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ORIGINAL ARTICLES / ORIGINALNI RADOVI

Sir Frederick Grant Banting (November 14, 1891–February 21, 1941) was a Canadian physician and scientist who co-discovered insulin along with Dr. Charles Best in 1922. For this discovery, he received the Nobel Prize in Medicine in 1923.

His birthday, November 14, was set to be World Diabetes Day (WDD) and is marked every year, beginning in 1991 when it was established by the International Diabetes Federation and the World Health Organization.

The theme for WDD 2019 is Family and Diabetes because of the impact that diabetes has on the family as well as the important role of the family in the prevention and treatment of diabetes.

Ser Frederik Grant Banting (14. novembar, 1891–21. februar, 1941) bio je kanadski lekar i naučnik koji je zajedno sa dr Čarlsom Bestom otkrio insulin 1922. godine. Za to otkriće, 1923. dobio je Nobelovu nagradu. Njegov rođendan, 14. novembar, određen je za Svetski dan dijabetesa i obeležava se svake godine, počevši od 1991, kada je ustanovljen od strane Međunarodne federacije za dijabetes i Svetske zdravstvene organizacije.

Tema ovogodišnjeg Svetskog dana dijabetesa je „Porodica i dijabetes" zbog uticaja koje dijabetes ima na porodicu, kao i zbog važne uloge porodice u prevenciji i lečenju dijabetesa.

General psychopathology and impulsivity as the pretreatment predictors of medical nutrition therapy outcome in the overweight and obese women

Opšta psihopatologija i impulsivnost kao pokazatelji potencijalnog ishoda medicinske nutritivne terapije kod predgojazih i gojaznih žena

Vesna Tepšić Ostojić*, Danijela Ristić-Medić[†], Tatjana Mraović[‡],
Zoran Bukumirić[§], Nadja Vasiljević[‖], Milan Latas[¶**]

Military Medical Academy, *Clinic for Psychiatry, [‡]Institute of Hygiene and Medical
Ecology, Belgrade, Serbia; University of Belgrade, Institute for Medical Research,
[†]Centre of Research Exellence in Nutrition and Metabolism, Belgrade, Serbia;
University of Belgrade, Faculty of Medicine, [§]Institute for Medical Statistics and
Informatics, [‖]Institute of Hygiene and Medical Ecology, Belgrade, Serbia; Clinical
Centre of Serbia, [¶]Clinic of Psychiatry, Belgrade, Serbia; University of Belgrade,
**Faculty of Medicine, Belgrade, Serbia

Abstract

Background/Aim. Obesity is the chronic disease and health threatening condition. The number of obese people in the world has taken epidemic conditions. Medical nutritional therapy is the first choice in the treatment of obesity, but it is also accompanied with a great percentage of attrition and a significant weight regain. The aim of our study was to evaluate if psychiatric and specific psychological factors (impulsivity) could be the predictors of successful weight loss. **Methods.** A study sample consisted of 84 consecutive overweight/obesity women, 20 to 40 years old, who were willing to adhere to the medical nutrition therapy after the initial anthropometric measurements were performed. All participants received a personalized nutritional counseling and a daily diet treatment with 20% caloric restriction from estimated daily energy requirement. At the beginning of the study, the 90-item Symptom Check-list (SCL-90) and the 30-item Barratt Impulsiveness scale (BIS-11) were administrated. At the end of six months of caloric restriction, the conventional diet therapy and control weight measurement, the patients were divided into two groups: the group I – 40 participants (48%) who lost ≥ 10% of their initial body weight (successful group) and the group II – 44 participants (52%) who lost < 10% of their initial weight (unsuccessful). **Results.** There were no significant differences between groups in the demographic data (age, education level, employment, marital status), baseline anthropometric measurements, and in the general psychopathology total score. Groups I and II were significantly different in the total BIS-11 score ($p < 0.001$) and in Factor II (motor impulsivity) ($p < 0.05$). **Conclusion.** Even though the successful and unsuccessful diet responded participatnts did not differ in the general psychopathology and symptom dimensions, our results pointed out impulsivity as a discriminative factor between them. The total impulsivity and Factor II (motor impulsivity) as an indicator of binge eating were higher in the unsuccessfully responding overweight/obese women on the conventional diet treatment. A focus on impulsivity in a psychotherapeutic work might leed to better outcomes in the medical nutrition therapy.

Key words:
body mass index; diet therapy; impulsive behaviour; obesity; overweight; surveys and questionnaires; treatment outcome.

Apstrakt

Uvod/Cilj. Gojazost je hronično oboljenje sa značajnim nepovoljnim efektima na zdravlje, a broj gojaznih osoba u svetu dostigao je epidemijske razmere. Medicinska nutritivna terapija je terapija izbora, ali je njena uspešnost ograničena zbog značajnog procenta odustajanja, kao i ponovnog dobijanja na težini koje je prate. Cilj ove studije je bio da se proceni da li psihijatrijski i specifični psihološki faktori (impulsivnost) mogu biti pokazatelji pozitivnog ishoda lečenja. **Metode.** Studijom je bilo obuhvaćeno ukupno 84 predgojaznih i gojaznih žena životnog doba 20–40 godi-

Correspondence to: Vesna Tepšić Ostojić, Military Medical Academy, Clinic for Psychiatry, Crnotravska 17, 11 000 Belgrade, Serbia.
E-mail: vesna.j.tepsic@gmail.com

na, koje su bile motivisane za medicinsku nutritivnu terapiju. Sve ispitanice su prošle individualno savetovanje i dobile dnevni plan ishrane koji je podrazumevao kalorijsku restrikciju od 20% u odnosu na procenjene dnevne energetske potrebe. Na početku studije sprovedeno je antropometrijsko merenje i primenjene su Lista simptoma od 90 pitanja (90-item Symptom Check-list) i Baratova skala impulsivnosti (Barratt Impulsiveness scale (BIS-11)). Nakon šest meseci primene kalorijski restriktivnog načina ishrane ponovljena su antropometrijska merenja. Ispitanice su podeljene u dve grupe: 40 ispitanica (48%) koje su izgubile ≥ 10% od početne telesne mase (uspešne) i 44 ispitanice (52%) koje su izgubile < 10% od početne telesne mase (neuspešne). **Rezultati.** Nije bilo značajne razlike između grupa ispitanica u odnosu na demografske karakteristike (godine života, stepen obrazovanja, zaposlenost, bračni status) i antropometrijske parametre na početku studije, kao i na skor opšte psihopatologije i dimenzije simpto-

ma. Statistički značajna razlika između uspešnih i neuspešnih predgojaznih/gojaznih ispitanica dobijena je za ukupni BIS-11 skor (*p* < 0.001) i za Faktor II (motorna impulsivnost) (*p* < 0.05). **Zaključak.** Ispitanice sa ponovljenim lošim odgovorom nisu se razlikovale po opštoj psihopatologiji od onih sa uspešnim odgovorom na nutritivnu terapju. Naši rezultati ukazuju na impulsivnost kao mogući pokazatelj ishoda lečenja. Ukupna impulsivnost i motorna impulsivnost kao indikator povremenog impulsivnog prejedanja bili su viši u grupi predgojazih/gojaznih ispitanica koje nisu postigle terapijski cilj. Psihoterapijski rad na impulsivnosti možda bi mogao doprineti boljem ishodu medicinske nutritivne terapije.

Ključne reči:
telesna masa, indeks; dijetetska terapija; ponašanje, impulsivno; gojaznost; telesna masa, prekomerna; ankete i upitnici; lečenje, ishod.

Introduction

Obesity is the chronic disease defined as excess body fat [1]. The basic pathophysiological mechanism of the accumulation of excess body fat is basically very simple – energy intake is higher than energy consumption [2]. In the light of this fact, the solution to the problem of obesity seems relatively easy – to reduce the intake, or increase the energy consumption. Even though the solution seems logical and easily feasible, the number of obese people in the world has taken epidemic conditions; 35% of the adult population is overweight, while 12% obese is considered as a "global pandemic" [3]. About 35% of the adult population in Serbia are overweight and 21% are obese, while in the self-evaluation only 16.9% of the population consider themselves to be obese [4]. Obesity is the health threatening condition. Empiric and scientific data confirmed its clear association with plethora of medical conditions (metabolic syndrome, type 2 diabetes, ischemic heart disease, stroke, inflammation, apnea, certain carcinoma, etc.) [1, 5–7] and decreased quality of life [8, 9]. If the current trend of increased morbidity and mortality due to obesity continues, the life expectancy in future could decrease for the first time in modern history [10]. However, it is important to point out that there is evidence that people with obesity are confronted with structural discrimination in their everyday life [11].

Even though obesity is considered to be an interplay of genes and environment [12], research focus has been moving from the metabolism, resting metabolic rate and energy expenditure. A recent research points out that the environmental variables are of greater importance in determining eating behavior than the biological ones [13, 14].

In the contemporary culture, a person's physical appearance exceeds the importance of aesthetic liking and becomes one of the main social markers of success, or stigma. On the opposite social pole, there is the extremely obesogenic environment with aggressively marketed cheap calories that are not only easy-to-get but also highly palatable and rewarding (saturated in fat, sugars and/or salt) [14] Hedonic eating is promoted while physiological and physical activity is discour-

aged by modern technology (cars, smart phones, etc.). But not everyone becomes obese despite the environmental temptations. The answer may be in the individual differences. Someone's reaction to the environmental conditions could be influenced by the psychiatric conditions such as anxiety and depression, personality traits, or other psychological factors such as impulsivity that they do, or do not possess [15]. All those factors play a role in the etiology and/or maintenance of obesity.

Treatment of obesity includes the medical nutritional therapy (MNT), physical activity, behavioral techniques, pharmacotherapy and bariatric surgery. MNT is the "gold standard" in the treatment of obesity [16, 17]. The conventional dietary treatment is based on an energy deficit, which can be achieved in many ways, but most experts agree that the optimal daily deficit is 500 to 1,000 kcal, which leads to a loss of about 0.5 to 1 kg per week that can be recommended for everyday practice [16]. This therapy yields good results in some patients, but it is also accompanied with a great percentage of attrition (20%–80%) and a significant weight regain [17, 18]. Differences in the individual results in the obese subjects integrated in the weight-loss treatment, lead to analyse the potential pre-treatment predictors of weight control [19].

Thus, the aim of our study was to evaluate if psychiatric and specific psychological factors (impulsivity) could be the predictors of successful weight loss.

Methods

Participants

This study was designed as a prospective cohort study with two measurements. It was realised from October 2015 to May 2016.

The anthropometric measurements and MNT was performed in the Centre of Research Excellence in Nutrition and Metabolism, Institute for Medical Research, Belgrade while the psychiatric interview and psychometric measurements were conducted in the Psychiatric Clinic, Clinical Center of Serbia, Belgrade.

The study enrolled 84 consecutive patients who were willing to treat obesity in the Centre of Research Excellence in Nutrition and Metabolism, the Institute for Medical Research in Belgrade. The inclusion criteria for our investigation were female gender, age between 20 and 40 years, overweight and obese class I with the body mass index (BMI) between 25 and 34.9 kg/m^2 and self-reported weight stable (± 2 kg) for 3 months before the start of the diet treatment. The exclusion criteria were: the history of psychiatric illness, inflammatory or infective diseases, hypertension, diabetes mellitus, cardiovascular, cerebrovascular and malignant disease.

The written informed consent was obtained from all participants prior to participation in the study, and only those who were volunteered to take part in the investigation were included. The study protocol was approved by the Ethics Committee, Faculty of Medicine, the University of Belgrade and carried out in accordance with the principles of the Declaration of Helsinki

Anthropometric parameters

The anthropometric parameters were measured at the baseline and at the end of six-month treatment. The body height and weight of participants wearing light clothes and without shoes were measured. The participants were weighed with a lever-actuated balance to the nearest 0.1 kg. The BMI was calculated as weight (kg)/height (m) squared. The same qualified nutritionist did the measurements for all participants.

Medical nutrition therapy

After completing the anthropometric measurements, the participants were referred to a diet therapist for MNT. All participants received a personalized nutritional counseling and the daily diet treatment with 20% caloric restriction from the estimated daily energy requirement was prescribed individually [20]. The dietary treatment was based on the conventional-type of intervention with the energy limitation and balanced macronutrient composition. The dietary intake was controlled by the size of portion, food choice and composition. The daily energy requirements were calculated according to the obesity treatment guidelines issued by the US National Institute of Health and Food and Nutrition Board [20].

After six months of caloric restriction, the convectional diet therapy and the control weight measurements, the patients were divided into two groups.The first group included 40 (48%) participants who lost ≥ 10% of their initial body weight (successful – group I), and second included 44 (52%) participants who lost < 10% of their initial weight (unsuccessful – group II).

Psychopathology measures

Before starting MNT, all patients were interviewed with a standard psychiatric interview by the same psychiatrist. The patients had 90 min in a quiet place to fulfill the questionnaires.

The demographic characteristic were investigated by the 4-item sociodemographic self-reported questionnaire designed for this study. Four items were related to age, education (elementary school, high school, college, university degree), employment (employed, unemployed, student), and marital status (married/with partner, single, divorced).

For the purpose of this study, the two self-administered questionnaires were used the 90-item Symptom Check-list (SCL-90)[21] and the 30-item Barratt Impulsiveness scale (BIS-11)[22]. The SCL-90 was administrated as the screening instrument for the assessment of psychiatric psychopathology as a possible predictor of the MNT outcome. The items were rated on a 5- point scale of distress (ranging from "not at all" to "extremely"). There are nine primary symptom dimensions: somatisation, obsession-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism.

The BIS-11 was developed to measure impulsiveness as seen by this author (motor aspect, an attentional aspect, and a planning aspect). It is one of the most used scales for the measurement of impulsiveness. It consists of 6 first- grade and 3 second-grade factors. The factor labels with definitions are as follows: Factor 1, attention – "focusing on the task at hand"; Factor 2 – motor impulsiveness "acting on the spur of the moment"; Factor 3, self-control – "planning and thinking carefully"; Factor 4, cognitive complexity – "enjoy challenging mental task"; Factor 5, perseverance – "a consistent life style"; Factor 6, cognitive instability – "thought insertions and racing thoughts". The second-grade factors combine two first-grade factors. Factor I, attentional impulsiveness combines attention and cognitive instability. Factor II, motor impulsiveness combines the first-grade factors, motor impulsiveness and perseverance. The factor of non-planning impulsiveness III is combined with cognitive complexity and self-control.

Statistical analysis

The statistical data analysis was performed using the IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA). The results were presented as the frequency (percentage) and mean ± SD. The χ^2 test was used to test the differences between the nominal data (frequencies). The *t*-test was used for the numeric data with a normal distribution. For the ordinal data, the Mann-Whitney *U* test was used. All *p*-values less than 0.05 were considered significant.

Results

The sociodemographic characteristics of participants are presented in Table 1. The majority of participants of both I and II groups had the high school degree (55% *vs* 50%, respectively) and were employed (48% *vs* 66%, respectively) and most commonly were married/with a partner (40% *vs* 47%, respectively). There were no significant differences between the groups regarding the age, employment and marital status.

The anthropometric parameters of participants are shown in Table 2. There were not significant differences between groups in the body weight before starting MNT and in the BMI at the baseline of the study, but after 6 months, the body weight and BMI were significantly different ($p < 0.001$) in the Group I and Group II with the average weight loss of 15.89 ± 4.65 kg and 2.63 ± 1.81 kg, respectively. The loss from the initial body weight in the Group I, expressed in percentage, was $19.17 \pm 4.78\%$ (range 30.79–11.60%) and in the Group II, it was $3.16 \pm 2.3\%$ (range 7.67–2.53%).

The average scores of particular domains obtained from the SCL-90 questionnaire are presented in Table 3. The results indicate that both groups had similar scores in nine primary symptom dimensions, and that there were not the significant differences found in the symptom dimensions and the total score.

In Table 4, the average scores of particular domains obtained from the BIS-11 questionnaire with the first- and second-grade factors are shown. There were the statistically significant differences observed between the Groups I and II in the Factor II ($p < 0.05$) and in the total BIS-11 score ($p < 0.001$).

Table 1
Sociodemographic characteristics of overweight/obesity women (n = 84)

Characteristics	Total	Group I	Group II	p
	84 (100%)	40 (48%)	44 (52%)	
Age (years), mean ± SD	30.38 ± 5.95	29.40 ± 6.10	31.27 ± 5.74	0.152
Education, n (%)				
elementary school	2 (2)	1 (2)	1 (2)	
high school	44 (53)	22 (55)	22 (50)	0.700
college	12 (14)	5 (13)	7 (16)	
university degree	26 (31)	12 (30)	14 (32)	
Employment, n (%)				
employed	48 (57)	19 (48)	29 (66)	
unemployed	16 (19)	11 (27)	5 (11)	0.125
student	20 (24)	10 (25)	10 (23)	
Marital status, n (%)				
married/with partner	36 (43)	16 (40)	20 (47)	
single	8 (10)	5 (13)	3 (8)	0.696
divorced	40 (47)	19 (47)	21 (45)	

Group I – participants who lost ≥ 10% of their initial body weight; Group II – participants who lost < 10% of their initial body weight; SD – standard deviation; $p < 0.05$ considered significant.

Table 2
Anthropometric parameters of patients

Characteristica	Total	Group I	Group II	p
	mean ± SD (min-max)	mean ± SD (min-max)	mean ± SD (min-max)	
Body height, m	1.67 ± 0.07 (1.51–1.84)	1.67 ± 0.04 (1.55–1.75)	1.65 ± 0.08 (1.51–1.84)	0.971
Body weight, kg				
initial	81.56 ± 9.77 (60.7–103.9)	82.34 ± 9.40 (60.7–98.1)	80.86 ± 10.14 (65.0–103.9)	0.491
final	72.68 ± 10.87 (50–100)	66.52 ± 8.28 (50–81.3)	78.29 ± 9.91 (63–100)	< 0.001
BMI, kg/m²				
initial	29.46 ± 2.69 (25.29–34.83)	29.58 ± 2.79 (25.29–34.83)	29.34 ± 2.62 (25.34–34.75)	0.682
final	26.71 ± 3.50 (19.85–34.75)	23.99 ± 2.62 (19.85–30.33)	28.50 ± 2.74 (23.97–34.75)	< 0.001
Weight loss, kg	8.95 ± 7.50 (0.00–29.50)	15.89 ± 4.65 (9.40–29.50)	2.63 ± 1.81 (0.00–7.10)	< 0.001
(%)	-10.78 ± 8.84 (-30.79–2.53)	-19.17 ± 4.78 (-30.79–11.60)	-3.16 ± 2.3 (-7.67–2.53)	< 0.001
Weight loss participant expected, kg	17.73 ± 6.20 (5–30)	17.78 ± 5.43 (7–30)	17.68 ± 6.88 (5–30)	0.945

BMI – body mass index.
For other abbreviations see under Table 1.

Table 3

Psychiatric psychopathology of participants

SCL – 90 symptom dimensions	Total mean ± SD	Group I mean ± SD	Group II mean ± SD	p
Somatization	1.50 ± 0.46	1.53 ± 0.47	1.48 ± 0.46	0.684
Obsessivity-compulsive	1.58 ± 0.57	1.63 ± 0.69	1.54 ± 0.45	0.458
Interpersonal sensitive	1.54 ± 0.60	1.50 ± 0.65	1.57 ± 0.55	0.632
Depression	1.53 ± 0.53	1.55 ± 1.62	1.51 ± 0.43	0.771
Anxiety	1.65 ± 0.62	1.69 ± 0.72	1.61 ± 0.51	0.589
Anger-hostility	1.35 ± 0.44	1.33 ± 0.47	1.36 ± 0.41	0.813
Phobic anxiety	1.17 ± 0.31	1.11 ± 0.20	1.22 ± 0.38	0.095
Paranoid ideation	1.50 ± 0.60	1.44 ± 0.54	1.56 ± 0.65	0.385
Psychoticism	1.20 ± 0.31	1.21 ± 0.39	1.19 ± 0.22	0.838
Total score	1.48 ± 0.42	1.48 ± 0.48	1.48 ± 0.37	0.939

SCL-90 – 90-item Symptom Check-list.
For other abbreviations see under Table 1.

Table 4

Impulsiveness in the participants

BIS factors	Total mean ± SD	Group I mean ± SD	Group II mean ± SD	p
Attention	15.13 ± 2.14	14.78 ± 2.42	15.45 ± 1.81	0.147
Motor impulsiveness	17.07 ± 3.10	16.6 ± 3.51	17.5 ± 2.65	0.198
Self-control	13.64 ± 2.65	13.73 ± 2.83	13.56 ± 2.50	0.789
Cognitive complexity	13.07 ± 1.54	13.00 ± 1.66	13.13 ± 1.44	0.688
Perseverance	8.13 ± 1.74	8.25 ±1.60	8.02 ± 1.87	0.553
Cognitive instability	7.40 ±1.93	7.15 ± 2.09	7.63 ± 1.77	0.252
FACTOR I	22.54 ± 3.22	21.92 ± 3.63	23.09 ± 2.72	0.097
FACTOR II	25.08 ± 3.65	24.10 ± 3.67	25.98 ±3.44	< 0.05
FACTOR III	26.38 ± 3.87	26.65 ± 3.52	26.14 ± 3.84	0.543
Total score	72.21 ± 9.06	68.57 ± 10.79	75.52 ± 5.42	< 0.001

BIS – Barratt Impulsiveness Scale.
For other abbreviations see under the Table 1.

Discussion

In the light of the fact that obesity has become the epidemic health threatening condition, its effects on physical health were extensively studied and well-documented [5–7, 16]. However, the relationship between the psychiatric and/or psychological factors and etiology and obesity treatment is complicated and not well-known.

Our study showed that the obese/overweight participants, who were apparently healthy and homogenous regarding age, education level, employment and marital status, had different outcomes adhering to the same MNT protocol for six months.

The participants did not differ in the general psychopathology and its symptom domains measured with the SCL-90. Our results pointed out impulsivity measured as the total impulsivity on the BIS-11 as a pretreatment predictor of the MNT outcome. The total impulsivity and its Factor II (motor impulsivity), being singificantly higher, and the Factor I (attentional impulsivity), was higher in the participants who lost < 10% of their initial weight (Group II).

The symptoms of general psychopathology and obesity in both groups shared similar risk factors like dietary habits, level of physical activity and sedentary lifestyle [23]. Although one might expect a positive correlation between general psy-

chopathology and etiology and negative response on obesity treatment, the research evidence was not entirely clear. It is not easy to detect causality i.e., which comes first: from obesity, or from psychopathology. Some studies showed the positive correlation between obesity and the general psychopathology. It was shown for the following domains: somatization [24], interpersonal sensitivity [25], depression [26, 27], anxiety [26, 27], anger-hostility [28] and psychoticism [25]. Also, in several studies, mood disorders are found to be frequent, especially in the morbidly obese patients seeking an obesity treatment [29]. As well, the overweight and obese patients are not rare among those who were diagnosed with mood disorders [29]. But, in contrast to these findings, a lot of studies found no relationship between obesity and general psychopathology [30, 31]. In several community studies, the mood disorders were not found among the obese persons [32]. There was some evidence that the levels of anxiety among the obese persons are equal with those who seek the general medical or surgical treatment and that required obesity treatment may be the actual cause of anxiety [30].

No differences in the SCL-90 scores between the Group I and Group II general psychopathology (i.e., somatization, obsession-compulsion, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism) could be explained by the fact that the women who will-

ingly began treatment of obesity were different from those who did not consider the general psychopathology levels. One could hypothesize that the overweight and obese women who participated in a weight-loss treatment had a lower level of general psychopathology when compared to those who did not, and therefore were more prone to start MNT. So, our results did not show that general psychopathology was a pretreatment predictive factor of medical nutrition outcome.

Impulsivity could be defined as "a predisposition toward rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of these reactions to the impulsive individual or to others"[33]. Therefore, it was seen as a multidimensional construct. The self-report questionnaires and behavioral measurements such as the task of inhibiting motor response or delaying discounting, measure a different aspect of impulsivity. Impulsivity measured by the self-reported questionnaires and behavioral measurements was a risk factor for a range of maladaptive behaviors, such as overeating, binge eating and substance abuse, or in bipolar disorder, or attention-hyperactivity disorder[33].

The BIS-11 scale used in this study measured total impulsivity and three second-grade factors: motor impulsiveness (act without thinking), attentional impulsiveness (no focus on the present task), and non-planning impulsiveness (lack of orientation to the future).

In our study, the women in the Group II had the higher scores of total impulsivity compared to the women in the Group I. Our findings were in accordance with the results of other studies that self-reported impulsivity was higher in the obese individuals[14, 15, 34]. Davis et al.[35] found the positive association of overeating and the preference for sweet and fatty food with impulsivity in the general population. Also, it was shown that those two factors were in a relationship with the BMI[32]. The relevant literature data provided evidence that the higher scores on impulsivity were found not only in the participants with negative dieting outcome[16], as it was in our study, but also in the participants exhibiting food cravings[36], emotional eating[37] and restraint eating[38]. All those phenomena are important for the development, maintenance and treatment of obesity[34, 37].

Attentional impulsivity measured with the BIS-11 subscales is the most often linked with overeating[15, 34, 37]. The women who did not achieve ≥ 10% of weight loss in our study had tendency to higher score for attentional impulsivity (attentional impulsiveness combined the first- grade factors attention and cognitive instability) than the ones who did it. Attentional impulsivity may be the reason why, in the participants engaged in the weight–loss program, the highly palatable food came to the focus and became a trigger for eating[15, 37].

In our study, the motor impulsivity score (motor impulsiveness combined the first-grade factors, motor impulsive-

ness and perseverance) was a significantly higher in the Group II (unsuccessful responders) than in the Group I (the successful restrictive diet treatment responders). The literature data suggest that motor impulsivity is less often linked to obesity itself[15]. The higher scores were found in the individuals that were engaged in the binge eating behavior and the higher scores on motor impulsivity were referred to bulimia nervosa and the anorexia nervosa binge/purge type, but not for the anorexia nervosa restrictive type[15, 38]. Nonplanning impulsivity was rarely linked to obesity, and our results were in accordance with the mentioned investigation. So, it seems that a combination of high attentional and motor impulsivity might be partly responsible for overeating as well as a clinically significant binge eating.

This study has some limitations. Firstly, we did not divide overweight and obese women into separate groups. However, there is evidence that the overweight and the obese women showed the same weight loss from the initial body weight during the same treatment period[39]. Secondly, the data on impulsivity must be more precise if the self-reported measures were accompanied with the behavioral ones (inhibiting motor response, or delaying discounting).

Conclusion

The results of our study showed that the obese/overweight women in both groups – the successful and unsuccessful responders, who were apparently healthy, did not differ in age, education level, employment and marital status, but had different outcomes of the same MNT protocol for six months. The participants, who lost 10%, and more or less than 10% of their initial body weight, did not differ in the general psychopathology. Even though the successful and unsuccessful diet responders did not differ in general psychopathology, our results pointed out that impulsivity could be the pretreatment predictor of the outcome. The overweight/obese women as the unsuccessful diet responders had the higher total impulsivity and motor impulsivity scores. Our data highlighted the role of total impulsivity and Factor II (motor impulsivity) as an indicator of binge eating that may contribute to a poor response to the conventional diet weight loss treatment. The focus on impulsivity in the psychotherapeutic work integrated into the weight-loss treatments might lead to the better outcome in MNT.

R E F E R E N C E S

1. Centers for Disease Control and Prevention. Defining overweight and obesity 2012. Available from: https://www.cdc.gov/obesity/adult/defining.html

2. *Tepšić V, Pavlović M, Ristić-Medić D, Ristić V, Lekić N, Tepšić J*, et al. Influence of dietary fats on serum phospholipid fatty acid composition and its relation to obesity in animals. Acta Vet 2008; 58(1): 33–41.

3. *World Health Organization*. Overweight and obesity 2015. Available from:
www.who.int/gho/ncd/risk_factors/overweight/en/

4. *Boričić K, Vasić M, Grozdanov J, Gudelj-Rakić J, Živković Šulović M, Jaćović-Knežević N*, et al. Results of the research on the health of the population of Serbia, 2013. Belgrade: Ministarstvo Zdravlja i Institut za Javno zdravlje Srbije "Dr Milan Jovanović-Batut" 2014. (Serbian)

5. *Mraović T, Radaković S, Ristić Medić D, Tepšić Ostojić V, Raden S, Hajduković Z*, et al. The effects of different caloric restriction diets on anthropometric and cardiometabolic risk factors in overweight and obese females. Vojnosanit Pregl 2018; 75(1): 30–8.

6. *Mraović T, Radaković S, Ristić Medić D, Tepšić Ostojić V, Raden S, Čairović A*, et al. The relationship between adiposity parameters and C-reactive protein values in overweight and obese women. Vojnosanit Pregl 2018; 75(2): 185–190.

7. *Markovic D, Ristic-Medic D, Vucic V, Mitrovic G, Nikolic Ivosevic J, Peric T*, et al. Association between being overweight and oral health in Serbian schoolchildren. Int J Paediatr Dent 2015; 25(6): 409–17.

8. *Taylor VH, Forhan M, Vigod SN, McIntyre RS, Morrison KM*. The impact of obesity on quality of life. Best Pract Res Clin Endocrinol Metab 2013; 27(2): 139–46.

9. *Vasiljevic N, Ralevic S, Kolotkin RL, Marinkovic J, Jorga J*. The Relationship Between Weight Loss and Health-related Quality of Life in a Serbian Population. Eur Eat Disord Rev 2012; 20(2): 162–8.

10. *Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J*, et al. A potential decline in life expectancy in the United States in the 21st century. N Engl J Med 2005; 352(11): 1138–45.

11. *Spahlholz J, Baer N, König HH, Riedel-Heller SG, Luck-Sikorski C*. Obesity and discrimination - a systematic review and meta-analysis of observational studies. Obes Rev 2016; 17(1): 43–55.

12. *Bouchard C, Pérusse L, Rice T, Rao DC*. Genetics of human obesity. In: *Bray GA, Bouchard C*, editors. Handbook of Obesity: Etiology and Pathophysiology. 2nd ed. New York, NY: Marcel Dekker; 2004. p. 157–200.

13. *Jansen A, Houben K, Roefs A*. A Cognitive Profile of Obesity and ItsTranslation into New Interventions. Front Psychol 2015; 6: 1807.

14. *Appelhans BM, French SA, Pagoto SL, Sherwood NE*. Managing temptation inobesity treatment: A neurobehavioral model of intervention strategies. Appetite 2016; 96: 268–79.

15. *Meule A, Platte P*. Facets of impulsivity interactively predict body fat andbinge eating in young women. Appetite 2015; 87: 352–7.

16. National Institutes of Health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults-The Evidence Report. Obes Res 1998; 6 Suppl 2: 51S–209S.

17. *Varady KA*. Intermittent versus daily calorie restriction: which diet regimenis more effective for weight loss? Obes Rev 2011; 12(7): e593–e601.

18. *Lasikiewicz N, Myrissa K, Hoyland A, Lawton CL*. Psychological benefits of weight loss following behavioural and/or dietary weight loss interventions. A systematic research review. Appetite 2014; 72: 123–37.

19. *Silva I*. Importance of emotional regulation in obesity and weight loss treatment. Fractal Rev Psicol 2015; 27(3): 286–90.

20. *Trumbo P, Schlicker S, Yates AA, Poos M*. Food and Nutrition Board of the Institute of Medicine, The National Academies. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002; 102(11): 1621–30.

21. *Derogatis LR, Lipman RS, Rickels K, Uhlenhuth EH, Covi L*. The Hopkins Symptom Checklist (HSCL): a self-report symptom inventory. Behav Sci 1974; 19(1): 1–15.

22. *Patton JH, Stanford MS, Barratt ES*. Factor structure of the Barratt impulsiveness scale. J Clin Psychol 1995; 51(6): 768–74.

23. *Dimitriadis D, Mamplekou E, Dimitriadis P, Komessidou V, Dimitriadis G, Papageorgiou C*. The Association between Obesity and Symptoms of Psychopathology and its Relationship with Sedentary Behavior and Mediterranean Diet. Isr J Psychiatry Relat Sci 2016; 53(3): 17–24.

24. *Papelbaum M, Moreira RO, Gaya CW, Preissler C, Coutinho WF*. Impact of body mass index on the psychopathological profile of obese women. Rev Bras Psiquiatr 2010; 32(1): 42–6.

25. *Mills JK*. A note on interpersonal sensitivity and psychotic symptomatology in obese adult outpatients with a history of childhood obesity. J Psychol 1995; 129(3): 345–8.

26. *Petry NM, Barry D, Pietrzak RH, Wagner JA*. Overweight and obesity are associated with psychiatric disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Psychosom Med 2008; 70(3): 288–97.

27. *Scott KM, McGee MA, Wells JE, Oakley Browne MA*. Obesity and mental disorders in the adult general population. J Psychosom Res 2008; 64(1): 97–105.

28. *Carmody TP, Brunner RL, St Jeor ST*. Hostility, dieting, and nutrition attitudes in overweight and weight-cycling men and women. Int J Eat Disord 1999; 26(1): 37–42.

29. *Lin HY, Huang CK, Tai CM, Lin HY, Kao YH, Tsai CC*, et al. Psychiatric disorders of patients seeking obesity treatment. BMC Psychiatry 2013; 13: 1.

30. *Scott KM, Bruffaerts R, Simon GE, Alonso J, Angermeyer M, de Girolamo G*, et al. Obesity and mental disorders in the general population: results from the world mental health surveys. Int J Obes (Lond) 2008; 32(1): 192–200.

31. *Lawlor DA, Hart CL, Hole DJ, Gunnell D, Davey Smith G*. Body mass index in middle life and future risk of hospital admission for psychoses or depression: findings from the Renfrew/Paisley study. Psychol Med 2007; 37(8): 1151–61.

32. *McElroy SL, Kotwal R, Malhotra S, Nelson EB, Keck PE, Nemeroff CB*. Are mood disorders and obesity related? A review for the mental health professional. J Clin Psychiatry 2004; 65(5): 634–51, quiz 730.

33. *Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC*. Psychiatric aspects of impulsivity. Am J Psychiatry 2001; 158(11): 1783–93.

34. *van den Akker K, Stewart K, Antoniou EE, Palmberg A, Jansen A*. Food Cue Reactivity, Obesity, and Impulsivity: Are They Associated? Curr Addict Rep 2014; 1(4): 301–8.

35. *Davis C, Patte K, Levitan R, Reid C, Tweed S, Curtis C*. From motivation to behaviour: a model of reward sensitivity, overeating, and food preferences in the risk profile for obesity. Appetite. 2007; 48(1): 12–9.

36. *Meule A, Westenhöfer J, Kübler A*. Food cravings mediate the relationship between rigid, but not flexible control of eating behavior and dieting success. Appetite 2011; 57(3): 582–4.

37. *Guerrieri R, Nederkoorn C, Jansen A*. The Effect of an Impulsive Personality on Overeating and Obesity: Current State of Affairs. Psychological Topics 2008; 17: 265–86.

38. *Meule A, Vögele C, Kübler A*. Restrained eating is related to accelerated reaction to high caloric foods and cardiac autonomic dysregulation. Appetite 2012; 58: 638–44

39. *Redman LM, Heilbronn LK, Martin CK, Alfonso A, Smith SR, Ravussin E*. Effect of calorie restriction with or without exercise on body composition and fat distribution. J Clin Endocrinol Metab 2007; 92: 865–72

Pulmonary exacerbations as a risk factor for lung function decline – experiences of the National Cystic Fibrosis Center

Egzacerbacije plućne bolesti kao faktor rizika od sniženja funkcije pluća – iskustva Nacionalnog centra za cističnu fibrozu

Bojana Z. Gojšina*, Milan Rodić[†], Jelena Višekruna[†], Goran Z. Trajković*[‡], Aleksandar D. Sovtić*[†], Predrag B. Minić*[†]

University of Belgrade, *Faculty of Medicine, Belgrade, Serbia; Mother and Child Health Institute "Dr Vukan Čupić", [†]Department of Pulmonology, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, [‡]Institute for Medical Statistics, Belgrade, Serbia

Abstract

Background/Aim. Pulmonary exacerbations have negative impact on clinical course of cystic fibrosis (CF) lung disease being associated with a steeper decline in the lung function, unfavorable prognosis and impaired quality of life. The aim of this study was to determine whether an increased number of exacerbations had influence on the lung function in the patients with CF, as well as to estimate the nutritional status, gender, presence of comorbid conditions and bacterial colonization of airways as predictive factors for pulmonary exacerbations. **Methods.** This retrospective cohort study included 83 pediatric and adult patients, treated from 2011–2015 in the Mother and Child Health Institute of Serbia „Dr Vukan Čupić". The best result of forced expiratory volume in the first second (FEV1) and forced vital capacity (FVC) in each year of follow-up was taken into account to calculate the five-year trend values of these indicators. The number of exacerbations per year of follow-up and its impact on the FEV1 decline was evaluated. **Results.** Mean annual decline of FEV_1 and FVC were 2.4% and 1.7% respectively. The malnourished patients had the lower initial values of FEV_1 and FVC, and more frequent exacerbations in comparison with the normal weight and overweight patients. The frequency of exacerbations was significantly higher in the patients chronically colonized with *Burkholderia cepacia* ($p = 0.023$). The increased number of exacerbation was proved to be the most important factor in a prediction of FEV_1 decline over time ($p = 0.013$). **Conclusion.** Pulmonary exacerbations lead to the more progressive lung function decline in the patients with CF. Malnourishment and chronic airway colonization with *Burkholderia cepacia* result in more frequent pulmonary exacerbations.

Key words:
burkholderia cepacia; cystic fibrosis; forced expiratory volume; lung diseases; recurrence; respiratory function tests.

Apstrakt

Uvod/Cilj. Egzacerbacije plućne bolesti imaju negativan uticaj na klinički tok cistične fibroze (CF), a njihova veća učestalost povezuje se sa izraženijim sniženjem vrednosti funkcije pluća i lošijim kvalitetom života. Cilj ovog istraživanja bio je da se utvrdi uticaj broja egzacerbacija na trend funkcije pluća kod obolelih od CF, kao i značaj stanja uhranjenosti i pola ispitanika, komorbidnih stanja i kolonizacije disajnih puteva patogenim bakterijama na trend funkcije pluća i učestalost egzacerbacija plućne bolesti. **Metode.** Istraživanjem su obuhvaćena 83 ispitanika, deca i odrasle osobe, obolela od CF, lečena u periodu od 2011. do 2015. godine u Institutu za zdravstvenu zaštitu majke i deteta Srbije „Dr Vukan Čupić". Najbolji rezultat forsiranog ekspiri-jumskog volumena u prvoj sekundi (FEV_1) i forsiranog vitalnog kapaciteta (FVC) svake godine praćenja uziman je u obzir pri računanju petogodišnjeg trenda vrednosti ovih pokazatelja. Procenjivan je broj egzacerbacija po godini praćenja i ukupan broj na kraju perioda praćenja. **Rezultati.** Prosečno smanjenje vrednosti FEV_1 bilo je 2,4%, a FVC 1,7% godišnje. Neuhranjeni ispitanici su imali niže vrednosti FEV_1 i FVC i veći broj egzacerbacija u odnosu na normalno i prekomerno uhranjene ($p = 0,001$). Učestalost egzacerbacija je bila statistički značajno viša kod ispitanika hronično kolonizovanih bakterijom *Burkholderia cepacia* ($p = 0,023$). Povećanje broja egzacerbacija bilo je statistički najznačajniji prediktivni činilac pogoršanja FEV_1 u posmatranom periodu ($p = 0,013$). **Zaključak.** Veća učestalost egzacerbacija plućne bolesti kod obolelih od CF dovodi do izraženijeg

Correspondence to: Predrag B Minić, Mother and Child Health Institute "Dr Vukan Čupić", Department of Pulmonology, Radoja Dakića 6, 11 000 Belgrade, Serbia. E-mail: pbminic@eunet.rs

smanjenja vrednosti parametara funkcije pluća. Niže vrednosti funkcije pluća imaju neuhranjeni bolesnici i bolesnici kolonizovani bakterijom *Burkholderia cepacia* čije prisustvo dovodi do češćih egzacerbacija plućne bolesti.

Introduction

Cystic fibrosis (CF) is the most frequent autosomal recessive disease in the Caucasians. In its typical form, CF is manifested by failure to thrive, repeated lung infections and impaired mucus clearance which leads to suppurative lung disease, characterized by a decline in the lung function during a lifetime, which gradually progresses to respiratory insufficiency [1]. Recent data show that the mean life expectancy in the USA is 40 years and in Serbia is around 30 years [2]. Many factors contributed to a significant life prolongation and improvements of quality of life. Among these factors, the major influence have an early diagnosis, especially by introduction of neonatal screening programmes, hypercaloric diet and efficient treatment of pulmonary exacerbations (PE) [3].

A clinical stage of the disease can be expressed by several indicators (biomarkers) whose standardization enables an objective assessment of a patient's condition and final outcome. The most commonly used biomarker is the value of the forced expiratory volume in the first second (FEV_1), which is influenced by the frequency and course of PE [4]. Although there is no widely used standardized definition of PE, the ideal definition should cover the objective clinical, biochemical and physiological factors. In clinical research settings, the hospitalization and intravenous use of antibiotics are listed as the undeniable indicators [5]. PE is characterized by the increased cough and sputum production, haemoptysis, fever, loss of appetite and weight loss, dyspnea, tachypnea, exercise intolerance and sinus discharge [6]. It was shown that more frequent exacerbations led to a steeper lung function decline, poorer quality of life and earlier fatal outcome [4, 7].

In the patients with CF, in an ideal clinical course, the annual decrease of FEV_1 is about 2% [8]. In a majority of patients, PE is associated with a significant reduction in lung function, with gradual, but often not complete recovery thereafter. In 25% of cases, the lung function decline persists, despite the aggressive systemic antimicrobial therapy [7].

A favorable clinical response to the treatment is FEV_1 recovery to ≥ 90% of the baseline value [9]. Certain risk factors can contribute to an unfavorable outcome and partial recovery besides initially lower lung function. These risk factors are: female gender, impaired glucose tolerance, chronic colonization of respiratory tract with specific bacterial pathogens [e.g. *Pseudomonas aeruginosa* (*P. aeruginosa*) or *Burkholderia cepacea* (*B. cepacia*) and initially lower FEV_1 values [9]. Despite the novel treatment modalities, the incidence of PE did not significantly decreased in the last two decades [8].

The aim of the study was to determine if an increased number of exacerbations during the five-years period had influence on the lung function in our cohort of patients with CF. Additionally, we evaluated if the nutritional status, presence of comorbid conditions and bacterial colonization of airways affect the lung function decline and if they can be used as the predictive factors for PE.

Methods

This retrospective cohort study included 110 pediatric and adult patients. All patients were treated at the national CF center – Mother and Child Health Institute of Serbia "Dr Vukan Čupić". The demographic data, current therapy, bacterial colonization of lower airways and lung function results were obtained from the European CF registry and patient's medical history files. The patients performed the regular physical therapy on daily basis during the study. The best annual values of FEV_1 and forced vital capacity (FVC) were taken into account in calculating the five-year trend value of these indicators. The number of exacerbations per year and the total number at the end of the monitoring period were evaluated for each patient. Nutritional status was estimated according to the Z score of body mass index (BMI). The patients were stratified into three groups: (underweight – BMI < 18.5 [or ≤ 1 standard deviation (SD)] kg/m^2 normal weight – BMI 18.5-24.9 (-1–+1 SD) and overweight – BMI > 24.9 (or ≥ 1 SD) kg/m^2.

The descriptive statistics, including mean and standard deviation of numerical variables, and frequencies and percentages of categorical variables were used to characterize the study sample. Differences between groups regarding the numerical variables were analyzed by use of the Kruskal-Wallis test, while the Fisher exact test or the Pearson's χ^2 test were used for the categorical variables. The linear regression models were used to assess relationship between the number of exacerbations, or changes of FEV1, as the dependent variables and the independent variables. The R environment for statistical computing (R Core Team, 2016) was used to conduct the statistical analyses. A significance level (alpha level) was set at 0.05.

Results

Data of 83 patients out of the 110 screened ones were included in the analysis. Others (27 patients) were excluded from the analysis due to the incomplete medical records. The average age of patients at the beginning of the follow-up period was 17.1 ± 7.1 years, and the average age at the time of diagnosis was 3.7 ± 4.3 years, with similar sex distribution. A majority of study participants had normal weight, with the average Z score of BMI of -0.97 ± 1.4 SD.

During the five year of follow-up, the subjects had an average of 0.6 exacerbations annually. Other demographic data are shown in Table 1.

Table 1

Demographic data of patients with cystic fibrosis

Variable	Value
Gender, n (%)	
male	42 (51)
female	41 (49)
Age (years), mean ± SD	17.1 ± 7.1
Age at diagnosis (years), mean ± SD	3.7 ± 4.3
BMI (kg/m^2), mean ± SD	18.1 ± 3.5
Z score BMI, mean ± SD	-0.97 ± 1.4
Colonization with *Pseudomonas aeruginosa*, n (%)	53 (62)
Colonization with *Burkholderia cepacia*, n (%)	10 (12)
Diabetes, n (%)	12 (14)
Asthma, n (%)	23 (27)
Liver disease, n (%)	17 (20)

**n – number of patients; BMI – body mass index;
SD – standard deviation.**

The average decrease of FEV_1 in the five-year period was 11.9% ± 14.5%, and annual decrease was 2.4% ± 2.9%. The average decrease of FVC was 8.6% ± 1.8%, and 1.7% ± 3.6% respectively. The annual decrease of FEV_1 and FVC was not different in regard to the patient's sex ($p = 0.2$ and $p = 0.7$, respectively).

The underweight patients had the significantly lower values of FEV_1 in comparison with the normal and overweight patients ($p = 0.001$). Similar difference was shown between the groups according to the FVC values ($p < 0.001$).

Furthermore, in the underweight subjects, exacerbations were more frequent over five years of follow-up compared to other two groups ($p = 0.02$) (Table 2).

It was shown that exacerbations were more frequent in the patients chronically colonized with *B. cepacia* (0.8 average annual exacerbations) than in those colonized with *P. aeruginosa*, or other pathogens ($p = 0.023$).

The presence of diabetes mellitus ($p = 0.796$) and regular use of recombinant DNase ($p = 0.282$) and hypertonic saline ($p = 0.791$), were not proven to be the risk factors for PE.

The univariant analysis with the number of exacerbations as the dependent variable showed that the presence of *B. cepacia* in the underweight patients significantly increased the number of exacerbations ($p = 0.018$) (Table 3).

Table 3

Regression models for the underweight patients with the number of exacerbations as a dependent variable

Variable	B	p
Burkholderia cepacia	0.90	0.018*
Pseudomonas aeruginosa	0.33	0.20
Diabetes mellitus	0.11	0.33
Hepatic cirrluosis	0.01	0.96

* – statistically significant.

The univariate linear regression had shown that the number of exacerbations ($p = 0.002$) and the BMI Z-score ($p = 0.028$) were the statistically significant predictors for FEV_1 decline in five years of follow-up ($dFEV_1$). Both variables were entered into the multiple regression model with $dFEV_1$ as the dependent variable. This analysis showed that there was a statistically significant association between the number of exacerbations and $dFEV_1$. With an increase in the number of exacerbations, there was a tendency for greater deterioration of FEV_1 in the observed period ($p = 0.013$) (Table 4).

Table 2

The lung function and frequency of exacerbations in the patients with cystic fibrosis according to the nutritional status

Parameter	Underweight (n = 40)	Normal weight (n = 38)	Overweight (n = 4)	p
FEV_1 (%), mean ± SD	64.0 ± 24.4	83.7 ± 24.4	91.5 ± 17.2	0.001
FVC (%), mean ± SD	71.2 ± 20	90.3 ± 19	98.3 ± 12.5	< 0.001
Average annual number of exacerbations during the study	0.8	0.5	0.0	0.02

**FEV_1 – forced expiratory volume in the first second; FVC – forced vital capacity; SD – standard deviation;
p – value for statistical significance defined as p < 0.05.**

Table 4

Regression models with dFEV1 as a dependent variable

Variable	Univariate regression models		Multiple regression models	
	B	p	B	p
Age at diagnosis	-0.21	0.587		
Initial FEV_1% value	0.02	0.796		
Number of exacerbations	-7.22	0.002*	-6.10	0.013*
Z score BMI	2.75	0.028*	1.77	0.158
Asthma	5.26	0.145		

**FEV_1 – forced expiratory volume in the first second; $dFEV_1$–FEV_1 – decline in the five-years follow-up;
BMI – body mass index.
* – statistically significant.**

Discussion

Exacerbations of lung disease are significant factor of morbidity that affects decline in the lung function in the patients with CF. We showed that the most significant risk factor for the exacerbation occurrence in our cohort of patients was chronic airway colonization with *B. cepacia*.

The natural course of CF lung disease is characterized by a gradual deterioration with the intermittent episodes of acute endobronchial infection [10]. The lung function mostly has steeper decline in the female patients from adolescence, which was not the case in our study. Exacerbations of pulmonary disease present a major burden for the patients and their families with a negative affection on quality of life. Due to inexistence of dedicated home care providers, all patients in Serbia are hospitalized for the intravenous antibiotic therapy and intensive physical rehabilitation. The burden is even more significant for the health system, as antimicrobial therapy in hospital significantly increases medical expenses [8]. In a large observational study, which involved more than 11,000 patients, 42% of patients had exacerbation during a six-months follow-up [11]. In the etiology of exacerbation, the respiratory viruses play an important role by reactivation of chronic bacterial infection in the lower respiratory tract with common CF pathogens such as *P. aeruginosa* or *Staphylococcus aureus*, which lead to prolongation of hospital stay [6, 12].

Despite the high prevalence of chronic *Pseudomonas* colonization in our cohort (62%), it was not shown to be a significant predictor of appearance of PE. The patients with the higher exacerbation score and shorter interval between it, had a greater overall FEV_1 decline [4]. Our research in the relatively heterogeneous CF population confirmed that the increased number of exacerbations correlated with a more significant loss of lung function. The annual FEV_1 decline in our cohort was 2.4%, which was significantly higher compared to the results of other studies (1.8%–2%) [8, 11]. Although the treatment of patients with CF in our country is mostly performed without delay in a specialized center according to the international guidelines, there are several reasons which may explain this negative trend.

The most likely reasonses are malnutrition and a high prevalence of chronic *Burkholderia* colonization, which is in accordance with previous studies [13, 14]. Colonization with *B. cepacia* is associated with higher mortality and morbidity, including more frequent exacerbations, weight loss and rapid lung function decline [15, 16]. The patients colonized with *B. cepacia* in our cohort had the higher exacerbation score. Association of chronic *B. cepacia* colonization with malnutrition, leads to even more frequent exacerbations compared to the patients with normal weight.

The results of large cohort study showed that the nutritional status and pulmonary function are the dependent variables in CF, which is in concordance with our results [17]. More frequent PE and steeper decline in the lung function in the malnourished patients is directly related to the poorer prognosis and unfavorable outcome.

Conclusion

Pulmonary exacerbations lead to a progressive lung function decline in the patients with CF over time. Malnourishment and chronic airway colonization with *B. cepacia* result in more frequent PE.

The objective assessment of the symptoms and signs of PE allows vigorous antimicrobial therapy. This usually leads to a favorable clinical course with preservation of the lung function, which is an important indicator of respiratory health in the patients with CF.

R E F E R E N C E S

1. *Amadori A, Antonelli A, Balteri I, Schreiber A, Bugiani M, De Rose V.* Recurrent exacerbations affect FEV(1) decline in adult patients with cystic fibrosis. Respir Med 2009; 103(3): 407−13.
2. *Zdravković D.* Problemi u pedijariji. Beograd: Zavod za udžbenike; 2011. (Serbian)
3. *O'Sullivan BP, Freedman SD.* Cystic fibrosis. Lancet 2009; 373(9678): 1891−904.
4. *Waters V, Stanojevic S, Atenafu EG, Lu A, Yau Y, Tullis E,* et al. Effect of pulmonary exacerbations on long-term lung function decline in cystic fibrosis. Eur Respir J 2012; 40(1): 61−6.
5. *Dakin C, Henry RL, Field P, Morton J.* Defining an exacerbation of pulmonary disease in cystic fibrosis. Pediatr Pulmonol 2001; 31(6): 436−42.
6. *Waters V, Ratjen F.* Pulmonary Exacerbations in Children with Cystic Fibrosis. Ann Am Thorac Soc 2015; 12 Suppl 2: S200−6.
7. *Waters V, Stanojevic S, Klingel M, Chiang J, Sonneveld N, Kukkar R,* et al. Prolongation of antibiotic treatment for cystic fibrosis pulmonary exacerbations. J Cyst Fibros 2015; 14(6): 770−6
8. *de Boer K, Vandemheen KL, Tullis E, Doucette S, Fergusson D, Freitag A,* et al. Exacerbation frequency and clinical outcomes in adult patients with cystic fibrosis. Thorax 2011; 66(8): 680−5
9. *Waters VJ, Stanojevic S, Sonneveld N, Klingel M, Grasemann H, Yau YC,* et al. Factors associated with response to treatment of pulmonary exacerbations in cystic fibrosis patients. J Cyst Fibros 2015; 14(6): 755−62.
10. *Bhatt JM.* Treatment of pulmonary exacerbations in cystic fibrosis. Eur Respir Rev 2013; 22(129): 205−16.
11. *Rabin HR, Butler SM, Wohl ME, Geller DE, Colin AA, Schidlow DV,* et al. Epidemiologic Study of Cystic Fibrosis. Pulmonary exacerbations in cystic fibrosis. Pediatr Pulmonol 2004; 37(5): 400−6.
12. *Elborn JS, Flume PA, Cohen F, Loutit J, VanDevanter DR.* Safety and efficacy of prolonged levofloxacin inhalation solution (APT-1026) treatment for cystic fibrosis and chronic Pseudomonas aeruginosa airway infection. J Cyst Fibros 2016; 15(5): 634−40.
13. *Vasiljevic ZV, Novovic K, Kojic M, Minic P, Sovtic A, Djukic S,* et al. Burkholderia cepacia complex in Serbian patients with cystic fibrosis: prevalence and molecular epidemiology. Eur J Clin Microbiol Infect Dis 2016; 35(8): 1277−84.
14. *Folescu TW, da Costa CH, Cohen RW, da Conceição Neto OC, Albano RM, Marques EA.* Burkholderia cepacia complex: clinical course in cystic fibrosis patients. BMC Pulm Med 2015; 15: 158.
15. *Martina P, Feliziani S, Juan C, Bettiol M, Gatti B, Yantorno O,* et al. Hypermutation in Burkholderia cepacia complex is mediated by DNA mismatch repair inactivation and is highly preva-

lent in cystic fibrosis chronic respiratory infection. Int J Med Microbiol 2014; 304(8): 1182−91.

16. *Tullis DE, Burns JL, Retsch-Bogart GZ, Bresnik M, Henig NR, Lewis SA,* et al. Inhaled aztreonam for chronic Burkholderia infection in cystic fibrosis: a placebo-controlled trial. J Cyst Fibros 2014; 13(3): 296−305.

17. *Steinkamp G, Wiedemann B.* Relationship between nutritional status and lung function in cystic fibrosis: cross sectional and longitudinal analyses from the German CF quality assurance (CFQA) project. Thorax 2002; 57(7): 596−601.

Correlation of focal neuroendocrine differentiation in prostate cancer with the parameters of predictive value

Korelacija fokalne neuroendokrine diferencijacije u karcinomu prostate sa parametrima od prediktivnog značaja

Milica Mijović*, Aleksandar Ćorac[†], Sonja Smiljić[‡], Sladjana Savić[§],
Predrag Mandić[‖], Leonida Vitković[§], Snežana Leštarević[§],
Snežana Janićijević Hudomal[¶]

University of Priština/Kosovska Mitrovica, Faculty of Medicine, *Institute of Pathology,
[†]Department of Preventive Medicine, [‡]Institute of Physiology, [§]Institute of Histology,
[‖]Institute of Anatomy, [¶]Institute of Pharmacology, Kosovska Mitrovica, Serbia

Abstract

Background/Aim. Neuroendocrine (NE) cells are one of the epithelial populations in the prostate. It is well-known that the focal neuroendocrine differentiation (FNED) in prostate cancer (PC) is an aggressive subtype that most commonly evolves from preexisting PC which does not respond to hormone therapy (androgen independed PC). The incidence and clinical importance of FNED in PC is not clearly understood because of conflicting results in the studies, and evaluation of FNED is not routinely performed in clinical practice. The aim of the present study is to determine the importance of FNED presence in the examined prostate changes with special reference to the relationship of FNED degree in PC with some parameters of predictive value [Gleason score, preoperative serum total prostata specific antigen (PSA) value, tumor volume and tumor stage]. **Methods.** The study included the biopsy material from 100 untreated consecutive prostate pathological changes: 70 PC, 20 prostatic intraepithelial neoplasia (PIN) and 10 benign prostatic hyperplasia (BPH). The patients with PIN and BPH were the control groups. A block containing part of the main bulk of pathological change was chosen as representative based on hematoxylin-eosin appearance, and a section of this block was immunohistochemically stained for the tissue PSA (to mark prostatic secretory cells) and chromogranin A, serotonin and synaptophysin (to mark NE cells). **Results.** We found a very pronounced degree of FNED differentiation in 16 (22.9%) PC. Ten (62.5%) of them had Gleason score $\geq$ 7, the average serum PSA level was 32.62 ± 30.80 ng/mL, average tumor volume was 43.18 ± 31.45 mL and 6 (37.5%) of this PC were detected in D clinical stage with distant hematogenous metastases. The FNED is negatively correlated with the serum PSA level, Gleason score and clinical stage positively correlated with the tumor volume, but without statistically significant differences. **Conclusion.** The FNED has no significant role in the prognosis of PC.

Key words:
prostatic neoplasms; neuroendocrinology; prostate-specific antigen; risk factors; neoplasm staging; prognosis.

Apstrakt

Uvod/Cilj. Neuroendokrine (NE) ćelije su deo epitelne populacije prostate. Dobro je poznato da fokalna neuroendokrina diferencijacija (FNED) u karcinomu prostate (KP) predstavlja agresivni subtip, koji obično nastaje iz već postojećeg KP koji nije pokazao nikakav odgovor na hormonsku terapiju (tzv. androgen nezavisni KP). Incidenca i klinički značaj FNED u KP nisu u potpunosti razjašnjeni zbog kontradiktornih rezultata studija i zbog toga što se evaluacija FNED ne primenjuje rutinski u kliničkoj praksi. Cilj ove studije bio je utvrđivanje značaja prisusutva FNED u ispitivanim patološkim promenama u prostati sa posebnim osvrtom na odnos stepena FNED u KP sa nekim parametrima od prediktivnog značaja [Gleason score, preoperativne vrednosti ukupnog serumskog prostata specifičnog antigena (PSA), tumorski volumen i klinički stadijum]. **Metode.** Studija je sprovedena na biopsijskom materijalu 100 uzastopnih patoloških promena prostate: 70 KP, 20 prostatičnih intraepitelnih neoplazija (PIN) i 10 benignih hiperplazija prostate (BHP). Bolesnici sa PIN i BHP činili su kontrolnu grupu. Kalupi sa najreprezentativnijim uzorcima patoloških pro-

Correspondence to: Milica Mijović, University of Priština/Kosovska Mitrovica, Faculty of Medicine, Institute of Pathology,
Anri Dinana, bb, 38 220 Kosovska Mitrovica, Serbia. E-mail: milicavancetovic@yahoo.com

mena dijagnostikovanih na rutinskim hematoksilin-eozin preparatima, imunohistohemijski su obojeni na tkivni PSA (za obeležavanje prostatičnih sekretornih ćelija) i hromogranin A, serotonin i sinaptofizin (za obeležavanje NE ćelija). **Rezultati.** Veoma izražen stepen FNED nađen je kod 16 (22,9%) KP. Među njima, kod 10 (62,5%) je dijagnostikovan Gleason score ≥ 7, prosečna vrednost serumskog PSA bila je 32,62 ± 30,80 ng/mL, prosečni tumorski volumen bio je 43,18±31,45 mL, dok su 6 (37,5%) KP otkrivene u D kliničkom stadijumu sa verifikovanim udaljenim hematogenim metastazama. FNED je bio u negativnoj korelaciji sa vrednostima serumskog PSA, Gleason skorom i kliničkim stadijumom i pozitivno je korelisan sa tumorskim volumenom, ali bez statistički značajne razlike. **Zaključak.** FNED nema značajnu ulogu u prognozi karcinoma prostate.

Ključne reči:
prostata, neoplazme; neuroendokrinologija; prostata, specifični antigen; faktori rizika; neoplazme, određivanje stadijuma; prognoza.

Introduction

Prostate cancer (PC) can be manifested in different forms from small slowly growing neoplasia to tumor with an aggressive metastasizing potential [1]. PC is the second most common epithelial malignant tumor in men. It is estimated that 1.1 million men all around the world had a diagnosis of PC in 2012, with almost 70% of cases (759,000) diagnosed in more developed countries. The rates are highest in Australia/New Zealand and Northern America [age-standardised rate (ASR) 111.6 and 97.2 per 100,000] and in the Western and Northern Europe. These data are expected according to the fact that the prostate specific antigen (PSA) testing and prostate biopsy became standard in mentioned regions [2]. Also, worldwide, there is an increasing proportion of men older than 65 years in whom PC is prevalent [3]. The incidence rates are also high in less developed regions such as the Caribbean (79.8), Southern Africa (61.8) and South America (60.1), but still low in Asian men with the estimate rates of 10.5 and 4.5 South-Central and in Eastern Asia. PC is the fifth cancer death cause in men (6.6% of all men deaths). The mortality rates are very high in predominantly black men, very low in Asia and intermediate in the Americas and Oceania [2]. According to the data from the Institute of Public Health of Serbia „Dr Milan Jovanovic – Batut" for 2014, PC is the second leading cause of morbidity and third among the causes of death with 1,748 new cases each year in Ser-bia [4]. Despite its increasingly frequent occurrence, the knowledge of the PC biology is not clear enough. Still, it is very difficult to predict the clinical course and the outcome of advanced PC. It is completely necessary to improve understanding of the PC development as well as the new credibile biomarkers are needed for a therapy planning with a relevant aim to avoid overtreatment, or undertreatment [1].

The age, ethnicity, family history, level of preoperative serum PSA, free/total PSA ratio and outcome of digital rectal examination (DRE) determine the risk of clinically significant PC [5]. PSA is not highly specific, but its combination with DRE is considered as the most commonly used clinical procedures for early detection of PC. The risk assessment of localized PC is related to the plasma PSA level, Gleason score and tumor-node-metastasis (TNM) classification [6]. The prostate cancer antigen 3 (PCA-3) has higher specificity, although its sensitivity is little bit weaker. But, it has an important role in predicting the patients who will benefit from a biopsy of prostate [7]. In addition, one of the standard diagnostic procedures in PC diagnose is also a transrectal ultrasound (TRUS) biopsy of prostate with the minimum of 10–12 cores [6]. An important step in predicting the outcome of invasive prostate carcinoma was the introduction of the Gleason's grading system. It has become a widely accepted pathological method with proven prognostic significance and reproducibility [8]. There was a considerable inter-observer variability in grading prostate cancer in some researches, which imposed the need for the additional prognostic parameters such as neuroendocrine differentiation (NED) [9–11].

The factors with the important role in the development of androgen independent PC, including NED, are still not clear enough, which is the reason of insufficient knowledge of the way to intervene, prevent, or delay the malignancy [3]. Neuroendocrine (NE) cells of the prostate were originally described by Pretl [12] in 1944. They are distributed in the prostate glands of all anatomic zones and consists less than 1% of normal glandular epithelium of prostate and have characteristic lateral dendritic processes spreading. The density of NE cells in peripheral prostatic acini is the highest in the neonatal period and after puberty, and this is possibly under the androgenic hormones influence. The NE cells probably play an important role in endocrine and neuronal regulation of normal prostate. However, their apparent function is not entirely clear [13]. NED in PC can occur in three different forms: focal NED (FNED) in conventional prostate adenocarcinoma, carcinoid tumor (according to the WHO marked as well differentiated neuroendocrine tumor), and small cell neuroendocrine carcinoma (according to the WHO marked as poorly differentiated neuroendocrine tumor) [14]. The histologic features seen in the NED subtype of PC are similar to neuroendocrine tumors of any other organs and consists of differently sized nests as well as the insular or trabecular patterns of mostly round cells with the low grade cytologic features and characteristics "salt and pepper" chromatin distribution [13]. NED is found in 30%–100% of all PC [15] but it is prominent in only 5%–10% of them [16]. In general, the most common histopathological pattern is focal NED in conventional adenocarcinomas of prostate [15]. PC with NED differs from conventional PC histologically by the presence of NE cells which do not express the generic PC markers like the prostate specific antigen (PSA), prostate specific acid phosphatase (PSAP), prostate specific membrane antigen (PSMA), androgen receptor (AR), P501S and the prostate specific androgen regulated homeobox gene protein (NKX3.1), but characteristically expresses the neuroendocrine markers such

as chromogranin A, synaptophysin, CD56 and neuron-specific enolase (NSE) [17, 18]. Today, it is widely accepted that the main product chromogranin A (CgA), is a distinguished marker of NE cell differentiation and is also a general marker of population of NE cell. Other commonly found secretory products include serotonin (5-HT), NSE, bombesin, calcitonin and other members of the calcitonin gene family, such as katacalcin, calcitonin-gene-related peptide, somatostatin, parathyroid hormone-related protein (PTHrP) and thyroid-stimulating-like peptide [3]. A diagnosis of PC with focal NED is mostly made on a needle biopsy or on the metastatic lesions biopsies with the low or negative PSA levels. Probably, it is a subset of PC which is usually related to the androgen receptor resistance and worse prognosis [19]. There are the conflicting data reported in the literature regarding the prognostic significance of NED in PC. Some researchers showed a significant correlation between NED, tumor grade and poor prognosis. In several studies, an increased number of NED tumor cells in the advanced tumor stages, high grade versus low-grade tumors and, especially after the androgen suppression therapy during the tumour progression, was reported [20]. On the other hand, other authors did not find a correlation between the number of NED tumor cells, tumor grade and prognosis. The controversial data of the prognostic significance of NED markers may be explained by the non-standardized patient cohorts, different methods, and other difficulties, such as the limited volume of tissue samples and irregularly distributied NE cells [21–23]. Focal NED is considered to be strongly related as well to poor prognosis in advanced PC as to androgen-indepedent tumors [3]. Some studies considered NED in the tumor, determined either with immunohistochemistry, or with the measuring of the tumor NE cells product concentracion in the peripherial blood, as a significant prognostic parameter associated with survival after the endocrine therapy [24, 25]. This highly agrressive form of PC is increasingly observed in the patients who failed the first- and second-line hormone therapy [26]. The standard therapeutic approaches for PC are ineffective. To date, no specific treatment for PC with focal NED has been found. The antiangiogenetic drugs represent the potential alternatives but are still in a process of clinical research [27].

The aim of the present study is to determine the importance of the focal NED presence in the most important prostate pathological changes with a special reference to the relationship of focal NED degree in PC with some parameters of predictive value (Gleason score, preoperative serum total PSA value, tumor volume and tumor stage).

Methods

The study included the biopsy material from 100 untreated consecutive prostate pathological changes [70 prostate cancer, 20 prostatic intraepithelial neoplasia (PIN) and 10 benign prostatic hyperplasia (BPH)] diagnosed at the Institute of Pathology, Faculty of Medicine, University of Priština-Kosovska Mitrovica and Institute of Pathology, Clinal Center Kragujevac, Kragujevac, Serbia. Diagnosis of PC was made on the core biopsies in 20 cases, the transurethral resection

specimens in 15 cases and the fine needle aspiration biopsies in 35 cases. The diagnosis of PIN and BPH was made on the core biopsies in all cases. The patients with PIN and BPH were the control groups. The tissue samples were fixed in 10% neutral buffered formalin solution. The formalin-fixed, paraffin-embedded sections, 4–5 μm in thickness, were clasically processed and stained with hematoxylin-eosin (HE). The original histological slides were reviewed by the author. The Gleason grading of the prostate carcinomas was carried out according to the official recommendations of the Urological Section of the Swedish Society of Pathology [28]. For each case, a block containing part of the main bulk of pathological change was chosen as representative based on the HE appearance, and a section of this block was immunohistochemically (IHC) stained. The antibodies to the following antigens were used: to mark prostatic secretory cells: anti PSA - DAKO Code No A0562, ER-PR8, dilution 1 : 1000 (as a positive control we used normal prostate tissue); to mark NE cells: anti-chromogranin A - DAKO Code No M0869 DAK-A3, dilution 1 : 800 (as a positive control we used tissue of carcinoid tumor); serotonin - DAKO Code No M0758 5HT-H209, dilution 1 : 20 (as a positive control we used normal gaster tissue); synaptophysin - DAKO Code No M0776 SY38, dilution 1 : 10 (as a positive control we used normal endocrine pancreas tissue). The selected sections of tissue were stained by means of the labeled streptavidin – biotin method (DAKO Cytomation; 1 : 100) [29]. The anti PSA IHC staining of cells was recorded into 4 groups: negative (< 10% of positive cells), weakly positive (+), (10%–40% positive cells), moderate positive (++), (40%–90% positive cells) and very positive (+++), (> 90% positive cells) [30]. At least 500 cells were counted on each slide. NED IHC staining was recorded as NED negative [≤ 10 positive NE cells per 10 high power fields - HPF (×400)] and NED positivite [> 10 positive NE cells per 10 high power fields - HPF (×400)]. Based on the immunoreactivity of one, two or all three antibodies, the FNED degree was classified as: low (1 NED IHC stain positive), moderate (2 NED IHC stains positive) and very pronounced (3 NED IHC stains positive) [16]. All IHC stainings slides were interpreted by three independent researaches and the final interpretation was the mean of their own IHC results. The IHC interpretation was blinded for the clinicopathological data. The PC staging was classifed by Whitmore-Jewel and TNM system. The clinical stage combined DRE, serum PSA levels, TRUS and MRI (magnetic resonance imaging). Determination of extraprostatic extension, surgical margin status, involvement of seminal vesicle and lymph node status were made by the histological examination. The tumor volume was determined by TRUS. PSA was measured by the chemiluminescent immunoassay method in all the patients. Normal levels of the laboratory were 0–4 ng/mL.

The data primarily obtained were analyzed by the descriptive statistical methods (absolute numbers, measures of central tendency – mean value, as well as the measures of variability – standard deviation), the methods for testing statistical hypotheses (the χ^2 test for testing the difference in the frequency among the groups; the Kruskal-Wallis and Mann-

Whitney test for testing the differences in the values of the characteristics among the groups), with the nonparametric correlation analysis – rank correlation and with the ROC (receiver operating characteristic) analysis. The statistical hypotheses were tested at a significance level of 0.05.

Results

Of the total number of 100 patients, 70 had PC, 20 had PIN and 10 had BHP. The patients with PIN and BPH were the control groups. The mean age of patients in the PC group was 71.8 ± 5.48, in the PIN group it was 69.8 ± 8.01 and in the BPH group it was 72.6 ± 6.13 (Table 1).

One of primary diagnostic procedures was also the determination of preoperative values of total serum PSA (ng/mL) in the patients with PC, PIN and BPH (Tables 2 and 3). The normal serum PSA levels were ≤ 4 ng/mL. The median PSA value was: in the PC group - 35.82 ng/mL, in the PIN group - 9.15 ng/mL and in the BPH group - 8.68 ng/mL.

The distribution value of serum PSA was statistically significant in the PC group compared to the control groups

($p < 0.0001$). Almost one half of all PC patients (47.1%) had the PSA levels > 40 ng/mL without a difference among the interval subgroups ($t = 0.49$; $p = 0.314$). There was a statistical significance in the interval subgroups in the PIN and BPH patients according to the PSA levels 5–10 and 11–20 ng/mL ($t = 5.96$; $p < 0.001$).

In the PC group with very pronounced focal NED, the average preoperative values of total serum PSA was 32.62 ± 30.80 ng/mL.

Immunostaining for the PSA and NED markers such as chromogranin A, serotonin and synaptophysin was performed on the representative tissue blocks of PC, PIN and BPH (Table 4).

There was a statistical significance for $> 90\%$ of the positive cells compared to 40%–90% of the positive cells subgroup on PSA immunostain (Figure 1) in all examined groups ($t = 4.22$; $p < 0.0001$). There was a respectable incidence of 40%–90% of the positive PSA cells at the PC group in almost 23% of cases, but without a statistical significance ($\chi^2 = 3.804$; $p = 0.149$).

Table 1
Age distribution of the patients with PC, PIN and BPH

Diagnosis	Total (n)	Min	Max	Mean	SD
PC	70	58	82	71.8	5.48
PIN[c]	20	53	80	69.8	8.01
BPH[c]	10	66	86	72.6	6.13

[c] – control group; PC – prostate cancer; PIN – prostatic intraepithelial neoplasia; BPH – benign prostate hyperplasia; SD – standard deviation.

Table 2
Preoperative values of total serum PSA (ng/ml) in the patients with PC, PIN and BPH

PSA (ng/mL)	PC[†]	PIN[c]	BPH[c]
	n (%)	n (%)	n (%)
5–10	7 (10.0)	12 (60.0)	7 (70.0)
11–20	10 (14.3)	6 (30.0)	1 (10.0)
21–30	12 (17.1)	2 (10.0)	1 (10.0)
31–40	8 (11.4)	0 (0.0)	1 (10.0)
> 40	33 (47.1)	0 (0.0)	0 (0.0)
Total	70 (100.0)	20 (100.0)	10 (100.0)

PC – prostate cancer; PIN – prostatic intraepitelial neoplasia; BPH – benign prostate hyperplasia;
PSA – prostate specific antigen; [c] – control group; [†] – statistical significance compared to the control groups ($p = 0.05$).

Table 3
Characteristics of preoperative values of serum PSA (ng/mL) in PC, PIN and BPH

Diagnosis	Total (n)	Min	Max	Median	Percentile	
					10%	90%
PC	70	6.00	960.40	35.82	10.54	266.97
PIN[c]	20	3.16	27.61	9.15	5.44	20.80
BPH[c]	10	0.80	31.20	8.68	1.05	30.90

PC – prostate cancer; PIN – prostatic intraepitelial neoplasia; BPH – benign prostate hyperplasia;
PSA – prostate specific antigen; [c] – control group.

Table 4

Immunohistochemical (IHC) staining in PC, PIN and BPH

Diagnosis	PSA (%)		CgA		Ser		Syn	
	40–90%[+]	> 90%[+]	$\leq 10^{+}$/10 HPF	> 10+/10 HPF	≤ 10+/10 HPF	> 10+/10 HPF	≤ 10+/10 HPF	> 10+/10 HPF
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
PC	16 (22.9)	54[†] (77.1)	42 (60.0)	28 (40.0)	18 (25.7)	52[†] (74.3)	31 (44.3)	39 (55.7)
PIN[c]	1 (5.0)	19[†] (95.0)	11 (55.0)	9 (45.0)	7 (35.0)	13 (65.0)	13 (65.0)	7 (35.0)
BPH[c]	1 (10.0)	9[†] (90.0)	10[†] (100.0)	0 (0.0)	9[†] (90.0)	1 (10.0)	10[†] (100.0)	0 (0.0)

PC – prostate cancer; PIN – prostatic intraepitelial neoplasia; BPH – benign prostate hyperplasia; PSA – prostate specific antigen; CgA – chromogranin A; Ser – serotonin; Syn – synaptophysin; [+] – IHC positive cells; [c] – control group; [†] – statistical significance compared to the subgroup within the IHC stain ($p = 0.05$).
10HPF – 10 high power field.
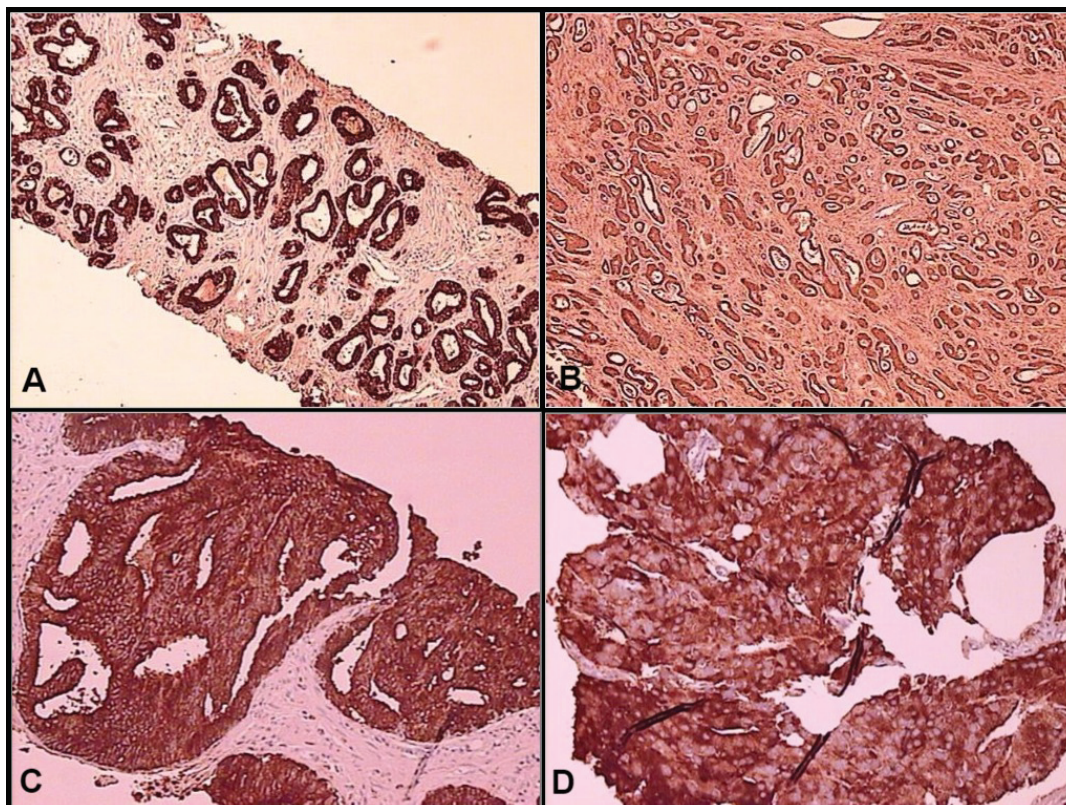
Fig. 1 – Prostate cancer (strong positive reactivity of > 90% cells, immunostain for prostate specific antigen):
A) Gleason grade 2 (×100); B) Gleason grade 3 (×50);
C) Gleason grade 4 (×200); D) Gleason grade 5 (×200).

Immunostain for chromogranin A (Figure 2) showed a statistically significant difference between PC, PIN and BHP ($\chi^2 = 6.625$; $p = 0.035$) concerning the presence of ≤ 10 positive cells per 10HPF in all the BPH cases. There was a respectable incidence of such cells at PC in 60% and PIN in 55% cases, but without a statistical significance (Mann-Whitney U = 78.00; $p = 0.690$). There was no statistical significance in the PC group based on the presence of ≤ 10 positive cells compared to > 10 positive cells per 10HPF ($t = 1.96$; $p = 0.05$), as well as in the PIN group ($t = 0.45$; $p = 0.32$).

Immunostain for serotonin (Figure 3) showed a statistically significant difference between PC, PIN and BHP ($\chi^2 = 15.964$; $p = 0.002$) concerning the presence of ≤ 10 positive cells per 10 HPF in 90% of BPH cases. There was a respectable incidence of such cells at PC in 25.7% and PIN in 35% of cases, but without a statistical significance. There was no statistical significance in the PIN group based on the presence of ≤ 10 positive cells compared to > 10 positive cells per 10 HPF ($t = 1.40$; $p = 0.08$). There was the statistically significant presence of > 10 positive cells compared to ≤ 10 positive cells per 10 HPF in the PC group ($t = 4.65$; $p < 0.001$) and BPH group ($t = 4.22$; $p < 0.0001$). In comparison with chromogranin A, there was a statistically significant representation of cases with 10 positive cells/10 HPF based on immunostain for serotonin suggesting that serotonin had better performances in this study ($t = 3.02$; $p = 0.001$).
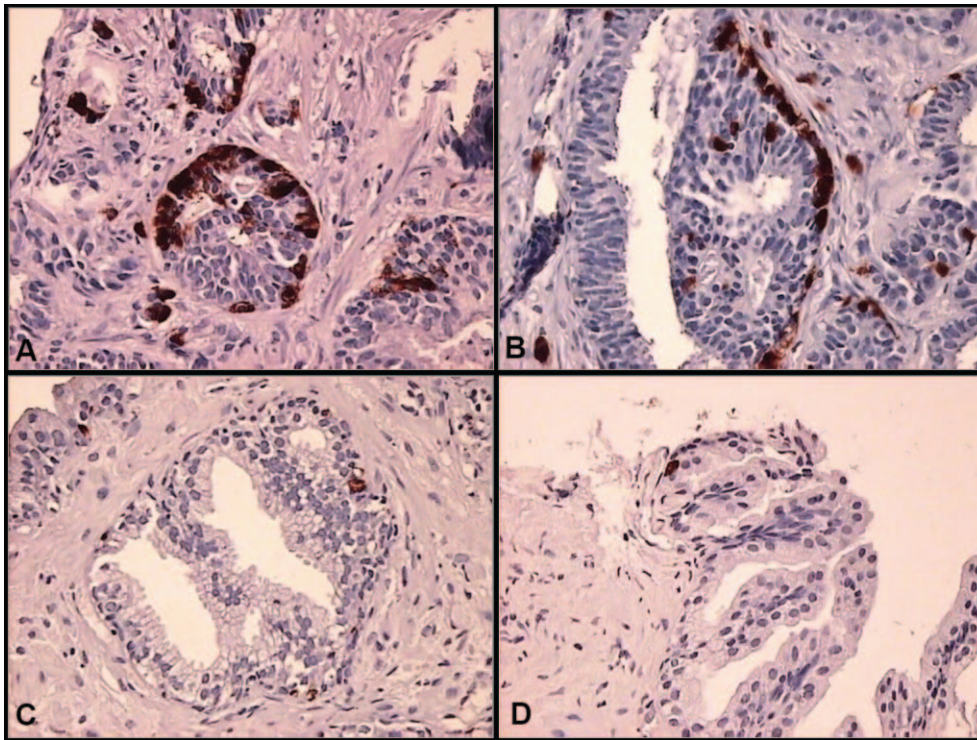
Fig. 2 – Immunostain for chromogranin A:
A) Prostate cancer, Gleason grade 4 (strong positive reactivity of > 10cells/10HPF, ×400);
B) Prostate cancer, Gleason grade 4 (≤ 10 positive cells/10HPF, ×400); C) HG prostatic intraepithelial
neoplasia (≤ 10 positive cells/10HPF, ×400); D) BPH (≤ 10 positive cells/10HPF, ×400);
BPH – benign prostatic hyperplasia; 10HPF – 10 high power fields benign prostatic hyperplasia.
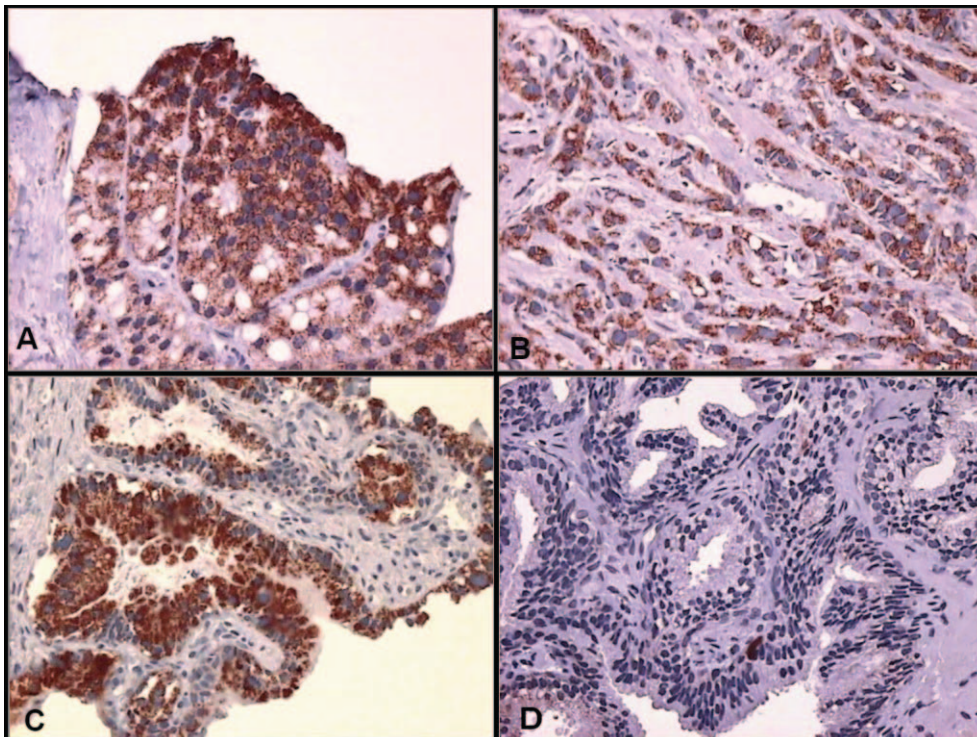
Fig. 3 – Immunostain for serotonin:
A) Prostate cancer, Gleason grade 4 (strong positive reactivity of > 10cells/10HPF, ×400);
B) Prostate cancer, Gleason grade 5 (strong positive reactivity of > 10cells/10HPF, ×400);
C) HG PIN (strong positive reactivity of >10cells/10HPF, ×400);
D) BPH (≤ 10 positive cells/10HPF, ×400).
PIN – prostatic intraepithelial neoplasia; BPH – benign prostatic hyperplasia; 10HPF – 10 high power fields.

Testing the differences in distribution, a degree of immunostain reactivity for synaptophysin (Figure 4) showed that there was a statistically significant difference between PC, PIN and BHP ($\chi^2 = 12.031$; $p = 0.002$) concerning the presence of ≤ 10 positive cells per 10 HPF in all cases of BPH. There was no statistical significance in the presence of > 10 positive cells per 10 HPF in the PC group ($t = 0.96$; $p = 0.17$) and the PIN group ($t = 1.40$; $p = 0.08$) compared to ≤ 10 positive cells per 10 HPF.

In order to improve the comparability of qualitative characteristics of each of the applied marker of neuroendocrine differentiation in the diagnosis of PC and PIN, the comparative qualitative values of these parameters were determined (Table 5).

Better diagnostic markers of focal NED were serotonin [odds ratio (OR) = 3.30] and synaptophysin (OR = 4.13) for PC, and chromogranin A (OR = 1.52) for PIN. Also, the additional ROC analysis showed that synaptophysin had the best NED diagnostic characteristics for prostate cancer considering the largest area under the ROC (AUC = 0.662; $p = 0.011$) compared to serotonin (AUC = 0.638; $p = 0.029$) and chromogranin A (AUC = 0.550; $p = 0.430$) (Figure 5 and Table 6).
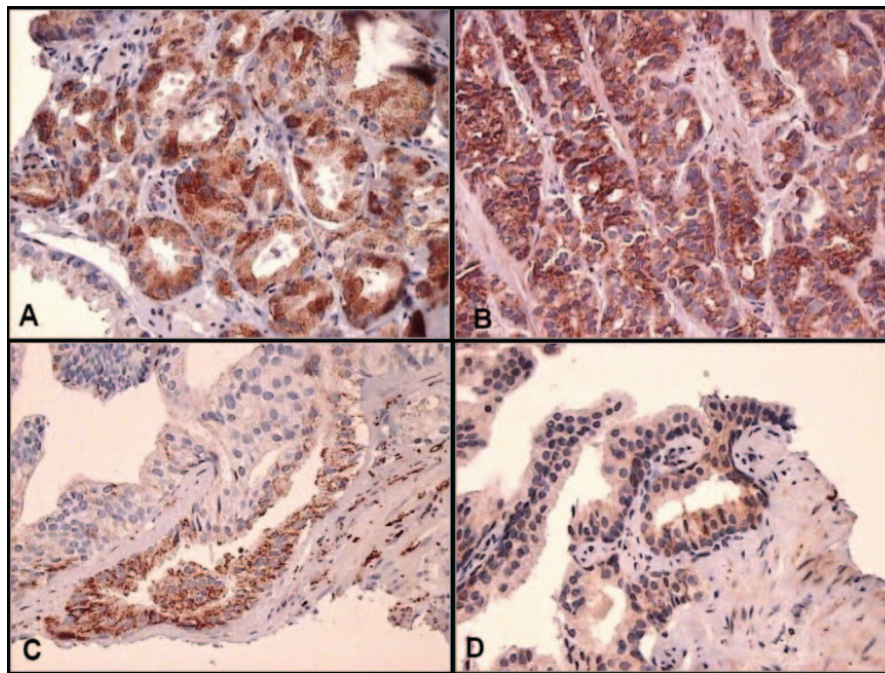
Fig. 4 – Immunostain for synaptophysin:
A) Prostate cancer, Gleason grade 3 (strong positive reactivity of >10cells/10HPF, ×400);
B) Prostate cancer, Gleason grade 4 (strong positive reactivity of >10cells/10HPF, ×400);
C) HG PIN (≤10 positive cells/10HPF, ×400); D) BPH (≤ 10 positive cells/10HPF, ×400).
For abbreviations see under Figure 3.

Table 5
Performance of markers of neuroendocrine differentiation (NED)

Marker of NED	Prostate cancer					Prostatic intraepithelial neoplasia				
	SE	SP	OR	PPV	NPV	SE	SP	OR	PPV	NPV
Chomogranin A	40.0%	70.0%	1.55	75.7%	33.3%	45.0%	65.0%	1.52	24.3%	82.5%
Serotonin	74.3%	53.3%	3.30	78.8%	53.5%	65.0%	33.8%	0.95	19.7%	79.4%
Synaptophysin	55.7%	76.7%	4.13	84.8%	42.6%	35.0%	51.2%	0.57	15.2%	75.9%

SE – sensitivity; SP – specificity; OR – odd ratio; PPV – positive predictive value; NPV – negative predictive value.

Table 6
The performance of neuroendocrine differentiation (NED) markers and focal NED degree in a prostate cancer diagnosis

Parameter	AUC	p	SE	SP
Chromogranin A	0.550	0.430	40.0%	70.0%
Serotonin	0.638	0.029[†]	74.3%	53.3%
Synaptophysin	0.662	0.011[†]	55.7%	76.7%
Focal NED degree	0.644	0.023[†]	62.9%	66.7%

SE – sensitivity; SP – specificity; AUC – area under curve; [†] – statistically significant ($p < 0.05$).

ROC
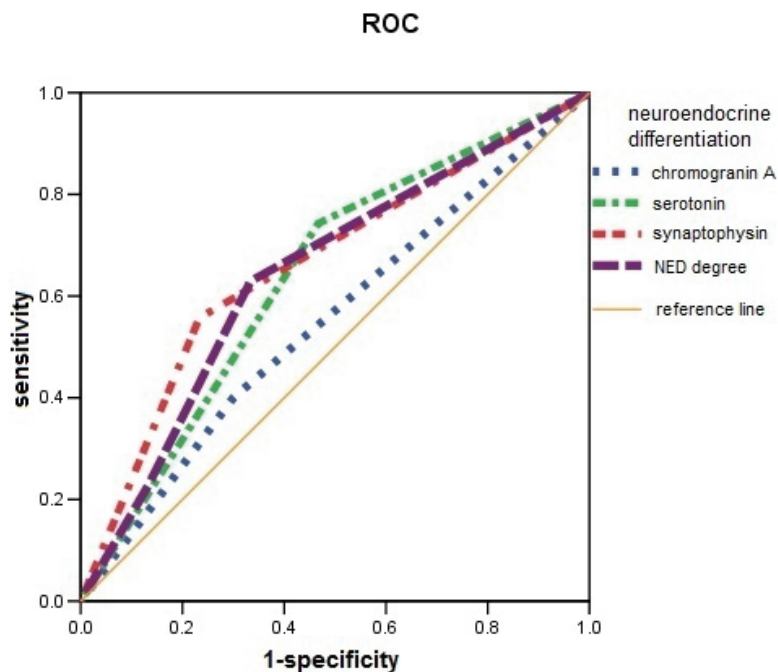
**Fig. 5 – Receiver operating characteristic (ROC) curve parameters
for neuroendocrine differentiation (NED) in prostate cancer.**

In order to better understand neuroendocrine differentiation, we determined the degree of focal NED according to the strong reactivity of at least 10 cells per 10HPF. The degree of focal NED divided into three groups was based on immunostain of only one (low), two (moderate), or all three (very pronounced) NED markers (Table 7).

There was a statistically significant difference compared BHP to PC and PIN concidering the apperance of low degree of focal NED in all cases ($p = 0.002$). There was no statistical significance between the PC and PIN group (Mann-Whitney U = 618.000; $p = 0.394$), nor in the subgroups inside PC ($t = 1.15$; $p = 0.12$) and PIN ($t = 0.78$; $p = 0.20$).

A Gleason score (GS) was based on primary and secondary on the Gleason pattern. We found GS 7 in almost one half of all 70 PC patients, more precisely, in 33 (47.1%) being statistically significant when compared to other Gleason scores ($t = 3.38$; $p = 0.03$). In the PC group with very pronounced focal NED we found the Gleason score to be ≥ 7

in 10 (62.5%) cases. GS was 3 and 4 in 3% of patients, 5 in 17% an 6 in 10% of patients.

The most common clinical stage in the PC patients was D2 – 31 (44.3%), having no statistical significance inside the groups ($t = 1.89$; $p = 0.058$). In the PC group with very pronounced focal NED, we found D2 in 6 (37.5%) cases. In the stage B_1, there was 1% of patients, in B_2 - 6%, in B_3 - 20%, in C_1 - in 6%, in C_2 - 19% and in C_3 - 4% of patients.

The average volume of all prostate cancers was 47.3 ± 30.39 mL (max = 183 mL; min = 10 mL). Most prostate cancer (51.4%) had the volume of 21–40 mL ($t = 3.81$; $p < 0.001$). In the PC group with very pronounced focal NED, the average volume was 43.18 ± 31.45 mL.

The results of correlation analysis of the examined parameters are shown in Table 8. The focal neuroendocrine differentiation is negatively correlated with the preoperative serum PSA level, the Gleason score and clinical stage and positively correlated with tumor volume, but without statistically significant differences.

Table 7

The degree of focal neuroendocrine differentiation (NED)

Degree of focal neuroendocrine differentiation	PC n (%)	PIN[c] n (%)	BPH[c] n (%)
low	26 (37.1)	10 (50.0)	10[†] (100.0)
moderate	28 (40.0)	6 (30.0)	0 (0.0)
very pronounced	16 (22.9)	4 (20.0)	0 (0.0)
Total	70 (100.0)	20 (100.0)	10 (100.0)

PC – prostate cancer; PIN – prostatic intraepitelial neoplasia; BPH – benign prostate hyperplasia; [c] – control group; [†] – statistical significance ($p = 0.05$).

Table 8
Correlation interdependence matrix of neuroendocrine differentiation (NED) and other histopathological parameters in the prostate cancer patients

Parameter		NED degree	PSA immunostain	Gleason score	PSA serum	Tumor volume	Clinical stage
NED degree	cc	1.000	0.126	-0.181	-0.197	0.083	-0.058
	p	.	0.298	0.135	0.102	0.494	0.636
PSA immunostain	cc	0.126	1.000	-0.017	0.083	-0.101	-0.132
	p	0.298	.	0.889	0.493	0.405	0.275
Gleason score	cc	-0.181	0.298	1.000	0.423**	0.195	0.317**
	p	0.135	-0.017	.	0.000	0.105	0.007
PSA serum	cc	-0.197	0.083	0.423**	1.000	0.284*	0.334
	p	0.102	0.493	0.000	.	0.017	0.002
Tumor volume	cc	0.083	-0.101	0.195	0.284	1.000	0.152
	p	0.494	0.405	0.105	0.017	.	0.210
Clinical stage	cc	-0.058	-0.132	0.317**	0.334	0.152	1.000
	p	0.636	0.275	0.007	0.002	0.210	.

PSA – prostate specific antigen.
cc – Spearman's correlation coefficient; $*p < 0.05$, $**p < 0.01$ – degree of significance.

Discussion

Neuroendocrine differentiation is found in almost all prostate cancers, but it is expressed in only 5%–10% of them [16]. It can occur either as individualy, or as a group of accumulated tumor cells [31–33]. The apparent function of NE cells is not entirely clear [13]. Although the neurosecretory granules tend to localize close to the plasma membrane of NE cells, their greatest density is within the cytoplasmic dendritic extensions, which is a characteristic of these cells. Neuroendocrine differentiation can be seen in three different forms in prostate cancer: focal NED in conventional prostate adenocarcinoma, carcinoid tumor and small cell neuroendocrine carcinoma [14]. It is now widely accepted that the main product, chromogranin A (CgA), is a distinguished marker of NE cell differentiation and is also the general marker of the population of NE cell. The most PC cells show immunoreactivity with CgA [3, 33], but some cells show synaptophysin and serotonin immunoreactivity. Usually, CgA positivity can be found in 31% and synaptophysin positivity in 8% of prostate cancer [34]. There are conflicting data reported in the literature regarding the prognostic significance of neuroendocrine differentiation in prostate cancer. Some authors believe that it has a negative effect on prognosis [35]. In several researches an increased number of NED tumor cells in the advanced tumor stages, high grade versus low-grade tumors and, especially after suppression by androgen drugs during the tumour progression was reported [20]. However, some researches do not find any link between focal NED and prognosis [21, 36]. In our study, to assess the presence and degree of focal NED in prostate cancer, so as in prostatic intraepithelial neoplasia and benign prostatic hyperplasia, the antibodies to chromogranin A, serotonin and synaptophysin were used. The majority of cases of PC showed positive immunostain of > 10 cells/10HPF on serotonin in 52 (74.3%) PC. There was no statistical significance when

chromogranin A and synaptophysin were applied. The obtained results were not in accordance with the data from the literature, which stated that the most cells are positive for chromogranin A [3, 33]. In most cases of PIN, there was a dominant finding of positive immunostain of ≤ 10 cells/10HPF on chromogranin A and synaptophysin. Only serotonin immunostain showed positivity of > 10 cells/10HPF in most cases. In all BHP cases there was a positive immunostain of ≤ 10 cells/10HPF on chromogranin A and synaptophysin, and in 9 (90.0%) of cases when serotonin immunostain was applied. There is a statistically significant difference in immunoreactivity of all the markers of neuroendocrine differentiation between prostate cancer and the control groups: for chromogranin A, for serotonin and for synaptophysin. On the basis of the mentioned above, serotonin proved to be the most sensitive marker of neuroendocrine differentiation.

In order to improve the comparability of qualitative characteristics of each applied NED marker, a special attention was paid to the comparative qualitative values of these parameters. According the sensitivity and specificity of NED markers and the odd ratio, synaptophysin had the best performance in diagnosis of prostate cancer with sensitivity of 55.7% and specificity of 76.7% (OR = 4.13) vs. serotonin (OR = 3.30) and chromogranin A (OR = 1.55). Nevertheless, chromogranin A had the best characteristics in the diagnosis of PIN with sensitivity of 45% and specificity of 65% (OR = 1.52) vs. synaptophysin (OR = 0.95) and serotonin (OR = 0.57). For the purpose of better understanding the characteristics of each of the applied neuroendocrine differentiation markers in the diagnosis of PC and PIN, the degree of focal NED was determined according to positive immunostain of > 10 cells/10HPF to only one (low focal NED), two (moderate focal NED), or all three (very pronounced focal NED) NED markers. Focal NED in PC was low in 26 (37.1%) cases, moderate in 28 (40%) cases and very pronounced in 16 (22.9%) cases, with no statistically significant

difference. Focal NED in PIN was low in 10 (50.0%) cases, moderate in 6 (30%) cases and very pronounced in 4 (20.0%) cases, with no statistically significant difference. Focal NED in BPH was low in all cases, which is in accordance with the literature data [33]. There was no statistically significant difference in the NED degree between PC and PIN.

A clinical stage was determined by the clinical and ultrasound examination in all patients with PC, and it was indexed by the alphabetical classification. The most of PC were diagnosed in the stage D – with verified distant hematogenous metastases mainly at the spine and ribs (in over 75% of patients), but without statistically significant differences compared to another clinical stages. The data are very disappointing because the majority of the patients visited the doctor at the time of the existence of distant hematogenous metastases, when there was almost no possibility of a cure. Such cases became rare in the countries with developed screening, where prostate cancer is usually discovered in the stages A or B [37, 38]. In the patients with distant metastasis, death is almost inevitable in about 15% within next 3 years, in 80% within next 5 years, in 90% within following 10 years. During the present study, we obtained the data regarding fatal outcome of 5 (16.13%) patients, among 31 patients with D stage, within the first 12 months from the time of diagnosis.

A histological grade, as a significant indicator of the survival of patients, is administered as a primary factor in almost all existing algorithms [39]. In this study, the most commonly diagnosed PC was that of the Gleason score 7 in almost one half of patients – 33 (47.14%), which is consistent with the findings of other authors [39]. The rarest diagnosed Gleason scores were 3 and 4, in the 2 (2.86%) cases, which is also in line with the data from the literature, according to which it is considered inadvisable to diagnose PC Gleason score 2–4 on prostate needle biopsies [40].

The volume of prostate cancer (mL) was determined by the ultrasound examination. Average PC volume was 47.29 ± 30.39 mL (max = 183 mL; min = 10 mL), which is consistent with findings of other authors [41]. Considering that theoretically lymph-node metastasis can be found only when the primary tumor volume is higher than 4 mL; 0.5 mL cancer would take approximately 12 years to reach 4 ml if its doubling time was 48 months [42]. Further development of the tumor is faster if the tumor at the time of diagnosis has a larger volume. The majority of patients in this study (51.4%) had PC of 21–40 mL, with a statistically significant difference compared to other interval sizes.

The correlation analysis of examined parameters showed there was a statistically significant positive coefficient of correlation between the preoperative serum PSA level on one side and the Gleason score (0.423), tumor volume (0.284) and clinical stage (0.334) on the other, but without a statistical significance. Most authors also find the good positive correlation of serum PSA level with the Gleason score, clinical stage and tumor volume [43]. Some authors consider PSAD (PSA density) [44] and PSAV (PSA velocity) [45] to be better prognostic parameters of serum PSA. The tumor volume and serum PSA were satisfactory positively correlated,

meaning that the higher PSA levels correspond to the larger tumors volumes, which is consistent with findings of the other authors [46, 47]. A clinical stage was positively correlated with several parameters. First of all, with the preoperative serum PSA levels < (0.334) and the Gleason score (0.317) with a statistical significance, which is consistent with the data from the literature [39, 43, 48]. The correlation analysis within the parameters of neuroendocrine differentiation showed that the most cells were positive for synaptophysin, with the significant level and high correlation (0.751), which gave it the importance of the most sensitive NED marker in this study. According to the results of the majority of authors, chromogranin A is the best NED marker [34, 49, 50]. Based on the demonstrated sensitivity, chromogranin A and serotonin are the NED markers whith the high and moderate correlation coefficients (0.677 and 0.545, respectively). The results of expression levels findings regarding immunoreactivity to serotonin and synaptophysin showed that they were well- connected with each other, with a statistically significant level, which pointed to the need to combine at least two, but optimally three markers of FNED. All the results of the NED markers expression are negatively correlated with the preoperative serum PSA levels (-0.197), as well as with the Gleason score, however, with statistical significance only for synaptophysin and the Gleason score (-0.280). This means that lower the Gleason score values correspond to a larger number of synaptophysin positive cells. These results are consistent with the data from the literature, according to which the focal NED is in good positive correlation with the Gleason score [16, 51]. The same authors state that focal NED does not correlate with the clinical stage, which was confirmed in this study, but without statistically significant differences (-0.058). There is a positive correlation between NED and tumor volume (0.083). Tumor volume was a significant predictor of biochemical recurrence (BCR) – free survival among the patients who underwent radical prostatectomy [52].

Conclusion

A large number of PC (77.1%) and almost all cases of PIN (95%) and BPH (90%) show a strong expression of tissue PSA, which is a confirmation of the important role of this marker in the diagnosis of PC and differential diagnosis of metastatic cancer of unknown origin. Prostate cancer and PIN show a significant positive reaction for chromogranin A, synaptophysin, serotonin as markers of focal neuroendocrine differentiation. BPH showed significantly less positive reaction for the same markers. Serotonin and synaptophysin proved to be more sensitive markers than chromogranin A in the diagnosis of FNED of PC. Very pronounced neuroendocrine differentiation was diagnosed in 16% PC and 4% PIN. There was no significant correlation between the degree of focal NED on one side, and the preoperative serum PSA, Gleason score and clinical stage, on the other hand. Based on the above, it can be concluded that the FNED has no significant role in the prognosis of the disease.

R E F E R E N C E S

1. *Tarján M*. Prognostic significance of focal neuroendocrine differentiation in prostate cancer: Cases with autopsy-verified cause of death. Indian J Urol 2010; 26(1): 41–5.

2. IARC. Section of Cancer Surveillance. 2012. Globcan; (7/11/2016). Lyon, France: International Agency for Research on Cancer; 2016. Available from: http://globocan.iarc.fr/old/FactSheets/cancers/prostate-new.asp.

3. *Vashchenko N, Abrahamsson P*. Neuroendocrine Differentiation in Prostate Cancer: Implications for New Treatment Modalities. Eur Urol 2005; 47(2): 147–55.

4. *Institut za javno zdravlje Srbije "Dr Milan Jovanović Batut"*. Incidencija i mortalitet od raka u Centralnoj Srbiji 2012. Izveštaj br. 14. Beograd: Registar za rak u Centralnoj Srbiji; 2014. Beograd, 2014. Avaliable from: www.batut.org.rs/download/publikacije/2012IncidencijaI MortalitetOdRaka.pdf (Serbian)

5. *Thompson IM, Ankerst DP, Chi C, Goodman PJ, Tangen CM, Lucia MS*, et al. Assessing Prostate Cancer Risk: Results from the Prostate Cancer Prevention Trial. J Natl Cancer Inst 2006; 98(8): 529–34.

6. *Parker C, Gillessen S, Heidenreich A, Horwich A*. ESMO Guidelines Committee.Cancer of the prostate: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2015; 26 Suppl 5: v69–77.

7. *Vlaeminck-Guillem V, Ruffion A, Andre J*. Value of urinary PCA3 test for prostate cancer diagnosis. Prog Urol 2008; 18(5): 259–65. (French)

8. *Egevad L*. Reproducibility of Gleason grading of prostate cancer can be improved by the use of reference images. Urology 2001; 57(2): 291–5.

9. *Amin M, Boccon-Gibod L, Egevad L, Epstein JI, Humphrey PA, Mikuz G*, et al. Prognostic and predictive factors and reporting of prostate carcinoma in prostate needle biopsy specimens. Scand J Urol Nephrol 2005; 39(Suppl 216): 20–33.

10. *Tarján M, Tot T*. Prediction of extracapsular extension of prostate cancer based on systematic core biopsies. Scand J Urol Nephrol 2006; 40(6): 459–64.

11. *Gancarczyk KJ, Wu H, McLeod DG, Kane C, Kusuda L, Lance R*, et al. Using the percentage of biopsy cores positive for cancer, pretreatment PSA, and highest biopsy Gleason sum to predict pathologic stage after radical prostatectomy: the center for prostate disease research nomograms. Urology 2003; 61(3): 589–95.

12. *Pretl K*. Zur Frage der Endokrinie der menschlichen vorsteherdrüse. Virch Arch Path Anat 1944; 312: 392–9.

13. *Parimi V, Goyal R, Poropatich K, Yang XJ*. Neuroendocrine differentiation of prostate cancer: A review. Am J Clin Exp Urol 2014; 2(4): 273–85.

14. *Eble JN, Sauter G, Epstein J, Sesterhenn I*. Pathology and Genetics of Tumours of the Urinary Sistem and Male Genital Organs. WHO Classification of Tumours. 3rd ed. Lyon, France: International Agency for Research on Cancer; 2002.

15. *Abrahamsson P*. Neuroendocrine differentiation in prostatic carcinoma. Prostate 1999; 39(2): 135–48.

16. *Cerović S, Brajušković G, Maletić-Vukotić V, Mičić S*. Neuroendocrine differentiation in prostate cancer. Vojnosanit Pregl 2004; 61(5): 513–8.

17. *Wang W, Epstein JI*. Small Cell Carcinoma of the Prostate: A morphologic and immunohistochemical study of 95 cases. Am J Surg Pathol 2008; 32(1): 65–71.

18. *Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY*, et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. Cancer Discov 2011; 1(6): 487–95.

19. *Parimi V, Goyal R, Poropatich K, Yang XJ*. Neuroendocrine differentiation of prostate cancer: a review. Am J Clin Exp Urol 2014; 2(4): 273–85.

20. *Abrahamsson PA, Falkmer S, Fält K, Grimelius L*. The course of neuroendocrine differentiation in prostatic carcinomas. An immunohistochemical study testing chromogranin A as an "endocrine marker". Pathol Res Pract 1989; 185(3): 373–80.

21. *Noordzij MA, van der Kwast TH, van Steenbrugge GJ, Hop WJ, Schröder FH*. The prognostic influence of neuroendocrine cells in prostate cancer: results of a long-term follow-up study with patients treated by radical prostatectomy. Int J Cancer 1995; 62(3): 252–8.

22. *Cohen MK, Arber DA, Coffield KS, Keegan GT, McClintock J, Speights VO Jr*. Neuroendocrine differentiation in prostatic adenocarcinoma and its relationship to tumor progression. Cancer 1994; 74(7): 1899–903.

23. *Abrahamsson PA, Cockett AT, di Sant'Agnese PA*. Prognostic significance of neuroendocrine differentiation in clinically localized prostatic carcinoma. Prostate Suppl 1998; 8: 37–42.

24. *Yamada Y, Nakamura K, Aoki S, Taki T, Naruse K, Matsubara H*, et al. An immunohistochemical study of chromogranin A and human epidermal growth factor-2 expression using initial prostate biopsy specimens from patients with bone metastatic prostate cancer. BJU Int 2007; 99(1): 189–95.

25. *Tamas EF, Epstein JI*. Prognostic Significance of Paneth Cell-like Neuroendocrine Differentiation in Adenocarcinoma of the Prostate. Am J Surg Pathol 2006; 30(8): 980–5.

26. *Berman-Booty LD, Knudsen KE*. Models of neuroendocrine prostate cancer. Endocr Relat Cancer 2015; 22(1): R33–49.

27. *Kretschmer A, Wittekind C, Stief CG, Gratzke C*. Neuroendocrine prostate cancer. Urologe A 2015; 54(12): 1779–83. (German)

28. *Egevad L*. Reproducibility of Gleason grading of prostate cancer can be improved by the use of reference images. Urology 2001; 57(2): 291–5.

29. Immunohistochemistry (IHC) Protocol: Staining Protocol. Available from: http://www.immunohistochemistry.us/index.php?page= ihc-staining-protocol.

30. *Leong A, Cooper K, Joel F, Leong WM*. Manual of diagnostic antibodies for immunohistology. Mol Pathol 2000; 53(1): 53.

31. *Amorino GP, Parsons SJ*. Neuroendocrine Cells in Prostate Cancer. Crit Rev Eukaryot Gene Expr 2004; 14(4): 287–300.

32. *Bonkhoff H*. Neuroendocrine differentiation in human prostate cancer. Morphogenesis, proliferation and androgen receptor status. Ann Oncol 2001; 12 Suppl 2: S141–4.

33. *di Sant'Agnese PA*. Neuroendocrine differentiation in prostatic carcinoma: an update on recent developments. Ann Oncol 2001; 12 Suppl 2: S135–40.

34. *Ather MH, Abbas F, Faruqui N, Israr M, Pervez S*. Correlation of three immunohistochemically detected markers of neuroendocrine differentiation with clinical predictors of disease progression in prostate cancer. BMC Urol 2008; 8: 21.

35. *Bollito E, Berruti A, Bellina M, Mosca A, Leonardo E, Tarabuzzi R*, et al. Relationship between neuroendocrine features and prognostic parameters in human prostate adenocarcinoma. Ann Oncol 2001; 12 Suppl 2: S159–64.

36. *Casella R, Bubendorf L, Sauter G, Moch H, Mihatsch MJ, Gasser TC*. Focal neuroendocrine differentiation lacks prognostic significance in prostate core needle biopsies. J Urol 1998; 160(2): 406–10.

37. *Bono AV*. The global state of prostate cancer: epidemiology and screening in the second millennium. BJU Int 2004; 94 Suppl 3: 1–2.

38. *Kim EH, Andriole GL*. Prostate-specific antigen-based screening: controversy and guidelines. BMC Med 2015; 13: 61.

39. *Humphrey PA*. Gleason grading and prognostic factors in carcinoma of the prostate. Mod Pathol 2004; 17(3): 292–306.

40. *Epstein JI*. Gleason score 2-4 adenocarcinoma of the prostate on needle biopsy: a diagnosis that should not be made. Am J Surg Pathol 2000; 24(4): 477–8.

41. *Dong F, Jones JS, Stephenson AJ, Magi-Galluzzi C, Reuther AM, Klein EA*. Prostate cancer volume at biopsy predicts clinically significant upgrading. J Urol 2008; 179(3): 896–900; discussion 900.

42. *Polascik TJ*. Imaging and focal therapy of early prostate cancer. In: *Klein EA*, editor. Current clinical urology. Philadelphia: Springer Science and Business Media LLC; 2012. p. 76.

43. *Haukaas SA, Halvorsen OJ, Daehlin L, Hostmark J, Akslen LA*. Is preoperative serum prostate-specific antigen level significantly related to clinical recurrence after radical retropubic prostatectomy for localized prostate cancer. BJU Int 2006; 97(1): 51–5.

44. *Bozkurt O, Çömez K, Gürboğa Ö, Demir Ö, Aslan G, Esen A*. Role of Prostate Specific antigen density for the prediction of radical therapy requirement in localized prostate cancer. Üroonkol Bül 2016; 15(3): 103–6. (Turkish)

45. *Pinsky PF, Andriole G, Crawford ED, Chia D, Kramer BS, Grubb R*, et al. Prostate-specific antigen velocity and prostate cancer gleason grade and stage. Cancer 2007; 109(8): 1689–95.

46. *Lepor H, Wang B, Shapiro E*. Relationship between prostatc epithelial volume and serum prostate-specific antigen levels. Urology 1994; 44(2): 199–205.

47. *Stamey TA, Johnstone IM, McNeal JE, Lu AY, Yemoto CM*. Preoperative serum prostate specific antigen levels between 2 and 22 ng/ml correlate poorly with post-radical prostatectomy cancer morphology: prostate specific antigen cure rates appear constant between 2 and 9 ng/ml. J Urol 2002; 167(1): 103–11.

48. *Gonzalez CM, Roehl KA, Antenor JV, Blunt LW, Han M, Catalona WJ*. Preoperative PSA level significantly associated with interval to biochemical progression after radical retropubic prostatectomy. Urology 2004; 64(4): 723–8.

49. *Ather MH, Abbas F, Faruqui N, Israr M, Pervez S*. Expression of pS2 in prostate cancer correlates with grade and Chromogranin A expression but not with stage. BMC Urol 2004; 4(1): 14.

50. *Deftos LJ, Nakada S, Burton DW, di Sant'Agnese PA, Cockett AT, Abrahamsson PA*. Immunoassay and immunohistology studies of chromogranin A as a neuroendocrine marker in patients with carcinoma of the prostate. Urology 1996; 48(1): 58–62.

51. *Ishida E, Nakamura M, Shimada K, Tasaki M, Konishi N*. Immunohistochemical analysis of neuroendocrine differentiation in prostate cancer. Pathobiology 2009; 76(1): 30–8.

52. *Kim TJ, Lee IJ, Song BD, Lee SC, Hong SK, Byun SS*, et al. Comparison of localized high volume tumor and locally advanced low volume tumor after radical prostatectomy according to risk classification. Korean J Urol Oncol 2016; 14(3): 165–71.

Periconceptional folic acid supplementation: knowledge, attitudes and counselling practice of Serbian pharmacists and pharmacy technicians

Perikoncepcijska suplementacija folnom kiselinom: znanja, stavovi i savetodavna praksa farmaceuta i farmaceutskih tehničara u Srbiji

Milica Zeković*, Dušanka Krajnović[†], Marina Nikolić*, Tatjana Stojković[†],
Mirjana Gurinović*, Marija Glibetić*

University of Belgrade, Institute for Medical Research, *Centre of Research
Excellence in Nutrition and Metabolism, Belgrade, Serbia; University of Belgrade,
Faculty of Pharmacy, [†]Department of Social Pharmacy and Pharmaceutical Legislation,
Belgrade, Serbia

Abstract

Background/Aim. The protective effect of periconceptional folic acid supplementation in reducing the risk of neural tube defects (NTDs) and other adverse pregnancy outcomes has been scientifically confirmed. The present study aimed to assess knowledge, attitudes, and practice (KAP) of community pharmacists and pharmacy technicians with regards to counselling women of reproductive age on adequate folate intake. **Methods.** The cross-sectional study was conducted among the nationwide stratified sample of pharmacists and pharmacy technicians, practicing in both private and state-owned community pharmacies in Serbia. The detailed literature review, expert consultation and pre-testing were employed in the questionnaire development. The descriptive and inferential statistical analyses were performed using the SPSS software ver. 22. **Results.** Among 730 survey participants, 96.6% correctly identified types of deformities that could be prevented by folic acid and 77.0% recognized recommended dosage for women capable of becoming pregnant. However, fewer were able to recognize the optimal timing for folic acid supplementation (61.1%) and the proper dose for the NTD recurrence prevention (42.9 %). While 43.2% of pharmacy technicians

thought that it was not their responsibility to provide a counselling concerning the measures for the prevention of congenital anomalies, only 4.7% of licensed pharmacists and none of the interns were of that opinion (χ^2 = 198.287; df = 4; p < 0.001). The majority of respondents (54.7%) reported provision of informative consultation concerning folic acid once a week, or few times a month, while only 10 stated to do so on a daily basis. In addition, based on self-report, only 36.7% of participants communicates information and advice regarding adequate folate status at their own initiative, while the rest addresses this topic only on the patients request, i.e., reactively. **Conclusion.** Although participants acknowledged the importance of their role in preconception health promotion, our findings revealed certain knowledge gaps as well as dominantly reactive counselling practice. Tailored educational interventions and professional support are needed to improve the engagement of community pharmacy personnel in this area of public health.

Key words:
community pharmacy services; pharmacists; pharmacy technicians; health knowledge, attitudes, practice; folic acid; pregnancy; serbia.

Apstrakt

Uvod/Cilj. Naučno je potvrđen zaštitni efekat primene suplemenata folne kiseline u perikoncepcijskom periodu u redukciji rizika od nastanka defekata nervne cevi (DNC) i drugih neželjenih ishoda trudnoće. Cilj ove studije bio je ispitivanje znanja, stavova i prakse farmaceuta i farmaceutskih tehničara zaposlenih u javnim apotekama u vezi sa savetovanjem žena reproduktivne dobi o adekvatnom unosu folata. **Metode.** Sprovedenom studijom preseka obuhvaćen je

nacionalni stratifikovani uzorak farmaceuta i farmaceutskih tehničara zaposlenih u državnim i privatnim javnim apotekama u Srbiji. Detaljan pregled literature, ekspertske konsultacije i pre-testiranje primenjeni su u procesu razvoja upitnika. Deskriptivna i inferencijalna statistička analiza realizovane su primenom SPSS softvera ver. 22. **Rezultati.** Od 730 ispitanika, 96,6% tačno je identifikovalo vrste deformiteta koji se mogu prevenirati primenom folne kiseline, a 77,0% prepoznalo je preporučenu dozu za žene sposobne da ostanu u drugom stanju. Ipak, manji udeo ispitanika prepoznao

Correspondence to: Milica Zeković, University of Belgrade, Institute for Medical Research, Centre of Research Excellence in Nutrition and Metabolism, Tadeuša Košćuška 1, 11 158 Belgrade, Serbia. E-mail: zekovicmilica@gmail.com

je optimalni vremenski okvir za suplementaciju (61,1%), kao i adekvatnu dozu za prevenciju rekurence DNC (42,9%). Dok je 43,2% farmaceutskih tehničara izrazilo uverenje da nije njihova obaveza da sprovode savetovanje o merama za prevenciju kongenitalnih anomalija, takvo mišljenje imalo je samo 4,7% licenciranih farmaceuta i nijedan farmaceut-stažer (χ^2 = 198.287; df = 4; p < 0,001). Većina učesnika studije (54,7%) izjavila je da pruža informativne konsultacije u vezi folne kiseline jednom nedeljno ili nekoliko puta mesečno, a samo 10 ispitanika tvrdilo je da to čini svako-dnevno. Dodatno, na osnovu sopstvenog izveštaja, samo 36,7% ispitanika pruža savete i informacije o adekvatnom statusu folata na sopstvenu inicijativu, dok ostatak ovu temu pominje samo na zahtev pacijenta tj. reaktivno. **Zaključak.** Iako su ispitanici prepoznali značaj sopstvene uloge u pro-mociji zdravlja u prekoncepcijskom periodu, naši rezultati ukazali su na određene propuste u znanju i dominantno re-aktivan pristup savetovanju. Prilagođene edukativne inter-vencije i profesionalna podrška neophodni su za unapre-đenje javnozdravstvenog angažovanja farmaceutskog tima iz javnih apoteka u ovoj oblasti.

Ključne reči:
farmaceutske službe, javne; farmaceuti; farmaceutski pomoćnici; zdravlje, znanje, stavovi, praksa; folna kiselina; trudnoća; srbija.

Introduction

Nutritive deficiencies are well-recognized preventable risk factors for the development of congenital anomalies and other adverse pregnancy outcomes. A protective effect of the pericon-ceptional folic acid supplementation in reducing the risk for both occurrence and recurrence of neural tube defects (NTDs) is sci-entifically confirmed through many observational and interven-tional studies [1]. Folate derivatives are involved as coenzymes in the single-carbon transfer of units in the metabolism of amino acids and synthesis and reparation of nucleic acids. Accordingly, the role of this water-soluble B_9 vitamin is of a special impor-tance for the intense cell maturation, division and proliferation during embryogenesis [2].

Teratogenic effect of folate deficiency is particularly in-tensive during the process of neurulation occurring in early gestation phases, namely, in the first 28 days after concep-tion [3]. Consequently, the timing for folic acid supplementa-tion is critical. The fact that over 40% of pregnancies world-wide are unplanned indicates that this process, essential for proper development of the foetal nervous system, occurs be-fore most women even become aware of the pregnancy [4]. That is the reason why great efforts have been made globally to create efficient public health strategies and interventions focused on folate intake and status optimization in all women of reproductive age [5–7].

Due to the problematic stability and limited bioavail-ability of folate native forms from natural sources, it is rec-ommended that all women capable of becoming pregnant should take 400 µg of the synthetic form of the vitamin, i.e., folic acid daily via oral supplements and/or fortified foods as an addition to varied and nutritionally balanced diet. Espe-cially high risk level is recognized in women with the NTD-affected pregnancies in their reproductive history, women us-ing anticonvulsive therapy and those with diagnosed diabe-tes. Thus, they are recommended to take substantially higher (medicinal) doses of folic acid, i.e. 4–5 mg and to be under medical supervision [8–10].

However, regardless the unequivocal epidemiological evidence, achieved scientific consensus on the folic acid benefits in ensuring healthy offspring, and the fact that many health authorities officially recommended the use of folic ac-id prior to conception and during early phases of pregnancy, numerous studies revealed suboptimal intake of this essential nutrient among the women of reproductive age [5, 11, 12].

The variability of the situation in different countries is affected by a number of factors such as the awareness of women of preven-tion measures for NTDs, the existence, application and coverage of strategies for improving folate intake and status in the targeted population group, the availability and affordability of folic acid sup-plements, and finally, the population exposure to folic acid – forti-fied foods [13–15]. In the absence of fortification, educational cam-paigns and programs directed towards the promotion of adequate periconceptional care are crucial, while the responsibility and role of healthcare professionals are especially emphasized.

Serbia, like the majority of European countries, has re-frained from establishing the mandatory folic acid fortifica-tion policy, and the availability of voluntarily fortified prod-ucts is quite modest. Recently conducted study among the Serbian women of childbearing potential pointed to the alar-mingly low level of folate intake. Based on an analysis of di-etary questionnaires (24h dietary recalls and food frequency questionnaire – FFQ), the folate intake was below recom-mended levels in more than 90% of studied women. Fur-thermore, folate concentration in erythrocytes associated with the optimal protection against neural tube defects (i.e., 906 nmol/L) was not recorded in any of the examinees. Pre-vious findings that less than 5% of women in reproductive age takes the folic acid supplements on regular basis was ad-ditionally confirmed [16, 17]. In such a context, the necessity of public health initiatives and efficient interventions directed to the optimization of nutritive status of women prior to their pregnancy and during it is clearly evident.

Healthcare professionals, being reliable members of the community, may significantly contribute to the improvement of maternal and newborns' health by the delivery of timely, accurate and evidence-based information and advice. Phar-macists and pharmacy technicians are ideally positioned within the health care system to promote the periconcep-tional folic acid supplementation and to participate in the health education of women of reproductive age. Unique ac-cessibility of community pharmacies provides a strategically significant platform for achieving the greater coverage of targeted population group. Dissemination of health-promotional messages regarding folic acid and stimulation of women to apply preventive measures is supported by the International

Pharmaceutical Federation (FIP). This organization, through policies and recommendations complying with the World Health Organization (WHO) Maternal, Newborn and Child Health Interventions, emphasizes the role and responsibility that pharmacists bear in the context of reaching child mortality and maternal health targets set forth by the United Nations Millennium Development Goals four and five [18, 19]. In order to efficiently address public health needs, it is essential for the community of pharmacy professionals to have positive perception of the importance of preconception care-related activities as well as the adequate knowledge, skills and motivation to integrate them into everyday work practice.

The aim of the present study was to assess the knowledge, attitudes, and practice (KAP) of Serbian community pharmacists and pharmacy technicians with regard to counselling the women of reproductive age on adequate folate intake.

Methods

Study design, setting and study participants

A questionnaire-based cross-sectional study was conducted from October 2015 to December 2016 among the nationwide stratified sample of licensed pharmacists and pharmacy technicians, working both in private and state-owned community pharmacies in Serbia.

To ensure that a number of survey participants is adequate to represent 5,803 registered pharmacists in Serbia, a sample size calculation procedure proposed by Krejcie and Daryle [20] was used. The required sample size as per formula (n = 385) was adjusted to the anticipated drop-out rate (d = 20%) using the equation $N = n/(1–d/100)$ resulting in a total of N = 481 subjects [21].

The Pharmaceutical Chamber of Serbia membership directory was used as a sampling frame and the geographical quotas were established in order to obtain the sample proportional to the actual distribution of community pharmacists on a national scale. Accordingly, the sample was divided into four strata corresponding to the regional Chamber branches: Belgrade, the capital city (37.92%), Vojvodina, the northern autonomous province (21.83%), Kragujevac, representing Central and Western Serbia (23.19%) and Niš with Kosovo and Metohija, representing the Southern and Eastern Serbia (17.04%). Participation in the study was offered to all pharmacy technicians and interns working in the same pharmacies as recruited pharmacists.

Data collection

After obtaining the consent of the director, or owner of the pharmacy, the self-administered questionnaires in a hard-copy format were hand-delivered to designated respondents in predefined time, so as not to disturb the regular daily operations. Participation in the study was voluntary, and the questionnaires were submitted anonymously. The survey packs included a cover letter, study information sheet, ethical statement and the copy of the survey instrument. During the questionnaires were filled-in, a researcher remained available in case of any further questions, or concerns. The informed consent was assumed by completion of the questionnaire and no incentives were offered to the respondents.

Questionnaire

The survey instrument, developed for the purpose of this study, was based on the detailed literature review and expert consultation. An expert group discussed the initial set of questions and selected the final assemblage in order to ensure that all relevant domains were covered. An eleven-member panel including five experienced pharmacists, four experts in the field of nutrition, psychologist and a statistician evaluated the face and content validity parameters of the questionnaire, i.e., item relevancy, comprehensibility, clarity and sequence; instrument organization, terminology and completeness [22]. For further refinement, the draft version was pre-tested among 10 pharmacists and 7 pharmacy technicians practicing in community pharmacies. Necessary amendments, dominantly wording and layout were made in accordance with their feed-back to ensure acceptability and proper understanding of the questions and instructions. A pilot study was then conducted with the convenience sample (n = 56) of pharmacists and pharmacy technicians to assess the study feasibility and instrument reliability. The Cronbach's alpha values yielded for both knowledge and attitudes sections of the questionnaire were satisfactory, i.e., above the apriory set threshold of 0.7 ($\alpha_{knowledge} = 0.823$; $\alpha_{attitudes} = 0.789$) indicating a good internal consistency.

The final version of the questionnaire consisted of 4 sections. Section 1 gathered the respondents' sociodemographic and professional data such as gender, age, highest professional credential, work experience, marital status and details regarding the practicing setting (pharmacy type, ownership and location). Knowledge was assessed by 13 multiple-choice questions with a single correct answer. Attitudes, explored by 14 statements, were captured using 5-point rating of the Likert scale (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree and 5 = strongly agree). The practice section of the questionnaire, containing 15 items with a combination of multiple-choice and the Likert-scale type response format covered the following topics: sources of information on folate, the current practice in advising women about an adequate intake of folate and the barriers to regular provision of information and advice about folate intake in community pharmacies.

Data processing and statistical analyses

The descriptive and inferential statistical analyses were performed using the Statistical Package for Social Science (SPSS) software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The numerical data were reported as frequencies for the categorical variables and mean ± standard deviation (SD) for the continuous variables. Every correct answer in the knowledge test was credited with one point. The total knowledge score, representing the summarised value for all the listed items, had a theoretical range from 0 to 13 points. The group differences for continuous variables were analysed using the Student's *t*-test and ANOVA

(with the Tukey's post hoc test), while the χ^2 test was employed for the categorical variables. A level of significance for all statistical tests was previously set at $p < 0.05$.

Ethical approval

The study was conducted in accordance with the principles of the Declaration of Helsinki and standards for health-related research involving human participants proposed by the World Health Organization [23] with the institutional review and ethical approval granted by the Ethics Committee for Clinical Trials, Faculty of Pharmacy – University of Belgrade (Dossier no. 261/2).

Results

Among 481 licensed pharmacists, 87 intern pharmacists and 256 pharmacy technicians who were approached during the recruitment stage 461, 77 and 218 respectively, voluntarily agreed to participate in the study, yielding 91.7% of consent rate. All the returned questionnaires were reviewed for completeness and those missing the data on the main outcome variables (n = 28) were excluded from further analyses resulting in a total number of 730 valid questionnaires (response rate = 88.6%; completeness rate = 92.9%). The final analytical cohort of pharmacists did not differ significantly with respect to the original Pharmaceutical Chamber directory with a reference to geographical distribution of participants ($\chi^2 = 4.810$; df = 3; $p = 0.186$).

Among the respondents, regardless the geographical region, there were more females than males ($p < 0.001$). The mean age of participants was 37.3 ± 10.3 years (range: 21.0–66.0 years) and, on average, they had 12.4 ± 9.9 years (range: 0.5–40.0 years) of working experience in community pharmacies. Within-stratum and overall summaries of the participants' sociodemographic and professional profile is presented in Table 1.

The mean score on the knowledge test for the entire study cohort was 7.27 ± 1.95 points with the empirical range of 1–12 points. The majority of participants (24.4%) had 7 correct answers and none achieved a maximum of theoretical score of 13 points. The analyses revealed that women were more knowledgeable than men (7.49 ± 1.91 vs. 6.31 ± 1.86; $t = 43.614$; $p < 0.0001$) just as were the respondents employed in the privately owned community pharmacies than those practising in the state-owned pharmacies (7.65 ± 2.01 vs. 6.84 ± 1.80; $t = 32.817$; $p < 0.001$). The participants who were parents scored higher on the knowledge test than those without children (7.66 ± 1.75 vs. 6.82 ± 2.07; $t = 35.679$; $p < 0.001$), while the respondents older than 50 years of age demonstrated the lower knowledge level (6.14 ± 1.79) than their younger colleagues: $M_{21–30 years} = 7.36 ± 2.16$; $M_{31–40 years} = 7.72 ± 1.97$; $M_{41–50 years} = 6.99 ± 1.29$ [$F(3.726) = 18.504$; $p < 0.001$]. The analyses of variance showed that the effect of professional status on knowledge was significant [$F(2.727) = 50.757$; $p < 0.001$]: the pharmacy technicians had lower average score (6.14 ± 1.97) than the intern pharmacists (8.00 ± 1.67) and the licensed pharmacists (7.60 ± 1.82). Corre-

spondingly, the lower level of educational attainment was associated with the lower knowledge scores [$F(2.727) = 59.391$; $p < 0.001$]. The participants with secondary education had notably the lower scores (6.18 ± 1.96) than the individuals with university (7.42 ± 1.76) and post-graduate education (8.26 ± 1.80). Furthermore, the marital status had a significant impact on knowledge regarding the folic acid supplementation [$F(3.715) = 26.280$; $p < 0.001$]; the married and the respondents living common law scored higher (7.61 ± 1.66 and 8.11 ± 2.67 respectively) than the single and divorced/separated participants (6.68 ± 2.03 and 6.09 ± 1.29 respectively). Distribution of correct response rates regarding folic acid supplementation across the professional groups of pharmacy staff is presented in Table 2.

The majority (n = 545; 74.6% of the total sample) of study participants had generally positive attitude towards health benefits of folic acid supplementation. Fewer pharmacy technicians (58.7%) than the licensed pharmacists (79.3%) and intern pharmacists (82.4%) acknowledged congenital anomalies as a major public health problem in view of their mortality and morbidity rates ($\chi^2 = 46.460$; df = 4; $p < 0.001$). All three groups of pharmacy staff expressed concern over the low level of informedness about the role of folic acid and proper use among the women of reproductive age in Serbia. Accordingly, a vast majority of respondents (95.7% females and 98.6% males) agreed that it was important to increase women's awareness regarding the folic acid value in preventing the foetal neural tube defects. While 43.2% of pharmacy technicians thought that it was not their responsibility to provide counselling concerning the measures for the prevention of congenital anomalies only 4.7% of licensed pharmacists and none of the interns were of that opinion ($\chi^2 = 198.287$; df = 4; $p < 0.001$). Similarly, the substantially higher percentage of pharmacy technicians (37.4%) than the licensed and intern pharmacists (13.1% and 0%, respectively) considered the patients in the community pharmacies uninterested in being educated about the dietary supplements that are used in preconception and prenatal care ($\chi^2 = 81.342$; df = 4, $p < 0.001$). More males than females (64.0% vs. 30.8%) reported discomfort with bringing up the issues of family planning and the prevention of neural tube defects in the community pharmacy setting ($\chi^2 = 60.552$; df = 2; $p < 0.001$). Furthermore, the male participants were more prone to believe that the use of folic acid-containing dietary supplements contributed to pregnancy medicalisation (53.3% vs. 37.1%; $\chi^2 = 40.167$; df = 2; $p < 0.001$).

The frequency distribution of respondents' attitudes across the professional groups of pharmacy staff is presented in Figure 1.

The majority of respondents (n = 399; 54.7% of the total sample) reported the provision of informative consultation concerning pre- and post-conception use of folic acid once a week, or few times a month, while only 10 (all licensed pharmacists) stated to do so on a daily basis. By contrast, 69.9% confirmed dispensing contraceptive preparations, vitamin and mineral dietary supplements, ovulation tests/fertility monitors and home pregnancy tests to women of reproductive age once daily or multiple times a day (Table 3).

Table 1

Sociodemographic characteristics and the professional profile of study participants

Parameters	Geographical region				Total sample (n = 730)
	Vojvodina (n = 166)	Belgrade (n = 240)	Central and Western Serbia (n = 174)	Southern and Eastern Serbia (n = 150)	
	n (%)	n (%)	n (%)	n (%)	n (%)
Gender					
male	30 (18.1)	38 (15.8)	44 (25.3)	27 (18)	139 (19)
female	136 (81.9)	202 (84.2)	130 (74.7)	123 (82)	591 (81)
Age (years)					
21–30	71 (42.8)	85 (35.4)	41 (23.6)	49 (32.7)	246 (33.7)
31–40	51 (30.7)	72 (30)	73 (42)	44 (29.3)	240 (32.9)
41–50	34 (20.5)	45 (18.8)	34 (19.5)	20 (13.3)	133 (18.2)
51+	10 (6)	38 (15.8)	26 (14.9)	37 (24.7)	111 (15.2)
Professional status					
licensed pharmacist	94 (56.7)	160 (66.7)	102 (58.6)	94 (62.7)	450 (61.6)
intern pharmacist	16 (9.6)	28 (11.7)	16 (9.2)	14 (9.3)	74 (10.1)
pharmacy technician	56 (33.7)	52 (21.7)	56 (32.2)	42 (28)	206 (28.2)
Marital status					
single	51 (30.7)	80 (33.3)	32 (18.4)	43 (28.7)	206 (28.2)
married	78 (47)	103 (42.9)	99 (56.9)	77 (51.3)	357 (48.9)
widowed	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
separated/divorced	13 (7.8)	24 (10)	26 (14.9)	16 (10.7)	79 (10.8)
living common law	24 (14.5)	33 (13.8)	17 (9.8)	14 (9.3)	88 (12.1)
Work experience					
less than 3 years	57 (34.3)	73 (30.4)	34 (19.5)	38 (25.3)	202 (27.7)
3–10 years	28 (16.9)	56 (23.3)	45 (25.9)	32 (21.3)	161 (22.1)
11–15 years	27 (16.3)	26 (10.8)	39 (22.4)	26 (17.3)	118 (16.2)
more than 15 years	54 (32.5)	85 (35.4)	56 (32.2)	54 (36)	249 (34.1)
Pharmacy ownership					
state-owned	62 (37.3)	110 (45.8)	99 (56.9)	78 (52)	349 (47.8)
privately-owned	104 (62.7)	130 (54.2)	75 (43.1)	72 (48)	381 (52.2)

Table 2

Distribution of correct response rates regarding folic acid supplementation across the professional groups of pharmacy staff

Knowledge domain	Licensed pharmacists (n = 450)	Intern pharmacists (n = 74)	Pharmacy technicians (n = 206)	Total sample (n = 730)
	n (%)	n (%)	n (%)	n (%)
Folic acid origin: natural vs. synthetic vitamin form	113 (25.1)	33 (44.6)	31 (15)	177 (24.2)
Folate natural food sources	285 (63.3)	41 (55.4)	86 (41.7)	412 (56.4)
Importance of folic acid supplementation as an addition to balanced diet in an optimal NTD risk reduction	327 (72.7)	60 (81.1)	130 (63.1)	517 (70.8)
Folic acid food fortification policy status in Serbia	95 (21.1)	28 (37.8)	79 (38.3)	202 (27.7)
Types of deformities prevented by folic acid	438 (97.3)	68 (91.9)	199 (96.6)	705 (96.6)
Folate recommended dietary allowances for adults (WHO)	351 (78)	50 (67.6)	88 (42.7)	489 (67)
Folate tolerable upper intake level (WHO)	178 (39.6)	35 (47.3)	37 (18)	250 (34.2)
Which women should take folic acid dietary supplements	367 (81.6)	62 (83.8)	133 (64.6)	562 (77)
Proper timing for folic acid supplementation	278 (61.8)	48 (64.9)	120 (58.3)	466 (63.8)
NTD timeline	276 (61.3)	39 (52.7)	96 (46.6)	411 (56.3)
Recommended dosage of folic acid for women capable of becoming pregnant	414 (92)	50 (67.6)	98 (47.6)	562 (77)
Recommended dosage of folic acid for women with history of NTD-affected pregnancy	187 (41.6)	46 (62.2)	80 (38.8)	313 (42.9)
Bioavailability: folate from natural food sources vs. folic acid	112 (24.9)	32 (43.2)	89 (43.3)	233 (31.9)

NTD – neural tube development; WHO – World Health Organization.

Frequency distribution of respondents' attitudes (%)

	Strongly disagree (1)	Disagree (2)	Neutral (3)	Agree (4)	Strongly agree (5)
Periconceptional use of folic acid-containing dietary supplements is of great importance in ensuring healthy offspring					
Licensed pharmacists	0	2.7	10.9	51.7	34.7
Intern pharmacists	0	1.4	4.1	32.4	62.1
Pharmacy technicians	1.9	14.1	42.2	30.6	11.2
Among women of reproductive age only those who are planning pregnancy should take folic acid dietary supplements					
Licensed pharmacists	19.1	24.9	22.4	25.1	8.5
Intern pharmacists	8.1	39.2	1.4	47.2	4.1
Pharmacy technicians	0	14.1	23.3	33	29.6
Pharmacists and pharmacy technicians are knowledgeable enough to provide appropriate counseling regarding adequate folate intake for reproductive-aged women					
Licensed pharmacists	0	7.1	36	52.4	4.5
Intern pharmacists	0	0	28.4	70.2	1.4
Pharmacy technicians	0	1.9	34	55.3	8.8
Congenital anomalies represent a major public health problem in view of their mortality and morbidity rates					
Licensed pharmacists	0	5.3	15.3	39.1	40.3
Intern pharmacists	0	10.8	6.8	50	32.4
Pharmacy technicians	0	6.3	35	41.7	17
Folic acid supplementation preconception and during pregnancy represents medicalization of pregnancy					
Licensed pharmacists	25.8	12.7	19.6	36.6	5.3
Intern pharmacists	51.4	24.3	5.4	17.5	1.4
Pharmacy technicians	21.8	21.4	13.1	28.5	15
Primary health care professionals play an important role in reproductive health promotion and the prevention of congenital anomalies					
Licensed pharmacists	0	1.8	5.8	30.4	62
Intern pharmacists	0	0	6.8	29.7	63.5
Pharmacy technicians	1.9	0	30.6	51.5	16
Women of reproductive age living in Serbia are well-informed about folic acid benefits and recommended level of intake					
Licensed pharmacists	22	54.7	20.6	1.8	0.9
Intern pharmacists	12.2	56.7	23	0	8.1
Pharmacy technicians	13.6	73.3	12.1	1	0

	Strongly disagree (1)	Disagree (2)	Neutral (3)	Agree (4)	Strongly agree (5)
It is important to increase women's awareness regarding the importance of folic acid in preventing foetal neural tube defects					
Licensed pharmacists	0	1.8	25.1	0	73.1
Intern pharmacists	0	0	5.4	37.8	56.8
Pharmacy technicians	1.9	0	13.2	49	35.9
Congenital anomalies do not pose a major public health problem in the context of social costs and human suffering					
Licensed pharmacists	54.7	32.8	9.8	1.8	0.9
Intern pharmacists	27	62.2	9.4	1.4	0
Pharmacy technicians	14.5	64.1	16.5	4.9	0
Patients in community pharmacies are not interested to be educated about dietary supplements that are used in preconception and prenatal care					
Licensed pharmacists	17.8	40	29.1	13.1	0
Intern pharmacists	6.7	54.1	39.2	0	0
Pharmacy technicians	0.5	48.5	13.6	23.8	13.6
It is not my responsibility to educate women about measures for the prevention of congenital anomalies					
Licensed pharmacists	46	20.9	28.4	2.9	1.8
Intern pharmacists	36.5	52.7	10.8	0	0
Pharmacy technicians	8.7	24.8	23.3	40.8	2.4
Pharmacists and pharmacy technicians should provide information about adequate folate intake only on patient's request, not on their own initiative					
Licensed pharmacists	29.1	25.8	23.8	18.9	2.4
Intern pharmacists	43.2	35.1	9.5	12.2	0
Pharmacy technicians	8.3	20.8	19.9	37.4	13.6
When interacting with women of reproductive age I feel uncomfortable to bring up the topic of family planning and the prevention of neural tube defects					
Licensed pharmacists	26.4	23.8	16	30	3.8
Intern pharmacists	6.7	29.7	20.3	41.9	1.4
Pharmacy technicians	7.3	35	15.4	24.3	18
Community pharmacists and pharmacy technicians, being health professionals most accessible to general public, play an important role in health promotion and disease prevention					
Licensed pharmacists	0.9	1.8	2.7	24.2	70.4
Intern pharmacists	0	0	5.4	25.7	68.9
Pharmacy technicians	0	1.9	15	34.6	48.5

Fig. 1 – Frequency distribution of respondents' attitudes across the professional groups of pharmacy staff.

Table 3

Frequency distribution of participants' answers to practice-related questions

Practice domain	Options	Number of participants	Total (n = 756)
Frequency of providing information and advice concerning pre- and post-conception use of folic acid for the prevention of neural tube defects and other congenital anomalies	Every day	10	1.4
	Few times a week	124	17
	Once a week	226	31
	Few times a month	173	23.7
	Once a month	87	11.9
	Rarely	110	15.1
	Never	0	0
Dispensing frequency of the following products to women of reproductive age: contraceptive preparations, vitamin and mineral dietary supplements, ovulation tests/ fertility monitors, and home pregnancy tests	Multiple times a day	191	26.2
	Every day	319	43.7
	Few times a week	197	27
	Once a week	20	2.7
	Few times a month	3	0.4
	Once a month or rarer	0	0
Last access to information on recommended level of intake, role and importance of folic acid	More than 5 years ago	115	15.8
	4–5 years ago	129	17.7
	2–3 years ago	278	38.1
	6 months–1 year ago	136	18.7
	3–6 months ago	59	8.1
	In the past 3 months	11	1.5
Main sources of information on recommended level of intake and health benefits of folic acid*	Medical representatives	317	43.4
	Continuing medical education courses	345	47.3
	Websites of relevant institutions and health authorities	377	51.6
	Scientific journals and textbooks	298	40.8
	Media (TV, radio, public press, internet etc.)	285	39
	Professional meetings and conferences	261	35.8
Barriers to regular provision of information and advice about folate intake in community pharmacies*	Lack of time and/or adequate space	432	59.2
	Lack of patients' interest	259	35.5
	Lack of remuneration for these activities/services	253	34.7
	Feeling uncomfortable to bring up the issue of birth defects	319	43.7
	Insufficient folic acid-relevant knowledge among pharmacy staff	243	33.3
	Low motivation level among pharmacy staff	384	52.6

*Note: Percentage may sum over 100 since multiple answers were permitted; 461 licensed pharmacists, 77 intern pharmacists, 218 pharmacy technicias (total n = 756).

The pharmacists (49.2%) were more likely than the pharmacy technicians (32.5%) to report a prior training on folic acid health protective benefits ($\chi^2 = 9.496$; df = 2; $p < 0.01$), and the vast majority of subjects (n = 630; 91.9%) expressed an interest in receiving the additional education on this issue. The main sources of information, used by the respondents to expand and update their folic acid-related knowledge are listed in Table 3. Among the survey participants 254 (34.8%) confirmed availability of informative materials (e.g., brochures, posters, flyers, etc.) about folic acid and/or recommended vitamin/mineral intake preconception and during pregnancy in the pharmacy. Based on the self-report, 36.7% of respondents, on most occasions, communicated information and advice concerning folic acid at their own initiative, while 63.3% dominantly did it reactively, i.e., in response to the patients' questions. The communication strategy was significantly associated with gender ($\chi^2 = 12.43$; df = 2; $p < 0.001$) and the current professional status ($\chi^2 = 10.289$; df = 4; $p < 0.01$). The females were more likely to act proactively than the males (39.8% vs. 23.7%), so as were the licensed and intern pharmacists (40.2% and 42.5% respectively) compared to the pharmacy technicians (27.7%). The participants' perceptions of the most common barriers to regular provision of advice and information regarding the folate intake are presented in Table 3.

Discussion

Being highly accessible and trusted health professionals, community pharmacists have both professional responsibility and opportunity to participate actively in disease prevention and health promotion [24]. Additionally, the evolving role of pharmacy technicians may support community pharmacists in providing advanced services, including those related to the public health promotion [25, 26]. During the routine interactions with women of reproductive age members of pharmacy staff can recognize and address biomedical and behavioural risk factors that may jeopardize women's health and cause adverse pregnancy outcomes [8]. Despite conclusive scientific evidence regarding the importance of adequate folate status prior to conception and in the early phases of pregnancy, the folate insufficiency among women remains a significant public-health concern worldwide. In countries lacking the mandatory folic acid food fortification policies, like Serbia, the strategies to optimize the folate status at the population level are focused on the folic acid supplementation [27]. The main challenge of this approach comes from the fact that folic acid is effective in the NTD prevention only during the neurulation, i.e., by 28th day post-conception. Due to a high rate of unplanned pregnancies, the interventions aiming to increase awareness of the folic acid benefits and compliance with the recommendations should be directed towards all women of childbearing age. By provision of health-education materials, informative consultation and advice pharmacists and pharmacy technicians may give their contribution in public-health efforts to combat neural tube defects and other preventable congenital anomalies [8]. To the best of our knowledge, this study was the first one that ex-

plore knowledge, attitudes and practice (KAP) of the Serbian pharmacists and pharmacy technicians with regards to counseling women of reproductive age on adequate folate intake.

The finding that the vast majority of survey participants (96.6%) correctly identified the types of deformities which could be prevented by folic acid indicates a high level of general awareness of this important issue. These observations were in accordance with previous studies conducted among the pharmacists, pharmacy students and obstetrician-gynecologists [28–30]. Nevertheless, the study revealed certain gaps in the knowledge regarding some particular aspects of folate nutrition and the folic acid supplementation. Even though more than two-thirds of surveyed pharmacy staff members knew the recommended dietary allowances for adults (67%) and a dosage of folic acid for women capable of becoming pregnant (77%), less than one-half properly identified a recurrence dose and a dose for women with an increased risk of having infants with NTDs. The folic acid origin, bioavailability issues and current food fortification policy in Serbia were targeted as knowledge domains that elicited the lowest correct response rates. It is worrisome that almost 40% of surveyed health professionals did not recognize that folic acid should be administered prior to pregnancy to ensure the optimal NTD prevention. This finding was directly coupled with a poor knowledge regarding the neural tube closure timeline. Even more significant deficiency in knowledge on these issues was observed among the obstetricians and specialists in women's health in the Northern China, physicians in the Southern Israel, health professionals in the Northwestern Ethiopia and Chilean primary health workers [31–34]. The participants in the present study had the comparable knowledge level to the Ohio community pharmacists and similar gaps were identified [28, 35]. Furthermore, consistent with other studies conducted among different groups of healthcare personnel, the professional category and educational attainment level significantly affected folic acid-related knowledge [31, 33, 36]. Based on the test results, the pharmacy technicians were less knowledgeable than both intern and licensed pharmacists concerning the majority of analyzed domains. The reasons for the identified knowledge deficiencies should be explored and the curriculum modifications, refreshment trainings and specific guidance should be created accordingly.

The majority of respondents acknowledged the importance of folic acid favourable effects on pregnancy outcomes and almost all expressed support to raising awareness of this issue among women of childbearing age. Although more than 40% of participants thought that folic acid contributed to pregnancy medicalization, in line with findings from study conducted in the Netherlands, that did not seem to affect their support to this NTD preventive measure [37]. Only 2.7% of respondents believed that the women in Serbia are sufficiently informed about the role of folate status in achieving a healthy offspring. Such observations of pharmaceutical staff members are supported with findings of previously conducted studies indicating a very small proportion of Serbian women regularly taking the folic acid dietary supplements [16, 17]. Similarly, alarmingly poor knowledge regarding benefits of

timely use of folic acid and a high rate of suboptimal peri-conceptional practises were found in a large multinational survey of more than 22,000 European women of reproductive age, clearly reflecting unmet needs in preconception care [38]. In the mentioned study, based on the participants' self-report, the lack of awareness and absence of appropriate counselling were identified as the main reasons for not taking folic acid preconceptionally and/or during pregnancy. It is noteworthy that more than 90% of surveyed licensed and intern pharmacists agreed that the primary health care professionals were of particular importance for preconception health promotion. Furthermore, comparable with the studies conducted among their Dutch and Australian colleagues, almost all responding pharmacists regarded it as their professional duty to inform women about folic acid and advocate for preconception care [37]. The analyses of reported attitudes, however, revealed that 37.1% of surveyed community pharmacy personnel expressed discomfort with discussing the issues of family planning and prevention of congenital anomalies, with this problem being especially accentuated among the male providers. In addition, 21.3%, 12.2% and 51.0% of licensed pharmacists, interns, and pharmacy technicians, respectively, were of the opinion that these topics should be addressed only on patients request, i.e., reactively. Consistently with other studies, the pharmacists' concern about being too intrusive by imposing information and advice discourage them from acting proactively, which causes under-utilisation of full potential of community pharmacy setting in the prevention of folic acid-sensitive congenital malformations [37–41].

In compliance with the United Nation Global Strategy for Women's and Children's Health, International Pharmaceutical Federation (FIP) encourages active involvement of pharmacists in the preservation and improvement of maternal, newborn and children health through a variety of evidence-informed interventions, screening, health-education, facilitation of health-care services and provision of appropriate products [18]. Nevertheless, the current study demonstrated a large discrepancy between the opportunities for preconception counselling and implemented activities, highlighting the gap between the ideal and actual level of pharmacy staff contribution. Based on the self-report, more than one-half of respondents provided information and advice regarding the folic acid value in the prevention of congenital abnormalities once a week, or few times a month, while only 10 pharmacists stated to perform these activities on daily basis. Conversely, approximately 70% of respondents reported dispensing products associated with the general and reproductive female health to women of childbearing age once daily, or even multiple times a day, which revealed a myriad of missed occasions for the informative consultation [40]. The fact that a healthcare provider recommendation was recognized as the single most important reason women would commence folic acid supplementation underscores the significance of responsible and consistent provision of counseling [38]. Understanding the key obstacles to the adequate delivery of such activities is a prerequisite for addressing suboptimal involvement of the community pharmacy health professionals.

The main barriers perceived by the surveyed pharmacists and pharmacy technicians were consistent with the findings from the studies done elsewhere and included the time and space constraints, low motivation level among personnel, discomfort with raising the issue of birth defects, lack of financial compensation, uncooperative patients and knowledge deficiencies [39, 42–44]. In order to overcome the low level of self-assessed competency and motivation, initiate behavioral change and improve current and future performance of pharmacy staff, implementation of targeted training programmes is required.

The theory of Planned Behaviour, as an extension of the Theory of Reasoned Action, has been widely applied in the analyses and prediction of health professionals' behaviour [45–48]. This theoretical framework postulates that intention, capturing the motivational factors which influence behaviour, is the crucial antecedent and reasonably accurate predictor of an individual's actions [49]. Based on this model, behavioural intention is influenced by attitudes, subjective norms (i.e., social pressure), and perceived control over the behaviour with each of these variables being driven with a corresponding type of salient beliefs (behavioural, normative and control). If these social-cognitive constructs were transferred to pharmacy practice, we might assume that the favourable attitudes toward preconception counselling, subjective norms supporting these activities and strong perceived behavioural control are positive and significant predictors of community pharmacists' and pharmacy technicians' intentions to involve proactively in dissemination of evidence-informed, health-promotional messages about folic acid. Advancement of self-perceived knowledge, skills and efficacy would empower pharmacists and pharmacy technicians to embrace more active roles in providing the patient-centered preconception care in accordance with their professional responsibilities and scope of practice [18].

Certain limitations of the present study should be taken into consideration when interpreting the results. The recruitment strategy applied to pharmacy technicians might have caused participation bias and influenced generalizability of the obtained results. However, the rationale behind the selected sampling protocol was based on the fact that the pharmacy technicians perform pharmaceutical health care activities in the community pharmacies only in the presence of licensed pharmacists and under their supervision. Furthermore, considering geographical distribution, no statistically significant difference was found between the structure of the analyzed cohort of pharmacy technicians and the Serbian Chamber of Nurses and Medical Technicians membership directory ($\chi^2 = 4.315$; df = 3; $p = 0.229$). Another limitation refers to the fact that the evaluation of counseling practice was based on the self-reported behaviour, and therefore, it was a subject of inherent imperfections of that method.

Conclusion

Given the widespread inadequacy of folate intake, a low level of public awareness concerning the importance of preconception care and high rate of unplanned pregnancies, in-

novative, sustainable and effective public health strategies are needed to reduce the risk of devastating folic acid-preventable congenital malformations. Due to the unique accessibility and expertise community pharmacy health professionals may give indispensable contribution to this agenda.

Nevertheless, the results of the present study provide evidence that tailored educational interventions and professional support are needed to improve their engagement in this area of public health. Although participants demonstrated positive general attitude toward folic acid value for maternal and foetal health, and acknowledged the importance of their role in preconception health promotion, our findings revealed certain knowledge gaps as well as dominantly reactive counselling practice. Being first to explore knowledge, attitudes and practice regarding folic acid supplementation among the pharmacists and pharmacy technicians in Serbia, this study provides valuable reference data for addressing the barriers to behavioural change, the practice optimisation and establishment of proactive advocacy of folic acid benefits.

Acknowledgements

R E F E R E N C E S

1. Safi J, Joyeux L, Chalouhi GE. Periconceptional folate deficiency and implications in neural tube defects. J Pregnancy 2012; 2012: 295083.
2. Lucock M. Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab 2000; 71(1–2): 121–38.
3. Blom HJ. Folic acid, methylation and neural tube closure in humans. Birth Defects Res A Clin Mol Teratol 2009; 85(4): 295–302.
4. Singh S, Sedgh G, Hussain R. Unintended pregnancy: worldwide levels, trends, and outcomes. Stud Fam Plann 2010; 41(4): 241–50.
5. Eichholzer M, Tönz O, Zimmermann R. Folic acid: a public-health challenge. Lancet 2006; 367(9519): 1352–61.
6. Cornel MC, de Smit DJ, de Jong-van den Berg LT. Folic acid--the scientific debate as a base for public health policy. Reprod Toxicol 2005; 20(3): 411–5.
7. World Health Organization, Food and Agriculture Organization of the United Nations. The Rome Declaration on Nutrition. ICN2 Second International Conference on Nutrition better nutrition, better lives; Rome, Italy; 2014 November 19–21. Rome: Food and Agriculture Organization of the United Nations; 2014.
8. DiPietro Mager NA. Fulfilling an Unmet Need: Roles for Clinical Pharmacists in Preconception Care. Pharmacotherapy 2016; 36(2): 141–51.
9. Gomes S, Lopes C, Pinto E. Folate and folic acid in the periconceptional period: recommendations from official health organizations in thirty-six countries worldwide and WHO. Public Health Nutr 2016; 19(1): 176–89.
10. Chitayat D, Matsui D, Amitai Y, Kennedy D, Vohra S, Rieder M, et al. Folic acid supplementation for pregnant women and those planning pregnancy: 2015 update. J Clin Pharmacol 2016; 56(2): 170–5.
11. McNulty H, Scott JM. Intake and status of folate and related B-vitamins: considerations and challenges in achieving optimal status. Br J Nutr 2008; 99 Suppl 3: S48–54.
12. Ray JG, Singh G, Burrows RF. Evidence for suboptimal use of periconceptional folic acid supplements globally. BJOG 2004; 111(5): 399–408.
13. De-Regil LM, Peña-Rosas JP, Fernández-Gaxiola AC, Rayco-Solon P. Effects and safety of periconceptional oral folate supplementation for preventing birth defects. Cochrane Database Syst Rev 2015; (12): CD007950.
14. Bailey LB, Stover PJ, McNulty H, Fenech MF, Gregory JF 3rd, Mills JL, et al. Biomarkers of Nutrition for Development-Folate Review. J Nutr 2015; 145(7): 1636S–80S.
15. Obeid R, Pietrzik K, Oakley GP Jr, Kancherla V, Holzgreve W, Wieser S.Preventable spina bifida and anencephaly in Europe. Birth Defects Res A Clin Mol Teratol 2015; 103(9): 763–71.
16. Zekovic M, Djekic-Ivankovic M, Nikolic M, Gurinovic M, Krajnovic D, Glibetic M. Validity of the Food Frequency Questionnaire Assessing the Folate Intake in Women of Reproductive Age Living in a Country without Food Fortification: Application of the Method of Triads. Nutrients 2017; 9(2): pii: E128.
17. Odalovic M, Vezmar Kovacevic S, Ilic K, Sabo A, Tasic L. Drug use before and during pregnancy in Serbia. Int J Clin Pharm 2012; 34(5): 719–27.
18. FIP. Statement of Policy on the effective utilization of pharmacists in improving maternal, newborn and child health (MNCH). FIP, International Pharmaceutical Federation; 2013. Available from: www.fip.org/statements
19. World Health Organization. European strategic approach for making pregnancy safer: improving maternal and perinatal health. Denmark, Copenhagen: World Health Organization; 2008.
20. Krejcie R, Daryle M. Determining sample size for research activities. Educ Psychol Meas 1970; 30(3): 607–10.
21. Sakpal TV. Sample size estimation in clinical trial. Perspect Clin Res 2010; 1(2): 67–9.
22. Bolarinwa OA. Principles and methods of validity and reliability testing of questionnaires used in social and health science researches. Niger Postgrad Med J 2015; 22(4): 195–201.
23. World Health Organization. Standards and operational guidance for ethics review of health-related research with human participants. Switzerland, Geneva: World Health Organization; 2011.
24. Kelling SE, Rondon-Begazo A, DiPietro Mager NA, Murphy BL, Bright DR. Provision of Clinical Preventive Services by Community Pharmacists. Prev Chronic Dis 2016; 13: E149.
25. Royal Society for Public Health. Building capacity: realising the potential of community pharmacy assets for improving the public's health. London, UK: RSPH; 2016.
26. Deeks LS, Cooper GM, Currie MJ, Martin SJ, Parker RM, Del Rosario R, et al. Can pharmacy assistants play a greater role in public health programs in community pharmacies? Lessons from chlamydia screening study in Canberra, Australia. Res Social Adm Pharm 2014; 10(5): 801–6.
27. McNulty H. The role of food fortification: the example of folic acid. In: Biesalski HK, Drenowski A, Dwyer JT, Strain J, Weber P, Eggersdorfer M, editors. Sustainable nutrition in a changing world. Cham: Springer International Publishing; 2017. p. 371–88.
28. Rodrigues CR, Dipietro NA. Knowledge of folic acid and counseling practices among Ohio community pharmacists. Pharm Pract (Granada) 2012; 10(3): 168–72.

29. *Lynch SM.* Assessment of student pharmacists' knowledge concerning folic acid and prevention of birth defects demonstrates a need for further education. J Nutr 2002; 132(3): 439–42.

30. *Power ML, Holzman GB, Schulkin J.* Knowledge and clinical practice regarding folic acid among obstetrician-gynecologists. Obstet Gynecol 2000; 95(6 Pt 1): 895–8.

31. *Li T, Zhu J, Zeng Z, Wang Y, Liang J, Yuan P.* Study of KAP with regard to taking folic acid supplements and factors affecting the recommendation and prescription of those supplements among obstetricians and specialists in women's health in six provinces of Northern China, 2009. Biosci Trends 2011; 5(3): 104–10.

32. *Abu-Hammad T, Dreiher J, Vardy DA, Cohen AD.* Physicians' knowledge and attitudes regarding periconceptional folic acid supplementation: a survey in Southern Israel. Med Sci Monit 2008; 14(5): CR262–7.

33. *Demilew YM, Asres Nigussie A.* Knowledge of Health Professionals on Folic Acid Use and Their Prescribing Practice in Bahir Dar City Administration, Northwest Ethiopia: Cross-Sectional Study. PLoS One 2017; 12(1): e0170116.

34. *Pardo R, Lay-Son G, Aranda W, Recabal P, Navarrete M, Tenhamm T, et al.* Chilean primary health workers' knowledge about folic acid supplementation for the prevention of neural tube defects. Am J Med Genet A 2006; 140(11): 1238–40.

35. *Boykin CM, DiPietro Mager NA.* Ohio pharmacy students' knowledge of folic acid and neural tube defects. Curr Pharm Teach Learn 2015; 7(2): 273–6.

36. *Williams JL, Abelman SM, Fassett EM, Stone CE, Petrini JR, Damus K, et al.* Health care provider knowledge and practices regarding folic acid, United States, 2002-2003. Matern Child Health J 2006; 10(Suppl 7): 67–72.

37. *De Jong-van den Berg LT, Van der Zee AH, Schaafsma E, De Smit D, Anderson C, Cornel MC.* Counselling women about periconceptional use of folic acid: The role of the community pharmacist can be improved. Int J Pharm Pract 1999; 7(3): 138–142.

38. *Bitzer J, von Stenglin A, Bannemerschult R.* Women's awareness and periconceptional use of folic acid: data from a large European survey. Int J Womens Health 2013; 5: 201–13.

39. *Eades CE, Ferguson JS, O'Carroll RE.* Public health in community pharmacy: a systematic review of pharmacist and consumer views. BMC Public Health 2011; 11: 582.

40. *Anderson C, Rajyaguru R.* The role of community pharmacists and medicines counter assistants in health promotion: reflections from a folic acid campaign. Int J Pharm Pract 2002; 10(1): 17–22.

41. *Meijer WM, de Smit DJ, Jurgens RA, Lolkje TV, de Jong-van den Berg LT.* Pharmacists' role in improving awareness about folic acid: a pilot study on the process of introducing an intervention in pharmacy practice. Int J Pharm Pract 2004; 12(1): 29–35.

42. *Laliberté MC, Perreault S, Damestoy N, Lalonde L.* Ideal and actual involvement of community pharmacists in health promotion and prevention: a cross-sectional study in Quebec, Canada. BMC Public Health 2012; 12: 192.

43. *Bixenstine PJ, Cheng TL, Cheng D, Connor KA, Mistry KB.* Association Between Preconception Counseling and Folic Acid Supplementation Before Pregnancy and Reasons for Non-Use. Matern Child Health J 2015; 19(9): 1974–84.

44. *Erku DA, Mersha AG.* Involvement of community pharmacists in public health priorities: A multi-center descriptive survey in Ethiopia. PLoS One 2017; 12(7): e0180943.

45. *Gavaza P, Brown CM, Lawson KA, Rascati KL, Wilson JP, Steinhardt M.* Examination of pharmacists' intention to report serious adverse drug events (ADEs) to the FDA using the theory of planned behavior. Res Social Adm Pharm 2011; 7(4): 369–82.

46. *Fleming ML, Barner JC, Brown CM, Shepherd MD, Strassels S, Novak S.* Using the theory of planned behavior to examine pharmacists' intention to utilize a prescription drug monitoring program database. Res Social Adm Pharm 2014; 10(2): 285–96.

47. *Watson MC, Johnston M, Entwistle V, Lee AJ, Bond CM, Fielding S.* Using the theory of planned behaviour to develop targets for interventions to enhance patient communication during pharmacy consultations for non-prescription medicines. Int J Pharm Pract 2014; 22(6): 386–96.

48. *Tan CL, Hassali MA, Saleem F, Shafie AA, Aljadhay H, Gan VB.* Building intentions with the theory of planned behaviour: a qualitative assessment of salient beliefs about pharmacy value added services in Malaysia. Health Expect 2016; 19(6): 1215–25.

49. *Ajzen I.* The theory of planned behavior. Organ Behav Hum Decis Process 1991; 50(2): 179–211.

Assessment of periodontal health among the inpatients with schizophrenia

Procena periodontalnog zdravlja hospitalizovanih bolesnika sa shizofrenijom

Vladan Djordjević*[†], Mila Jovanović[‡], Vesna Stefanović*, Nataša Nikolić Jakoba[‡], Gorica Djokić*, Ivana Stašević Karličić*[§], Ljubomir Todorović[†]

*Clinic for Psychiatric Disorders "Dr. Laza Lazarević", Belgrade, Serbia; University of Travnik, [†]Faculty of Pharmacy and Health, Bosnia and Herzegovina; University of Belgrade, [‡]Faculty of Dental Medicine, Belgrade, Serbia; University of Priština/Kosovska Mitrovica, [§]Faculty of Medicine, Kosovska Mitrovica, Serbia

Abstract

Background/Aim. Many studies on oral health of psychiatric inpatients reported schizophrenia as the most common psychiatric disorder among their sample population. The available evidence suggests the higher prevalence and severity of periodontal disease among the psychiatric inpatients. The aim of this study was to evaluate periodontal health among the inpatients with schizophrenia and to consider possible risk factors for their current periodontal diseases. **Methods.** This cross-sectional study comprised 190 inpatients with schizophrenia at the Clinic for Psychiatric Disorders "Dr Laza Lazarevic" in Belgrade, and 190 mentally healthy patients at the Clinic for Periodontology and Oral Medicine, Faculty of Dental Medicine, University in Belgrade. The Community Periodontal Index for Treatment Needs (CPITN) and sociodemographic characteristics were registered in both groups as well as the characteristics of the primary disease among the inpatients with schizophrenia. **Results.** The patients in the study group had significantly higher scores of the CPITN (2.24 ± 0.98) than the patients in the control group (1.21 ± 1.10). Most of the patients in the study group had supra- , or subgingival calculi (46.8%), in contrast to the control group patients, who had in most cases gingival bleeding (45.8%). The periodontal pockets where detected in 35.8% of schizophrenic inpatients. The linear regression analysis showed that the gender and age were statistically significant predictors of the CPITN value among the inpatients with schizophrenia. **Conclusion.** The results of this study generally indicate the need for continuous research of psychiatric patients' oral health, in order to determine the modes of its improvement. Similar studies should elucidate significance of psychiatric patients' periodontal health and sensitize psychiatrists and psychiatric nurses to the oral problems of their patients.

Key words:
age factors; hospitals, psychiatric; oral health; periodontal diseases; periodontal index; risk factors; schizophrenia; sex.

Apstrakt

Uvod/Cilj. Mnogobrojna istraživanja oralnog zdravlju hospitalizovanih psihijatrijskih bolesnika pokazala su da je shizofrenija najčešći psihijatrijski poremećaj među njima. Dostupni podaci ukazuju na veliku prevalenciju i težinu periodontopatije kod bolesnika sa psihijatrijskim poremećajima. Cilj ove studije bio je da se proceni periodontalno zdravlje hospitalizovanih osoba sa shizofrenijom, kao i da se ukaže na moguće faktore rizika od oboljenja potpornog aparata zuba tih bolesnika. **Metode.** Studijom preseka obuhvaćeno je 190 osoba sa shizofrenijom, hospitalizovanih u Klinici za psihijatrijske bolesti „Dr Laza Lazarević" u Beogradu i 190 mentalno zdravih osoba, pacijenata Klinike za parodontolo-giju i oralnu medicinu Stomatološkog fakultet, Univerziteta u Beogradu. U obe grupe ispitanika registrovane su vrednosti Zajenički periodontni indeks potreba tretmana – *Community Periodontal Index for Treatment Needs* (CPITN), kao i sociodemografska obeležja, dok su karakteristike primarne bolesti beležene u studijskoj grupi. **Rezultati.** Ispitanici studijske grupe su imali znatno veće vrednosti CPITN (2,24 ± 0,98) u odnosu na ispitanike kontrolne grupe (1,21 ± 1,10). Kod većine ispitanika studijske grupe registrovano je prisustvo supra- i subgingivalnog kamenca (46,8%), za razliku od ispitanika kontrolne grupe koji su češće imali gingivalno kr-varenje (45,8%). Periodontalni džepovi su registrovani kod 35,8% hospitalizovanih osoba sa shizofrenijom. Linearna regresiona analiza pokazala je da su pol i starost ispitanika

Correspondence to: Vladan Djordjević, Clinic for Psychiatric Disorders "Dr. Laza Lazarević", Višegradska 26, 11 000 Belgrade, Serbia.
E-mail: drvladandjordjevic@gmail.com

bili statistički značajni prediktori vrednosti CPITN hospita-lizovanih osoba sa shizofrenijom. **Zaključak.** Rezultati ovog istraživanja ukazuju na postojanje potrebe za kontinui-ranim istraživanjem stanja oralnog zdravlja psihijatrijskih bolesnika, kako bi se odredili načini za njegovo unapređenje. Slične studije bi trebalo da razjasne značaj peridodontalnog zdravlja psihijatrijskih bolesnika, kako bi se psihijatrima i

medicinskim sestrama ukazalo na postojanje problema oral-nog zdravlja njihovih bolesnika.

Ključne reči:
životno doba, faktor; bolnice, psihijatrijske; usta, zdravlje; periodontalne bolesti; periodontalni indeks; faktori rizika; shizofrenija; pol.

Introduction

Mental health is a state of well-being in which an indi-vidual realizes how can cope with usual life stresses with his or her own abilities, how can work productively and fruit-fully, and make a contribution to his or her community [1]. Several studies on oral health of psychiatric inpatients re-ported schizophrenia as the most common psychiatric disor-der among their sample population [2–4]. Schizophrenia is a mental disorder characterized by a disintegration of thinking processes and emotional responsiveness [5]. The disease oc-curs in 1% of the general population and it is one of ten lead-ing causes of disability in the population between 15 and 44 years of age [6].

Oral health is a significant part of general health and should not be separated from mental health [7]. The available evidence suggests the higher prevalence and severity of peri-odontal disease among the psychiatric inpatients [3, 8–13]. A bacterial role in the initiation and progression of periodontal disease is essential, but most of the nonbacterial risk factors appear to act as the disease modifiers that may alter the clini-cal effects of bacterial challenge [14]. There are many factors associated with the poor periodontal status among the psy-chiatric inpatients: sociodemographic characteristics like age [8, 15, 16], gender [10, 15] and educational level [15]; characteris-tics of schizophrenia like duration of disease [16], length of hospitalization [8] and psychotropic medications [15]; and, the oral health habits like frequency of tooth brushing [10], tooth brush technique [15] and smoking [10].

In Serbia, no research has been conducted on periodon-tal health of this vulnerable group of psychiatric inpatients, although the prevalence of the disease is 1% of the whole population [6]. Therefore, the aim of the present study was to evaluate periodontal health among the inpatients with schi-zophrenia, and to consider possible risk factors that might contribute to the current periodontal health status of this group of psychiatric inpatients.

Methods

Study population

The study was conducted as an observational cross-sectional study. It was adjusted to the Strengthening the Re-porting of Observational Studies in Epidemiology (STROBE) statement for improving the quality of observational studies [17]. The approval for the study was obtained from the Ethics Committee of the Clinic for Psychiatric Disorders "Dr. Laza Lazarević" in Belgrade, Serbia (No. 7221) and the Faculty of

Dental Medicine, University of Belgrade, Serbia (No. 36/10). The research was conducted in accordance with the Declara-tion of Helsinki [18].

Each subject participated voluntarily and was informed, through a special brochure, of the type of the research and the data collection procedure. The written consent was ob-tained from all subjects, or their legal representatives to use the personal data for the research purposes.

The study group size was determined according to the prevalence of schizophrenia in the general population in the Republic of Serbia, with 95% confidence level. Conse-quently, the study group comprised 190 randomly selected inpatients with schizophrenia (95 males and 95 females, aged 19 to 67 years; mean age 43.59 ± 11.96 years) hospitalized at the Clinic for Psychiatric Disorders "Dr. Laza Lazarević" in Belgrade, Serbia. The inclusion criteria for entering the study were that a patient was hospitalized, older than 18 years and diagnosed with schizophrenia (according to the 10th Revi-sion of the International Classification of Diseases) [19] at least two years prior to the study. The exclusion criteria were: a primary diagnosis of another mental disorder, the inpatients diagnosed with schizophrenia in the period shorter than two years from the time of the survey, simultaneous presence of severe somatic illnesses or severe disability, and inability to communicate, or refuse to cooperate.

The control group comprised of 190 patients suffering from localized or generalized chronic periodontitis [20] (95 males and 95 females, aged 19 to 72 years; mean age 43.20 ± 11.89 years), without any psychiatric or somatic illness, who were consecutively recruited from the pool of patients at the Department of Periodontology and Oral Medicine, Faculty of Dental Medicine, University of Belgrade, Serbia. The pa-tients in the control group did not use any medication that could affect oral health [21]. Both, the study and the control groups were age and gender matched.

A questionnaire for both groups was designed in order to record the sociodemographic characteristics (gender, age, educational level, marital status and residence), oral health habits (frequency of brushing teeth and tooth brushing tech-nique) and smoking habits. The data about schizophrenia in the study group were taken from the medical records and in-cluded the duration of schizophrenia, number of hospitaliza-tions and current psychotropic medication.

Clinical examination

All patients were subjected to the thorough dental clini-cal examination in accordance with the criteria recom-mended by the World Health Organization[22]. The dental

clinical examinations were carried out by two trained and calibrated examiners (V.Dj. and M.J.) at the dental office at the Clinic for Psychiatric Disorders "Dr. Laza Lazarević" in Belgrade, Serbia, and the Department of Periodontology and Oral Medicine, Faculty of Dental Medicine, University of Belgrade, Serbia. The examiners were calibrated twice, before and during the study, by assessing the Community Periodontal Index for Treatment Needs (CPITN) [23], with a degree of agreement with ± 1 mm of 94%. The clinical measurements were performed by using the periodontal probe graded in mm (WHO 621 Trinity probe) on the sextants, scoring on the scale from 0 to 4. In each sextant, all teeth were examined and only the highest value for each sextant was scored and recorded.

Statistical analysis

All collected data were organized and evaluated using the dedicated software (SPSS 17.0 Inc, Chicago, IL, USA) and were analysed by the descriptive statistical parameters and regression models. The descriptive statistical methods were represented by the measures of central tendency (mean and median), measure of variability (standard deviation and var-
iation interval) and were expressed in the percentages. The methods for testing the difference of numerical data (age and CPITN) were represented by the t-test of independent groups. For testing the data of different categories (gender, education level, employment status, marital status, residence, smoking, brushing teeth, tooth brush technique), the χ^2-test was used. The level of significance was set at $p \leq 0.05$.

Results

The groups where comparable in terms of age ($p = 0.747$ for the t-test of independent groups) and gender ($p = 1.000$ for χ^2-test). The distribution of sociodemographic characteristics and oral health habits of the enrolled subjects are shown in Table 1. The statistically significant differences between the groups were observed for all sociodemographic characteristics and oral health habits (Table 1). The educational structure of inpatients with schizophrenia was lower than of the control group patients. Furthermore, the percentage of employees among the inpatients with schizophrenia was significantly lower than in the study group. Most of the patients of the study group were smokers (74.7%) as opposed to the control group patients (39.5%).

Table 1
Sociodemographic characteristics, smoking habits, and oral health hygiene among the participants

Variables	Study group	Control group	p
	n (%)	n (%)	(χ^2 test)
Sociodemographic characteristics			
Education			
elementary school	40 (21.1)	6 (3.2)	
junior high school	109 (57.4)	93 (48.9)	0.000
high school	16 (8.4)	33 (17.4)	
college	25 (13.2)	58 (30.5)	
Employment			
unemployed	117 (61.5)	99 (52.1)	
employed	10 (5.3)	73 (38.4)	0.000
invalid retirement	37 (19.5)	3 (1.6)	
age or survivor retirement	26 (13.7)	15 (7.9)	
Marital status			
married	21 (11.1)	67 (35.3)	
divorced / detached	30 (15.8)	31 (16.3)	0.000
unmarried / alone	131 (68.9)	86 (45.3)	
widow	6 (4.2)	6 (3.2)	
Residence			
own property	64 (33.7)	100 (52.6)	
parents property	101 (53.2)	40 (21.1)	0.000
rent or other	25 (13.2)	50 (26.3)	
Smoking habits			
yes	142 (74.7)	75 (39.5)	0.000
no	48 (25.3)	115 (60.5)	
Oral hygiene habits			
Frequency of brushing teeth			
no	104 (54.7)	0 (0)	
yes, several times per month	21 (11.1)	0 (0)	0.000
yes, once a day	38 (20.0)	28 (14.2)	
yes, twice or more per day	27 (14.2)	163 (85.8)	
Tooth brushing technique			
correct	52 (27.4)	102 (53.7)	0.000
incorrect	138 (72.6)	88 (46.3)	

In the study group, schizophrenia lasted, on average, 14.69 ± 9.61 years (range 2 to 45 years), and the average number of hospitalizations was 8.52 ± 5.71 (range 1 to 30 hospitalizations). The patients were treated with an average of 3.54 ± 0.87 psychotropic medications (range 2 to 6). The characteristics of primary disease in the study group are shown in Table 2.

The patients in the study group mostly did not brush their teeth (54.7%), unlike the patients in the control group. Among the inpatients with schizophrenia, even 72.6% were not familiar with a correct tooth brushing technique, as opposed to the patients in the control group (46.3%).

A statistical significance was observed in the CPITN values between the examined groups (Table 3). The patients in the study group had the significantly higher scores of the CPITN (2.24 ± 0.98, range 0 to 4) than the patients in the control group (1.21 ± 1.10, range 0 do 4). The patients in the study group had supra- or subgingival calculi more often (46.8%) than the control group patients, who demonstrated gingival bleeding more frequently (45.8%). Moreover, the periodontal pockets where detected in 35.8% of schizo-

phrenic inpatients, much more frequently than in the control group patients (13.7%).

Analysing the mean of the CPITN in relation to the sociodemographic characteristics and oral health habits of subjects in both groups (Table 3), a statistically significant difference in the study group was observed in terms of gender, age, and tooth brushing. The older male inpatients with schizophrenia, who did not brush teeth, had the highest value of CPITN. In the control group, a statistically significant difference in the CPITN values was observed in terms of marital status and tooth brush technique (Table 4). The widowed patients in the control group, who used an incorrect tooth brush technique, had the highest value of CPITN.

In terms of characteristics of the primary disease, a statistically significant difference in the value of CPITN among the study group patients was observed in terms of number of hospitalizations (Table 5).

The impact of sociodemographic characteristics and oral health habits, as well as the characteristics of the disease, the CPITN values among the inpatients with schizophrenia was examined by the linear regression model (Table 6).

Table 2
Clinical characteristics of schizophrenia

Characteristics	Patients, n (%)
Duration of mental illness (years)	
≤ 10	67 (35.3)
≥ 11	123 (64.7)
Number of psychiatric hospitalizations	
≤ 10	129 (67.9)
≥ 11	61 (32.1)
Medications	
antipsychotics	
first generation	116 (61.1)
second generation	74 (38.9)
mood stabilizers	
no	55 (28.9)
yes	135 (71.1)
hypnotics	
no	127 (66.8)
yes	63 (33.2)
anxiolytics	
no	30 (15.8)
yes	160 (84.2)
antidepressants	
no	175 (92.1)
yes	15 (7.9)
antiparkinsonics	
no	80 (42.1)
yes	110 (57.9)

Table 3
The Community Periodontal Index for Treatment Needs (CPITN) values among the patients in the study and control groups

Groups	CPITN codes				
	0	1	2	3	4
Study (n = 190), n	9	25	89	46	21
Control (n = 190), n	50	87	22	15	11

Table 4

The Community Periodontal Index for Treatment Needs (CPITN) values in the patients of the study and control groups, in terms of sociodemographic characteristics, smoking habits and oral hygiene habits

Variables	CPITN index values			
	study group		control group	
	mean ± SD	p	mean ± SD	p
Sociodemographic characteristics				
Gender				
male	2.37 ± 0.96	0.040 [a]	1.21 ± 1.08	0.944 [a]
female	2.11 ± 0.98		1.22 ± 1.12	
Age (years)				
≤ 30	1.85 ± 0.70	0.001 [b]	1.15 ± 1.09	
31–40	1.96 ± 0.90	(1:2) 0.565 [a]; (2:3) 0.010 [a]	1.26 ± 1.14	
41–50	2.40 ± 0.90	(1:3) 0.002 [a]; (2:4) 0. 006 [a]	1.12 ± 1.18	0.457 [b]
≥ 51	2.52 ± 1.11	(1:4) 0.001 [a]; (3:4) 0.591 [a]	1.30 ± 0.97	
Education				
elementary school	2.14 ± 1.04		2.00 ± 1.10	
junior high school	2.18 ± 1.01		1.18 ± 0.98	
high school	2.25 ± 0.78		1.36 ± 1.17	0.173 [b]
college	2.08 ± 0.81	0.523 [b]	1.09 ± 1.22	
Employment				
unemployed	2.16 ± 0.95		1.21 ± 1.11	
employed	2.10 ± 0.88		1.08 ± 1.08	
invalid retirement	2.30 ± 1.05		1.33 ± 0.58	0.071 [b]
age or survivor retirement	2.54 ± 1.03	0.393 [b]	1.80 ± 1.08	
Marital status				
married	2.57 ± 0.98		1.42 ± 1.18	0.008 [b]
divorced / detached	2.37 ± 0.89		1.32 ± 0.91	(1:2) 0.855 [a]; (2:3) 0.018 [a]
unmarried / alone	2.15 ± 0.97		0.97 ± 1.05	(1:3) 0.007 [a]; (2:4) 0.247 [a]
widow	2.25 ± 1.39	0.277 [b]	1.83 ± 1.17	(1:4) 0.311 [a]; (3:4) [a]0.050 [a]
Residence				
own property	2.38 ± 1.00		1.27 ± 1.17	
parents property	2.01 ± 0.98		1.03 ± 0.89	
rent or other	2.11 ± 0.60	0.243 [b]	1.27 ± 1.10	0.942 [b]
Smoking hybits				
yes	2.23 ± 0.99		1.31 ± 0.77	
no	1.98 ± 0.96	0.101 [a]	1.08 ± 0.75	0.853 [a]
Oral hygiene hobits				
Frequency of brushing teeth				
no	2.37 ± 0.93	0.007 [b]	/	
yes, several times per month	2.05 ± 1.02	(1:2) 0.219 [a]; (2:3) 0.804 [a]	/	
yes, once a day	2.00 ± 0.84	(1:3) 0.032 [a]; (2:4) 0.230 [a]	1.28 ± 0.89	0.220 [b]
yes, twice or more per day	1.70 ± 1.14	(1:4) 0.002 [a]; (3:4) 0.123 [a]	0.91 ± 0.74	
Tooth brushing technique				
correct	1.87 ± 1.01		0.76 ± 0.71	
incorrect	2.28 ± 0.94	0.020 [a]	1.43 ± 0.79	0.020 [a]

[a] **Mann-Whitney test;** [b] **Kruskal Wallis test.**

Table 5

The value of the Community Periodontal Index for Treatment Needs (CPITN) in the study group, in terms of medical characteristics

Clinical characteristics	CPITN index mean ± SD	p (Mann-Whitney test)
Duration of mental illness (years)	2.01 ± 0.83	
≤ 10	2.24 ± 1.04	0.146
≥ 11		
Number of hospitalizations		
≤ 10	2.05 ± 0.91	
≥ 11	2.41 ± 1.07	0.016
Antipsychotics		
first generation	2.16 ± 1.01	
second generation	2.18 ± 0.93	0.914
Mood stabilizers		
no	2.38 ± 0.93	
yes	2.07 ± 0.98	0.079
Hypnotics		
no	2.11 ± 0.99	
yes	2.27 ± 0.95	[a]0.241
Anxiolytics		
no	2.27 ± 1.11	
yes	2.14 ± 0.95	0.444
Antidepressants		
no	2.14 ± 0.99	
yes	2.47 ± 0.83	0.307
Antiparkinsonics		
no	2.09 ± 1.00	
yes	2.22 ± 0.96	0.459

The univariate regression analysis showed a statistical significance of the CPITN among the inpatients with schizophrenia in terms of gender ($p = 0.044$) and age ($p = 0.018$), brushing teeth (0.001), a tooth brush technique ($p = 0.009$), the duration of schizophrenia ($p = 0.002$), the number of hospitalizations (0.004) and the use of mood stabilizers ($p = 0.048$).

However, in the multivariate regression analysis, only gender ($p = 0.013$) and age ($p = 0.010$) were the statistically significant predictors of the CPITN value among the inpatients with schizophrenia.

Discussion

The presented study was conducted to assess the periodontal health and possible risk factors that might influence periodontal health among the inpatients with schizophrenia. The principal finding in this study was a high prevalence of periodontal disease among the inpatients with schizophrenia. The average value of the CPITN in the inpatients with schizophrenia was a significantly higher than that of the control group, which is in accordance with similar study [15].

The CPITN revealed poor periodontal health of inpatients with schizophrenia, whereas the healthy periodontium was observed in only 4.7% of subjects, which is even lower in comparison to the previous studies [10, 15, 24–26]. The presence of calculi (46.8%) was the most common finding in the study group, in contrast to gingival bleeding (45.8%) which was observed in the control group.

Previous studies reported a significantly higher occurrence of calculi in the psychiatric inpatients (range 71.8% to 94.2%) [24, 25, 27]. The presence of shallow pockets was observed in 24.2% of patients in the study group, much more than in the control group (7.9%). Furthermore, the deep periodontal pockets were detected in 11.1% in the study group, while in the control group they were detected in 5.8% of cases. The occurrence of deep periodontal pockets in the study group is in accordance with previous studies [10, 11, 16, 24].

Table 6

The value of the Community Periodontal Index for Treatment Needs (CPITN) among the study group examined by the linear regression models

Observed characteristics	Univariate linear regression analysis		Multivariate linear regression analysis	
	[#]B (95%CI)	p	[#]B (95%CI)	p
Gender	-0.284	0.044	-0.334	0.013
Age	0.024	0.018	0.018	0.010
Education	-0.087	0.273	/	/
Employment	0.050	0.420	/	/
Marital status	-0.115	0.238	/	/
Residence	-0.094	0.386	/	/
Smoking cigarettes	-0.246	0.131	/	/
Brushing teeth	-0.209	0.001	-0.118	0.072
Tooth brushing technique	0.410	0.009	0.273	0.097
Duration of disease	0.022	0.002	-0.003	0.771
Number of hospitalizations	0.036	0.004	0.026	0.113
Antipsychotics	0.021	0.888	/	/
Antiepileptics	-0.308	0.048	-0.206	0.160
Hypnotics and sedatives	0.160	0.290	/	/
Benzodiazepines	-0.123	0.528	/	/
Antidepressants	0.330	0.210	/	/
Antiparkinsonics	0.131	0.363	/	/

[#] – Uni-standardized coefficient B.

Higher occurrence of CPITN values among the inpatients with schizophrenia can be explained by several facts. First of all, in the present study, more than one half of inpatients with schizophrenia (54.7%) did not brush their teeth, which is in contrast to previous studies [2, 15, 24]. It has been already described that the neglected oral hygiene increases the accumulation of dental plaque, which leads to the gingival inflammation and periodontal disease [28]. The highest value of the CPITN was observed in the patients who did not brush their teeth, compared to those who brushed their teeth once a day, or twice, or more times per day. Likewise, 72.6% of inpatients with schizophrenia did not know how to perform a correct tooth-brushing technique.

In the present study, 74.2% of inpatients with schizophrenia were smokers. It is well-known that stress (like in the people with psychiatric diseases) can cause a behaviour modification, such as smoking, and may have an immunosuppressant effect (decreased leukocyte count, altered helper T1 cell/T2 cell ratio), which can result in more frequent recurrence of periodontal disease [28]. Smoking appear to be a periodontal disease modifier – do not cause periodontal disease, but it can increase the rate of progression of the disease, by determining the age of clinical diagnosis and the severity at given age [14]. The risk for periodontal disease is 2.5 to 7 times higher in smokers than in non-smokers [29]. Smokers have a clinically less pronounced inflammation and less gingival bleeding [29]. Vasoconstriction caused by nicotine reduces blood flow, leading to oedema and clinical signs of inflammation [29], which may be an explanation for lower rate of bleeding on probing in the study group.

Schizophrenia in the study group lasted 14.69 ± 9.61 years, on average, which is not in line with previous study [30]. The present study showed a large number of hospitalizations per patient (8.52 ± 5.71, range 1–30), which points to the fact that the patients were hospitalized for a proportionally long period of time, which is in accordance with the already published data [2, 4, 10, 11]. The inpatients with schizophrenia were treated by several psychotropic drugs. Previous study [4] reported some oral side-effects of psychotropic drugs on buccal mucosa, and revealed that antipsychotics, benzodiazepines, antidepressants and mood stabilizers were related to xerostomia, because they interfere with the salivary glands' function. Furthermore, another study [26] concluded that xerostomia was recognized as a high risk factor for development of periodontal disease in the patients with schizophrenia due to the decrease in the salivary flow rate. Xerostomia can also increase accumulation of dental plaque [21], which is one of the important causes of periodontal disease [27]. All these facts may explain the higher CPITN values in the study group.

In the present study, 50% of inpatients with schizophrenia were the females. Therefore, the gender could not influence the difference in the CPITN values between the patients in the study and control groups in this study, although it was noticed that the CPITN values were higher in the males [10, 15], probably due to the observed ignorance toward oral hygiene among the males [31]. Concerning the age, the mean age of patients in the study group was in line with the previous study [8]. The inpatients younger than 30 years had the lower CPITN than the patients older than 50 years of age, which is in accordance with the previous studies [16, 24]. The increased severity of periodontal disease in older age is probably related to the length of time of periodontal tissues exposure to dental plaque, which reflects the individual cumulative oral history [32].

By the stepwise multiple regression analysis, it was demonstrated that the gender and age of inpatients with schizophrenia could influence the CPITN values. The higher CPITN values were observed in the males compared to the females, and in the older compared to the younger inpatients with schizophrenia. This is in accordance with the previous findings [8, 10, 15, 16, 24]. In the present study, the sociodemographic characteristics, oral health habits and medical characteristics did not influence the mean value of the CPITN in the inpatients with schizophrenia, which is not in line with the previous studies [8, 10, 15, 16]. Our findings suggest that the underlying disease affects oral health indirectly, reducing the patients' motivation for the oral health maintenance.

Certain limitations should be considered when interpreting the results of this study. All subjects in the study group were hospitalized at the Clinic for Psychiatric Disorders, which possesses a dental office enabling dental care within the easy patients' reach. Therefore, it can be assumed that in other psychiatric hospitals, in country, the inpatients with schizophrenia could exhibit even poorer periodontal health. Also, the control group patients were the outpatients coming to the Department of Periodontology and Oral Medicine, Faculty of Dental Medicine, University of Belgrade, Serbia, who were very much familiar with the tooth brushing technique and oral hygiene maintenance. This fact could contribute to the differences between the CPITN values of the study and the control groups in this study.

Conclusion

The high CPITN values of inpatients with schizophrenia indicate a need for continuous considering the treatment needs of their oral and periodontal health improvement, especially during the hospitalization periods. Also, the results of this study indicate the need for a continuous research of psychiatric patients' oral health, in order to determine the modes of its improvement. Similar studies should elucidate a significance of psychiatric patients' periodontal health and sensitize the psychiatrists and psychiatric nurses to the oral problems of their patients.

R E F E R E N C E S

1. *World Health Organization.* Promoting mental health: concepts, emerging evidence, practice: summary report / a report from the World Health Organization, Department of Mental Health and Substance Abuse in collaboration with the Victorian Health Promotion Foundation and the University of Melbourne. Geneva, Switzerland: World Health Organization; 2004.
2. *Bertaud-Gounot V, Kovess-Masfety V, Perrus C, Trohel G, Frederique R.* Oral health status and treatment needs among psychiatric

inpatients in Rennes, France: a cross-sectional study. BMC Psychiatry 2013; 13: 227.

3. *Morales-Chavez MC, Rueda-Delgado YM, Pena-Orozco DA*. Prevalence of bucco-dental pathologies in patients with psychiatric disorders. J Clin Exp Dent 2014; 6(1): e7–e11.

4. *Farhadmollashahi L, Lashkaripour K, Bakhshani N, Faghihinia M*. Dental Health Status in Hospitalized Psychiatric Patients in Sistan and Baluchestan Province, Iran. Health Scope 2014; 3(4): e21626.

5. Maastricht University Library. "Schizophrenia" Concise Medical Dictionary. Oxford: Oxford University Press; 2010.

6. Republic of Serbia, Ministry of Health. National guideline of good clinical practice for diagnostic and therapy of schizophrenia. 2013. Available from:
http://www.zdravlje.gov.rs/showpage.php?id=145.

7. Report of BSDH Working Group. *Griffiths J, Jones V, Leeman I, Patel K, Wilson K, Blankenstein R*. Oral health care for people with mental health problems: Guidelines and recommendations. Registered Charity No: 1044867. London: British society for disability and oral health; 2000.

8. *Gopalakrishnapillai AC, Iyer RR, Kalantharakath T*. Prevalence of periodontal disease among inpatients in a psychiatric hospital in India. Spec Care Dentist 2012; 32(5): 196–204.

9. *Jayakumar HL, Jyothi D, Manesch CK, Pallavi HN*. Periodontal health among elderly psychiatric patients in Bangalore city-India. Pak Oral Dent J 2011; 31(1): 128–36.

10. *Gurbuz O, Alatas G, Kurt E, Dogan F, Issever H*. Periodontal health and treatment needs among hospitalized chronic patients in Istanbul, Turkey. Community Dent Health 2011; 28(1): 69–74.

11. *Chu K, Yang N, Chou P, Chiu H, Chi L*. Comparison of oral health between inpatients with schizophrenia and disabled people or the general population. J Formos Med Assoc 2012; 111(4): 214–9.

12. *Denis F, Millot I, Abello N, Carpentier M, Peteuil A, Soudry-Faure A*. Study protocol: a cluster randomized controlled trial to assess the effectiveness of a therapeutic educational program in oral health for persons with schizophrenia. Int J Ment Health Syst 2016; 10: 65.

13. *Wey MC, Loh S, Doss JG, Abu Bakar AK, Kisely S*. The oral health of people with chronic schizophrenia: A neglected public health burden. Aust N Z J Psychiatry 2016; 50(7): 685–94.

14. *Kornman KS*. Commentary: periodontitis severity and progression are modified by various host and environmental factors. J Periodont 2014; 85(12): 1642–5.

15. *Kebede B, Kemal T, Abera S*. Oral health status of patients with mental disorders in southwest ethiopia. PLoS ONE 2012; 7(6): e39142.

16. *Chandu G, Shafiulla M, Kumar M*. Oral health status and treatment needs in institutionalized psychiatric patients: One year descriptive cross sectional study. Indian J Dent Res 2006; 17(4): 171.

17. *von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP*. STROBE Initiative. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. BMJ 2007; 335(7624): 806–8.

18. *World Medical Association*. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 2013; 310(20): 2191–4.

19. *World Health Organisation*. The ICD-10 classification of mental and behavioral disorders. Geneva, Switzerland: World Health Organisation; 1992.

20. *Armitage GC*. Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000. 2004; 34: 9–21.

21. *Scully C, Bagan JV*. Adverse drug reactions in the orofacial region. Crit Rev Oral Biol Med 2004; 15(4): 221–39.

22. Oral Health surveys: basic methods. 5th ed. Geneva, Switzerland: World Health Organisation; 2013.

23. *Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J*. Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J 1982; 32(3): 281–91.

24. *Ujaoney S, Motwani MB, Khairkar PH, Dewgekar SS, Bang G*. Oral health status and dental treatment needs in institutionalized versus non-institutionalized psychiatric patients. J Disabil Oral Health 2010; 11(4): 163–70.

25. *Friedlander AH, Friedlander IK, Marder SR*. Bipolar I disorder: psychopathology, medical management and dental implications. J Am Dent Assoc 2002; 133(9): 1209–17.

26. *Eltas A, Kartalcı Ş, Eltas ŞD, Dündar S, Uslu MO*. An assessment of periodontal health in patients with schizophrenia and taking antipsychotic medication. Int J Dent Hyg 2012; 11(2): 78–83.

27. *Hasan A, Palmer RM*. A clinical guide to periodontology: Pathology of periodontal disease. Br Dent J 2014; 216(8): 457–61.

28. *Malathi K, Sabale D*. Stress and periodontitis: A review. IOSR J Dent Med Sci 2013; 9(4): 54–7.

29. *Aurer A*. Risk factors of systemic disease and periodontitis. Medix 2003; 50: 117–8.

30. *Tani H, Uchida H, Suzuki T, Shibuya Y, Shimanuki H, Watanabe K*, et al. Dental conditions in inpatients with schizophrenia: A large-scale multi-site survey. BMC Oral Health 2012; 12: 32.

31. *Albandar JM, Kingman A*. Gingival recession, gingival bleeding, and dental calculus in adults 30 years of age and older in the united states, 1988-1994. J Periodontol 1999; 70(1): 30–43.

32. *Loe H, Anerud A, Boysen H, Morrison E*. Natural history of periodontal disease in man. Rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. J Clin Periodontol 1986; 13(5): 431–45.

Herpes zoster – is there a need for new treatment recommendations?

Herpes zoster – da li su potrebne nove preporuke za lečenje?

Uroš Karić*, Nataša Katanić*‡, Sanja Peruničić*, Nikola Mitrović*,
Nataša Nikolić*, Marko Marković*, Ksenija Bojović*†, Jovan Malinić*†,
Jasmina Poluga*†, Jasmina Simonović Babić*†

Clinical Center of Serbia, *Clinic for Infectious and Tropical Diseases, Belgrade, Serbia;
University of Beograde, †Faculty of Medicine, Belgrade, Serbia; University of Priština/
Kosovska Mitrovica, ‡Faculty of Medicine, Kosovska Mitrovica, Serbia

Abstract

Background/Aim. The reactivation of the *varicella zoster* virus results in herpes zoster. Acyclovir is currently recommended over 7 to 10 days for herpes zoster treatment and should be started within 72 hours of rash eruption. This study analyses whether a therapy delay and/or shorter courses of treatment are associated with adverse outcomes. **Methods.** We identified 292 patients treated at the Clinic for Infectious and Tropical Diseases in Belgrade for herpes zoster in a five-years period. The data on these patients were analyzed using the descriptive statistics, the χ^2 test, the Mann-Whitney U-test and the multiple logistic regression analysis. **Results.** The average time from rash eruption to the first dose of acyclovir was 4.07 ± 2.64 days. The patients received acyclovir for 6.83 ± 2.45 days. Seventy-one patients had disseminated herpes zoster, 100 had cranial nerve involvement, 86 had complications other than postherpetic neuralgia and one patient died. In cases where therapy was delayed there was no significant association with complications ($\chi^2 = 0.031$; $p = 0.86$). Our logistic regression model was not able to predict who was treated less than 7 days. An association between the HZ complications and abbreviated acyclovir regimens was not demonstrated ($\chi^2 = 1.109$; $p = 0.326$). We conducted the PubMed search on February 1st, 2017 and found no proof for the need to apply at least 7 days of acyclovir therapy for herpes zoster in the studies that have been published so far. **Conclusion.** We were unable to prove an association between therapy delay and unfavorable outcomes. The same was true for shorter than recommended acyclovir courses.

Key words:
varicella zoster virus infection; recurrence; drug users; treatment outcome.

Apstrakt

Uvod/Cilj. Rektivacija *varicela zoster* virusa dovodi do herpesa zostera (HZ). Prema aktuelnim preporukama terapija ove bolesti podrazumeva 7 do 10 dana primene aciklovira. Terapiju treba započeti unutar 72 časa od pojave ospe. Ova studija imala je za cilj da proceni da li je kašnjenje u započinjanju terapije i/ili skraćeno trajanje terapije aciklovirom povezano sa lošim tokom i ishodom HZ. **Metode.** Sprovedena je analiza 292 bolesnika koji su lečeni zbog herpes zostera u Klinici za infektivne i tropske bolesti u Beogradu u petogodišnjem periodu. Podatke o ovim bolesnicima analizirali smo pomoću metoda deskriptivne statistike, χ^2- testa, Man-Vitni U-testa i multiplom logističkom regresijom. **Rezultati.** Vreme od pojave ospe do prve doze aciklovira iznosilo je 4,07 ± 2,64 dana. Bolesnici su lečeni aciklovirom prosečno 6,83 ± 2,45 dana. Sedamdeset jedan bolesnik imao je diseminovanu bolest, kod 100 su bili zahvaćeni kranijalni nervi, a kod 86 su se razvile komplikacije. Jedan bolesnik je preminuo. Slučajevi kod kojih je došlo do kašnjenja u započinjanju terapije nisu bili statistički značajno povezani sa komplikacijama ($\chi^2 = 0.031$; $p = 0.86$). Logistička regresija nije uspela da predvidi ko je bio lečen aciklovirom više ili manje od sedam dana. Nije dokazana povezanost između komplikacija HZ i terapijskih režima kraćih od preporučenih ($\chi^2 = 1.109$; $p = 0.326$). Pregledom *PubMed* baze podataka učinjenim 1. februara 2017. godine, u do tada objavljenim studijama nismo pronašli dokaze o tome da je neophodno najmanje sedam dana terapije aciklovirom za herpes zoster. **Zaključak.** Nije dokazano da je kašnjenje u započinjanju terapije aciklovirom povezano sa nepovoljnim ishodima. Isto se može reći i za kraće terapijske kure.

Ključne reči:
infekcija, varičela-zoster virus; recidiv; aciklovir; lekovi, korišćenje; lečenje, ishod.

Correspondence to: Uroš Karić, Clinical Center of Serbia, Clinic for Infectious and Tropical Diseases, Bulevar oslobođenja 16, 11 000 Belgrade, Serbia. E-mail: uroskaric@gmail.com

Introduction

Reactivation of the *varicella zoster* virus results in a vesiculous rash that follows a dermatomal distribution usually accompanied by pain or itching, and is known as herpes zoster (HZ) or shingles [1].

The incidence rate of HZ is 4 to 4.5 per 1000 person/years. The cumulative lifetime incidence of HZ is 10%–20% [2]. Approximately 50% of 85-year-olds have experienced at least a single episode of HZ in their lifetime [3, 4]. HZ rarely occurs in younger patients [5]. The immunocompromised patients are at a high risk for HZ with a cumulative lifetime higher than 50%. HZ is generally considered to have no gender predilection, although some types demonstrated a higher prevalence of HZ in women [6, 7].

HZ is rarely fatal in the immunocompetent patients, whereas the mortality rate in the immunocompromised patients with disseminated disease is 5% to 15% [3].

HZ involving the ophthalmic branch of the trigeminal nerve is called herpes zoster ophtalmicus (HZO) [3]. The prevalence of HZO was reported to be 8% and 56% among the people with HZ [8]. HZO is important because it often affects the structures of the eye.

The otorhinolaryngology specialists can occasionally encounter HZ that involves the geniculate ganglion of the facial nerve producing earache, external auditory meatus rash, vestibulocochlear dysfunction and even disorders of taste. This clinical entity is called herpes zoster oticus. The Ramsay-Hunt syndrome (RHS) is defined as herpes zoster oticus with a facial nerve palsy. RHS has an incidence rate of 5 per 100,000 person-years [9].

The most common complication of HZ is postherpetic neuralgia (PHN). No clear official consensus exists, but the majority of the authors define PHN as persistent or recurrent pain lasting between one and six, or more months (depending on the author) after the beginning of the eruptive phase of HZ [10, 11]. PHN is recorded in 9% to 45% of those who had a bout of HZ [12, 13].

Other neurologic complications, ophthalmologic complications and bacterial superinfections also occur although they are less common. The neurologic complications other than PHN are aseptic meningitis, encephalitis, myelitis, peripheral neuropathies and the Guillain-Barre syndrome [14].

Immunosuppression carries a greater risk of complications in the patients with HZ. In this setting, the skin lesions may disseminate and cause multiple dermatomes, sometimes crossing over the midline or even taking on a chickenpox-like form. Cases of pneumonia, encephalitis and hepatitis were also reported [15].

The patient should start the antiviral therapy if he/she is 50 years of age, or older, has disseminated HZ, atopic dermatitis, intense pain, involvement of the face or complications of HZ, or immunocompromised [1, 16, 17]. Of the recommended antivirals only acyclovir is available in Serbia.

Shorter rash evolution, reduced duration of viral shedding, shorter pain duration and lower incidence of PHN were all reported in the patients started antivirals within the first 72 hours after the skin lesions first appear [1, 3, 16]. While most experts suggest a 72-hours deadline to start a therapy, others recommend prescribing antivirals even at later stage of the disease, especially if new lesions still form [16, 18, 19].

Intravenous acyclovir (10 mg/kg three times a day over 7 to 10 days) should be administered if the patients are immunocompromised, have disseminated HZ, cranial nerve involvement, or develop certain complications other than PHN [17, 20, 21]. Since intravenous acyclovir should be administered in an inpatient setting, the above mentioned subgroups of patients are also the ones who require hospitalization. In most studies, the hospitalization was found to be warranted in about 3% of patient with HZ [22, 23]. Some authors treated the patients with antivirals until clinical resolution was achieved, or no new lesions observed [24, 25].

In this study, our goal was to answer the question of whether shorter courses of therapy, or therapy delays were associated with the unfavorable outcomes of HZ. Expanding on this query, we tried to find evidence of the need for at least 7 days of antiviral therapy in the research conducted by other authors around the world.

Methods

We conducted a single cohort retrospective study. We searched the paper-based medical records of all the patients treated from January 1st, 2011 to December 31st, 2015 at the Respiratory and Skin Infection Ward of the Clinic for Infectious and Tropical Diseases, the Clinical Center of Serbia in Belgrade. The patients that were treated within that time frame for HZ as a primary diagnosis were included in the study and demographic, epidemiologic, clinical and laboratory data were obtained.

We found that 292 people were treated at our hospital for herpes zoster in the five year period analyzed in the study. The mean age of the patients was 65.07 ± 17.19 years with the youngest patient being 3 years old and the oldest one 94. One hundred and fifty-eight women (54.1%) and 134 men (45.9%) were included in the study. The majority of patients (91.1%) were residents of Belgrade.

We considered the antiviral therapy warranted if the patents had HZ-like rash that lasted less than 72 hours, or HZ-like rash and any of the following: more than, or equal to 50 years of age, disseminated HZ, immunosuppression, atopic dermatitis, involvement of the face and complications of HZ other than PHN.

The severity of pain and the severity of rash could not be determined from the medical histories of the patients, therefore these parameters were not included, although many authors included them [22].

We considered hospitalization justified if any of the following was present: complications of HZ other than PHN, disseminated HZ, immunocompromised state of the patient and cranial nerve involvement.

The patients who had chemotherapy and/or radiotherapy, allogenic hematopoietic stem cell transplant and the solid organ transplant recipients, human immunodeficiency virus (HIV) positive individuals and the patients on chronic corticosteroid or immunomodulatory therapy (e.g., the patients with rheumatologic disorders, inflammatory bowel disease, multiple sclerosis, etc.) were considered to be immunocompromised.

The compiled data were included in the descriptive statistics analysis using the IBM SPSS Statistics version 16.0. Only the patients in whom the hospitalization was objectively indicated were subjected to further analysis. The χ^2 test (categorical variables with more than 5 observations in a single cell) and the Mann-Whitney U (MWU) test (continuous variables with a non-normal distribution, or heterogeneous variances) were used in an attempt to assess the possible association between the treatment delay and/or a shorter treatment course and unfavorable outcomes. We used the multiple logistic regression model in order to identify the predictors of abbreviated antiviral treatment.

The research was conducted in accordance with the ethics standards of the Declaration of Helsinki.

Results

The age frequency distribution histogram illustrates a clearly non-normal distribution (Shapiro-Wilk statistic = 0.885; $p < 0.001$) and a negative skew (skewness = -1.392 ± 0.143). One hundred and eighty patients (61.6%) were 65 years of age, or older and 252 (86.3%) were 50 years of age, or older (Figure 1). The women were overrepresented in our study but this was not found to be statistically significant ($p = 0.178$).
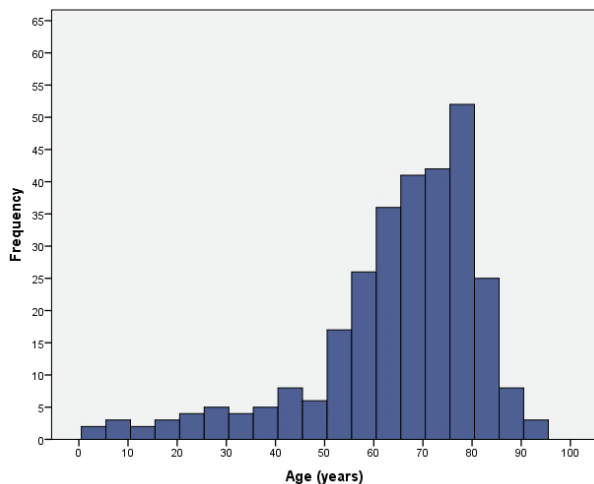
Fig. 1 – Patient age distribution histogram.

Figure 2 illustrates the seasonal variations of HZ. Even though Figure 2 seems to illustrate that the significant seasonal variations of HZ exist, the χ^2 test failed to demonstrate a statistically significant difference ($p = 0.059$).
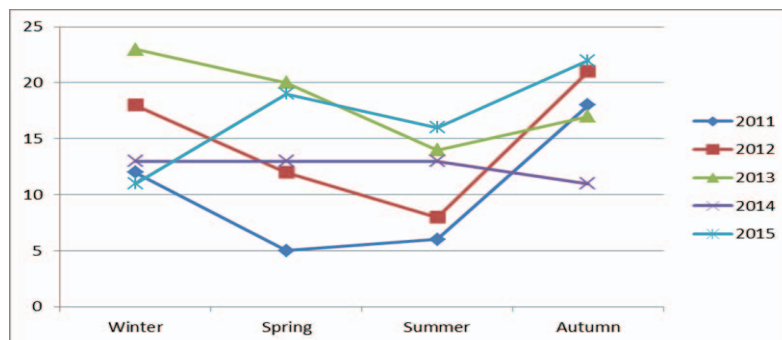
The clinical characteristics of HZ are presented in Table 1. The cranial nerves were most commonly affected by HZ. The ophthalmologic complications were the most frequent at 18.5%.

Table 1
Clinical characteristics of herpes zoster

Parameters	Patients, n (%)
Affected nerve	
cranial nerves	97 (33.2)
cervical spinal nerves	46 (15.8)
thoracic spinal nerves	96 (32.9)
lumbar and sacral spinal nerves	53 (18.2)
Complications	
HZO with eye involvement	54 (18.5)
neuroinfection	4 (1.4)
impetiginisation	37 (12.7)
pneumonia	2 (0.7)
PHN	12 (4.1)

HZO – herpes zoster opthalmicus;
PHN – postherpetic neuralgia.

The average time from the eruption of the rash to the first dose of acyclovir was 4.07 ± 2.64 days (range 0–15). Some 50.8% received acyclovir even though the rash lasted longer than 72 hours before the initiation of therapy.

Thirty-two patients were immunocompromised (15 were on immunosuppressant drugs, 14 were undergoing chemotherapy or radiation therapy and 3 were organ transplant recipients), 71 had disseminated HZ, 100 had the cranial nerve involvement and 86 had complications other than PHN. A single patient had atopic dermatitis. A single patient died and 5 patients were discharged against medical advice. We found that 5 patients (1.7%) did not require treatment with antiviral drugs at all. The hospitalization had objectively been indicated in 188 (64.4%) patients. The results presented in the subsequent paragraphs concern these 188 patients.

The patients received acyclovir for 6.83 ± 2.45 days (range 1–16 days), with 61 (32.4%) patients treated for less than 7 days (Figure 3). Eight patients (4.3%) were treated for more than 10 days and 48 (25.5%) were treated for more than 7 days. No data on treatment duration was available for 31 (16.5%) patients.

Fig. 2 – Seasonal incidence of herpes zoster spanning 5 years.
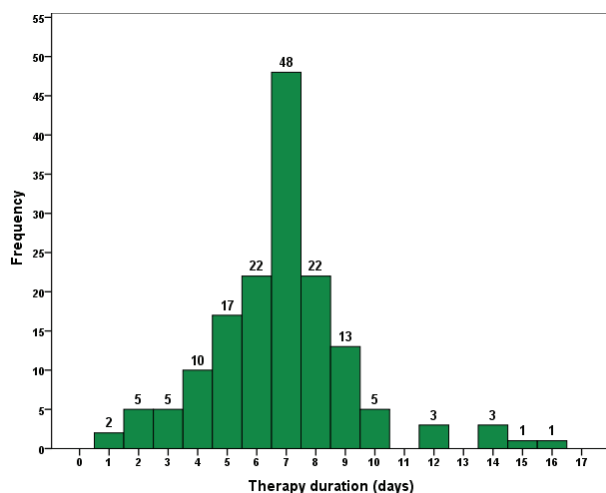
**Fig. 3 – Histogram of acyclovir treatment duration
(days).**

All patients were discharged on the completion of antiviral therapy so the hospital stay was exactly equal to the duration of antiviral therapy.

In cases in which therapy was instituted 72 hours after the HZ rash first appeared, there was no significant association with the complications ($\chi^2 = 0.031$; $p = 0.86$). There was also no association with the overall duration of acyclovir therapy (MWU = 2645; $p = 0.131$).

The gender, patient age, longer than 3 days from the rash eruption to treatment, presence of disseminated disease, immunosuppression and cranial nerve involvement as well as the presence of complications other than PHN were entered into the multiple logistic regression model and it proved not to be able to predict who amongst our patients would be treated for less than 7 days ($\chi^2 = 6.84$, $p = 0.446$).

No association between the HZ complications and an acyclovir therapy regimen, or shorter than 7 days ($\chi^2 = 1.109$; $p = 0.326$) was demonstrated. The therapy duration was not significantly different between the patients with and without the HZ dissemination (MWU = 2780; $p = 0.941$), complications (MWU = 1456; $p = 0.341$), immunodeficiency (MWU = 1456; $p = 0.102$), or the patients with and without the cranial nerve involvement (MWU = 2699; $p = 0.189$). The association with the fatal outcomes in the cases of shorter antiviral treatment regimens was not analyzed due to the rarity of lethal outcomes in our cohort.

No other antivirals beside acyclovir were given to our patients. Local acyclovir ointment was prescribed to 38 (13%) patients. One hundred thirty-one (54.2%) patients received local or systemic antibiotics. The majority of patients, that is, 149 (51%), were treated with nonsteroidal anti-inflammatory drug (NSAID), while 43 patients (14.7%) were prescribed opioids, and another 14 (4.8%) needed antiepileptics in order to achieve an adequate pain control. Diclofenac was most commonly utilized, around the clock, for pain management in our study, with 144 patients (49.3%) receiving the drug.

We conducted a search in the PubMed database on February 1st 2017 using the formula [(day) OR days] AND ["Herpes Zoster/therapy" (Major) AND acyclovir] with the

filter set to human. Two researchers independently reviewed the papers that were identified by the search algorithm in the search to prove the need for at least 7 days of acyclovir therapy for herpes zoster. Our search in the PubMed database yielded 190 results. Upon reviewing all the papers we found no evidence of the need for at least 7 days of acyclovir therapy. The oldest study designs even used 5 days of acyclovir therapy as a standard regimen.

Discussion

The majority of the authors have found HZ to be a disease of the elderly and the same is true of this study [3, 4]. Although some demonstrated a predomination of women amongst the patients with HZ, we failed to demonstrate any significant difference between the genders when it comes to HZ [7]. According to the 2011 census, Belgrade has 1,659,440 inhabitants, and our study indicates that the average number of patients hospitalized every year for HZ is 53.2. We can extrapolate that the incidence rate of hospitalization is at least 3.2 patients per 100,000 person-years [26]. This is significantly less than what had been reported by other countries. A possible explanation for this anomaly is the fact that a certain number of patients are hospitalized in other hospitals, or in other wards of our clinic, thereby underestimating the true incidence of HZ related hospitalizations.

Despite the evidence of seasonal variations in the incidence of HZ that are thought to be related to changes in atmospheric temperature and insolation, our study failed to corroborate such claims [27]. Paradoxically, we even demonstrated that the incidence of HZ was lowest in summer months (this was statistically nonsignificant), the opposite of what other authors found to be true [28].

We showed that a number of patients with HZ were hospitalized without the clear indications. The fact that more than half of our patients received acyclovir after more than 72 hours after the rash appearance is also worrisome. Yet, there seemed to be no association between the therapy delay and unfavorable outcomes like the development of complications, or longer hospital stay. A possible association with a lethal outcome could not be reliably analyzed due to the fact that a single patient in our cohort had died.

The frequency of herpes zoster oticus was less than expected in our study [9]. Cases of HZ associated with neuroinfection were also rare, due to the fact that these patients required a treatment by the intensive care specialists and were treated in the ICU ward of our clinic [14, 29].

The researchers in Iceland found that no patients younger than 50 years of age had any serious pain, while 6% of patients older than 60 yeras of age reported the severe pain a month after HZ, and 4% reported the severe pain three months after HZ [30]. The frequency of PHN was lower in our study in comparison with the data published by other authors [10]. We speculate that this is a consequence of the outpatient management of PHN as well as management by a neurologist.

The average length of inpatient acyclovir treatment in our cohort was similar to that reported by other centers [31, 32]. Around a quarter of the patients in our study were treated

longer than one week. Our logistic regression model could not predict which patients would be treated with acyclovir shorter than 7 days. Also, we found no significant differences in acyclovir treatment duration between those with and those without the dissemination, complications, immunodeficiency and cranial nerve involvement. Consequently, we cannot say that we were able to understand the logic that led the treating physicians to prescribe neither longer nor shorter courses of acyclovir therapy. The acyclovir shortages, that were sadly commonplace in the five year period analyzed, were definitely the most important factor that influenced the decision to utilize abbreviated antiviral regiments.

Viewed another way, the lack of association between the presence of complications and abbreviated acyclovir treatment regimens could be interpreted as a proof of safety of therapy courses shorter than 7 days. Therefore, the acyclovir shortages, though unfortunate, offered a chance to observe the outcomes of shortened acyclovir treatment in all, except the most severe cases of HZ. The conclusion we present in the first sentence of this paragraph cannot be generalized to all patients with HZ since those with severe pneumonia requiring the mechanical ventilation, and/or the central nervous system infections were not analyzed.

Even more intriguingly, we were unable to find a conclusive proof that less than 7 days of acyclovir treatment is associated with unfavorable outcomes in any of the 190 papers we reviewed.

We feel that a major limitation of this study was the lack of objective measures of pain intensity and rash severity. There are also no data on the skin lesion progression that could potentially be useful for determining if there is a need for an extended course of acyclovir therapy. Also, on more than one occasion, there was an acyclovir shortage which might have forced the treating physicians to shorten treatment even though they might have not felt this had been justified from a medical perspective. On the other hand, this can hardly be considered relevant since none of these patients had an unfavorable outcome, despite what is considered a subpar therapy. As noted earlier, few patients with the HZ associated neuroinfections were included in the study.

Conclusion

Our study demonstrated that an unacceptably high number of patients with HZ were hospitalized without clear indications pointing to a need for comprehensive guidelines for the HZ management in Serbia. While the majority of authors recommend at least 7 days of acyclovir therapy, we found no proof, both in the published literature and in this study, that this approach is superior to shorter treatment courses. We believe that the new, evidence-based recommendations regarding the HZ management are necessary in order to avoid the unwarranted hospitalizations and unreasonably long treatment courses, thereby cutting down the HZ-related costs and sparing the patients from the psychological stress associated with hospitalization.

R E F E R E N C E S

1. Dworkin RH, Johnson RW, Breuer J, Gnann JW, Levin MJ, Backonja M, et al. Recommendations for the Management of Herpes Zoster. Clin Infect Dis 2007; 44 Suppl 1: S1–26.
2. Straus SE, Ostrove JM, Inchauspé G, Felser JM, Freifeld A, Croen KD, et al. . Varicella-Zoster Virus Infections: Biology, Natural History, Treatment, and Prevention. Ann Intern Med 1988; 108(2): 221–37.
3. Gnann JW Jr, Whitley RJ. Clinical practice. Herpes zoster. N Engl J Med 2002; 347(5): 340–6.
4. Nagasako EM, Johnson RW, Griffin DR, Elpern DJ, Dworkin RH. Geographic and racial aspects of herpes zoster. J Med Virol 2003; 70 Suppl 1: S20–3.
5. Hope-Simpson RE. The nature of herpes zoster: a long-term study and a new hypothesis. Proc R Soc Med 1965; 58(1): 9–20.
6. Donahue JG, Choo PW, Manson JE, Platt R. The Incidence of Herpes Zoster. Arch Int Med 1995; 155(15): 1605–9.
7. Insinga RP, Itzler RF, Pellissier JM, Saddier P, Nikas AA. The incidence of herpes zoster in a United States administrative database. J Gen Intern Med 2005; 20(8): 748–53.
8. Pavan-Langston D. Herpes zoster ophthalmicus. Neurology 1995 ;45(12 Suppl 8): S50–1.
9. Uscategui T, Dorée C, Chamberlain IJ, Burton MJ. Antiviral therapy for Ramsay Hunt syndrome (herpes zoster oticus with facial palsy) in adults. Cochrane Database Syst Rev 2008; (4): CD006851.
10. Johnson RW, Rice AS. Clinical practice. Postherpetic neuralgia. N Engl J Med 2014; 371(16): 1526–33.
11. Yawn BP. Post-shingles neuralgia by any definition is painful, but is it PHN? Mayo Clin Proc 2011; 86(12): 1141–2.
12. van Wijck AJ, Opstelten W, Moons KG, van Essen GA, Stolker RJ, Kalkman CJ, et al. The PINE study of epidural steroids and local anaesthetics to prevent postherpetic neuralgia: a randomised controlled trial. Lancet 2006; 367(9506): 219–24.
13. Pasqualucci A, Pasqualucci V, Galla F, De Angelis V, Marzocchi V, Colussi R, et al. Prevention of post-herpetic neuralgia: acyclovir and prednisolone versus epidural local anesthetic and methylprednisolone. Acta Anaesthesiol Scand 2000; 44(8): 910–8.
14. Gilden DH, Kleinschmidt-DeMasters BK, LaGuardia JJ, Mahalingam R, Cohrs RJ. Neurologic complications of the reactivation of varicella-zoster virus. N Engl J Med 2000; 342(9): 635–45.
15. Gnann JW Jr. Varicella-zoster virus: atypical presentations and unusual complications. J Infect Dis 2002; 186 Suppl 1: S91–8.
16. Cohen JI. Clinical practice: Herpes Zoster. N Eng J Med 2013; 369(3): 255–63.
17. Gross G, Schöfer H, Wassilew S, Friese K, Timm A, Guthoff R, et al. Herpes zoster guideline of the German Dermatology Society (DDG). J Clin Virol 2003; 26(3): 277–89; discussion 291–3.
18. Cohen JI, Brunell PA, Straus SE, Krause PR. Recent Advances in Varicella-Zoster Virus Infection. Ann Intern Med 1999; 130(11): 922–32.
19. Wood MJ, Shukla S, Fiddian AP, Crooks RJ. Treatment of acute herpes zoster: effect of early (< 48 h) versus late (48-72 h) therapy with acyclovir and valaciclovir on prolonged pain. J Infect Dis 1998; 178 Suppl 1: S81–4.
20. Gupta S, Jain A, Gardiner C, Tyring SK. A rare case of disseminated cutaneous zoster in an immunocompetent patient. BMC Fam Pract 2005; 6: 50.
21. Gomez E, Chernev I. Disseminated cutaneous herpes zoster in an immunocompetent elderly patient. Infect Dis Rep 2014; 6(3): 5513.

22. *Yawn BP, Gilden D.* The global epidemiology of herpes zoster. Neurology 2013; 81(10): 928–30.

23. *Esteban-Vasallo MD, Domínguez-Berjón MF, Gil-Prieto R, Astray-Mochales J, Gil de Miguel A.* Temporal trends in herpes zoster-related hospitalizations in Madrid (Spain), 2003-2013. J Infect 201l;71(1): 85–92.

24. *Sarialioğlu F, Buyukpamukcu M, Cevik N, Kutluk MT, Akyol H, Kansu E.* Short-time and low-dose intravenous acyclovir therapy in varicella zoster infections with malignant disease in children receiving combined chemotherapy. Pediatr Hematol Oncol 1986; 3(3): 267–71.

25. *Rolston KV, Manzullo E, Elting L, Frisbee-Hume S, Rodriguez S, Rubenstein EB.* Ambulatory management of varicella-zoster virus infection in immunocompromised cancer patients. Support Care Cancer 1998; 6(1): 57–62.

26. Republički zavod za statistiku. Popis u Srbiji 2011. Preuzimanje popisnih rezultata. 2011. [cited 2016 Jun 12]. Available from: http://popis2011.stat.rs/?page_id=2162

27. *Yang Y, Chen R, Xu J, Li Q, Xu X, Ha S,* et al. The effects of ambient temperature on outpatient visits for varicella and herpes zoster in Shanghai, China: a time-series study. J Am Acad Dermatol 2015; 73(4): 660–5.

28. *Zak-Prelich M, Borkowski JL, Alexander F, Norval M.* The role of solar ultraviolet irradiation in zoster. Epidemiol Infect 2002; 129(3): 593–7.

29. *Weitzman D, Shavit O, Stein M, Cohen R, Chodick G, Shalev V.* A population based study of the epidemiology of Herpes Zoster and its complications. J Infect 2013; 67(5): 463–9.

30. *Helgason S, Petursson G, Gudmundsson S, Sigurdsson JA.* Prevalence of postherpetic neuralgia after a first episode of herpes zoster: prospective study with long term follow up. BMJ 2000; 321(7264): 794–6.

31. *Gabutti G, Serenelli C, Cavallaro A, Ragni P.* Herpes zoster associated hospital admissions in Italy: review of the hospital discharge forms. Int J Environ Res Public Health 2009;6(9): 2344–53.

32. *Schmidt SA, Kahlert J, Vestergaard M, Schønheyder HC, Sørensen HT.* Hospital-based herpes zoster diagnoses in Denmark: rate, patient characteristics,and all-cause mortality. BMC Infect Dis 2016; 16: 99.

Fecal sST2 correlates with the disease severity of ulcerative colitis

Fecesni sST2 korelira sa težinom ulceroznog kolitisa

Marina Jovanović*, Nevena Gajović†, Milena Jurišević‡, Bojana Simović Marković†,
Veljko Marić§, Milan Jovanović‖¶, Nebojša Arsenijević†, Nataša Zdravković*

University of Kragujevac, Faculty of Medical Sciences, *Department of Internal Medicine,
†Center for Molecular Medicine and Stem Cell Research, ‡Department of Pharmacy, Kragujevac, Serbia;
University of East Sarajevo, Faculty of Medicine, §Department of Surgery, Foča, Bosnia and Herzegovina;
Military Medical Academy, ‖Department of Abdominal Surgery, Belgrade, Serbia;
University of Defence, ¶Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia

Abstract

Background/Aim. Ulcerative colitis (UC) is a chronic, relapsing inflammatory disease affecting the distal colon and rectum with complex pathogenesis and diagnosis, indicating the need for new diagnostic and prognostic markers. The aim of this study was to determine the fecal values of TNF-α, IL-17, IL-10 and soluble protein ST2 (sST2) in the patients with UC and their relationship with clinicopathological aspects. **Methods.** The samples of stool of 80 patients with UC were analyzed. Concentrations of TNF-α, IL-17, IL-10 and sST2 were measured by ELISA. **Results.** Concentrations of TNF-α, IL-17 and sST2 were significantly increased in the feces of patients with the higher endoscopic, clinical and total Mayo score, as well as in the patients with an intense crypt destruction, erosion of the mucous membranes, architectural changes, neutrophil infiltration and eosinophil infiltration. The local value of anti-inflammatory cytokine IL-10 in liquid fraction of feces was increased in the patients with an advanced endoscopic stage of UC. The moderate positive correlation between the fecal sST2/IL-17 and the clinical and histological parameters of disease severity and also the strong correlation between sST2 and IL-17 was also observed in the feces of patients with UC. The analysis of receiver operating characteristic (ROC) curves showed that the optimal cut-off value for sST2 of 624.0 pg/g allows the discrimination of clinical stages of UC. **Conclusion.** The increased fecal value of sST2 in the UC patients with a higher endoscopic, clinical and histological stage of disease may be considered as a sign of the disease severity. The fecal values of sST2 can be used as a valuable marker for UC severity.

Key words:
colitis, ulcerative; biomarkers; disease progression; feces; tumor necrosis factor-alpha; interleukin-10; interleukin-17; il1rl1 protein, human.

Apstrakt

Uvod/Cilj. Ulcerozni kolitis (UC) je hronična, relapsirajuća inflamacijska bolest koja zahvata distalni kolon i rektum sa kompleksnom patogenezom i dijagnozom, ukazujući na potrebu za novim dijagnostičkim i prognostičkim markerima. Cilj studije bio je utvrđivanje vrednosti TNF-α, IL-17, IL-10 i rastvorljivog proteina ST2 (sST2) fecesu bolesnika sa UC kao i njihovog odnosa sa klinikopatološkim aspektima bolesti. **Metode.** Analizirani su uzorci stolice 80 bolesnika sa ulceroznim kolitisom. Koncentracije TNF-α, IL-17, IL-10 I sST2 merene su korišćenjem senzitivnog ELISA (*Enzyme-linked immunosorbent assay*) testa. **Rezultati.** Koncentracije TNF-a, IL-17 i sST2 značajno su bile povećane u fecesu bolesnika sa većim endoskopskim, kliničkim i ukupnim Mayo skorom, kao i kod onih sa izraženim oštećenjem kripti, erozijom sluzokože, strukturnim promenama tkiva, neutrofilnom infiltracijom i eozinofilnom infiltracijom. Lokalna vrednost antiinflamacijskog citokina IL-10 u tečnoj frakciji fecesa bila je povećana kod bolesnika sa uznapredovalim endoskopskim stadijumom bolesti. Takođe je nađena umerena pozitivna korelacija između vrednosti sST2/IL-17 u fecesu i kliničkih i histoloških parametara težine bolesti, kao i snažna korelacija između vrednosti sST2 i IL-17 u fecesu bolesnika sa UC. Analizom ROC krive ustanovljeno je da granična vrednost za sST2 od 624,0 pg/g determiniše potencijalno veći rizik za razvoj klinički teže forme UC. **Zaključak.** Povećane vrednosti sST2 u fecesu bolesnika sa UC sa uznapredovalim endoskopskim, kliničkim i histološkim stadijumom bolesti može se smatrati znakom težine bolesti. Vrednosti sST2 u fecesu mogu se koristiti kao marker procene težine UC.

Ključne reči:
kolitis, ulcerativni; biološki pokazatelj; bolest, progresija; stolica; faktor nekroze tumora; interleukin-10; interleukin-17; il1rl1 protein, humani.

Correspondence to: Nevena Gajović, University of Kragujevac, Faculty of Medical Sciences, Center for Molecular Medicine and Stem Cell Research, Svetozara Markovića 69, 34 000 Kragujevac, Serbia. E-mail: gajovicnevena@yahoo.com

Introduction

Inflammatory bowel disease (IBD) presents a chronic, relapsing and progressive inflammation of gastrointestinal tract [1]. The two most studied entities of IBD are the ulcerative colitis (UC) and Crohn`s disease (CD). UC is a chronic, relapsing inflammatory disease affecting the distal colon and rectum, limited to mucosa and associated with continuous, submucosal inflammation and the formation of ulcers [1–2]. The pathogenesis of disease is complex. The immune system seems to be the major mediator in it [3]. It is usually based on imbalance between pro- and anti- inflammatory cytokines in the intestinal mucosa and subsequent chronic inflammation [4–5]. The secretion of type 1 (TNF-α, IFN-γ), type 2 (IL-4, IL-10) and type 17 cytokines (IL-17) as well as a response to self-antigens in mucosa are predominant factors in the genesis and development of UC [6–9]. Although UC seems to be predominantly Th2 disease [10], recent data revealed an important role of type 1 and type 17 innate and acquired immune responses in the onset and progression of disease [11]. Local chronic inflammation, typical for UC, often leads to the clinical symptoms and signs such as diarrhea, rectal bleeding, abdominal pain, fever, anemia and body weight loss [2]. The disease activity can be determined by using the clinical and endoscopic scores, serum or fecal biomarkers [12–14]. The endoscopic findings of mucosa were classified as ones of the major parameters in estimating the disease severity [13–15]. A disadvantage of using the clinical and endoscopic scores for the disease severity evaluation is usage of invasive and costly procedures [15]. This has led to the need for noninvasive tests regarding the disease evaluation. There has been a sustained interest in the identification of state biomarkers for the UC severity [11, 15]. New markers should contribute to the prediction of prognosis.

The aim of the study was to compare the accuracy of selected biomarkers in assessing the disease severity. Concentrations of biomarkers TNF-α, IL-17, IL-10 and soluble ST2 protein (sST2) were assessed in the stool samples of patients with UC. We tested how reliably these biomarkers reflect the clinical and endoscopic scores and the histopathological characteristics of affected tissue. For more sensitive markers, we determined cut- off levels for the clinically and endoscopically estimated severe disease.

Methods

Ethic approvals

The study was conducted at the Center for Gastroenterology, Clinical Center of Kragujevac and the Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia. The ethics approvals for this study were obtained from relevant Ethics Committees of the Clinical Center Kragujevac, Kragujevac, Serbia, and Faculty of Medical Sciences, University of Kragujevac, Serbia. All experiments were performed according to the relevant guidelines and regulations. The informed consent was obtained from all patients in writing.

Patients

Eighty patients, between 21 and 80 years of age diagnosed as the UC cases were recruited in this study. A diagnosis was made on basis of established clinical, endoscopic and histological criteria [16]. The study did not include the patients with no well-defined pathology, no adequate clinical document available, or with previously diagnosed coexisting cardio-pulmonary, renal, hepatic, allergy and rheumatic disease, who were treated with anti-inflammatory drugs. The stool samples were taken before the surgery and stored at -80°C.

Disease activity index

The clinical activity of the disease was evaluated according to the data available from the patients' records. Three clinical variables were graded: frequency of evacuation, amount of blood in the stool, and a physician's global assessment. The Mayo clinical subscore 0 was defined as remission, 1–3 as mildly active disease (I), 4–6 as moderately active disease (II), and ≥ 7 as severely active disease (III) [17]. The endoscopic findings were scored according to the Mayo endoscopic subscore, graded as normal (0), mild (I), moderate (II), or severe (III) disease activity. Finally, the full Mayo Score was calculated on basis of four parameters: stool frequency, rectal bleeding, endoscopic evaluation and a physician's global assessment [18]. The score 0–I was defined as remission and the score II–III as active disease [19].

Histological activity

The histological activity was scored according to the Geboes Score (GS), considering the presence of architectural changes, neutrophils, eosinophils, crypt destruction and erosion of the mucous membranes [20].

Measurement of TNF-α, IL-17, IL-10 and sST2 in feces

Stools (1–10 g) were collected in the sterile containers and weighed. They were divided into 1 g aliquots and then emulsified in 5 mL of protease inhibitor cocktail (SIGMA, P83401), diluted 1:100, and centrifuged for 5 minutes at 400 G, at 4°C, as previously described [21, 22]. The supernatant fluid was collected and stored at -80°C until ELISA.

The fecal concentrations of cytokines were measured, as described [9], using the sensitive enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN,) specific for human cytokines according to the manufacturer's instructions. Briefly, the 96-well plates were coated with capture antibody, overnight. The plates were washed with a washing buffer (0.05% Tween-20 in PBS) and incubated with blocking buffer (1% bovine serum albumin in PBS) for 1 hour at room temperature. The serum/fecal samples, or standard recombinant TNF-α/IL-17/IL-10/sST2 were introduced to the plates for 2 hours before the application of biotinylated detection antibody for 1 hour at room temperature. After introduction of streptavidin peroxidase for 1 hour, the plates were developed with substrate reagent for 20 minutes. The reaction was stopped by adding 4 mol/L sulfuric acid, and the absorbance was read at 495 nm by a microplate reader. We measured the exact concentration of the men-

tioned biomarkers by intrapolation of a standard curve made by a series of well-known concentrations as per manufacturer's instruction. The values of measured cytokines are presented as pg/g of feces.

Statistical analysis

The statistical analyses were performed using the SPSS 20.0 software. The results were reported as mean and standard error (SE). The statistically significant difference between the means of two groups was determined using the Student's t-test for the independent samples if the data had normal distribution, or the Mann-Whitney U-test for data without normal distribution. The Pearson's correlation evaluated the possible relationship between the cytokines and disease severity and progression in the patients with UC. The strength of correlation was defined as negative, or positive, weak (-0.3 to -0.1 or 0.1 to 0.3), moderate (-0.5 to -0.3, or 0.3 to 0.5), or strong (-1.0 to -0.5 or 1.0 to 0.5). The p-value of 0.05 was considered statistically significant.

Results

Eighty patients, between 21 and 80 years of age diagnosed and histologically confirmed as UC were recruited in this study. There was no significant difference in gender distribution. The clinical and pathologic characteristics of these patients are presented in Table 1. We have assessed the concentration of pro- and anti-inflammatory cytokines as well as sST2 in the fecal liquid fraction of all patients. The patients with UC were classified into two groups based on the endoscopic, clinical and total Mayo score, respectively: I – scores 0 and 1; II – scores 2 and 3. The representative images of endoscopic Mayo subscores in patients with UC are shown in Figure 1 A and B. Further, the patients were divided according to the histological characteristics (crypt destruction, erosion of the mucous membranes, architectural changes, neutrophil infiltration and eosinophil infiltration) into two groups: I: scores 0 and 1; II: scores 2 and 3. We analyzed the values of previously defined markers of interest between the defined groups.

Table 1

Baseline characteristics of patients

Parameters	Values
Gender (male/female), n	46/34
Age (years), mean (range)	50 (21–80)
Endoscopic score (0/I/II/III), n	0/41/26/13
Clinical score (0/I/II/III), n	0/43/24/13
Mayo score (0/I/II/III), n	0/43/23/14
Crypt destruction (0/I/II/III), n	6/38/14/22
Erosion of the mucous membranes (0/I/II/III/IV), n	20/21/10/14/15
Architectural changes (0/I/II/III), n	0/38/22/20
Neutrophil infiltration (0/I/II/III), n	7/32/13/28
Eosinophil infiltration (0/I/II/III), n	17/25/20/18

Fecal concentrations of TNF-α, IL-17 and IL-10 are associated with the clinical activity, endoscopic and histo-pathologic characteristics of ulcerative colitis

Our evaluation revealed a significantly higher level of TNF-α in the group of patients with the advanced endoscopic, clinical as well as total Mayo score ($p < 0.05$), Figure 2A. We also noticed a significant increment of fecal level of TNF-α in the group of patients with an intense crypt destruction, erosion of the mucous membranes, architectural changes, neutrophil infiltration and eosinophil infiltration ($p < 0.05$), Figure 2B.

The patients with advanced endoscopic, clinical and total Mayo score revealed significantly higher IL-17 in feces in comparison to the patients with lower endoscopic and clinical scores ($p < 0.05$), Figure 3A. IL-17 was higher in the stool of patients with an intense crypt destruction, erosion of the mucous membranes, architectural changes, neutrophil infiltration and eosinophil infiltration ($p < 0.05$), Figure 3B.

As shown in Figure 4, the UC patients with higher endoscopic, clinical and total Mayo score appear to have a higher fecal level of IL-10, although this difference reached a statistical significance for endoscopic score only ($p = 0.006$).
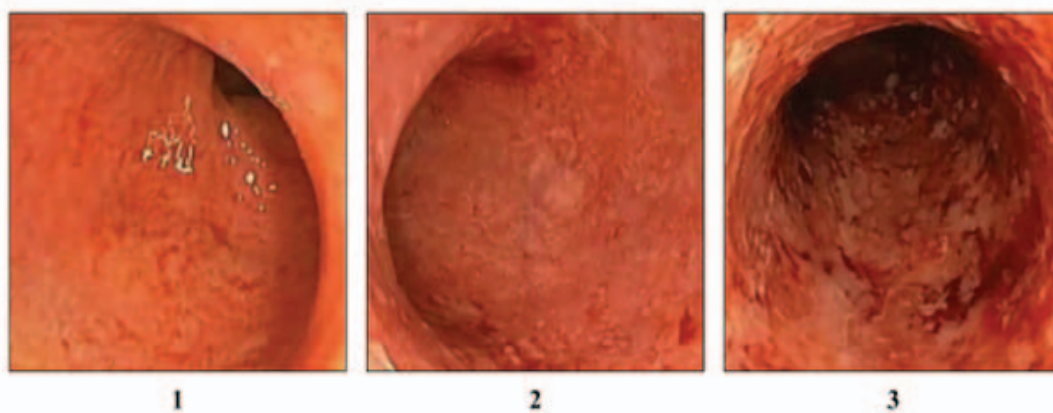
Fig. 1 – Mayo 1: mild activity (erythema, decreased vascular pattern, mild friability);
Mayo 2: moderate activity (marked erythema, lack of vascular pattern, friability, erosions);
Mayo 3: severe activity (spontaneous bleeding, large ulcerations).
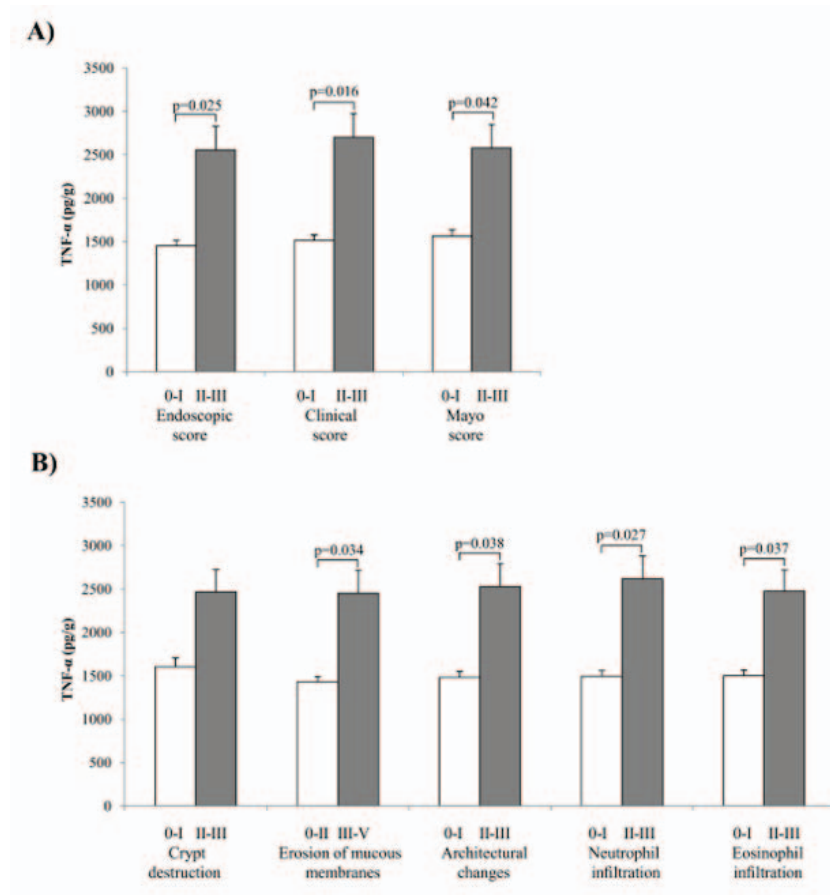
Fig. 2 – Distribution of fecal levels of TNF-α in the groups of patients with different Mayo and histological scores.
A) The patients with ulcerative colitis were divided into two groups, based on the endoscopic, clinical and total Mayo score. A fecal level of TNF-α was determined by ELISA; B) The patients with ulcerative colitis were divided into two groups, according to several histological characteristics group I – scores 0–I; group II – scores II–III.
A statistical significance was tested by the Mann–Whitney Rank Sum test.
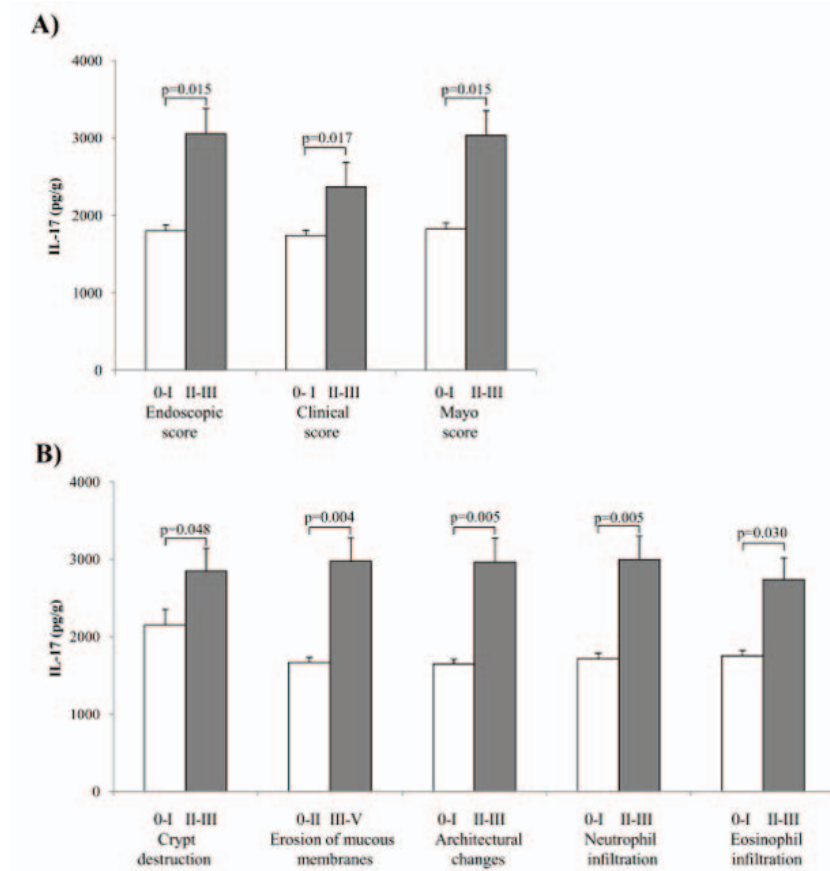
Fig. 3 – The analysis of fecal IL-17 in the ulcerative colitis patients according to the endoscopic, clinical and histopathological activity:
A) The patients with UC were divided into two groups, based on the endoscopic, clinical and total Mayo score, respectively. A fecal level of IL-17 was determined by ELISA; B) The patients with UC were divided into two groups according to several histological characteristics group I – scores 0–I; group II – scores II–III.
A statistical significance was tested by the Mann–Whitney Rank Sum test.
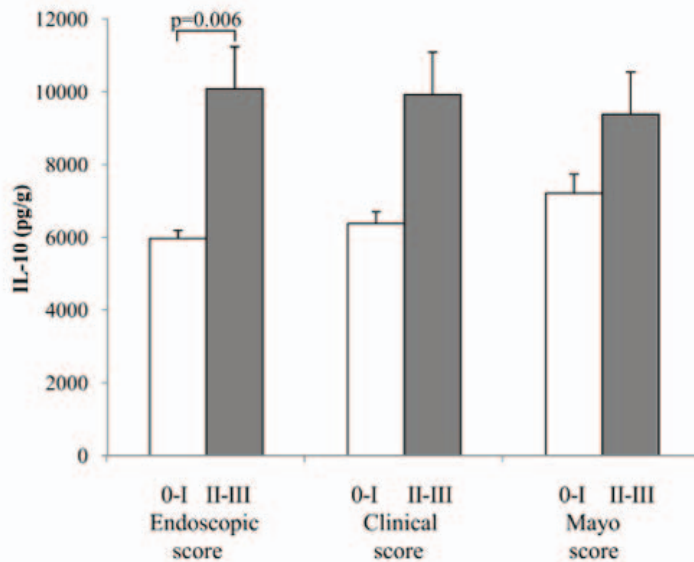
Fig. 4 – The increased concentration of IL-10 in feces of patients with the higher Mayo score. The patients with ulcerative colitis were divided into two groups, based on the endoscopic, clinical and total Mayo score group I – scores 0–I; group II – scores II–III. A fecal level of IL-10 was determined by ELISA. A statistical significance was tested by the Mann–Whitney Rank Sum test.
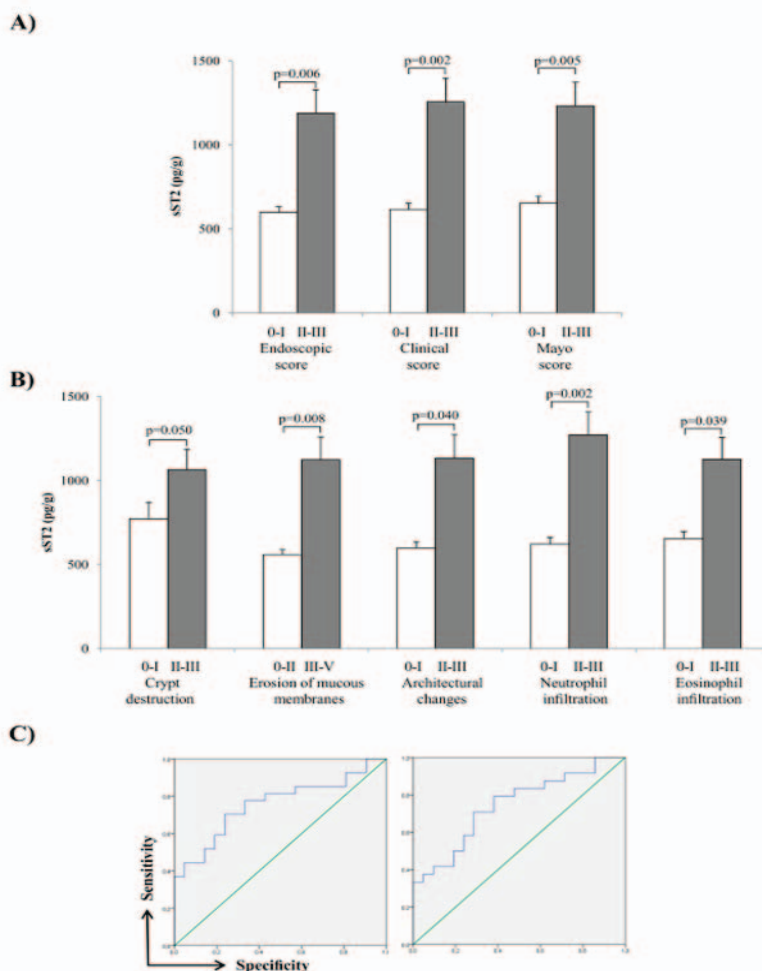
Fig. 5 – Differences in fecal sST2 in the patients according to the change in the endoscopic, clinical and histological scores:
A) The patients with ulcerative colitis were divided into two groups, based on the endoscopic, clinical and total Mayo score, respectively. A fecal level of sST2 was determined by ELISA;
B) The patients with UC were divided into two groups according to several histological characteristics, respectively. A statistical significance was tested by the Mann-Whitney Rank Sum test;
C) Reciever operating characteristic (ROC) curves illustrate the specificity and sensitivity of fecal sST2 comparing the clinical score and the total Mayo score.

Clinical activity, endoscopic and histo-pathologic characteristics of ulcerative colitis are associated with higher fecal sST2

We noticed a higher fecal level of sST2 in the patients with advanced endoscopic, clinical and total Mayo score ($p < 0.05$), Figure 5A. Further, sST2 was significantly increased in the patients with a strong crypt destruction, erosion of the mucous membranes, architectural changes, neutrophil infiltration and eosinophil infiltration ($p < 0.05$), Figure 5B. The analysis of receiver operating characteristic (ROC) curves of fecal sST2 for various stages and parameters of UC found that the sST2 level in feces could predict the disease severity. The analysis showed that sST2 can be a valuable marker for

distinguishing the clinical score (sensitivity 77.8%, specificity 66.7%) and the total Mayo score (sensitivity 79.2%, specificity 61.9%). The optimal cut-off value estimated for sST2 that allows the discrimination of stages of UC progression was 624.0 pg/g.

Fecal sST2 and IL-17 concentrations significantly correlated with disease severity

The relationship between the fecal sST2/IL-17 and the clinico-pathological parameters of patients with UC were summarized in Table 2. The analysis revealed positive correlation between the fecal sST2/IL-17 and parameters and markers of disease severity. There is the moderate positive correlation between fecal sST2/IL-17 and the endoscopic score (r = 0.390; p = 0.005/ r = 0.330; p = 0.014), clinical score (r = 0.441; p = 0.002/ r = 0.330; p = 0.016), total Mayo score (r = 0.442; p = 0.004 r = 0.356; p = 0.013), crypt destruction (r = 0.267; p = 0.049 r = 0.269; p = 0.047), erosion of the mucous membranes (r = 0.376; p = 0.006 r = 0.407; p = 0.003), architectural changes (r = 0.300; p = 0.038 r = 0.409; p = 0.003), neutrophil infiltration (r = 0.480; p = 0.001 r = 0.411; p = 0.002) and eosinophil infiltration (r = 0.304; p = 0.038 r = 0.305; p = 0.028). Finally, we found the strong positive correlation between fecal sST2 and IL-17 (r = 0.771; p = 0.001).

Table 2
Correlation between the fecal level of sST2 and IL-17 and the parameters of disease severity in the patients with ulcerative colitis

Variables	Spearman`s rho	p value	Spearman`s rho	p value
	sST2		IL-17	
Endoscopic score	0.390	0.005	0.330	0.014
Clinical score	0.441	0.002	0.330	0.016
Mayo score	0.422	0.004	0.356	0.013
Crypt destruction	0.267	0.049	0.269	0.047
Erosion of the mucous membranes	0.376	0.006	0.407	0.003
Architectural changes	0.300	0.038	0.409	0.003
Neutrophil infiltration	0.480	0.001	0.411	0.002
Eosinophil infiltration	0.304	0.038	0.305	0.028
IL-17	0.771	0.001		

Note: A statistical significance was tested by the Spearman correlation coefficient.

Discussion

Recently, studies have pointed on a significance of measuring biomarkers in feces [23–27]. These kinds of tests have a significant promise. In this way, proteins and molecules produced by intestinal mucosa are measured, which reflects the intestinal inflammation [27]. Recent studies have considered the potential as well as the diagnostic accuracy of different non-invasive fecal markers in the detection of IBD and as indicators of the therapy response [23–27].

Our results revealed that the levels of all measured biomarkers in feces reflected the disease activity. A domination of type 1 response, manifested by a significantly higher fecal level of TNF-α, was detected in the patients with the advanced endoscopic score, higher clinical and total Mayo score and intense crypt destruction, erosion of the mucous membranes, architectural changes, neutrophil and eosinophil infiltration, respectively. This indicates that TNF-α correlates with the severity of UC. TNF-α is already established as master cytokine implicated in the pathogenesis of various inflammatory conditions, particularly IBD [28, 29]. Its importance has been confirmed in many studies using anti-TNF antibodies in controlling the disease activity [30, 31]. The main sources of TNF-α are the activated macrophages and Th1 cells [32, 33]. An elevated level of TNF-α in the sera, stool or intestinal tissue were detected in the UC patients [34, 35], while the systemic values of TNF-α correlate with the clinical activity of UC [36].

Current publications indicate that the type 17 immune response also play an important role in the biology of UC [37, 38]. The type 17 response is mediated mainly by IL-17, produced by the Th-17 cells and CD8$^+$T cells, during chronic inflammation [39]. Our data revealed a significantly increased fecal level of IL-17 in the patients with more severe stage of UC (the advanced endoscopic and clinical score and histological grade). We also obtained the positive correlation between fecal IL-17 and disease severity (the advanced endoscopic score, higher clinical and total Mayo score). Moreover, the fecal level of IL-17 is in a positive correlation with histologically advanced and more severe disease (intense cript destruction, erosion of the mucous membranes, architectural changes, neutrophil and eosinophil infiltration, respectively). Although many studies support the pro-inflammatory role of IL-17 in the genesis and progression of UC [38, 40], some demonstrated a protective role of IL-17 in IBD [41]. IL-17 can mediate a protective function in an experimental model of UC [42]. In humans, recent publications showed that the IL-17 levels were increased in the UC patients, and correlated with the disease severity [37, 38, 40, 43].

Interleukin 10 is one of the most important anti-inflammatory cytokines known to suppress an inflammation in mucosa [44]. It is produced mainly by the macrophages, dendritic cells, Th2 and Treg cells [45]. We found the significant increment of IL-10 in feces of patients with the advanced endoscopic score, and this trend was detected in the patients

with the higher clinical and total Mayo score (did not reach statistical significance). Based on our results, we believe that the relatively enhanced fecal level of IL-10 in the patients with severe UC is a way to compensate for an intense inflammation and the enhanced type 1 and type 17 immune response.

Interleukin 33 presents the alarmin and cytokine that plays a role in the polarization of immune response toward type 2 [46]. Soluble ST2 is a decoy receptor for IL-33 [24]. The increased systemic values of sST2 were detected in the patients with the chronic inflammatory diseases [47, 48] and correlate with the disease severity [49]. The serum sST2 is proposed as a potential biomarker of the UC activity [49]. Our goal was to determinate whether fecal sST2 correlated with the severity of UC. We demonstrated, for the first time, a significantly increased fecal level of sST2 in the patients with more severe stage of UC (higher endoscopic, clinical and total Mayo score, respectively). Moreover, fecal sST2 was significantly increased in the patients with the intense cript destruction, erosion of the mucous membranes, architectural changes, neutrophil and eosinophil infiltration, respectively. Also, it was demonstrated that fecal sST2 correlated closely with the endoscopic, clinical and total Mayo score as well as with the histopathological parameters of affected tissue, and might be used to evaluate the severity of UC. This observation is in line with previous reports indicating a direct association between the systemic sST2 and endoscopic score of UC as well as the histopathological stage [49]. To our knowledge, this is the first study revealing direct association between fecal sST2 and the degree of endoscopic, clinical and histopathological activity of UC. Further in this study, we envisaged the possible role of fecal sST2 as a biomarker in preceding the disease severity and progression. The analysis of the ROC curves of sST2 and the disease parameters revealed that sST2 could predict the advanced endoscopic and clinical score, at good sensitivity and specificity. According to our findings, fecal sST2 could be a valuable marker for the UC severity and activity. The best cut-off point for the sST2 concentration was obtained at a threshold of 624.0 pg/g, predicting the active disease.

Another novel finding in this study was a strong positive correlation between IL-17 and sST2 in feces of the UC patients. Both biomarkers correlated closely with the parameters of disease severity. Little is known about the IL-17/sST2 relation. Recent studies of inflammatory diseases, demonstrate that IL-33 suppresses the type 17 immune response and subsequent production of IL-17 [50, 51]. Taken together, the enhanced local values of sST2 can neutralize and reduce concentration of present IL-33 and thus suppress the IL-33 inhibition of IL-17.

Conclusion

The increased local values of sST2, reflected trough higher fecal concentration, in the UC patients with the higher endoscopic, clinical and total Mayo score, as well as the histopathological parameters of affected tissue may be considered as a sign of the disease progression and, consequently, of a poor prognosis for the patients. An increment of sST2 may facilitate the IL-17 production, via the IL-33 reduction, implicating immunomodulatory role of sST2 in enhancing the ongoing proinflammatory processes. Furthermore, the fecal values of sST2 can be used as a valuable marker for the UC severity. These observations support the possible role of fecal sST2 as an activity marker of UC and its potential use as a therapeutic target.

Declaration of interest

The authors declare that they have no conflict of interests.

R E F E R E N C E S

1. *Matkowskyj KA, Chen ZE, Rao MS, Yang G.* Dysplastic lesions in inflammatory bowel disease: molecular pathogenesis to morphology. Arch Pathol Lab Med 2013; 137(3): 338–50.
2. *Podolsky DK.* Inflammatory bowel disease. N Engl J Med 2002; 347(6): 417–29.
3. *Raza A, Yousaf W, Giannella R, Shata MT.* Th17 cells: interactions with predisposing factors in the immunopathogenesis of inflammatory bowel disease. Exp Rev Clin Immunol 2012; 8(2): 161–8.
4. *Xavier RJ, Podolsky DK.* Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007; 448(7152): 427–34.
5. *Neurath MF.* Cytokines in inflammatory bowel disease. Nat Rev Immunol 2014; 14(5): 329–42.
6. *Sandborn WJ, Hanauer SB.* Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results, and safety. Inflamm Bowel Dis 1999; 5(2): 1119–33.
7. *Ito R, Shin-Ya M, Kishida T, Urano A, Takada R, Sakagami J,* et al. Interferon-gamma is causatively involved in experimental inflammatory bowel disease in mice. Clin Exp Immunol 2006; 146(2): 330–8.
8. *Műzes G, Molnrar B, Tulassay Z, Sipos F.* Changes of the cytokine profile in inflammatory bowel diseases. World J Gastroenterol 2012; 18(41): 5848–61.
9. *Jovanovic M, Zdravkovic N, Jovanovic I, Radosavljevic G, Gajovic N, Zdravkovic N,* et al. TGF-β as a marker of ulcerative colitis and disease severity. Ser J Exp Clin Res 2017; DOI: 10.1515/sjecr-2017-0019.
10. *Fuss IJ, Heller F, Boirivant M, Leon F, Yoshida M, Fichtner-Feigl S,* et al. Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis. J Clin Invest 2004; 113(10): 1490–7. 1
11. *Mańkowska-Wierzbicka D, Swora-Cwynar E, Poniedziałek B, Adamski Z, Dobrowolska A, Karczewski J.* Usefulness of selected

laboratory markers in ulcerative colitis. Eur Cytokine Netw 2015; 26(2): 26–37.

12. *Schoepfer AM, Vavricka S, Zahnd-Straumann N, Straumann A, Beglinger C.* Monitoring inflammatory bowel disease activity: Clinical activity is judged to be more relevant than endoscopic severity or biomarkers. J Crohns Colitis 2012; 6(4): 412–8.

13. *Cooney RM, Warren BF, Altman DG, Abreu MT, Travis SP.* Outcome measurement in clinical trials for ulcerative colitis: towards standardisation. Trials 2007; 8: 17.

14. *Satsangi J, Silverberg MS, Vermeire S, Colombel JF.* The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006; 55(6): 749–53.

15. *Hiraoka S, Inokuchi T, Nakarai A, Takashima S, Takei D, Sugihara Y,* et al. Fecal immunochemical test and fecal calprotectin results show different profiles in disease monitoring for ulcerative colitis. Gut Liver 2018; 12(2): 142–8.

16. *Dignass A, Eliakim R, Magro F, Maaser C, Chowers Y, Geboes K,* et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis Part 1: Definitions and diagnosis. J Crohns Colitis 2012; 6(10): 965–90.

17. *Gomollón F, García-López S, Sicilia B, Gisbert JP, Hinojosa J.* Grupo Espa~nol de Trabajo en Enfermedad de Crohn y Colitis Ulcerosa . Therapeutic guidelines on ulcerative colitis: A GRADE methodology based effort of GETECCU. Gastroenterol Hepatol 2013; 36(2): 104–14.

18. *Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J,* et al. Infliximab for Induction and Maintenance Therapy for Ulcerative Colitis. N Eng J Med 2005; 353(23): 2462–76.

19. *D'Haens G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ,* et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology 2007; 132(2): 763–86.

20. *Geboes K, Riddell R, Ost A, Jensfelt B, Persson T, Löfberg R.* A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. Gut 2000; 47(3): 404–9.

21. *Heilmann RM, Cranford SM, Ambrus A, Grützner N, Schellenberg S, Ruaux CG,* et al. Validation of an enzyme-linked immunosorbent assay (ELISA) for the measurement of canine S100A12. Vet Clin Pathol 2016; 45(1): 135–47.

22. *Prakash N, Stumbles P, Mansfield C.* Initial validation of cytokine measurement by ELISA in canine feces. Open J Vet Med 2013; 3(6): 282–8.

23. *Langhorst J, Elsenbruch S, Kölzer J, Rueffer A, Michalsen A, Dobos GJ.* Noninvasive Markers in the Assessment of Intestinal Inflammation in Inflammatory Bowel Diseases: Performance of Fecal Lactoferrin, Calprotectin, and PMN-Elastase, CRP, and Clinical Indices. Am J Gastroenterol 2008; 103(1): 162–9.

24. *Vermeire S, van Assche G, Rutgeerts P.* Laboratory markers in IBD: Useful, magic, or unnecessary toys? Gut 2006; 55(3): 426–31.

25. *Kopylov U, Rosenfeld G, Bressler B, Seidman E.* clinical utility of fecal biomarkers for the diagnosis and management of inflammatory bowel disease. Inflamm Bowel Dis 2014; 20(4): 742–56.

26. *Lewis JD.* The utility of biomarkers in the diagnosis and therapy of inflammatory bowel disease. Gastroenterology 2011; 140(6): 1817–26.e2.

27. *Boon GJ, Day AS, Mulder CJ, Gearry RB.* Are faecal markers good indicators of mucosal healing in inflammatory bowel disease? World J Gastroenterol 2015; 21(40): 11469–80.

28. *Murch SH, Braegger CP, Walker Smith JA, MacDonald TT.* Location of tumour necrosis factor alpha by immunohistochemistry in chronic inflammatory bowel disease. Gut 1993; 34(12): 1705–9.

29. *Zdravkovic ND, Jovanovic IP, Radosavljevic GD, Arsenijevic AN, Zdravkovic ND, Mitrovic SL,* et al. Potential dual immunomodulatory role of VEGF in ulcerative colitis and colorectal carcinoma. Int J Med Sci 2014; 11(9): 936–47.

30. *Willrich MA, Murray DL, Snyder MR.* Tumor necrosis factor inhibitors: clinical utility in autoimmune diseases. Trans Res 2015; 165(2): 270–82.

31. *Colombel J, Sandborn WJ , Ghosh S, Wolf Douglas C , Panaccione R, Feagan B,* et al. Four-year maintenance treatment with adalimumab in patients with moderately to severely active ulcerative colitis: data from ULTRA 1, 2 and 3. Am J Gastroenterol 2014; 109(11): 1771–80.

32. *Bradley JR.* TNF-mediated inflammatory disease. Journal of Pathology 2008; 214(2): 149–60.

33. *Begue B, Wajant H, Bambou J, Dubuquoy L, Siegmund D, Beaulieu J,* et al. Implication of TNF-related apoptosis-inducing ligand in inflammatory intestinal epithelial lesions. Gastroenterology 2006; 130(7): 1962–74.

34. *Braegger CP, Nicholls S, Murch SH, MacDonald TT, Stephens S.* Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. Lancet 1992; 339(8785): 89–91.

35. *Maeda M, Watanabe N, Neda H, Yamauchi N, Okamoto T, Sasaki H,* et al. Serum tumor necrosis factor activity in inflammatory bowel disease. Immunopharmacol Immunotox 1992; 14(3): 451–61.

36. *Reimund JM, Wittersheim C, Dumont S, Muller CD, Baumann R, Poindron P,* et al. Mucosal inflammatory cytokine production by intestinal biopsies in patients with ulcerative colitis and Crohn's disease. J Clin Immunol 1996; 16(3): 144–50.

37. *Abraham C, Cho J.* Interleukin-23/Th17 pathways and inflammatory bowel disease. Inflamm Bowel Dis 2009; 15(7): 1090–100.

38. *Öhman L, Dahlén R, Isaksson S, Sjöling Å, Wick M, Sjövall H,* et al. Serum IL-17A in newly diagnosed treatment-naive patients Laboratory markers in ulcerative colitis 35 with ulcerative colitis reflects clinical disease severity and predicts the course of disease. Inflamm Bowel Dis 2013; 19(11): 2433–9.

39. *Shen W, Durum SK.* Synergy of IL-23 and Th17 Cytokines: new light on inflammatory bowel disease. Neurochem Res 2010; 35(6): 940–6.

40. *Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y,* et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut 2003; 52(1): 65–70.

41. *Karczewski J, Mazur M, Karczewski M.* Dual role of Th17 cells in Crohn's disease. Central Eur J Immunol 2012; 37: 286–9

42. *O'Connor W Jr, Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y,* et al. A protective function for interleukin 17A in T cell–mediated intestinal inflammation. Nat Immunol 2009; 10(6): 603–9.

43. *Kobayashi T, Okamoto S, Hisamatsu T, Kamada N, Chinen H, Saito R,* et al. IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. Gut 2008; 57(12): 1682–9.

44. *Fiorentino DF, Bond MW, Mosmann TR.* Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989; 170(6): 2081–95.

45. *Paul G, Khare V, Gasche C.* Inflamed gut mucosa: downstream of interleukin-10. Eur J Clin Invest 2011; 42(1): 95–109.

46. *Pavlović S, Zdravković N, Radosavljević G, Arsenijević N, Lukić M, Jovanović I.* Interleukin-33/ST2: A new signaling pathway in immunity and immunopathology. Vojnosanit Pregl 2012; 69(1): 69–77.

47. *Kuroiwa K, Arai T, Okazaki H, Minota S, Tominaga S.* Identification of Human ST2 Protein in the Sera of Patients with Autoimmune Diseases. Biochem Biophys Res Commun 2001; 284(5): 1104–8.

48. *Mok MY, Huang FP, Ip WK, Lo Y, Wong FY, Chan EY,* et al. Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus. Rheumatology (Oxford) 2010; 49(3): 520–7.

49. *Díaz Jiménez D, Núñez LE, Beltrán CJ, Candia E, Suazo C, Alvarez Lobos M,* et al. Soluble ST2: A new and promising activity marker in ulcerative colitis. World J Gastroenterol 2011; 17(17): 2181–90.

50. *Lv R, Zhao J, Lei M, Xiao D, Yu Y, Xie J.* IL-33 Attenuates sepsis by inhibiting il-17 receptor signaling through upregulation of socs3. Cell Physiol Biochem 2017; 42(5): 1961–72.

51. *Jiang H, Milovanović M, Allan D, Niedbala W, Besnard A, Fukada SY,* et al. IL-33 attenuates EAE by suppressing IL-17 and IFN-γ production and inducing alternatively activated macrophages. Eur J Immunol 2012; 42(7): 1804–14.

Psychometric properties of the Headache Under-Response to Treatment (HURT) questionnaire and the Migraine Disability Assessment Test (MIDAS) translated to Serbian

Psihometrijske karakteristike prevoda HURT (*Headache Under-Response to Treatment*) i MIDAS (*Migraine Disability Assessment Test*) upitnika na srpski jezik

Radica Živković Zarić*, Slobodan M. Janković*, Éva Csépány[†], Tamás Gyüre[†], Csaba Ertsey[‡], Marija Andjelković*

University of Kragujevac, *Faculty of Medical Sciences, Kragujevac, Serbia;
Semmelweis University, [†]János Szentágothai Doctoral School of Neurosciences,
[‡]Department of Neurology, Budapest, Hungary

Abstract

Background/Aim. The Headache Under-Response to Treatment (HURT) questionnaire and the Migraine Disability Assessment Test (MIDAS), which are intended for assessing the headache-related disability, impact (MIDAS) and management (HURT), were not yet translated to Serbian and validated in the population of Serbia. The aim of this study was to translate the HURT and MIDAS from English to Serbian, to make necessary cultural adaptations and to test their psychometric properties in a sample of outpatients with the headache. **Methods.** The HURT and MIDAS questionnaires were translated and adapted according to the internationally accepted guidelines, and then tested on a sample of Serbian patients with various headache types. Internal consistency was checked through the calculation of Cronbach's alpha for the questionnaires, and by correlation of each question with the corrected total score. The criterion validity of the translation was tested by correlating scores of individual items, domains and whole questionnaire with the headache characteristics (severity, duration, frequency), and convergent validity was tested by correlating the abovementioned scores with results of an instrument for measurement of headache-related quality of life. **Results.** There were 171 (79.2%) females and 45 (20.8%) male study participants. The mean age of the patients was 42.3 years, (standard deviation – SD 13.35; range 18–75); 27 (12.5%) suffered from a migraine and 189 (87.5%) from the episodic tension-type headache (TTH). The Serbian translation of HURT and MIDAS questionneires showed excellent internal consistency, with high values of the Cronbach's alpha: 0.764 and 0.731, respectively. The validity of the instruments in all aspects (criterion, convergent and discriminant validity) was also excellent for the whole sample and for the subgroup of patients with TTH, while the results for the patients with the migraine were less favorable. The factor analysis suggested the existence of one domain of MIDAS and two domains of HURT questionneires. **Conclusion.** The Serbian translations of HURT and MIDAS could be used as the reliable and valid specific instruments for measuring a headache-related disability, impact (MIDAS) and management (HURT) in the patients with TTH and probably in the patients with the migraine.

Key words:
headache; migraine disorders; analgesics; treatment outcome; serbia; translating; surveys and questionnaires.

Apstrakt

Uvod/Cilj. Nedovoljan efekat terapije na glavobolju (HURT) i Test procene nesposobnosti usled migrene (MIDAS), upitnici namenjeni proceni nesposobnosti kod bolesnika sa glavoboljom, nisu do sada bili prevedeni na srpski jezik, niti je ispitana njihova punovažnost u srpskoj populaciji. Cilj ovog rada bio je da se prevedu HURT i MIDAS upitnici sa engleskog na srpski jezik, da se učine neo- phodna kulturološka prilagođavanja i da se ispitaju psiho- metrijska svojstva prevoda na grupi vanbolničkih bolesnika se glavoboljom. **Metode.** HURT i MIDAS su prevedeni na srpski jezik i prilagođeni prema međunarodno prihvaćenim vodičima, zatim ispitani na uzorku bolesnika iz Srbije sa različitim tipovima glavobolje. Unutrašnja konzistencija je bila ispitana kroz izračunavanja Kronbahovog koeficijenta alfa za upitnike kao celine, kao i korelacije svakog od pitanja iz upitnika sa ukupnim skorom bez tog pitanja (korigovani

Correspondence to: Radica Živković Zarić, University of Kragujevac, Faculty of Medical Sciences, Svetozara Markovića, 69, 34 000 Kragujevac, Serbia. E-mail: radica_zivkovic@yahoo.com

ukupni skor). Punovažnost prevoda upitnika prema određenim kriterijumima je bila ispitana kroz izračunavanje korelacije skorova za pojedina pitanja, domenu upitnika i upitnike kao celine sa karakteristikama glavobolje kod bolesnika (težina, trajanje, učestalost), kao i sa rezultatima upitnika za merenje kvaliteta života kod bolesnika sa glavoboljom (konvergentna punovažnost). **Rezultati.** U studiji su bili uključeni 171 (79,2%) žena i 45 (20,8%) muškaraca. Prosečna starost bolesnika je bila 42,3 godine, (standardna devijacija – SD 13,35; opseg 18–75); 27 (12,5%) bolesnika je imalo migrenu i 189 (87,5%) povremene tenzione glavobolje. Prevodi HURT MIDAS upitnika na srpski jezik su pokazali odličnu unutrašnju konzistenciju, sa visokim vrednostima Kronbah alfa: 0,764 i 0,731. Punovažnost ovih upitnika u svim pogledima (prema kriterijumima, konvergentna i diskriminatorna) je bila, takođe, odlična za čitav uzorak grupe bolesnika sa tenzionom glavoboljom, dok su rezultati kod bolesnika sa migrenom bili manje povoljni. Faktorska analiza je pokazala da MIDAS upitnik ima jedan domen, a HURT dva domena. **Zaključak.** Prevodi HURT i MIDAS upitnika na srpski jezik se mogu koristiti kao pouzdani i punovažni specifični instrumenti i za merenje nesposobnosti izazvane glavoboljom (MIDAS) i uticaja terapije (HURT), pre svega kod bolesnika sa tenzionom glavoboljom i verovatno kod bolesnika sa migrenom.

Ključne reči:
glavobolja; migrena; analgetici; lečenje, ishod; srbija; prevođenje; ankete i upitnici.

Introduction

High prevalence of headache was recorded in almost all age groups all over the world. Within the adult population, one-year prevalence of migraine is 10%–18%, and that of tension-type headache (TTH) 31%–90% [1]. A headache is also frequently encountered among children: the mean prevalence of headache in children and adolescents from 32 countries was reported to be 54.4% for any kind of headache and 9.1% for the migraine [2, 3]. A headache is causing a significant disability in the patients, which is further translated to job absenteeism, social withdrawal, decreased quality of life and disturbed family relations. When treating a patient with a headache, the physicians should be interested not only in the decrease of pain, but also in the withdrawal of disability and return of the patient to his/her normal life [4].

When treating a patient with a headache, in order to prescribe a suitable therapy we need to know the extent of disability caused by the disorder and whether it was appropriately managed. Beside several specific instruments for measuring quality of life in the patients with the migraine and other headache types with excellent psychometric properties, like Comprehensive Headache-related Quality of Life Questionnaire (CHQQ) [5–7], there are also the validated short and simple instruments for measuring disability, the Migraine Disability Assessment Test (MIDAS) for the patients with the migraine [8] and evaluating both disability and response to treatment, the Headache Under-Response to Treatment (HURT) questionneires for all headache types [9]. Currently, there are no available Serbian translations with trans-cultural adaptations of instruments measuring a headache-related disability and response to treatment, so clinicians in Serbia use only basic clinical tools when managing the patients with the headache [10].

In response to the current needs, the aim of our study was to translate the HURT questionnaire and the MIDAS from English to Serbian, to make necessary cultural adaptations and to test their psychometric properties in a sample of outpatients with a headache.

Methods

Patients

The study included outpatients of primary care Health Center in Kragujevac, Serbia, who consecutively visited their general practitioners from February to June 2015, with a headache as the main complaint and who had either diagnosis of migraine with and without aura (episodic type; The Intenational Classification of Headache Disorders-2nd – ICHD-II codes 1.1 and 1.2) or a TTH (episodic type; ICHD-IIcodes 2.1–2.2) [11]. The patients with the following diagnoses were excluded from the study: rare adult migraine subtypes, probable migraine, probable tension-type headache, analgesic abuse, moderate to severe liver or kidney failure, untreated hypertension, refractive disorders and chronic pain syndromes.

The study was approved by the Ethics Committee of the Health Center in Kragujevac, Serbia, where the study took place.

Translation and cultural adaptation of the original MIDAS and HURT questionnaires

The translation and cultural adaptation of the MIDAS and the HURT questionnaires were made according to the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) guidelines [12]. The permission for the translation and cultural adaptation was obtained from Professor Walter F. Stewart (the Johns Hopkins School of Public Health, Baltimore) for MIDAS and from Professor Timothy J. Steiner (the Department of Neuroscience, the Norwegian University of Science and Technology, Trondheim) for HURT. The original questionnaires were at first translated to Serbian by Radica Živković Zarić and Slobodan Janković, working independently. The translations were then harmonized to one Serbian version at the meeting of the study investigators. The harmonized Serbian version was then translated back to English by Dr. Žan Friščić, a native English speaker, born in Australia. When translating back to English, he was not aware of the original English versions of the MIDAS and the HURT questionnaiers. The back-translation to English was then compared with the original English versions by the study investigators, and at a separate meeting the final

Serbian versions of the MIDAS and HURT questionnaiers were agreed on. The final translations of the MIDAS and the HURT to Serbian were then probed on 9 patients with a headache (at the Health Center in Kragujevac, Serbia) for clarity and comprehension. Only a few minor changes were made after the pilot test, and then final Serbian versions were used for the psychometric testing.

Data recording

The patients completed the Serbian versions of CHQQ, HURT and MIDAS questionnaires by themselves in the Health Center, after their visit to the general practitioners, who administered the questionnaires. The general practitioners noted the clinical characteristics of the patients during the visit, using a checklist prepared by the study investigators. The patients rated severity of their headaches on a visual analogue scale (VAS; 0–100 mm). Exact diagnosis of the headache type had been previously made by the neurologists from the Clinical Center Kragujevac. The patients were not tested for anxiety, or depression.

Statistics

The responses to the questions from the MIDAS were not transformed, while the answers to questions from the HURT questionnaires were rated from 1 to 5; the total scores for both questionnaires were obtained by summation. The reliability of the Serbian versions of the MIDAS and the HURT questionnaires was tested by measuring the internal consistency through calculation of the Cronbach's alpha. The Cronbach's alpha was calculated both for the instruments and their dimensions. The criterion validity of the Serbian versions of the MIDAS and the HURT questionnaires was tested by correlating the patient's headache characteristics with the individual items, dimensions and total score of the questionnaires. The convergent validity was assessed through correlating (Spearman's rank correlation) the individual items, dimensions and total scores with the total score of the CHQQ instrument and with each other. The discriminative validity was tested by comparing the results of the Serbian versions of the MIDAS and the HURT questionnaires in the two study groups, the patients with the migraine and tension-type headache, using the Mann–Whitney test.

The confirmatory factorial analysis of the Serbian translations of the MIDAS and the HURT questionnaires was made by the principal components method [13]. First, the suitability of the questionnaires and the sample for the factorial analysis was tested by the Kaiser-Meyer-Olkin measure of sampling adequacy and by the Bartlett's test of sphericity. Then, the factors were extracted at first without rotation, under the condition that Eigen values had to be greater than 1, and using the Scree-plot (the extracted factors were above the "elbow" of the graph). Second, the referent axes were rotated by orthogonal Varimax method, and another extraction of the factors was made, using the same criteria as for the un-rotated solution. The extracted factors were then compared with the dimensions of the original MIDAS and HURT questionnaires, and named accordingly.

All calculations in this study were performed by the SPSS software, version 18. The level of significance was set at $p < 0.05$.

Results

Completing the questionnaires

There were 216 patients who completed the questionnaires. The average time spent for filling-in the questionnaires was less than 25 minutes, and there were no complaints from the patients' side. The questionnaires were completely filled, so the answers of all 216 patients were used for the statistical calculations.

Patient characteristics

There were 171 (79.2%) female and 45 (20.8%) male patients, all of Caucasian origin. The mean age was 42.3 years, [standard deviation (SD) 13.35; range 18–75]. As to the occupation, 168 (78.2%) patients were employed, 21 (9.7%) were unemployed, 15 were retired (6.9%) and 11 (5.1%) were students.

Among the study patients 27 (12.5%) had a migraine and 189 (87.5%) an episodic TTH. The headache attack frequency was higher in the patients with the migraine (mean 11.9 attacks in 3 months, SD 9.2, versus 9.8 in 3 months, SD 14.2; $p > 0.05$), as well as the pain intensity (mean 7.9 points on a scale from 1 to 10, SD 1.7 in the migraineurs, versus 5.5 points, SD 2.2 in the TTH patients; $p < 0.01$) and the duration of attack (mean 619.6 minutes, SD 1002.6 minutes in the migraineurs, versus 281.7 minutes, SD 524.3 minutes in the TTH patients; $p < 0.01$). The distribution of sexes was similar among the patients with the migraine and among those with the TTH (2:25 versus 43:146; the Fisher's exact test; $p = 0.078$). Three patients with TTH reported depression as a concomitant condition.

Reliability

For testing the reliability the first 5 questions of MIDAS and the first 6 questions of the HURT questionnaire were taken into account (the seventh question of HURT questionnaire is measuring fear from the adverse drug effects and it did not significantly correlate with both other questions individually and with the corrected total score of the HURT – the data available on request). The reliability of the MIDAS and the HURT taking the whole study sample was very good: the Cronbach's alpha was 0.764 for the HURT and 0.731 for the MIDAS; when tested on the patients with the tension headache only, the Cronbach's alpha was 0.727 for the MIDAS and 0.821 for the HURT. The same parameter was 0.709 for the MIDAS in the group of patients with the migraine, and 0.484 for the HURT. The first dimension of the HURT questionnaire (disability) also showed the satisfactory reliability both in the whole study sample and in the diagnostic subgroups, while the second dimension worked well in the TTH group (Table 1).

Table 1

Internal consistency of the questionnaires and their dimensions (Cronbach's alpha values)

Item or score	Whole study sample		Patients with TTH		Patients with migraine	
	MIDAS	HURT	MIDAS	HURT	MIDAS	HURT
Total score	0.731	0.764	0.727	0.821	0.709	0.484
Disability dimension	N/A*	0.892	N/A	0.884	N/A	0.937
Treatment response dimension	N/A	0.491	N/A	0.732	N/A	0.028

*Not applicable, because MIDAS has only one dimension.
MIDAS – Migraine Disability Assessment Test; HURT – Headache Under-Response to Treatment questionnaire; TTH – tension-type headache.

Table 2

Testing criterion validity: the correlations between the items, dimensions and total score of the instrument with the clinical characteristics

Item or score	Group	Frequency of headache attacks (in the last 3 months)	Headache severity (VAS)	Mean attack length (minutes)	Disease length (years)
MIDAS					
Job absenteeism	ALL	**0.279**	**0.279**	0.086	-0.022
Productivity loss at job	ALL	**0.323**	**0.316**	0.091	0.097
Incapable of housekeeping	ALL	**0.520**	**0.353**	**0.144**	**0.164**
Productivity loss at home	ALL	**0.545**	**0.359**	**0.232**	0.102
Avoiding social interaction	ALL	**0.517**	**0.453**	**0.246**	0.109
Total score MIDAS	ALL	**0.642**	**0.490**	**0.255**	**0.171**
	TTH	**0.624**	**0.470**	**0.221**	0.136
	Migraine	**0.481**	0.108	0.259	-0.088
HURT					
Headache frequency	ALL	**0.739**	**0.421**	**0.164**	0.062
Productivity loss	ALL	**0.663**	**0.395**	**0.215**	**0.150**
Avoiding social interaction	ALL	**0.636**	**0.492**	**0.197**	**0.181**
Taking analgesic	ALL	**0.600**	**0.464**	**0.285**	0.130
Efficacy of analgesic	ALL	**0.256**	**0.330**	**0.232**	0.026
Control of headache	ALL	**0.320**	**0.329**	**0.237**	0.084
Disability dimension	ALL	**0.784**	**0.519**	**0.255**	**0.150**
Treatment response dimension	ALL	**0.333**	**0.365**	**0.257**	-0.062
Total score HURT	ALL	**0.704**	**0.530**	**0.292**	0.070
	TTH	**0.688**	**0.532**	**0.247**	0.026
	Migraine	**0.624**	0.167	0.326	-0.157

*ALL – whole sample; TTH – subgroup of patients with tension-type headache; Migraine – subgroup of patients with migraine; MIDAS – Migraine Disability Assessment Test; HURT – Headache Under-Response to Treatment questionnaire.
Values of Spearman's correlation coefficients given in bold are significant ($p < 0.05$).

Validity

The criterion validity: When taking the whole sample, five items from the MIDAS and 6 items from the HURT, the total scores of both questionnaires, and the scores of disability and treatment response dimensions of the HURT were significantly and positively correlated with the headache frequency and headache severity. It was similar with the mean attack length, with the exception of the items 1 and 2 of the MIDAS (job absenteeism and productivity loss at job) where the correlation was not significant. On the other hand, the disease length correlated only with the total score of the MIDAS, the item "incapable of housekeeping" from the MIDAS, disability dimension of the HURT questionnaires and with two items from the HURT: "productivity loss" and "avoiding social interaction". The results were similar in the subgroup of patients with the tension-type headache. However, in the subgroup of patients with the migraine, the total

scores of the MIDAS and the HURT questionnaires correlated positively and significantly only with the headache frequency (Table 2).

The convergent validity: the MIDAS and the HURT instruments showed excellent convergent validity for the whole sample and for the subgroup of patients with the tension-type headache (Table 3). All items, the total scores of the MIDAS and the HURT questionnaires and the scores of the HURT's dimensions correlated negatively and significantly (with high correlation coefficients) with the CHQQ score. All items, the total score of the HURT and the scores of HURT's dimensions correlated positively and significantly with the MIDAS score, and vice versa. The results were somewhat less favorable for the subgroup of patients with the migraine, where the score for the MIDAS did not correlate with the CHQQ score (Table 3).

The discriminative validity: The comparison of headache-related disability and treatment response as measured

by the MIDAS and the HURT questionnaires among two subgroups (migraine and TTH) showed the higher scores in the group of patients with migraine. The difference was sig-nificant for the 3 of 5 MIDAS items, the 3 the of 6 HURT items, for the whole questionnaires and for each of the two HURT's dimensions (Table 4).

Table 3
Testing convergent validity: the correlations between the items, dimensions and total score of the HURT, MIDAS and CHQQ

Item or score	Group	MIDAS	HURT	CHQQ
MIDAS				
Job absenteeism	ALL	**1.00**	**0.392**	**-0.309**
Productivity loss at job	ALL	**1.00**	**0.403**	**-0.364**
Incapable of housekeeping	ALL	**1.00**	**0.508**	**-0.582**
Productivity loss at home	ALL	**1.00**	**0.587**	**-0.582**
Avoiding social interaction	ALL	**1.00**	**0.593**	**-0.573**
Total score MIDAS	ALL	**1.00**	**0.697**	**-0.690**
	TTH	**1.00**	**0.666**	**-0.692**
	Migraine	**1.00**	**0.632**	-0.211
HURT				
Headache frequency	ALL	**0.581**	**1.00**	**-0.486**
Productivity loss	ALL	**0.665**	**1.00**	**-0.603**
Avoiding social interaction	ALL	**0.651**	**1.00**	**-0.640**
Taking analgesic	ALL	**0.590**	**1.00**	**-0.530**
Efficacy of analgesic	ALL	**0.382**	**1.00**	**-0.389**
Control of headache	ALL	**0.355**	**1.00**	**-0.400**
Disability dimension	ALL	**0.721**	**1.00**	**-0.660**
Treatment response dimension	ALL	**0.403**	**1.00**	**-0.444**
Total score HURT	ALL	**0.697**	**1.00**	**-0.672**
	TTH	**0.666**	**1.00**	**-0.675**
	Migraine	**0.632**	**1.00**	**-0.396**

*ALL – whole sample; TTH – subgroup of patients with a tension-type headache; Migraine – subgroup of patients with a migraine;; MIDAS – Migraine Disability Assessment Test; HURT – Headache Under-Response to Treatment; CHQQ – Comprehensive Headache-related Quality of Life Questionnaire.
The values of Spearman's correlation coefficients given in bold are significant ($p < 0.05$)

Table 4
Discriminative validity: scores for the individual items, dimensions and whole MIDAS and HURT questionnaires in the tension-type headache group versus the migraine group

Item or score	TTH (mean ± SD)	Migraine (mean ± SD)	p*
MIDAS			
Job absenteeism	1.16 ± 3.42	2.96 ± 4.90	**0.008**
Productivity loss at job	3.12 ± 8.14	4.24 ± 4.21	**0.017**
Incapable of housekeeping	3.63 ± 5.28	5.00 ± 5.63	0.185
Productivity loss at home	4.09 ± 6.13	5.60 ± 5.76	0.094
Avoiding social interaction	2.03 ± 3.62	5.56 ± 4.81	**0.000**
Total score MIDAS	13.88 ± 19.16	23.12 ± 17.47	**0.001**
HURT			
Headache frequency	2.59 ± 0.98	2.93 ± 0.73	**0.037**
Productivity loss	2.25 ± 0.97	2.63 ± 0.88	0.065
Avoiding social interaction	2.19 ± 0.90	2.59 ± 0.89	**0.015**
Taking analgesic	2.36 ± 1.00	2.70 ± 0.95	0.055
Efficacy of analgesic	2.18 ± 1.03	3.63 ± 3.86	**0.001**
Control of headache	2.59 ± 1.12	3.11 ± 1.37	0.050
Disability dimension	9.50 ± 3.33	10.85 ± 3.18	**0.023**
Treatment response dimension	4.77 ± 2.00	6.74 ± 4.11	**0.003**
Total score HURT	14.29 ± 4.44	17.59 ± 5.75	**0.002**

*TTH – subgroup of patients with the tension-type headache; MIDAS – Migraine Disability Assessment Test;
HURT – Headache Under-Response to Treatment questionnaire; SD – standard deviation.
*Mann-Whitney tests; significant differences ($p < 0.05$) are marked in bold.

Dimensions of the questionnaire

In order to check whether dimensions of the questionnaires defined in the English versions corresponded to the dimensions (factors) in Serbian versions, we performed the confirmatory factor analysis of the whole sample. For the MIDAS questionnaire the Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.669 and the Bartlett's Test of Sphericity was highly significant ($p = 0.000$).

Only one factor was extracted, explaining 51.3% of variability. For the HURT questionnaire the Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.812 and the Bartlett's Test of Sphericity was highly significant ($p = 0.000$). After orthogonal Varimax rotation, 2 factors were extracted: the first explaining 49.6% of variance and the second explaining 23.4%. The first factor consisted of items 1–4, and it explained the headache-related disability. The second factor consisted of items 5 and 6, explaining the treatment response.

Discussion

The Serbian translations of the MIDAS and the HURT questionnaires showed a satisfactory internal consistency as whole scales (the first 5 questions of the MIDAS and the first 6 questions of the HURT) when tested of the whole sample and on the subgroup of patients with TTH. The Disability dimension of the HURT questionnaires was also highly consistent, while the Treatment response dimension did not work well in the whole sample. The validity of the instruments in all aspects (criterion, convergent and discriminant validity) was also satisfactory when whole sample and the subgroup of patients with TTH were analyzed; only the items 1 (job absenteeism) and 2 (productivity loss at job) of the MIDAS questionnaires did not correlate with the attack length, which could be explained by social differences. The majority of patients in Serbia work in still un-reformed public sector for minimal wages, where the productivity is not an issue, therefore they were not familiar with this aspect of loss [14]. This was probably the reason why these two items did not correlate with the attack lenght well, and why their impact on the disability estimate was small.

While the MIDAS questionnaire showed good internal consistency in the subgroup of patients with the migraine, the HURT one did not perform well, but this was not the consequence of inherent weakness of the HURT. First of all, the MIDAS questionnaire was originally constructed for measuring a headache-related disability only in the migreneurs [8], and second, probably the small number of patients with the migraine (only 27) precluded the true estimate of the HURT's potential in this category of headache. The disability domain of the HURT questionnaire showed an excellent internal consistency in the migreneurs with the Cronbach's alpha of 0.937, what would likely happen if the testing was conducted on a larger sample of patients with the migraine.

When compared to the original English version [15], the Serbian translation of the HURT questionnaire is not inferior (alpha of the original is 0.85) and performs similarly on primary care patients as the Arabian translation (alpha 0.74) [16]. The Serbian translation of the MIDAS questionnaire also has somewhat lower internal consistency than the English original (0.73 vs. 0.84) [17]. However, the criterion validity of both instruments was good and the convergent validity was impressive in the whole Serbian sample, since i the inverse correlation with the CHQQ was mostly higher than 0.5, as well as the direct correlation between the MIDAS and the HURT themselves. The discriminant validity was good except for the questions about the productivity loss, which again shows that the full meaning of the term "productivity" is not yet clear to the majority of Serbian patients (as a matter of fact, this English term was just taken into Serbian language, since there was no appropriate Serbian word). Overall, the Serbian translations of the MIDAS and the HURT instruments could be considered as reliable and valid measurement of headache-related disability and treatment response in the patients with TTH, and probably with the migraine.

The original HURT questionnaire has two dimensions (disability and treatment efficacy) [9], while dimensions of the MIDAS questionnaire were not reported [8]. The factor analysis of the Serbian translation of the HURT questionnaire confirmed the same two dimensions, with the same distribution of items among the dimensions as in the original. On the other hand, the factor analysis of the MIDAS questionnaire found only one factor (dimension). This was additional confirmation of usefulness of Serbian translations of the two instruments.

The main limitation of this study was the small proportion of patients with migraine in the study sample, which precluded gathering a complete picture about the HURT and MIDAS performance in the patients with migraine. This limitation was caused by the site where the study was performed – the primary healthcare facility – which reflects the higher prevalence of TTH than that of migraine in the general population [1, 18].

Conclusion

In conclusion, the Serbian translations of the MIDAS and the HURT questionnaire appear to be reliable and valid specific instruments for measuring the headache-related disability and impact (MIDAS) and disability, impact and management (HURT) in the patients with TTH and probably in the patients with migraine. Further studies are necessary to confirm the usefulness of this instrument in the Serbian patients suffering from migraine, and to explore its suitability for other types of headache.

Acknowledgements

R E F E R E N C E S

1. *Zebenholzer K, Andree C, Lechner A, Broessner G, Lampl C, Luthringshausen G,* et al. Prevalence, management and burden of episodic and chronic headaches-a cross-sectional multicentre study in eight Austrian headache centres. J Headache Pain 2015; 16: 531.

2. *Albers L, Straube A, Landgraf MN, Filippopulos F, Heinen F, von Kries R.* Migraine and tension type headache in adolescents at grammar school in Germany – burden of disease and health care utilization. J Headache Pain 2015; 16: 534.

3. *Wöber-Bingöl C.* Epidemiology of migraine and headache in children and adolescents. Curr Pain Headache Rep 2013; 17(6): 341.

4. *Freitag FG, Schloemer F.* Medical management of adult headache. Otolaryngol Clin North Am 2014; 47(2): 221–37.

5. *Manhalter N, Bozsik G, Palásti A, Csépány E, Ertsey C.* The validation of a new comprehensive headache-specific quality of life questionnaire. Cephalalgia 2012; 32(9): 668–82.

6. *Ertsey C, Magyar M, Csépány É, Kellermann I, Balogh E, Juhász N,* et al. Validation of the comprehensive headache-related quality of life questionnaire (CHQQ) in cluster headache: preliminary data. Cephalalgia 2015 35(6 Suppl): 85.

7. *Gyüre T, Csépány É, Hajnal B, Kellermann I, Balogh E, Nagy Z,* et al. The comprehensive headache-related quality of life questionnaire shows significant improvement after withdrawal treatment in medication overuse headache: a pilot study. Ideggyogy Sz 2014; 67(5–6): 169–76; discussion 177.

8. *Stewart WF, Lipton RB, Dowson AJ, Sawyer J.* Development and testing of the Migraine Disability Assessment (MIDAS) Questionnaire to assess headache-related disability. Neurology 2001; 56(6 Suppl 1): S20–8.

9. *Buse DC, Sollars CM, Steiner TJ, Jensen RH, Al Jumah MA, Lipton RB.* Why HURT? A review of clinical instruments for headache management. Curr Pain Headache Rep 2012; 16(3): 237–54.

10. *Stojimirovic B, Milinkovic M, Zidverc-Trajkovic J, Trbojevic-Stankovic J, Maric I, Milic M,* et al. Dialysis headache in patients undergoing peritoneal dialysis and hemodialysis. Ren Fail 2015; 37(2): 241–4.

11. *Headache Classification Committee of the International Headache Society (IHS).* The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia 2013; 33(9): 629–808.

12. *Wild D, Grove A, Martin M, Eremenco S, McElroy S, Verjee-Lorenz A,* et al. ISPOR Task Force for Translation and Cultural Adaptation. Principles of Good Practice for the Translation and Cultural Adaptation Process for Patient-Reported Outcomes (PRO) Measures: report of the ISPOR Task Force for Translation and Cultural Adaptation. Value Health 2005; 8(2): 94–104.

13. *Carleton RN, Thibodeau MA, Teale MJ, Welch PG, Abrams MP, Robinson T,* et al. The center for epidemiologic studies depression scale: a review with a theoretical and empirical examination of item content and factor structure. PLoS One 2013; 8(3): e58067.

14. *Stošić S, Karanović N.* Health care economics in Serbia: current problems and changes. Vojnosanit Pregl 2014; 71(11): 1055–61.

15. *Westergaard ML, Steiner TJ, MacGregor EA, Antonaci F, Tassorelli C, Buse DC,* et al. The Headache Under-Response to Treatment (HURT) Questionnaire: assessment of utility in headache specialist care. Cephalalgia 2013; 33(4): 245–55.

16. *Al Jumah M, Al Khathaami A, Tamim H, Al Owayed A, Kojan S, Jawhary A,* et al. HURT (Headache Under-Response to Treatment) questionnaire in the management of primary headache disorders: reliability, validity and clinical utility of the Arabic version. J Headache Pain 2013; 14: 16.

17. *Stewart WF, Lipton RB, Kolodner K, Liberman J, Sawyer J.* Reliability of the migraine disability assessment score in a population-based sample of headache sufferers. Cephalalgia 1999; 19(2): 107–14; discussion 74.

18. *Vlajinac HD, Dzoljic ED, Sipetic SB, Kostic VS.* Hereditary patterns of Belgrade university female students with migraine and nonmigraine primary headache. J Neurol 2004; 251(8): 973–6.

Correlation of sociodemographic variables and interpersonal sources of stress at work among miners

Odnos sociodemografskih varijabli i interpersonalnih izvora stresa na radu kod rudara

Ljiljana Kulić*, Milivoje Galjak*, Jovana Jovanović[†]

University of Priština/Kosovska Mitrovica, *Faculty of Medicine, Kosovska Mitrovica,
Serbia; University of Niš, [†]Faculty of Medicine, Niš, Serbia

Abstract

Background/Aim. Some professions are somewhat more exposed to stress and have been the subject of research for a long time, and research of occupational stress among employees at workplaces with a special health risk is of a special significance. That is the case with miners. The aim of the study was an assessment of the relation between the sociodemographic variables and interpersonal sources of stress at work among miners. **Methods.** The study was designed as a cross-sectional study and covered 170 respondents, classified into two groups: a study group comprised of miners (n = 142) and a control group (n = 28) comprised of administrative workers. The research was conducted at the Occupational Medicine Service of the Health Center Zvečan and at the Institute of Occupational Medicine in Niš. The questionnaire on the basic sociodemographic indicators of respondents, the Intrapersonal and Interpersonal Related Stressors Questionnaire (IRS), the Work Ability Index Questionnaire (WAI), the General Health Questionnaire (GHQ), the Copenhagen Burnout Inventory (CBI) and the Questionnaire on the stressors were used in this research. **Results.** Statistically, the miners consumed alcohol significantly more than the respondents from the control group (68.30% vs. 25.00%; $p < 0.001$). Relative to age, there was a statistically significant difference in the subscales: demands ($p = 0.037$), control ($p = 0.010$), relations ($p = 0.009$) and change ($p = 0.008$). The control score values increased with age. The relations values were highest among the oldest respondents. The values of change decreased with age. Relative to exposed years of service (ERS), there was a statistically significant difference in the subscale relations; the miners with ERS longer than 15 years had a statistically significantly higher relations score ($p = 0.003$). The values of subscales control ($p < 0.001$), manager support ($p = 0.010$), relations ($p < 0.001$) and work role ($p < 0.001$) were statistically significantly lower among the miners compared to the control group. The values of subscales peer support ($p = 0.002$) and change ($p < 0.001$) were statistically significantly higher among the miners compared to the control group. **Conclusion.** The results indicate the correlation of sociodemographic variables with interpersonal sources of stress at work among the miners. The following prevention measures are proposed: adequate professional orientation, professional selection, professional adaptation, organizational measures, progressive development of knowledge and skills, development of good interpersonal relationships, strengthening the family-organization-company relation, relaxation techniques at the workplace, stress control, application of physiological techniques, application of cognitive techniques.

Key words:
miners; sociological factors; interpersonal relations; stress, psychological; risk factors; occupational exposure; surveys and questionaires.

Apstrakt

Uvod/Cilj. Neke profesije su nešto više izložene stresu i već duže vreme su predmet istraživanja, a poseban značaj ima istraživanje profesionalnog stresa kod zaposlenih na radnim mestima sa posebnim rizikom po zdravlje, što je slučaj sa rudarima. Cilj rada bio je procena odnosa sociodemografskih varijabli i interpersonalnih izvora stresa na radu kod rudara. **Metode.** Istraživanje je dizajnirano kao studija preseka i njime je obuhvaćeno 170 ispitanika, svrstanih u dve grupe: ispitivana grupa, koju su činili rudari (n = 142) i kontrolna grupa (n = 28) koju činili adminstrativni radnici. Istraživanje je sprovedeno u Domu zdravlja Zvečan, u Službi medicine rada u Zvečanu i u Institutu za medicinu rada u Nišu. U istraživanju su korišćeni: Upitnik o osnovnim sociodemografskim pokazateljima ispitanika, Upitnik o interper-

Correspondence to: Ljiljana Kulić, University of Priština/Kosovska Mitrovica, Faculty of Medicine, Kosovska Mitrovica, Serbia, Ul. Blagoja Parovića 102V, 11 000 Beograd, Serbia. E-mail. ljiljana.kulic@yahoo.com

sonalnim i intrapersonalnim stresorima, Upitnik indeksa radne sposobnosti, Upitnik opšteg zdravlja, *Copenhagen Burnout Inventory* i Upitnik o stresorima. **Rezultati.** Rudari su statistički značajno češće konzumirali alkohol u odnosu na ispitanike kontrolne grupe (68,30% vs. 25,00%; $p < 0,001$). U odnosu na starosne grupe postojala je statistički značajna razlika u supskalama: zahtevi ($p = 0,037$), kontrola ($p = 0,010$), relacija ($p = 0,009$) i promena ($p = 0,008$). Vrednosti skora kontrola rasle su sa starošću. Vrednosti skora relacije bila je najveći kod najstarijih ispitanika. Vrednosti skora promena opadale su sa starosti. U odnosu na efektivan radni staž (ERS) postojala je statistički značajna razlika u supskali relacije; rudari sa ERS dužim od 15 godina imali su statistički značajno veći skor relacije ($p = 0,003$). Vrednosti supskala kontrola ($p < 0,001$), podrška rukovodioca ($p = 0,010$), relacije ($p < 0,001$) i radna uloga ($p < 0,001$) statistički je bila značajno manja kod rudara u odnosu na kon-

trolnu grupu. Vrednosti supskala podrška kolega ($p = 0,002$) i promene ($p < 0,001$) statistički vila je značajno veća kod rudara u odnosu na kontrolnu grupu. **Zaključak.** Rezultati ukazuju na povezanost pojedinih sociodemografskih varijabli sa interpersonalnim izvorima stresa na radu kod rudara. Predlažu se sledeće mere prevencije: adekvatna profesionalna orijentacija, profesionalna selekcija, profesionalna adaptacija, organizacione mere, postepeno razvijanje znanja i sposobnosti, razvijanje dobrih međuljudskih odnosa, jačanje veza porodica-organizacija-preduzeće, tehnike relaksacije na radnom mestu, kontrola stresa, primena fizioloških tehnika, primena saznajnih tehnika.

Ključne reči:
rudari; socijalni faktori; međuljudski odnosi; stres, psihički; faktori rizika; profesionalna izloženost; ankete i upitnici.

Introduction

Studies oriented at identifying dangers and risks at the workplace and working environment which can cause injury at work and/or occupational disease are always actual. These studies must be continuous, because some dangers are not known enough, workplace hazards change and new ones appear. One of the most serious workplace dangers is stress. Some professions are somewhat more exposed to stress and for a long time they are the subject of research, such as managers, pilots, flight controllers, rescuers, journalists, actors, miners, construction workers, health workers, etc. The study of occupational stress among employees at workplaces with a special health risk is of great importance, which is the case with miners [1–3]. According to the European Agency for Safety and Health at Work (EU-OSHA) study, workplace stress is present in almost every third employee in the European Union (EU). Stress at work in the EU includes 28% of employees, or 41.2 million people. The result of occupational stress is 50%–60% of all lost working days, but also about 5 million accidents at work and a loss of at least 20 billion euros per year. In the EU, 12 million people complained that they were offended by management staff (8%); 6 million (4%) complained of physical violence, and 3 million (2%) of sexual abuse [4,5].

The aim of this study was to examine the connection of the Interpersonal Relationship Scale (IRS) subscales with other examined scores, which follow the types of stressors, impact of stress at work on the general health condition of miners, occupational burnout and their working ability through appropriate tests, and to evaluate the correlation of sociodemographic variables and interpersonal sources of stress at work at miners.

Methods

The study covered 170 respondents. There were two groups of respondents: the tested group (n = 142), consisting of miners, and the control group (n = 28), consisting of administrative staff of the company that deals with the installing of quality standards. Unlike miners, administrative workers do not have contact with risks and they work a steady job

without stress. The research was designed as a cross-sectional study. The criteria for inclusion in the study were: for the tested group – the Trepča Mines miners who come to the periodical control examinations at the Occupational Medicine Service of the Health Center Zvečan, and for the control group – administrative workers who come to the examinations at the Institute of Occupational Medicine in Niš. The oral consent of participating in the research obtained from all respondents. The respondents who refused to participate were excluded from the study. After giving the consent to participate in the study, the respondents were explained the goals and purpose of the research. After the questionnaire was distributed and a brief explanation was given, the respondents filled in the questionnaire themselves, or with the help of a nurse, or researcher. The respondents were guaranteed privacy through their anonymous and voluntary participation. The research was conducted at the Occupational Medicine Service of the Health Center Zvečan, before miners' periodical control examinations, and at the Institute of Occupational Medicine in Niš, from February to April 2017. Researchers used the Questionnaire on basic sociodemographic indicators of respondents, the Interpersonal Relationship Scale Questionnaire – IRS, the Work Ability Index Questionnaire – WAI, the General Health Questionairre – GHQ, the Copenhagen Burnout Inventory – CBI and the Questionnaire on stressors.

The Questionnaire on basic sociodemographic indicators of respondents contains general information, basic information about sex, age, marital status, habitation, type of work, eventual use of alcohol, cigarettes and sedatives, etc.

The IRS is used for evaluating interpersonal sources of stress at work. The respondent should read each claim carefully and mark the answer that best describes how much each event is present at his workplace. The scale has 42 items, and the response format is the Likert five point scale ("almost never", "rarely", "sometimes", "often" and "almost always"). It contains seven subscales that evaluate various sources of stress in the organization, such as: job demands, control, manager support, peer support, relations and working atmosphere, work role and change.

The WAI is a standardized questionnaire of the Finnish Institute of Occupational Health. It is used to test the working ability in relation to job demands. The WAI is expressed numerically and calculated according to the instructions of the Finnish Institute of Occupational Health. The questionnaire contains seven items: 1. subjective assessment of current work ability compared to the best work ability in life (grade 1–10); 2. subjective assessment of work ability in relation to the physical and mental requirements of the work position (grade 1–10); 3. number of diagnosed diseases (injuries, diseases of the musculoskeletal system, diseases of the cardiovascular and respiratory system, psychological disorders, neurological and sensory diseases, gastrointestinal system diseases, urogenital system diseases, tumors, endocrine diseases, blood diseases, etc.); 4. subjective determination of the disease impact on work (grade 1–6); 5. sick leave during the previous year (scale 1–5; 5 – no sick leave at all; 4 – a maximum of 9 days; 3 – 10–24 days; 2 – 25–99 days; and 1 – 100–365 days); 6. personal forecast of work ability for the next two years; 7. questions about mental health and satisfaction. The score ranges from 7 to 49 points. A higher score indicates better work ability. According to the number of points, WAI is ranked in four categories: poor (7 to 27 points), indicating poor working ability; good, (from 28 to 36 points), meaning moderate working ability; very good (from 37 to 43 points), marking good working ability; excellent (from 44 to 49 points), for excellent working ability.

The GHQ measures the sense of tension, depression, inability to defend, anxiety based insomnia, lack of self-confidence and self-esteem and other symptoms of mental disorders. There are four variants of this questionnaire, and the GHQ-12 variant used in this study is recommended for measuring psychological distress. The test contains 12 items (points) that offer four responses (better than usual, same as usual, less than usual, much less than usual). In this study, the bimodal scoring method (0-0-1-1) was used in accordance with the official manual. The maximum score (number of points on the test) is 12, and the possible range is 0–12. A score of 4, or more, means a possible presence of mental distress, and a score of 8, or more, indicates certain presence of various symptoms of stress-related psychological disorders.

The CBI is a questionnaire that has been in use since 2004. It consists of 19 items and includes three scales: personal burnout, occupational burnout and client burnout. In this research, a part of the scale related to occupational burnout will be used and it consists of 7 questions, to which the respondents can choose one of the five offered answers. For the first three questions, the respondents choose one of the following possible answers: a very high level, a high level, here and there, low level and very low level. For the other four questions the following answers are offered: always, often, occasionally, rarely and never/almost never.

The Questionnaire on stressors assesses the causes of stress on specified work position, i.e., the respondent is asked to explain 37, the most frequently represented offered stressors. The questions are answered by marking the number of offered answer that best describes the respondent's opinion on the existence of an appropriate stressor, using the

scale for rating from 1 to 5. In this case, 1 means not stressful at all, 2 marks rarely stressful, 3 indicates sometimes stressful, 4 – stressful and 5 – extremely stressful.

The statistical data analysis

Data is presented in the form of an arithmetic mean and standard deviation, i.e., in the form of absolute and relative numbers. The comparison of continuous variables between two or more groups was conducted using the ANOVA (Analysis of variance), or the Kruskal-Wallis test, depending on the data distribution. The comparison of continuous variables between the two groups was performed by the Student *t*-test, or the Mann-Whitney U test, depending on the data distribution. The comparison of categorical variables was done by the χ^2 test or the Fisher's exact test. The correlation of the tested subscales was tested by the Pearson's correlation coefficient. The multiple linear regression analysis was used to test the effects of sociodemographic and clinical parameters on the IRS score subscales. The hypothesis was tested with a threshold of significance of $p < 0.05$. The data analysis was performed using the SPSS 16.0 software package.

Results

The average age of miners was 46.00 ± 11.68 years of age (min 22, max 64 years) and the average age of the control group respondents was 46.19 ± 8.98 years of age (min 31, max 66 years). There was a statistically significant difference in the structure by sex between miners and control group ($p < 0.001$). Most miners were married (71.80%). Most of them had two children (36.60%) and lived in a rented apartment (50.00%). Cigarettes were consumed by one-third of miners (33.80%), 68.30% consumed alcohol, and 9.60% consumed sedatives (Table 1). The subjects in the control group had statistically significantly more children than the miners (29.60% vs. 9.20%, $p = 0.011$). The miners were statistically significantly more likely to live in a rented apartment compared to the control group (50.00% vs. 10.70%; $p < 0.001$). The control group respondents statistically significantly more often lived in their apartment than the miners (78.60% vs. 34.50%; $p < 0.001$). The miners were statistically significantly more likely to consume alcohol than those of the control group (68.30% vs. 25.00%; $p < 0.001$). The length of years of service was uniform compared to the examined groups ($p = 0.659$).

Relative to the age groups, there were statistically significant differences in the subscales: demands ($p = 0.037$), control ($p = 0.010$), relations ($p = 0.009$) and change ($p = 0.008$). The respondents aged 45–54 years had the lowest score of demands, while the youngest respondents had the highest demands score. The values of control score increased with age. The values of relations score were the highest among the oldest respondents. The values of change decreased with age. Relative to the total years of service (URS), there were statistically significant differences in the subscales manager support ($p = 0.020$) and relations ($p = 0.008$). The score of manager support was statistically significantly higher in the respondents with fewer years of

service, while the relations score was statistically significantly higher in the respondents with more years of service. Relative to the exposed years of service (ERS), there was a statistically significant difference in the subscale relations, the miners with the ERS longer than 15 years had the statistically significantly higher relations score ($p = 0.003$) (Table 2).

Table 1

Demographic characteristics

Parameter	Miners	Control group	p
Sex (male/female), n	142/0	19/9	< 0.001[1]
Age (years), mean ± SD	46.00 ± 11.68	46.19 ± 8.98	0.734[2]
Marriage, n (%)			
single	11 (7.70)	4 (14.80)	0.453[1]
married	102 (71.80)	21 (77.80)	0.911[1]
cohabitation	12 (8.50)	0	0.219[3]
divorced	11 (7.70)	0	0.270[3]
widower	2 (1.40)	1 (3.70)	0.419[1]
Number of children, n (%)			
without children	46 (32.40)	7 (25.90)	0.583[1]
1 child	13 (9.20)	8 (29.60)	0.011[1]
2 children	52 (36.60)	11 (40.70)	0.957[1]
3 children	27 (19.00)	1 (3.70)	0.051[3]
4 children	4 (2.80)	0	1.000[3]
Type of housing, n (%)			
with parents	22 (15.50)	3 (10.70)	0.770[3]
own apartment	49 (34.50)	22 (78.60)	< 0.001[1]
rented apartment	71 (50.00)	3 (10.70)	< 0.001[1]
Consume, n (%)			
cigarettes	48 (33.80)	8 (28.60)	0.750[1]
alcohol	97 (68.30)	7 (25.00)	< 0.001[1]
sedatives	14 (9.60)	2 (7.10)	0.923[1]
URS (mean ± SD)	15.00 ± 7.35	18.21 ± 11.73	0.659[4]
ERS (mean ± SD)	13.00 ± 7.63		

URS –total years of service; ERS – exposed years of service.
[1]χ^2 test; [2]Student t-test; [3]Fisher's exact test; [4]Mann-Whitney test.

Table 2

Analysis of subscales of Interpersonal relationship scale (IRS) according to the sociodemographic characteristics

Parameter	Demands mean ± SD	Control mean ± SD	Manager support mean ± SD	Peer support mean ± SD	Relations mean ± SD	Work role mean ± SD	Change
Age category (years)							
< 25	23.20 ± 4.09	12.80 ± 1.64	21.40 ± 2.19	19.20 ± 1.92	11.40 ± 2.79	12.50 ± 1.00	16.20 ± 1.78
25–34	22.74 ± 3.88	14.70 ± 2.78	21.26 ± 3.51	21.04 ± 4.02	11.13 ± 2.47	12.74 ± 2.00	16.17 ± 1.82
35–44	22.77 ± 3.73	15.89 ± 3.20	22.39 ± 4.89	23.12 ± 3.82	11.00 ± 3.50	12.43 ± 2.43	14.58 ± 2.10
45–54	20.61 ± 2.88	16.16 ± 2.65	21.81 ± 5.37	20.55 ± 4.74	12.73 ± 4.11	12.66 ± 2.84	14.46 ± 1.79
55–64	22.56 ± 3.32	16.92 ± 3.31	21.05 ± 4.90	20.86 ± 4.98	13.71 ± 3.46	12.11 ± 2.99	14.76 ± 2.17
p-value[1]	0.037	0.010	0.800	0.084	0.009	0.884	0.008
Marriage							
single	22.95 ± 3.91	16.51 ± 3.68	21.59 ± 3.81	20.16 ± 4.17	12.16 ± 3.68	12.43 ± 2.34	14.92 ± 2.74
married	21.82 ± 3.33	15.73 ± 2.83	21.67 ± 5.05	21.73 ± 4.55	12.29 ± 3.64	12.47 ± 2.68	14.92 ± 1.78
p-value[2]	0.117	0.178	0.922	0.061	0.857	0.936	0.999
Number of children							
without children	21.56 ± 2.99	15.96 ± 3.34	21.60 ± 4.28	20.80 ± 4.18	12.29 ± 3.98	12.37 ± 3.68	14.64 ± 1.87
with children	22.41 ± 3.73	15.94 ± 3.00	21.68 ± 4.96	21.55 ± 4.64	12.24 ± 3.48	12.50 ± 2.55	15.05 ± 2.15
p-value[2]	0.149	0.975	0.925	0.341	0.943[3]	0.793[3]	0.254[3]
Type of housing							
with parents	22.19 ± 4.40	15.05 ± 2.67	21.45 ± 4.78	19.57 ± 4.51	12.45 ± 3.65	12.35 ± 2.72	15.20 ± 2.38
own apartment	21.67 ± 2.96	16.02 ± 2.58	21.75 ± 5.09	21.57 ± 4.01	12.41 ± 3.52	12.63 ± 2.97	14.96 ± 1.87
rented apartment	22.44 ± 3.61	16.15 ± 3.50	21.64 ± 4.53	21.53 ± 4.73	12.28 ± 3.76	12.38 ± 2.29	14.81 ± 2.12
p-value[4]	0.621	0.405	0.961	0.165	0.913	0.898	0.806
URS (years)							
≤ 15	21.68 ± 3.68	15.83 ± 3.48	22.57 ± 4.74	21.59 ± 4.43	11.45 ± 3.40	12.25 ± 2.50	14.94 ± 2.24
> 15	22.61 ± 3.31	16.06 ± 2.68	20.71 ± 4.57	21.03 ± 4.56	13.07 ± 3.70	12.68 ± 2.65	14.90 ± 1.89
p-value[3]	0.117	0.666	0.020	0.468	0.008	0.332	0.900
ERS (years)							
≤ 15	22.07 ± 3.81	15.82 ± 3.36	22.05 ± 4.70	21.30 ± 4.35	11.52 ± 3.38	12.41 ± 2.47	15.02 ± 2.14
> 15	22.25 ± 3.01	16.15 ± 2.63	20.98 ± 4.76	21.32 ± 4.74	13.46 ± 3.74	12.55 ± 2.78	14.75 ± 1.94
p-value[3]	0.762	0.514	0.205	0.977	0.003	0.764	0.442

URS – total years of service; ERS – exposed years of service; SD – standard devation;
[1]ANOVA; [2]t-test; [3]Mann Whitney test; [4]Kruskal-Wallis test.

Table 3

Connection of the IRS subscales with other examined scores

Subscales	WAI		GHQ		Stressors		CBI	
	r	p	r	p	r	p	r	p
Demands	0.062	0.466	0.279	0.001	0.491	<0.001	0.066	0.438
Control	-0.064	0.454	0.033	0.698	0.111	0.188	0.041	0.630
Manager support	0.118	0.168	-0.140	0.103	-0.196	0.021	-0.217	0.011
Peer support	0.143	0.095	-0.277	0.001	-0.019	0.825	-0.186	0.030
Relations	-0.231	0.007	0.350	< 0.001	0.094	0.274	0.349	< 0.001
Work role	-0.082	0.338	-0.019	0.825	0.183	0.032	0.117	0.172
Change	0.035	0.683	-0.099	0.248	-0.037	0.663	-0.009	0.919

WAI – Work Ability Index; GHQ – General Health Questionnaire; CBI – Copenhagen Burnout Inventory.
r – correlation coefficient.

The correlation analysis showed that the WAI score was statistically significantly related to the relations subscale ($r = -0,231$; $p = 0.007$). The GHQ score was related to the demands subscale ($r = 0.279$; $p = 0.001$), the peer support subscale ($r = -0.277$; $p = 0.001$) and the relations subscale ($r = 0.350$; $p < 0.001$). The stressors scale was associated with the demands subscale ($r = 0.491$; $p < 0.001$), manager support subscale ($r = -0.196$; $p = 0.021$) and the work role subscale ($r = 0.183$; $p = 0.032$). The CBI scale correlated with the manager support scale ($r = -0.217$; $p = 0.011$), peer support subscale ($r = -0.186$; $p = 0.030$) and the relations subscale ($r = 0.349$; $p < 0.001$) (Table 3).

There was a statistically significant influence on the demands subscale regarding age (Beta = -0.385; $p = 0.002$), URS (Beta = 0.363; $p = 0.039$), GHQ scale (Beta = 0.304; $p = 0.002$), and stressors (Beta= 0.440; $p < 0.001$). The stressors had the strongest impact on this subscale. There was a statistically significant influence on the control subscale regarding age (Beta = 0.705; $p < 0.001$) and marital status (Beta = -0.224; $p = 0.010$). There was a statistically significant influence on the manager support subscale regarding URS (Beta = 0.455; $p = 0.029$) and CBI (Beta = -0.242; $p = 0.045$). There was a statistically significant influence on the peer support subscale regarding GHQ (Beta = -0.303; $p = 0.010$). None of the tested parameters had a statistically significant influence on the relations subscale. There was a statistically significant influence on the work role subscale regarding the age (Beta = -0.320; $p = 0.031$), GHQ (Beta = -0.252; $p = 0.035$) and stressors (Beta = 0.220; $p = 0.019$). There was a statistically significant influence on change subscale regarding age (Beta = 0.364; $p = 0.017$) (Table 4).

The values of the subscale demands were equal in comparison to the examined groups ($p = 0.848$). The values of the subscales control ($p < 0.001$), manager support ($p = 0.010$), relations ($p < 0.001$) and working role ($p < 0.001$) were statistically significantly lower in the miners compared to the control group (Table 5, Table 6, Figure 1). The values of subscales peer support ($p = 0.002$) and changes ($p < 0.001$) were statistically significantly higher among the miners compared to the control group.

Table 5

Subscale values relative to the examined groups

IRS subscale	Miners mean ± SD	Control group mean ± SD	p[1]
Demands	22.14 ± 3.52	21.88 ± 2.82	0.848
Control	15.94 ± 3.10	20.65 ± 3.93	< 0.001
Manager support	21.65 ± 4.73	23.35 ± 3.07	0.010
Peer support	21.31 ± 4.49	18.50 ± 2.66	0.002
Relations	12.26 ± 3.63	14.92 ± 2.35	< 0.001
Work role	12.46 ± 2.58	15.85 ± 2.20	< 0.001
Change	14.92 ± 2.06	11.92 ± 2.54	< 0.001

IRS – Interpersonal Relationship Scale; [1]Mann-Whiteney test; SD – standard devation.

Table 6

Subscale values relative to examined groups (male controls)

IRS subscale	Miners mean ± SD	Male control mean ± SD	p[1]
Demands	22.14 ± 3.52	22.35 ± 2.78	0.771
Control	15.94 ± 3.10	21.53 ± 4.17	< 0.001
Manager support	21.65 ± 4.73	24.12 ± 2.50	0.002
Peer support	21.31 ± 4.49	19.41 ± 2.32	0.009
Relations	12.26 ± 3.63	14.35 ± 1.84	< 0.021
Work role	12.46 ± 2.58	15.24 ± 2.08	0.001
Change	14.92 ± 2.06	11.53 ± 2.55	< 0.001

IRS – Interperosnal Relationship Scale; SD – standard devation; [1]Mann-Whiteney test.

Discussion

The miners belong to a group of workers who work at the workplaces with increased health risks and are exposed to a large number of stress factors during their work. On one hand, there are complex work tasks and often poor working conditions with the presence of physical and chemical harmful noxae, and on the other, there is a demand for almost absolute precision, corresponding effect and achievement of

Table 4

Multiple regression analysis of factors associated with Interpersonal Relationship Scale (IRS)

Parameter	Demands			Control			Manager support			Peer support			Relations			Work role			Change		
	B	Beta	p	B	Beta	p	B	Beta	p	B	Beta	p	B	Beta	p	B	Beta	p	B	Beta	p
Age	-0.117	-0.385	0.002	0.189	0.705	0.000	0.102	0.251	0.093	-0.049	-0.127	0.396	0.030	0.097	0.503	-0.072	-0.320	0.031	-0.065	-0.364	0.017
URS	0.174	0.363	0.039	-0.132	-0.313	0.113	-0.295	-0.455	0.029	-0.112	-0.184	0.380	0.061	0.124	0.540	0.043	0.120	0.564	0.096	0.340	0.106
ERS	-0.052	-0.113	0.530	-0.059	-0.146	0.475	0.112	0.179	0.404	0.222	0.378	0.079	-0.012	-0.025	0.906	0.036	0.105	0.622	-0.055	-0.201	0.356
WAI	0.044	0.116	0.193	0.007	0.020	0.845	0.005	0.010	0.924	0.020	0.041	0.712	0.004	0.010	0.920	-0.031	-0.111	0.317	-0.014	-0.064	0.556
GHQ	0.311	0.304	0.002	-0.054	-0.060	0.585	0.071	0.052	0.655	-0.400	-0.303	0.010	0.223	0.212	0.058	-0.189	-0.252	0.036	-0.079	-0.131	0.263
Stressors	0.108	0.440	0.000	0.019	0.088	0.307	-0.043	-0.131	0.153	0.035	0.112	0.218	-0.019	-0.076	0.389	0.039	0.220	0.019	-0.002	-0.015	0.869
Marriage	-0.363	-0.046	0.542	-1.546	-0.224	0.010	-0.436	-0.041	0.657	1.703	0.169	0.064	-0.429	-0.053	0.551	0.069	0.012	0.898	0.175	0.038	0.682
Children	0.752	0.070	0.340	0.371	0.039	0.639	1.162	0.081	0.361	0.367	0.027	0.759	0.693	0.063	0.462	0.204	0.026	0.771	0.533	0.085	0.344
CBI	-0.013	-0.082	0.409	-0.011	-0.077	0.497	-0.052	-0.242	0.045	-0.009	-0.042	0.719	0.026	0.157	0.177	0.028	0.237	0.054	0.015	0.165	0.174
R^2	0.619		<0.001	0.440		0.001	0.347		0.050	0.377			0.426		0.002	0.329		0.092	0.301		0.182
Adjusted R^2	0.383			0.194			0.121			0.142			0.181			0.108			0.027		

B – unstandardized regression coefficient. Beta – standardized regression coefficient; URS – total years of service; ERS – exposed years of service; WAI – Work Ability Index; GRQ – General Health Questionnaire; CBI – Copenhagen Burnout Inventory.
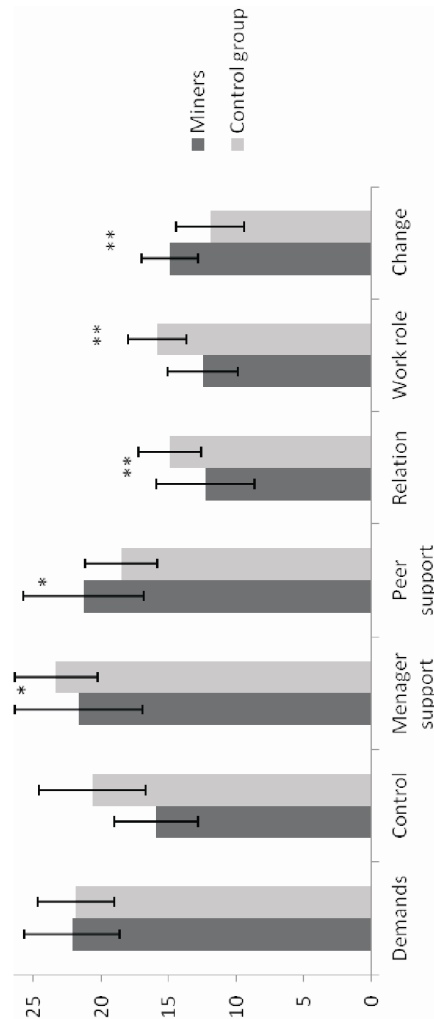

Fig. 1 – The Interpersonal Relationship Scale (IRS) subscales relative to examined groups ($*p < 0.05$; $**p < 0.001$).

work results that are standardized and related to economic compensation, whereby any mistake can lead to the severe consequences for the health and life of workers. The miners have a worse social status and most of them do not have an adequately resolved housing issue, which is one of the stressful factors.

Relative to the age groups, there is a statistically significant difference in the IRS subscales demands, control, relations and changes. In the demands subscale, the higher the score, the higher the job demands, and this research shows that the minimum score is related with the respondents aged 45–54, and the highest with the youngest respondents. In the subscale control, the higher the score, the more control over own work, and in our research the value of the control score increases with age. The impact of stress on human health and labor productivity has been studied for many years and studies showed that the level of stress increases as job demands increase and the decision-making level decreases, whereby stress at work is not the result of only one factor, but the sum of increased demands and low level of decision-making, or control [6, 7]. The high job demands with a small work control are considered as high work stressors [8, 9]. Older employees have more problems with accepting the permanent changes and new requirements in the field of informatics application in their everyday work activities. The rapid deprivation of knowledge, skills and techniques at work is a widespread phenomenon, which tails many professions. Workers are required to continuously monitor business and technology innovation and adopt them quickly. Mismatch between job requirements and individual potentials, i.e., too low, or too high job demands, jobs that are below, or above individual potentials, frustrate and make the perpetrator dissatisfied, and that mismatch as such can be the source of stress [10, 11]. In the subscales relations and working atmosphere, the higher the score, the worse the relations and the working atmosphere are, and the values of the relations are highest among the oldest respondents. In the changes subscale, the higher the score, the more the stressors related to changes at work there are, and this research shows that the values of the changes score decrease with age.

The organizational and financial issues are at a high level as stress factors, which is confirmed by various studies [12]. In addition, modern technologies enable electronic monitoring, i.e., continuous control of employees' work using cameras, which besides the undoubted benefits for efficient and quality work, also represents another difficult requirement for most employees. Abuse at work, or mobbing, is very actual in recent years. An unclear working role arises when a worker does not have adequate information about what he/she is expected to do, and the scope and type of work responsibilities can also be unclear. Disturbed employee relations and frequent conflicts cause the inevitable stressful effects manifested by various symptoms [13–15]. In this research, in the manager support subscale, the higher score, the more manager support there is; in the peer support subscale, the higher the score, the more peer support is present; and in the work role subscale, the higher the score, the more stressors related to the work role there are. The length of

years of service is uniform compared to both examined groups. Relative to the total years of service (URS), there are the statistically significant differences in the subscales manager support and relations, and the manager support score is statistically significantly higher in the respondents with fewer years of service, while the relations score is statistically significantly higher in the respondents with more years of service, and the higher relations score, the worse working atmosphere is. Relative to the exposed years of service (ERS), there is a statistically significant difference in the subscale relations, the miners with the ERS longer than 15 years have a statistically significantly higher relations score, which indicates the poor relations and working atmosphere in this group of respondents, as confirmed by some studies [16–19].

The correlation analysis showed that the WAI score is statistically significantly related to the relations subscale, and the higher WAI score means better working ability, in the older respondents and ones with the ERS longer than 15 years. The GHQ score is related to the demands subscale, the peer support subscale and the relations subscale. The GHQ was used in this research to measure the psychological distress and presence of various symptoms of stress-related psychical disorders, and the more score, the greater presence of these symptoms is, which is associated with the increased demands at work, peer support, relations with colleagues and working atmosphere. The CBI scale correlates with the manager support scale, peer support subscale and the relations subscale. The occupational burnout is associated with the poor manager support, poor peer support and poor work relations and working atmosphere. The stressors scale is associated with the following subscales: demands, manager support and work role. The age, URS, GHQ scale and esspecially stressors have a statistically significant influence on the demands subscale. The age and marital status have a statistically significant influence on the control subscale. The URS and CBI have a statistically significant influence on the manager support subscale. The GHQ has a statistically significant influence on the peer support subscale. None of the tested parameters has a statistically significant influence on the relations subscale. The age, GHQ and stressors have a statistically significant influence on the work role subscale. Furthermore, the age has a statistically significant influence on the changes subscale. These results coincide with the results of other authors [19–22].

The values of the demands subscale are uniform in comparison to the tested groups. The values of the subscales control, manager support, relations and working role, are statistically significantly lower among the miners compared to the control group. The values of the subscales peer support and changes are statistically significantly higher in the miners compared to the control group. The most common causes of stress at the workplace are a fear of losing the job, overstrain, the short deadlines, a lack of manager support, the inability to control their own time and output, or influence the work methods, the sense of alienation from the management, feeling of overwhelming exploitation or dormancy, idling, monotony, numerous physical, biological and chemical ef-

fects [9]. Job satisfaction and good interpersonal relations can have protective treatment on the stress impacts [13–15]. As the workplace related demands (work standardization, longer working hours, shift work and work at night), the quality of social life aggravates, and the economic status is lower than the status of some other professions. The social component is also disturbed, less time remains for the quality development of family relations, satisfying their interpersonal needs and needs that are not related to professional engagement [19–21]. It was shown that the miners are statistically significantly more likely to consume alcohol than those of the control group. Smoking, excessive alcohol and coffee drinking, drug use, poor appetite, high need for food, physical passivity, excessive sports activity and overwork are the result of wrong reaction to stress [22–24].

Conclusion

The results indicate the correlation of some sociodemographic variables and interpersonal sources of stress at work among miners. Relative to the age groups, there is a statistically significant difference in the subscales demands, control, relations and changes. The youngest respondents have the highest score, while the minimum score is noted in those aged 45–54 years, and the stress level increases as job demands increase and the level of decision-making decreases.

The value of the control and relations score increases with age, and the value of the change score decreases. Relative to the total years of service, the manager support score is statistically significantly higher in the respondents with less years of service, while the relations score is statistically significantly higher for those with more years of service. The miners with the exposed years of service longer than 15 years have a statistically significantly higher relations score. The values of the control, manager support, relations and working role subscales are statistically significantly lower among miners compared to the control group. The values of the subscales peer support and changes are statistically significantly higher among the miners compared to the control group.

The following prevention measures are proposed: adequate professional orientation, professional selection, professional adaptation, organizational measures, progressive development of knowledge and skills, development of good interpersonal relationships, strengthening the family-organization-company relation, relaxation techniques at the workplace, stress control (personal strategy, organizational strategy, change of lifestyle, i.e. changes in habits related to nutrition, physical activity, smoking, alcoholism, etc.), application of physiological techniques (meditation, resting, spiritual entertainment, i.e., theater, cinema, TV, concerts, etc.), application of cognitive techniques (positive attitude, self-command, acceptance of what cannot be changed).

R E F E R E N C E S

1. *Dmitrović I, Grubić-Nešić L.* Stress and stressors in the working enviroment. Novi Sad: Zbornik radova Fakulteta tehničkih nauka; XXVI, 2011; 9/11.
2. *Lojić R.* Stress management and mobbing prevention. Belgrade: Vojni glasnik; 2010. (Serbian)
3. *Mihailović D.* Psychology in organization. Belgrade: University of Belgrade, Faculty of Organizational Sciences; 2008. (Serbian)
4. *Babić B.* The stress and consequences of stress at work Belgrade: Vojno delo; 2011. p. 329–45. (Serbian)
5. *Milczarek M, Schneider E, Gonzalez ER.* European Agency for Safety and Health at Work, European risk observatory report, OSH in figures: Stress at work - facts and figures. Luxembourg: Office for Official Publications of the European Communities; 2009.
6. *Karasek RA, Theorell TGT, Schwartz J, Pieper C, Alfredsson L.* Job, psychological factors and coronary heart disease, Swedish prospective findings and US prevalence findings using a new occupational inference method. Adv Cardiol 1982; 29: 62–7.
7. *Karasek RA, Theorell T, Schwartz JE, Schnall PL, Pieper CF, Michela JL.* Job characteristics in relation to the prevalence of myocardial infarction in the US Health Examination Survey (HES) and the Health and Nutrition Examination Survey (HANES). Am J Public Health 1988; 78(8): 910–8.
8. *Aasland OG, Olff M, Falkum E, Schweder T, Ursin H.* Health complaints and job stress in Norwegian physicians: The use of an overlapping questionnaire design. Soc Sci Med 1997; 45(11): 1615–29.
9. *Snelgrove SR.* Occupational stress and job satisfaction: a comparative study of health visitors, district nurses and community psychiatric nurses. J Nurs Manag 1998; 6(2): 97–104.
10. *Visser MR, Smits EM, Oort FJ, de Haes MG.* Stress, satisfaction and burnout among Dutch medical specialist. CMAJ 2003; 168(3): 271–85.

11. *Chong A, Killeen O, Clarke T.* Work-related stress among paediatric non consultant hospital doctors. Ir Med J 2004; 97(7): 203–5 ; discussion 205.
12. *Pavičević L, Bobić J.* Stress at work In: *Šarić M, Žuškin E,* editors. Occupational and Environmental Medicine. Zagreb: Medicina rada i okoliša; 2002. p. 530–7. (Croatian)
13. *Levi L, Sauter SL, Shimomitsu T.* Work-related stress-it's time to act. J Occup Health Psychol 1999; 4(4): 394–6.
14. *Finset KB, Gude T, Hem E, Tyssen R, Ekeberg O, Vaglum P.* Which young physicians are satisfied with their work? A prospective nationwide study in Norway. BMC Med Educ 2005; 5(1): 19.
15. *Elfering A, Grebner S, Semmer NK, Gerber H.* Time control, catecholamines and back pain among young nurses. Scand J Work Environ Health 2002; 28(6): 386–93.
16. *Stafyla A, Spyridis N.* Gender differences in work stress, related to organizational conflicts and organizational constraints: An empirical research. Int J Econ Sci Appl Res 2013; 6(1): 91–101.
17. *Weyman AK, Clarke DD.* Investigating the influence of organizational role on perceptions of risk in deep coal mines. J Appl Psychol 2003; 88(3): 404–12.
18. *Barger LK, Cade BE, Ayas NT, Cronin JW, Rosner B, Speizer FE, et al.* Extended Work Shifts and the Risk of Motor Vehicle Crashes among Interns. N Engl J Med 2005; 352(2): 125–34.
19. *Edwards A.* Workplace stress in South African mineworkers. In: INRS Occupational Health Research Conference2012: Health risks associated with mixed exposures. South Africa, Brummeria, Pretoria: Centre for Mining Innovation, Council for Scientific and Industrial Research; 2012.
20. *Philibert I.* Sleep Loss and Performance in Residents and Non-physicians: A Meta-Analytic Examination. Sleep 2005; 28(11): 1392–402.

21. *Boscolo P*. Effects of occupational stress and job insecurity on the immune response. G Ital Med Lav Ergon 2009; 31(3): 277–80. (Italian)
22. *Kishore K, Khan NA*. A Report on GIT Disorders and Related Complications in Coal Miners of Shaktinagar, Sonebhadra, Uttar Pradesh. Arch Appl Sci Res 2010; 2(3): 231–7.
23. *Stefanović V, Jovanović J, Jovanović M*. The influence of occupational stress on the lipid status of drivers in road traffic. Med Pregl 2010; 63(1–2): 57–61. (Serbian)
24. *Veličković D, Mladenović P, Jovanović J*. Professional stressors in public enterprises Profesionalni stresori u javnim preduzećima. Stud Med Glas 2010; 1(1): 1–5.

Factors associated with depression in the patients with diabetes mellitus type 2

Faktori povezani sa depresijom kod bolesnika sa dijabetesom melitusom tip II

Admir Šabanović*, Nataša Maksimović[†], Mirjana Stojanović-Tasić[‡],
Marijan Bakić [§], Anita Grgurević[†]

*Primary Health Center Bijelo Polje, Bijelo Polje, Montenegro; University of Belgrade,
Faculty of Medicine, [†]Institute of Epidemiology, Serbia; University of Priština –
Kosovska Mitrovica, [‡]Faculty of Medicine, Kosovska Mitrovica, Serbia;
[§]Institute for Public Health of Montenegro, Podgorica, Montenegro

Abstract

Background/Aim. The assessment of association of depression and diabetes mellitus type 2 using the Patient Health Questionaire (PHQ-9) has not been done in Montenegro. The aim of this study was to assess the prevalence of depression in the patients with type 2 diabetes mellitus, and to identify the risk factors associated with the presence of depression. **Methods.** A cross-sectional study was conducted at the General Hospital in Bijelo Polje, from July to September, 2015. It included 70 patients over 35 years of age with the diagnosis of diabetes for at least six months. For the assessment of depression presence and intensity PHQ–9 was used. All variables associated with the presence of depression at a significance level of $p < 0.05$ were included into the final method of the multivariate logistic regression analysis. **Results.** Comorbidities were statistically significant more frequent among patients with depression ($\chi^2 = 5.40$; $p = 0.020$). Duration of diabetes over five years was significantly associated with depression ($\chi^2 = 12.48$; $p < 0.001$). Depression occurred more frequently among physically inactive subjects ($\chi^2 = 10.74$; $p = 0.005$). The presence of diabetic polyneuropathy ($\chi^2 = 6.04$; $p = 0.014$) and cataract ($\chi^2 = 5.351$; $p = 0.021$) were also significantly associated with depression. A multivariate logistic regression analysis showed that the duration of diabetes over five years and presence of cataract were independently associated with depression. **Conclusion.** The risk factors for depression among the subjects with diabetes were disease duration more than five years and the presence of cataract. Since depression is a serious disease and can be a risk factor for many chronic diseases, the best way of prevention is its early detection and treatment.

Key words:
depression; diabetes mellitus, type 2; patient health questionnaire; montenegro.

Apstrakt

Uvod/Cilj. Merenje povezanosti depresije i dijabetesa melitusa tip 2, primenom *Patient Health Questionaire 9* (PHQ-9) upitnika, nije do sada rađeno u Crnoj Gori. Cilj ovog rada je bio da se proceni prevalencija depresije kod bolesnika sa dijabetesom tip 2 i da se identifikuju faktori rizika povezani sa prisustvom depresije. **Metode.** Studija preseka sprovedena je u Opštoj bolnici Bijelo Polje, u periodu od jula do septembra 2015. godine. Studijom je bio obuhvaćeno 70 osoba starijih od 35 godina sa dijagnozom dijabetesa od najmanje šest meseci. Za procenu prisustva depresije korišćen je upitnik PHQ-9. Sve varijable koje su bile značajno povezane sa prisustvom depresije ($p < 0,05$) ušle su u model multivarijantne logističke regresije. **Rezultati.** Komorbiditeti su se značajno češće javljali kod osoba sa depresijom ($\chi^2 = 5,40$; $p = 0,020$). Trajanje dijabetesa više od pet godina bilo je značajno povezano sa prisustvom depresije ($\chi^2 = 12,48$; $p < 0,001$). Depresija je bila značajno češća kod fizički neaktivnih osoba ($\chi^2 = 10,74$; $p = 0,005$). Prisustvo komplikacija dijabetesa u vidu dijabetične polineuropatija ($\chi^2 = 6,04$; $p = 0,014$) i katarakte ($\chi^2 = 5,351$; $p = 0,021$) je takođe bilo statistički značajno povezano sa prisustvom depresije. Multivarijantna logistička regresija je pokazala da su trajanje bolesti preko pet godina i katarakta nezavisni prediktori depresije. **Zaključak.** Istraživanjem je ustanovljeno da su faktori rizika od pojave depresije bili trajanje dijabetesa preko pet godina i prisustvo katarakte. Kako je depresija ozbiljno oboljenje i faktor rizika od pojave mnogih hroničnih bolesti, najbolja prevencija su njena rana detekcija i lečenje.

Ključne reči:
depresija; dijabetes melitus, tip 2; upitnik o zdravstvenom stanju bolesnika; crna gora.

Correspondence to: Admir Šabanović, Primary Health Center Bijelo Polje, Bijelo Polje, Montenegro E-mail: admir0903@gmail.com

Introduction

Depression is the most common psychiatric disorder and one of the most common diseases in general [1]. It is estimated that about 350 million people suffer from depression worldwide [2]. According to the forecasts of the World Health Organization (WHO) experts, in 2030, depression would be the most common cause of morbidity in the world, indicating the increasing importance of investing efforts in the promotion and preservation of mental health [3].

Depression is a common comorbidity among patients with diagnosis of chronic somatic diseases [3]. Reasons are complex and usually depend on the nature of disease, complications, length of survival, and partially, on the patient's perception of the disease [4]. Depression has been shown to be a common co-morbidity in diabetes, affecting 10% to 30% of the diabetic population [4, 5]. In the patients with diabetes, depression may worsen the overall situation, accelerate the development of complications and increased mortality [4]. A course of depression in the patients with type 2 diabetes is a chronic and a large number of them (80%) lead to exacerbation of depression for a period of five years [4]. The patients with diabetes who have developed major depression are 1.5 to twofold in a higher risk of developing the cardiovascular complications than those who do not have major depression [4, 5]. Depression can also be a risk factor for degenerative changes in the small blood vessels if not adequately treated [6]. In the patients with cerebrovascular complications, depression is associated with the permanent ischemic changes in subcortical brain mass (vascular depression) [7].

Depression remains unrecognized and untreated in about the two-thirds of patients with the type 2 diabetes [8, 9]. Women are at higher risk than men [10]. The results of the impact of psychological factors on glycemic control in men and women research showed that the main predictors of poor glycemic control in women are depressed mood, low sexual desire and poorer quality of life, and in men, the way they perceive their illness ("emotional experiences" of hyperglycemia) [10]. The pathophysiological mechanisms that link depression and diabetes are still not completely understood. Some studies have shown that in the depressed patients with and without diabetes, there is an increase of blood glucose level and insulin resistance on the oral glucose tolerance test (OGTT) [10].

Connection between depression and chronic diseases, including type 2 diabetes mellitus, using the Patient Health Questionaire (PHQ-9) was tested around the world [11, 12], but similar study has not been done in Montenegro. These studies are important because the chronic patients are at the increased risk of developing depression. PHQ-9 is a brief, standardized questionnaire for the rapid screening, diagnosing, monitoring and measuring the severity of depression in primary health care [11].

The aim of this study was to assess the prevalence of depression in the patients with type 2 diabetes mellitus as well as to identify the risk factors associated with the presence of depression in the patients with type 2 diabetes.

Methods

In order to investigate the potential risk factors for depression in the patients with type 2 diabetes mellitus, a cross-sectional study was conducted at the General Hospital in Bijelo Polje, Montenegro, from July 1st until September 1st, 2015. The study included 70 patients older than 35 years of age who were treated in the Department of Endocrinology in the General Hospital in Bijelo Polje, with at least a six-months diagnosis of diabetes. Exclusion criteria were: suffering from malignant disease, severe neurological disorders (dementia, epilepsy, muscular dystrophy) and psychosis.

All participants were interviewed using an epidemiological questionnaire made for the study purposes. The questionnaire consisted of several parts: patients' sociodemographic characteristics (age, gender, place of residence, dwelling place, occupation, marital status), physical activity (physically inactive – less than 30 minutes per day; moderate physically active – 30 minutes per day; intesive physically active – more than 30 minutes per day), body mass index (BMD), data related to the diabetes type 2: duration of disease, complications (diabetic ketoacedosis, ketogenic coma, diabetic polyneuropathy, diabetic nephropathy, diabetic retinopathy, cerebrovascular disease, cataract, diabetic foot), and comorbidities (hypertension, osteoarthritis, heart failure, chronic obstructive pulmonary disease, benign prostatic hyperplasia, osteoporosis and cholelithiasis). The data related to the complications of diabetes and comorbidities were obtained from the medical records.

For the assessment of presence and intensity of depression, we used the PHQ-9. The PHQ-9 is a standardized questionnaire which consists of 9 questions. Each question has four response categories ("not at all", "several days", "more than half the days" and "nearly every day") corresponding to scores of 0, 1, 2 and 3, respectively. As a severity measure, the PHQ-9 score ranges from 0 to 27 and a score greater than 4 indicates the existence of depression. Based on the total score of the PHQ-9, the values from 0 to 4 indicate the absence of depression; from 5 to 9 mild depression; from 10 to 14 moderate depression; from 15 to 19 moderately severe depression and over 20 severe depression. Only those patients who gave their written consent were included in the study. The Institutional Review Board for Human Subject Investigations reviewed and approved the study.

The data analysis was performed using the descriptive statistics, that is, the Pearson's χ^2 test and Student t-test. All variables associated with the presence of depression at a significance level of $p < 0.05$ were included into the final method of the multivariate logistic regression analysis to assess the independent association of these variables with the presence of depression. The tatistical analysis was done in the SPSS 21.0 software package.

Results

The study included 70 patients with type 2 diabetes, 39 men and 31 women. The basic demographic characteristics of the respondents in relation to the presence of depression,

including the physical activity, body mass index, duration of disease and comorbidities are shown in Table 1. Fourty-four (62.9%) patients had the symptoms of depression, of which the largest percentage of respondents manifested the symptoms of mild depression (38.6%). By analyzing the depression patients in relation to sex, age, place of residence, dwelling place, level of education, marital status and occupation, no significant differences were found among the studied groups. The presence of comorbidities was a statistically significant more frequent among those with depression compared with those without it ($\chi^2 = 5.40$; $p = 0.020$). However, this variable in the multivariate logistic analysis did not occur as an independent risk factor for depression. By analyzing depression in relation to the duration of the disease, duration more than five years was a statistically significantly associated with depression ($\chi^2 = 12.48$; $p < 0.001$). Regarding

physical activity, depression occurred more frequently among physically inactive subjects ($\chi^2 = 10.74$; $p = 0.005$). There was no statistically significant difference in the presence of depression depending on the body mass index. By analyzing depression in relation to the presence of complications of diabetes statistically significant differences between the studied groups were found in relation to the presence of diabetic polyneuropathy ($\chi^2 = 6.04$; $p = 0.014$) and cataract ($\chi^2 = 5.351$; $p = 0.021$) (Table 2).

To examine the independent impact of potential risk factors for depression, the multivariate logistic regression analysis was performed (Table 3). By performing the multivariate logistic regression analysis, we found that the duration of disease over five years and the presence of cataract as complications of diabetes were significantly independently associated with depression.

Table 1

Characteristics of patients according to the presence of depression

Characteristics of patients	With depression n (%)	No depression n (%)	p (χ^2 test)
Sex			
male	23 (59.0)	16 (41.0)	0.451
female	21 (67.7)	10 (32.3)	
Age (years)			
< 60	20 (55.6)	16 (44.4)	0.193
≥ 60	24 (70.6)	10 (29.4)	
Place of residence			
apartment	10 (71.4)	4 (28.6)	0.458
house	34 (60.7)	22 (39.3)	
Dwelling place			
rural	8 (66.7)	4 (33.3)	
urban	19 (63.3)	11 (36.7)	0.936
semi urban	17 (60.7)	11 (39.3)	
Level of education			
incomplete primary school, primary school and secondary school	39 (63.9)	22 (36.1)	
college and university degree	5 (55.6)	4 (44.4)	0.627
Employment			
employed	23 (67.6)	11 (32.4)	0.420
unemployed	21 (58.3)	15 (41.7)	
Marital status			
married	28 (56.0)	22 (44.0)	0.060
widows/widowers, unmarried/single; divorced	16 (80.0)	4 (20.0)	
Physical activity			
inactivity	16 (80.0)	4 (20.0)	
moderate activity	26 (60.4)	20 (39.6)	0.005
intensive activity	2 (28.6)	5 (71.4)	
Duration of the disease (years)			
< 5	13 (40.6)	19 (59.4)	< 0.001
≥ 5	31 (81.6)	7 (18.4)	
Boddy mass index (kg/m^2)			
< 25	9 (75.0)	3 (25.0)	0.299
≥ 25	33 (58.9)	23 (41.1)	
Comorbidity			
with comorbidity	40 (69.0)	18 (31.0)	0.020
no comorbidity	4 (33.3)	8 (66.7)	

Table 2

Complication of the diseases in subjects in relation to the presence of depression

Complication of the disease	With depression	No depression	p
	n (%)	n (%)	(χ^2 test)
Diabetic ketoacidosis			
yes	2 (100.0)	0 (0.0)	0.264
no	41 (61.2)	26 (38.8)	
Ketogenic coma			
yes	4 (100.0)	0 (0.0)	0.109
no	39 (60.0)	26 (40.0)	
Diabetic polyneuropathy			
yes	19 (82.6)	4 (17.4)	0.014
no	24 (52.2)	22 (47.8)	
Diabetic nefropathy			
yes	4 (80.0)	1 (20.0)	0.397
no	39 (60.9)	25 (39.1)	
Diabetic retinopathy			
yes	21 (75.0)	7 (25.0)	0.072
no	22 (53.7)	19 (46.3)	
Cerebrovascular disease			
yes	5 (100.0)	0 (0.0)	0.071
no	38 (59.4)	26 (40.6)	
Cataract			
yes	16 (84.2)	3 (15.8)	0.021
no	27 (54.0)	23 (46.0)	
Diabetec foot			
yes	4 (66.7)	2 (33.3)	0.818
no	39 (61.9)	24 (38.9)	

Table 3
Factors related to the presence of depression according to the multivariate logistic regression analysis

Variable	OR	95% CI	p
Duration of the disease > 5 years	7.27	2.37–22.84	0.001
Cataract	4.84	1.11–20.99	0.036

OR – odds ratio; CI – confidence interval.

Discussion

In our survey, 62.9% of respondents, according to the PHQ-9 questionnaire, had depression and most of them met criteria for mild depression (38.6%), while the criteria for severe depression had 11,4% of respondents. According to the results of a study made by Raval et al. [13], in which 300 subjects with type 2 diabetes were included, depression was present in 41% of respondents, while 23% of them met criteria for severe depression. In a study conducted in Canada, 19.6% of subjects showed the symptoms and signs of depression and 8.7% among them were severe depressed [14]. According to the results of our study, there was no significant difference in the presence of depression between males and females. Also, no statistically significant difference was found in the presence of depression in relation to age. Roy et al. [15], in the cross-sectional study in Bangladesh, found a significantly higher prevalence of depression in older age groups (over 50 years). However, in the studies by Das Mun-

shi et al. [16] and Miyaoka et al. [17] it was not found that age can be a risk factor for depression in the patients with type 2 diabetes.

By examining the levels of education, we found no significant difference between the examined groups. In a prospective cohort study Mezuk et al. [18], which aims were to determine the association between depression and type 2 diabetes, it was observed that the people with lower education have a higher risk of developing depression compared to the people with higher level of education. The analysis of marital status in relation to the presence of depression showed no significant difference among the studied groups, which is in agreement with other studies.

By analyzing depression in relation to the presence of comorbidity, we found a statistically significant difference between the treated groups. Depression was more common among the respondents who had comorbidities compared to those who did not have them. The most common reported comorbidity among the subjects with type 2 diabetes was hypertension (41.7%). The heart failure and disorders of joint function was reported by 8.3% of respondents, and 6.9% of them reported benign prostatic hyperplasia. Shmitz et al. [14] found a significantly higher percentage of patients with type 2 diabetes who developed the major depressive episode among the respondents who had two or more comorbidities compared to those who had no comorbidities, or those who had only one comorbidity. However, comorbidity, in our study, in the multivariate logistic analysis, did not appear as an independent risk factor for depression.

By analyzing depression in relation to the duration of the diabetes we found statistically significant difference between the treated groups. Depression was more common among subjects whose illness lasted longer than 5 years. One Australian survey corroborated this association [19]. By analyzing depression in relation to physical activity, there was a statistically significant difference between the observed groups. Depression was significantly more common among the physically inactive subjects (72.7%), or moderately active (52.4%), while much less intensively occurs among the physically active subjects (28.6%). Physical inactivity appeared in other studies as a predictor of depression among the people with type 2 diabetes and other chronic diseases. Von Korff et al. [20] concluded that depression was significantly more common among the respondents who were physically inactive (40.7%) compared to those who were moderately physically active (2–3 times per week, 25.7%), and among the respondents who were physically active more than 4 times per week (33.7%). There were studies in which physical activity did not appear as a significant risk factor for depression in the patients with type 2 diabetes [21].

By analyzing depression in relation to the body mass index, in the patients with type 2 diabetes, we found no significant difference between the observed groups ($p = 0.299$). The body mass index ≥ 30 kg/m^2 in the study of Nasser et al. [21] was a significant predictor of depression in the patients with type 2 diabetes, as so in a study of Reddy et al. [19]. But, in the study of Roy et al. [15] the body mass index was not found as a risk factor for depression in the patients with type 2 diabetes.

When it comes to the complications of diabetes mellitus, according to our research, the univariate analasys showed that the diabetic polyneuropathy and cataract were associated with depression. The presence of cataract was significantly and independently associated with depression. The significant number of studies indicated that the presence of diabetes complications acted as a risk factor for depression. According to the study developed in Bahrain, complications of diabetes associated with depression were nephropathy and ischemic heart disease [21]. According to the results of Schmitz et al. [14] a number of present complications was significantly associated with severe depression. Major depression was more frequent among the subjects who developed two or more complications than those with one, or without any complication.

Beside that depression often occurred as a comorbidity in the people with chronic illnesses, depression was often mentioned as a possible risk factor for chronic diseases, including type 2 diabetes. The conclusions of a prospective study conducted at several thousand people pointed to twofold higher incidence of type 2 diabetes in the people with depression, independent of other risk factors (obesity, age, physical inactivity, chronic somatic diseases and family history of diabetes) [22]. According to the results of a meta-analysis of nine longitudinal studies, the depressed, adult patients had a 37% higher risk of developing diabetes [23]. With the results of meta-analysis, Cosgrove et al. [24] confirmed that depression was accompanied by the subsequent development of type 2 DM . According to the results of Brown et al. [25], the presence of depression increased the risk for diabetes for about 23%, but only in the younger patients (from 20–50 years of age).

Our study has several limitations. Firstly, it was the absence of a control group which in this case entailed the persons who were not suffering from chronic diseases. Secondly, the selected sample of respondents was not large (70 patients), which could have reduce the accuracy of the estimates of the association of independent variables with the outcome variable, and thus affected the validity of the findings and the generalization of results. Thirdly, in our study there was not the "gold standard", so there was no comparison of the results obtained by the PHQ-9 questionnaire with a specific relevant psychiatric approach to prove, or refute the existence of depression in the patients, because the PHQ-9 questionnaire contains questions about specific symptoms that might be part of the clinical picture of certain somatic diseases. And fnally, certain biochemical tests, such as the testing blood glucose or glycosylated hemoglobin in the patients with type 2 diabetes were not included in this study. Such tests would require significantly greater financial resources and participation of doctors of other specialties. Despite the above mentioned shortcomings, the significance of our study is that this is the first study that examines the connection between depression and type 2 diabetes in the population of Montenegro, where the PHQ-9 questionnaire to assess depression of respondents was used.

Conclusion

In our study, we found that the risk factors for depression among the subjects with type 2 diabetes were duration of disease more than 5 years and the presence of cataract as a complication of diabetes. In this regard, it is necessary to treat the underlying disease and prevent the occurrence of complications because the presence of complications is a predictor of poor disease control. Since the depression can be a high risk factor for many chronic and serious diseases, as well as for suicide, the most effective way of prevention is its early detection and adequate treatment.

Disclosure statement

No potential conflicts of interest were disclosed.

R E F E R E N C E S

1. World Health Organization. What is depression. Available from:
   www.who.int/mental_health/management/depression/definition/en

2. World Health Organization. Depression, fact sheets. Available from: www.who.int. depression/factsheets

3. Republička stručna komisija za izradu i implementaciju vodiča dobre kliničke prakse. Nacionalni vodič dobre kliničke prakse za dijagnostiku i lečenje depresije. Beograd: Ministarstvo zdravlja Republike Srbije; 2011.

4. *Stanković Ž, Jašović-Gašić M.* Comorbidity of depression and type 2 diabetes: Risk factors and clinical significance. Vojnosanit Pregl 2010; 67(6): 493–500.

5. *Li C, Ford ES, Strine TW, Mokdad AH.* Prevalence of depression among U.S. adults with diabetes: findings from the 2006 behavioral risk factor surveillance system. Diabetes Care 2008; 31(1): 105–7.

6. *de Groot M, Anderson R, Freedland KE, Clouse RE, Lustman PJ.* Association of depression and diabetes complications: a meta-analysis. Psychosom Med 2001; 63(4): 619–30.

7. *Sneed JR, Culang-Reinlieb ME.* The vascular depression hypothesis: an update. Am J Geriatr Psychiatry 2011; 19(2): 99–103.

8. *Schmitz N, Gariépy G, Smith KJ, Malla A, Wang J, Boyer R,* et al. The pattern of depressive symptoms in people with type 2 diabetes: a prospective community study. J Psychosom Res 2013; 74(2): 128–34.

9. *Janssen EP, Köhler S, Stehouwer CD, Schaper NC, Dagnelie PC, Sep SJ,* et al. The Patient Health Questionnaire-9 as a Screening Tool for Depression in Individuals with Type 2 Diabetes Mellitus: The Maastricht Study. J Am Geriatr Soc 2016; 64(11): e201–6.

10. *Larijani B, Khoram M, Bayat S, Gorgani KM, Bandarian F, Akhondzadeh S,* et al. Association between depression and diabetes. German J Psychiatry 2004; 7: 62–5.

11. *Willborn RJ, Barnacle M, Maack B, Petry N, Werremeyer A, Strand MA.* Use of the 9-item patient health questionnaire for depression assessment in primary care patients with type 2 diabetes. J Psychosoc Nurs Ment Health Serv 2016; 54(1): 56–63.

12. *van der Zwaan GL, van Dijk SEM, Adriaanse MC, van Marwijk HWJ, van Tulder MW, Pols AD,* et al. Diagnostic accuracy of the Patient Health Questionnaire-9 for assessment of depression in type II diabetes mellitus and/or coronary heart disease in primary care. J Affect Disord 2016; 190: 68–74.

13. *Raval A, Dhanaraj E, Bhansali A, Grover S, Tiwari P.* Prevalence and determinants of depression in type 2 diabetes patients in a tertiary care centre. Indian J Med Res 2010; 132: 195–200.

14. *Schmitz N, Messier L, Nitka D, Ivanova A, Gariepy G, Wang J,* et al. Factors associated with disability and depressive symptoms among individuals with diabetes: a community study in Quebec. Psychosomatics 2011; 52(2): 167–77.

15. *Roy T, Lloyd CE, Parvin M, Mohiuddin KG, Rahman M.* Prevalence of co-morbid depression in out-patients with type 2 diabetes mellitus in Bangladesh. BMC Psychiatry 2012; 12: 123.

16. *Das-Munshi J, Stewart R, Ismail K, Bebbington PE, Jenkins R, Prince MJ.* Diabetes, common mental disorders, and disability: findings from the UK National Psychiatric Morbidity Survey. Psychosom Med 2007; 69(6): 543–50.

17. *Miyaoka Y, Miyaoka H, Motomiya T, Kitamura S, Asai M.* Impact of sociodemographic and diabetes-related characteristics on depressive state among non-insulin-dependent diabetic patients. Psychiatry Clin Neurosci 1997; 51(4): 203–6.

18. *Mezuk B, Eaton WW, Golden SH, Ding Y.* The influence of educational attainment on depression and risk of type 2 diabetes. Am J Public Health 2008; 98(8): 1480–5.

19. *Reddy P, Philpot B, Ford D, Dunbar JA.* Identification of depression in diabetes: the efficacy of PHQ-9 and HADS-D. Br J Gen Pract 2010; 60(575): e239–45.

20. *Von Korff M, Katon W, Lin EH, Simon G, Ludman E, Oliver M,* et al. Potentially modifiable factors associated with disability among people with diabetes. Psychosom Med 2005; 67(2): 233–40.

21. *Nasser J, Habib F, Hasan M, Khalil N.* Prevalence of depression among people with diabetes attending diabetes clinics at primary health settings. Bahrain Med Bull 2009; 31: 1–7.

22. *Kawakami N, Takatsuka N, Shimizu H, Ishibashi H.* Depressive symptoms and occurrence of type 2 diabetes among Japanese men. Diabetes Care 1999; 22(7): 1071–6.

23. *Knol MJ, Twisk JW, Beekman AT, Heine RJ, Snoek FJ, Pouwer F.* Depression as a risk factor for the onset of type 2 diabetes mellitus. A meta-analysis. Diabetologia 2006; 49(5): 837–45.

24. *Cosgrove MP, Sargeant LA, Griffin SJ.* Does depression increase the risk of developing type 2 diabetes? Occup Med (Lond) 2008; 58(1): 7–14.

25. *Brown LC, Majumdar SR, Newman SC, Johnson JA.* History of depression increases risk of type 2 diabetes in younger adults. Diabetes Care 2005; 28(5): 1063–7.

Four varieties of diabetes mellitus in acute myocardial infarction

Četiri tipa šećerne bolesti u akutnom infarktu miokarda

Goran Koraćević*[†], Slobodan Obradović[‡§], Tomislav Kostić*[†],
Milan Pavlović*[†], Marija Kutlešić[||], Milovan Stojanović[¶],
Sladjana Vasiljević**, Maja Koraćević[†,††]

Clinical Center Niš, *Department for Cardiovascular Diseases,
Obstetrics and Gynecology Clinic, [||]Center of Anesthesiology, Niš, Serbia; University
of Niš, [†]Faculty of Medicine, [††]Innovation Center, Niš, Serbia; Military Medical
Academy, [‡]Clinic of Internal Emergency Medicine, Belgrade, Serbia; University of
Defence, [§]Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia;
[¶]Institute for Treatment and Rehabilitation Niška Banja, Niška Banja, Serbia; Mother
and Child Health Care Institute „Dr Vukan Čupić", **Department of Anesthesiology
and Intensive Care, Belgrade, Serbia

Introduction

It is believed that 387 million patients (> 8% of the global population) have diabetes mellitus (DM), and almost one half of them are unaware of their diagnosis [1]. DM is very important risk factor for acute myocardial infarction (AMI) [2]. The patients with DM have a two- to four-fold increased risk of developing cardiovascular (CV) disease [3], three-fold for the acute coronary syndrome (ACS) [4], and they experience the CV events 15 years earlier than the general population [4, 5]. The patients with DM were believed to have as the high risk for the new AMI as the patients with previous myocardial infarction (MI), the "coronary artery disease (CAD) equivalent" [6–8]. This is an overestimation as shown by the meta-analysis [9]. The Euro Heart Survey and other registries/studies found that a majority of ACS patients had dysglycaemia, including DM, which was not previously diagnosed [1, 8, 10, 11]. The patients with MI have the incidence of insulin resistance twice as often as the individuals with no history of myocardial infarction. Therefore, some authors consider a MI to be a pre-DM equivalent [1, 8], or a DM risk equivalent [12], or a pre-DM risk eqivualent [13]. Out of 2,036 DM-naïve CAD patients who were followed up for at least one year, AMI significantly increased the risk of "new-onset" DM after adjusting the covariates [hazard ratio (HR), 1.54; 95% confidence interval (CI), 1.14–2.07; $p < 0.01$] [14].

Nevertheless, a short-term [4] and a long-term mortality risk in the AMI patients with DM is almost doubled in comparison with the nondiabetic AMI patients [2, 4, 7]. A systematic review and meta-regression of 1,614,174 AMI, or ACS patients showed that the patients with DM (n = 432,066) had the odds ratio (OR) [95% CI] of 1.66 [1.59–1.74] ($p < 0.0001$) for early mortality, and of 1.86 [1.75–1.97] ($p < 0.0001$) for 6–12 months mortality in comparison with 1,182,108 nondiabetic patients [2]. The mortality risk after a 10-year follow-up in the patients with CAD and DM exceeds 70% [15]. Glycemia on admission of 108–126 mg/dL (6–7 mmol) in the AMI patients with DM is associated with 3 times higher mortality vs. the AMI patients without DM [16, 17]. In AMI, the risk for the repeated myocardial infarctions, heart failure, cardiogenic shock and stroke is also greater in the patients with concomitant DM, as compared to the AMI patients without DM [4, 18]. Increased mortality in the AMI patients vs. those without DM has remained constant over time (from 1970 to 2011), despite the important therapeutic advantages [2]. The survival curves were persistently diverging for 20 years between the AMI patients with and without DM and the median survival was less than 3.3 years ($p < 0.0001$) in the DM patients following the AMI [5]. DM confers increased in-hospital mortality risk both in the ST-elevation myocardial infarction (STEMI) and the non-STEMI (NSTEMI) patients [19].

Correspondence to: Goran Koraćević, Clinical Center Niš, Department for Cardiovascular Diseases, 18 000 Niš, Serbia.
E-mail: gkoracevic@yahoo.com

Moreover, this dismal prognosis of patients with AMI and DM is not related to the body mass index, i.e., „obesity paradox" is not relevant to them (in contrast to the AMI patients without DM)[20]. Indeed, a meta-analysis of 21,759 DM patients (~29% of them were insulin-treated) revealed that both short-term and long-term mortality, and the incidence of new AMI, target lesion revascularization, major adverse cardiac effect (MACE), and stent thrombosis were significantly more frequent in the insulin-treated DM patients[21]. Also, among 243,861 patients with AMI the in-hospital mortality risk was higher in the insulin-treated DM patients (n = 20,051) vs. the DM patients who did not require insulin (n = 25,364)[19]. DM is prevalent in AMI, with usuallly quoted figures between 20%–30%[7, 22–24], or 30%–40%[25], and the incidence and the prevalence of DM are expected to grow further[2, 26]. Another 300 million individuals are at a risk of developing DM[26]. Even higher actual prevalence was published and a few recent papers reported almost doubled DM prevalence in AMI (47%)[27].

Diagnosing DM in AMI has been clearly suboptimal

In a recent research paper, of the 3,778 AMI patients who had no history of DM before admission, 18.7% had the criteria for DM during hospitalization: fasting glucose level of at least 126 mg/dL (7 mmol/L), random plasma glucose (RPG) of at least 200 mg/dL (11.1 mmol/L), or glycated hemoglobin [HbA1c] level of at least 6.5%. Out of the AMI patients with the criteria for a new-onset DM, only 30% were clinically diagnosed (as having DM) in the hospital and were treated instantly[27]. Similarly, in a study of 1,566 patients, insulin, or oral agents were prescribed at discharge for 80% of patients with known DM and only 25.4% of patients with newly diagnosed DM[1]. Therefore, the great majority of newly diagnosed DM remained without an appropriate hypoglycemic treatment, which is very important finding. Indeed, the AMI patients who met the criteria for DM, but were not diagnosed, had a significantly higher risk for a MACE, 1 year following discharge, compared with the patients without previous diagnosis of DM (OR, 1.5; 95% CI, 1.3–1.7; p < 0.0001). On the other hand, there was no a statistically significant difference between the patients with properly newly diagnosed DM and the patients without DM (OR, 1.3; 95% CI, 0.9–1.7; p = 0.15)[27]. The authors divided their DM patients into only three groups: 1) with a history of DM (34%); 2) without a history of DM, diagnosed during hospitalization (4%); with the criteria for DM, but undiagnosed during their intrahospital stay (9%)[27].

How many kinds of DM can be observed in AMI?

We believe that it is important to recognize that there were actually four kinds of DM in ACS: A) Previously diagnosed DM[1]; B) Newly diagnosed (previously present, but not diagnosed until this admission) DM with HbA1c > 6.5%[1, 28]; C) New-onset DM with HbA1c < 6.5% and with either C1. Fasting blood glucose (FBG) ≥ 126 mg/dL (7 mmol/L), or C2. A RPG ≥ 200 mg/dL (11.1 mmol/L), or C3.

The positive oral glucose tolerance test (OGTT) before discharge with 2-hr plasma glucose (PG) ≥ 200 mg/dL (11.1 mmol/L)[1]; D) Undetected DM, i.e., some of the four 2016 American Diabetes Association (ADA) criteria [in terms of FBG ≥ 126 mg/dL (7 mmol/L), or a RPG ≥ 200 mg/dL (11.1 mmol/L) or HbA1c ≥ 6.5% (48 mmol/mol), or 2-hr PG ≥ 200 mg/dL (11.1 mmol/L) during OGTT (75 g)] not fulfilled and the other analysis not performed[29].

Some frequent mistakes in categorizing DM and stress hyperglycemia in AMI

The forth group (D) is missing in the aforementioned work[27] and with this group, the number of DM patients in AMI would be even higher. Consequently, as these patients were neither diagnosed nor treated – the real number of adequately diagnosed and treated DM patients in AMI may be therefore even lower than 30%. This should call to action. Regarding the terminology used in writing on this topic, there is a mistake with labeling "newly diagnosed" DM as "new-onset". In AMI, the patients without known (previously diagnozed) DM can be considered as having "new-onset", which frequently is not true, because they may have unrecognized DM for some time. It is not difficult to distinguish "new onset DM" from undiagnosed DM, because HbA1c is normal in the first and elevated in the second case.

Common methodological error for decades was to use the same cut-off in the AMI patients for the subgroup with the glycometabolic disease (DM) and without it. This single cut-off was arteficially low for the DM patients (and high for non-DM ones) and decreased somewhat the predicitive accuracy of stress hyperglycemia (SH). It is particularly true for the DM patients, because they are less represented in AMI, so that their cut-off value for SH is more remote from the artificial single cut-off of the whole AMI group[30]. Hyperglycemia is common, valid both for risk stratification and treatment initiation and adjustments, but is often the underestimated parameter in critical illnesses, including AMI[31]. The importance of hyperglycemia in AMI stems from two facts: AMI is one of the most common lethal diseases and glycemia is undoubtely one of the basic parameters in general and in AMI[17]. Hyperglycemia in AMI has different cut-offs for the prognostic and therapeutic purposes. The common mistake is to take therapeutic treshold e.g., 11 mmol/L (198 mg/dl) for the prognostic one, because the AMI patients without DM have far less prognostic cut-off ~8 mmol/L (144 mg/dl)[32]. Post-prandial hyperglycaemia contributed more to the CAD genesis as compared to fasting hyperglycemia[33]. No less than 84% of AMI patients with abnormal glucose tolerance had normal plasma glucose (FPG)[34].

Comparison of the most important tools to detect DM in AMI (in addition to FPG and RPG)

HbA1c is a marker of an increased CV risk in the patients with and without DM[35]. ADA recommended the HbA1c with a threshold of 6.5%, to diagnose DM, due to its preanalytical stability, convenience (fasting not required),

and less day-to-day variability [28]. Moreover, HbA1c is currently used to guide the management decisions [4]. The ACS patients with HbA1c of 6.0%–6.4% should have an OGTT 6–8 weeks after discharge [4]. HbA1c reflects the average glycemia, including postprandial spikes during last 3 months [36]. The relation of postprandial hyperglycemia and a risk of CV diseases was demonstrated in a meta-analysis of 95,783 individuals [3, 37]. HbA1c has a low intraindividual variability and is not influenced by the stress caused by ACS [25, 36]. A single measurement of HbA1c is not sufficient to diagnose DM [38]. Using HbA1c, FPG, and RPG newly diagnosed DM was found in ~1/5 of all AMI patients and pre-DM in 14% [39].

OGTT has been often recommended for the AMI patients [23, 25, 26]. But neither from ADA [28] nor from NICE [40], OGTT is a valid screening tool for both DM and a high CV risk [41]. OGTT in the patients with ACS has comparable accuracy to that in the general population. Therefore, it is sound to perform OGTT in the ACS patients to improve search for such an important disease as DM [42]. Bronisz et al. [43] reported that a substantial proportion of AMI patients with the abnormal result of OGTT soon after AMI, can have a normal glucose tolerance 3 months after AMI. To the contrary, a meta-analysis found that < 10% of ACS patients diagnosed with DM by means of an OGTT before discharge will have a different result at the follow-up OGTT [42]. Moreover, HbA1c is more expensive, it is not availabile so widely, and does not correlate adequately with the average glycemia in certain individuals [44].

Comparing all three glycemic parameters, FPG, OGTT and HbA1c simultaneously for mortality and CV disease risk reveals that the association is strongest for 2-hour plasma glucose concentration (2hPG) in OGTT [26]. In addition to FPG and HbA1c, OGTT reveals much more cases of DM, both in the general population and CAD [45]. A portion of AMI patients with new-diagnosed AMI by means of OGTT was not negligible [8]. The OGTT showed that 27.4% of CAD patients without known DM at admission actually had DM. Moreover, 33.5% were found to have impaired glucose tolerance (IGT) and another 11.2% were found to have both IGT and impaired fasting glucose (IFG) [46]. On one hand, HbA1c ≥ 6.5% can predict the DM values on OGTT (2hPG value ≥ 11.1 mmol/L) with the positive predictive value of no less than 100% and could, therefore, replace OGTT to diagnoze DM following ACS [25]. To the contrary, OGTT or HbA1c may not diagnose the same patients; evidence of discrepancies between the two modalities to classify abnormal glyco-

regulation accumulated [25]. OGTT is likely needed, but there is a dilemma when to perform it, during the initial hospitalization, or later (e.g., within 30 days, or at three months).

Variable practice reflects directly the lack of consensus – some authors used to perform OGTT: as early as on the day one of hospitalization [47], on the day three from the admission [34, 48, 49]; on the day four of hospitalization [50] (the ESC guidelines also recommend delaying the test for 4 to 5 days after ACS to minimize the false positive results [51, 52], because the results of OGTT could be somewhat falsified by stress hyperglycemia [52]); from one to 3 days following the hospital discharge [53]; from 7 to 28 days after ACS [25]; or 3 months after discharge [42, 46].

OGTT at the discharge performed in the patients with AMI detected a high proportion of patients with previously unknown abnormal glycoregulation that was significantly and independently related to a dismal long-term prognosis [41]. Within 7 days following AMI, OGTT can detect many patients with previously unknown either newly detected DM or IGT, indicating a high risk for the CV events in the next decade. OGTT was a better prognosticator as compared to FBG or HbA1c [41].

Characteristics of most important methods detection of DM in AMI patients are given in Table 1.

The combination of HbA1c and OGTT to diagnose DM in the AMI patients

To obtain the diagnosis of DM as soon as possible, HbA1c and FBG should be analysed during the first days of hospitalization, but both of them will leave an undetected group of patients with glucometabolic abnormalities [41]. Indeed, OGTT should be performed when HbA1c and FPG are inconclusive [51]. Moreover, a combination of tests (both HbA1c and OGTT) in addition to simple FBG can be used to the better risk-stratification of AMI patients. The OGTT is more sensitive than fasting plasma glucose and HbA1c. The AMI patients categorized as newly diagnosed DM by OGTT, although HbA1c < 6.5%, have a poor long-term prognosis compared to the patients with HbA1c < 6.5%, and an IFG, or normal glucose tolerance (NGT)/IGT by OGTT [37]. The combination of HbA1c and OGTT seems sound. For example, in the AMI patients treated invasively with IGT and newly diagnosed DM (detected by OGTT), an increase of HbA1c was one of the strongest independent risk markers of death [54].

Table 1

Detection of diabetes mellitus (DM) in the acute myocardial infarction patients

Method	Advantage
RPG > 11.1 mmol/L	routine, always available [1, 27]
FBG > 7 mmol/L	routine, always available [1, 46]
HbA1C > 6.5%	widely available, within 24 hours [1, 28]
OGTT 2-hr PG ≥ 11.1 mmol/L	improves DM detection additionally [26, 45]
All above	provides optimal result in DM detection [36, 41, 51]

RPG – random plasma glucose; FBG – fasting blood glucose; HbA1C – glycated haemoglobin A1c; OGTT – oral glucose tolerance test; PG – plasma glucose.

OGTT in a combination with HbA1c provides the additional prognostic information on all-cause mortality, as this identifies a group of high-risk patients, who would remain undetected if using an OGTT, or HbA1c only [38]. Regretably, in practice, the HbA1c levels were not available in about 3/4 of AMI patients without DM [19]. Moreover, the frequency of performing HbA1c varied widely; it was quite different among hospitals (capturing from 7.7% to 87.6% of hospitalized patients) [55].

The real world underutilization of evidence-based therapies for DM may contribute to worse outcome of patients with DM and ACS [7]. Early diagnosis and treatment of dysglycemia may slow down, or even reverse the adverse effects on the CV system [11]. Improved glycaemic control in the DM patients following the AMI results in the reduced long-term mortality [55]. A greater benefit could be obtained from treating the ACS patients with newly diagnosed DM more intensively [4]. Some of the important advantages of measuring HbA1c and performing OGTT are timely detection of abnormal glucose regulation (during the AMI hospitalization, or shortly after it) which could give rise to the prevention strategies, such as lifestyle and pharmacological interventions that can help to prevent DM [45, 46], and the detection of pre-DM which can be used to avoid drugs known to impair glucoregulation, such as diuretics, non-vasodilatory and non-selective beta blockers, some types of statins, etc. [56–58]. When DM is diagnosed, the appropriate diet, anti-DM drugs, exercise programs, etc., can be planed to improve quality of life and prognosis [29]. Moreover, in the newly diagnozed DM, the prevention of CV diseases can be improved, such as introduction/intensification of treatment (e.g., renin-angiotensin system inhibitor, statin, aspirin, etc.), as a risk category changes substantially with the diagnosis of DM. Timely made DM diagnosis can improve even the mode of reperfusion, as the diabetic status influence the choice between a coronary artery by-pass graft (CABG) and a stent, and further – the choice of stent, as well as antiplatelet therapy. For example, < 20% of the patients with dysglycemia, detected by OGTT, received drug-eluting stents, since they were treated as the non-diabetic patients at the time of percutaneous coronary intervention (PCI) [1, 11]. In the multivariate analysis, the ACS patients with pre-DM (OR, 1.58, 95%:1.08–2.31) and undiagnosed DM (OR, 1.51, 95%:1.01–2.26) also had reduced kidney function more frequently, in comparisson with the AMI patients who had normal glycoregulation [23].

Consequently, the detection of pre-DM and previously undiagnozed DM could enable us to pay more attention to the kidney function and prevent its deteoration. It may be useful to have information about pre-DM and newly detected DM prior to imaging techniques requiring contrast, in order to better prevention of contrast induced nephropathy, i.e., acute kidney injury. Moreover, an additional care should be taken to avoid potentially nephrotoxic drugs, such as, e.g., aminoglycosides.

Conclusion

There are four different DM varieties in ACS. The real number of the DM patients in AMI, who are adequately diagnosed and treated, could be even less then 30%. It is important to measure HbA1c on admission to detect the unrecognized patients with glycometabolic abnormalities. OGTT before discharge, or within the next 3 months is recommended in the majority of guidelines, but not in all. It is reasonable not to omit OGTT at least if HbA1c is not conclusive. DM and pre-DM are important prognostically, they can be prevented, or treated only if detected. There is no excuse to avoid at least one of such routine tests as it frequently occurred in practice with probable serious clinical consequence (see Table 1).

Acknowledgement

R E F E R E N C E S

1. *Aggarwal B, Shah GK, Randhawa M, Ellis SG, Lincoff AM, Menon V.* Utility of glycated hemoglobin for assessment of glucose metabolism in patients with st-segment elevation myocardial infarction. Am J Cardiol 2016; 117(5): 749–53.
2. *Bauters C, Lemesle G, de Groote P, Lamblin N.* A systematic review and meta-regression of temporal trends in the excess mortality associated with diabetes mellitus after myocardial infarction. Int J Cardiol 2016; 217: 109–21.
3. *Madsbad S.* Impact of postprandial glucose control on diabetes-related complications: How is the evidence evolving? J Diabetes Complications 2016; 30(2): 374–85.
4. *Canadian Diabetes Association Clinical Practice Guidelines Expert Committee.* Canadian Diabetes Association 2013 clinical practice guidelines for the prevention and management of diabetes in Canada. Methods. Can J Diabetes 2013; 37(Suppl 1): S1–212.
5. *Patel PA, Cubbon RM, Sapsford RJ, Gillott RG, Grant PJ, Witte KK,* et al. An evaluation of 20 year survival in patients with diabetes mellitus and acute myocardial infarction. Int J Cardiol 2016; 203: 141–4.
6. *Witt DM, Clark NP, Kaatz S, Schnurr T, Ansell JE.* Guidance for the practical management of warfarin therapy in the treatment of venous thromboembolism. J Thromb Thrombolysis 2016; 41(1): 187–205.
7. *Wei CC, Shyu KG, Cheng JJ, Lo HM, Chiu CZ.* Diabetes and Adverse Cardiovascular Outcomes in Patients with Acute Coronary Syndrome - Data from Taiwan's Acute Coronary Syndrome Full Spectrum Data Registry. Acta Cardiol Sin 2016; 32(1): 31–8.
8. *Mazurek M, Kowalczyk J, Lenarczyk R, Zielinska T, Sedkowska A, Pruszkowska-Skrzep P,* et al. The prognostic value of different glucose abnormalities in patients with acute myocardial infarction treated invasively. Cardiovasc Diabetol 2012; 11: 78.
9. *Bulugahapitiya U, Siyambalapitiya S, Sithole J, Idris I.* Is diabetes a coronary risk equivalent? Systematic review and meta-analysis. Diabet Med 2009; 26(2): 142–8.
10. *Bartnik M, Ryden L, Ferrari R, Malmberg K, Pyorala K, Simoons M,* et al. The prevalence of abnormal glucose regulation in patients with coronary artery disease across Europe. The Euro

Heart Survey on diabetes and the heart. Eur Heart J 2004; 25(21): 1880–90.

11. *Kuhl J, Jörneskog G, Wemminger M, Bengtsson M, Lundman P, Kalani M.* Long-term clinical outcome in patients with acute coronary syndrome and dysglycaemia. Cardiovasc Diabetol 2015; 14: 120.

12. *Usami M, Sakata Y, Nakatani D, Suna S, Matsumoto S, Hara M,* et al. Clinical impact of acute hyperglycemia on development of diabetes mellitus in non-diabetic patients with acute myocardial infarction. J Cardiol 2014; 63(4): 274–80.

13. *Mozaffarian D, Marfisi R, Levantesi G, Silletta MG, Tavazzi L, Tognoni G,* et al. Incidence of new-onset diabetes and impaired fasting glucose in patients with recent myocardial infarction and the effect of clinical and lifestyle risk factors. Lancet 2007; 370(9588): 667–75.

14. *Park CS, Chung WB, Choi YS, Kim PJ, Lee JM, Baek K,* et al. Acute Myocardial Infarction Is a Risk Factor for New Onset Diabetes in Patients with Coronary Artery Disease. PLoS One 2015; 10(8): e0136354.

15. *Tavares CA, Wajchjenberg BL, Rochitte C, Lerario AC.* Screening for asymptomatic coronary artery disease in patients with type 2 diabetes mellitus. Arch Endocrinol Metab 2016; 60(2): 143–51.

16. *Ishihara M.* Acute hyperglycemia in patients with acute myocardial infarction. Circ J 2012; 76(3): 563–71.

17. *Koracevic GP.* Proposal of a new approach to study and categorize stress hyperglycemia in acute myocardial infarction. J Emerg Med 2016; 51(1): 31–6.

18. *Gomez-Arbelaez D, Sánchez-Vallejo G, Perez M, Garcia RG, Arguello JF, Peñaherrera E,* et al. Hyperglycaemia is associated with worse outcomes in Latin-American individuals with acute myocardial infarction. Clin Investig Arterioscler 2016; 28(1): 9–18. (Spanish)

19. *Rousan TA, Pappy RM, Chen AY, Roe MT, Saucedo JF.* Impact of diabetes mellitus on clinical characteristics, management, and in-hospital outcomes in patients with acute myocardial infarction (from the ncdr). Am J Cardiol 2014; 114(8): 1136–44.

20. *Colombo MG, Meisinger C, Amann U, Heier M, von Scheidt W, Kuch B,* et al. Association of obesity and long-term mortality in patients with acute myocardial infarction with and without diabetes mellitus: results from the MONICA/KORA myocardial infarction registry. Cardiovasc Diabetol 2015; 14(1): 24.

21. *Bundhun PK, Li N, Chen M.* Adverse cardiovascular outcomes between insulin-treated and non-insulin treated diabetic patients after percutaneous coronary intervention: a systematic review and meta-analysis. Cardiovasc Diabetol 2015; 14(1): 135.

22. *Arnold SV, Spertus JA, Lipska KJ, Tang F, Goyal A, McGuire DK,* et al. Association between diabetes mellitus and angina after acute myocardial infarction: analysis of the TRIUMPH prospective cohort study. Eur J Prev Cardiol 2015; 22(6): 779–87.

23. *Liu H, Zhou C, Yu J, Jiang Q, Hu D.* Reduced kidney function in acute coronary syndrome patients with undiagnosed diabetes or pre-diabetes. Nephrology (Carlton) 2013; 18(4): 263–8.

24. *Koracevic GP, Petrovic S, Damjanovic M, Stanojlovic T.* Association of stress hyperglycemia and atrial fibrillation in myocardial infarction. Wien Klin Wochenschr 2008; 120(13–14): 409–13.

25. *Vergès B, Avignon A, Bonnet F, Catargi B, Cattan S, Cosson E,* et al. Diabetes and cardiovascular Disease study group of the Société francophone du diabète (SFD), in collaboration with the Société française de cardiologie (SFC). Consensus statement on the care of the hyperglycaemic/diabetic patient during and in the immediate follow-up of acute coronary syndrome. Diabetes Metab 2012; 38(2): 113–27.

26. *Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N,* et al. Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC); European Association for the Study of Diabetes (EASD). ESC guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD - summary. Diab Vasc Dis Res 2014; 11(3): 133–73.

27. *Le VT , Knight S, Anderson JL, Lappé DL, May HT, Horne BD,* et al. Undiagnosed diabetes at the time of acute myocardial infarction is frequent and associated with poor cardiovascular outcomes. Circulation 2015; 132: A15117.

28. *American Diabetes Association.* Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2010; 33(Suppl 1): S62–9.

29. *American Diabetes Association.* Standards of medical care in diabetes. Diabetes Care 2016; 39(Suppl 1): S1-S106.

30. *Koracevic GP.* Using a single cut-off for stress hyperglycemia in myocardial infarction decreases its prognostic value particularly in diabetes. Am J Emerg Med 2014; 32(1): 103–4.

31. *Koraćević G, Vasiljević S, Veličković-Radovanović R, Sakac D, Obradović S, Damjanović M,* et al. Stress hyperglycemia in acute myocardial infarction. Vojnosanit Pregl 2014; 71(9): 858–69.

32. *Koracevic GP.* Various admission glucose cut-offs for prognostication and for therapeutic threshold in acute myocardial infarction. Am J Emerg Med 2015; 33(1): 108–9.

33. *Henareh L, Agewall S.* 2-h postchallenge plasma glucose predicts cardiovascular events in patients with myocardial infarction without known diabetes mellitus. Cardiovasc Diabetol 2012; 11: 93.

34. *George A, Bhatia RT, Buchanan GL, Whiteside A, Moisey RS, Beer SF,* et al. Impaired glucose tolerance or newly diagnosed diabetes mellitus diagnosed during admission adversely affects prognosis after myocardial infarction: an observational study. PLoS One 2015; 10(11): e0142045.

35. *Naito R, Miyauchi K, Ogita M, Kasai T, Kawaguchi Y, Tsuboi S,* et al. Impact of admission glycemia and glycosylated hemoglobin A1c on long-term clinical outcomes of non-diabetic patients with acute coronary syndrome. J Cardiol 2014; 63(2): 106–11.

36. *Pararajasingam G, Høfsten DE, Løgstrup BB, Egstrup M, Henriksen FL, Hangaard J,* et al. Newly detected abnormal glucose regulation and long-term prognosis after acute myocardial infarction: Comparison of an oral glucose tolerance test and glycosylated haemoglobin A1c. Int J Cardiol 2016; 214: 310–5.

37. *Coutinho M, Gerstein HC, Wang Y, Yusuf S.* The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care 1999; 22(2): 233–40.

38. *American Diabetes Association.* Standards of medical care in diabetes - 2012. Diabetes Care 2012; 35(Suppl 1): S11–63.

39. *Abdullatef WK, Al-Aqeedi RF, Dabdoob W, Hajar HA, Bener A, Gehani AA.* Prevalence of unrecognized diabetes mellitus in patients admitted with acute coronary syndrome. Angiology 2013; 64(1): 26–30.

40. *Senthinathan A, Kelly V, Dzingina M, Jones D, Baker M, Longson D.* Hyperglycaemia in acute coronary syndromes: summary of NICE guidance. BMJ 2011; 343: d6646.

41. *Ritsinger V, Tanoglidi E, Malmberg K, Näsman P, Rydén L, Tenerz Å,* et al. Sustained prognostic implications of newly detected glucose abnormalities in patients with acute myocardial infarction: long-term follow-up of the Glucose Tolerance in Patients with Acute Myocardial Infarction cohort. Diab Vasc Dis Res 2015; 12(1): 23–32.

42. *Ye Y, Xie H, Zhao X, Zhang S.* The oral glucose tolerance test for the diagnosis of diabetes mellitus in patients during acute coronary syndrome hospitalization: a meta-analysis of diagnostic test accuracy. Cardiovasc Diabetol 2012; 11(1): 155.

43. *Bronisz A, Kozinski M, Magielski P, Fabiszak T, Gierach J, Swiatkiewicz I,* et al. Value of oral glucose tolerance test in the acute phase of myocardial infarction. Cardiovasc Diabetol 2011; 10(1): 21.

44. *American Diabetes Association (ADA).* Standards of medical care in diabetes Diabetes Care 2015; 38(Suppl 1): S1–S90.

45. *de la Hera JM, García-Ruiz JM, Martínez-Camblor P, Martín M, Tellería AL, Corros C,* et al. Real incidence of diabetes mellitus in a coronary disease population. Am J Cardiol 2013; 111(3): 333–8.

46. *Colomo N, Linares F, Rubio-Martín E, Moreno MJ, de Mora M, García AM,* et al. Stress hyperglycaemia in hospitalized patients with coronary artery disease and type 2 diabetes risk. Eur J Clin Investig 2013; 43(10): 1060–8.

47. *Knudsen EC, Seljeflot I, Abdelnoor M, Eritsland J, Mangschau A, Arnesen H,* et al. Abnormal glucose regulation in patients with acute ST- elevation myocardial infarction-a cohort study on 224 patients. Cardiovasc Diabetol 2009; 8: 6.

48. *Gierach J, Gierach M, Świątkiewicz I, Woźnicki M, Grześk G, Sukiennik A,* et al. Admission glucose and left ventricular systolic function in non-diabetic patients with acute myocardial infarction. Heart Vessels 2016; 31(3): 298–307.

49. *Bronisz A, Kozinski M, Magielski P, Fabiszak T, Bronisz M, Swiatkiewicz I,* et al. Stress hyperglycaemia in patients with first myocardial infarction. Int J Clinl Pract 2012; 66(6): 592–601.

50. *Gitt A, Bramlage P, Towae F, Papp A, Deeg E, Senges J,* et al. Newly diagnosed diabetes and prediabetes in patients after acute myocardial infarction predicts adverse outcomes during a three-year follow-up: results of the sweetheart registry. JACC 2013; 61(10): E176.

51. Task Force Members. *Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N,* et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). Eur Heart J 2013; 34(39): 3035–87.

52. Task Force of the SEC for the ESC Guidelines on Diabetes, Prediabetes and Cardiovascular Disease; Expert Reviewers for the ESC Guidelines on Diabetes, Prediabetes And Cardiovascular Disease; Guideliness Committee of the SEC. Comments on the ESC guidelines on diabetes, prediabetes, and cardiovascular diseases developed in collaboration with the european society for the study of diabetes. Rev Esp Cardiol (Engl Ed) 2014; 67(2): 87–93.

53. *Monteiro CM, Oliveira L, Izar MC, Helfenstein T, Santos AO, Fischer SM,* et al. Early glucometabolic profile in patients with acute coronary syndromes and metabolic syndrome. Arq Bras Cardiol 2009; 92(2): 89–99. (English, Portuguese, Spanish)

54. *Kowalczyk J, Mazurek M, Zielinska T, Lenarczyk R, Sedkowska A, Swiatkowski A,* et al. Prognostic significance of HbA1c in patients with AMI treated invasively and newly detected glucose abnormalities. Eur J Prev Cardiol 2015; 22(6): 798–806.

55. *Snir A, Dabin B, Hyun K, Yamen E, Ryan M, Aliprandi-Costa B,* et al. Glycosylated haemoglobin assessment in diabetic patients with acute coronary syndromes. Intern Med J 2016; 46(5): 574–82.

56. *Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M,* et al. Task Force for the Management of Arterial Hypertension of the European Society of Hypertension and the European Society of Cardiology. 2013 ESH/ESC Practice Guidelines for the Management of Arterial Hypertension. Blood Press 2014; 23(1): 3–16.

57. *Sternlicht H, Bakris GL.* Hydrochlorothiazide as the Diuretic of Choice for Hypertension: Time to Kick the Habit. J Am Coll Cardiol 2016; 67(4): 390–1

58. *Navarese EP, Buffon A, Andreotti F, Kozinski M, Welton N, Fabiszak T,* et al. Meta-analysis of impact of different types and doses of statins on new-onset diabetes mellitus. Am J Cardiol 2013; 111(8): 1123–30.

VOJNOSANITETSKI PREGLED

Using respiratory polygraphy in diagnosing obstructive sleep apnea – our experiences

Primena respiratorne poligrafije u dijagnostikovanju opstruktivnog poremećaja disanja tokom spavanja – naša iskustva

Dobrivoje Novković*[†], Gordana Cvetković*[†], Slobodan Aćimović*[†],
Rade Milić*[†], Sanja Šarac*[†], Radmila Urošević[‡]

Military Medical Academy, *Clinic for Pulmonology, Belgrade, Serbia;
University of Defence, [†]Faculty of Medicine of the Military Medical Academy, Belgrade,
Serbia; [‡]General Hospital Jagodina, Jagodina, Serbia

Abstract

Bacground/Aim. Obstructive sleep apnea (OSA) involves repeated episodes of cessation of breathing that occur due to a decrease in pharyngeal muscle tone. This disorder is more common in men and represents a significant risk factor for serious cardiovascular and cerebrovascular events. The gold standard in the diagnosis of this disorder represents a polysomnography (PSG), which is technically a complex and multidisciplinary method. Respiratory polygraphy (RP) may constitute an adequate replacement for most uncomplicated cases of obstructive sleep apnea. The aim of the study was to examine the efficacy of using respiratory polygraphies in diagnosing of obstructive sleep apnea. **Methods.** On all the patients with suspected obstructive sleep apnea, RP and a retrospective analysis of the obtained results were performed. **Results.** By completing our examination, we proved that there was a positive correlation between the results obtained by using RP and the predictors of obstructive sleep apnea such as the Epworth sleepiness scale (EPWORTH) score, neck circumference and body mass index. **Conclusion.** Respiratory polygraphy represents a cheaper and simpler replacement for PSG, especially with uncomplicated obstructive breathing disorders during sleep.

Key words:
sleep apnea syndrome; diagnostic techniques and procedures; respiratory function tests; risk factors.

Apstrakt

Uvod/Cilj. Opstuktivni poremećaj disanja tokom spavanja podrazumeva ponovljene epizode prestanka disanja koji nastaju zbog smanjenja tonusa mišića faringsa. Ovaj poremećaj češći je kod muškaraca i predstavlja značajan faktor rizika od ozbiljnih kardio- i cerebrovaskularnih događaja. Zlatni standard u postavljanju dijagnoze ovog poremećaja predstavlja polisomnografija (PSSG) koja je tehnički zahtevna i multidisciplinarna metoda. Respiratorna poligrafija (RP) može predstavljati adekvatnu zamenu kod većine nekomplikovanih slučajeva opstruktivne *sleep apneae*. Cilj rada bio je ispitati efikasnost primene RP u dijagnostikovanju opstuktivnog poremećaja disanja tokom sna. **Metode.** Kod svih ispitanika sa sumnjom na opstuktivni poremećaj disanja tokom sna, učinjena je respiratorna poligrafija i retrospektivna analiza dobijenih rezultata. **Rezultati.** Učinjenim ispitivanjem dokazali smo da postoji pozitivna korelacija između rezultata dobijenih RP prediktora kao što su *Epworth Sleepinness scale* (EPWORTA skor), obim vrata, indeks telesne mase. **Zaključak.** Respiratorna poligrafija predstavlja jeftiniju i jednostavniju zamenu za PSG naročito za nekomplikovane opstruktivne poremećaje disanja tokom sna.

Ključne reči:
apneja u snu, sindromi; dijagnostičke tehnike i procedure; respiratorna funkcija, testovi; faktori rizika.

Introduction

Obstructive sleep apnea (OSA) is regarded as continuing episodes of obstruction of upper airways which cause lowered saturation of blood with oxygen and interrupted sleep. More precisely, OSA implies more than five breathing abortions and/or significant reduction of ventilation during one hour of sleeping. These episodes are quantified with the apnea/hypopnea index (AHI). Breathing disorder is the consequence of reduction muscle pharynx's tone and the cause of specific day-night symptoms which significantly decrease the quality of life and increase the risk of lethal cerebral and cardiovascular events [1, 2].

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

In the general adult population, 4% of males and 2% of females have OSA.

Physical constitution and age are tightly connected with occurrence of OSA. Obese and middle aged people are more likely to have OSA, particularly patients with the body mass index over 35 kg/m^2, where incidence is between 7% and 11% [3–5].

In this disorder, the main pathophysiological change consists of continuing episodes of hypoxemia which cause complex inflammatory mechanisms and accelerate pathological processes in organism, such as arteriosclerosis [3, 6, 7].

Interrupted sleep, intermittent hypoxia, systemic inflammation and chronically raised tone of sympathetic nerve system caused by OSA make many cardiovascular disorders, such as arterial hypertension, ischemic heart disease, congestive heart failure and disturbance of heart rhythm [8, 9].

Except the negative effect on brain and cardiovascular diseases, OSA makes already existing lung diseases such as bronchial asthma, chronic obstructive pulmonary diseases significant and deepens the respiratory insufficiency [10].

OSA leaves serious consequences on the gastrointestinal tract and endocrine system which are manifested through the gastroesophageal reflux disease – GERD, and disturbance of liver and pancreatic functions [11].

The diagnosis of this disorder is based on the typical anamnestic data, results gathered from various questionnaires at the end and on the specific examination – recording respiratory acts during sleeping.

Initially, the hetero-anamnestic data, obtained from family members often has a crucial part in the diagnostic process of this disorder [12, 13].

This way, many diagnostic procedures contribute to raising the number of diagnosed cases in real conditions by increasing the number of the diseased [14].

The Epworth's sleepiness scale (ESS) is one of the most frequently used tests for estimating somnolence which consistsed of 8 questions focused on problem intensity caused by interrupted sleep. Answers are graded with points from 0 to 3. According to this test, a score of 10 or more points is regarded as an indication for the examination [15].

In order to verify a sleeping disorder, the standards require usage of polysomnography (PSG) with registering several vigilance and somnolence parameters (electroencephalogram, electrooculogram, electromyogram as well as cardiorespiratory (airflow, oximetry, effort, respiratory movement, etc.) and the visual parameters.

Polysomnography is technically complex and expensive, which makes it often an unavailable method. Because of its complicatedness, the need for a faster, simpler and less expensive method has aroused [16].

Respiratory polygraphy (RP) is a technically simpler and much cheaper method which can play an important role in the diagnosis of obstructive sleep apnea.

The aim of the study was to show the efficiency of using RP in diagnosing obstructive sleep apnea and examine the correlation between apnea-hypopnea index (AHI) index obtained using RP and scoring achieved by using the ESS, body mass index and neck circumference, as well as risk factors for the existence of sleep apnea.

Methods

The examination included 61 patients, 52 men and 9 women, age from 30 to 76 years old, with suspicion of having a breathing sleep disorder. All of them completed the ESS test after the patients' history was gathered and physical examination performed. Everyone got their neck circumference measured as well as their body mass index calculated. After that, the patients who had positive anamnestic data and the result on the ESS of at least 10 did a RP. We conducted a retrospective analysis of the results.

Results

By analyzing the distribution of patients by sex and age, we found that among examined patients (52 mean and 9 women) most of them were middle-aged and elderly men with average age of 59 years.

On further analysis, we performed an examination of the correlation of the AHI index and parameters which represent known risk factors, such as the ESS, neck circumference and body mass index.

The results obtained from the ESS were in a positive correlation with the AHI index acquired by RP ($p < 0.0001$; R = 0.53, n = 61) (Figure 1).

We found that the patients with the increased neck circumference had a higher AHI index ($p < 0.0001$; r = 0.46, n = 49). The correlation between the raised body mass index and the higher AHI index was: $p < 0.0001$; r = 0.49, n = 61 (Figures 2 and 3).
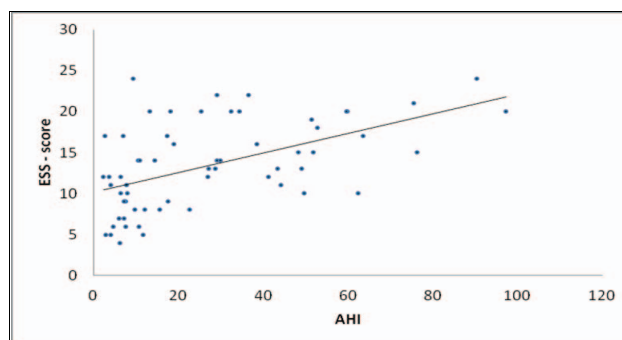

Fig. 1 – Correlation between the ESS score and AHI index.
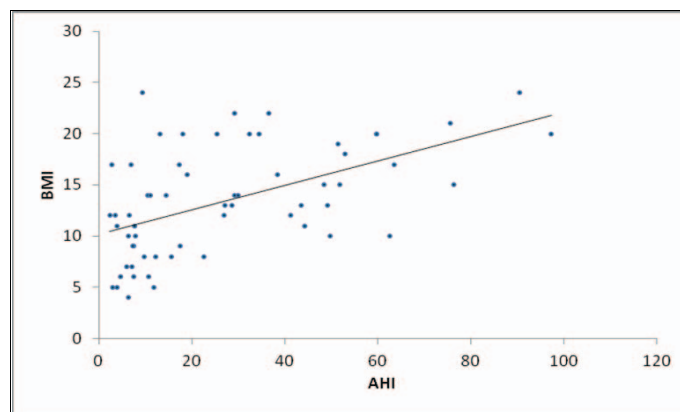**ESS – Epworth sleepiness scale; AHI – apnea-hypopnea index.**

Fig. 2 – Correlation between the BMI and AHI index.
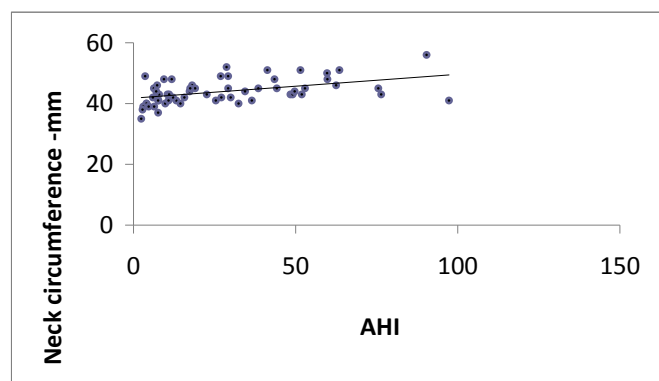**BMI – body mass index in kg/m^2; AHI – apnea-hypopnea index.**

Fig. 3 – Correlation between neck circumference and AHI index.
AHI – apnea-hypopnea index.

Discussion

Verification of obstructive sleep apnea is the most important step in the process of diagnosis and successful treatment. Although PSG is recommended as a "gold standard", there is a growing interest in the alternative diagnostic methods that could quickly and easily give the same or similar data.

In support of this fact is an increasingly frequent application of RP, especially in the pediatric population [17].

Besides accepting RP by the clinician, a need for the new diagnostic methods has been recognized by the relevant world organizations.

Based on the number of measured variables, the American Academy of Sleep Medicine (AASM) classified the sleep study into four main types out of which the three of them can be performed with the portable monitoring [18].

Despite the fact that the portable devices measured the same parameters as PSG, there are authors who believe that the portable monitoring has variable sensitivity and specificity for OSA.

These attitudes largely result in the lack of research on this type of devices [19].

The existence of conflicting opinions did not reduce the interest for RP. Consequently, numerous studies confirmed the compatibility of the AHI index obtained after PSG and RP.

In one big study, Iber et al. [20] showed that portable devices displayed a very low degree of failure in diagnosing apnea.

Other studies proved the acceptable sensitivity and specificity of portable devices with a very low percentage of falsely positive results (about 3%) [21].

In a randomized study, Campbell and Neill [22] pointed that a portable diagnosis without supervision was proven to be an adequate PSG alternative.

This knowledge encouraged us to perform our study whose aim was to examine the effectiveness of RP in the diagnosis of OSA.

The results we obtained mostly confirmed well- known views on the possibilities of RP.

The population of our examinees had the typical demographic characteristics of people with obstructive sleep apnea, having been largery made up of elderly and middle-aged men, which is consistent with pervious findings.

By examining the correlation between the results obtained by the ESS and the AHI index determined by RP, we demonstrated a positive correlation between these two parameters. In other words, the higher the score on the test, the higher the AHI index.

We examined the relationship between the neck circumference and AHI index and we also got a positive correlation.

Similarly happened when we compared the value of body mass index with the AHI index obtained by RP because

we proved a positive correlation between these two parameters. The greater body mass index was associated with the higher index of AHI.

Our results showed that there was no inferiority of RP compared to PSG in diagnosing the obstructive disorders of breathing during sleep.

Of course, our conclusions, and the conclusions of other authors are primarily related to OSA in conditions where there are no significant comorbidities [23, 24].

Conclusion

Although PSG is the "gold standard" in the diagnosis of obstructive sleep apnea, RP can be an adequate substitute especially when it comes to the obstructive disorders.

The significance of this method is even greater if we take into account the fact that RP is significantly more favorable financially, technically simpler and therefore more accessible for wider use.

R E F E R E N C E S

1. *Kryger MH.* Diagnosis and management of sleep apnea syndrome. Clin Cornerstone 2000; 2(5): 39–47.
2. *Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S.* The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993; 328(17): 1230–5.
3. *Young T, Peppard PE, Taheri S.* Excess weight and sleep-disordered breathing. J Appl Physiol (1985). 2005; 99(4): 1592–9.
4. *Tishler PV, Larkin EK, Schluchter MD, Redline S.* Incidence of sleep-disordered breathing in an urban adult population: the relative importance of risk factors in the development of sleep-disordered breathing. JAMA 2003; 289(17): 2230–7.
5. *Newman AB, Foster G, Givelber R, Nieto FJ, Redline S, Young T.* Progression and regression of sleep-disordered breathing with changes in weight: the Sleep Heart Health Study. Arch Intern Med 2005; 165(20): 2408–13.
6. *Lavie L.* Oxidative stress and inflammation in OSA. Eur Respir Mon 2010; 50: 360–80.
7. *Almendros I, Montserrat JM, Ramírez J, Torres M, Duran-Cantolla J, Navajas D,* et al. Intermittent hypoxia enhances cancer progression in a mouse model of sleep apnoea. Eur Respir J 2012; 39(1): 215–7.
8. *Shamsuzzaman A, Amin RS, Calvin AD, Davison D, Somers VK.* Severity of obstructive sleep apnea is associated with elevated plasma fibrinogen in otherwise healthy patients. Sleep Breath 2014; 18(4): 761–6.
9. *Kendzerska T, Gershon AS, Hawker G, Leung RS, Tomlinson G.* Obstructive sleep apnea and risk of cardiovascular events and all-cause mortality: a decade-long historical cohort study. PLoS Med 2014; 11(2): e1001599.
10. *Marin JM, Soriano JB, Carrizo SJ, Boldova A, Celli BR.* Outcomes in patients with chronic obstructive pulmonary disease and obstructive sleep apnea: the overlap syndrome. Am J Respir Crit Care Med 2010; 182(3): 325–31.
11. *Gami AS, Olson EJ, Shen WK, Wright RS, Ballman KV, Hodge DO,* et al. Obstructive sleep apnea and the risk of sudden cardiac death: a longitudinal study of 10,701 adults. J Am Coll Cardiol 2013; 62(7): 610–6.
12. *Byrne TJ, Parish JM, Somers V, Aqel BA, Rakela J.* Evidence for liver injury in the setting of obstructive sleep apnea. Ann Hepatol 2012; 11(2): 228–1.
13. *Kopitović I, Jovančević-Drvenica M, Vukoja M, Okiljević Z.* Breathing disorder during sleep. Časopis udruženja pulmologa Republike Srpske 2011; 11(1): 310–5. (Serbian)
14. *Young T, Peppard PE, Gottlieb DJ.* Epidemiology of obstructive sleep apnea: a population health perspective. Am J Respir Crit Care Med 2002; 165(9): 1217–39.
15. *Abrishami A, Khajehdehi A, Chung F.* A systematic review of screening questionnaires for obstructive sleep apnea. Abrishami A, Khajehdehi A, Chung F. A systematic review of screening questionnaires for obstructive sleep apnea. Can JAnesth 2010; 57(5): 423–38.
16. *Lloberes P, Sampol G, Levy G, Aristizabal D, Sagales T, De la Calzada M,* et al. Influence of setting on unattended respiratory monitoring in the sleep apnoea/hypopnoea syndrome. Eur Respir J 2001; 18(3): 530–4.
17. *Marcus CL, Brooks LJ, Draper KA, Gozal D, Halbower AC, Jones J,* et al. Diagnosis and Management of Childhood Obstructive Sleep Apnea Syndrome. Pediatrics 2012; 130(3): 576–84.
18. *Ferber R, Millman R, Coppola M, Fleetham J, Murray CF, Iber C,* et al. Portable recording in the assessment of obstructive sleep apnea. ASDA standards of practice. Sleep 1994; 17(4): 378–92.
19. *Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK,* et al. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. J Clin Sleep Med 2012; 8(5): 597–619.
20. *Iber C, Redline S, Kaplan Gilpin AM, Quan SF, Zhang L, Gottlieb DJ,* et al. Polysomnography performed in the unattended home versus the attended laboratory setting-Sleep Heart Health Study methodology. Sleep 2004; 27(3): 536–40.
21. *Portier F, Portmann A, Czernichow P, Vascaut L, Devin E, Benhamou D,* et al. Evaluation of home versus laboratory polysomnography in the diagnosis of sleep apnea syndrome. Am J Respir Crit Care Med 2000; 162(3 Pt 1): 814–8.
22. *Campbell AJ, Neill AM.* Home set-up polysomnography in the assessment of suspected obstructive sleep apnea. J Sleep Res 2011; 20(1 Pt 2): 207–13.
23. *Rosen CL, Auckley D, Benca R, Foldvary-Schaefer N, Iber C, Kapur V,* et al. A Multisite Randomized Trial of Portable Sleep Studies and Positive Airway Pressure Autotitration Versus Laboratory-Based Polysomnography for the Diagnosis and Treatment of Obstructive Sleep Apnea: The HomePAP Study. Sleep 2012; 35(6): 757–67.
24. *Kuna ST, Gurubhagavatula I, Maislin G, Hin S, Hartwig KC, McCloskey S,* et al. Noninferiority of Functional Outcome in Ambulatory Management of Obstructive Sleep Apnea. Am J Respir Crit Care Med 2011; 183(9): 1238–44.

Rectal syphilis - A case report

Rektalni sifilis

Dimitrije Damjanov*[†], **Tatiana Jocić**[†], **Olgica Latinović-Bošnjak***[†],
Dragomir Damjanov*[†], **Željka Savić***[†], **Dijana Kosijer***[†], **Vladimir Vračarić***[†],
Tihomir Orlić[†], **Žarko Krnetić***[†]

**University of Novi Sad, Faculty of Medicine, *Department of Internal Medicine,
Novi Sad, Serbia; Clinical Centre of Vojvodina, [†]Clinic for Gastroenterology
and Hepatology, Novi Sad, Serbia**

Abstract

Introduction. Syphilis rarely affects anorectal region. The symptoms are nonspecific and are commonly disregarded in our country. Therefore, they pose a difficulty both for a diagnosis and for a treatment. We presented a patient with the clinical, laboratory, endoscopic and histological characteristics of rectal siphilis who was initially suspected to have inflammatory bowel disease. **Case report.** A 29-year-old man was hospitalized with a suspected inflammatory bowel disease, with symptoms such as frequent blood-stained diarrhea, lower abdominal pain and a loss of appetite. The physical examination showed maculopapular skin rash on the body. The ileocolonoscopic examination revealed finely granulated rectal mucosa, the loss of vascular pattern, and at 3 cm from the anal verge, an exulcerated submucosal lesion 1.2 cm in diameter, with two smaller, similar looking lesions. The histological examination of biopsies showed diffuse inflammatory-cell infiltration, with cryptitis, Paneth cell metaplasia with granuloma without caseous necrosis, which was highly suggestive of Crohn's disease. The *Treponema pallidum* test results were positive [hemagglutination assay (TPHA)] with a titer 1 : 2,560 and the rapid plasma reagin test (RPR) with a titer 1 : 16. The ensuing detailed anamnesis on the patient's sexual behavior showed that the patient had unprotected anal sexual relation with another man and the diagnosis of secondary syphilis was confirmed. After the treatment with benzathine penicillin G once a week, during a three-week period, the patient had no symptoms and had normal inflammatory markers, with a significant decrease of RPR titre and normal mucosa on rectosigmoidoscopy. **Conclusion.** Taking in consideration the variable clinical and endoscopic manifestations of this disease, it is necessary to take a detailed history of sexual behavior, since it can be crucial for determining the diagnosis and differential diagnosis of syphilis.

Key words:
diagnosis, differential; homosexuality; inflammatory bowel diseases; rectal diseases; sexually transmitted diseases; syphilis; treatment outcome.

Apstrakt

Uvod. Zahvatanje rektuma sifilisom je retko. Simptomi ove infekcije nisu specifični i u našoj sredini se često na njih ne misli. Zbog toga ona predstavlja dijagnostički i terapijski izazov. Prikazan je bolesnik sa kliničkim, laboratorijskim, endoskopskim i histološkim karakteristikama rektalnog sifilisa kod koga se inicijalno sumnjalo na inflamatornu bolest creva. **Prikaz bolesnika.** Bolesnik star 29 godina je hospitalizovan pod sumnjom na zapaljensku bolest creva, sa tegobama u vidu učestalih stolica sa primesama krvi, bolova u donjem delu trbuha i gubitka apetita. Fizikalnim pregledom zapažena je makulopapulozna ospa po koži. Ileokolonoskopijom je viđena fino granulisana sluznica rektuma bez vaskularne šare i na 3 cm od anokutane granice egzulcerisana submukozna lezija veličine 1,2 cm, sa dve manje lezije sličnog izgleda. Patohistološkim pregledom biopsija opisan je difuzni inflamatorni infiltrat, sa kriptitisom, metaplazijom Panetovih ćelija, sa prisustvom granuloma bez kazeozne nekroze, što je bilo visoko suspektno na Krono-vu bolest. *Treponema Pallidum Hemaglutination Assay* (TPHA) test je bio pozitivan u titru 1 : 2 560 i *Rapid Plasma Reagin* (RPR) test pozitivan u titru 1 : 16. Nakon detaljno uzete anamneze o seksualnom ponašanju, utvrđeno je da je bolesnik imao nezaštićen analni seks sa drugim muškarcem i potvrđena je dijagnoza sekundarnog sifilisa. Nakon terapije benzatin penicilinom jednom nedeljno u trajanju od tri nedelje, bolesnik je bio bez tegoba, sa normalnim laboratorijskim nalazima, značajnim padom titra RPR testa a rektosigmoidoskopijom je viđena normalna sluznica. **Zaključak.** Uzimajući u obzir raznolike kliničke i endoskopske manifestacije sifilisa, neophodno je detaljno uzeti podatke o seksualnom ponašanju, što može biti presudno prilikom postavljanja dijagnoze.

Ključne reči:
dijagnoza, diferencijalna; homoseksualnost; creva, zapaljenske bolesti; rektum, bolesti; venerične bolesti; sifilis; lečenje, ishod.

Correspondence to: Dimitrije Damjanov, University of Novi Sad, Faculty of Medicine, Hajduk Veljkova 3, Novi Sad, Serbia. E-mail: DIMITRIJE.DAMJANOV@mf.uns.ac.rs

Introduction

Sexually transmitted diseases may spread to parts of gastrointestinal tract. The most common causes of the diseases of anorectal region are chlamydia, gonorrhea, herpes simplex virus (HSV) and syphilis [1]. *Treponema pallidum* infection is the third cause of symptomatic infection of anorectal region in men who have sex with men, after the HSV infection and gonorrhea. Syphilis rarely affects anorectal region. The symptoms of these infections are nonspecific and are commonly disregarded in our country. Therefore, they pose a difficulty both for a diagnosis and for a treatment [1–3].

We presented a patient with rectal syphilis with the clinical, laboratory, endoscopic and histological characteristics which suggested Crohn's disease.

Case report

A 29-year-old man was hospitalized with suspected inflammatory bowel disease, with the symptoms such as frequent blood-stained diarrhea, lower abdominal pain and a loss of appetite. The physical examination of the systems was negative, except for the presence of maculopapular skin rash on the body. He denied previous diseases or risky sexual behavior.

The laboratory test results revealed the increased inflammatory markers – leucocytes 12.15×10^9/L (reference range $4.0–10.0 \times 10^9$/L), C-reactive protein 58.1 mg/L (reference range 0.0–5.0 mg/L) and erythrocyte sedimentation rate 80 mm/h (reference range 3–8 mm/h). The parameters of liver and renal function were within the range, without anemia or hypoproteinemia. The stool sample tests on *Clostridium difficile* toxin A and B, bacteria, parasites and protozoa were negative. Ileocolonoscopic examination revealed finely granulated rectal mucosa, the loss of vascular pattern, and at 3 cm from the anal verge, an exulcerated submucosal lesion, 1.2 cm in size was found, with two smaller, similar looking lesions, 4–5 mm in size (Figure 1).

The pathohistological examination of rectal biopsies showed diffuse inflammatory-cell infiltration, with cryptitis, Paneth cell metaplasia, with granuloma without caseous necrosis, which was highly suggestive of Crohn's disease. The computed tomography (CT) scan of the pelvis revealed the enlargement of inguinal lymph nodes, up to 16 mm in diameter. Due to the maculopapular skin rash, and with the goal of investigating the other granulomatosis, the additional examinations were performed. While the anti-neutrophil cytoplasmic antibodies (ANCA), antinuclear antibodies (ANA) and angiotensin converting enzyme (ACE) tests were negative, the *Treponema pallidum* hemagglutination assay (TPHA) results were positive with a titer 1 : 2,560 and the rapid plasma reagin test (RPR) with a titer 1 : 16). The ensuing detailed anamnesis on the patient's sexual behavior showed that the patient had unprotected anal sexual relation with another man. Upon consultation with a dermatovenereologist, the diagnosis of secondary syphilis was confirmed. Because of common co-occurrence with other sexually transmitted diseases, the additional tests were performed, including the

HIV and hepatitis C antibodies, HBs antigen and urethral swab for chlamydia and gonorrhea, which were all negative.
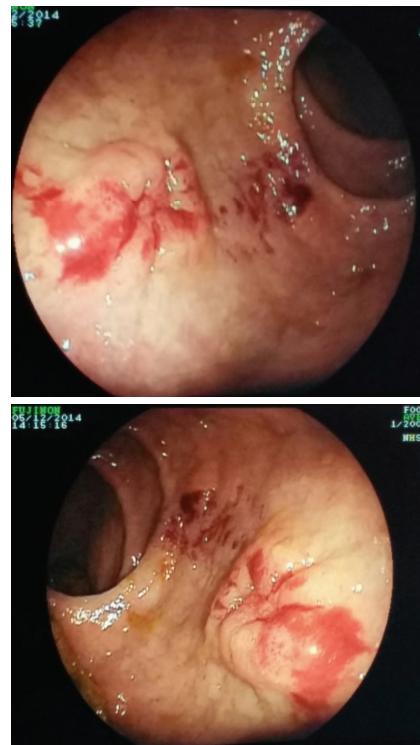

Fig. 1– Endoscopic image of the rectal ampulla with finely granulated mucosa, the loss of vascular pattern and exulcerated submucous lesion with two minor lesions.

The patient was treated with intramuscular therapy of 2.4 million units of benzathine penicillin G once a week, during a three-week period. One month after commencing the treatment, the patient had no symptoms and had normal inflammatory markers with a significant decrease of RPR titre. Control rectosigmoidoscopy revealed normal mucosa of the rectosigmoid colon.

Discussion

Syphilis is a sexually transmitted systematic disease caused by the *Treponema pallidum* spirochete. The disease develops through the phase of early syphilis, which lasts up to two years, and late syphilis. Early syphilis includes primary, secondary and early latent stages, while late syphilis includes late latent and tertiary stages [4].

Primary syphilis is characterized by the presence of solid chancre (ulcus durum) at the place of inoculation, most commonly found at genitals with regional lymphadenopathy. At least 5% of such lesions are extragenital, so that the ulcus may pass unnoticed if located in the anorectum, cervix, or in the oral cavity [5, 6]. It is usually solitary, although the multiple ulcerations may also occur. In some untreated patients the chancre does not epithelize, and may be present in the secondary stages of the disease, as in our patient's case. Secondary syphilis is characterized by maculopapular skin rash,

plaques, ulceration, erosion and papula on the mucosa, as well as by the systemic manifestations (generalized lymphadenopathy, mild form of hepatitis, splenomegaly, uveitis, arthritis, parotitis, glomerulonephritis), which is the reason why syphilis is often called "the great imitator" [6].

Due to its nonspecific symptoms, rectal syphilis represents a diagnostic challenge, although the insight into the relevant literature reveals that, despite being rare, it is recognizable in comparison to other localizations of the disease [7]. Only several dozens of cases of primary and secondary anorectal syphilis was described in the past several decades. Most cases described involve the homosexual and bisexual men, mainly coming from the underprivileged social groups in larger cities. Among the homosexual male population, it has been found in 2% of the patients with rectal symptoms. 25%–50% cases of syphilis co-occur with the HIV infection [1].

The patients usually complain about defecation disorders, diarrhea with blood and mucus, tenesmus, urgency of defecation, the symptoms which are not specific and suggest all benign diseases of anorectal region and tumors [1, 2, 8].

The endoscopic image of rectal syphilis includes the inflammatory, infiltratory vegetant lesions, ulceration and pseudotumors, which are not located in the proximal segments of the colon. Therefore, the differential diagnostic specter is rather wide, including the inflammatory bowel diseases, lymphomas, viral ulcerations [cytomegalo virus (CMV) and HSV], lymphogranuloma venerum, solitary rectal ulcer and rectal carcinoma, which necessitates multiple biopsies. In case of our patient, endoscopy revealed finely granulated rectal mucosa, the loss of vascular pattern, with an exulcerated submucous lesion and two minor satellite changes, which may suggest Crohn's disease, also confirmed by the histological results of the granuloma in biopsies. Except in Crohn's disease, granuloma without caseous necrosis can also be present in other diseases, such as sarcoidosis, vasculitis, lymphogranuloma venererum, but also in syphilis, although its histological image is most commonly nonspecific and corresponds to chronic inflammation [2, 7].

For diagnosing syphilis, the most important tests are nonspecific [veneral disease research laboratory (VDRL) and RPR] and specific serological (TPHA) tests [6].

The treatment recommendation of all stages of syphilis is penicillin (benzathine penicillin G, benzyl penicillin). In the patients with sensitivity to penicillin and depending on the stage and form of syphilis, the treatment may include doxycycline, ceftriaxone or azithromycin [4].

Syphilis is a serious health threat world-wide. In the past 15 years there was an increase in its incidence and prevalence, especially of the anorectal form in the men who had sex with men. It may occur on its own, or together with the HIV infection, or other sexually transmitted diseases [8, 9].

Conclusion

Taking into consideration the variable clinical and endoscopic manifestations of this disease, it is necessary to take a detailed history of sexual behavior, since it can be crucial for determining the diagnosis and differential diagnosis of siphilis.

R E F E R E N C E S

1. *Hamlyn E, Taylor C.* Sexually transmitted proctitis. Postgrad Med J 2006; 82(973): 733–6.
2. *Zhao WT, Liu J, Li YY.* Syphilitic proctitis mimicking rectal cancer: A case report. World J Gastrointest Pathophysiol 2010; 1(3): 112–4.
3. *Pisani Ceretti A, Virdis M, Maroni N, Arena M, Masci E, Magenta A,* et al. The Great Pretender: Rectal Syphilis Mimic a Cancer. Case Rep Surg 2015; 2015: 434198.
4. *Janier M, Hegyi V, Dupin N, Unemo M, Tiplica GS, Potočnik M,* et al. 2014 European guideline on the management of syphilis. J Eur Acad Dermatol Venereol 2014; 28(12): 1581–93.
5. *Chapel TA, Prasad P, Chapel J, Lekas N.* Extragenital syphilitic chancres. J Am Acad Dermatol 1985; 13(4): 582–4.
6. *Golušin Z.* Venerology. Novi Sad: Faculty of Medicine, University of Novi Sad; 2014. (Serbian)
7. *Cha JM, Choi SI, Lee JI.* Rectal syphilis mimicking rectal cancer. Yonsei Med J 2010; 51(2): 276–8.
8. *Adachi E, Koibuchi T, Okame M, Sato H, Imai K, Shimizu S,* et al. Case of secondary syphilis presenting with unusual complications: syphilitic proctitis, gastritis, and hepatitis. J Clin Microbiol 2011; 49(12): 4394–6.
9. *Yilmaz M, Memisoglu R, Aydin S, Tabak O, Mete B, Memisoglu N,* et al. Anorectal syphilis mimicking Crohn's disease. J Infect Chemother 2011; 17(5): 713–5.

Aortic coarctation and associated cardiac lesions – optimal therapeutic approach: report of 2 cases

Koarktacija aorte i pridružene lezije srca – optimalni terapijski pristup

**Miloš Velinović*[†], Radmila Karan*[†‡], Nataša Kovačević-Kostić*[†‡],
Biljana Obrenović-Kirćanski*[§], Milica Stojimirov[‖], Vladimir Milićević[†],
Dejan Nikolić*[‖], Dragan Milić[¶]****

University of Belgrade, *Faculty of Medicine, Belgrade, Serbia; Clinical Center of
Serbia, [†]Cardiac Surgery Clinic, [‡]Center for Anesthesiology, [§]Clinic for Cardiology,
[‖]Clinic for Vascular and Endovascular Surgery, Belgrade, Serbia; University of Niš,
[¶]Faculty of Medicine, Niš, Serbia; Clinical Center of Niš, [††]Clinic for Cardovascular
Surgery, **Clinic for Cardiovascular Surgery, Niš, Serbia

Abstract

Introduction. Aortic coarcation is a congenital condition mostly detected and treated during childhood. Adult patients with coarctation and associated cardiac lesions represent a challenge and a subject of debates concerning adequate treatment. We report 2 patients with aortic coartation when a surgical treatment was necessary. **Case report.** First patient was a 61-year-old male with previous mechanical aortic valve implantation. He underwent one stage surgical reconstruction of aortic coarctation and surgical repair of aneurysm of ascending aorta. The second patient was a 49-year-old female with aortic aneurysm, bicuspid aortic valve, severe aortic insufficiency and coarctation of aorta below branching of subclavian artery. She underwent the Bentall procedure and surgical repair of coarctation by bypass where the proximal anastomosis was made between the terminal part of Dacron graft and the lateral part of graft used for Bentall, while the distal anastomosis was made between the terminal part of Dacron graft and the lateral part of descending aorta below coarctation. The postoperative course and the follow-up of the patients of 3 and 1 year, respectively, were without complications. **Conclusion.** Single operation is a better choice in the patients with concomitant pathology such as the aortic aneurysm, or aortic valve disease. Each patient should be analyzed on a single basis, and a decision about a surgical technique and surgical course brought accordingly.

Key words:
aortic aneurysm; aortic coarctation; aortic valve insufficiency; echocardiography; cardiac surgical procedures; treatment outcome.

Apstrakt

Uvod. Koarktacija aorte je kongenitalno stanje koje se najčešće otkriva i leči u detinjstvu. Odrasli bolesnici sa koarktacijom i pridruženim srčanim oboljenjima predstavljaju pravi izazov i temu za stručne polemike oko adekvatnog načina lečenja. Prikazana su dva bolesnika sa koarktacijom aorte kod kojih je hiruški tretman bio neophodan. **Prikaz bolesnika.** Prvi bolesnik bio je muškarac star 61 godinu sa prethodno ugrađenom mehaničkom aortnom valvulom. U jednom aktu je urađena rekonstrukcija koarktacije aorte i operativno zbrinjavanje aneurizme ascendentne aorte. Drugi bolesnik bila je 49-godišnja žena sa aneurizmom aorte, bikuspidnom aortnom valvulom, teškom aortnom insuficijencijom i koarktacijom aorte ispod odvajanja arterije supklavije. Urađena je Bental operacija i operacija bajpasa između ascendentne i torakalne aorte. Kod oba bolesnika je postoperativni tok protekao bez komplikacija. U periodu praćenja od 1 i 3 godine nije bilo komplikacija. **Zaključak.** Operacija u jednom aktu je bolji izbor kod bolesnika sa pridruženom patologijom kao što je aneurizma aorte i bolest aortne valvule. Svakog bolesnika treba pojedinačno razmotriti i doneti odluku o najpogodnijoj hirurškoj intervenciji.

Ključne reči:
aorta, aneurizma; aorta, koarktacija; zalistak, aortni, insuficijencija; ehokardiografija; hirurgija, kardijalna, procedure; lečenje, ishod.

Correspondence to: Miloš Velinović, Clinical Center of Serbia, Clinic for Cardiac Surgery, Koste Tiodorovića 8, 11 000 Belgrade, Serbia.
E-mail: velinovicsurg@gmail.com

Introduction

Aortic coarctation is a congenital condition mostly detected and treated during childhood. A diagnosis of coarctation in adulthood is usually incidental one, made during the diagnostic evaluation for other cardiac conditions [1, 2]. The adult patients with coarctation and associated cardiac lesions represent a challenge even in the era of percutaneous stent procedures, and are the subject of many professional debates concerning the most adequate treatment [3]. Based on the underlying morphology, the age of patient, and presence or absence of other lesions, multidisciplinary team of surgeons, a cardiologist, interventionists and an anesthesiologist should decide on the right course of treatment.

We presented 2 patients with aortic coarctation when the surgical treatment was necessary.

Case report

Case 1

A 61-year-old male was admitted to our hospital for surgical reconstruction of aortic coarctation and aneurysm of ascending aorta. Four years before that, he underwent the surgery to implant the mechanical aortic valve due to the aortic valve insufficiency. This previous aortic valve replacement surgery was not performed in our Clinic and no prior documentation was available to us. We have no knowledge if the diagnosis of coarctation was established then and, if yes, why it was not treated during the aortic valve replacement surgery. Transthoracic echocardiography (TTE) showed the aneurysmatic dilatation of ascending aorta at bulbus level 74 mm. Mitral valve regurgitation of 1-2+/4 was present into the enlarged (50 mm) left atrium. The left ventricle was normal with the preserved systolic function. The artificial aortic valve showed the normal excursions with 1+/4 transvalvular regurgitation. The right ventricle was normal with tricuspid regurgitation of 1+/4 into the dilated right atrium and the right ventricular systolic pressure (RVSP) 31 mmHg. Multislice computed tomography (MSCT) with aortography showed the aneurysmatic dilatation of aortic root and the ascending aorta with the lumen diameter at the level of aortic bulbus 74×73 mm, aortic arch 50×49 mm as well as a spindle like narrowing of aorta after the branching of left subclavian artery with poststenotic dilatation of the descending aorta (Figure 1). It was not possible to perform coronary angiography due to an extreme dilatation of the ascending aorta. Comorbidities included the arterial hypertension, paroxysmal atrial fibrillation. The patient underwent a repair of aneurysm by interposition of Dacron graft as well as a repair of coarctation by the bypass ascending aorta to the descending aorta with Dacron graft. The approach to the femoral artery cannulation was achieved by establishing the retrograde cardiac pulmonary bypass. Being that it was a cardiac surgery requiring resternotomy (REDO) surgery, adhesiolysis was performed, after which the aorta was cannulated and subsequently clamped. A resection of the aneurysm was performed. The Dacron graft 30 mm was implanted – the prox-imal anastomosis was sutured to the previously implanted aortic valve, the coronary arteries ostia were detached and re-implanted on the graft. The posterior pericardium was then cut-opened, the posterior side of pericardium was cut-opened and the desceding aorta was accessed and clamped distally as well as proximally. After the clamping of the aorta, the Dacron graft 12 mm was sutured terminal-laterally to the descending aorta, followed by the terminal lateral anastomosis between the 12 mm Dacron graft and Dacron graft at the position of the ascedenting aorta (Figure 2). The graft that was used to treat coarctation was positioned from the ascending aorta graft to the thoracic aorta on the left side. In this case, the approach of positioning the graft behind the vena cava was not used, being that this was a REDO surgery and the numerous adhesions made it technically impossible. The postoperative course and the follow-up of 36 months were without complications.

Case 2

A 49-year-old female, with a history of arterial hypertension and hyperlipidemia, was admitted for a surgical repair of the aortic aneurysm and coarctation of the aorta. Two months prior the surgery, the patient felt heart pounding, and later, the same day, the chest pain propagating to the left upper arm. The intensity of pain was 6/10, and it lasted for two hours. She was admitted to the regional hospital for the evaluation, and a few days later, the patient was transferred to the Emergency Department at our Clinical Center under the suspicion of possible aortic dissection or myocardial infarction. Performed aortography showed the ascending aortic aneurysm, circulatory diameter of around 70 mm and 150 mm lenght, aortic regurgitation 3+/4. Coronary angiogrphy was not possible due to the unachievable coronary cannulation. Coarctation of the aorta was also visualized. TTE showed the dilated aorta root 39 mm, ascending part 66 mm, aortic arch 46 mm. The descending, thoracic and abdominal aorta were of normal size. Stenosis (coarctation) was evident just below the branching of subclavian artery. The aortic valve was bicuspid and aortic regurgitation of 3+/4 was present. The left ventricle diastolic diameter was enlarged (63 mm). There was hypokinesia of a small part of basal segment of inferior wall, but the global left ventricle ejection fraction was preserved (70%). The structure resembling the intimal flap was visualized in the initial part of ascending aorta. MSCT angiography of the thoracic and abdominal aorta and iliac artery revealed the dilated ascending aorta diameter of 70 mm. Coarctation with internal kinking was spotted at the beginning of descending aorta. There were no signs of dissection. The patient underwent the Bentall surgical procedure and the reparation of coarctation by suturing the terminal part of 10 mm Dacron graft with the lateral part of the Dacron graft that was used for the Bentall procedure. Other part of 10 mm Dacron graft was sutured to the lateral part of descending aorta below coarctation. In this case, there was no need for a Dacron graft of diameter greater than 10 mm, because the patient did not have the classical symptoms for coarctation and she developed collateral circulation.

Fig. 1 – Presurgical multislice computed tomography showing aneurysmatic dilatation of aortic root and ascending aorta. A spindle like narrowing of aorta after the branching of left subclavian artery with poststenotic dilatation of the descending aorta. Visible mechanical aortic valve.
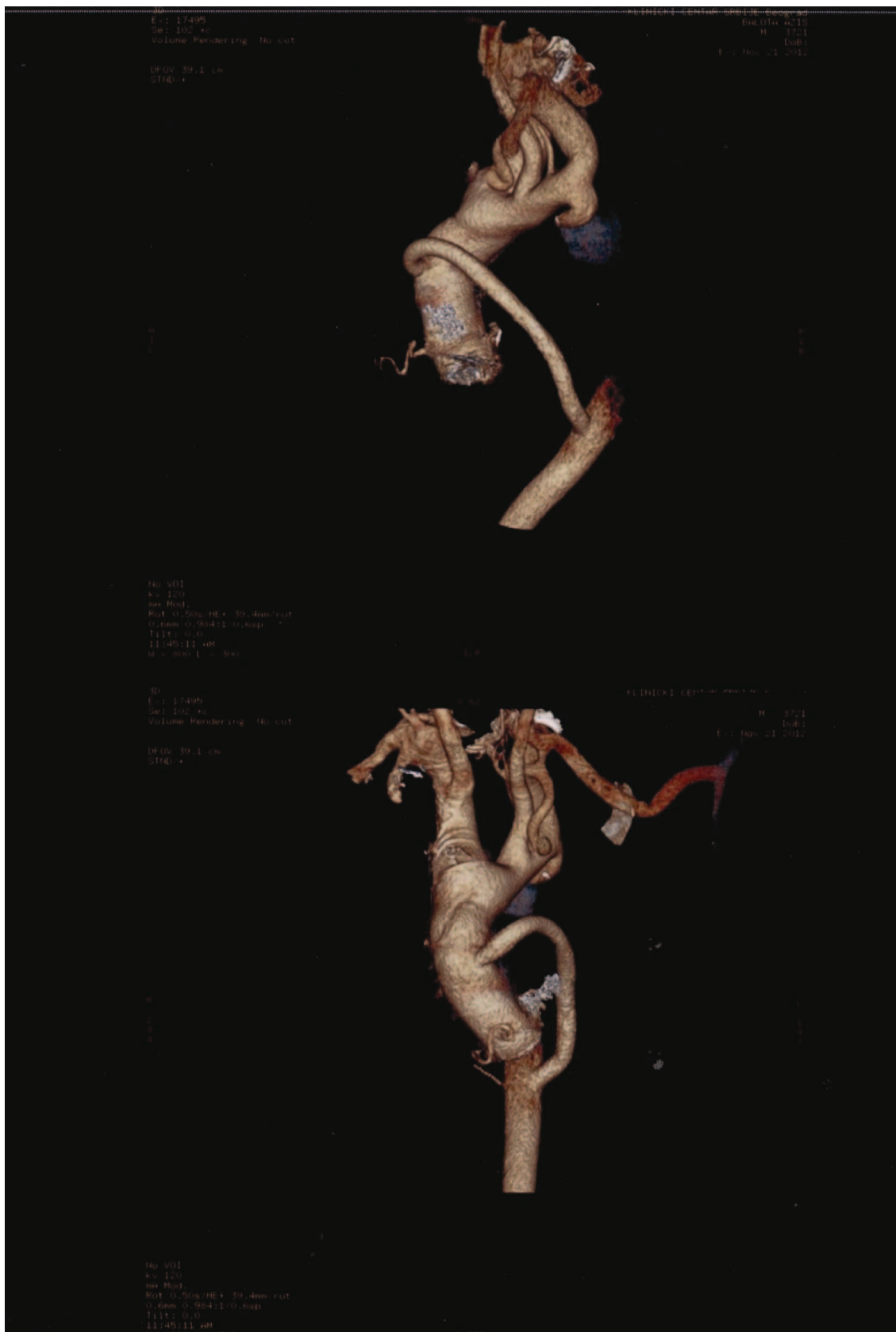
Fig. 2 – Postsurgical multislice computed tomography showing repaired aneurysm with interposition of Dacron graft. Repaired coarctation with extraanatomic bypass (ascending to descending aorta).

Also, the anastomosis was sutured below coarctation enabling the partial native blood flow through coarctation itself. The postoperative pressure gradient in the descending aorta below coarctation was 10 mmHg. The patient was discharged with no postoperative complications. During the period of one-year follow-up, she was without complications.

Discussion

Surgery as a treatment of coarctation of the aorta still has its place, even today in the era of percutaneous procedures. The surgical management of coarctation of the aorta presents technical controversy, especially if other cardiac pathology is involved as well. There is an ever-present question about the choice of the right technique, single-stage or two stage procedure, and which pathology to repair first [3].

The advantages of a single stage approach are: a number of surgical procedures and shorter duration of hospitalization. Many authors do not prefer this approach due to its negative side, which includes the hemodynamic instability and myocardial hypoperfusion due to a sudden decrease in afterload [4]. Also, a single stage procedure with one large incision is not preferred because the exposure is poor and there is an increased risk of bleeding. A single stage operation with sternotomy and thoracotomy simultaneously may increase the postoperative pain and risk of atelectasis [5]. The surgical repair of coarctation carries a risk of spinal cord ischemia and consequent paralysis [6, 7]. The lung compression, chylothorax, uncontrollable bleeding, phrenic nerve and laryngeal nerve injury may also occur [7, 8].

We think that the modified a single stage approach with the extra anatomic bypass technique (ascending to descending bypass) has advantage because the pericardial approach decreases a risk of the above mentioned complications. The use of partial aortic cross clamp allows perfusion distally, decreases possibility of mesenteric ischemia, spinal cord ischemia and consequent paralysis especially in the older patients. Some authors find this to be the technique of choice for the adult patients with the ascending aortic aneurysm, aortic valve or coronary disease associated with coarcta-

tion [7, 9, 10]. Even though repairing coarctation in the first stage facilitates the blood pressure control and decreases afterload, a substantial risk of aneurysm rupture still remains between two stage approach operations. A single stage procedure is preferred in these patients [3]. The two-stage procedure includes the correction of coarctation and associated cardiac lesion [4]. There is an ongoing debate about which pathology should be corrected first. Repairing coarctation in the first stage allows the relief of proximal hypertension and safer aortic cannulation for the second stage procedure [11]. Operating on first the cardiac lesions enables the coronary and myocardial flow redistribution and prevents left ventricular ischemia caused by sudden decrease in afterload. Of course, each approach carries its disadvantages as well. The patients whose cardiac lesions are repaired in the first stage have a risk of bleeding and hemodynamic instability due to the increase in the afterload, as well as occurrence of atrial fibrillation and ischemia in the dilated left ventricle. Anticoagulation and renal perfusion complications may occur [3, 12].

If aortic dissection of ascending aorta occurs in a patient with coarctation, it is rational to manage surgically the more urgent problem, either to repair the ascending aorta first, then repair coarctation in a single stage using one incision, or two incisions (median sternotomy and thoracotomy), which is a great trauma for the patient. All patients treated for aortic coarctation should have regular follow-ups, because of the significant incidence of recoarctation, aneurysm, stenosis or incompetence of aortic valve, and coronary artery disease [13].

Conclusion

The choice of surgical technique depends on skills and experience of a surgical team, and the surgical equipment as well. Single operation is a better choice for the patients with concomitant pathology such as the aortic aneurysm, or the aortic valve disease. Each case should be analyzed on a single basis and decision about the surgical technique and surgical course brought accordingly.

R E F E R E N C E S

1. *Koletsis E, Ekonomidis S, Panagopoulos N, Tsaousis G, Crockett J, Panagiotou M.* Two stagehybrid approach for complex aortic coarctation repair. J Cardiothorac Surg 2009; 4: 10.
2. *Maron BJ, Humphries JO, Rowe RD, Mellits ED.* Prognosis of Surgically Corrected Coarctation of the Aorta: A 20-Year Postoperative Appraisal. Circulation 1973; 47(1): 119–26.
3. *Ugur M, Alp I, Arslan G, Temizkan V, Ucak A, Yilmaz AT.* Four different strategies for repair of aortic coarctation accompanied by cardiac lesions. Interactive Interact Cardiovasc Thorac Surg 2013; 17(3): 467–71.
4. *Mulay AV, Ashraf S, Watterson KG.* Two-Stage Repair of Adult Coarctation of the Aorta With Congenital Valvular Lesions. Ann Thorac Surg 1997; 64(5): 1309–11.
5. *Horai T, Shimokawa T, Takeuchi S, Okita Y, Takanashi S.* Single-Stage Surgical Repair of Type II Acute Aortic Dissection Associated With Coarctation of the Aorta. Ann Thorac Surg 2007; 83(3): 1174–5.

Korkmaz AA, Guden M, Onan B, Tarakci SI, Demir AS, Sagbas E, et al. New technique for single-staged repair of aortic coarctation and coexisting cardiac disorder. Tex Heart Inst J 2011; 38(4): 404–8.
6. *Connolly HM, Schaff HV, Izhar U, Dearani JA, Warnes CA, Orszulak TA.* Posterior pericardial ascending-to-descending aortic bypass: an alternative surgical approach for complex coarctation of the aorta. Circulation 2001;104(12 Suppl 1): 133–7.
7. *Sun LZ, Luo XJ, Liu YM.* Single-stage treatment of aortic coarctation and aortic valve disease. Asian Cardiovasc Thorac Ann 2003; 11(3): 208–12.
8. *Ramnarine I.* Role of surgery in the management of the adult patient with coarctation of the aorta. Postgrad Med J 2005; 81(954): 243–7.
9. *Wu Q, Chen X, Li H, Wang L, Xue H.* Ascending-to-descending aortic bypass via posterior pericardium for complex coarctation of aorta. J Card Surg 2009; 24(2): 167–9.

10. *Rufilanchas JJ, Villagra F, Maroñas JM, Tellez G, Agosti J, Juffe A,* et al. Coarctation of the aorta and severe aortic insufficiency: What to repair first? Am J Surg 1977; 134(3): 428–30.

11. *Morris RJ, Samuels LE, Brockman SK.* Total simultaneous repair of coarctation and intracardiac pathology in adult patients. Ann Thorac Surg 1998; 65(6): 1698–702.

12. *Celermajer DS, Greaves* K. Survivors of coarctation repair: fixed but not cured. Heart 2002; 88(2): 113–4.

13. *Celermajer DS, Greaves* K. Survivors of coarctation repair: fixed but not cured. Heart 2002; 88(2): 113–4.

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

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

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