard one circulating or total plasma volume (TPV)] was removed, and the remaining cellular components were resuspended in an equivalent/appropriate replacement fluid (human albumin in normal saline) and re-transfused [11].

Parameters monitored and analyzed during TPE procedures were as follows: 1) quantity of processed blood; 2) volume of removed plasma (one TPV); 3) replacement fluid amount and balance; 4) anticoagulant [acid-citrate-dextrose (ACD) formula B – ACD-B] quantity and proportion; 5) vascular access protection, blood flow rate, and single TPE procedure duration; 6) degree of plasma constituent and/or platelet (Plt) loss. Permanent monitoring of blood pressure, pulse, and cardiac rhythm was indicated only in "medically unstable" patients [10, 12–16].

In general, replacing one volume of circulating plasma removes approximately 45–55% of the pathological substrate present in the patient's plasma. A larger exchanged/removed plasma volume may result in coagulopathy, a higher risk of citrate-related adverse events (AEs), as well as plasma protein or electrolyte dysbalance [1, 13, 16]. For the above reasons, in our study, the quantity of removed plasma by a single TPE was standardized and equal to one TPV. Total blood volume (TBV) was defined by Spectra-Optia® software. The value of TPV was calculated by the following formula: TPV = TBV × (1.0–hematocrit), as previously described [12]. The whole (complete) apheresis treatment for these patients was carried out using a constant five single TPE procedures.

Vascular access was typically obtained across a central venous catheter or, rarely, using antecubital veins. Patients were anticoagulated by ACD-B (United States Pharmacopeia – USP XX; 1.8% citrate concentration). The ACD-B to WB ratio was 1:10. The removed plasma was replaced by 20% human albumin in combination with normal saline (removed vs. replaced fluid ratio = 1.0) [12, 13].

Following each reduction of autoantibody intravascular concentration by TPE, they will migrate from the extravascular into the intravascular space (equilibration phase is around 18 hrs). Furthermore, a decrease of antibody plasma concentration due to TPEs can lead to elevated synthesis, followed by higher antibody levels compared to pre-apheresis grade ("rebound" effect), resulting in inferior TPE effectiveness [1–4]. For this reason, TPE procedures in our study were conducted every second day, based on previous experiences, as well as accepted guidelines of the ASFA [10–13, 17, 18].

The plasma collection/removal efficacy (PCRE) was calculated as a ratio by dividing the quantity of removed plasma by the plasma volume processed in the blood cell separator, using the formula PCRE [%] = $(TPV_{removed} : TPV_{processed}) \times 100$. The Plt loss was determined by the quantity of Plts found in the waste bag expressed as the percentage of the initial Plt number in WB, as explained earlier [12, 15].

Thus, the most important attributes and properties of standardized TPE protocol applied in the treatment of our patients were as follows: 1) the whole apheresis treatment consisted of five TPE procedures; 2) single TPE procedures were performed every other (second) day; 3) by using single TPE procedure, one TPV was removed/replaced and with the whole apheresis treatment, TPV of five patients was removed/replaced.

TPE procedure was conducted in patients with acute exacerbation of disease in MS and MG, or in the first attack in APRN. Before starting the TPE protocol, MS and MG patients were treated with immunosuppressive therapy, but without an adequate positive response. Evaluation of the effectiveness of comprehensive or complete IMND treatment included: 1) monitoring of clinical improvement/status (based on the disability scale, muscle weakness or neural conduction deficit or disease relapse) and 2) laboratory data research: blood cell count analysis, biochemical parameter testing (aspartate transferase, alanine aminotransferase, C-reactive protein – CRP, total protein and albumin, sodium, potassium, calcium levels), as well as hemostatic activity investigation (prothrombin time, activated partial thromboplastin time), fibrinogen, etc. Quantitative analysis was done by comparing initial (before TPE) and final (after TPE) values in the Expanded Disability Status Scale (EDSS) in MS patients, the Osserman score of improvement in MG patients, and the Hughes Motor Scale (HMS) in APRN patients.

AE was considered severe if it was life-threatening or led to irreversible consequences with organ failure.

Statistical analysis

Statistical analysis was conducted using SPSS software. The normality of data distribution was tested using the Shapiro-Wilk test, given that the sample consisted of 32 patients. For analyzing the procedure's success in relation to patient admission laboratory values, the Student's *t*-test for independent samples or the Mann-Whitney *U* test was applied, depending on whether the data followed a normal distribution.

Results

The investigated sample was composed of IMND patients with an average age of 43.09 ± 14.54 years. Gender, blood group, and patient diagnoses (type of IMNDs) are shown in Table 1.

There was clear evidence of beneficial therapeutic effects with positive clinical outcomes in patients treated with a complete apheresis protocol (five single TPEs on 84.4% of

cases from our total IMND patient pool, with an evident therapeutic-benefit rate of 89.3%). In 15.6% of cases, treatment was not completed due to patients' severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (followed by coronavirus disease 19 – COVID-19) and their transport to a COVID center for adequate treatment. In MS patients, there was an improvement ranging from evident regression of symptoms to complete remission (reduction in EDSS) compared to the onset of disease relapse, particularly concerning walking and vision ability. In patients with APRN and MG, improvement was related to the improvement of muscle strength in all affected muscle groups. It was seen that APRN patients had a considerable improvement in neurological deficits. Moreover, peripheral neural conduction was improved in all APRN patients after TPE treatment.

In the group of nine MG patients, seven went through the whole TPE treatment (five procedures). All of them were estimated as grade III (three patients) and IV (four patients) (after Osserman's classification). The improvement was observed in six patients; in four patients, there was improvement from grades III and IV to grades I and II (by Osserman). In two patients, we recorded complete remission. There was no positive therapy response in one patient. Among the 11 MS patients (10 of them went through the whole TPE treatment), positive therapy response was observed in 7, in different degrees. All patients were in acute exacerbation before starting TPE, with an EDSS score between 5 and 6.5. After TPE treatment, nine patients had a positive clinical response with a reduction of 2–3 degrees in EDSS. One patient was without a positive clinical response after TPE. Twelve APNR patients started the TPE procedure within 7 days after the onset of the acute disease attack. Two patients did not finish the whole cycle due to COVID-19. Four patients were in grade III (walking 5 m with support), four patients were in grade IV (relying on a bed or wheelchair), and two patients were in grade V (requiring ventilatory support), according to HMS. In nine patients, a moderate to significant improvement was observed. TPE resulted in significant walking improvement in grade III patients, two grade IV patients improved their walking ability to grade II, and one of them resulted in HMS grade III. One patient who required ventilator support had a positive response to TPE in terms of clinical improvement in spontaneous breathing ability, but with no walking ability. Unfortunately, one patient in the HMS grade V had no positive therapy response (which led to death due to numerous comorbidities).

In the follow-up period (six months after TPE treatment), there were no relapses or significant worsening in terms of an increase in EDSS or decrease in muscle strength in all groups of IMND patients.

Data related to specific apheretic parameters (processed WB, removed plasma and replacement fluid volumes, fluid balance, and TPE duration) for whole apheresis treatments (five single TPE procedures) are shown in Table 2.

Table 1

IMND patients treated by apheresis procedures

Parameters	n (%)
Gender	
female	18 (56.3)
male	14 (43.7)
Blood groups	
O RhD positive	12 (37.5)
A RhD positive	16 (50.00)
B RhD positive	3 (9.38)
O RhD negative	1 (3.13)
Diagnosis	
myasthenia gravis	9 (28.13)
multiple sclerosis	11 (34.38)
APNR	12 (37.5)

IMND – immune-mediated neurologic disease; APNR – acute polyradiculoneuropathy.
Values are given as numbers (percentages).

Table 2

Parameters for single TPE treatments of IMND patients

Procedure parameters	Single TPE procedures				
	1th	2nd	3rd	4th	5th
Removed plasma volume (mL)	2,777 ± 214	2,881 ± 224	2,974 ± 159	3,027 ± 281	2,941 ± 196
Replacement fluid volume (mL)	2,585 ± 196	2,566 ± 321	2,504 ± 323	2,768 ± 124	2,884 ± 288
Processed blood (mL)	6,002 ± 452	5,804 ± 613	5,986 ± 751	5,826 ± 582	5,393 ± 548
ACD-B (mL)	418 ± 32	449 ± 36	437 ± 58	389 ± 49	437 ± 55
Removed vs. replaced fluid ratio	1.05	9.80	1.0	1.04	1.08
Procedure duration (min)	90 ± 18	94 ± 22	89 ± 24	90 ± 16	86 ± 23

TPE – therapeutic plasma exchange; IMND – immune-mediated neurologic disease; ACD-B – anticoagulant solution acid-citrate-dextrose formula B (United States Pharmacopeia – USP XX; 1.8% citrate concentration).
All values are given as mean ± standard deviation, except for fluid ratio, which is shown as a number.

As presented, the value of the processed blood across the device, i.e., the cell separator, ranged from 5,393 ± 548 mL to 6,002 ± 452 mL, with an evident decrease during the last single TPE. The volume of plasma removed/exchanged ranged from 2,777 ± 214 mL to 3,027 ± 281 mL and increased over time with treatments, but with the noted reduction during the fifth single TPE.

The replacement fluid quantity showed a slight decrease in the third single TPE (2,504 ± 323 mL). The fluid balance during a single TPE was constant and amounted to practically 1.0 (range 1.08–9.80). The amount of ACD-B solution used ranged from 389 ± 49 mL to 449 ± 36 mL. The duration of the procedure ranged from 86 ± 23 min to 94 ± 22 min, and this value decreased from the first to the last single TPE. There were no significant differences in the investigated apheresis parameters.

The mean PCRE in this study (using Spectra-Optia®) was high: 83.2 ± 5.2% (range 77–88%), and the Plt loss into the waste bag was minor: 2.1 ± 1.6% on average (range 1.2–4.9%).

Descriptive statistical analysis was done using the minimum and maximum values, as well as mean values ± standard deviation. The impact of the applied procedure on the laboratory values at discharge was analyzed using the Student's *t*-test for paired samples or the Wilcoxon test, depending on whether the data followed a normal distribution. The results of laboratory blood testing at the start and the end of the single TPE procedures are shown in Table 3.

There was a significant decrease in values for RBCs [t = 4.280; df = 21; $p < 0.005$; mean initial value (M1) = 4.56; mean final value (M2) = 3.99], hemoglobin (t = 4.014; df = 21; $p < 0.005$; M1 = 129.91; M2 = 115.04), hematocrit (t = 4.014; df = 21; $p < 0.005$; M1 = 0.40; M2 = 0.35), platelets (t = 2.831; df = 21; $p < 0.05$; M1 = 240.95; M2 = 197.23), total proteins (t = 5.320; df = 21; $p < 0.005$; M1 = 60.36; M2 = 52.05), potassium (t = 2.538; df = 21; $p < 0.05$; M1 = 4.06; M2 = 3.85), and calcium (Z = 3.064; $p < 0.05$; M1 = 2.29; M2 = 2.15), respectively.

As presented, most of the laboratory values decreased. Using the Student's *t*-test for independent samples, we detected a significant difference (initial vs. final values) for red blood cell (RBC) parameters (especially for hematocrit) in patients in whom TPE treatment was realized completely, with subsequent high-quality clinical effects (superior recovery of neurologic deficiency and peripheral nerve conduction). In the treatment of patients in whom apheresis was interrupted (due to COVID-19), the partially realized TPE protocol was unproductive, and laboratory data for them were disqualified and excluded.

The impact of the applied procedure on the laboratory values at discharge was analyzed using the Student's *t*-test for paired samples or the Wilcoxon test, depending on whether the data followed a normal distribution (Table 3).

The influence of laboratory values at patient admission on procedure parameters is shown in Table 4. By applying the correlation and regression method, i.e., by interpreting the values of the Pearson and Spearman correlation coefficients, a significant and moderately strong to strong correlation was established between the creatinine value and the quantity of removed plasma (r = 0.494; $p < 0.05$), replacement fluid substitution (r = 0.517; $p < 0.05$), processed blood (r = 0.493; $p < 0.05$), and procedure duration (r = 0.434; $p < 0.05$).

In this study, we had only a few mild AEs, such as transitory hypotension (two patients), citrate toxicity (one patient) due to hypocalcaemia (corrected promptly by calcium gluconate), and one patient with an allergic reaction (urticaria) that had been solved with antihistaminic and corticosteroid therapy.

Table 3

The influence of the applied procedure on patients' laboratory data at discharge

Parameters	Initial values	Final values	Stat.	*df*	*p*
WBCs, $\times 10^9$/L	8.76 ± 4.25	11.23 ± 5.11	Z = 1.246	-	0.177
RBCs, $\times 10^{12}$/L	4.68 ± 0.97*	3.86 ± 0.65*	t = 4.280	21	< 0.05
Hemoglobin, g/L	131.22 ± 15.50*	114.88 ± 28.45*	t = 4.014	21	< 0.05
Hematocrit, L/L	0.41 ± 0.04*	0.34 ± 0.04*	t = 4.014	21	< 0.05
Platelets, $\times 10^9$/L	236.85 ± 72.62*	207.32 ± 65.70*	t = 2.831	21	< 0.05
Prothrombin time, s	13.17 ± 1.24	14.65 ± 3.7	t = 1.836	21	0.087
aPTT, s	29.65 ± 8,23	30.05 ± 5.82	Z = 0.416	-	0.655
Fibrinogen, g/L	3.15 ± 1.03	2.67 ± 0.88	Z = 1.511	-	0.138
Glucose, mmol/L	5.61 ± 2.16	6.46 ± 2.46	Z = 1.350	-	0.204
Urea, mmol/L	6.04 ± 2.08	5.45 ± 1.87	Z = 1.296	-	0.158
Creatinine, μmol/L	71.91 ± 12.50	64.92 ± 15.54	t = 0.543	21	0.492
ESR, mm/hr	26.73 ± 8.44	34.92 ± 18.82	t = 0.754	21	0.48
Total proteins, g/L	62.15 ± 8.82*	54.06 ± 6.22*	t = 5.320	21	< 0.05
Albumin, g/L	39.89 ± 5.40	32.91 ± 4.21	t = 0.466	21	0.685
AST, U/L	32.2 ± 18.67	31.58 ± 16.19	Z = 0.134	-	0.899
ALT, U/L	50.48 ± 32.06	44.76 ± 34.31	Z = 0.427	-	0.715
Potassium, mmol/L	4.16 ± 0.30*	3.73 ± 0.32*	t = 2.538	21	< 0.05
Sodium, mmol/L	138.45 ± 6.44	117.65 ± 8.29	Z = 0.361	-	0.788
Calcium, mmol/L	2.47 ± 0.23*	1.92 ± 0.31*	Z = 3.064	-	< 0.05
CRP, mg/L	7.82 ± 6.05	14.68 ± 12.54	Z = 2.121	-	1.125

WBCs – white blood cells; RBCs – red blood cells; aPTT – activated partial thromboplastin time; ESR – erythrocyte sedimentation rate; AST – aspartate aminotransferase; ALT – alanine aminotransferase; CRP – C-reactive protein.
Values are given as mean ± standard deviation. *$p < 0.05$.

Table 4

The influence of patients' laboratory data upon admission to the hospital on the parameters of the procedure

Parameters	Removed plasma		Replacement fluid		Processed blood		Procedure duration	
	r/ρ	*p*	*r*/ρ	*p*	*r*/ρ	*p*	*r*/ρ	*p*
WBCs	-0.216	0.335	-0.115	0.612	-0.171	0.447	0.102	0.651
RBCs	0.033	0.885	0.143	0.527	0.099	0.661	0.178	0.427
Hemoglobin	0.087	0.701	0.205	0.359	0.160	0.477	0.303	0.170
Hematocrit	-0.043	0.851	0.108	0.634	0.142	0.530	0.020	0.931
Platelets	0.106	0.640	0.075	0.740	0.060	0.791	0.100	0.658
Prothrombin time	-0.083	0.715	-0.018	0.935	-0.278	0.210	0.078	0.729
aPTT	0.422	0.050	0.301	0.174	0.286	0.197	-0.229	0.306
Fibrinogen	0.017	0.942	0.027	0.904	0.110	0.625	-0.064	0.778
Glucose	0.028	0.899	0.019	0.935	-0.020	0.931	-0.034	0.882
Urea	0.057	0.802	0.156	0.488	0.105	0.641	0.372	0.088
Creatinine	0.494	0.020*	0.517	0.014*	0.493	0.020*	0.434	0.043*
eGFR	0.225	0.315	0.151	0.502	0.279	0.208	0.271	0.223
Total proteins	-0.248	0.266	-0.318	0.149	0.109	0.630	-0.171	0.446
Albumin	0.052	0.820	-0.030	0.894	0.205	0.360	-0.378	0.083
AST	0.088	0.698	0.127	0.573	0.137	0.544	0.043	0.850
ALT	0.082	0.718	-0.021	0.927	-0.097	0.667	0.130	0.565
Potassium	0.098	0.663	0.071	0.753	0.165	0.464	-0.086	0.704
Sodium	0.091	0.688	0.067	0.766	0.010	0.966	-0.056	0.804
Calcium	0.109	0.629	-0.056	0.806	0.221	0.323	-0.155	0.492
CRP	-0.024	0.915	-0.169	0.453	0.180	0.423	-0.016	0.942

eGFR – estimated glomerular filtration rate. For other abbreviations, see Table 3. *$p < 0.05$.

Discussion

As mentioned, the rationale for the use of TPE in the treatment of IMNDs is based on the acceptance of the fact that numerous autoimmune and inflammatory diseases result from immune system dysregulation or malfunctioning, leading to the production of destructive autoantibodies or excessive inflammation [1, 14]. Through the depletion of these pathogenic factors in the bloodstream, TPE interrupts disease development/progress and offers therapeutic benefits. This study aimed to provide an answer as to whether the use of TPE results in an improvement in the overall clinical condition and laboratory parameters of IMND patients. The precise assessment of the advantageous effects of TPE procedures was definitely affected by the incomplete understanding of the pathogenesis of the majority of IMNDs and the lack of simple and well-established laboratory tests to quantify the pathogenic substrate in the patient's blood and/or removed plasma [1, 11, 13].

Although TPE is a relatively invasive procedure compared to intravenous immunoglobulin (IVIG), the rationale for choosing TPE over IVIG can vary depending on the specific clinical setting and the patient's general condition. Various factors may impact this choice in favor of TPE. TPE has a quicker action, which is important in the acute attack of the disease. Moreover, patients with renal or cardiovascular conditions are more appropriate for TPE than for IVIG treatment. Finally, TPE can be an alternative in patients who did not respond adequately to IVIG [19–23].

Thus, Liu et al. [20] reported that TPE treatment showed better short-term clinical effectiveness than IVIG therapy in patients with MG. The results of TPE published by Kumar et al. [21] also confirmed tremendous improvement in patients with MG and in those who experience exacerbations despite treatment with steroids and oral immunosuppressive medications.

In the study by Tombak et al. [24], 19 out of 21 MG patients had improvements with TPE, with 14 in the "complete responses" group (the neurological deficit was improved completely after TPE) and 5 in the "partial responses" group (some response, but the neurological deficit did not disappear completely). Two patients were in the "nonresponse" group (there was no response after the performed TPE treatment), possibly because they were admitted too late after the onset of symptoms. They died shortly after the start of the procedure due to respiratory failure. In this study, the overall response rate of TPE in IMNDs was 81%, with mild to moderate and manageable side effects. Comparable data are presented in a study by Yeh and Chiu [25] for 30 MG patients using the double filtration plasmapheresis method. According to them, the success rate in the TPE treatment of MG patients ranges from 55% to 100%.

A certain number of authors have described the functional impact of TPE on the immune system in patients with different immune-mediated disorders, such as lupus, MS, acute disseminated encephalomyelitis, or APRN. It causes a modification of lymphocyte proliferation capacities and modulation of antibody production [26–28]. Furthermore, it induces the reconstitution of certain subpopulations of lymphocytes (regulatory T cells) [28, 29] and the improvement of the functional capacities of monocytes and macrophages [30]. In brief, TPE is an extracorporeal blood purification technique allowing the removal of pathogenic macromolecules from the blood. It has been successfully used for several decades in managing IMNDs.

Together, these therapeutic approaches form part of a comprehensive system for treating severe IMNDs, intending to reduce disease activity and enhance patient clinical outcomes.

Thanks to the increased number of studies, indications are based not only on assumptions but also on facts.

Our pilot study confirms the positive clinical effects (recovery of neurologic deficiency and peripheral nerve conduction) of the TPE treatment used in IMND patients. Namely, favorable effects of treatment were manifested by the return of weakened reflexes, normalization of motor functions, disappearance of speech, and swallowing dysfunction, with positive findings in certain paraclinical (laboratory) tests. Overall, 89.3% of patients with positive therapy responses after TPE had different degrees of improvement, which is comparable with results revealed by several previous studies [31, 32].

According to our study, TPE is a potent and well-tolerated method for treating IMNDs. Beneficial therapeutic effects were observed in patients undergoing five single TPE procedures (without interruption). TPEs were completed in 84.4% of patients (the therapeutic-benefit rate was 89.3%). Superior treatment efficacy in our study was observed in patients with significantly inferior RBC parameters (primarily hematocrit levels) as a result of an elevated ratio of the plasma vs. blood cell volume and higher PCRE values (up to 88%). It is comparable to our earlier results and data from the literature for PCRE [12, 15]. With increased creatinine values on admission, the values of all considered procedure parameters rose. Considering the normal WBC values during TPE, we can assume that this was a transient increase in CRP values due to the placement of a central venous catheter or urinary catheter, which can cause a slight increase in CRP. Therefore, we believe these values are not directly associated with the TPE procedure.

The timing of the TPE is also important for some IMNDs – the TPE treatment started as early as possible (within 7 days after the onset of the disease) and was followed by antibody reduction and superior clinical improvement in APRN patients [11]. The importance of optimized apheresis timing was determined as a significant factor of the TPE efficacy in our earlier studies and also in data from the literature [9–11, 13]. In the therapy of a certain number of patients (15.6%), TPE treatment was not completed, as mentioned, due to patients' SARS-CoV-2 infection, followed by COVID-19, and their transport to a COVID center for proper treatment.

Despite the benefits of TPE, several studies have evaluated some potential AEs and complications, including those related to replacement fluids or anticoagulant solutions, cardiovascular vulnerability, vascular access, normal plasma constituents, or Plt reduction/loss, etc. [11, 15–18]. Finally, the use of TPE procedures is not possible without updated medical education of personnel (apheresis team members) and experience related to work with extracorporeal circulation [11, 14].

In this study, we observed only a small number of mild AEs, such as transitory hypotension in two patients, citrate toxicity in one patient (with mild tingling in the legs as a result of hypocalcemia), and mild to moderate urticaria in one patient. There were no serious AEs related to TPE treatment during this study.

Conclusion

The effectiveness of immunomodulatory drugs plus TPE therapy varies and, to some degree, depends on the type of immune-mediated neurological disorders and the patient's condition. Reduced levels of red blood cell parameters (particularly hematocrit levels) were associated with an increased TPE effectiveness, primarily due to an increased plasma-to-blood cell volume ratio, followed by superior plasma collection/removal efficacy values. Other paraclinical and laboratory findings did not correlate positively with TPE efficacy. The patient tolerated the TPE treatment well without severe adverse events. No relapses were found within a 6-month follow-up period. For definitive conclusions, further randomized, controlled, and larger clinical trials are needed.

Conflict of interest

The authors declare no conflict of interest.

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Demographic characteristics and spectrum of comorbidities in patients with muscle tension dysphonia: a retrospective cross-sectional study

Demografske karakteristike i spektar komorbiditeta kod osoba sa mišićnom tenzionom disfonijom: retrospektivna studija preseka

Jelena Sotirović*†, Maša Petrović‡§, Milanko Milojević*†, Nenad Baletić*†, Ognjen Čukić*, Ivan Zaletel‖

***Military Medical Academy, Clinic for Otorhinolaryngology, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; University of Belgrade, ‡Faculty of Medicine, ‖Institute for Histology and Embryology "Aleksandar Đ. Kostić", Belgrade, Serbia; §Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia**

Abstract

Background/Aim. Muscle tension dysphonia (MTD) is one of the most common voice disorders. The aim of the study was to examine demographic characteristics and the prevalence of comorbidities in patients diagnosed with MTD. **Methods.** A retrospective cross-sectional study included an analysis of demographic characteristics and comorbidities in 87 patients diagnosed with MTD during a one-year period. **Results.** The mean age of the patients was 49.2 years (range 18–84 years), and 79.3% were younger than 65 years. The female gender comprised 73.6%. No statistically significant difference was found in smoking status between men and women ($\chi^2 = 0.624$; $p > 0.05$). Out of the 87 patients, 43 (49.4%) were professional voice users. Among these patients, 20 (46.5%) were school teachers, 8 (18.6%) sales-related occupations, 5 (11.6%) professional singers, 3 (7.0%) kindergarten teachers, 3 (7.0%) lecturers, 2 (4.6%) psychologists, 1 (2.3%) speech-language pathologist, and 1 (2.3%) fitness instructor. Secondary MTD was diagnosed in 36 (41.4%) patients. The most common clinical findings diagnosed in those with secondary MTD were vocal cord nodules in 24 patients (66.7%). The prevalence of comorbidities were as follows: mild to moderate hearing loss (9.2%), postnasal drip (31.0%), hypersensitivity to common inhalant allergens (31.0%), asthma or chronic obstructive pulmonary disease (18.4%), thyroid dysfunction (17.2%), and history of gastroesophageal reflux disease (21.8%). **Conclusion.** MTD is more frequent in females and professional voice users. Due to the high prevalence of comorbidities in MTD patients, a detailed history and additional examinations are necessary in order to determine the final treatment plan.

Key words:
comorbidity; demography; dysphonia; prevalence; serbia.

Apstrakt

Uvod/Cilj. Mišićna tenziona disfonija (MTD) je jedan od najčešćih poremećaja glasa. Cilj rada bio je da se ispitaju demografske karakteristike i prevalencija komorbiditeta kod pacijenata kojima je postavljena dijagnoza MTD. **Metode.** Retrospektivnom studijom preseka analizirane su demografske karakteristike i komorbiditeti kod 87 pacijenata kojima je postavljena dijagnoza tokom jednogodišnjeg perioda. **Rezultati.** Prosečna starost pacijenata bila je 49,2 godine (opseg 18–84 godina), a 79,3% je bilo mlađe od 65 godina. Ženski pol činio je 73,6%. Nije utvđena statistički značajna razlika u pušačkom statusu između muškaraca i žena ($\chi^2 = 0{,}624$; $p > 0{,}05$). Od 87 pacijenata, 43 (49,4%) su bile osobe koje se bave profesijama koje zahtevaju intenzivnu upotrebu glasa. Među ovim pacijentima, 20 (46,5%) su bili nastavnici u školi, 8 (18,6%) prodavci, 5 (11,6%) profesionalni pevači, 3 (7,0%) vaspitači u vrtiću, 3 (7,0%) predavači, 2 (4,6%) psiholozi, 1 (2,3%) logoped i 1 (2,3%) *fitness* instruktor. Sekundarna MTD dijagnostikovana je kod 36 (41,4%) pacijenata. Najčešći klinički nalazi dijagnostikovani kod osoba sa sekundarnom MTD bili su čvorići na glasnim žicama kod 24 pacijenta (66,7%). Prevalencija komorbiditeta bila je sledeća: blago do umereno oštećenje sluha (9,2%), postnazalna sekrecija (31,0%), preosetljivost na uobičajene inhalatorne alergene (31,0%),

Correspondence to: Jelena Sotirović, Military Medical Academy, Clinic for Otorhinolaryngology, Crnotravska 17, 11 000 Belgrade, Serbia. Email: j.sotirovic@gmail.com

astma ili hronična opstruktivna bolest pluća (18,4%), poremećaj rada štitaste žlezde (17,2%) i anamnestički podaci o gastroezofagealnoj refluksnoj bolesti (21,8%). **Zaključak.** MTD se češće javlja kod žena i osoba koje se bave profesijama koje zahtevaju intenzivnu upotrebu glasa. Zbog visoke prevalencije komorbiditeta kod pacijenata kojima je postavljena dijagnoza MTD, neophodni su detaljna anamneza i dodatna ispitivanja kako bi se odredio konačan plan lečenja.

Ključne reči:
komorbiditet; demografija; glas, poremećaji; prevalenca; srbija.

Introduction

Muscle tension dysphonia (MTD) refers to an excessive tension of extrinsic and intrinsic laryngeal muscles, which can be identified by surface electromyography and leads to voice change, throat pain, and vocal fatigue [1]. It represents one of the most frequent voice disorders, with an estimated prevalence of up to 40% of presenting disorders in voice clinics [2, 3]. MTD can be categorized as primary or secondary depending on whether the underlying organic conditions are present (secondary) or absent (primary). Diagnosis is done with a case history, an inspection of the hard glottal attack and any elevation of the larynx, palpation of the strap muscles to identify any increased tension, and stroboscopy [4, 5]. As previously mentioned in the definition of the condition, surface electromyography is a valuable diagnostic modality, but it is not routinely available in clinical practice. Although surgery is often necessary in secondary MTD, the cornerstone of therapy is conservative treatment. Voice therapy is fundamental, and MTD is one of the most common reasons for a voice therapy referral [6].

MTD is thought to be a compensatory manifestation of an underlying disturbance in laryngeal structure and/or function [1]. In our opinion, establishing all the contributing factors in the pathophysiology of MTD will result in the proper choice of therapeutic approach.

The aim of this study was to examine demographic characteristics and the prevalence of comorbidities in patients with MTD, as the recognition of all of the underlying etiologic factors may be crucial to determining the adequate treatment modality.

Methods

The study was performed in accordance with the International Code of Medical Ethics of the World Medical Association, Declaration of Helsinki (1964). It was a part of the regular assessment and treatment of patients at the Phoniatric Department of the Clinic for Otorhinolaryngology and Maxillofacial Surgery, Faculty of Medicine of the University of Belgrade, Serbia (No. 16-UFO-01).

Our retrospective cross-sectional study included 87 patients diagnosed with MTD during their first visit to the Phoniatric Department. A retrospective chart review of these patients was performed over a period of one year. The MTD diagnosis was clinically determined by the same senior laryngologist who reviewed the detailed case histories and physical examinations.

Our diagnostic algorithm was used to identify comorbidities that could potentially affect laryngeal muscle tension. Clinical examination protocol included: pure tone audiometry, nasendoscopy, and skin prick test for common inhalant allergens. Hearing loss was characterized as > 25 decibels (dB) of hearing thresholds at 0.5, 1, 2, and 4 kilohertz (kHz) in the worse hearing ear. Anamnestic data on demographic characteristics, smoking status, occupational vocal load, thyroid status, gastroesophageal reflux disease (GERD), and pulmonary disease were included in the analysis. According to Koufman and Isaacson [7], patients' vocal load was classified into professional voice users (PVUs), levels I–III, and nonprofessional voice users, level IV. To analyze the influence of age on MTD, patients were divided into those above and below 65 years of age [8].

Exclusion criteria encompassed premalignant and malignant laryngeal lesions, laryngeal papillomatosis, previous radiotherapy in the head and neck region, previous laryngeal surgery, and neurologic and muscle diseases.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA) and the R statistical software (R Core Team, 2019). Descriptive statistics were calculated to summarize demographic and clinical characteristics of the study population. For categorical variables, frequencies and percentages were calculated, while continuous variables were reported as means with standard deviations.

To examine associations between categorical variables, Pearson's Chi-square test was employed. For comparisons between continuous variables across different MTD groups, Student's independent *t*-test was used. Spearman's rank correlation coefficient was calculated to assess correlations between comorbidity parameters and MTD status. A *p*-value < 0.05 was considered statistically significant.

Results

The mean age of the patients was 49.2 ± 16.7 years (range 18–84). Females represented 73.6% [64 patients with a mean age of 46.7 ± 15.9 years (range 18–76)] of the cohort. The proportion of patients aged < 65 years [69 (79.3%)] was higher than those aged ≥ 65 years [18 (20.7%)]. In the female group, 8 were smokers and 56 were non-smokers, while in the male group, 2 were smokers and 21 were non-smokers (Table 1). Statistical analysis showed

no difference in smoking status between males and females to the level of statistical significance ($\chi^2 = 0.624$; $p > 0.05$).

Out of the 87 patients, 43 (49.4%) were PVUs. The majority of PVUs were school teachers (20; 46.5%), followed by sales-related occupations (8; 18.6%) (Figure 1).

Out of the 87 patients, 51 (58.6%) had primary MTD, while 36 (41.4%) were diagnosed with secondary MTD. Of the 36 patients with secondary MTD, the majority were females, 31 (86.1% vs. 13.9%). Secondary MTD was statistically more frequent in females than males ($\chi^2 = 4.972$; $p < 0.05$). The mean age of patients with primary MTD and secondary MTD was 54.86 ± 14.7 years and 41.28 ± 16.16 years, respectively. There was a statistically significant difference between the primary and secondary MTD groups ($t = 4.074$; $df = 85$; $p < 0.001$). The most common findings in patients with MTD were vocal fold nodules [24/36 (66.7%)]. Structural findings in secondary MTD are summarized in Figure 2.

Pure tone audiometry showed abnormal pure-tone thresholds in 8 (9.2%) patients with mild to moderate hearing loss. Twenty-seven (31.0%) patients had a postnasal drip on nasoendoscopy. Skin prick test revealed that 27 (31.0%) patients were sensitive to common inhalant allergens. Anamnestic data demonstrated that 16 (18.4%) patients had asthma or chronic obstructive pulmonary disease (COPD). Fifteen (17.2%) patients had thyroid hormone replacement therapy or Hashimoto thyroiditis. Nineteen (21.8%) patients had a history of previously treated GERD. Figure 3 summarizes the total number of comorbidities, considering that some patients had multiple comorbidities.

Using Spearman's rank correlation coefficient, we were unable to find a correlation between the observed comorbidities and primary and secondary MTD. Likewise, there was no statistically significant difference in the frequency of these comorbidities in primary versus secondary MTD.

Table 1

Demographic characteristics of patients with muscle tension dysphonia

Parameters	Female	Male	Total
Patients, years	64 (73.6)	23 (26.4)	87 (100.0)
< 65	55 (85.9)	14 (60.9)	69 (79.3)
≥ 65	9 (14.1)	9 (39.1)	18 (20.7)
Smokers	8 (9.2)	2 (2.3)	10 (11.5)
Non-smokers	56 (64.4)	21 (24.1)	77 (88.5)
Age	46.7 ± 15.9 (18–76)	56.4 ± 17.1 (21–84)	49.2 ± 16.7 (18–84)

Values are given as numbers (percentages) except for the age parameter, which is expressed as mean ± standard deviation (minimum–maximum).

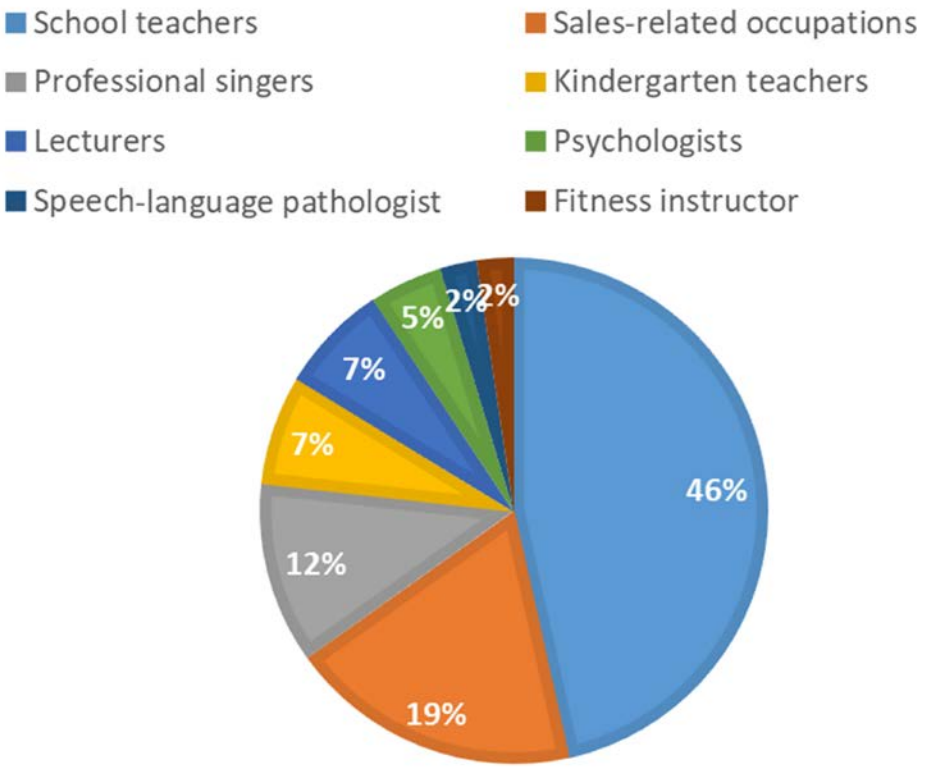

Fig. 1 – Distribution of professions with vocal load (n = 43).

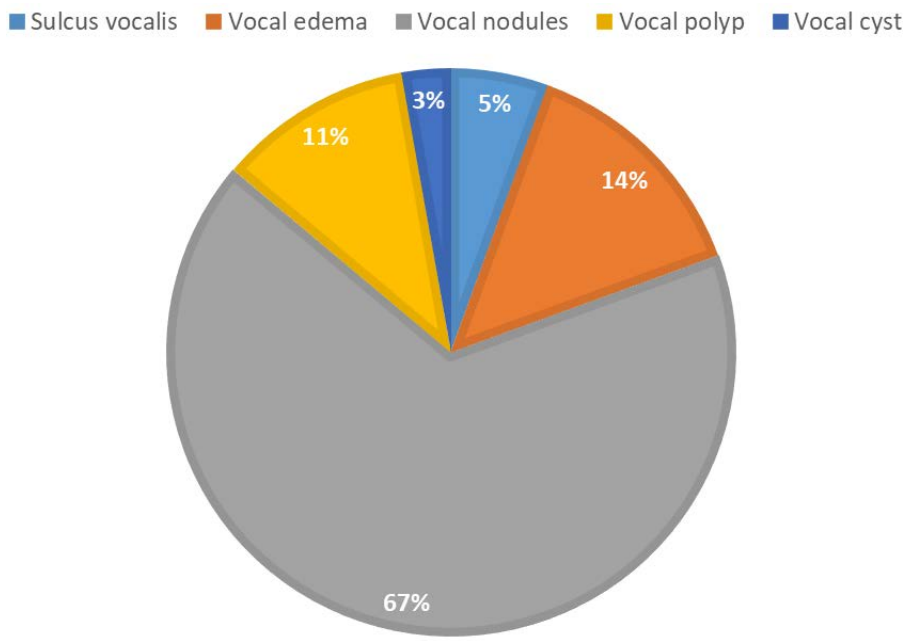

Fig. 2 – Structural changes in secondary muscle tension dysphonia (n = 36).

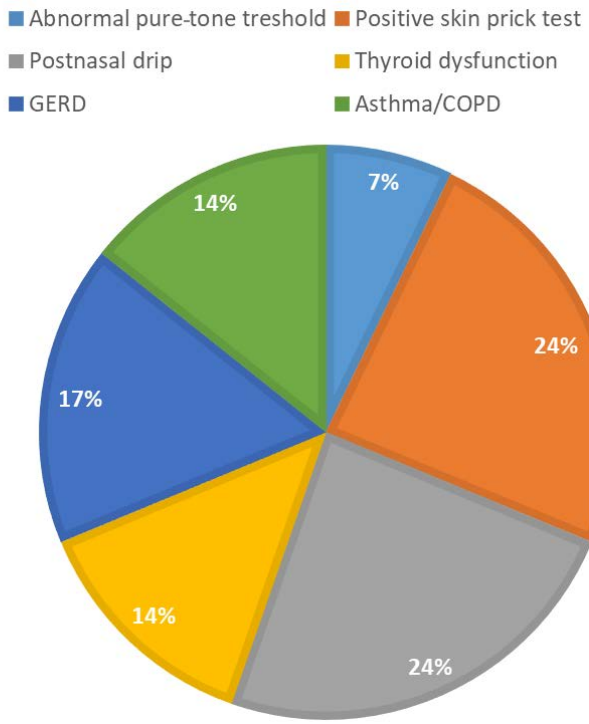

Fig. 3 – Total number of comorbidities in patients with muscle tension dysphonia (n = 87). GERD – gastroesophageal reflux disease; COPD – chronic obstructive pulmonary disease. Note: each particular comorbidity is presented according to the total number of comorbidities, considering that some patients had multiple comorbidities.

Discussion

Identifying the nature of the voice impairment is the fundamental purpose of the voice evaluation. Previous studies suggest that several etiological factors could play a role in patients with MTD, including psychological/personality factors, vocal abuse/misuse, underlying factors such as organic fold lesions, laryngopharyngeal reflux, altered hormonal status, aging of the larynx, and upper respiratory tract infection [1, 9]. Stroboscopic examination reveals posterior glottal chink, supraglottic contraction, and/or anterior-posterior contraction. Stroboscopy is highly important in establishing the diagnosis of MTD by providing a more detailed

examination of the vocal fold vibratory properties and any subtle abnormalities which can be easily missed by mirror laryngoscopy in general otorhinolaryngologists' practice, hence, patients with MTD can be underdiagnosed [6].

Analysis of patients' age data revealed that the majority (79.3%) of patients with MTD are part of the working population under the age of 65. This highlights the impact of MTD on employees' work performance and the significance of the timely initiation of appropriate treatment in order to minimize sick leave.

Voice change is detrimental for all patients, especially for voice professionals, given that dysphonia can be crucial to job performance. Our study findings showed that 49.4% of participants were PVUs, with teachers and sales-related occupations being the most prevalent. As previously reported, PVUs are at a significant risk of developing hyperfunctional voice disorders [9, 10]. Moreover, in PVUs, patient-perception of voice impairment can be influenced by occupational demand, which probably leads to earlier evaluation of voice changes [11]. The high prevalence of PVUs with MTD in our study suggests the necessity of further studies of voice training programs and effective treatment planning in professions with a vocal load.

On the other hand, aging promotes structural and functional changes of laryngeal structures, which can lead to voice change [12]. Belafsky et al. [13] showed that persons with underlying glottal insufficiency in the face of presbylaryngis are 17 times more likely to exhibit abnormal compensatory hyperkinetic laryngeal behaviors. In our study, 20.7% of patients were 65 or older. Voice disorders in the elderly population frequently bring fear of cancer and a negative impact on quality of life, hence, adequate therapy of MTD in the elderly will have a significant impact on their ability to maintain social activities, as well as functional and emotional well-being [14].

According to our results, a relatively small proportion of patients with MTD (11.5%) were smokers. In a large cross-sectional study with 821 adult participants, smoking was not associated with functional voice disorders [15].

The majority (73.6%) of patients with MTD in our study were females, which is consistent with other studies [16]. Female preponderance in MTD encompasses multiple factors including physiologic (lesser amount of hyaluronic acid in the superficial layer of the *lamina propria*; shorter vocal folds with higher frequency vibration) and psychological/personality factors (higher level of stress; higher effort in background noise), which could make them more vulnerable to phonotrauma [3, 6, 17, 18]. Our findings also showed that secondary MTD is more frequent in female patients (86.1% vs. 13.9%). Multiple factors influencing female voice may be related to voice disturbances, and special consideration must be taken when evaluating females for MTD.

Previous studies demonstrate that hearing loss affects voice production [19, 20]. Our analysis revealed that 8 (9.2%) patients with MTD also had audiometrically proven hearing loss. Own-voice perception is a result of hearing ability and can interfere with voice production. It is reasonable to assume that individuals with hearing loss are more likely to compensate with inappropriate vocal techniques, resulting in MTD. Hengen et al. [20] also indicated that hearing aids can affect vocal satisfaction. In our opinion, hearing evaluation in selected cases can reveal a subset of patients who will benefit from hearing aids along with other therapeutic strategies.

In our study, the prevalence of patients with inhalant allergy, postnasal drip, and asthma/COPD was 31.0%, 31.0%, and 18.4%, respectively. According to the 2019 Serbian National Health Survey published by the Statistical Office of the Republic of Serbia, the prevalence of allergies in the general population is 7.3%. The prevalence of asthma and COPD in the Serbian population is 3.6% and 3.5%, respectively [21].

Dysphonia, vocal fatigue, throat clearing, and cough are among the most common complaints in patients with allergic rhinitis, allergy, and asthma, and also in patients taking inhaled steroids [22–25]. It is possible that symptoms like runny nose, itchy eyes, shortness of breath, and cough are more prominent and therefore distract from observing voice changes in these patients. Nonetheless, irritation in the upper airways and gradual voice deterioration might lead to voice disorders and a significantly worsening quality of life.

The lifetime prevalence of allergic rhinitis in the United States is estimated between 11% and 33%, and between 10% and 41% in Europe [26]. The results of one Swedish study showed that patients with confirmed allergic rhinitis from birch pollen experienced voice symptoms during both the pollen and non-pollen season [27]. Given that population-based studies have shown increases in allergic rhinitis prevalence in the adult population in recent decades, it is important to exclude allergies when assessing patients with MTD. Findings of airborne allergies could significantly influence the treatment plan for these patients.

In our study, 15 (17.2%) patients had a history of clinical hypothyroidism with levothyroxine replacement therapy or Hashimoto's thyroiditis with normal thyroid hormone levels but elevated levels of antibodies to thyroglobulin and thyroid peroxidase. Clinical manifestations of hypothyroidism, among others, include voice change, profound fatigue, dry mouth, and lethargy [28]. However, voice change in thyroid disease might be less obvious compared to other, more severe symptoms. According to the published literature, a significant number of patients who reach biochemical treatment targets with thyroid hormone replacement therapy have persistent complaints. There is a subset of patients in which symptoms persist despite the patients' euthyroid status while receiving hormone substitution. Persistent symptoms respond only partly to adequate thyroid hormone substitution and are thought to be related to autoimmune disease rather than to hypothyroidism [29–32]. Furthermore, Carta et al. [33] found a link between the presence of the antithyroid peroxidase autoantibodies and the diagnosis of mood or anxiety disorder, which is independent of gender and age. In our opinion, patients with thyroid dysfunction deserve special attention when evaluated for MTD. Voice symptoms in patients with hormone replacement therapy and/or

antithyroid autoantibodies might be atypical or subtler since not all patients benefit from levothyroxine supplementation. Further investigations might be needed to determine the role of thyroid dysfunction in voice disorders.

Our data showed that 19 (21.8%) patients had a history of GERD without presentation of laryngopharyngeal reflux (LPR) clinical findings. Hoarseness can be one of the extraesophageal symptoms of GERD [34]. One possibility is that increased laryngeal muscle tension and associated voice change might have occurred as a result of previous gastric reflux. The other possible mechanism may include nasal symptoms. A review by Hanna and Wormald [35] concluded that reflex response between the esophagus and nose can produce postnasal drip in patients with GERD. Although not to the level of statistical significance, the authors proved that gastroesophageal reflux leads to nasal congestion and increased mucus secretion. Our data suggest that in MTD patients with a history of GERD, it seems justifiable to include diet and lifestyle changes in the treatment plan to prevent the potential effects of reflux. Considering that LPR often coexists with GERD, patients with MTD and a history of GERD could benefit from further investigations for LPR, including impedance-pH metry, and pepsin and trypsin detections.

In addition, it is worth pointing out that our study is limited by its retrospective nature. Further research is needed to examine the potential benefits of treating comorbidities in patients with MTD.

Conclusion

Muscle tension dysphonia is most commonly diagnosed in females and professional voice users. Voice specialists should consider multiple potential etiologies for altered muscular tension of extrinsic and intrinsic laryngeal muscles. The reported comorbidities in patients with muscle tension dysphonia may influence their response to treatment. The further assessment of how hearing loss, inhalant allergy, postnasal drip, asthma/chronic obstructive pulmonary disease, thyroid dysfunction, and history of gastroesophageal reflux disease interact with muscle tension dysphonia remains to be further studied. Although voice therapy plays an important role in treating patients with muscle tension dysphonia, in treatment planning, it is rational to perform a more detailed history and assessment, revealing all the potential contributing disorders.

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The relationship between the apnea-hypopnea index, oxygen desaturation index, average oxygen saturation, and body mass index in patients with obstructive sleep apnea

Odnos između indeksa apneja-hipopneja, indeksa desaturacije kiseonikom, prosečne saturacije kiseonikom i indeksa telesne mase kod bolesnika sa opstruktivnom apnejom tokom spavanja

Dobrivoje Novković*, Nela Popović†, Nikola Novković‡, Jelena Lukić§, Vesna Begović Kuprešanin‖, Martin Popević§

Military Medical Academy, *Clinic for Pulmonology, ‡Clinic for Emergency and Clinical Toxicology, §Institute of Occupational Medicine, ‖Clinic for Infectious and Tropical Diseases, Belgrade, Serbia; †University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Obstructive sleep apnea (OSA) involves a range of conditions manifested as various forms of breathing disorders with intermittent complete breathing interruptions caused by obstruction in the upper airways. The disorder is more common in adult men, and obesity is a significant predisposing factor. The apnea-hypopnea index (AHI) is the main diagnostic criterion that reflects the frequency and duration of apneic phases. Besides the AHI, other parameters, such as the oxygen desaturation index (ODI), average oxygen saturation, and body mass index (BMI), may have diagnostic value. The aim of the study was to examine the correlation between AHI and ODI, AHI and average oxygen saturation, and AHI and BMI. **Methods.** A retrospective study included 200 patients, 166 men and 34 women, aged between 18 and 65, in whom OSA was proven by respiratory polygraphy. Depending on the AHI values, they were divided into three groups: Group I (AHI 5–15 events *per* hour), Group II (AHI 15–30 events *per* hour), and Group III (AHI > 30 events *per* hour). **Results.** There was a significant correlation between AHI and ODI in all groups, with the strongest correlation in Group III, where ODI also had predictive value for severe forms of apnea. Average oxygen saturation and BMI were significantly correlated with AHI only in Groups II and III. **Conclusion.** In addition to AHI, known as the main diagnostic parameter for OSA, ODI, average oxygen saturation, and BMI play a significant role in assessing apnea. With its strong correlation with AHI, as well as predictive value for more severe forms of apnea, ODI has the same importance as AHI in diagnosing and assessing the severity of this disorder.

Key words:
body mass index; oxygen saturation; prognosis; sleep apnea, obstructive.

Apstrakt

Uvod/Cilj. Opstruktivni poremećaj disanja tokom spavanja (*obstructive sleep apnea* – OSA) uključuje niz stanja koja se manifestuju raznim oblicima poremećaja disanja sa povremenim potpunim prekidima disanja, uzrokovanim opstrukcijom u gornjim disajnim putevima. Poremećaj je češći kod odraslih muškaraca, a gojaznost je značajan predisponirajući faktor. Indeks apneja-hipopneja (*apnea-hypopnea index* – AHI) glavni je dijagnostički kriterijum, koji govori o učestalosti i trajanju apneičnih faza. Osim AHI, i drugi parametri, kao što su indeks desaturacije kiseonikom (*oxygen desaturation index* – ODI), prosečno zasićenje (saturacija) kiseonikom (SK) i indeks telesne mase (ITM), mogu imati dijagnostičku vrednost. Cilj rada bio je da se ispita korelacija između AHI i ODI, AHI i prosečne SK kao i AHI i ITM. **Metode**. Retrospektivnom studijom obuhvaćeno je 200 bolesnika, 166 muškaraca i 34 žena, starosti između 18 i 65 godina, kod kojih je respiratornom poligrafijom dokazano postojanje OSA. U zavisnosti od vrednosti AHI, bolesnici su podeljeni u tri grupe: grupa I (AHI 5–15 prekida disanja na sat), grupa II (AHI 15–30 prekida disanja na sat) i grupa III (AHI > 30 prekida disanja na sat). **Rezultati.** Utvrđena je značajna korelacija između AHI i ODI u svim grupama, a najizrazitija korelacija ispoljena je u grupi III, gde je ODI imao i prediktivnu

Correspondence to: Dobrivoje Novković, Military Medical Academy, Clinic for Pulmonology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: dobrivojenovkovic@yahoo.com

vrednost za teške oblike apneje. Prosečna SK i ITM bili su u značajnoj korelaciji sa AHI samo u grupama II i III. **Zaključak.** Pored AHI, koji je poznat kao glavni dijagnostički parametar za OSA, značajnu ulogu u proceni apneje imaju ODI, prosečna SK i ITM. Snažnom korelacijom sa AHI, kao i prediktivnom vrednošću za teže oblike apneje, ODI ima jednak značaj kao AHI u dijagnostikovanju i proceni težine tog poremećaja.

Ključne reči:
telesna masa, indeks; kiseonik, zasićenost; prognoza; apneja u snu, opstruktivna.

Introduction

Obstructive sleep apnea (OSA) encompasses a range of conditions, from simple snoring to hypoventilation and apnea. Even though sleep breathing disorders (SBD) were identified much earlier, the first studies in this field began in the 1970s [1]. Depending on the pathophysiological cause and clinical manifestations, central, obstructive, and mixed types of apnea are distinguished. Although today this disorder is diagnosed in 6% of men and 4% of women, the prevalence in the general adult population is estimated to be significantly higher, ranging from 9% to 38% [2, 3].

In terms of frequency, the most common clinical form of apnea is OSA, which constitutes 90–95% of all breathing disorders during sleep [4].

OSA is a condition characterized by repeated episodes of partial or complete cessation of breathing during sleep. These episodes cause a series of adverse effects on the respiratory, digestive, cardiovascular, nervous, and endocrine systems. OSA occurs due to relaxation of the throat muscles during sleep, leading to a reduction or cessation of airflow through the upper airways [5].

Despite the epidemiological data mentioned, the number of diagnosed and confirmed cases of OSA is significantly lower. It is estimated that 75% of people with SBD with suspected sleep apnea remain undiagnosed for various reasons [6].

OSA is confirmed by using polysomnography (PSG), which represents the "gold standard" for diagnosing this disorder. Depending on the number of registered signals, there are various levels of testing. Limited PSG, or respiratory polygraphy (RP), is a level III diagnostic method that is simpler, more accessible, and sufficient for accurately recording obstructive SBD [7].

Episodes of hypopnea and apnea in OSA last from 10 to even 60 seconds or more. It is said that individuals with more than 5 hypopnea/apnea episodes *per* hour have sleep apnea, which is determined using the apnea-hypopnea (AH) index – AHI. In severe forms of OSA, AHI can be as high as 100 episodes *per* hour (100/hr) [8].

OSA is significantly associated with excessive body weight, which is an independent risk factor for this disorder. When talking about the impact of obesity, it primarily refers to central obesity and obesity characterized by an increased body mass index (BMI) [9].

In addition to obesity, tongue hypertrophy, short lower jaw, and short neck represent key risk factors for upper airway collapse in OSA.

Upper airway obstruction in OSA causes several critical pathophysiological conditions, such as decreased blood oxygen saturation, increased carbon dioxide concentration, changes in intrathoracic pressure, and increased sympathetic activity.

The main disorder in OSA is intermittent hypoxia, which triggers a cascade of events responsible for worsening cardiovascular diseases [10]. By using PSG and RP, we obtained information about the frequency and duration of AH episodes (AHI) while learning about the severity of the respiratory disorder caused by these episodes from the oxygen saturation or oxygen desaturation index (ODI).

ODI is defined as the average number of desaturation episodes *per* recording hour, where blood oxygen saturation is decreased by at least 3% from the basal value [11].

Given the facts stated, there is a need to consider other parameters that will indicate a disorder in oxygenation levels or hypoxemia caused by OSA.

The aim of the study was to examine the relationship between AHI and ODI, AHI and average oxygen saturation, and AHI and BMI in individuals with OSA.

Methods

Our retrospective study included 200 patients, 18 to 65 years old. Among those patients, 166 were male and 34 were female, with OSA diagnosed by RP. All patients predominantly had OSA and an AHI greater than 5/hr. In all patients, ODI, AHI, BMI, and average blood oxygen saturation, expressed in percentages, were measured during an overnight RP. Depending on the AHI, patients were classified into three groups: Group I (AHI 5–15/hr), Group II (AHI 15–30/hr), and Group III (AHI more than 30/hr). ODI was considered significant if desaturation was > 3% from the basal value. Descriptive methods expressed through frequency and percentage were used to present demographic and anthropometric data.

The relationship between AHI and ODI, AHI and BMI, and AHI and average saturation was analyzed using Spearman and Pearson correlation coefficients. Receiver operating characteristic (ROC) curves were used to determine the existence of threshold values (cut-offs) between the given parameters.

Results

The study included 200 subjects, 166 men and 34 women. Group I consisted of 57 respondents, Group II of 52 respondents, and Group III of 91 respondents. The average age for men was 52.15 years, and for women, it was 52.75 years. The results of the study showed that in the first group of subjects, only ODI showed a statistically significant correlation with AHI ($p < 0.05$, $r = 0.320$), while the other parameters

were not significantly correlated. In the second group, only ODI maintained a statistically significant positive correlation with AHI, which was stronger than in the previous group (p = 0.01, r = 0.453). In the last group, the third one, ODI showed a strong, positive correlation with AHI (p < 0.01, r = 0.842) (Figure 1).

In this group, which had the highest AHI, average oxygen saturation showed a statistically significant negative correlation with AHI (p < 0.01, r = 0.375, n = 91) (Figure 2). In contrast, BMI showed a statistically significant positive correlation with AHI (p < 0.01, r = 0.470, n = 91) (Figure 3).

Further analysis revealed that ODI has a predictive value for AHI in the group with AHI > 30/hr, a determined cut-off value ≥ 21, and a sensitivity of 92% and specificity of 88% (Figure 4).

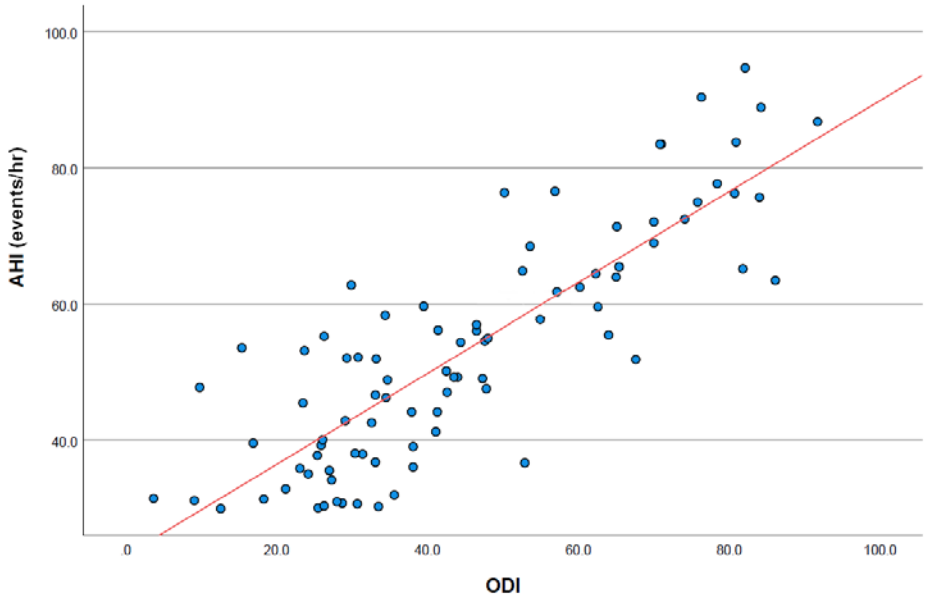

Fig. 1 – Correlation between AHI greater than 30/hr and ODI in patients with OSA (p < 0.01, r = 0.842, n = 91). AHI – apnea-hypopnea index; ODI – oxygen desaturation index; OSA – obstructive sleep apnea; n – number of respondents. Note: on the abscissa, ODI represents the number of times desaturation events occur *per* hour of sleep.

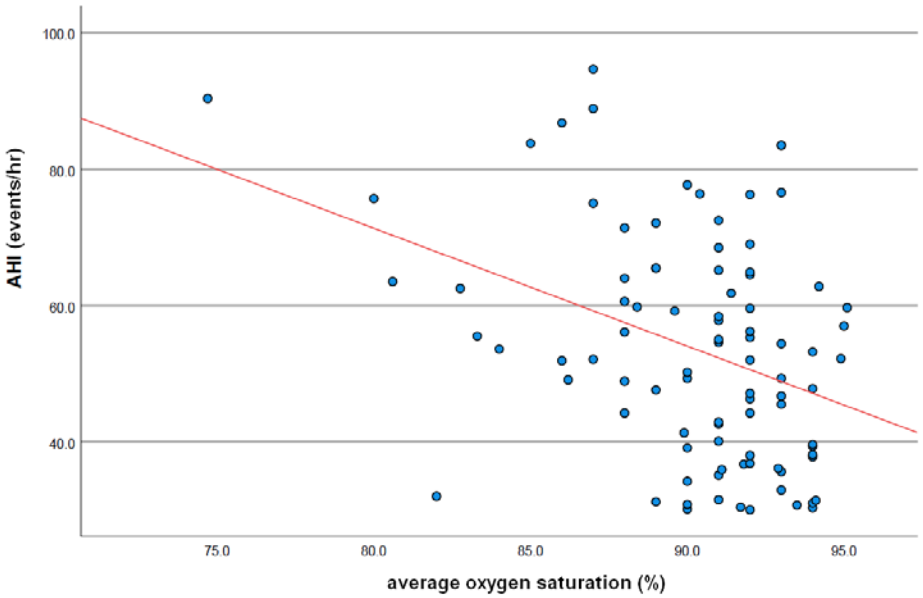

Fig. 2 – Correlation between AHI greater than 30/hr and average oxygen saturation in patients with OSA (p < 0.01, r = 0.375, n = 91). For abbreviations, see Figure 1.

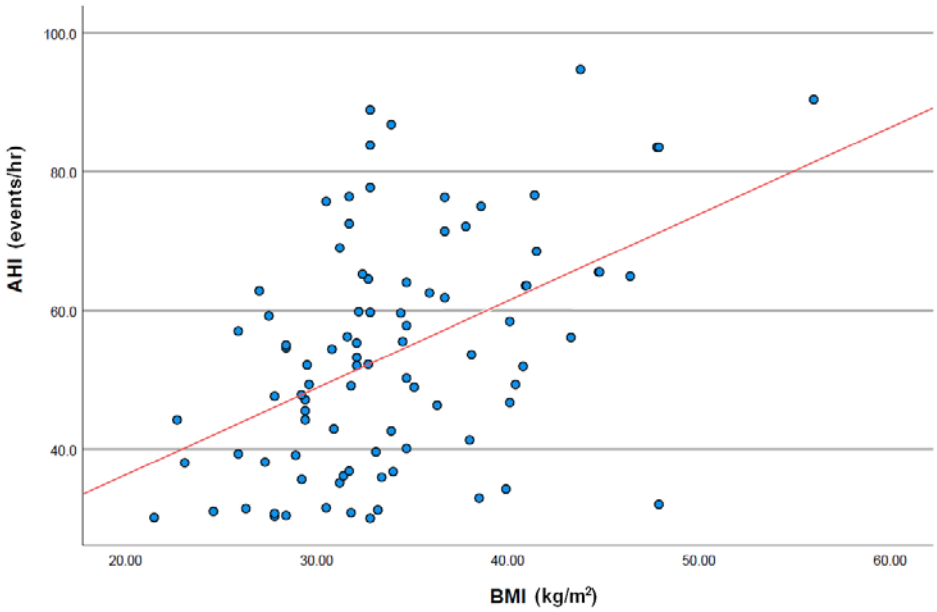

Fig. 3 – Correlation between AHI greater than 30/hr and BMI in patients with OSA ($p < 0.01$, $r = 0.470$, n = 91).
BMI – body mass index.
For other abbreviations, see Figure 1.

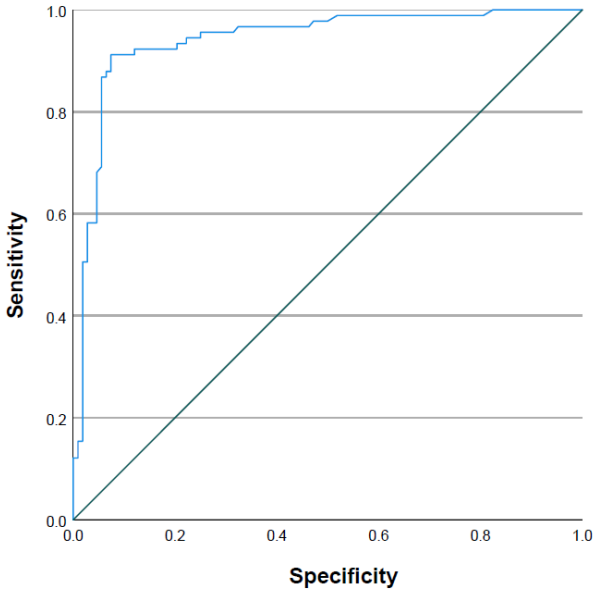

Fig. 4 – Cut-off value for ODI in the group with AHI > 30/hr
For abbreviations, see Figure 1.

Discussion

Previous studies have shown that the harmful effects of OSA were more pronounced in individuals who had a more severe form of this disorder, i.e., where AHI was greater than 15/hr or 30/hr. In recent years, the number of newly revealed patients with severe forms of the disease has been steadily increasing. Recent data indicate that over 400 million people worldwide between 30 and 70 years of age with OSA have a moderate to severe form of the disease [12]. Although blood oxygenation disorders are more pronounced in severe forms of apnea with higher AHI, this parameter does not provide enough information to indicate how OSA causes harmful effects on systems and organs. Chronic intermittent hypoxia, interrupted sleep, and initiation of inflammation are the main pathophysiological mechanisms in OSA [13]. These conditions are also associated with the appearance of cognitive and neurological symptoms such as reduced attention, poor concentration, irritability, anxiety, and depression [14].

In our study, we examined the correlation between AHI and ODI, AHI and average oxygen saturation, and AHI and BMI in patients with OSA. Among the 200 subjects in our study, we found a statistically significant positive correlation between AHI and ODI, as well as between AHI and BMI. On the other hand, we found a negative correlation between AHI and average oxygen saturation. When we analyzed the correlation of these parameters by groups that we formed according to the AHI amount, we found that in groups with AHI from 5–15/hr and 15–30/hr, only ODI showed a significant positive correlation, while average oxygen saturation and BMI did not significantly correlate with AHI. Since the groups in question had mild and moderate forms of the disorder, the results we obtained were expected. After all, the results are in accordance with the authors' previous study [8].

Unlike the previous groups, a significant positive correlation was observed in the group with AHI greater than 30/hr between AHI and ODI, as well as between AHI and BMI. In contrast, a significant negative correlation was found between AHI and average oxygen saturation, which is in line with basic pathophysiological mechanisms.

In addition, Temirbekov et al. [15] indicated the importance of the parameters we examined and their mutual correlation. They found that hypoxia during the apnea period is closely related to oxygen desaturation and suggested that ODI has the same value as AHI in diagnosing and evaluating OSA syndrome.

Further analysis revealed that ODI in the group AHI > 30/hr also has predictive value for assessing the severity of apnea, indicating that this parameter can have the same role as AHI. The diagnostic predictive significance of ODI has also been suggested in papers by Chung et al. [8], whose conclusions were confirmed in our research.

In addition to ODI indicating hypoxia during apnea or the severity of this disorder, studies have shown that this parameter also plays a role as a predictor of the success of other therapeutic procedures in the treatment of OSA [16]. This primarily refers to surgical methods such as uvulopalatopharyngoplasty or velopharyngoplasty. In this sense, Davanian et al. [17] found in their research that the degree of desaturation has a more accurate prognostic value than AHI in individuals who are candidates for surgical treatment methods.

The facts about ODI indicate that this parameter is gaining increasing importance, both in the diagnosis and assessment of the severity of obstructive apnea and in choosing an appropriate therapeutic approach to OSA.

Conclusion

As the most significant parameter in individuals with obstructive sleep apnea, the apnea-hypopnea index positively correlates with oxygen desaturation index and body constitution, expressed through body mass index or obesity. The apnea-hypopnea index is in a significant negative correlation with average oxygen saturation, because a higher apnea-hypopnea index, i.e., a more severe form of obstructive sleep apnea, causes lower saturation. In severe forms of obstructive sleep apnea characterized by an apnea-hypopnea index greater than 30/hr, in addition to a strong positive correlation, the oxygen desaturation index also has predictive significance for the apnea-hypopnea index. This study provides valuable insights, but the gender balance limitations of the sample indicate the need for additional research with more balanced samples.

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Seroprevalence of arboviruses in members of United Nations peacekeeping missions in the Central African Republic

Seroprevalencija arbovirusnih infekcija kod pripadnika mirovnih misija Ujedinjenih nacija u Centralnoafričkoj Republici

Sonja Atanasievska Kujović*, Elizabeta Ristanović*, Nikoleta Djordjevski*, Maja Živić†, Milena Krstić†‡, Nenad Ljubenović†, Jovan Mladenović†, Sofija Trifunović‡, Sonja Marjanović‡

Military Medical Academy, *Institute of Microbiology, †Institute of Epidemiology, Belgrade, Serbia; ‡University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Members of the Serbian Armed Forces participating in the United Nations (UN) Multidimensional Integrated Stabilization Mission in the Central African Republic (CAR) – MINUSCA, are exposed to the bites of the *Aedes* genus mosquitoes, which are transmitters of some (or certain) arboviruses. The aim of the study was to determine the exposure, risk factors, and prevention and protection measures against infection with arboviruses in members of peacekeeping missions (PKM). **Methods.** The study included 136 members of UN PKM in CAR in two rotations during 2022/2023, lasting 8 and 6 months. Each respondent had to answer the questions of the designed epidemiological questionnaire for the returnees from PKM. The subjects' blood sera were tested for specific antibodies against Dengue, Zika, and Chikungunya viruses using an enzyme-linked immunosorbent assay (ELISA) test. **Results.** The number of seropositive individuals for arboviruses was 48 (35.3%). Most members, 23 (16.9%), were seropositive for the Dengue virus, slightly fewer for the Chikungunya virus, 14 (10.3%), and the fewest were seropositive for the Zika virus, 11 (8.1%). Our research shows that impregnating uniforms with repellent plays a significant role in preventing mosquito bites and the Zika virus infection. The number of days spent in the mission and the factors of gender and age did not show an influence on the occurrence of arbovirus infections. **Conclusion.** The members of PKM in the CAR have come into contact with Dengue, Zika, and Chikungunya viruses. This indicates that it is necessary to work on the constant improvement of prevention measures and protection of their health, including further serological tests, but also tests for the presence of various infectious microorganisms in this endemic area.

Key words:
arbovirus infections; central african republic; culicidae; hospitals, military; insect repellents; preventive health services; risk factors; surveys and questionnaires; united nations.

Apstrakt

Uvod/Cilj. Pripadnici Vojske Srbije koji učestvuju u Multinacionalnoj operaciji Ujedinjenih nacija (UN) u Centralnoafričkoj Republici (CAR) – MINUSCA, izloženi su tokom boravka u misiji ubodima komaraca roda *Aedes* koji su prenosioci nekih (ili pojedinih) arbovirusa. Cilj rada bio je da se utvrdi izloženost, faktori rizika i mere prevencije i zaštite od infekcije arbovirusima kod pripadnika mirovnih misija. **Metode.** U studiju je uključeno 136 pripadnika mirovnih operacija UN u CAR iz dve rotacije tokom 2022/2023 godine u trajanju od 8 i 6 meseci. Svaki ispitanik morao je da odgovori na pitanja koja su data u dizajniranom epidemiološkom upitniku za povratnike iz mirovnih misija. Krvni serumi ispitanika testirani su *enzyme-linked immunosorbent assay* (ELISA) testom na prisustvo specifičnih antitela na viruse *Dengue*, *Zika* i *Chikungunya*. **Rezultati.** Utvrđeno je 48 (35,3%) seropozitivnih ispitanika na arboviruse. Najviše seropozitivnih pripadnika, 23 (16,9%), bilo je na *Dengue* virus, nešto manje na *Chikungunya* virus, 14 (10,3%), dok je najmanje bilo pozitivno na *Zika* virus, 11 (8,1%). Naše istraživanje pokazuje da impregnacija uniforme repelentom ima značajnu ulogu u prevenciji uboda komaraca i sprečavanju infekcije *Zika* virusom. Broj dana provedenih u misiji, kao i faktor pola i godine starosti nisu pokazali uticaj na pojavu infekcija arbovirusom. **Zaključak.** Pripadnici mirovnih misija u CAR došli su u

Correspondence to: Sonja Atanasievska Kujović, Military Medical Academy, Institute of Microbiology, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: sonja.atanasievska@gmail.com

kontakt sa virusima *Dengue, Zika* i *Chikungunya*. To ukazuje da je neophodno raditi na stalnom unapređenju mera prevencije i zaštite njihovog zdravlja, uključujući serološka ispitivanja, ali i ispitivanja prisustva različitih infektivnih mikroorganizama tog endemskog područja.

Ključne reči:
infekcija, arbovirus; centralna afrička republika; komarci; bolnice, vojne; repelenti; preventivno-medicinska zaštita; faktori rizika; ankete i upitnici; mirovne snage ujedinjenih naroda.

Introduction

Dengue, Zika, and Chikungunya viruses are arboviruses transmitted by mosquitoes of the genus *Aedes*. These viruses are mainly found in tropical and subtropical regions, but in recent years, an expansion of their geographical range towards temperate zones has been observed. This happens because of the spread of vectors to new areas due to climate change and global warming, so the epidemics caused by these viruses occur on almost all continents [1, 2].

Dengue fever is the most common arbovirus infection, occurring in over 100 tropical and subtropical countries, with approximately 400 million cases reported annually [3, 4]. The infection is caused by one of the four serotypes of the Dengue virus. Symptoms can range from mild to severe (hemorrhagic fever and shock syndrome) and can be potentially lethal. This virus occurs seasonally, with a peak during and after the rainy season when the mosquito population increases [3–5].

The Zika virus was named after the Zika area in Uganda, where it was first isolated in 1947. A person can become infected with the Zika virus through a mosquito bite, but also through sexual contact. The symptoms are mild and last two to seven days. However, infection with this virus during pregnancy can cause microcephaly and lead to congenital brain damage in the fetus. It has also been linked to Guillain-Barre syndrome, neuropathy, and myelitis in children and adults [6, 7].

The Chikungunya virus was first isolated in Tanzania in 1952. In addition to increased fever, the most common symptom of this infection is pain in the joints, which can occur repeatedly throughout a person's life and have a progressive degenerative course in the form of chronic arthritis. Muscle pain, headaches, nausea, and skin rash are also present. In addition to Africa and Asia, imported cases have been registered in Europe and parts of the American continent [8–10].

Infections with Dengue, Zika, and Chikungunya viruses are often asymptomatic or have mild clinical symptoms, such as fever, headache, muscle and joint pain, nausea, and skin rash. Since these diseases have similar symptoms, they can resemble each other, and if there is no laboratory confirmation, patients can often be misdiagnosed. As patients with a mild clinical infection do not report to the doctor, a large number of cases go undetected [2, 11, 12].

The treatment of these diseases is symptomatic. There is specific prophylaxis for Dengue fever only, and that in the form of the quadrivalent vaccine Dengvaxia®, while the vaccines for Zika and Chikungunya are still at the clinical trial stage [13, 14].

Considering the increase in the incidence of arbovirus infections, the question of effective prevention of these diseases arises. Prophylaxis and disease control aim to prevent or reduce the transmission of viruses through vector control and prevention of human-vector contact. This could be achieved by implementing personal protective measures, general hygiene measures, specific prophylaxis (where available), as well as by performing continuous educational work among the population in endemic areas [3, 15].

Members of the Ministry of Defence and the Serbian Armed Forces of the Republic of Serbia have been engaged in a United Nations (UN) Multidimensional Integrated Stabilization Mission in the Central African Republic (CAR) – MINUSCA since September 2014. This country and region are endemic for arboviruses. The climate of the CAR is predominantly tropical, with a rainy season from June to September in the northern regions of the country and from May to October in the south. The maximum annual rainfall is around 1,800 mm in the upper part of the Ubangi region. Across the country, annual average temperatures range from 23 °C in the south to 26 °C in the north [16]. There are not many papers in the literature examining the presence of arboviruses in peacekeeping mission (PKM) participants. Various studies from the literature show the results of different geographical, climatic, and epidemiological areas for members of PKM who come from various countries and have different genetic predispositions [2, 3, 11].

The aim of our study was to examine the extent to which members of PKM are exposed to the risk of infection with arboviruses, which risk factors increase the possibility of infection, as well as the effectiveness of the prevention and protection measures implemented to reduce the risk of infection.

Methods

A total of 136 participants from two rotations of the UN PKM in the CAR during 2022/2023 took part in the study. The first rotation lasted 8 months (66 participants), while the second lasted 6 months (70 participants). As part of the preparation for PKM, the participants were informed about the risks of contracting diseases occurring on the territory of the CAR and introduced to the measures for its prophylaxis and prevention. The participants were vaccinated with the prescribed and recommended vaccine (yellow fever) for entry into the CAR territory.

After returning from PKM, the health status of the participants was monitored for 21 days. On the tenth day upon returning, a blood sample was taken from each individual for further microbiological testing. On this occasion, they completed the specifically designed questionnaire at the Institute of Epidemiology, Military Medical Academy (MMA), Belgrade, Serbia, intended for individual risk assessment. From these questionnaires, the following data were collected: whether and how many mosquito bites they had, whether they used repellents for personal protection and room protection, nets on doors and windows, and whether their uniforms were impregnated with repellent. The research has been approved by the Ethics Committee of the Faculty of Medicine of the MMA (No. 1/11/2024).

The serological detection of Dengue, Zika, and Chikungunya viruses was carried out at the Institute of Microbiology (Department of Microbe Genetics and Immunology) of the MMA. An enzyme-linked immunosorbent assay (ELISA) test (EUROIMMUN, Lübeck, Germany) was used for the detection of specific immunoglobulin (Ig) M and IgG antibodies against Dengue, Zika, and Chikungunya viruses [positive results IgM > 1.1 relative units (RU)/mL, IgG > 22 RU/mL].

Descriptive and analytical statistical methods were used for statistical data processing. Quantitative characteristics are presented as mean ± standard deviation, while absolute numbers with percentages were used for categorical characteristics. The Chi-square test was used to determine the statistically significant difference in the distribution of categorical characteristics, and the Student's *t*-test was used to determine the difference in quantitative characteristics. The program package SPSS version 26.0.0.0 (IBM Corporation, USA) was used for statistical data processing.

Results

Out of the total number of respondents, 60 were women and 76 were men, and the average age of the members of both rotations was 47.2 years. The presence of specific IgM and IgG antibodies against Dengue, Zika, and Chikungunya viruses is shown in Table 1. Out of the total of 136 returnees, antibodies were found in 48 (35.3%) members. The number of seropositive members for the Dengue virus was 23 (16.9%), for the Zika virus, it was 11 (8.1%), and for the Chikungunya virus, it was 14 (10.3%) members.

The presence of IgM, IgG, or both classes of antibodies against two viruses simultaneously in the same person was also observed. The number of these individuals is shown in Table 2. In two persons seropositive for Dengue and Zika, one was positive for IgM antibodies to both viruses, while the other was IgM positive for Dengue and IgG positive for the Zika virus. In the case of co-infection with Zika and Chikungunya viruses, one person had positive IgM antibodies. Among the four samples seropositive for Dengue and Chikungunya, two had positive IgG antibodies, one IgM, while one was positive for both classes of antibodies. Likewise, two individuals in the second rotation were seropositive for all three infections in IgM antibodies.

Out of the 23 members seropositive for the Dengue virus in both rotations, 8 (34.8%) were female, while 15 (65.2%) were male. No statistically significant difference was found ($p = 0.323$). As for age, the average age of seropositive individuals with the Dengue virus was 45.43 ± 7.95 years, while the average age of seronegative individuals was 47.54 ± 10.09 years, with no statistical significance ($p = 0.347$) (Table 3).

Table 1

Presence of specific IgM and IgG antibodies against Dengue, Zika, and Chikungunya viruses in members of the peacekeeping mission

Rotation	Number of tested	Dengue positive			Zika positive			Chikungunya positive		
		IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total
First	66 (48.5)	2 (3.0)	4 (6.0)	6 (9.0)	7 (10.6)	0 (0)	7 (10.6)	3 (4.5)	0 (0)	3 (4.5)
Second	70 (51.5)	12 (17.1)	5 (7.1)	17* (24.2)	3 (4.3)	1 (1.4)	4 (5.7)	7 (10.0)	4 (5.7)	11 (15.7)
Total	136 (100)	14 (10.3)	9 (6.6)	23* (16.9)	10 (7.3)	1 (0.7)	11 (8.1)	10 (7.3)	4 (2.9)	14 (10.3)

Ig – immunoglobulin.
All values are given as numbers (percentages).
Note: * one person had both IgM and IgG antibodies present.

Table 2

Presence of simultaneous seropositivity for Dengue, Zika, and Chikungunya viruses in members of the peacekeeping missions

Rotation	Seropositive			
	Dengue and Zika	Dengue and Chikungunya	Zika and Chikungunya	Dengue, Zika, and Chikungunya
First	2 (3.0)	0 (0)	0 (0)	0 (0)
Second	0 (0)	4 (5.7)	1 (1.4)	2 (3.0)
Total	2 (1.5)	4 (2.9)	1 (0.7)	2 (1.5)

All values are given as numbers (percentages).

Out of the 11 seropositive members for the Zika virus observed in both rotations, 6 (54.5%) were female, while 5 (45.5%) were male, with no statistical significance ($p = 0.467$). In terms of age, the average age of those seropositive for the Zika virus was 49.64 ± 11.90 years, while the average age of those seronegative was 46.97 ± 9.59 years, with no statistical significance found ($p = 0.387$) (Table 4).

Out of the 14 members seropositive for the Chikungunya virus in both rotations, 5 (35.7%) were female, while 9 (64.3%) were male, with no statistically significant difference ($p = 0.504$). As for age, the average age of those seropositive for the Chikungunya virus was 46.29 ± 8.93 years, while the average age of those seronegative was 47.29 ± 9.89 years. In this case, no statistical significance was found ($p = 0.717$) (Table 5).

Table 3

Statistical association of risk factors and preventive measures with Dengue virus seropositivity

Parameters	Dengue virus yes (n = 23)	Dengue virus no (n = 113)	OR (95% CI)	*p*
Gender, n (%)				
female	8 (34.8)	52 (46.0)	ref.	0.323
male	15 (65.2)	61 (54.0)	1.60 (0.63–4.07)	
Age (years), mean ± SD	45.43 ± 7.95	47.54 ± 10.09	/	0.347
Number of days in the mission, mean ± SD	207.74 ± 29.55	221.76 ± 31.73	/	0.053
Symptoms, n (%)	9 (39.1)	16 (14.2)	3.90 (1.45–10.49)	0.005
Mosquito bite, n (%)	20 (87.0)	80 (70.8)	2.75 (0.77–9.89)	0.109
Repellents for personal protection, n (%)	22 (95.7)	100 (88.5)	2.86 (0.36–23.02)	0.303
Room protection, n (%)	22 (95.7)	105 (92.9)	1.68 (0.20–14.09)	0.631
Impregnation of uniforms, n (%)	8 (34.8)	50 (44.2)	0.67 (0.26–1.71)	0.403

SD – standard deviation; n – number; OR – odds ratio; CI – confidence interval; ref. – reference.

Table 4

Statistical association of risk factors and preventive measures with Zika virus seropositivity

Parameters	Zika virus yes (n = 11)	Zika virus no (n = 125)	OR (95% CI)	*p*
Gender, n (%)				
female	6 (54.5)	54 (43.2)	ref.	0.467
male	5 (45.5)	71 (56.8)	0.63 (0.18–2.19)	
Age (years), mean ± SD	49.64 ± 11.90	46.97 ± 9.59	/	0.387
Number of days in the mission, mean ± SD	231.0 ± 26.51	218.34 ± 32.02	/	0.206
Symptoms, n (%)	4 (36.4)	21 (16.8)	2.83 (0.76–10.54)	0.108
Mosquito bite, n (%)	11 (100)	89 (71.2)	/	/
Repellents for personal protection, n (%)	9 (81.8)	113 (90.4)	1.82 (0.37–8.84)	0.452
Room protection, n (%)	10 (90.9)	117 (93.6)	0.68 (0.08–6.03)	0.731
Impregnation of uniforms, n (%)	5 (45.5)	53 (42.4)	0.06 (0.01–0.23)	< 0.001

For abbreviations, see Table 3.

Table 5

Statistical association of risk factors and preventive measures with Chikungunya virus seropositivity

Parameters	Chikungunya virus yes (n = 14)	Chikungunya virus no (n = 122)	OR (95% CI)	*p*
Gender, n (%)				
female	5 (35.7)	55 (45.1)	ref.	0.504
male	9 (64.3)	67 (54.9)	1.48 (0.47–4.67)	
Age (years), mean ± SD	46.29 ± 8.93	47.29 ± 9.89	/	0.717
Number of days in the mission, mean ± SD	206.21 ± 26.87	220.89 ± 31.97	/	0.101
Symptoms, n (%)	2 (14.3)	23 (18.9)	0.72 (0.15–3.43)	0.676
Mosquito bite, n (%)	11 (78.6)	89 (73.0)	1.36 (0.36–5.18)	0.652
Repellents for personal protection, n (%)	13 (92.9)	109 (89.3)	1.55 (0.19–12.84)	0.682
Room protection, n (%)	14 (100)	113 (92.6)	/	/
Impregnation of uniforms, n (%)	7 (50.0)	51 (41.8)	1.39 (0.46–4.21)	0.557

For abbreviations, see Table 3.

The frequency of seropositive respondents to arboviruses with the use of preventive measures is shown in Tables 3–5. Preventive measures include the use of repellents for personal protection, the protection of rooms from mosquitoes (use of repellents for rooms and protective nets on windows and beds), and the impregnation of uniforms.

Out of the 23 returnees seropositive for the Dengue virus, 22 (95.7%) reported daily use of repellents for personal use (OR: 2.86; 95% CI: 0.36–23.02; $p = 0.303$). Protection of rooms with repellent and use of protective nets on windows and beds was performed by 22 (95.7%) out of 23 seropositive returnees (OR: 1.68; 95% CI: 0.20–14.09; $p = 0.631$). Eight (34.8%) seropositive returnees impregnated their uniform with repellent (OR: 0.67; 95% CI: 0.26–1.7; $p = 0.403$) (Table 3).

Out of the 11 Zika seropositive returnees, 9 (81.8%) reported daily use of repellent for personal use (OR: 1.82; 95% CI: 0.37–8.84; $p = 0.452$). Protection of rooms with repellent and use of protective nets on windows and beds was performed by 10 (90.9%) out of 11 seropositive returnees (OR: 0.68; 95% CI: 0.08–6.03; $p = 0.731$). Five (45.5%) seropositive returnees impregnated their uniforms with repellent (OR: 0.06; 95% CI: 0.01–0.23; $p < 0.001$). Out of the 125 seronegative members, 53 (42.4%) impregnated their uniforms. In this case, the statistical significance of the association between the use of the uniform impregnation measure to prevent the Zika virus infection was found (Table 4).

Out of the 14 seropositive Chikungunya returnees, 13 (92.9%) reported using repellents daily for personal use (OR: 1.55; 95% CI: 0.19–12.84; $p = 0.682$). All 14 (100%) seropositive returnees used repellents and protective nets on windows and beds. Seven (50.0%) seropositive returnees impregnated their uniforms with repellent (OR: 1.39; 95% CI: 0.46–4.21; $p = 0.557$) (Table 5).

The frequency of Dengue, Zika, and Chikungunya seropositivity with the number of days spent in PKM is shown in Tables 3–5. Returnees from PKM seropositive for the Dengue virus spent 207.74 ± 29.55 days in the mission ($p = 0.053$), indicating a result at the limit of statistical significance. Seronegative returnees spent 221.76 ± 31.73 days in the mission (Table 3).

Seropositive PKM returnees for the Zika virus spent 231.00 ± 26.51 days in the mission ($p = 0.206$), which is not statistically significant. Seronegative returnees spent 218.34 ± 32.02 days in the mission (Table 4).

The Chikungunya virus seropositive PKM returnees spent 206.21 ± 26.87 days in the mission ($p = 0.101$). Again, we did not reach statistical significance in this case. Seronegative returnees spent 220.89 ± 31.97 days in the mission (Table 5).

Based on the questionnaire, Table 6 shows the most common symptoms in the arbovirus seropositive individuals. Out of the total of 23 members seropositive for the Dengue virus, 9 (39.1%) reported symptoms during their stay in the mission. The same symptoms were reported by 16 (14.2%) of the 113 seronegative members of PKM (OR: 3.90; 95% CI: 1.45–10.49; $p = 0.005$) (Table 3).

Out of the total of 11 members seropositive for the Zika virus, 4 (36.4%) reported symptoms during their stay in the mission. The same symptoms were reported by 21 (16.8%) out of the 125 seronegative PKM members (OR: 2.83; 95% CI: 0.76–10.54; $p = 0.108$) (Table 4).

Out of the total of 14 members seropositive for the Chikungunya virus, 2 (14.3%) reported symptoms during their stay in the mission. The same symptoms were reported by 23 (18.9%) of 122 seronegative PKM members (OR: 0.72; 95% CI: 0.15–3.43; $p = 0.676$) (Table 5).

Discussion

Viral infections transmitted by arboviruses are increasingly common diseases worldwide. Vegetation, temperature, and precipitation are some of the factors that influence the arthropod vector and its distribution. In recent years, social, demographic, and climatic changes, population migration, and urbanization have strongly influenced the spread of infections with these viruses, which are of increasing global concern [3, 11].

A large number of infected people on the African continent go unrecognized because of the insufficiently developed health system. More precisely, there is a lack of laboratories and a shortage of health personnel to diagnose a disease. Infected people are often discovered as imported cases through subsequent tests when they return from an endemic area [17]. This is also largely the case with returnees from PKM. Establishing the correct diagnosis is essential for preventing complications, severe clinical manifestations, and chronic forms of the disease.

In our research, 48 (35.3%) returnees were seropositive for arboviruses, i.e., one in three respondents had contact with mosquitoes infected with Dengue, Zika, or Chikungunya viruses. When answering the epidemiological questionnaire questions, the members themselves often stated that there were many mosquitoes during their stay in the CAR. Most members were seropositive for the Dengue

Table 6

The presence of the most common symptoms in seropositive members of the peacekeeping missions (two rotations)

Rotation	Symptoms						Total
	fever	headache	weakness	muscle and bone pain	skin rash	red and itchy eyes	
First	2 (4.17)	1 (2.08)	3 (6.25)	2 (4.17)	1 (2.08)	0 (0)	9 (18.75)
Second	5 (10.41)	5 (10.41)	3 (6.25)	3 (6.25)	0 (0)	2 (4.17)	18 (37.50)
Total	7 (14.58)	6 (12.50)	6 (12.50)	5 (10.42)	1 (2.08)	2 (4.17)	27 (56.25)

All values are given as numbers (percentages).

virus, 23 (16.9%), slightly fewer for the Chikungunya virus, 14 (10.3%), and the fewest members were seropositive for the Zika virus, 11 (8.1%). Our results show that the number of antibodies identified for Dengue and Chikungunya viruses increased approximately 3-fold and 3.7-fold, respectively, in the second rotation compared to the first rotation, while it decreased approximately 2-fold for the Zika virus. In addition, for all three arboviruses, more subjects were found to have positive IgM antibodies (34 subjects) compared to IgG antibodies detected (15 subjects), which may indicate that there is a failure in the use of preventive measures in PKM members towards the end of their stay in the mission.

The Dengue virus infections have occurred more frequently in soldiers and military personnel around the world. The United States (US) military is frequently involved in a variety of PKM. Therefore, the problem of the growing risk of Dengue fever among these members has been recognized, which leads to a significant increase in medical costs and potential impact on combat readiness, jeopardizing and compromising the potential success of these operations. A study conducted in 2017 investigated the presence of antibodies in members of PKM deployed in an endemic area for the Dengue virus. Overall, one thousand samples were analyzed, and neutralizing antibodies against all four serotypes were detected in 7.6% of the subjects. The study showed that better epidemiologic surveillance and rapid determination of the correct infection diagnosis are necessary for preventing infections [18].

In a study conducted on Mongolian PKM members deployed in South Sudan between 2012 and 2013, 632 members were tested for possible seroconversion of the Chikungunya virus. Although there are areas on the South Sudan borders where the Chikungunya virus is endemic, with a low prevalence rate (< 2%), no sample showed seroconversion for this virus [19].

In another study conducted in Central America in 2017, different results were obtained. A cohort of 124 Dutch soldiers was deployed to Belize, Curacao, and Saint Martin for an average of eight weeks each. The soldiers were tested for signs and symptoms of the Chikungunya infection at least fourteen days after their return. The 19 members were tested, and virus-specific IgG antibodies were detected in one member who was on Saint Martin, where an epidemic of this virus was reported in 2013 and 2014 [20, 21].

Few papers in the literature investigate the presence of the Zika virus in PKM participants. One of the few published studies involved 1,420 members of the US military who were in areas where this virus is endemic. ELISA and polymerase chain reaction (PCR) tests were performed on all members. Eleven (0.8%) participants were positive for the Zika virus by PCR test, while 26 (1.8%) were positive by serologic analysis [22].

Dengue, Zika, and Chikungunya viruses are usually detected by laboratory tests – ELISA and PCR. The cross-reactivity between Dengue and Zika viruses, which belong to the same *Flaviviridae* family, can be problematic in serological tests. There is also cross-reactivity between Chikungunya and other alphaviruses. A definitive diagnosis is made using PCR tests, but only in acute infections due to the short duration of viremia [11, 23].

In the first rotation, two members were seropositive for Dengue and Zika viruses. In the second rotation, there was no such combination. Still, four people were seropositive for Dengue and Chikungunya viruses, one for Zika and Chikungunya viruses, and two for all three viruses simultaneously. A similar phenomenon was observed in a retrospective study conducted by a hospital in the Indian district of Kolhapur. Kolhapur district is an endemic area for Dengue, where recurrent epidemics occur annually. The aim of the study was to determine the seropositivity rate for Dengue and Chikungunya infections. The study lasted from January 2021 to August 2022, during which time 3,285 samples were tested for IgM antibodies against Dengue, and 1,823 samples were tested for IgM antibodies against Chikungunya. The antibody positivity for Dengue was 29.4%, and 18.4% for Chikungunya. No significant increase in Dengue seropositivity was observed (29% in 2021, up to 30% in 2022), but 17 samples in 2021 and 12 samples in 2022 were positive for both Dengue and Chikungunya [24].

If we look at the distribution of seropositivity by gender and age, our results show no significant correlation. Studies around the world linking gender and age to seropositivity are conducted with a large number of respondents in all age groups from 6 to 65 years [2, 3, 11]. As our study refers to a military population with a small age range and a similar representation of both sexes, obtaining such data was impossible. Similar to our results, a cross-sectional study of 1,003 respondents in northeastern Tanzania did not identify gender or age as statistically significant predictors of seropositivity. In this study, the influence of risk factors on the incidence of Dengue and Chikungunya virus infections was investigated based on the seropositivity of the subjects. The results showed that environmental factors such as living in a house with uncovered containers had a higher probability of Chikungunya IgM positivity (OR 2.89; 95% CI: 1.76–4.76). People who keep ungulates at home or live near lush vegetation are also at a higher risk of being infected. Similar to our results, the analysis by gender and age in this study showed no statistically significant impact on infection risk [25].

The symptoms and clinical manifestations caused by Dengue, Zika, and Chikungunya viruses can be similar. Some symptoms are not specific to these infections alone, so a number of other infectious diseases can also be suspected. The possibility of co-infections and potential serologic cross-reactions should also not be ruled out. Our results show that out of the 48 seropositive members, 27 (56.25%) reported some of the symptoms characteristic of Dengue, Zika, and Chikungunya virus infections. Had the PKM members been tested when the symptoms appeared, a laboratory analysis would have provided a definitive diagnosis. Unfortunately, these analyses were not carried out during the PKM, so making an accurate diagnosis is impossible. Some of the symptoms are likely due to other infections, most commonly malaria, coronavirus disease 2019, or West Nile virus.

During the outbreak of Chikungunya infections in 2006 and 2007 on the island of La Reunion (in the Indian

Ocean, French territory), it was shown that the risk of infection for French military police officers was equivalent to that of the local population. Out of 770,000 inhabitants, 35.0% were infected in the first six months. The French military police formed a cohort of 662 respondents. They were young or middle-aged men, with an average age of 40. Based on the questionnaires they completed, 23.9% reported symptoms related to the infection. Objectively, 19.3% had IgM or IgG antibodies or both simultaneously, which is almost twice as high as our results (10.3%) [21, 26]. Among the seropositives in the aforementioned study, 3.2% of asymptomatic cases of infection were recorded, whereas in our study, the number of asymptomatic infections was significantly higher and amounted to 14.3%.

If we look at the length of stay of PKM members in correlation with seropositivity, there is no statistically significant difference for any arbovirus. Both rotations spent some time in the CAR in both the rainy and dry seasons, so this risk factor was equal for both rotations, considering that the peak of the mosquito population is in the rainy season. The total number of seropositive members in the first rotation was 16 (24.2%), while in the second rotation, the number of seropositive members was twice as high, 32 (45.7%). The first rotation lasted eight months, i.e., two months longer than the second one. Therefore, an opposite result would have been expected due to the duration of exposure. This leads to the conclusion that other risk factors have a much greater influence than the time spent in the risk area.

In contrast to our results, studies of Dengue seroprevalence in US soldiers serving in Puerto Rico correlate to some extent with duration of exposure and seropositivity. The study used sera from 500 US soldiers serving in Puerto Rico from January through July 2015, collected from the military blood bank. The study was stratified by age and place of birth/residence in endemic areas or non-endemic areas prior to residing in Puerto Rico (87.2% of respondents were born or lived in endemic areas). The study showed that respondents who were not born/lived in endemic areas before arriving in Puerto Rico had a very low risk of being exposed to the Dengue virus [adjusted odds ratio (aOR) = 0.28, $p = 0.001$]. Among them, the risk of exposure to the virus was observed to increase with each year of military service in Puerto Rico (aOR = 1.58, $p = 0.06$). The correlation with age was not significant. Among respondents who were born/lived in endemic areas before coming to Puerto Rico, it was observed that age (aOR = 1.04, $p = 0.02$) and not the duration of military service was associated with the occurrence of Dengue seropositivity. This indicates previous lifetime exposure to the virus [27].

Preventive measures to prevent contact of mission members with the vector include personal protection measures using repellents for personal use, protection of rooms from mosquitoes (use of repellents for the rooms, use of protective nets on windows and beds), and wearing a uniform impregnated with a 0.1% permethrin solution [11]. Further measures to reduce the number of mosquitoes in the area of the camp where the mission members are staying are implemented by the Prevention Department team. These are general hygiene controls, drinking water controls, kitchen controls, proper waste disposal controls, disinfection, disinsection, and pest control measures, both in the camp and the hospital. The presence of animals in the warehouse could also be a risk factor. It has been proven that the presence of hoofed animals (goats, sheep, cows) and lush vegetation near houses is associated with the proliferation of mosquitoes. A very important risk factor is water retention, like rainwater on plants and artificial vegetation, where mosquitoes lay their eggs. Containers with stagnant water, like decorative containers with water, are also common places where mosquito larvae can be found. Places with improper disposal of waste and food are abundant with mosquitoes [28].

The statistical analysis of risk factors and preventive measures in relation to arbovirus seropositivity showed no significant associations in any category, except for the use of uniform repellent impregnation, which was significantly associated with a reduced risk of Zika virus infection. A cross-sectional study conducted in northeastern Tanzania yielded similar results. Of the risk factors, they examined the influence of the use of nets around the bed ($p = 0.63$), the use of nets on the windows ($p = 0.18$), and the number of people sleeping in the room ($p = 0.80$ for two people, $p = 0.13$ for three people, $p = 0.80$ for four or more people). No statistical significance was demonstrated in their study either [25].

Another study tracked the association between the seroprevalence of IgG antibodies to Dengue, Chikungunya, and West Nile viruses and their association with risk factors in Madagascar from 2011 to 2013. The study showed that the risk factors for the occurrence of seropositivity for Dengue and Chikungunya are living near lush vegetation and forests, and daily work in the fields. A protective effect for Chikungunya was exerted by a program that included spraying houses against mosquitoes in the last twelve months ($p < 0.001$) [28].

Considering the potential exposure of our members of PKM to arboviruses in endemic and high epidemiologic risk areas, and based on the results of other investigations, it is necessary in the coming period to strengthen epidemiologic surveillance measures and expand the scope of research to other infectious agents for a longer period of time. In addition to the aforementioned personal and general protection measures, in order to achieve an even better prevention effect, the following must be done: paying attention to the education of the defence personnel about risk factors and the method and importance of protection; strengthening epidemiological surveillance in the warehouses and hospitals; removing animals, dense vegetation, ornamental shrubs, and artificial plants from the warehouses; keeping water containers and flower pots outside of the warehouses and hospitals. In the future, one could think about introducing a vaccine against the Dengue virus. As Dengue and Zika viruses have become increasingly widespread in our geographical area in recent years, a seroepidemiological study should be carried out on their presence in our country and the risks to the military population.

Conclusion

The members of the peacekeeping missions in the Central African Republic have come into contact with Dengue, Zika, and Chikungunya viruses. This shows a need for improving prevention and protection of their health, including serologic testing for the presence of various infectious microorganisms in this endemic area. Our research shows that impregnating uniforms with a repellent plays an important role in preventing mosquito bites and infection with the Zika virus.

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APOE gene polymorphism as a potential predictor of postoperative cognitive dysfunction in colon cancer surgery under general anesthesia

Polimorfizam APOE gena kao potencijalni prediktor postoperativne kognitivne disfunkcije u operaciji raka debelog creva u opštoj anesteziji

Dalibor Bošković*, Sanja Marić†‡, Nenad Lalović‡§, Srdjan Mašić‖, Nikolina Elez-Burnjaković¶, Rade Miletić‡§, Dragan Spaić‖, Jelena Krunić**

***Hospital East Sarajevo, Department of Anesthesiology and Intensive Care, East Sarajevo, Republic of Srpska, Bosnia and Herzegovina; University Hospital, †Department of Anesthesiology and Intensive Care, §Clinic of Surgery, Foča, Republic of Srpska, Bosnia and Herezgovina; University of East Sarajevo, Faculty of Medicine, ‡Department of Surgery, ‖Department of Primary Health Care and Public Health, ¶Department of Cell Biology and Human Genetics, **Department of Dental Pathology, Foča, Republic of Srpska, Bosnia and Herzegovina**

Abstract

Background/Aim. Postoperative cognitive dysfunction (POCD) occurs very often in operated patients. This increasingly frequent complication compromises the recovery of operated patients, impairs the quality of life of patients and their families, prolongs the length of hospital stay, and increases the cost of treatment. The aim of the study was to examine the association between the apolipoprotein E (APOE) ε4 allele and sociodemographic and clinical characteristics with the occurrence of POCD seven days and three months after colon cancer surgery (CCS) under general anesthesia (GA). **Methods.** A total of 113 patients aged 18 to 87 years who underwent CCS under GA in the period from 2021 to 2023 participated in the study. Preoperative preparation, anesthesia, and postoperative treatment were uniform and standardized for all patients. The assessment of cognitive status was conducted using the Mini Mental State Examination psychometric test on the day before surgery, on the seventh postoperative day, and three months after surgery. **Results.** Seven days after surgery, a statistically significant impairment of cognitive functions was found in patients with the APOE ε4 allele in their genotype ($p = 0.007$). Patients 65 years old or above were more likely to have POCD three months after surgery compared to younger patients (80.0% vs. 52.9%; $p = 0.003$). **Conclusion**. The presence of the APOE ε4 allele is a potential predictor of the occurrence of POCD seven days after surgery, and age is a significant sociodemographic factor for the occurrence of POCD three months after CCS is performed under GA.

Key words:
anesthesia, general; cognitive dysfunction; colonic neoplasms; prognosis; psychological tests.

Apstrakt

Uvod/Cilj. Postoperativni kognitivni poremećaj (*postoperative cognitive dysfunction* – POCD) se veoma često javlja kod operisanih bolesnika. Ta sve češća komplikacija negativno utiče na oporavak operisanih bolesnika, narušava kvalitet života kako bolesnika tako i njihovih porodica, produžava dužinu hospitalizacije i uvećava troškove lečenja. Cilj rada bio je da se ispita povezanost ε4 alela apolipoproteina E (APOE), sociodemografskih i kliničkih karakteristika sa pojavom POCD sedam dana i tri meseca posle operacije karcinoma debelog creva (KDC) u opštoj anesteziji (OA). **Metode.** U istraživanju je učestvovalo 113 bolesnika starosne dobi od 18 do 87 godina koji su bili podvrgnuti operaciji KDC u OA u periodu od 2021. do 2023. godine. Preoperativna priprema, anestezija i postoperativno lečenje bili su ujednačeni i standardizovani kod svih bolesnika. Procena kognitivnog statusa urađena je na osnovu psihometrijskog testa *Mini Mental State Examination* jedan dan pre operacije, sedmog postoperativnog dana i tri meseca posle operacije. **Rezultati**. Sedam dana posle operacije, utvrđen je statistički značajan poremećaj kognitivnih funkcija kod bolesnika koji su u svom genotipu imali ε4

Correspondence to: Sanja Marić, University of East Sarajevo, Faculty of Medicine, Studentska 5, 73 300 Foča, Republic of Srpska, Bosnia and Herzegovina. E-mail: dalibor.boskovic@bolnicasrbija.com

alel APOE ($p = 0,007$). Bolesnici od 65 godina i stariji češće su imali POCD tri meseca posle operacije u poređenju sa mlađim bolesnicima (80,0% vs. 52,9%; $p < 0,003$). **Zaključak**. Prisustvo ε4 alela APOE predstavlja potencijalni prediktor nastanka POCD sedam dana posle operacije, a godine starosti predstavljaju značajan sociodemografski faktor nastanka POCD tri meseca posle operacije KDC u OA.

Ključne reči:
anestezija, opšta; saznanje, disfunkcija; kolon, neoplazme; prognoza; psihološki testovi.

Introduction

Postoperative cognitive dysfunction (POCD) is one of the most significant neurological morbidities after surgery and anesthesia, which is a significant increase on a global scale. The estimated current prevalence of POCD in a short period, seven days after the surgery, is approximately 3–53% [1]. POCD is particularly associated with age and complex operations. As such, this problem has a great impact on the quality of life of the patient and family. At the same time, it causes high social and personal costs [1, 2]. POCD is defined using one or more standardized psychometric tests, such as the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment (MoCA), the Clock Drawing Test (CDT), as well as a range of other psychometric tests [3–7]. The MMSE is one of the most commonly used tests for examining cognitive state. It includes testing different domains of cognition, such as concentration, visual-spatial abstraction, attention, and memory [7].

The majority of studies focused on examining predisposing risk factors for the occurrence of POCD, including neurological disorders, surgical interventions, level of education, sex, age, types of anesthesia, and drugs used in anesthesia [2,8].

Due to the global trend of increased life expectancy and the growing number of the elderly population, the need for operative treatment has also increased significantly, which eventually leads us to the understanding that POCD could become a problem of epidemiological proportions [8]. Over the last decade, an increasing number of studies have been done examining the role of genetic risk factors in the development of POCD [9].

Apolipoprotein E (APOE) alleles are perhaps the most significant genetic determinants that have been investigated and linked to the development of POCD [10]. The mentioned gene for human APOE is located on chromosome 19 and contains three polymorphic alleles: ε2, ε3, and ε4. There are three heterozygotes (ε2/ε3, ε2/ε4, and ε3/ε4) and three homozygotes (ε2/ε2, ε3/ε3, and ε4/ε4). The APOE ε4 allele, which plays a leading role in the deposition of amyloid plaque, is the most significant risk factor for the development of Alzheimer's disease and POCD [10, 11]. The expression and effects of the APOE ε4 genotype are the result of a complex interaction between genetic predispositions and external influences [12].

A larger number of studies dealt with the connection between surgical interventions and POCD, where it was observed that they were positively correlated [13]. A more recent meta-analysis showed a significant association between the APOE ε4 allele and POCD seven days and three months after various surgical interventions. When studies involving patients who underwent cardiovascular surgery were excluded from the analysis, no significant association between POCD and the APOE ε4 allele was observed [9].

Up to now, little has been known about the association of POCD in patients who underwent colon and gastric cancer surgery [8, 14, 15]. Some studies examined the association of POCD in patients who underwent brain cancer surgery [16]. However, in the available literature, there is no data on the association between POCD and the APOE ε4 allele in patients who underwent colon cancer (CC) surgery – CCS under general anesthesia (GA).

The aim of our study was to examine the association of the APOE ε4 allele and sociodemographic and clinical variables with the occurrence of POCD seven days and three months after the CCS under GA.

Methods

The study was conducted according to the valid permission of the Ethics Committee of the University Hospital of Foča, Bosnia and Herzegovina (No. 1-1051/2, from 13 July 2021). The entire study was conducted following the principles of the Declaration of Helsinki, by the law on medical research involving human subjects. The patients gave their written consent for the study. The study was conducted from January 2021 to August 2023.

Study population

This prospective observational study included patients undergoing open CCS under GA. A total of 113 patients of both sexes, aged 18 to 87 years, were recruited for this study.

The criteria for inclusion in this study were as follows: patients over 18 years of age diagnosed with CC based on pathohistological (PH) findings after colonoscopy and patients without cognitive impairment before surgery, whose cognitive status was checked based on the MMSE. The following were excluded from the study: patients who did not provide informed consent or were incapable of doing so; those with a history of central nervous system disease, such as dementia and other neurodegenerative diseases, cerebrovascular disease, and liver or kidney failure; patients with visual or sensory disorders; patients who have language difficulties or significant hearing impairment; patients who have used antipsychotic medication or drugs affecting cognitive functions; patients diagnosed with psychiatric diseases, delirium or drug, alcohol, or opioid abuse.

For each participant, sociodemographic data (age, sex, level of education, marital status), clinical data related to the

primary disease [PH findings, tumor location, type of surgery, duration of anesthesia, body mass index (BMI), and blood transfusions], and medical history [presence of comorbidities, such as diabetes mellitus (DM), anemia, and hypertension] were collected. Additionally, the results of hematological, biochemical, and genetic analyses, as well as information on operative and postoperative complications, were documented seven days after surgery.

All patients were subjected to the same preoperative, operative, and postoperative anesthetic procedures. Based on the diagnosis, the appropriate surgical procedure was performed under endotracheal GA.

Psychometric testing

Prior to inclusion in the study, all subjects were psychometrically tested on the seventh day and three months after surgery. MMSE was used to assess POCD. The test includes the assessment of six domains through eleven questions: time orientation (5 points), place orientation (5 points), registration (3 points), attention and calculation (5 points), memory (3 points), and language (9 points). MMSE scale ranges from 0 to 30 points; a score of 23 points or less was defined as cognitive disorder [3, 7].

APOE genotyping

Genomic DNA was isolated from 200 μL of whole blood using a commercial kit (GeneJET Genomic DNA Purification Kit, Thermo Fisher Scientific, Waltham, MA, USA). A polymerase chain reaction (PCR) was performed in a final volume of 25 μL containing 0.5 mM forward F: 5'- TAA GCT TGG CAC GGC TGT CCA AGG A -3' and the reverse R: 5'- ACA GAA TTG GCC CCG GCC TGG TAC AC -3' primers [17]. PCR amplification consisted of 30 cycles of 30 sec at 90 °C, 20 sec at 72 °C, and 20 sec at 72 °C. PCR products [244 base pairs (bp)] were confirmed by 1% agarose gel electrophoresis and digested using HhaI (Biolabs New England, Ipswich, MA, USA). The digested products were analyzed on a 4% agarose gel. The fragment lengths were as follows: for e2/e2 genotype 94 bp and 81 bp; for e2/e3 genotype 94 bp, 81 bp, and 66 bp; for e2/e4 genotype 94 bp, 81 bp, 66 bp, and 58 bp; for e3/e3 genotype 94 bp and 58 bp; for e3/e4 genotype 94 bp, 66 bp, and 58 bp; for e4/e4 genotypes 66 bp and 58 bp.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 25 software. Numerical data are presented as means or medians with corresponding measures of variability (ranges and standard deviations). Categorical data are presented as absolute numbers with frequencies. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test normal distribution. The Chi-square test, McNemar test, Wilcoxon, and Mann-Whitney *U* test were used as statistical tests. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

Results

The sociodemographic and clinical characteristics of the patients are given in Table 1. A total of 113 patients participated in this study, and 51 (45.1%) were women. The average age was 66.62 ± 11.28 years. Of 113 patients, 74 (65.5%) were highly educated, and 110 (97.3%) were married. The average BMI was 26.24 ± 3.71 kg/m². The following comorbidities were present: 23 (20.4%) had DM, and 63 (55.8%) had hypertension. The most frequently diagnosed malignancy was sigmoid CC, identified in 42 (37.2%) patients, while the least common was descending CC, observed in 8 (7.1%) patients. Each patient received an average of 439.82 ± 193.10 mL of blood during the operation and an average of 200.44 ± 189.08 mL after the operation.

Table 1

Sociodemographic and clinical characteristics of 113 patients who underwent colon cancer surgery

Variables	Values
Age (years)	66.62 ± 11.28
Sex	
male	62 (54.9)
female	51 (45.1)
Level of education (years)	
≤ high school	74 (65.5)
> high school	39 (34.5)
Marital status	
married	110 (97.3)
single	3 (2.7)
BMI (kg/m²)	26.24 ± 3.71
Diabetes mellitus	
yes	23 (20.4)
no	90 (79.6)
Hypertension, n (%)	
yes	63 (55.8)
no	50 (44.2)
Duration of surgery (hrs)	4.43 ± 0.87
Total treatment time (days)	13.47 ± 1.52
Postoperative treatment time (days)	10.55 ± 1.41
Diagnosis	
ascending colon cancer	24 (21.2)
transvers colon cancer	17 (15.0)
descending colon cancer	8 (7.1)
sigmoid colon cancer	42 (37.2)
rectal cancer	22 (19.5)
Amount of blood during surgery (mL)	439.82 ± 193.10
Amount of blood after surgery (mL)	200.44 ± 189.08
Amount of blood all day (mL)	640.27 ± 281.01
APOE genotypes	
ε2/ε4	27 (23.9)
ε3/ε4	67 (59.3)
ε2/ε2	1 (0.9)
ε3/ε3	14 (12.4)
ε4/ε4	0 (0)
ε2/ε3	0 (0)
Not detected*	4 (3.5)

BMI – body mass index; APOE – apolipoprotein E.
Values are given as numbers (percentages) or mean ± standard deviation.
***could not be analyzed.**

The average duration of anesthesia was 4.43 ± 0.87 hrs, and the average length of hospital stay was 13.47 ± 1.52 days. At least one APOE ε4 allele was found in 94 (83.2%) patients. The most common allele combination was ε3/ε4, observed in 67 (59.3%) subjects, while no subjects carried the APOE ε2/ε3 or APOE ε4/ε4 allele combination.

Based on the MMSE, 105 (92.9%) patients had POCD seven days postoperatively, while POCD was found in 45 (39.8%) patients three months after surgery (Table 2).

Among patients with POCD seven days after surgery, 88 (89.8%) had at least one APOE ε4 allele, while this allele was present in 6 (54.5%) patients without POCD. This difference was statistically significant ($p = 0.007$). However, this difference was not observed three months postoperatively, where 36 (81.8%) patients with POCD and 58 (89.2%) patients without POCD carried at least one APOE ε4 allele ($p = 0.270$). Three months postoperatively, the incidence of POCD was significantly related only to older patients. About 36 (80.0%) patients aged 65 years and older exhibited POCD ($p = 0.003$). Other examined risk factors for the occurrence of POCD, such as level of education, BMI, duration of anesthesia, length of hospitalization, pain, and amount of prescribed blood during and after surgery, did not show statistical significance seven days or three months after surgery. Furthermore, no significant difference was found in hematological and biochemical parameters between patients with and without POCD seven days after the surgical intervention (Table 3).

Table 2

The incidence of POCD in patients seven days and three months after CCS according to the MMSE score

Time after surgery	MMSE score (points)	
	patients with POCD (0–23)	patients without POCD (24–30)
7 days	105 (92.9)	8 (7.1)
3 months	45 (39.8)	68 (60.2)

CCS – colon cancer surgery; MMSE – Mini Mental State Examination; POCD – postoperative cognitive dysfunction.
Values are given as numbers (percentages).

Table 3

Sociodemographic, clinical, and genetic characteristics of patients with and without POCD seven days and three months after CCS

Risk factors	7 days after CCS			3 months after CCS		
	Patients without POCD	Patients with POCD	*p*	Patients without POCD	Patients with POCD	*p*
Age (years)						
< 65	5 (62.5)	36 (34.3)	0.110	32 (47.1)	9 (20.0)	0.003
≥ 65	3 (37.5)	69 (65.7)		36 (52.9)	36 (80.0)	
Level of education (years)						
≤ high school	6 (75.0)	68 (64.8)	0.712	46 (67.6)	28 (62.2)	0.686
> high school	2 (25.0)	37 (35.2)		22 (32.4)	17 (37.8)	
BMI (kg/m^2)						
0–19	0 (0)	3 (2.9)	0.532	2 (2.9)	1 (2.2)	0.946
20–24	2 (25.0)	44 (41.9)		27 (39.7)	19 (42.2)	
> 24	6 (75.0)	58 (55.2)		39 (57.4)	25 (55.6)	
Duration of anesthesia (hrs)						
0–4	3 (37.5)	65 (61.9)	0.262	39 (57.4)	29 (64.4)	0.557
> 4	5 (62.5)	40 (38.1)		29 (42.6)	16 (35.6)	
Duration of hospitalization (days)						
0–10	0 (0)	3 (2.9)	1.000	3 (4.4)	0 (0)	0.275
> 10	8 (100)	102 (97.1)		65 (95.6)	45 (100)	
Amount of blood during surgery (mL)						
0–600	6 (75.0)	81 (77.1)	1.000	53 (77.9)	34 (75.6)	0.821
> 601	2 (25.0)	24 (22.9)		15 (22.1)	11 (24.4)	
Total amount of blood during hospitalization (mL)						
0–600	5 (62.5)	49 (46.7)	0.476	33 (48.5)	21 (46.7)	1.000
> 601	3 (37.5)	56 (53.3)		35 (51.5)	24 (53.3)	
Intraoperative fluid replacement (mL)						
0–2,000	0 (0)	8 (7.6)	0.629	7 (10.3)	1 (2.2)	0.249
2,001–4,000	8 (100)	94 (89.5)		59 (86.8)	43 (95.6)	
> 4,000	0 (0)	3 (2.9)		2 (2.9)	1 (2.2)	

Table 3 (continued)

Risk factors	7 days after CCS			3 months after CCS		
	Patients without POCD	Patients with POCD	*p*	Patients without POCD	Patients with POCD	*p*
Postoperative fluid replacement (mL)						
0–2,000	0 (0)	2 (1.9)	0.889	1 (1.5)	1 (2.2)	0.687
2,001–4,000	8 (100)	102 (97.1)		66 (97.1)	44 (97.8)	
> 4,000	0 (0)	1 (1.0)		1 (1.59)	0 (0)	
APOE ε4 allele						
yes	6 (54.5)	88 (89.8)	0.007	58 (89.2)	36 (81.8)	0.270
no	5 (45.5)	10 (10.2)		7 (10.8)	8 (18.2)	

Values are given as numbers (percentages). For abbreviations, see Tables 1 and 2.
Chi-squared test; $p < 0.05$ statistical significance.

Discussion

To our knowledge, this is the first study to investigate the relationship between the APOE ε4 allele and POCD in CCS patients under GA. In addition, the influence of other sociodemographic and clinical factors on POCD incidence was assessed. We found that the APOE ε4 allele and age could significantly increase the incidence of POCD seven days and three months after CCS under GA, respectively.

The primary approach for treating patients with CC is surgical treatment. Considering that the incidence of CC increases in the aging population, an increase in the incidence of POCD can be expected since older patients are more susceptible to the effects of surgery and anesthesia, which was confirmed in our study. Activation of the immune system and inflammation during surgery are key factors in the development of POCD [18, 19]. These mechanisms are triggered during the surgical procedure and anesthesia, leading to the release of peripheral inflammatory cytokines that can directly compromise the integrity of the blood-brain barrier while also increasing the infiltration of inflammatory factors and macrophages into the brain. This ultimately leads to microglial activation and the initiation of an inappropriate inflammatory response in the hippocampus, contributing to POCD. This cascade of events can subsequently cause synaptic damage and neuronal death [20]. Another important factor associated with the development of POCD is anesthesia. The effects of anesthetics during surgery can lead to mitochondrial damage, further inducing oxidative damage to neurons [21]. As a result, microglial activation occurs, leading to neuroinflammation and neuronal apoptosis, which significantly contributes to POCD [20]. However, the multifactorial etiology of POCD has been proposed. It is essential to identify risk factors for POCD so that the success rate of surgical interventions can be improved and complications prevented. Recently, genetic risk factors associated with POCD have garnered significant research interest [22, 23]. Therefore, the present study investigates the possible effect of the APOE ε4 allele on POCD incidence following CCS under GA, besides the patient's sociodemographic and clinical factors. In this study, the MMSE was chosen to assess POCD. This test is one of the most widely used tools for measuring cognitive function, including cognitive function measurement after surgical procedures, due to its simplicity and ease of administration, with sensitivity from 80% to 95% and specificity from 86% to 100% [24–26].

In our study of patients undergoing CCS under GA, the incidence of POCD seven days and three months after surgery was 92.9% and 39.8%, respectively. The prevalence of POCD after various surgical interventions varies significantly across studies. Specifically, the incidence of POCD following noncardiac surgery ranges from 41% to 75% seven days after surgery and from 18% to 45% three months after surgery. Conversely, higher rates of POCD are observed following cardiac surgery [26–29]. POCD obtained in the present study is higher than that found in other types of noncardiac surgery studies, especially in the early postoperative period (seven days). The possible explanation for this variation in the results of present and previous studies can be partially explained by some methodological differences, such as the tool used to define POCD, time of POCD testing, primary diagnosis, type of surgical intervention, as well as sample characteristics. The sample in this study is characterized by a higher percentage of older patients and those with a lower level of education. The incidence of POCD decreases over time, as demonstrated in this study. This reduction may be attributed to the overall improvement in the patient's health status following the resolution of the primary disease and its associated symptoms.

Of all patients carrying at least one APOE ε4 allele (94 out of 113 in total), the largest proportion of participants, i.e., 67 patients (59.3%), had the ε3/ε4 allele, 27 (23.9%) had the ε2/ε4 allele, while no participants had the ε4/ε4 allele. The present study showed a significant relationship between the APOE ε4 allele and POCD seven days after surgery ($p = 0.007$) but not three months after surgery ($p = 0.270$). Some studies found that the APOE ε4 allele is a risk factor for POCD one week after surgery. In contrast, others found a significant relationship between the APOE ε4 allele and POCD three months after surgery [30, 31]. Other authors found no association between POCD and APOE ε4 allele status one week or three months after surgery [32]. The meta-analysis conducted by Cao et al. [10], which included nine independent studies, found a significant association between the APOE ε4 allele and POCD one week after surgery; however, no such association was observed one to three months or one year following surgery. A possible explanation for the predominantly short-term effect of the APOE ε4 allele on POCD is that the incidence of POCD is

notably higher in the first seven days following surgery. Additionally, the APOE ε4 allele may exacerbate the negative effects of surgery by influencing neuronal repair mechanisms, altering neuronal susceptibility to injury, or increasing embolic load. A recent meta-analysis including 22 studies showed that the APOE ε4 allele was significantly associated with POCD within one week and one to three months after surgery, but not one year after surgery [9]. Furthermore, this meta-analysis included trial sequential analysis according to the surgery type to strengthen the conclusiveness of the results. After excluding patients who underwent cardiovascular surgery, it was found that POCD was not significantly associated with the APOE ε4 allele. Furthermore, excluding both studies that included participants with cognitive impairment as well as retrospective studies, this sensitive analysis revealed a significant association between POCD and the APOE ε4 allele only one week after surgery, which is consistent with the findings of the present study. Several mechanisms might be responsible for the APOE ε4 allele-related POCD. Older APOE ε4 allele carriers may experience reduced cerebral blood flow, which could contribute to the risk of developing POCD [33]. In addition, the clearance of amyloid-β, an essential factor in Alzheimer's disease, is reduced in APOE ε4 allele carriers, predisposing the individuals to POCD. Moreover, the APOE ε4 allele has been linked to the impairment of the blood-brain barrier in Alzheimer's patients, a mechanism that can be significant in the context of POCD [11].

The current understanding is that the risk of developing POCD increases with age [2]. In this study, patients aged 65 years or above had a significant risk of POCD three months after surgery ($p = 0.003$). This result is consistent with previous studies, which indicate that age is one of the most important factors for POCD [34]. The possible reason for the fact that age has only a medium-term effect (three months) in the present study can be explained by the fact that we recruited older patients (mean age 66.6). Although POCD can affect surgical patients in all age groups on a short-term basis, it resolves faster in the younger age group [35]. A decrease in brain volume and cerebral blood flow, with subsequent reduced oxygen delivery and metabolism, advancing age, and age-induced central nervous system apoptosis, may increase the incidence of POCD in elderly patients [36]. Moreover, older patients may experience the progression of their underlying medical comorbidities or develop new health problems between the two testing periods.

Other clinical and sociodemographic factors previously associated with the incidence of POCD, including gender, educational level, duration of surgery and anesthesia, blood loss, and length of hospitalization, did not demonstrate a significant relationship with POCD in this study. The most plausible explanations for this lack of association may stem from the overall higher incidence of POCD observed in both investigated time intervals and the limited sample size.

This study has several limitations that should be taken into account. Firstly, the findings may be limited to patients undergoing CCS under GA. The potential application of laparoscopic techniques should be considered a less stressful option. Additionally, we did not assess which specific cognitive domains (memory, attention, information processing, visuospatial skills, and cognitive flexibility) were most affected in the postoperative period. Furthermore, the relatively small sample size of our study restricts a comprehensive analysis of the relationship between the APOE ε4 allele and POCD. Future prospective studies with larger cohorts and more detailed psychometric assessments are warranted to enhance our understanding of the factors contributing to the development of POCD.

Conclusion

The present study indicates that the presence of the apolipoprotein E ε4 allele may serve as a potential risk predictor for the occurrence of postoperative cognitive dysfunction seven days after surgery. Additionally, age emerges as a significant sociodemographic factor influencing the incidence of postoperative cognitive dysfunction three months after colon cancer surgery performed under general anesthesia. These findings highlight the importance of genetic and demographic factors in assessing the risk of postoperative cognitive dysfunction. Targeted interventions and monitoring may be beneficial for the population at risk. Further research is needed to explore the underlying mechanisms and develop strategies for reducing the impact of these risk factors on cognitive outcomes after surgery.

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CASE REPORTS

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Clinically significant anti-Wr^a antibody: a report of two successfully managed patients

Klinički značajno anti-Wr^a antitelo: prikaz dva uspešno vođena pacijenta

Nevenka Bujandrić*†, Neda Kocić*, Radovan Dinić‡, Jasmina Grujić*†

***Blood Transfusion Institute of Vojvodina, Novi Sad, Serbia; †University of Novi Sad, Faculty of Medicine, Department of Transfusiology, Novi Sad, Serbia; ‡University Clinical Center of Serbia, Emergency Center, Department of Pretransfusion, Hospital Blood Bank, Belgrade, Serbia**

Abstract

Introduction. Wr^a is an antigen of the Diego blood group system. Anti-Wr^a antibody can be found in the sera of healthy individuals (naturally occurring anti-Wr^a) or can be immunostimulated after transfusion or after exposure to foreign erythrocytes during pregnancy. Commercial antibody identification panels do not routinely contain Wr^a antigen-positive erythrocytes. We present two cases of patient blood management in complex situations, such as antibody appearance to low-frequency Wr^a antigen. **Case report.** In the first presented patient, who received multiple blood transfusions, anti-Wr^a antibody was detected while solving cross-match incompatibility. In the second patient, anti-Wr^a antibody was identified during routine antierythrocyte antibody screening during pregnancy. Immunohematological testing included blood typing and antibody screening, cross-matching, antibody identification, indirect antiglobulin test (IAT), and direct antiglobulin test (DAT). Column agglutination technology with microtubes containing gel and standard tube test methodology were used in IAT and DAT. **Conclusion.** Due to the limitations of the screening test in detecting antibodies to clinically significant low-frequency blood group antigens, such as Wr^a, the recommendation when selecting erythrocytes or transfusion is to use units that are cross-match compatible by the IAT test at 37 ºC. In the case of anti-Wr^a antibody, as well as in cases of other antibodies to low-frequency antigens, immunohematology findings should be confirmed in a national or international reference laboratory that has the structure, organization, as well as technical and professional capacities to provide this service.

Key words:
antigens; diagnosis; erythrocyte; erythrocyte transfusion; pregnancy; transfusion reaction.

Apstrakt

Uvod. Wr^a je antigen Dijego sistema krvnih grupa. Anti-Wr^a antitelo se može naći u serumu zdravih osoba (prirodno anti-Wr^a) ili može biti stimulisano imunski-posredovanim mehanizmima nakon transfuzije ili nakon izlaganja stranim eritrocitima tokom trudnoće. Komercijalni paneli za identifikaciju antitela rutinski ne sadrže Wr^a antigen pozitivne eritrocite. Prikazujemo dva slučaja transfuziološkog zbrinjavanja pacijenata u kompleksnim okolnostima, kao što je pojava antitela na Wr^a antigen niske učestalosti. **Prikaz bolesnika.** Kod prvog prikazanog pacijenta, koji je više puta primio transfuziju, anti-Wr^a antitelo je otkriveno tokom rešavanja unakrsne nepodudarnosti. Kod drugog pacijenta je anti-Wr^a antitelo identifikovano tokom rutinskog skrininga antieritrocitnih antitela tokom trudnoće. Imunohematološko testiranje je uključivalo tipizaciju krvi i skrining antitela, test unakrsnog podudaranja, identifikaciju antitela, indirektni antiglobulinski test (IAT) i direktni antiglobulinski test (DAT). Tehnologija aglutinacije u karticama sa gel mikroepruvetama i standardna metodologija ispitivanja u epruveti korišćene su i u IAT-u i u DAT-u. **Zaključak.** Zbog ograničenja skrining testa u otkrivanju antitela na klinički značajne antigene krvnih grupa niske učestalosti, kao što je Wr^a, preporuka pri odabiru jedinica eritrocita za transfuziju jeste korišćenje jedinica čija je unakrsna podudarnost pokazana IAT-om na 37 ºC. U slučaju anti-Wr^a antitela, kao i u slučajevima drugih antitela na niskofrekventne antigene, imunohematološki nalaz treba da bude potvrđen u nacionalnoj ili međunarodnoj referentnoj laboratoriji koja raspolaže strukturom, organizacijom, kao i tehničkim i stručnim kapacitetima za obezbeđivanje ove usluge.

Ključne reči:
antigeni; dijagnoza; eritrociti; transfuzija eritrocita; trudnoća; transfuzija, reakcija.

Correspondence to: Jasmina Grujić, Blood Transfusion Institute Vojvodina, Hajduk Veljkova 9A, Novi Sad 21 000, Serbia. E-mail: jasmina.grujic@mf.uns.ac.rs

Introduction

Although often life-saving, blood transfusion carries some risk. Some red blood cell (RBC) transfusion recipients may encounter the development of alloantibodies. Alloantibodies can potentially lead to acute or delayed hemolytic transfusion reactions (HTR), cause difficulty finding compatible RBC units for future transfusion, or potentially result in hemolytic disease of the fetus and newborn (HDFN). Although finding compatible blood should not be difficult for patients with antibodies to lower-frequency antigens, these antibodies may have to be considered when multiple specificities are present or if they have the potential to cause HDFN. The specificity of antibodies, as well as how urgently blood is required, the immunological status of the patient, class and subclass of the immunoglobulin, strength and thermal amplitude of the antibody, etc., will determine whether or not antigen-negative blood is required for transfusion or whether a child runs a risk of developing HDFN.

The Wr^a blood group RBC antigen is expressed in less than 0.01% of blood donors, making it a low-prevalence antigen. The first description was made by Holman [1] in 1953. In 1995, it was assigned to the Diego system [2]. The Diego blood group system is composed of 23 antigens, including pairs of antithetical antigens: the Wright^a (Wr^a) antigen and the Wright^b (Wr^b) antigen, differing by one amino acid on the AE1 glycoprotein. Anti-Wr^a antibody is termed clinically significant because it is capable of causing acute intravascular hemolysis, resulting in HTR or HDFN [3–6]. Therefore, the detection and determination of their specificity (identification) are essential for pretransfusion testing or prenatal antibody screening. Unfortunately, current antibody screening tests cannot detect all clinically significant antibodies against low-incidence antigens, for instance, antibodies such as anti-Wr^a are likely to be missed. Anti-Wr^a antibody is often found in the sera of healthy individuals (naturally occurring anti-Wr^a) or can be immunostimulated (after transfusion of Wr^a antigen-positive donor RBC or after pregnancy-related RBC exposure). Anti-Wr^a antibody can be found in the serum of 1% of blood donors [7, 8].

Anti-Wr^a antibody in healthy donors is predominantly immunoglobulin (Ig) M. In pregnant or previously transfused patients, it can be IgG or IgM plus IgG type, with the potential to cause severe immediate or delayed HTR and severe HDFN [7, 9, 10]. Those event-causing antibodies may remain unknown because the reagent RBC panel used for antibody identification rarely expresses Wr^a antigen [11]. If the specificity of the antibody has been determined, compatible blood units can be found without difficulty because Wr^a antigen-negative RBCs are practically always available within the blood stock [12–14].

We report on the effectiveness of our clinical practice in complex situations, such as antibody appearance to low-frequency antigens. Our initial focus was based on the fact that detailed quality records of a particular case could provide adequate insight into resolving a problem and provide vital information to enhance awareness of blood safety issues. Thus, we report two cases of anti-Wr^a antibody identified for the first time in the Serbian population. The first case was detected while solving cross-match incompatibility in the repeatedly transfused patient, and the second was identified during routine RBC antibody screening during pregnancy. Both patients were from the West Bačka District of northern Serbia.

Case report

Case I

A 53-year-old female with headaches and weakness was admitted to the General Hospital Sombor, Serbia, because of the clinical manifestation of anemia: hemoglobin 79 g/L, RBC count 2.07 10^{12}/L, hematocrit 24.9% (normal references in adult non-pregnant women: hemoglobin levels 120–160 g/L, RBC count 4.2–5.4 10^{12}/L, hematocrit 36–48%). Anemia in this patient occurred as a complication of Myelodysplastic syndrome. The patient was a repeatedly transfused person who received a total of fifteen RBC units over the past four years and who had been transfused within the last 3 months.

Prior to the anemia correction of this patient, Blood Bank Sombor performed pretransfusion compatibility testing. The testing involved the patient's blood group and cross-match. The patient's blood group was determined as blood type A, RhD-positive, Rh phenotype ccDEe, Kell-negative. The complete cross-match (with a 37 °C incubation and antiglobulin phase) was performed on commercial cards with a gel matrix containing anti-IgG, anti-IgM, and anti-C3d (Cellbind Screen, Sanguin Reagens, the Netherlands), and it was positive. The results of subsequent testing were as follows: negative direct antiglobulin test (DAT), negative indirect antiglobulin test (IAT) (Screening set 1+2, Sanguin Reagens, the Netherlands), negative RBC antibody screening with enzyme-treated cells, and negative cold agglutinins blood test. There were no records of a positive cross-match in history. The sample was sent to the Blood Transfusion Institute Vojvodina (BTIV) for further testing.

BTIV performed antibody screening by the IAT method using commercial reagent RBCs (ID-DiaCell I+II, Bio-Rad, DiaMed GmbH, Switzerland) and commercial Liss-Coombs cards (DiaMed AG 1785 Cressier, Switzerland). IAT was negative as well as screening with enzyme-treated, same reagent RBCs. DAT was also negative. The antibody screening was continued using IAT, a gel technique with commercial reagent RBC panel (Column panel 16, Sanguin Reagens, the Netherlands), and an irregular anti-Wr^a antibody was identified. The strength of agglutination was 4+ (grading 1–4). The autocontrol was negative. Antisera was available for testing, so typing of the patient's RBC showed that she was Wr^a antigen-negative. We provided Wr^a antigen-negative RBC for transfusion, and cross-matches were negative. After receiving a blood transfusion, the patient did not experience hemolytic reactions. The incompatible donation from Blood Bank Sombor was not tested to confirm the presence of Wr^a antigen.

Case II

A 25-year-old female who was pregnant for the second time was admitted to the General Hospital Sombor for a routine RBC antibody screening. She was in the eighth month of pregnancy. She had never had any transfusion. However, the patient had a history of one abortion because of an unwanted pregnancy at the age of eighteen (with a different partner). It was determined that she had a blood group type A, RhD-negative, Rh phenotype ccddee. The routine antenatal antibody screening using commercial RBC reagent (Screening set 1+2, Sanguin Reagens, The Netherlands) detected that the pregnant woman had an irregular antibody. Identification of unexpected antibodies was negative. It was determined that the partner had a blood group type B, RhD positive.

Freshly collected venous samples from both the pregnant woman and the presumptive father were sent to BTIV for further testing. Antibody screening of the mother's sample using the IAT method [a gel technique with commercial reagent RBC (ID-DiaCell I+II, Bio-Rad, DiaMed GmbH, Switzerland)] was negative. No antibodies were detected by the same reagent RBC, i.e., bromelin-treated. Further antibody screening by the IAT method with the commercial reagent RBCs panel (Column panel 16, Sanguin Reagens, the Netherlands) was 2+ positive and revealed anti-Wr^a antibody. The paternal RBC was non-reactive by IAT with anti-Wr^a antibody in maternal plasma. The paternal was typed for the Wr^a antigen and found to be Wr^a antigen-negative.

The pregnancy was not complicated, and the woman delivered a full-term male newborn without jaundice. DAT for IgG antibody was negative. Antigen typing showed the newborn to be negative for Wr^a antigen.

Discussion

In the first reported case of anti-Wr^a antibody in patients, an irregular antibody was detected during anti-human globulin (AHG) cross-match. After the anti-Wr^a antibody was successfully identified, compatible blood was easily found for the patient. As the risk of HTR due to anti-Wr^a antibody was removed, the testing algorithm seemed optimal. If the reagent RBC panel had not identified the anti-Wr^a antibody, AHG cross-match negative blood would have been transfused, also without any post-transfusion issues. Contrary to the aforementioned, antibody screening as the sole part of pre-transfusion testing would be a suitable solution only if the RBC reagent contained low-frequency Wr^a antigen, on the basis of which the antibody would be detected and identified. Suppose the RBC reagent does not contain a corresponding potentially clinically significant antigen, such as the Wr^a antigen, because of its low incidence in the population. In such a case, pretransfusion testing will not reveal anti-Wr^a antibody, and the consequences are uncertain.

Many studies have focused on comparing what is more efficient during pretransfusion testing, cross-matching or antibody screening [13–15]. Unfortunately, the presence of antibodies to a low-frequency antigen can also be discovered after an incompatible transfusion, which has resulted in an HTR [16, 17].

If the AHG cross-match is part of pretransfusion testing, irregular antibodies will be detected after an unexpected positive cross-match occurs. Regrettably, the identification RBC panel without Wr^a antigen cannot reveal anti-Wr^a antibody. On the other hand, the identification RBC panel with Wr^a antigen will identify anti-Wr^a antibody and allow patients with this relatively common naturally occurring antibody to receive a blood transfusion with a low risk of receiving a non-compatible unit and HTR [14, 18].

In the second case, the irregular antibody was detected during routine prenatal screening. After identification of anti-Wr^a antibody, we were pleased to establish that the presumptive father was Wr^a antigen-negative and concluded that the child was not at risk of HDFN due to anti-Wr^a antibody. As the next purchased screening and identification panels did not have an anti-Wr^a antibody in their composition, some questions remained open, such as anti-Wr^a antibody future proving, as well as the possibility of prenatal risk assessment, prenatal monitoring, and pre- and postnatal diagnosis.

Anti-Wr^a antibody is present in pregnant women and crosses the placenta and enters the fetus, where it initiates immune destruction of fetal erythroid cells. It is restricted to IgG (mostly IgG1 and IgG3), as antibodies of other classes are not transported across the placental barrier. If the anti-Wr^a antibody is not recognized during pregnancy, the pregnancy may be at risk of HDFN, but prenatal risk assessment cannot be done, and the titer cannot be monitored. A positive DAT in a newborn should pay attention to the presence of maternal antibodies directed against antigens inherited from the father. The problem can occur in a child born with HDFN, ranging from mild to severe, whose mother has a negative antibody screening test [5, 6].

The Wr^a antigen is present in less than 1% of the population. The other study has reported a frequency as high as 1 in 1,500 in the European population and 1 in 785 in the Spanish population [3, 4]. Globally, the percentage of alloimmunized patients in the general population ranges from 1–6% after a single transfusion [3, 4, 9]. A higher percentage occurs among multitransfused patients, while the risk of alloimmunization is lower in patients receiving dialysis [19]. The frequency of Wr^a antigen and anti-Wr^a antibody in the Serbian population is unknown.

About 50% of the anti-Wr^a antibodies have the potential to be clinically significant. The use of antigen-negative RBCs ensures the safety of blood transfusion in these patients. Given the increased risk of hemolysis in patients with sickle cell disease, if the patient develops anti-Wr^a antibody, then Wr^a antigen negative units should be provided, along with a match for the full expanded phenotype.

Conclusion

One of the limitations of the screening test is the inability to detect antibodies to clinically significant low-frequency blood group antigens such as Wr^a. Therefore, when selecting red blood cells for transfusion, units that are cross-match compatible with the indirect antiglobulin test at 37 ºC are recommended. It should be pointed out that anti-

Wr^a antibody is not routinely included in antibody screening cells, but it is capable of causing severe hemolytic disease of the fetus and newborn.

In the case of anti-Wr^a antibody, as well as in cases of other antibodies to low-frequency antigens, the immunohematology findings should be confirmed in a national or international reference laboratory that has the structure, organization, technical, and professional capacities to provide this service.

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Written consent was obtained from the patients for publishing the study.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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Bilateral emphysematous pyelonephritis and emphysematous cystitis: a rare case report

Obostrani emfizematozni pijelonefritis i emfizematozni cistitis

Irfan Šabotić*†, Ahmo Habibović‡, Irfan Ćorović§, Selma Habibović‖

***University of Niš, Faculty of Medicine, Niš, Serbia; General Hospital of Novi Pazar, †Department of Urology, ‡Department of Radiology, §Department of Internal Medicine, Novi Pazar, Serbia; ‖Institute of Public Health Novi Pazar, Department of Microbiology, Novi Pazar, Serbia**

Abstract

Introduction. Emphysematous pyelonephritis (EPN) is a rare type of severe kidney infection characterized by gas formation in the renal parenchyma, collecting system, or surrounding perinephric tissues. Emphysematous cystitis (EC) is a rare, potentially fatal condition characterized by the presence of gas in the bladder wall and lumen. We present a rare case of EPN with EC in a patient with diabetes mellitus (DM). **Case report**. A 38-year-old female patient with poorly regulated DM was referred to the Department of Urology at the General Hospital in Novi Pazar, Serbia, with malaise, fever, and flank and suprapubic pain. After physical examination, laboratory analyses, and radiological diagnostics, the diagnosis of bilateral EPN with EC was established. Throughout the hospitalization, the patient remained hemodynamically stable with preserved diuresis. The patient's urine and blood cultures were negative. The patient was treated with intravenous fluids and antibiotics (metronidazole, vancomycin, and meropenem). No other invasive form of treatment was needed due to successful conservative treatment. **Conclusion.** A timely diagnosis and rapid inclusion of specific antibiotic therapies enables the avoidance of the need for invasive forms of treatment in patients with EPN and EC. The conservative management of the presented patient was successful.

Key words:
cystitis; diabetes mellitus, tip 1; emphysema; pyelonephritis; treatment outcome.

Apstrakt

Uvod. Emfizematozni pijelonefritis (EP) je retka i teška infekcija bubrega koja dovodi do stvaranja gasova u bubrežnom parenhimu, sabirnom sistemu ili perirenalnom tkivu. Emfizematozni cistitis (EC) je retko i potencijalno fatalno stanje, koje karakteriše nakupljanje gasa u zidu i lumenu mokraćne bešike. Prikazan je slučaj EP sa EC kod osobe obolele od dijabetesa melitusa (DM). **Prikaz bolesnika**. Bolesnica stara 38 godina, sa loše regulisanim DM, hospitalizovana je na Odeljenju urologije u Opštoj bolnici Novi Pazar, Srbija, sa tegobama u vidu malaksalosti, povišene telesne temperature, bola u slabinskim regijama i donjem delu stomaka. Nakon kliničkog pregleda, laboratorijskih analiza i radiološke dijagnostike postavljena je dijagnoza obostranog EP sa EC. Sve vreme tokom hospitalizacije bolesnica je bila hemodinamski stabilna, sa očuvanom diurezom. Urinokultura i hemokultura bile su negativne. Bolesnica je lečena intravenskim rastvorima i antibioticima (metronidazol, vankomicin i meropenem). Nije bilo potrebe za drugim invazivnim oblikom lečenja, jer je konzervativna terapija bila uspešna. **Zaključak.** Blagovremenim uspostavljanjem dijagnoze i brzim uključivanjem antibiotske terapije omogućuje se izbegavanje invazivnog oblika lečenja kod bolesnika sa EP i EC. Primenjena konzervativna terapija imala je povoljan ishod kod prikazane bolesnice.

Ključne reči:
cistitis; dijabetes melitus, tip 1; emfizem; pijelonefritis; lečenje, ishod.

Introduction

Emphysematous pyelonephritis (EPN) is a rare type of severe kidney infection characterized by gas formation in the renal parenchyma, collecting system, or surrounding perinephric tissues [1]. Emphysematous cystitis (EC) is a rare, potentially fatal condition characterized by the presence of gas in the bladder wall and lumen [2]. Bilateral EPN combined with EC in the same patient is a very rare case. It is caused by gram-negative microorganisms, most commonly *Esche*

Correspondence to: Irfan Šabotić, General Hospital in Novi Pazar, Department of Urology, Dubrovačka 406/19, 36 300 Novi Pazar, Serbia. E-mail: irfan_sabotic@hotmail.com

richia coli (*E. coli*), and other anaerobes such as *Proteus spp* and *Klebsiella* [3]. Kelly and MacCallum [4] reported the first case of gas-forming renal infection in 1898. EPN is commonly associated with poorly controlled diabetes mellitus (DM) and urinary tract obstruction or polycystic kidneys [5, 6]. The gold standard for diagnosis is computed tomography (CT). Treatment of EPN with EC can be conservative, based on appropriate antibiotic therapy, or invasive, including emergency nephrectomy or percutaneous drainage [7].

Huang and Tseng [5] created a classification system based on the CT scan: class 1 – gas in the pyeloureteral system only; class 2 – gas forming in the renal parenchyma without extension to parararenal tissues; class 3 – accumulation of gas to perirenal tissues; class 4 – solitary kidney with EPN or bilateral EPN.

We present a patient with poorly controlled DM who acquired EC and bilateral EPN. Written informed consent for participating in the study was obtained from the patient.

Case report

A 38-year-old female patient with type 1 DM experienced malaise, suprapubic pain, fever, and flank pain for 4 days before admission to the Department of Urology, General Hospital of Novi Pazar, Serbia, in September 2023 (day 0). She had been suffering from DM, and she had been receiving insulin therapy. She did not consume alcohol or cigarettes.

A physical examination on admission revealed the following: blood pressure of 110/80 mmHg; body temperature of 37.5 °C, heart rate of 80 beats *per* minute, Glasgow Coma Scale (GCS) of 15 (the highest possible GCS score of 15 means fully awake, responsive, and without any problems concerning thinking ability or memory), and respiration rate of 17 breaths *per* minute. Bilateral costovertebral angle tenderness was present without palpable masses. The remainder of the physical examination was unremarkable.

At the time of the patient's admission, laboratory analyses showed raised inflammatory markers: C-reactive protein (CRP) 398.7 mg/L [normal range (NR) < 5 mg/L], leukocytes 29.5 × 10^9 /L (NR 4.0–10.0 × 10^9 /L) and neutrophils 92% (NR 34–71%). Laboratory analyses also showed a raised blood glucose of 24.1 mmol/L (NR 2.6–6.1 mmol/L), values of glycated hemoglobin A1c (HbA1c) of 12.1%, serum creatinine 154 µmol/L (NR 53–124 µmol/L), and blood urea 9.6 mmol/L (NR 2.5–8.3 mmol/L). Hypoalbuminemia was detected at 22 g/L (NR 35–55 g/L). Urine analysis confirmed the presence of glycosuria, ketonuria, and pyuria.

An abdominal ultrasound (US) showed evidence of gas on both kidneys (Figure 1) and the bladder wall (Figure 2). An urgent CT scan of the abdomen and pelvis was performed. CT confirmed the presence of extensive gas accumulation in both kidneys (Figure 3), with gas inside and around the bladder wall (Figure 4). According to Huang and Tseng [5], this EPN with EC was categorized as class 4.

The patient was hospitalized and started on empirical antibiotics along with other supportive measures. The patient's blood and urine cultures were negative. On the second day of hospitalization (day 2), due to the complexity of the treatment, we decided to refer the patient for further treatment to the Clinic for Urology at the Clinical Center of Serbia, Belgrade, Serbia. The patient was treated with intravenous (i.v.) fluids, antibiotics (metronidazole, vancomycin, and meropenem), and rapid-acting insulin. In addition, the electrolyte imbalance was treated. No other invasive form of treatment was needed. Urine cultures were repeated several

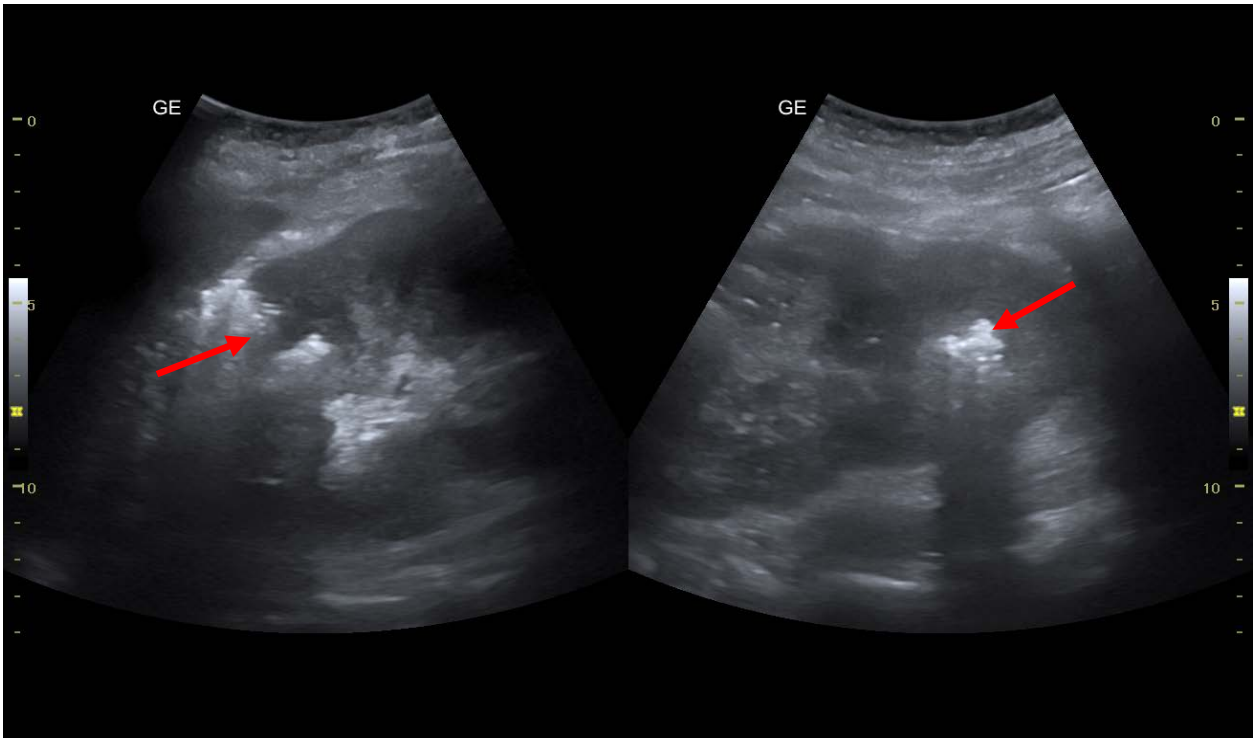

Fig. 1 – Ultrasound of the abdomen showed the presence of gas accumulation in both kidneys (arrows).

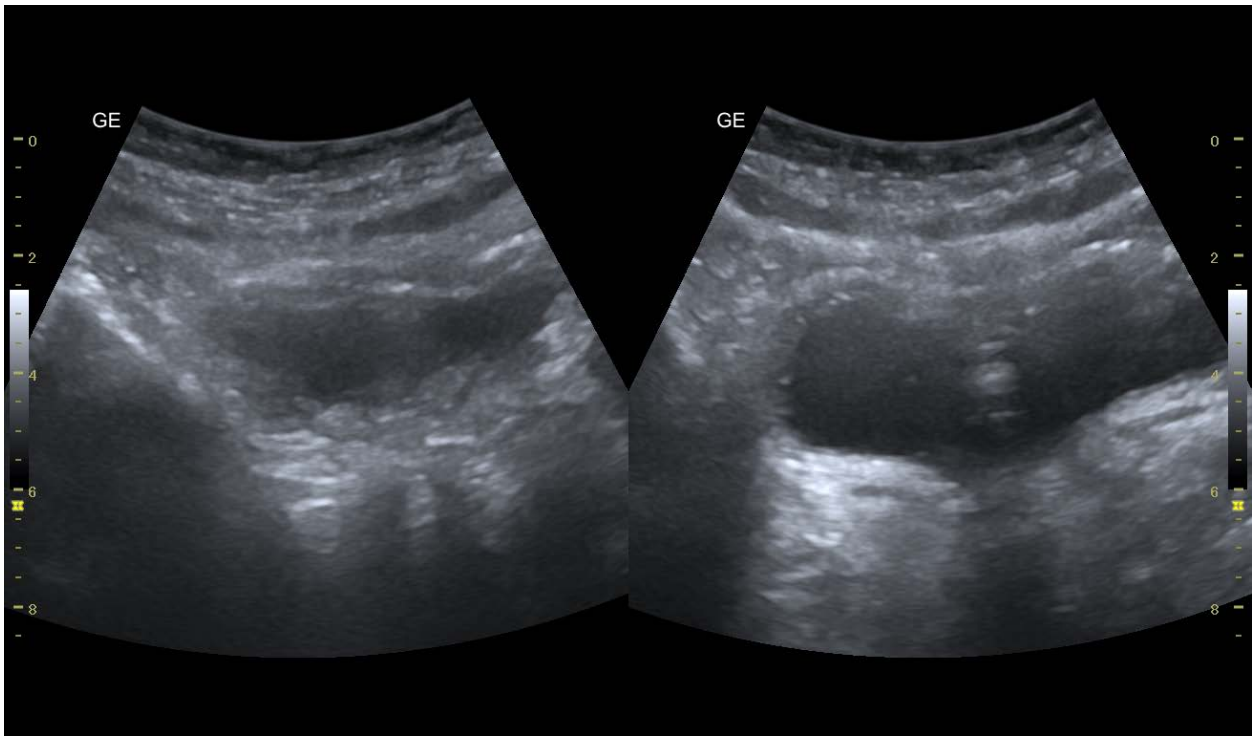

Fig. 2 – Ultrasound of the abdomen showed gas accumulation in the bladder wall (acoustic shadowing around the bladder wall).

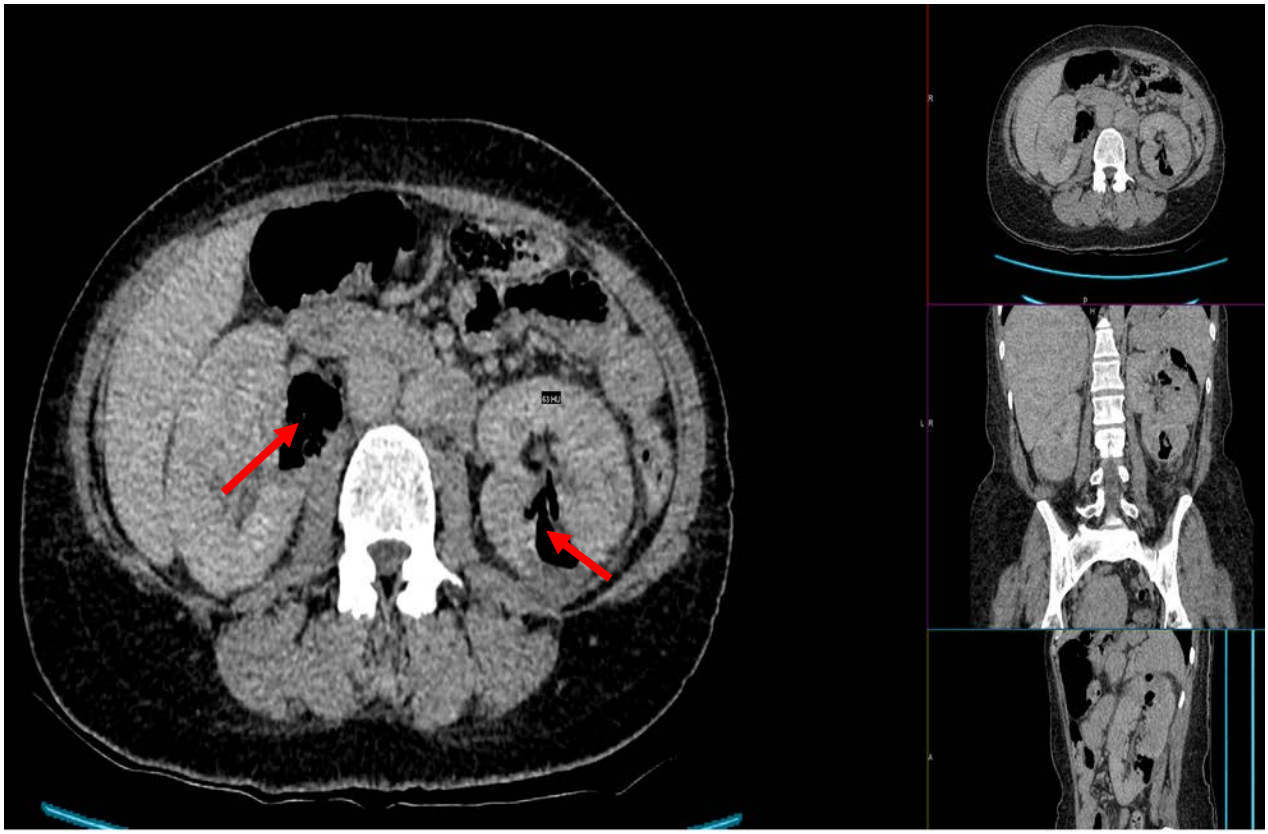

Fig. 3 – Computed tomography showing diffusely enlarged kidneys and gas in the kidneys bilaterally, indicative of bilateral emphysematous pyelonephritis (class 4 emphysematous pyelonephritis) (arrows).

times during hospitalization and were always negative. Likewise, the blood culture was also negative. Throughout the hospitalization, the patient remained hemodynamically stable with preserved diuresis. On day 15 after admission, the patient was afebrile. On day 25 of admission, a second CT scan was performed, which showed a complete resolution of the right renal and bladder emphysema and the presence of a small gas accumulation in the left renal parenchyma. After 28 days of inpatient care, the patient was discharged with oral antibiotics. US of the abdomen 7 days after discharge showed a complete resolution of right renal and bladder emphysema and the presence of a small amount of gas in the upper pole of the left kidney (Figure 5). Laboratory analyses had normalized by discharge (leucocytes 9.3 x 10^9/L, urea 7,1 mmol/L, creatinine 89 μmol/L, and CRP 4 mg/L).

Laboratory analyses performed 20 days after discharge showed increased CRP 13 mg/L and leukocytes 11.2 × 10^9/L (other values were expected for DM patients) (Table 1).

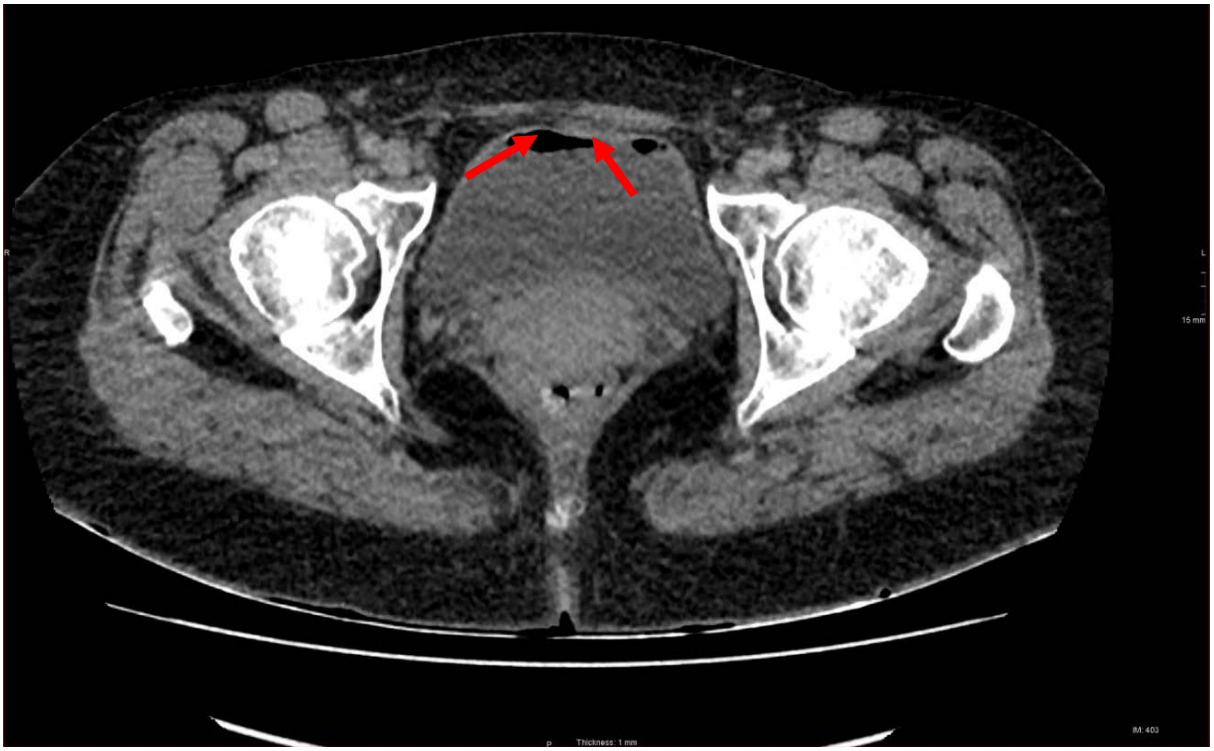

Fig. 4 – Computed tomography image of gas accumulation in the bladder wall (arrows).

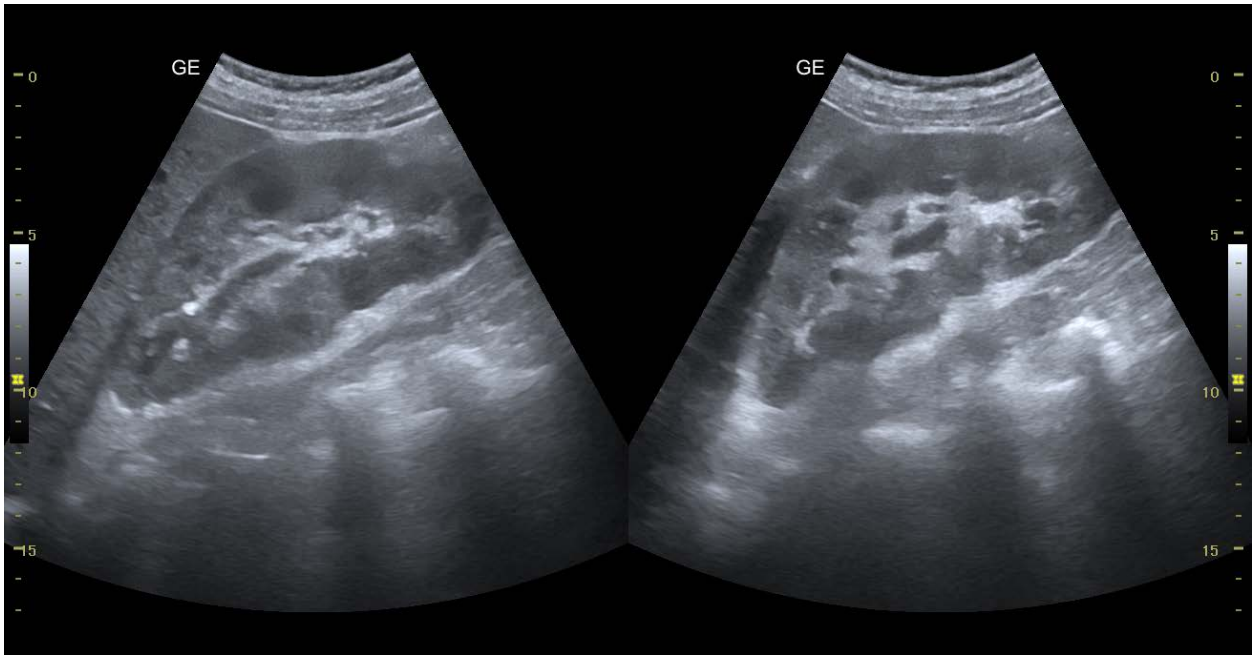

Fig. 5 – Ultrasound imaging – disappearance of gas in the right renal parenchyma four weeks after initiation of treatment, and a small amount of gas in the left kidney.

Table 1

Patient laboratory test results 20 days after discharge from the hospital

Parameters	Values
White blood count, × 10^9 /L	11.2
C-reactive protein, mg /L	13
Serum creatinine, μmol/L	65.5
Serum urea, mmol/L	4.0
HbA1c, %	7.7
Hemoglobin, g/L	117
Serum glucose, mmol/L	11.8
Serum albumin, g/L	43

HbA1c – glycated hemoglobin A1c.

Discussion

Descriptions of patients with bilateral EPN and EC in the literature are scarce. It is believed that EPN is more common in females because they have urinary tract infections more commonly [8]. The most common risk factor for the development of EPN associated with EC is DM. Stapleton [9] reports that a high glucose concentration in the tissue contributes to the growth and proliferation of gas-producing bacteria. Moreover, a high tissue glucose concentration inhibits the function of leukocytes and impairs the response to infection. Our patient had a poorly controlled DM with an HbA1c value of 12.1% on admission. Due to the fermentation of glucose and lactate, gas is produced, which is then accumulated at the site of inflammation. As mentioned above, EPN is caused by gram-negative microorganisms, most commonly *E. coli*, and other anaerobes such as *Proteus spp* and *Klebsiella* [3]. In our case, no definitive causative organism was identified from urine or blood culture. We assume it is because our patient used antibiotics prior to urine collection, which suppresses bacterial growth and leads to false-negative results.

In patients with EPN or EC, the mortality rate is between 8.7% and 21%. This infection is life-threatening [10, 11]. Lu et al. [12] reported that high mortality was present in EPN patients who require emergency hemodialysis, have present shock on initial presentation, have altered mental status, severe hypoalbuminemia, inappropriate antibiotic therapy, or polymicrobial infection. Likewise, inadequate empirical antibiotic and polymicrobial infections were associated with high mortality. Our patient did not require emergency hemodialysis because she did not have altered mental status or shock on the initial presentation. In a series of 19 patients with EPN, Khaira et al. [13] also reported that shock was an independent poor prognostic risk factor.

Over the years, the treatment of EPN with EC has evolved from an invasive to a conservative approach. The major treatment methods are conservative management, percutaneous drainage, or emergency nephrectomy.

Angulo et al. [14] suggested that conservative treatment or percutaneous drainage should be performed in patients with EPN in a solitary kidney, bilateral EPN, localized EPN, or if the patient cannot tolerate general anesthesia. In a study conducted in Taiwan, ten patients with EPN received only antibiotics, and the mortality rate was 20% (two patients died) [12]. Our patient was diagnosed with class 4 disease, leukocytosis, azotemia, and hypoalbuminemia on admission. Aboumarzouk et al. [15] show that patients with EPN and EC have an overall mortality rate of about 18%. Furthermore, this study shows that conservative treatment and percutaneous drainage are associated with significantly higher survival rates than emergency nephrectomy. Emergency nephrectomy should be applied when the patient does not improve despite previous treatment methods. Empirical antibiotics are the first line for the treatment of EPN with EC [13]. Our patient was managed in accordance with the current evidence-based protocols, where she was treated with empirical antibiotics and other supportive therapy. The most successful treatment was a combination of i.v. antibiotics and nephrostomy insertion, with a mortality rate of around 13% [16].

Conclusion

The best diagnostic method for emphysematous pyelonephritis and emphysematous cystitis is computed tomography, which also aids in classifying emphysematous pyelonephritis. The conservative management of our patient was successful. We emphasize that timely diagnosis and specific antibiotic treatment can prevent the need for surgery. The risk of mortality was reduced because our patient did not manifest shock, mental disorder, thrombocytopenia, or polymicrobial infection.

Conflict of interest

The authors declare no conflict of interest.

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Primeri referenci:

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Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom 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.

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

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

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