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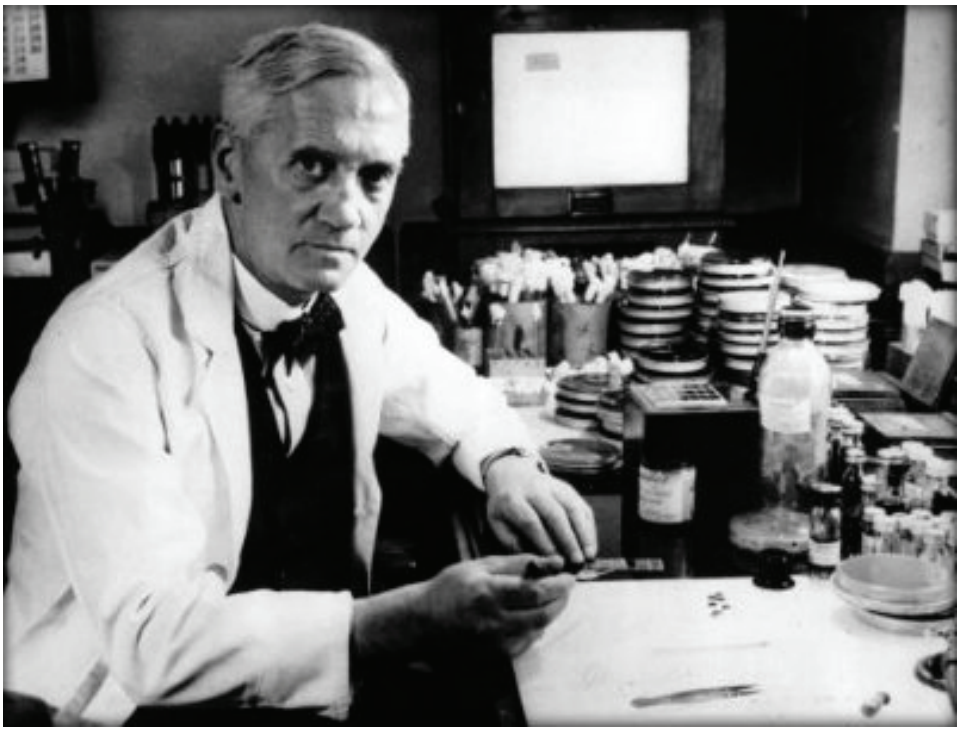

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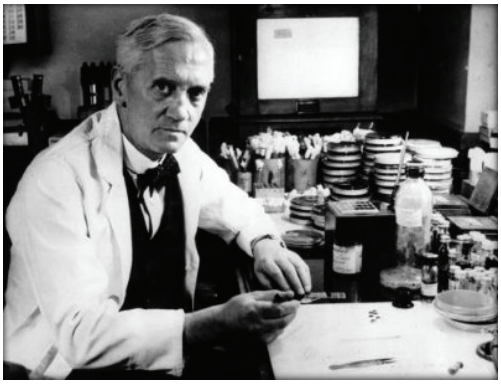

Alexander Fleming (6 August, 1881–11 March, 1955), was a Scottish bacteriologist who discovered penicillin, the world's first antibiotic, in September 1928. He had left Petri dishes with *staphylococcus* culture in a laboratory during his holidays and when he returned, he found that mould (*Penicillium notatum*) had accidentally developed on this culture, creating a bacteria-free circle around itself. He experimented further and found that the mould culture prevented growth of *staphylococci*, even when diluted 800 times. The active substance isolated from the mould, which he named penicillin, marked the beginning of the antibiotic era in therapy of infectious diseases. Fleming shared the Nobel Prize for Physiology or Medicine in 1945 with Ernst Chain and Howard Florey, who both continued Fleming's work.
This September marks 90 years since discovery of penicillin.

Aleksandar Fleming (6. avgust, 1886–11. mart, 1955), bio je škotski bakteriolog koji je u septembru 1928. godine otkrio penicilin, prvi antibiotik na svetu. On je u svojoj laboratoriji ostavio Petrijevu šolju sa kulturom stafilokoka za vreme odmora, a kada se vratio ustanovio je da se na njima slučajno razvila plesan (*Penicillium notatum*) oko koje se formirala zona bez prisustva bakterija. Kasnijim eksperimentima ustanovio je da je kultura te plesni sprečavala rast stafilokoka čak i u 800 puta većem razređenju. Aktivnu supstancu izolovanu iz plesni nazvao je penicilin i ona je označila početak antibiotske ere u lečenju infektivnih bolesti. Za to otkriće Fleming je, zajedno sa Ernestom Čejnijem i Hauardom Florijem koji su nastavili njegov rad, dobio Nobelovu nagradu za medicinu 1945. godine.
Ovog septembra navršava se 90 godina od otkrića penicilina.

ORIGINAL ARTICLES

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Depressive symptoms among patients with schizophrenia in acute and remission phases

Simptomi depresivnosti kod bolesnika obolelih od shizofrenije u akutnoj fazi i u remisiji

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Abstract

Bacground/Aim. Researchers suggest that among people with schizophrenia, the prevalence of depressive symptoms ranges from 7% to 80%. The rate of depressive symptoms among people with schizophrenia varies widely because of the phase of the disease, type of study applied, rating scale for depressive symptoms and diagnostic criteria. The aim of this research was to determine the prevalence of depressive symptoms and the clinical correlation of depressive symptoms with other clinical parameters (type and severity of psychotic symptoms, severity of illness, insight and global functioning) among patients with schizophrenia in acute and remission phases. **Methods.** This prospective clinical study enrolled 100 consecutive patients with schizophrenia both in acute and remission phases. Psychometric assessments were made using the Positive and Negative Syndrome Scale (PANSS) for rating the symptoms of schizophrenia, Scale to Assess the Unawareness of Mental Disorder (SUMD), Calgary Depression Scale for Schizophrenia (CDSS), and Global Assessment of Functioning Scale. **Results.** The prevalence of depressive symptoms among patients with schizophrenia in the acute phase was 23% at the study group, while in the remission phase it was 13%. In the acute phase, the CDSS scale correlated with a depressive and positive subscale of the PANSS scale as well as SUMD scale. In the remission phase, the CDSS scale correlated only with a depressive subscale of the PANSS scale. The CDSS scale did not correlate with the negative subscale of the PANSS scale. The subjective nature of depressive symptoms is more pronounced in the remission phase. **Conclusion.** Our findings showed that depressive symptoms were more pronounced in the acute psychotic phase than in the remission phase of schizophrenia. Targeted, patient oriented, and algorithm-based approach for treatment management, with taking into account different phenotypic expressions of the disorder (patients with and without affective symptoms) is warranted in patients with schizophrenia.

Key words:
depression; schizophrenia; prevalence; acute disease; remission induction.

Apstrakt

Uvod/Cilj. Istraživanja pokazuju da se kod bolesnika koji boluju od shizofrenije, prevalenca depresivne simptomatologije kreće od 7% do 80%. Varijabilnost u studijama potiče od faze bolesti u kojoj su se bolesnici nalazili u trenutku opservacije, metoda procene, vrste mernih instrumenata kao i različitih dijagnostičkih kriterijuma za shizofreniju i depresiju. Cilj rada bio je da se ispita prevalenca depresivnih simptoma i korelacije depresivnih simptoma sa drugim kliničkim parametrima (vrsta i težina psihotičnih simptoma, težina bolesti, uvid, opšta funkcionalnost) kod bolesnika obolelih od shizofrenije u akutnoj fazi bolesti i u remisiji. **Metode.** Istraživanje predstavlja kliničku prospektivnu studiju kod 100 konsekutivnih bolesnika obolelih od shizofrenije u odnosu na fazu bolesti (faza akutnog pogoršanja i faza remisije). Psihometrijske procene težine bolesti i prisutne psihopatologije vršile su se korišćenjem Skale za procenu pozitivnog i negativnog sindroma shizofrenije (PANSS), Skale za procenu nedostatka uvida u mentalni poremećaj (SUMD), Kalgari skale za procenu depresije u shizofreniji (CDSS) i Skale za opštu procenu funcionisanja (GAF). **Rezultati.** Prevalenca depresivne simptomatologije kod bolesnika obolelih od shizofrenije u akutnoj fazi bolesti

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u studijskoj grupi iznosila je 23%, a u fazi remisije 13%. U akutnoj fazi, Kalgari skala za procenu depresije u shizofreniji korelirala je sa depresivnom i pozitivnom podskalom PANSS kao i sa skalom uvida, dok u remisiji samo sa depresivnom podskalom PANSS. Kalgari skala nije korelirala sa negativnom podskalom PANSS. Subjektivni korelati depresije bili su izraženiji u remisiji u odnosu na akutnu fazu. **Zaključak.** Depresivni simptomi u shizofreniji izraženiji su tokom akutne psihotične faze u odnosu na fazu remisije. Prepoznavanje depresivne simtomatologije otvara mogućnosti ciljane farmakoterapije, psihoterapije i socioterapijskih intervencija, što može voditi ka boljem terapijskom ishodu.

Ključne reči:
depresija; shizofrenija; prevalenca; akutna bolest; remisija, indukcija.

Introduction

Depressive symptoms are frequently observed in people with schizophrenia. Although they can be observed during the course of schizophrenia [1], depressive symptoms are frequently not considered during diagnostics of schizophrenia, except as post-psychotic depression.

During the eighties and nineties of the XX century, scientists were intensively engaged in studying depressive symptoms in schizophrenia to construct a unique nosological entity. Despite several studies, the unified nosological entity remains undefined. Recent attempts have been made for conducting cross-sectional and longitudinal assessments using new research tools for better understanding of this syndrome. Concerning the nosological debate on depression in schizophrenia, recent work of Gaebel and Wolwer [2] concluded that depressive symptoms among patients with schizophrenia seem to reflect a subjective impression of affective flattening combined with unspecific depressive symptoms. This is in contrast to the more specific depressive symptoms, such as depressive mood or inhibition, in major depression [3, 4]. From a phenomenological point of view, there are three different meanings of depression in schizophrenia: depression as a reaction to schizophrenia, depression as an integral part of it, and depression as an independent disorder [5]. Moreover, the differential diagnosis of depressive symptoms is often difficult among patients with schizophrenia. There were several reasons reported and they included organic factors, negative symptoms of schizophrenia, consequence or adverse effects of treatment with neuroleptics (neuroleptic-induced dysphoria, akinesia, or akathisia) [6], reaction to disappointment, or stress resulting from the disease [7].

The emergence of depressive symptoms among patients with schizophrenia was associated with an increased rate of relapse [8], longer hospitalization [9], weak response to pharmacotherapy [10], disturbed social activities, and feeling of hopelessness, which is an important risk factor for suicide [11, 12].

The aim of this study was to determine the prevalence of depressive symptoms among the patients with schizophrenia in the acute and remission phases. We also sought to determine the clinical correlation of depressive symptoms with other clinical parameters (type and severity of psychotic symptoms, severity of illness, insight and global functioning) in the acute and remission phases.

Methods

Patients

Patients who were admitted to the Institute of Mental Health were recruited consecutively. Study inclusion criteria were as follows: diagnosis of acute phase of schizophrenia based on the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) [13]; age 18–65 years. The follow-up was done in the remission phase. Patients were excluded if they had any other DSM-IV axis I or axis II disorder, or any comorbid medical disease. They were also excluded if they had been treated with antidepressants at the time of the first assessment. Sociodemographic and clinical data were collected by trained psychiatrists.

Design and assessments

This was a prospective clinical study which was carried out at the Institute for Mental Health, Belgrade between 2011 and 2013. The study was approved by the Ethics Committee and written informed consent was obtained from all participants prior to participation in the study. The study was a part of a study that examines, using the same model, patients from the Psychiatric Hospital of Attica (Athens, Greece) as well as patients from psychiatric hospitals at St. Pancras Hospital, Highgate Mental Health Hospital and St Luke's Hospital (London, United Kingdom).

The patients were observed in two different phases of the disease: 1) the acute phase during hospitalization and 2) the stage of initial remission (approximately after six weeks of treatment as outpatients after they had been discharged). A cut-off score of $\leq 50\%$ for the Positive and Negative Syndrome Scale (PANSS) for rating the symptoms of schizophrenia was considered to indicate that remission was achieved [14].

Psychometric assessments of the severity of the disease and existing psychopathological symptoms were made both at the admission and end of the inpatient treatment using the Structured Clinical Interview for Mental Disorders (SCID-I) [13], PANSS scale [15], Scale to Assess the Unawareness of Mental Disorder (SUMD) [16], Calgary Depression Scale for Schizophrenia (CDSS) [17], and Global Assessment of Functioning Scale (GAF) [18].

The PANSS scale is used for assessing positive, negative and depressive symptoms as well as general psychopathology. It comprises 30 items, each item rated 0–7. We used four subscales to assess positive symptoms (the

PANSS-P total score, 49), negative symptoms (the PANSS-N total score, 49), depressive symptoms (the PANSS-D total score, 28), and general psychopathology (the PANSS-G total score, 112). A cut-off score of □16 was used to define depression as measured by the PANSS-D subscale. Based on other studies, remission was defined by reducing the scale value by ≤ 3 on each item [14].

The SUMD scale (abbreviated version) is in the form of semi-structured interview developed to assess insights into mental illness [16]. The scale ranges from 0 to 15. A score of ≤ 9 represents the existence of insight into the mental illness.

The CDSS scale is used to assess depression among patients with schizophrenia, avoiding significant overlap between extrapyramidal, negative and depressive symptoms [17]. The scale includes nine items and each item is scored from 0 to 3. In accordance with previous studies, a value higher than six was the cut-off score for depression diagnosis. Similar to previous studies, we used a score higher than six on the CDSS scale to indicate the presence of depression symptoms. The CDSS scale was shown as a more specific instrument than the HAMD-D and Beck Depression Inventory in the evaluation of depression in schizophrenia [17].

The GAF Scale assigns a clinical judgment in a numerical fashion to an individual's overall functioning level [19]. It considers psychological, social and occupational functioning on a hypothetical continuum of mental health illness. It is divided into 10 intervals of 10 points and a count from 1 to 100, where 100 indicate superior performance.

Statistical analysis included parametric and non-parametric descriptive statistics, depending on the nature of data. Further analysis included inferential statistical methods (unifactorial analysis of variants, Students' *t*-test, Mann–Whitney's U-test, Pearson's χ^2-test of independence, and Spearman's rank correlation). The Statistical Package for Social Sciences –SPSS for Windows, Version 19.0 (SPSS Inc. Chicago, IL) was used for this analysis.

Results

The original sample comprised 109 consecutive patients who were admitted to the Institute of Mental Health. Nine patients were excluded because of failure of remission or noncompliance; therefore, the final sample comprised 100 patients with schizophrenia. The age range of patients was between 19 and 63 years, with male comprimising 55% of the sample. The majority of patients (86%) were unemployed and 90.7% were single. All patients received antipsychotic treatment (71.7% were atypical antipsychotics).

Our findings showed that the prevalence of depressive symptoms among patients with schizophrenia was 23% in the acute phase. After hospital treatment, in the remission phase, the prevalence of depressive symptoms was 13%. From a total of 23% patients with depressive symptoms in the acute phase, 12% achieved complete remission, whereas 11% remained with depressive symptoms. Two (2%) patients from the group without depressive symptoms in the acute phase developed depressive symptoms at the end of the treatment.

We have identified a significant difference between the patients with and without depressive symptoms relative to the time of admission, regarding clinical correlates and psychopathology [Wilks' $\Lambda = 0.74$, $F(8.91) = 4.07$, $p < 0.01$]. An additional univariate analysis revealed the differences in the PANNS-P and PANNS-D subscales and SUMD scale (Table 1).

The data analysis indicated one significant discriminant function [Wilks' $\Lambda = .75$, χ^2 (3, N = 100) = 27.48, $p < 0.001$]. The canonical correlation analysis was 0.49. Correlations between the discriminant function and variables showed the highest projection for insight (r = 0.80) and less for the PANSS-D1 subscale (r = −0.49) and PANSS-P1subscale (r = 0.44). The significant discriminant function indicated that the patients without depression (0.31) were at the positive extreme and the patients with depression (−1.04) were at the negative extreme.

Table 1

Depressive symptoms in the acute stage among patients with schizophrenia compared to clinical parameters at the admission

Questionnaires	Non-depressive		Depressive		df_b	df_w	F	*p*
	M	SD	M	SD				
PANSS-P1[a]	21.57	5.41	18.57	3.89	1	98	6.13	.015
PANSS-N1[b]	23.13	8.40	24.74	7.32	1	98	0.69	.409
PANSS-G1[c]	58.34	8.78	58.13	7.80	1	98	0.01	.919
PANSS-Total1[d]	102.64	16.70	101.43	13.56	1	98	0.10	.753
SUMD1[e]	10.90	3.11	7.43	3.53	1	98	20.65	.000
PANSS-D1[f]	15.90	3.47	18.13	3.00	1	98	7.77	.006
GAF1[g]	30.70	9.29	32.13	11.91	1	98	0.37	.546

[a]Subscale for positive symptoms from The Positive and Negative Syndrome Scale for Rating the Symptoms of Schizophrenia (PANSS) in acute phase; [b]Subscale for negative symptoms from the PANSS in acute phase; [c]Subscale for general psychopathology from the PANSS in acute phase; [d]Total score of the PANSS in acute phase; [e]Scale to Assess the Unawareness of Mental Disorder (SUMD) in acute phase; [f]Subscale for depressive symptoms from the PANSS in acute phase; [g]Global Assessment of Functioning Scale (GAF1) in acute phase; M – мean score; SD – standard deviation.

Table 2

Depressive symptoms in remission among patients with schizophrenia compared to clinical parameters at the remission

Questionnaires	Non-depressive		Depressive		df_b	df_w	F	*p*
	M	SD	M	SD				
PANSS-P2[a]	11.29	2.66	10.08	2.36	1	98	2.40	0.125
PANSS-N2[b]	14.22	5.73	14.62	5.72	1	98	0.05	0.816
PANSS-G2[c]	31.13	5.26	31.00	3.83	1	98	0.01	0.934
PANSS-Total2[d]	57.11	10.53	55.69	7.20	1	98	0.22	0.640
SUMD2[e]	6.29	2.28	5.69	2.05	1	98	0.78	0.378
PANSS-D2[f]	8.06	1.78	9.46	1.85	1	98	6.96	0.010
GAF2[g]	56.28	9.01	58.23	7.87	1	98	0.55	0.461

[a]Subscale for positive symptoms from The Positive and Negative Syndrome Scale for Rating the Symptoms of Schizophrenia (PANSS) in remission; [b]Subscale for negative symptoms from the PANSS in remission; [c]Subscale for general psychopathology from the PANSS in remission; [d]Total score of the PANSS in remission; [e]Scale to Assess the Unawareness of Mental Disorder (SUMDR) in remission; [f]Subscale for depressive symptoms from the PANSS in remission; [g]Global Assessment of Functioning Scale (GAF2) in remission; M – mean score; SD – standard deviation.

Table 3

Mean scores and the proportion on items on the Calgary Depression Scale for Schizophrenia (CDSS)

CDSS Items	Acute phase		Remission	
	M ± SD	%	M ± SD	%
Depression	0.67 ± 0.54	29.8	1.01 ± 0.71	14.6
Hopelessness	0.60 ± 0.42	18.1	0.91 ± 0.86	13.2
Self depreciation	1.02 ± 0.72	17.6	0.84 ± 0.66	12.4
Guilty ideas of reference	0.76 ± 0.70	7.8	0.67 ± 0.54	5.7
Pathological guilt	0.64 ± 0.68	4.9	0.54 ± 0.56	3.1
Morning depression	0.82 ± 0.76	10.6	0.71 ± 0.67	8.0
Early wakening	1.11 ± 0.96	19.3	0.80 ± 0.48	9.1
Suicide	0.61 ± 0.72	1.8	0.13 ± 0.37	1.2
Observed depression	0.23 ± 0.94	20.7	0.66 ± 0.72	11.9

M – mean; SD – standard deviation.

We identified a statistically significant difference between the patients with and without depressive symptoms relative to the time of remission, regarding clinical correlates and psychopathology [Wilks' Λ= 0.85, F (8,91) = 2.00, p = 0.055]. An additional univariate analysis revealed the differences in the PANNS-D subscales (Table 2).

The proportions on each item and the mean scores on the CDSS scale in the acute and remission phases are shown in Table 3.

The correlation among depressive symptoms (total score of the CDSS scale and PANSS-D subscale) and other scales in the acute and remission phases were examined. During the acute phase, the CDSS scale had a high positive correlation with the PANSS-D subscale (r = 0.38, p < 0.0.01) as well as high negative correlation with the PANSS-P subscale (r = -0.37, p < 0.01) and SUMD scale (r = -0.47, p < 0.01). There was no correlation with the negative factor of the PANSS-N, PANSS–G and PANSS-total subscales.

Correlation between the CDSS scale and PANSS-D subscale with other clinical parameters in the acute phase did not change during remission.

Discussion

Our findings showed that the prevalence of depressive symptoms among patients with schizophrenia was 23% patients in the acute phase of the disease. After hospital treatment, the prevalence of patients with depressive symptoms decreased in remission phase. In addition, we also found that the insight was related not only to the positive psychotic symptoms but also to depressive symptoms in schizophrenia. Moreover, the results indicated the difference in the profile of depressive symptoms in the different phases of the disease.

The rate of patients with schizophrenia who exhibited depressive symptoms during their lifetime ranged from 25% to 80% [20]. Depressive symptoms were more frequent in the acute phase with a point of prevalence ranging from 20% to 80% [21]. However, our findings are in accordance with a review of Siris and Bench [7] who showed a modal rate of 25%.

Lower prevalence was found in the chronic phase of the disease with a point of prevalence as low as 4% and as high as 15% [22]. This finding led some authors to conclude that there was a specific relationship between affective symptoms and positive symptoms of schizophrenia [7]. Our rate of depressive symptoms after hospital treatment was 13% in the remission phase.

The rate of depressive symptoms among people with schizophrenia varies widely because of the phase of the disease, type of study that was applied (cross-sectional vs. longitudinal), depressive rating scale, diagnostic criteria, and whether depressive symptoms, depressive syndrome, or a depressive disorder as a whole are being considered [23]. Additionally, few studies explored the prevalence of depressive

symptoms in a patient with schizophrenia using the CDSS scale. It was shown that the CDSS scale had greater validity in patients with schizophrenia than other assessment tools, such as the Hamilton Depression scale and Montgomery-Asberg scale [24].

Evaluation of depressive symptoms in a sample of 249 patients with schizophrenia with acute exacerbation by Schennach-Wolff using the CDSS scale registered a prevalence of depressive symptoms of 36% at admission, with 23% remaining depressed at discharge [24]. Maggini and Raballo [25] in 2006 evaluated a sample of 161 outpatients with chronic schizophrenia in remission and determined a prevalence of depression of 30%. Majadas et al. [26] in the Spanish sample of 95 patients with stable schizophrenia found a prevalence of depression of 31%. However, Gorna et al. [27] used the same scale in a sample of 74 patients with remission and found a higher prevalence of depression of 45.9%. Roche et al. [28], in a sample of 165 patients with the acute phase of the first episode of schizophrenia, registered low prevalence of 10.4%. The differences between our findings and those previously mentioned may be related not only to cultural differences in perception of depressive symptoms but also to the differences in patient's selection, inclusion of other similar diagnostic groups (schizoaffective or schizophreniform disorder), sample size, and treatment applied.

During hospital treatment, in both groups of our patients, there was a reduction of psychotic symptoms. Our study indicated that the improvement was independent of the decrease in depressive symptoms. Moreover, we observed that depressive symptoms persisted in almost half of patients during the remission phase. However, the inclusion of more patients with schizophrenia and a longer follow-up period might be of informative additional value in determining differences among those with and without depressive symptoms in schizophrenia. Few studies tried to answer the question of "treatment-resistant" depressive symptoms within schizophrenia. Schennach-Wolf et al. [24] found that a greater number of patients with multiple, recurrent episodes were treatment-resistant compared to patients experiencing their first episode. This was later confirmed by Arranz et al. [29] in a sample of nonaffective acute remitting psychosis as well as by our study.

The rate of patients who developed depressive symptoms in the course of treatment was lower in our study than that in a study by Möller and von Zerssen [30] (14% in a sample of 280 hospitalized patients).

Apart from the moderate rate of depressive symptoms in schizophrenia observed in our study, our results also highlight the importance of insight and positive symptoms and depressive symptoms in schizophrenia. The subjects with depressive symptoms in the acute phase had psychometric properties related to the less severe form of schizophrenia. However, in remission, the same group of patients exhibited a similar clinical profile to those with no depressive symptoms, apart from pronounced depressive symptoms on the subscales of PANSS.

We found that the CDDSS scale scores had a negative correlation with the PANSS-P subscale which might be correlated with the intensity of positive symptoms. Some studies showed a low but significant positive correlation between depressive symptoms and positive symptoms [27] while other studies did not find this association [31, 32]. The explanation of the positive correlation can be associated with less intensity of positive symptoms in the observed sample.

There is still a debate on whether depression within schizophrenia is an autonomous domain, or is a part of the negative symptoms. Some authors found a correlation between negative symptoms and depressive symptoms [33] while other studies did not confirm this relationship [20, 34]. As with the prevalence of depressive symptoms, the association between depressive and negative symptoms may be caused by tools of assessments as well as the phase of the disease at the time of observation. In our study, the correlation between the CDSS scale and the PANSS-N subscale was not found, which is in line with the findings of other authors who stated that depressive and negative symptoms existed as two separate syndromes within schizophrenia [35]. Additionally, if we observe the percentage of answers on items of CDSS scale, we will see that in the acute phase, the most common were depression, morning depression, early awakening, feelings of hopelessness and self-depreciation. Moreover, the lowest rates were ideas based on guilt and suicide. The remission phase of our patients included persisting symptoms of depression, feelings of hopelessness and self-depreciation. Therefore, the nature of the symptoms in remission was more subjective in nature, whereas the acute phase included biological symptoms. This finding of the absence of a correlation with the PANSS-N subscale is in accordance with Siris's view that the major difference between depressive and negative symptoms is a "blue mood" [7].

In the literature, there is little data on the correlation between the CDSS scale and the SUMD. Sim et al. [36] in a sample of 66 patients with the first psychotic episode in schizophrenia found that patients with depressive symptoms hada greater insight into their mental illness. In our sample of patients with depressive symptoms, there was a negative correlation between the CDSS and the SUMD which implies better insight into mental illness.

This study has several limitations as well as strengths to be considered in the interpretation of the results. The study sample was relatively small to allow for an analysis of more complex variables, especially if there is a division of a sample according to the presence of depressive symptoms. Evaluation of interplay between pharmacotherapy and depressive symptoms is not explored in the study, but should be taken into account in interpretation of the results. In addition, the short follow-up period did not allow a complete analysis of the development of depressive symptoms from the acute phase to remission. However, we overcame the limitations of previous studies and analyzed depressive symptoms in schizophrenia both in the acute and remission phases, in representative sample of clinical patients, excluding those with poor treatment response as well as those with potential cognitive problems. Moreover, and unlike the majority of previous studies in the assessment of depressive symptoms we used CDSS, an instrument specifically developed to evaluate depressive symptoms among patients with schizophrenia.

Conclusion

By searching the literature, we noticed that this has been the first study in Serbia so far, to assess the rate of depressive symptoms among the patients with schizophrenia. Our findings clearly show that depressive symptoms are more pronounced during the acute psychotic phase than in remission in patients with schizophrenia. Although the rate of depressive symptoms in remission is low, these symptoms can persist or occur during hospital treatment in some patients.

Based on these findings, we cannot say with certainty whether each patient suffering from schizophrenia may be located on a point of the continuum of depressive symptoms during the course of schizophrenia, or, whether, on the other side, there is a clear categorical distinction between the depressive and non-depressive groups. However, it is certain that the distinction between these two groups and the recognition of depressive symptoms in patients suffering from schizophrenia have clinical and therapeutic importance. Recognizing depressive symptoms raises the possibility of targeted interventions and consequently better therapeutic outcome.

R E F E R E N C E S

1. *Krabbendam L, van Os J.* Affective processes in the onset and persistence of psychosis. Eur Arch Psychiatry Clin Neurosci 2005; 255(3): 185–9.
2. *Gaebel W, Wölwer W.* Facial expressivity in the course of schizophrenia and depression. Eur Arch Psychiatry Clin Neurosci 2004; 254(5): 335–42.
3. *Peralta V, Cuesta MJ.* Negative parkinsonian, depressive and catatonic symptoms in schizophrenia: a conflict of paradigms revisited. Schizophr Res 1999; 40(3): 245–53.
4. *Cheniaux E, Landeira-Fernandez J, Lessa Telles L, Lessa JL, Dias A, Duncan T,* et al. Does schizoaffective disorder really exist? A systematic review of the studies that compared schizoaffective disorder with schizophrenia or mood disorders. J Affect Disord 2008; 106(3): 209–17.
5. *Skodlar B.* Three different meanings of depression in schizophrenia. A phenomenological perspective. Psychiatr Danub 2009; 21 Suppl 1: 88–92.
6. *Kantrowitz JT, Citrome L.* Schizoaffective disorder: a review of current research themes and pharmacological management. CNS Drugs 2011; 25(4): 317–31.
7. *Siris SG, Bench C.* Depression and schizophrenia. In*: Hirsch SR, Weinberger D,* editors. Schizophrenia. 2nd ed. Oxford, UK: Blackwell Publishing Ltd; 2003.
8. *Conley RR, Ascher-Svanum H, Zhu B, Faries D, Kinon BJ.* The burden of depressive symptoms in the long-term treatment of patients with schizophrenia. Schizophr Res 2007; 90(1–3): 186–97.
9. *Heald A, Morris J, Soni SD.* Characterisation of depression in patients with schizophrenia. Indian J Med Res 2008; 127(6): 544–50.
10. *Morrissette DA, Stahl SM.* Affective symptoms in schizophrenia. Drug Discovery Today: Therapeutic Strategies 2011; 8 (1–2): 3–9.
11. *Pruß L, Wiedl KH, Waldorf M.* Stigma as a predictor of insight in schizophrenia. Psychiatry Res 2012; 198(2): 187–93.
12. *Staring AB, Van der Gaag M, Van den Berge M, Duivenvoorden HJ, Mulder CL.* Stigma moderates the associations of insight with depressed mood, low self-esteem, and low quality of life in patients with schizophrenia spectrum disorders. Schizophr Res 2009; 115(2–3): 363–9.
13. *First MB, Spitzer RL, Gibbon M, Williams JB.* Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV). Washington, D.C.: American Psychiatric Press, Inc.; 1996.
14. *Andreasen NC, Carpenter WT Jr, Kane JM, Lasser RA, Marder SR, Weinberger DR.* Remission in schizophrenia: proposed criteria and rationale for consensus. Am J Psychiatry 2005; 162(3): 441–9.
15. *Kay SR, Fiszbein A, Opler LA.* The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987: 13(2): 261–76.
16. *Amador XF, Strauss DH, Yale SA, Flaum MM, Endicott J, Gorman JM.* Assessment of insight in psychosis. Am J Psychiatry 1993; 150(6): 873–9.
17. *Addington D, Addington J, Maticka-Tyndale E.* Specificity of the Calgary Depression Scale for schizophrenics. Schizophr Res 1994; 11(3): 239–44.
18. *Jones SH, Thornicroft G, Coffey M, Dunn G.* A brief mental health outcome scale-reliability and validity of the Global Assessment of Functioning (GAF). Br J Psychiatry 1995; 166(5): 654–9.
19. *El Yazaji M, Battas O, Agoub M, Moussaoui D, Gutknecht C, Dalery J,* et al. Validity of the depressive dimension extracted from principal component analysis of the PANSS in drug-free patients with schizophrenia. Schizophr Res 2002; 56(1–2): 121–7.
20. *Baynes D, Mulholland C, Cooper SJ, Montgomery RC, MacFlynn G, Lynch G,* et al. Depressive symptoms in stable chronic schizophrenia: prevalence and relationship to psychopathology and treatment. Schizophr Res 2000; 45(1–2): 47–56.
21. *Lançon C, Auquier P, Reine G, Bernard D, Addington D.* Relationships between depression and psychotic symptoms of schizophrenia during an acute episode and stable period. Schizophr Res 2001; 47(2–3): 135–40.
22. *Hausmann A, Fleischhacker WW.* Differential diagnosis of depressed mood in patients with schizophrenia: a diagnostic algorithm based on a review. Acta Psychiatr Scand 2002; 106(2): 83–96.
23. *Ramirez N, Sarro S, Arranz B, Sanchez J, Gonzalez J, San L.* Instruments of depression assessment in schizophrenia. Actas Esp Psiquiatr 2001; 29(4): 259–68. (Spanish)
24. *Schennach-Wolff R, Obermeier M, Seemuller F, Jager M, Messer T, Laux G,* et al. Evaluating depressive symptoms and their impact on outcome in schizophrenia applying the Calgary Depression Scale. Acta Psychiatr Scand 2011; 123(3): 228–38.
25. *Maggini C, Raballo A.* Exploring depression in schizophrenia. Eur Psychiatry 2006; 21(4): 227–32.
26. *Majadas S, Olivares J, Galan J, Diez T.* Prevalence of depression and its relationship with other clinical characteristics in a sample of patients with stable schizophrenia. Compr Psychiatry 2012; 53(2): 145–51.
27. *Gorna K, Jaracz K, Wrzyszczynska L, Rybakowski F.* Quality of life and depression in schizophrenic patients. Adv Med Sci 2007; 52(Suppl 1): 108–11.
28. *Roche E, Clarke M, Browne S, Turner N, McTuige O, Kamali M*, et al. Prevalence and clinical correlates of depression in acute phase of first episode schizophrenia. Irish J Psych Med 2010; 27(1): 15–8.

29. *Arranz B, San L, Ramírez N, Dueñas RM, Perez V, Salavert J*, et al. Clinical and serotonergic predictors of non-affective acute remitting psychosis in patients with a first-episode psychosis. Acta Psychiatr Scand 2009; 119(1): 71–7.
30. *Möller HJ, von Zerssen DV*. Depressive states during the clinical treatment of 280 schizophrenic inpatients (author's transl). Pharmacopsychiatria 1981; 14(5): 172–9. (German)
31. *Reine G, Lançon C, Di Tucci S, Sapin C, Auquier P*. Depression and subjective quality of life in chronic phase schizophrenic patients. Acta Psychiatr Scand 2003; 108(4): 297–303.
32. *Sarro S, Dueñas RM, Ramírez N, Arranz B, Martínez R, Sánchez JM*, et al. Cross cultural adaptation and validation of the Spanish version of the Calgary Depression Scale for Schizophrenia. Schizophr Res 2004; 68(2–3): 349–56.
33. *Müller MJ, Szegedi A, Wetzel H, Benkert O*. Depressive factors and their relationships with other symptom domains in schizophrenia, schizoaffective disorder, and psychotic depression. Schizophr Bull 2001; 27(1): 19–28.
34. *Bottlender R, Strauss A, Möller HJ*. Prevalence and background factors of depression in first admitted schizophrenic patients. Acta Psychiatr Scand 2000; 101(2): 153–60.
35. *Rocca P, Bellino S, Calvarese P, Marchiaro L, Patria L, Rasetti R,* et al. Depressive and negative symptoms in schizophrenia: different effects on clinical features. Compr Psychiatry 2005; 46(4): 304–10.
36. *Sim K, Mahendran R, Siris SG, Heckers S, Chong SA*. Subjective quality of life in first episode schizophrenia spectrum disorders with comorbid depression. Psychiatry Res 2004; 129(2): 141–7.

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Nitric oxide as prediction factor of gingival inflammation in orthodontic patients

Azot oksid kao prediktivni faktor inflamacije gingive kod ortodontskih bolesnika

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Abstract

Background/Aim. Nowadays therapy with fixed orthodontic appliances is widely spread, having in mind positive effects it has in malocclusion treatments The side effect is increased gingivial inflammation in treated patients. The aims of this research are to estimate the inflammatory condition of gingiva in the first 6 months of orthodontic therapy on the basis of clinical parameters of sulcus bleeding index, plaque index, gingival crevicular fluid and salivary nitric oxide concentration, and to investigate role of nitric oxide as predicting factor of gingival inflammation in orthodontic patients. **Methods.** In this study, 30 patients of the Dental Clinic treated with fixed orthodontic appliances (11 males and 19 females), aged 15–22 years, were examined. Clinical parameters were evaluated and gingival crevicular fluid and saliva were collected, before the beginning of orthodontics therapy, and then, three and six months after it. **Results.** The approximate values of clinical parameters, gingival crevicular fluid and salivary nitric oxide concentration progressively increased. Low statistical significance of correlations among gingival crevicular fluid and salivary nitric oxide concentration and the measured clinical parameters were found. There is a statistically significant correlation between gingival crevicular fluid and salivary nitric oxide concentration. **Conclusion.** According to the obtained results, we can conclude that gingival crevicular fluid and saliva are reliable mediums for monitoring of the gingival inflammatory condition. More studies are needed to investigate a potential role of nitric oxide as predicting factor of gingival inflammation in orthodontic patients.

Key words:
orthodontics, corrective; nitric oxide; inflammation; gingival crevicular fluid; saliva.

Apstrakt

Uvod/Cilj. Ortodontska terapije fiksnim aparatima je široko rasprostranjena u terapiji malokluzija. Jedna od negativnih strana ove terapije je pojava zapaljenja gingive tretiranih bolesnika. Cilj rada bio je procena stanja zdravlja gingive u prvih šest meseci ortodontske terapije na osnovu vrednosti kliničkih parametara krvarenja gingive, plak indeksa, kao i koncentracije azot monoksida u pljuvački i sulkusnoj tečnosti. Drugi cilj bio je utvrđivanje stepena korelacije koncentracija azot monoksida u pljuvački i sulkusnoj tečnosti u toku prvih šest meseci terapije. **Metode.** Studijom je bilo obuhvaćeno 30 bolesnika Klinike za stomatologiju lečenih fiksnim ortodontskim aparatima (11 muškog, 19 ženskog pola), starosti 15–22 godine. Određivani su parametri, a pljuvačka i sulkusna tečnost su sakupljani pre početka, kao i tri i šest meseci posle početka terapije. **Rezultati.** Utvrđen je statistički značajan porast vrednosti kliničkih parametara i koncentracije azot monoksida u toku prvih šest meseci ortodontske terapije. Nađeni su nizak nivo statističke značajnosti korelacije merenih kliničkih parametara i koncentracije azot monoksida u pljuvački i sulkusnoj tečnosti, kao i statistički značajna korelacija koncentracija azot monoksida u pljuvački i sulkusnoj tečnosti u toku prvih šest meseci terapije. **Zaključak.** I gingivalna sulkusna tečnost i pljuvačka su pouzdani medijumi za praćenje stanja zdravlja gingive kod ortodontskih pacijenata. Potrebno je sprovesti još studija koje bi rasvetlile mogućnost korišćenja azot monoksida kao faktora za praćenje stanja zdravlja gingive kod ortodontskih bolesnika.

Ključne reči:
ortodoncija, korektivna; zapaljenje; gingivalna sulkusna tečnost; pljuvačka.

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Introduction

Nowadays orthodontic therapy with fixed orthodontic appliances is widely spread, having in mind positive effects it has in malocclusion treatments [1]. The side effect of therapy is that the constant presence of orthodontic brackets in mouth cavity prevents adequate oral hygiene, increases the number of retaining locations where dental plaque can be acumulated and provokes mechanical irritations of oral mucosa. Numerous studies have confirmed the presence and increased gingivial inflammation within the patients having the orthodontic braces [2–6].

Periodontal disease is an inflammatory process of the periodontal tissues (gingiva, alveolar bone, cement, periodontal fibres), affecting single or multiple locations [7].

Progression of periodontal disease is at large scale conditioned by a patient's individual characteristics [8].

Microbiological basis of periodontal diseases was verified long ago. The microorganisms of the oral biofilm operate in two ways: they directly aggravate the tissue of the host and provoke the release of numerous biological mediators which could lead to the tissue destruction. The mediators being a part of organism's reaction to bacterial infection and, as such, leading to the tissue decay are: proteinases, cytokines and prostaglandins [9].

Traditional diagnostical clinical methods, such as assessing the pockets' depth, bleeding on probing, determing the clinical loss of the ephitelial lining, plaque index and radiography are more useful for the determination of the presence and consequences of a periodontal disease rather than its activity. There is a need to develop some new diagnostical tests that might indicate the presence of active disease, its progression and effects it has on the applied therapy.

Gingival crevicular fluid (GCF) represents an exudate found in gingival sulcus. It consists of serum, although its composition may vary depending on the neighbouring gingival tissue and bacterial presence, so that it may contain immunoglobulins, toxins, cells, microorganisms and numerous enzymes [10]. GCF has recently been used as the medium for measuring and quantifying various molecules and bacteria presence in the mouth cavity as well as in the periodontal ligament space [11–13]. Markers of the bone remodelation in orthodontic patients may also be detected [14].

Nitric oxide (NO) is a free radical with many biological functions. It is an intercellular signaling molecule involved in many physiological functions in organism: regulation of vascular tonus, intestinal motility, aggregation and adhesion of thrombocytes, the formation and destruction of bones, numerous immunological functions, apoptosis, and neurotransmission. It is a highly-reactive molecule, easily diffusing through a cell membrane [15]. Endothelial and neuronal cells produce NO constitutively, but it can be also produced by macrophages and other inflammatory cells in pathological conditions. The most important stimuli for NO synthesis are bacterial products. In inflammatory processes of periodontium, the positive effects of NO are related to bacteria destruction, while negative effects involve the damage of the tissue through the mechanisms of oxidation, nitric reactions, enzyme inhibitions, DNA distraction, metalloproteinase activation. As other free radicals, NO participates in neutrophile procolagenase activation, in the supression of protheoglycans and collagen synthesis, thus contributing to gingival damage advancement. The findings from the different studies suggest that NO levels are increased in GCF and serum in subjects with periodontal disease, compared to the healthy controls. In the research conducted on 90 persons [16], the authors concluded that the increase in GCF NO levels was directly proportional to the severity of periodontal disease. Skaleric et al. [17] examined GCF of 18 diabetic patients and their results showed the increased GCF NO level in patients with more severe gingival inflammation. In the literature there are no data about GCF NO as the marker of gingival inflammatory condition in patients with fixed orthodontic appliances.

The aim of this study was to estimate the inflammatory condition of gingiva in the first six months of orthodontic therapy on the basis of clinical parameters of Sulcus Bleeding Index (SBI), Plaque Index (PI), GCF and salivary NO production, so that the degree of correlation between clinical parameters and the NO production can be established with a possibility to show the periodontic inflammatory condition.

Methods

Study population

This study included 30 orthodontic patients (11 males and 19 females) of the Dental Clinic, aged 15–22 years, treated with fixed orthodontic appliances.

The selected patients fulfilled the next criteria: 1) need for non extraction orthodontic treatment with fixed appliances, 2) absence of approximal caries or approximal fillings on permanent molars and second premolars, 3) good systemic health of patient (absence of chronic diseases history), 4) absence of use of anti-inflammatory and antibiotic therapy three months before the beginning of the treatment.

Exclusion terms were: 1) mouth breathing patients, 2) patients with severe crowding 3) patients with dentofacial deformities, 4) treatment by dental hygienist any time during the first six months of therapy, 5) use of anti-inflammatory and antibiotic therapy during first six months of the orthodontic treatment, 6) smoking habits.

Informed consent and approval document for the participation in the study were signed by the patients or their parents if patients were younger than 18 years. The study protocol was approved by the Ethical Committee No 01-890-6.

Clinical procedure

Specialist of orthodontics was evaluating clinical parameters (PI), (SBI) and collecting GCF and saliva.

At the beginning of the treatment, the patients were given detailed instructions about oral hygiene procedures that they should follow out during orthodontic treatment. The patients were not allowed to use anti plaque substances during first six months of treatment. During first six months of the therapy only nivelation of dental arches was done, using round NiTi arch wires 0.12, 0.14 and 0.16 at the end. The patients using antibiotics

and antiinflammatory drugs during first six months of therapy were excluded from the study.

Supra- and subgingival ultrasound cleaning was conducted in all patients two weeks before the beginning of orthodontic treatment.

All patients from the study were treated using fixed orthodontic appliances, following basic principles of technique of straight arch. Control checkups were conducted precisely in one month periods.

Evaluation of clinical parameters

Clinical parameters of gingival inflammation Silness-Löe PI and Muhlemann-Son SBI [18] were evaluated using periodontal probe and respecting World Health Organization (WHO) criteria, before, three and six months after the beginning of the orthodontics therapy during regular orthodontic controls. We selected particular time points in the study, since we found these time periods minimal for clear expression of gingival inflammation in orthodontic patients.

Before starting investigation, the necessary calibrations were performed to provide validity of the results. To test intra-examiner agreement, examiner re-measured PI and SBI in 30 persons two weeks after initial measurements.

Kappa statistics was used to evaluate the consistency of intra-examiner agreement.

GCF collection

GCF was collected using paper strips before, three and six months after the beginning of orthodontic therapy. The teeth to be sampled were isolated with cotton rolls in order to avoid saliva contamination. Supragingival dental plaque was removed. The paper strip was inserted into the crevice on vestibular surface of first permanent molars, second premolars, canines and central incisors until mild resistance is felt and it was left there for 30 sec. It means that eight paper strips from every patient were collected at the end. Paper strips contaminated with blood because of gingival irritation were excluded from study. All collected samples of GCF were stored in sterile eppendorfs at –80 ºC until the next step in their elaboration at the Institute of Biochemistry.

Saliva collection

After making the subjects rinse their mouths thoroughly with water, salivary samples were collected in sterile containers by instructing them to allow saliva to collect naturally in mouth and to expectorate it into the containers. All collected samples of saliva were stored at -80 ^{o}C until the next step in their elaboration at the Institute of Biochemistry.

Biochemical analyses

After deproteinization, the production of NO was evaluated by measuring $NO_2^- + NO_3^-$ concentrations. Nitrates were transformed into nitrites by cadmium reduction, before the measuring of total $NO_2^- + NO_3^-$ concentration [19]. Nitrites were assayed directly spectrophotometrically at 543 nm, using the colorimetric method of Griess. Salivary and GCF NO concentrations are rendered by proteins.

Statistical analysis

The results from the study are presented in tables and figures. The values of examined parameters are represented with mean values (r), standard deviations (SD), 95% confidence intervals (95% CI), medians (MD) and interquartile ranges (IQR). The distributions of the continuous variables were assessed for normality by Shapiro-Wilk test. A paired *t*-test was used in case of two related observations with a normal distribution, and Wilcoxon Signed-Ranks if a distribution of data was not normal. Depending on the distribution of normality Pearson (r), or Spearman (ρ) correlation coefficients were used to analyze associations between continuous variables. Statistical data analysis was done with the SPSS software package (Version 18) where significance level was $p < 0.05$.

Results

The kappa values of the intra-examiner reproducibility for PI and SBI were 0.82 and 0.86, respectively.

When these values were analysed, almost perfect agreement was obtained for both PI and SBI.

There was a statistically significant increase of values of clinical parameters (PI and SBI) three and six months after the beginning of the orthodontic therapy in comparison to the values before beginning of the therapy, ($p < 0.001$). There was also a statistically significant increase of GCF and salivary NO concentration three and six months after the beginning of therapy in comparison to the values before the beginning of the therapy ($p < 0.001$) (Figures 1–4).

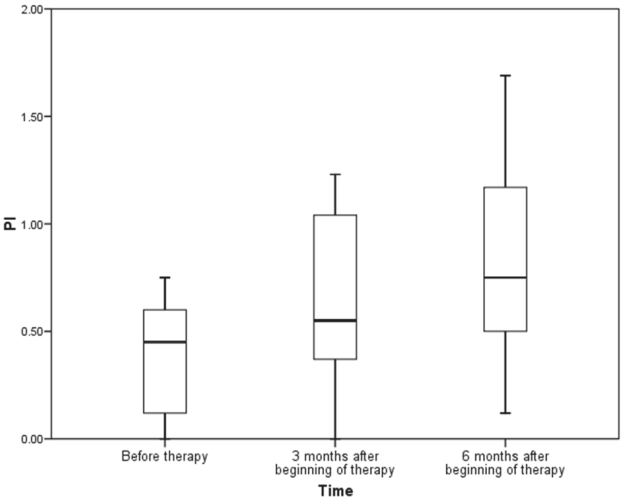

Fig. 1 – Box plots with medians and interquartile ranges of Plaque Index (PI) before and three and six months after the beginning of the orthodontic therapy.

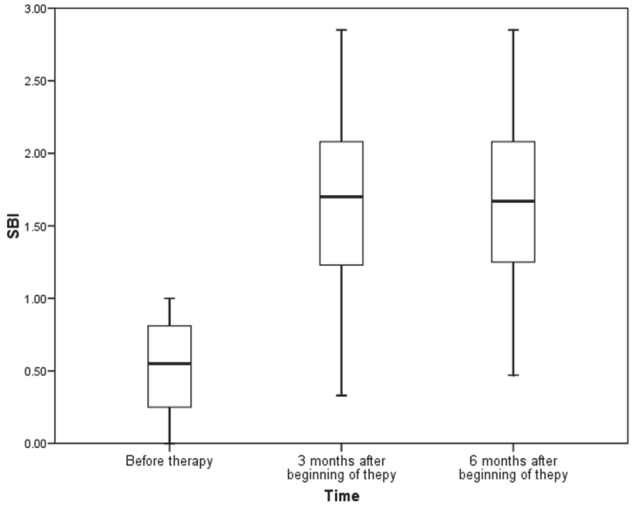

Fig. 2 – Box plots with medians and interquartile ranges of Sulcus Bleeding Index (SBI) before, and three and six months after the beginning of the orthodontic therapy.

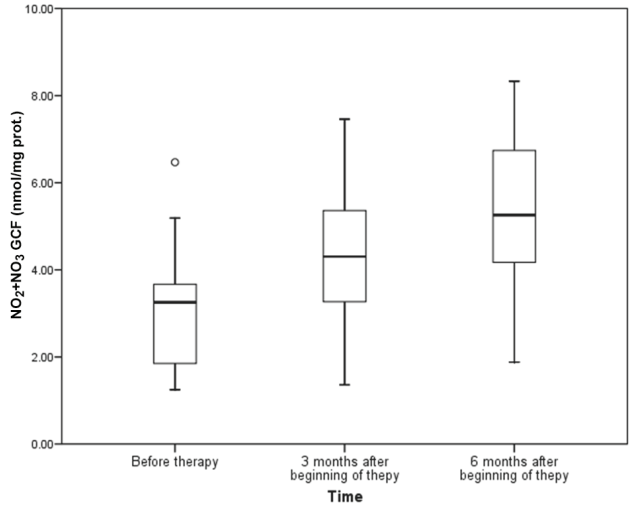

Fig. 3 – Box plots with medians and interquartile ranges of gingival crevicular fluid (GCF) NO_2^- + NO_3^- concentration before, 'and three and six months after the beginning of the orthodontic therapy. NO_2^- – nitrite; NO_3^- – nitrate.

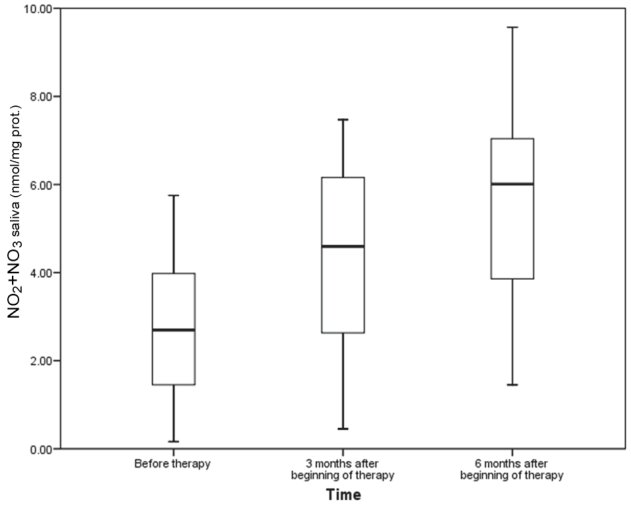

Fig. 4 – Box plots with medians and interquartile ranges of salivary NO_2^- + NO_3^- concentration before, and three and six months after the beginning of the orthodontic therapy. NO_2^- – nitrite; NO_3^- – nitrate.

There was a positive statistically significant correlation between clinical parameters of the gingiva (PI and SBI) in the period of first three months of the orthodontic therapy ($p < 0.05$) and even bigger significance after six months of the therapy ($p < 0.01$) (Table 1).

There was a positive statistically significant correlation between GCF and salivary NO_2^- + NO_3^- concentration before beginning of the orthodontic therapy ($p < 0.01$), 3 months after the beginning of the therapy ($p < 0.05$) and even bigger significance six months after the beginning of the therapy ($p < 0.01$) (Table 1).

There was a negative statistically insignificant correlation between GCF and salivary NO_2^- + NO_3^- concentrations with both clinical parameters of gingiva PI and SBI except between SBI and salivary NO_2^- + NO_3^- concentration before the therapy and between PI and salivary NO_2^- + NO_3^- concentration six months after the beginning of the therapy ($p < 0.05$) (Table 1).

Table 1

Correlations of examined variables during orthodontic therapy

Variable	Before therapy	3 months after beginning of therapy	6 months after beginning of therapy
PI and SBI	0.46*	0.41*	0.46**
PI and NO_2^- + NO_3^- GCF	-0.20	-0.17	-0.10
PI and NO_2+NO_3 saliva	-0.19	-0.27	-0.41*
SBI and NO_2+NO_3 GCF	-0.08	0.15	0.05
SBI and NO_2+NO_3 saliva	-0.42*	-0.30	-0.24
NO_2+NO_3 GCF and NO_2+NO_3 saliva	0.53**	0.43*	0.71***

***p < 0.05; **p < 0.01, ***p < 0.001.**

PI – Plaque Index; SBI – Sulcus Bleeding Index; NO_2^- – nitrite; NO_3^- – nitrate; GCF – gingival crevicular fluid.

Table 2

Changes of PI, SBI, GCF and salivary $NO_2^- + NO_3^-$ concentration during orthodontic therapy (changes *vs* values before therapy)

Changes of variable	3 months after beginning of therapy	6 months after beginning of therapy
PI	0.27 ± 0.26 (0.17–0.36) 0.28 (0.00–0.49)	0.42 ± 0.35 (0.29–0.55)*** 0.39 (0.00–0.62)
SBI	1.04 ± 0.52 (0.85–1.24) 1.07 (0.54–1.50)	1.17 ± 0.53 (0.97–1.36)* 1.21 (0.74–1.46)
$NO_2^- + NO_3^-$ GCF (nmoL/mg prot.)	1.34 ± 0.99 (0.97–1.71) 1.06 (0.3 –1.94)	2.20 ± 1.22 (1.74–2.65)** 1.81 (1.51–3.41
$NO_2^- + NO_3^-$ saliva (nmoL/mg prot.)	1.60 ± 1.08 (1.20–2.00) 1.16 (0.75–2.53)	2.89 ± 1.50 (2.33–3.45)*** 2.60 (1.83–3.98)

Data are given as means ± standard deviation (95% confidence interval); medians (interquartile ranges).
***$p < 0.05$, ****$p < 0.01$, *****$p < 0.001$.**
For abbreviations see under Table 1.

Table 3

Correlations of PI, SBI , GCF and salivary $NO_2^- + NO_3^-$ concentration changes during the orthodontic therapy

Variable	3 months after the beginning of the therapy	6 months after the beginning of the therapy
PI and SBI	0.27	0.31
PI and $NO_2^- + NO_3^-$ GCF	0.02	0.11
PI and $NO_2^- + NO_3^-$ saliva	–0.19	0.03
SBI and $NO_2^- + NO_3^-$ GCF	0.13	0.03
SBI and $NO_2^- + NO_3^-$ saliva	–0.25	–0.04
$NO_2^- + NO_3^-$ GCF and $NO_2^- + NO_3^-$ saliva	0.31	0.59***

*****$p < 0.001$.**
For abbreviations see under Table 1.

Changes of initial values of all examined parameters six months after the beginning of the therapy in comparison to those three months after the beginning of the therapy were significantly higher, SBI ($p < 0.05$), GCF $NO_2^- + NO_3^-$ ($p < 0.01$), PI and salivary $NO_2^- + NO_3^-$ ($p < 0.001$) (Table 2).

There was a positive, but statistically insignificant correlation of changes between PI and SBI during first three and six months of the therapy.

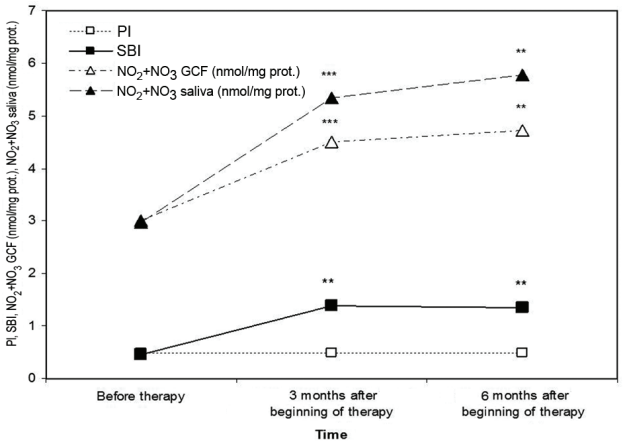

Fig. 5 – Medians of PI, SBI, GCF and salivary $NO_2^- + NO_3^-$ concentration in patients with constant values of PI before, three and six months after the beginning of the orthodontic therapy ($p < 0.01$, ***$p < 0.001$).**
For abbreviations see under Table 1.

There was also a positive but statistically insignificant correlation of changes between CGF and salivary $NO_2^- + NO_3^-$ concentration after three months of the therapy, while it became strong and statistically significant after six months of the therapy ($p < 0.001$) (Table 3).

Eight patients of the examined group did not have changes in PI values during the first six months of the therapy, but there was a statistically significant increase of values of the clinical parameter SBI, three and six months after the beginning of the therapy compared to the values before the beginning of the therapy ($p < 0.01$). In the same group, there was also a statistically significant increase of GCF and salivary $NO_2^- + NO_3^-$ concentration three ($p < 0.001$) and six months after the beginning of the therapy ($p < 0.01$) (Figure 5).

Discussion

In our research, the important findings are statistically significant increase of the values of PI and SBI, three and six months after the beginning of the therapy compared to those before the therapy. The results given indicate the presence of the gingival inflammation and worsening of the inflammatory processes within the first six months of the therapy. All these results are in agreement with the results of numerous studies [3–6, 20].

Although periodontal disease can have difficult diagnosis and treatment, this article evaluates gingivitis and it is important to note that gingivitis is easily diagnosed through clinical observation.

Out of 30 patients, eight of them did not have any changes in PI after three and six months of the therapy, when the results were compared to those obtained before the therapy was applied. Such results suggest adequate oral hygiene in spite of the circumstances that include the orthodontic braces in mouth cavity. During the therapy, there was, however, the increase of the values of SBI, GCF and salivary NO production compared to those before the therapy. These results indicate that among the patients having orthodontic braces, gingival inflammation, apart from plaque presence, is influenced by some other factors, such as bonding, bracket gluing, and mechanical irritation of gingiva. Corbacho de Melo et al. [5] pointed the fact that positioning of the bracket edge below the gingival margin provoked gingival inflammation to a large extent.

There are very few researchers who examined the role of NO in the process of a bone remodeling during the initial phases of orthodontic teeth movement [21]. They suggest that NO is involved in the regulation of the second messenger system formation, in the regulation of osteoblasts and osteoclasts functions and the blood flow in the pulp. It has been determined that NO increases microvascular permeability and, as a such, can acquire a crucial role in the first stages of bone remodeling due to the fact that the blood vessels monocytes become the basis of a bone remodeling later during the orthodontic therapy [22]. Being familiar with the role of NO in the process of bone remodeling during the orthodontic teeth movements is very important for the interpretation of our results. Samples of GCF in our study were always taken one month after the previous control and the prospective application of orthodontic force and always before taking the next step in the therapy. In this way, we have tried to reduce the influence of the orthodontic force on NO production and make it, as much as possible, a measure of gingival inflammation caused by the presence of the orthodontic braces in the mouth cavity.

Our results point out the statistically significant increase of GCF and salivary $NO_2^- + NO_3^-$ concentration three and six months after the beginning of the orthodontic therapy. Changes in NO production during this period, as our research shows, may indicate tendency of gingiva inflammation increase among some patients. Still, there is a low correlation between clinical parameters of gingival condition (PI and SBI) and GCF and salivary $NO_2^- + NO_3^-$ concentrations during the-six-month-long-therapy. This fact indicates that GCF NO production in the orthodontic patients might be influenced not only by gingival inflammation but also by some other factors related to the nature of orthodontic treatment and the process of bone remodeling it is followed by.

NO is generated by the nitric oxide synthase (NOS) enzymes from the oxygen and the amino acid L-arginine. There are three isoforms of NOS: a neuronal form (nNOS), an endothelial form (eNOS) and an inducible form (iNOS). High levels of eNOS are detected in the endothelial cells of the blood vessels. NO is important factor responsible for the relaxation of the blood vessels smooth muscles in compressed areas during tooth movement [23, 24]. Periodontal hyperaemia is the initial phase that leads to complex processes of bone remodeling during orthodontic movement of teeth.

D'Attillio et al. [25] examined eNOS and iNOS levels of gingival tissue in patients treated with fixed orthodontic appliances. In their study, canine undergoing treatment for distal movement served as the test tooth whereas its contralateral canine was used as the control tooth. Two weeks after beginning of the therapy both eNOS and iNOS levels were significantly higher in gingiva of the test tooth than those of the control tooth. They concluded that gingival tissue, surrounding a moving teeth, did not undergo resorption, but was compressed and retracted. The role of gingival eNOS, iNOS and NO during the early phases of orthodontic treatment in humans is of significant importance, as obvious.

Two major cell types responsible for bone remodeling are osteoclasts, which resorb bone, and osteoblasts, which form new bone. There are many studies that indicate NO role in promoting osteoclasts differentiation and bone resorption [26, 27].

During orthodontic tooth movement, there is also an increase in number and activity of macrophage-like cells in resorptive areas or periodontal tissue undergoing more intensive mechanical stress [27]. Macrophages remove necrotic periodontal tissue and during the process of cell interaction, they release NO [28]. Gaspirc et al. [29] showed detectable levels of iNOS in macrophages of gingival tissue.

Briefly, GCF NO concentration during orthodontic tooth movements depends on the activity of several cell types in bone, periodontium and gingiva. Beside its main role in initial phase of bone remodeling, there are different complicated processes that could influence GCF NO concentration even one month after starting with orthodontic force. That could explain the lack of statistically significant correlations between GCF, salivary NO production and clinical parameters of gingival inflammation in our study. On the other hand, the clinical parameters are more useful in the determination of the presence and consequences of periodontal disease rather than its activity, so missing of the correlation among the PI, SBI and GCF NO production in our study can be expected, having in mind that many authors have suggested NO as a marker of inflammation activity.

The question that arises is whether GCF and saliva can be equally reliable parameters for monitoring of periodontal conditions. Saliva sampling, compared to that of GCF one, is a pretty simple procedure and thus much larger quantities of it can be available. Certain authors consider GCF to be a more reliable source for identifying and monitoring periodontal diseases. The reason lies in the fact that GFC is solely under the influence of the neighbouring periodontal tissues. On the other hand, saliva is primarily formed by the secretion of salivary glands that are also responsible for NO production. One more reason that makes saliva and its diagnostic potential a less reliable parameter in identifying the periodontal disease is that it can easily reflect both systemic inflammatory and infectious conditions [10].

The results we obtained indicate statistically significant increase of NO production in saliva and GCF three and six months after the beginning of the orthodontic treatment. It is also proven that there is a statistically significant correlation between the va-

lues of GCF and salivary NO production before, three and six months after the beginning of the orthodontic treatment.

There is a positive but at the same time statistically insignificant correlation in the change of salivary and GCF $NO_2^- + NO_3^-$ concentrations that occurred three months after the treatment started. However, there is a strong statistically significant correlation in the changes of the salivary and GCF $NO_2^- + NO_3^-$ concentrations that occurred six months after the beginning of the orthodontic treatment. All these facts lead to a conclusion that GCF and saliva can be used as a reliable medium for periodontal conditions monitoring. The given results coincide with the results achieved by Topcu et al. [30]. On the other hand, Poorsattar Bejeh Mir et al. [31] concluded that detecting NO biomarker and its end metabolites in saliva was of more value to assess the periodontal health when comparing to GCF.

Conclusion

According to the measurements of the values of the clinical parameters, PI and SBI, related to the gingival inflammatory status as well as the measurements of GCF and salivary NO concentration, it can be concluded that there is the increase of gingival inflammation during the first six months of the orthodontic therapy with fixed appliances. In spite of the fact that our results indicate a statistically significant increase of GCF and salivary NO concentration during the first six months of orthodontic therapy, one should be very cautious because there is a low correlation with the clinical parameters.

According to the results obtained in this study, we can suggest that both GCF and saliva are reliable mediums for monitoring the gingival inflammatory condition.

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REFERENCES

1. *Ireland AJ, Mcdonald F.* The orthodontic patient: treatment and biomechanics. London: Oxford University Press; 2003. p. 129–30.
2. *Thornberg MJ, Riolo CS, Bayirli B, Riolo ML, Van Tubergen EA, Kulbersh R.* Periodontal pathogen levels in adolescents before, during, and after fixed orthodontic appliance therapy. Am J Orthod Dentofacial Orthop 2009; 135(1): 95–8.
3. *Alexander SA.* Effects of orthodontic attachments on the gingival health of permanent second molars. Am J Orthod Dentofacial Orthop 1991; 100(4): 337–40.
4. *Matić S, Ivanović M, Nikolić P.* Evaluation of a prevention programme efficiency for patients with fixed orthodontic appliances. Vojnosanit Pregl 2011; 68(3): 214–9.
5. *Corbacho de Melo MM, Cardoso MG, Faber J, Sobral A.* Risk factors for periodontal changes in adult patients with banded second molars during orthodontic treatment. Angle Orthod 2012; 82(2): 224–8.
6. *Rego RO, Oliveira CA, Santos-Pinto A, Jordan SF, Zambon JJ, Cirelli JA*, et al. Clinical and microbiological studies of children and adolescents receiving orthodontic treatment. Am J Dent 2010; 23(6): 317–23.
7. *Page RC.* The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 1991; 26(3 Pt 2): 230–42.
8. *Dumitrescu AL.* Etiology and pathogenesis of periodontal disease. Heidelberg: Springer. 2010.
9. *Zia A, Khan S, Bey A, Gupta ND, Mukhtar-Un-Nisar S.* Oral biomarkers in the diagnosis and progression of periodontal diseases. Biol Med 2011; 3(2): 45–52.
10. *Cimasoni G.* Crevicular fluid updated. Monographs in oral science. Basel: S Karger Pub; 1983.
11. *Armitage GC.* Analysis of gingival crevice fluid and risk of progression of periodontitis. Periodontology 2000 2004; 34: 109–19.
12. *Grenier G, Gagnon G, Grenier D.* Detection of herpetic viruses in gingival crevicular fluid of patients suffering from periodontal diseases: Prevalence and effect of treatment. Oral Microbiol Immunol 2009; 24(6): 506–9.
13. *Khosravi R, Tran SD, Lambert M, Loughlin JO, Kâ K, Feine JS,* et al. Adiposity and gingival crevicular fluid tumour necrosis factor-alpha levels in children. J Clin Periodontol 2009; 36(4): 301–7.
14. *Lamster IB, Novak MJ.* Host mediators in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. Crit Rev Oral Biol Med 1992; 3(1–2): 31–60.
15. *Matejka M, Partyka L, Ulm C, Solar P, Sinzinger H.* Nitric oxide synthesis is increased in periodontal disease. J Periodontal Res 1998; 33(8): 517–8.
16. *Menaka KB, Ramesh A, Thomas B.* A multifaceted molecule, nitric oxide: Its possible role in periodontitis. J Oral Health Res 2011; 2(4): 112–7.
17. *Skaleric U, Gaspirc B, McCartney-Francis N, Masera A, Wahl SM.* Proinflammatory and antimicrobial nitric oxide in gingival fluid of diabetic patients with periodontal disease. Infect Immun 2006; 74(12): 7010–3.
18. *Wolf HE, Hassell TM.* Indices. In: *Wolf HE, Hassell TM*, editors. Color atlas of dental hygiene. Periodontology. Stuttgart, New York: Thieme; 2006. p. 67–76.
19. *Navarro-Gonzálvez JA, García-Benayas C, Arenas J.* Semiautomated measurement of nitrate in biological fluids. Clin Chem 1998; 44(3): 679–81.
20. *Gong X, Chen W, Gong Y, Zhou L.* Clinical analysis of PLI, GI and SBI in patients with fixed orthodontic appliances. Shanghai Kou Qiang Yi Xue 2006; 15(4): 367–9.
21. *Shirazi M, Nilforoushan D, Alghasi H, Dehpour A.* The role of nitric oxide in orthodontic tooth movement in rats. Angle Orthod 2002; 72(3): 211–5.
22. *Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P.* Molecular biology of the cell. 4th ed. New York: Garland Science; 2002. p. 838-39 1304–7.
23. *Nathan C.* Nitric oxide as a secretory product of mammalian cells. Faseb J 1992; 6(12): 3051–64.
24. *Vandevska-Radunovic V, Kvinnsland S, Kvinnsland IH.* Effect of experimental tooth movement on nerve fibres immunoreactive to calcitonin gene-related peptide, protein gene product 9.5, and blood vessel density and distribution in rats. Eur J Orthod 1997; 19(5): 517–29.

25. *D'Attillio M, di Maio F, D'Arcangela C, Filippi MR, Felaco M, Lohinai Z*, et al. Gingival endothelial and inducible nitric oxide synthase levels during orthodontic treatment: A cross-sectional study. Angle Orthod 2004; 74(6): 851−8.
26. *Mancini L, Becherini L, Benvenuti S, Brandi ML*. Bioeffects of a nitric oxide donor in a human preosteoclastic cell line. Int J Clin Pharmacol Res 1997; 17(2−3): 93−6.
27. *Mentaverri R, Kamel S, Wattel A, Prouillet C, Sevenet N, Petit JP,* et al. Regulation of bone resorption and osteoclast survival by nitric oxide: Possible involvement of NMDA-receptor. J Cell Biochem 2003; 88(6): 1145−56.
28. *Brudvik P, Rygh P*. Non-clast cells start orthodontic root resorption in the periphery of hyalinized zones. Eur J Orthod 1993; 15(6): 467−80.
29. *Gaspirc B, Masera A, Skaleric U*. Immunolocalization of inducible nitric oxide synthase in localized juvenile periodontitis patients. Connect Tissue Res 2002; 43(2−3): 413−8.
30. *Topcu AO, Akalin FA, Sahbazoglu KB, Yamalik N, Kilinc K, Karabulut E*, et al. Nitrite and nitrate levels of gingival crevicular fluid and saliva in subjects with gingivitis and chronic periodontitis. J Oral Maxillofac Res 2014; 5(2): e5.
31. *Poorsattar Bejeh-Mir A, Parsian H, Akbari Khoram M, Ghasemi N, Bijani A, Khosravi-Samani M*. Diagnostic Role of Salivary and GCF Nitrite, Nitrate and Nitric Oxide to Distinguish Healthy Periodontium from Gingivitis and Periodontitis. Int J Mol Cell Med 2014; 3(3): 138−45.

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Quality of life of the mechanically ventilated patients with community-acquired pneumonia

Kvalitet života posle mehaničke ventilacije kod bolesnika lečenih od pneumonije

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Abstract

Background/Aim. Patients with pneumonia who require mechanical ventilation (MV) are associated with several poor outcomes such as prolonged hospitalization, higher rate of mortality and increased spread of antibiotics-resistant pathogens. MV in patients with community-acquired pneumonia (CAP) could cause development of psychological symptoms, often neglected in the Intensive Care Units (ICU) as well as decreased quality of life after the withdrawal of the MV. The aim of the study was to evaluate the quality of life in patients with CAPs treated with MV in ICU. **Methods.** The study was designed as a cohort study of hospital-treated patients with CAP with prospective data collection. The quality of life was defined as the primary outcome, while the use of MV was assumed as the primary prognostic factor that adversely affected the outcome. The patients were recruited from the population of patients with CAPs who were hospitalized at the ICU, Clinical Center Kragujevac, Serbia, from January 2013 to January 2014. The experimental group consisted of patients who were on MV while the control group included patients who were treated for CPAs in the ICU, but were not subjected to MV. The quality of life was assessed by using patient-rated Euro Quality of Life (EuroQoL) Group-EQ-5D index. The calculation of the total EQ-5D-5L score values was performed by using the predefined, validated mapping key according to response combinations. Statistical analysis was performed by using χ^2 test, Student's *t*-test, univariate and multivariate logistic regression analyses. **Results.** The patients with MV had worse EQ5D-5L values in comparison to the control group for all 5 domains. Mobility, self-care and usual activities were negatively affected during the whole follow-up period. Pain or discomfort and anxiety or depression differed significantly between the study group and the control group at days 7 and 30. **Conclusion.** Patients with MV tend to have poorer quality of life, especially in 3 domains. The main reasons are the presence of chronic comorbidities in the population that require MV.

Key words:
respiration, artificial; pneumonia; critical care; quality of life; prognosis.

Apstrakt

Uvod/Cilj. Bolesnici sa pneumonijom koji zahtevaju mehaničku ventilaciju (MV) povezani su sa nekoliko loših ishoda, kao što su produžena hospitalizacija, veća stopa smrtnosti i povećano širenje patogena otpornih na antibiotike. MV kod bolesnika sa vanbolnički stečenom pneumonijom (*community-acquired pneumonia* – CAP) može izazvati razvoj psihološke simptomatologije, koje su često zanemarene u jedinicama intenzivnog lečanja (JIL), i smanjenjem kvaliteta života nakon prestanka MV. Cilj studije je bio da se proceni kvalitet života kod bolesnika sa CAP koji se leče primenom MV u JIL. **Metode.** Istraživanje je sprovedeno u obliku studije koja je obuhvatala hospitalizovane zbog CAP. Kvalitet života definisan je kao primarni ishod, dok je primena MV pretpostavljena kao primarni prognostički

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faktor koji negativno utiče na ishod. Bolesnici su regrutovani iz populacije bolesnika sa CAP koje su bile hospitalizovane u JIL u Kliničkom centru Kragujevac, Srbija, od januara 2013. do januara 2014. godine. Eksperimentalna grupa sastojala se od bolesnika koji su bili na MV, a kontrolna grupa bili su bolesnici sa CAP, kiji nisu bili podvrgnuti MV. Kvalitet života procenjen je pomoću EuroQoL Group-EQ-5D indeksa. Izračunavanje ukupne vrednosti EQ-5D-5L izvršeno je korišćenjem unapred definisanog, validiranog kartografskog ključa, u skladu sa kombinacijama odgovora. Statistička analiza obavljena je korišćenjem χ^2 testa, Studentovog *t*-testa, univarijantne i multivariantne logističke regresione analize. **Rezultati.** Bolesnici sa MV imali su lošije vrednosti EQ5D-5L u poređenju sa kontrolnom grupom za svih pet domena. Mobilnost, samozbrinjavanje i uobičajene aktivnosti bili su najlošije ocenjeni tokom čitavog perioda praćenja. Bol ili neugodnost i anksioznost ili depresija značajno su se razlikovali između studijske i kontrolne grupe u sedmom i tridesetom danu lečenja. **Zaključak.** Bolesnici sa MV imaju slabiji kvalitet života, naročito u tri domena. Glavni razlozi su prisustvo hroničnih komorbiditeta kod populacije koja zahteva primenu MV.

Ključne reči:
disanje, mehaničko; pneumonija; intenzivna nega; kvalitet života; prognoza.

Introduction

Community-acquired pneumonia (CAP) represents acute infection of lung parenchyma, associated with systemic of inflammatory response and with the presence of infiltrations on chest radiography in patients who were not hospitalized in the last 14 days before the onset of symptoms [1]. CAP is a frequent disease encompassing all ages, being more prevalent in patients with associated co-morbidities particularly chronic obstructive pulmonary disease (COPD), chronic heart failure (CHF), chronic liver and kidney diseases, Alzheimer's dementia, cystic fibrosis (CF), immunocompromised syndromes and in those who are smokers [2]. Approximately 0.5%–1% of people from the adult population are diagnosed with some form of CAP annually. Among them, 22%–42% need hospitalization, out of which 5% to 10% need to be treated in the intensive care unit (ICU) [3, 4].

Patients with pneumonia who require the mechanical ventilation (MV) treatment are at the increased risk for several poor outcomes such as prolonged hospitalization and colonization with pathogens resistant to antibiotics as well as a higher mortality rate [4]. In addition, the mechanically ventilated patients with CAP frequently develop central nervous system disturbances which could adversely affect the quality of life of survivors [5, 6]. The quality of life, as defined by the World Health Organization, is "the individual perception of their life position, in the context of culture and system values in which they live and in relation to their goals, standards, expectations and concerns." It estimates the gap between the expectations of people and their achievements and it reflects the satisfaction of the individual to his/her whole life [7].

Despite the high medical and economic burden imposed on societies throughout the world, the studies reporting its influence on the quality of life of the survivors are not common [8–11]. Initial studies investigated the quality of life of CAP patients mostly as a secondary outcome giving limited data about it such as the baseline values and the change trends during the course of the treatment [12]. In these times, researches revealed connections between pneumonia and disturbances of systemic homeostatic pathways on molecular levels (e.g., cytokine response), but their consequences on the quality of life were poorly understood [13, 14]. The interest for the topic has recently been raised, and in studies which appeared it was found that physical components, mobility, self-care and usual activities were the most affected domains of the quality of life of the patients recovering from pneumonia [15, 16]. In addition, our knowledge about the underlying biological mechanism of the poor quality of life is increasing rapidly, including the role of mediators of acute inflammation [17].

However, very little is known about the quality of life parameters within various clinical types of the disease in various patient population such as community-acquired, nosocomial and pneumonia treated in the intensive care units. There is a need for increasing our knowledge about the role of proposed predictors of poor quality of life outcomes including their influence within separate patients' subgroups by using different treatment strategies.

Therefore, we hypothesized that the MV represented the independent risk factor for the decreased quality of life of the patents who recovered after community-acquired pneumonia. In this study, we aimed to estimate the quality of life of the patients with CAP who were on MV, compared to those with CAP who were not on MV.

Methods

The research was designed as a cohort study in the hospital-treated patients with CAP and prospective data collection. The quality of life was considered as the primary outcome, while the use of MV was assumed as the primary prognostic factor that adversely affected the outcome. The quality of life was followed repeatedly according to the dynamics of the expected recovery from pneumonia in the course of three study visits.

Study population

Patients were recruited from the population of patients with CAP who were hospitalized in the Emergency Center, Intensive Care Unit, Clinic for Pulmonology and Clinic for Infectious Disease of the Clinical Center "Kragujevac", Kragujevac, Serbia, from January 2013 to January 2014. The experimental group consisted of patients who were treated and

mechanically ventilated while the control group were patients who were treated, but were not mechanically ventilated. The inclusion criteria were the following: female or male adults (≥ 18 years), patients who had CAP confirmed by microbiological, radiographic and laboratory tests, and those who gave voluntary informed consent for the participation in the study. The patients were excluded from the study if they had been mechanically ventilated more than 24 hours before their admission to the hospital, if they were mechanically ventilated for a disease other than CAP, pregnant and lactating women, patients from whom we could not get accurate data needed for the research at baseline (e.g., psychiatric patients with altered cognitive functions, patients with incomplete data in the available medical records patients for whom the adequate monitoring by the end of the study was unlikely at the time of screening) and patients who refused to give informed consent.

Only those patients who survived to the day 90 after MV stopping (experimental group) and their controls (matching the time of hospital stay) with the same period of surviving, were included in the study.

In total, there were 164 patients eligible for study participation at the screening time, but 17 (10.3%) of them refused to give their informed consent and therefore 147 (89.7%) patients were included in the study. During the study period, the fatal outcome was confirmed in 3 (2%) patients and 14 (9.5%) patients were lost to follow-up and they did not complete all study visits. Therefore, the data from 130 patients who completed all three study visits were included in the analysis. A total of 65 patients were treated in ICU (all subjects were in the experimental group), 21 subjects were treated in the semi-ICU and 44 patients were treated in the clinical wards.

Study variables

We measured the quality of life by using patient-rated Euro Quality of Life (QoL) Group-EQ-5D index [18]. It consisted of two parts: five domains (mobility, self-care, usual activities, pain/discomfort, anxiety/depression) and visual analog scale (VAS). The new version of the EQ-5D used in the study included five grading levels, in each of the five EQ-5D domains and it is called EQ-5D-5L. The calculation of the total EQ-5D-5L score value, according to response combinations, was performed by using the predefined, validated mapping key. Clinical, laboratory and socio-demographic data were considered as secondary variables.

Data collection

Baseline visit was done at the index day. For the experimental group, the index day was day 7 after stopping MV. For the control group, the index day was within the period of 7 days from his or her matching subject's index day in the experimental group. All study patients were in hospital at the baseline visit. The researchers retrieved in detail the patients' medical files and collected necessary data. At the same time, the patients were interviewed for EQ-5D-5L questionnaire data collection. The second visit was performed on day 30 after the baseline (index day). At the second visit, all study patients were discharged from hospital and were at their homes. Therefore, EQ-5D-5L data collection was performed during a phone interview through asking and answering the question-by-question. In addition, the researchers asked the patients if the significant changes in their comorbid disease (including drug treatment) had appeared since the time of their hospital stay. The third visit was performed on day 90 after the index day and the researchers collected data using the same approach as for the second visit.

Statistical analysis

We performed sample size calculation based on a presumed level of the mean of weighted EQ-5D-5L total index of 0.7, standard deviation of 0.2 and the difference of at least 15% between the study arms, presuming the poorer scores in the mechanically ventilated patients. The difference was considered clinically significant based on the quality of life estimations of patients with asthma or COPD of different severity types as the appropriate data in the patients with CAP or other types of pneumonia, using EQ-5D, at the time of designing our study were scarce [19]. According to these presumptions, alpha error of 0.05, study power of 0.8 and allocation ratio of 1:1, we calculated the sample size of at least 130 patients, 65 in each study group [20]. Data were analyzed by descriptive and analytical statistics. We also used χ^2 test and *t*-test for the analysis of influence of different demographic, clinical, laboratory factors between the compared groups. All data were expressed as means ± standard deviation or frequencies (percentages). Univariate and multivariate linear regression analyses were performed to characterize predictors associated with the total score of EQ-5D-5L. The factors that were present only during the treatment in hospital and whose influence, in terms of biological sense, could not be extrapolated to the days 30 and 90, were not analyzed in univariate or in a multivariable linear regression model. The probability level of significance was $p \leq 0.05$, with two-tailed approach.

Results

The study population consisted of 130 patients who were divided into two groups, 65 patients each. Both groups had similar socio-demographic and clinical characteristics (Table 1). Only marital status and previous surgeries significantly differed between the study groups.

The distribution of comorbidities among patients, who were mechanically ventilated and those who were not, was unequal. Significant differences were observed in the presence/absence of cardiomyopathy, cerebrovascular diseases, chronic kidney disease, hypertension and pulmonary emphysema (Table 2). Among the study population, the use of enoxaparin, spironolactone, carvedilol, amlodipine, methylprednisolone, salbutamol and aminophylline was significantly more frequent in patients who were mechanically ventilated, while angiotensin converting enzyme (ACE) in-

hibitors, amoxicillin and azithromycin were more frequent in the control group (Table 3). Regarding the findings of blood and serum biochemistry [the mean number of erythrocytes and leukocytes and the mean values of hematocrit, platelets, activated partial thromboplastin time (aPTT), creatinine, urea, C-reactive protein, procalcitonin, sodium, potassium, calcium, partial pressure of oxygen, carbondioxide and bicorbonate (pO_2, pCO_2 and HCO_3^-)], only platelet count was significantly higher in patients who were on MV, whereas glycemia was significantly higher in the control group.

Table 1

Demographic and social characteristics of study population

Variable	Experimental group (n = 65)	Control group (n = 65)	*p**
Age (years), mean ± SD	55.6 ± 14.7	42.6 ± 17.4	1.000[a]
Gender, n (%)			
male	32 (49.2%)	37 (56.9%)	0.380[b]
female	33 (50.8%)	28 (43.1%)	
Smoking, n (%)	22 (33.8%)	44 (67.7%)	0.852[b]
Education, n (%)			
elementary school	3 (4.6%)	5 (7.7%)	
high-school	46 (70.8%)	41 (63.1%)	0.804[b]
faculty	16 (24.6%)	19 (29.2%)	
Marital status, n (%)			
single	24 (36.9%)	7 (10.8%)	0.003[b]
married	34 (52.3%)	51 (78.5%)	
divorced	5 (7.7%)	5 (7.7%)	
widow/widower	2 (3.1%)	2 (3.1%)	
Employment status, n (%)			
unemployed	12 (18.5%)	8 (18.5%)	0.076[b]
employed	40 (61.5%)	36 (55.4%)	
student	5 (7.7%)	2 (3.1%)	
retirement	8 (12.3%)	19 (29.2%)	
Material status, n (%)			
good	33 (50.8%)	30 (46.2%)	0.845[b]
moderate	29 (44.6%)	33 (50.8%)	
bad	2 (3.1%)	2 (3.1%)	
excellent	1 (1.5%)	0 (0%)	
Alcohol intake, n (%)	34 (52.3%)	38 (58.5%)	0.480[b]
Allergies, n (%)			
no	44 (67.7%)	45 (69.2%)	
food allergy	21 (32.3%)	17 (26.2%)	0.200[b]
drug allergies	0 (0%)	3 (4.6%)	
Previous surgery, n (%)	36 (55.4%)	52 (80%)	0.003[b]

***p* – probability of a) – independent sample *t*-test or b) – χ^2 test; SD – standard deviation.**

Table 2

Underlying disease and comorbidities

Variable	Experimental group (n = 65)	Control group (n = 65)	*p**
	n (%)	n (%)	
Cardiomyopathy	26 (40.0)	15 (23.1)	0.038
Cerebrovascular disease	14 (21.5)	3 (4.6)	0.004
Liver disease	14 (21.5)	8 (12.3)	0.160
Chronic kidney disease	18 (27.7)	5 (7.7)	0.003
Diabetes mellitus 1	10 (15.4)	4 (6.2)	0.090
Diabetes mellitus 2	10 (15.4)	2 (3.1)	0.015
Bronchopneumonia	39 (60.0)	30 (46.2)	0.114
Lobar pneumonia	26 (40.0)	31 (47.7)	0.377
Severe influenza	5 (7.7)	10 (15.4)	0.170
COPD	15 (23.1)	10 (15.4)	0.266
Arterial hypertension	19 (29.2)	8 (12.3)	0.017
Pulmonary emphysema	14 (21.5)	5 (7.7)	0.025
Arrhythmias	9 (13.8)	3 (4.6)	0.069
Sepsis	1 (1.5)	4 (6.2)	0.171

***p* – probability of χ^2 test; COPD – chronic obstructive pulmonary disease.**

Table 3

The drugs with significantly different frequency of the prescription between study subgroups

Drug	Experimental group, (n = 65) n (%)	Control group, (n = 65) n (%)	*p*
Enoxaparin	12 (18.8)	3 (4.6)	0.015
Spironolactone	17 (26.6)	4 (6.2)	0.002
Carvedilol	19 (29.7)	8 (12.3)	0.015
Amlodipine	25 (39.1)	7 (10.8)	< 0.001
ACE inhibitors	16 (25.4)	58 (89.2)	0.031
Methylprednisolone	31 (47.7)	8 (12.3)	< 0.001
Amoxicillin	2 (3.1)	10 (15.4)	0.015
Azithromycin	15 (23.1)	26 (40)	0.038
Salbutamol	31 (47.7)	16 (24.6)	0.006
Aminophylline	27 (41.5)	11 (16.9)	0.002

***p* – probability of χ^2 test; ACE – angiotensin converting enzyme.**

The mechanically ventilated patients had worse EQ5D-5L values in comparison to the control group for all 5 domains. Mobility, self-care and usual activities were negatively affected over the whole follow-up period (on days 7, 30 and 90) (Figures 1, 2 and 3); pain/discomfort and anxiety/depression differed significantly between the study and the group on days 7 and 30 (Figures 4 and 5).

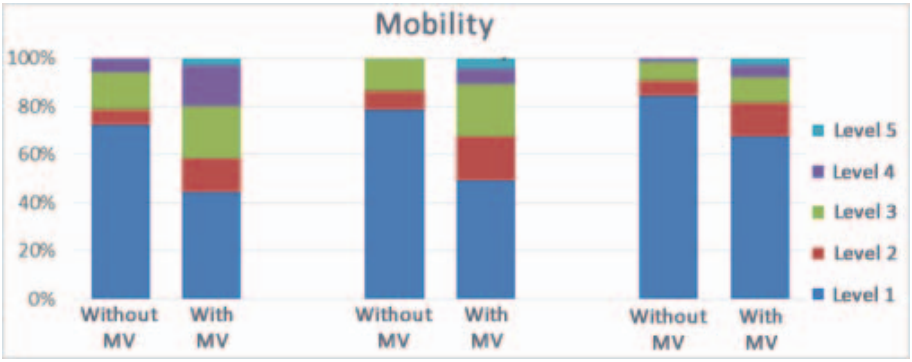

Fig. 1 – EQ-5D values for mobility domain.
MV – mechanical ventilation.

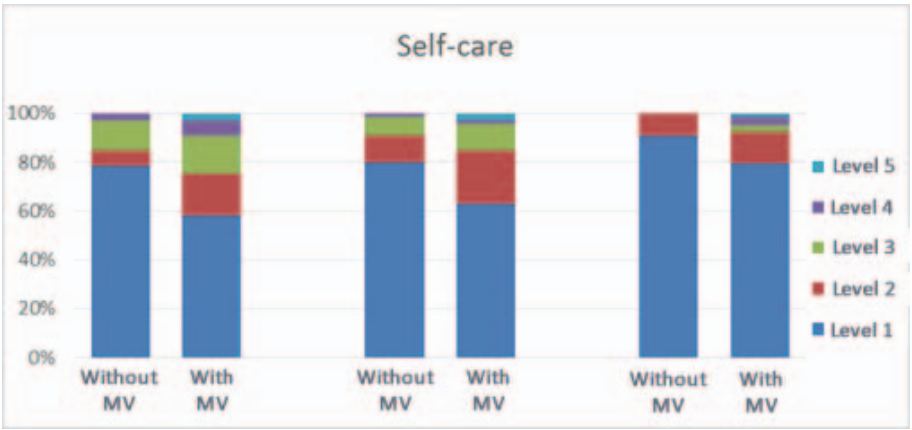

Fig. 2 – EQ-5D values for self-care domain.
MV – mechanical ventilation.

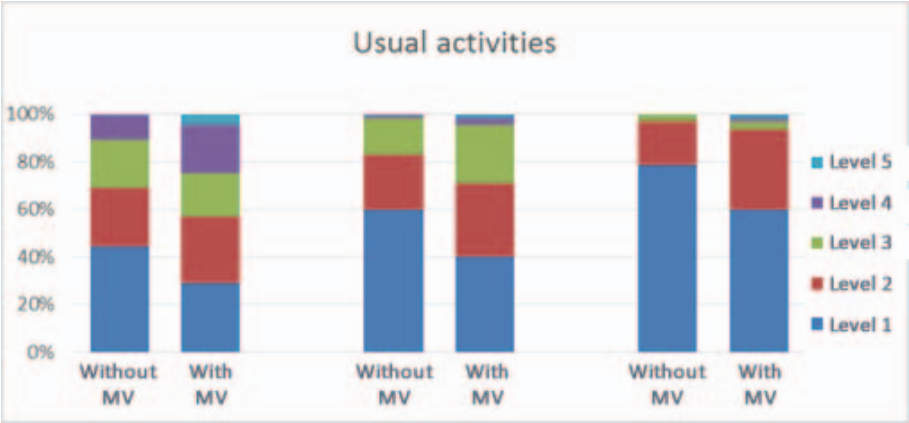

Fig. 3 – EQ-5D values for usual activities domain.
MV – mechanical ventilation.

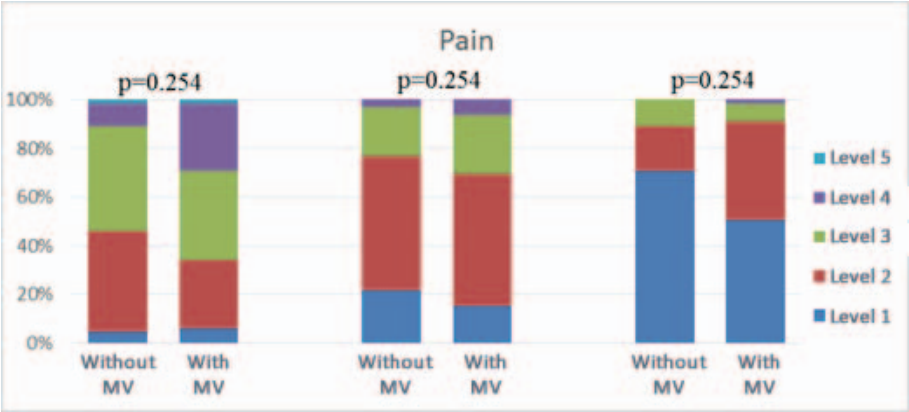

Fig. 4 – EQ-5D values for pain domain.
MV – mechanical ventilation.

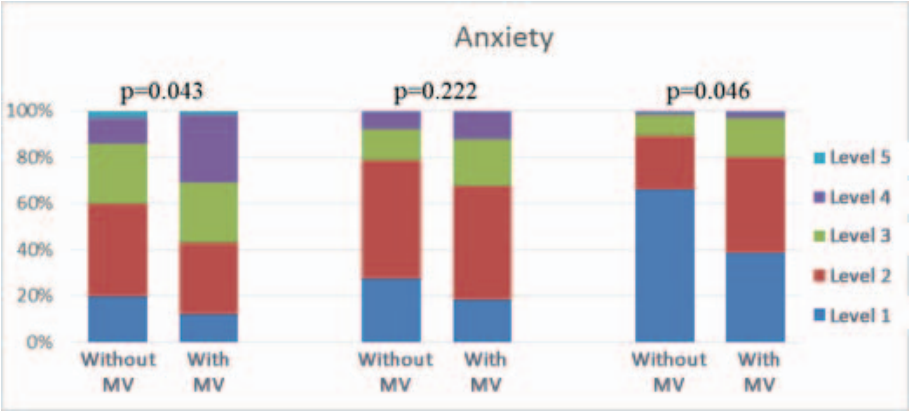

Fig. 5 – EQ-5D values for anxiety domain.
MV – mechanical ventilation.

Average values of EQ-5D VAS scores in patients who were mechanically ventilated during the study period are listed in the table (Table 4). Statistically significant differences in the mean values of EQ-5D VAS scores between the groups were after 7 days ($p = 0.003$), after 30 days ($p = 0.004$) and after 90 days ($p = 0.001$).

Overall, for both groups, there was a significant change in the values of EQ-5D VAS scores in time ($F = 411.406$, $p < 0.001$). There was a significant increase in the value of EQ-5D VAS during the study period (Figure 6). Generally, in the reporting period, there was a statistically significant difference in the values of EQ – 5D VAS scores between groups ($F = 10.010$, $p = 0.002$). There were no significant interactions between the groups and changes in the value of EQ-5D VAS scores during the study period ($F = 0.691$; $p = 0.450$).

Average values of EQ-5D index in the mechanically ventilated patients during the study period are listed in Table 4. Statistically significant differences in mean values of EQ-5D index between the examined groups were noted after 7 days ($p = 0.004$), after 30 days ($p = 0.011$) and after 90 days ($p = 0.003$). Overall, there was a statistically significant

change in the value of EQ-5D index in time (F = 92.598, $p < 0.001$) for both groups. There was a significant increase in the values of the EQ-5D index during the study period (Figure 7). Overall, in the reporting period, there was a significant difference in the values of EQ-5D index (F = 10.027, $p = 0.002$) between the groups. There were no significant interactions between the groups and changes in the values of EQ-5D index during the study period (F = 0.423; $p = 0.621$).

Table 4

The values of EQ-5D VAS domain and EQ-5D index domain in patients with and without mechanical ventilation

Day	Mechanical ventilation	EQ-5DVAS mean ± SD; median (range)	*p*	EQ-5DVAS mean ± SD; median (range)	*p*
7	No	68.4 ± 17.9; 70.0 (20.0–92.0)	0.003	0.70 ± 0.23; 0.78 (0.01–1.00)	0.004
	Yes	58.7 ± 18.2; 60.0 (20.0–90.0)		0.57 ± 0.28; 0.57 (0.05–1.00)	
30	No	76.8 ± 16.0; 80.0 (35.0–100.0)	0.004	0.79 ± 0.19; 0.86 (0.20–1.00)	0.011
	Yes	68.3 ± 17.0; 70 (20.0–95.0)		0.68 ± 0.27; 0.76 (-0.43–1.00)	
90	No	84.7 ± 14.4; 90.0 (45.0–100)	0.001	0.91 ± 0.14; 1.00 (0.54–1.00)	0.003
	Yes	76.0 ± 16.0; 80.0 (30.0–100)		0.80 ± 0.25; 0.86 (0.29–1.00)	

VAS – visual analogue scale; SD-standard deviation.

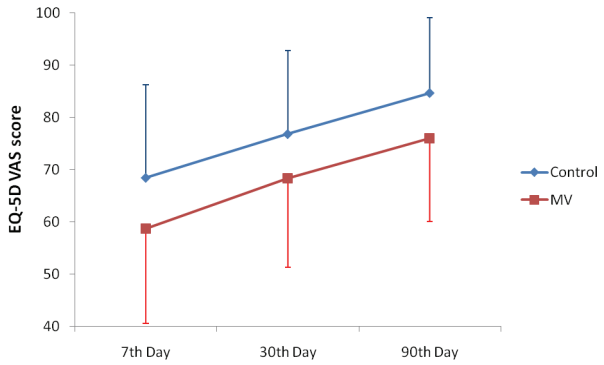

Fig. 6 – EQ-5D VAS scores in the mechanically ventilated (MV) and control group of patients. The points and the vertical bars represent the medians and the interquartile ranges, respectively.
VAS – visual analog scale.

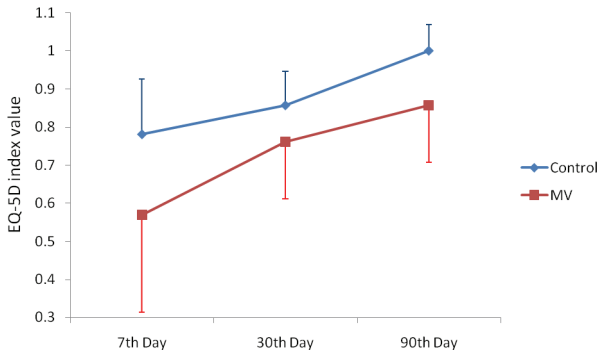

Fig. 7 – EQ-5D index values in the mechanically ventilated (MV) and control group of patients. The points and the vertical bars represent the medians and the interquartile ranges, respectively.

Using univariate linear regression model with the values of the total scores of EQ-5D index on days 7, 30 and 90 being dependent variables (outcome), we found that 15 variables (from the total number of 25 analyzed) were significantly associated with lower values of the EQ-5D index scores, at least at one time, indicating worse quality of life (Table 5). However, no variable consistently influenced the quality of life across all study visits, and, in general, the magnitudes of their effects were mostly mild. In three models of multivariate linear regression (corresponding to three study visits), only age was significantly associated with lower scores of EQ-5D-5L index over two visits. The presence of diabetes mellitus type 1, chronic kidney disease and the use of amlodipine and methylprednisolone were also significant predictors of lower quality of life scores, but their influences were time-limited. Bearing in mind the possibility of indirect associations and other indirect impacts (confounding factors), the impact of variables that were statistically significant was further analyzed by multivariable linear regression models.

The factors that were present only during the treatment in hospital and whose influence, in terms of biological sense, cannot be extrapolated to days 30 and 90, were not analyzed it in this or in a multivariable linear regression model. Approximately, it was assumed that the use of amlodipine, ACE inhibitors and aminophylline was used during the whole period for the treatment of hypertension and pulmonary obstruction, while the impact of methylprednisolone was limited to the first 7 days, while chronic use of oral corticosteroids was not used for such a long period. Therefore, only these four drugs were taken for the multivariate model (Table 6). In addition, the impact of pO_2 and pCO_2 outside the acute treatment period was also unlikely, so they were excluded from this table for days 30 and 90 and from a multivariate model.

Table 5

Variables significantly associated with a total score of EQ-5D in the model of univariate linear regression

Variables	7th day		30th day		90th day	
	B	*p*	B	*p*	B	*p*
Mechanical ventilation	-0.132	0.004	-0.105	0.011	-0.109	0.003
Gender	0.028	0.549	0.014	0.734	0.002	0.960
Age	-0.005	< 0.001	-0.005	< 0.001	-0.004	< 0.001
Previous surgery	-0.122	0.014	-0.123	0.005	-0.075	0.055
Cardiomyopathy	-0.005	0.916	-0.086	0.056	-0.113	0.004
Cerebrovascular disease	-0.073	0.295	-0.123	0.047	-0.107	0.050
Chronic kidney disease	-0.141	0.021	-0.191	< 0.001	-0.131	0.006
Hypertension	-0.133	0.021	-0.105	0.040	-0.080	0.079
Diabetes mellitus type 1	-0.177	0.018	-0.118	0.078	-0.083	0.165
Amlodipine	-0.229	< 0.001	-0.115	0.018	-0.116	0.006
ACE inhibitors	-0.126	0.041	-0.083	0.132	-0.093	0.055
Enoxaparin	-0.132	0.073	-0.239	< 0.001	n.a.	
Azithromycin	0.063	0.213	0.095	0.034	n.a.	
Aminophylline	-0.103	0.045	-0.078	0.087	-0.096	0.017
Methylprednisolone	-0.170	0.001	-0.091	0.046	-0.051	0.206

B – beta coefficient; *p* – probability; n.a. – not applicable (a variable excluded from the model); ACE – angiotensin converting enzyme.

Table 6

Variables significantly associated with a total score of EQ-5D in the models of multivariable linear regression

Variables	7th day		30th day		90th day	
	B	*p*	B	*p*	B	*p*
MV	0.019	0.703	-0.026	0.549	-0.038	0.324
Age	-0.002	0.157	-0.003	0.018	-0.003	0.049
Previous surgery	0.010	0.848	-0.020	0.674	n.a.	n.a.
Cardiomyopathy	n.a.	n.a.	n.a.	n.a.	-0.044	0.298
Cerebrovascular disease	n.a.	n.a.	-0.024	0.717	-0.030	0.606
Chronic kidney disease	-0.088	0.175	-0.129	0.044	-0.053	0.310
Hypertension	-0.018	0.760	0.012	0.828	n.a.	n.a.
Diabetes mellitus type 1	-0.153	0.032	n.a.	n.a.	n.a.	n.a.
Amlodipine	-0.140	0.016	-0.030	0.563	-0.041	0.368
ACE inhibitors	-0.049	0.431	n.a.	n.a.	n.a.	n.a.
Aminophylline	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Methylprednisolone	-0.0157	0.003	n.a.	n.a.	n.a.	n.a.

B – beta coefficient; *p* – probability; n.a. – not applicable (a variable excluded from the model); MV – mechanical ventilation; ACE – angiotensin converting enzyme.

Discussion

The results of this study showed that people with community-acquired pneumonia who had been treated in hospital suffered for weeks after recovery from poor quality of life, due to the acute disease, particularly if they were managed with mechanical ventilation and within an intensive care unit setting. Other researchers have also reported similar findings but, as a rule, with mixed intensive-care patient population, suffering from a variety of diseases that caused acute lung injury and/or respiratory distress syndrome [15, 21, 22]. Only recently, the researchers focused exclusively on the quality of life measurement in patients with pneumonia because such studies had been rare in the past [12].

Other studies are different from our research in some important methodological points. For example, one study included only subjects suffering from severe influenza pneumonia and another one investigated patients with serious lower respiratory tract infections, particularly interested in pneumococcal pneumonia [23, 24]. They assessed the quality of life with the same rating instrument as we did, but only cross-sectionally, omitting the prospective data collection during the longer period. In addition, these and other similar studies, having been conducted so far, did not pay particular attention either to the effects of mechanical ventilation or to the subjects treated within intensive care unit settings as it was the case in our study. The magnitude and the pattern of the change of the quality of life in our study, both within and between subgroups, could be considered clinically important based on the comparisons with the existing data in the field [12, 19, 21–24].

The existing knowledge suggest that pro-inflammatory response, and, to some extent, brain dysfunction are probably the main factors deteriorating the quality of life of the mechanically ventilated CAP patients [17]. It is well known that the activation of immunological cells and the release of inflammatory mediators during pneumonia contributed to both the elimination of the invaders and the injury of the patient, depending on the balance of favorable and harmful factors [25]. Researchers found that in the mechanically ventilated patients with pneumonia there was an large increase of serum and local cytokines like interleukin 6 (IL-6), IL-8 and IL-10 [13, 14]. In addition, the patients with severe

CAP could develop brain dysfunction, and in some of them investigators revealed a significant deterioration of cerebral blood vessels [26, 27]. Cytokine effects, synaptic dysfunction of brain neurotransmitters (particularly dopaminergic, serotonergic, glutamatergic and opioid synapses), disruption of circadian rhythm and the disturbances of neurotrophic mediators (e.g., brain derived neurotrophic factor) are proposed biological mechanisms contributing to fatigue, pain, emotional and social functioning which constitute domains of quality of life perception [17].

There were several demographic and clinical factors, such as age, the presence of chronic kidney disease and diabetes mellitus type 1 that were slightly, but independently associated with the unfavorable outcome in our research. Some researchers identified the pattern of radiological pulmonary findings, prolonged hospital stay, older age, poor functioning at the baseline and persistent weakness during ICU treatments as the risk factors for poor quality of life after surviving pneumonia and/or other severe pulmonary disease requiring mechanical ventilation [22, 28]. Obviously, the existing knowledge about the putative, independent risks is limited and further studies investigating the issue are required.

In our study, univariate analysis revealed many additional factors which were associated with poor quality of life, either in the experimental or the control group, regarding marital status, previous surgery, cardiomyopathy, cerebrovascular disease, diabetes mellitus type 1, hypertension and pulmonary emphysema as well as near a dozen of drugs. However, it is very likely that these factors represent rather the cofounders than the independent predictors of lower scores of the quality of life measurements in our study populations. Indeed, it is well documented that prevalent chronic diseases caused enduring, negative impact on the patient's quality of life resulting in significant medical and socio-economic burden of the modern societies, particularly respiratory, cardiovascular, musculoskeletal, cerebrovascular and mental illnesses [29-34]. These comorbidities are also linked with the use of drugs that were identified in our study as putative risks like amlodipine and methylprednisolone. Researchers had already proved that the use of both, calcium channel blockers and corticosteroids in the treatment of chronic disease, in fact, increased patients' quality of life [35-37]. Therefore, it seems that the quality of life in the mechanically ventilated CAP patients is influenced rather by the complex milieu of numerous subtle-acting, highly interconnected, intrinsic and acquired factors than by the profound effects of leading causes.

Results of EQ5D-5L questionnaire showed that the use of MV as a part of CAP treatment in our study was associated with poorer outcome in terms of mobility, self-care and usual activities as well as to lesser extent, pain/discomfort and anxiety/depression domains. The others just reported that the patients with CAP had the decrease of both the total EQ-5D weighted index and the total VAS score, indicating poor quality of life in general sense; but, mobility, self-care and usual activities were much affected, similarly to our observations [21]. In intensive-care patients with pneumonia and/or sepsis the high Simplified Acute Physiology Score (SAPS) II predicted low scores on EQ-5D dimensions, particularly of physical components that were in good agreement with our results [15]. In our study, the negative trend for the first three domains was maintained during 7, 30 and 90 days after the hospitalization, and for the last other two only on days 7 and 90. In general, similar findings were observed in a previous study which showed that the use of MV was linked with the poorer quality of life, far beyond the end of active treatment, at 3 and 12 months after discharge from ICU [28]. There were suggestions that the presence of symptoms of CAP beyond 28 days and any impairment in quality of life was a reflection of age and comorbidity rather than persistent effects of pneumonia itself [38]. However, the effects of other risk factors that were not followed in this study, but acting independently on the quality of life could not be excluded yet.

The main limitations of our study are moderate-sample size, the use of a single quality of life instrument, reliance primarily on variables used in routine health-care and the possibility of existence of unidentified significant, independent risks as well as selection bias. We powered our study to detect the difference of weighted EQ-5D-5L index of presumed clinical significance between the study arms at prespecified level only in the survivors after the treatment as the intubation for MV precluded baseline measurements. The details about biological basis of the outcomes observed in our research remained poorly understood and this requires further prospective research studies, probably with combination of experimental and clinical approaches. For example, novel biomarkers, pro-adrenomedullin and pro-atrial natriuretic peptide have been recently found superior to conventional laboratory parameters like leukocyte numbers, C-reactive protein and procalcitonin in prediction of poor quality of life of patients with CAP [21].

Our study included only the survivors who could provide us with reliable data at weeks after the hospital treatment. Consequently, selection bias, and, to some extent, bias due to the missing data about newly emerged comorbidities and their treatments could not be completely excluded. However, selection bias is often avoidable in published studies that investigate the quality of life, as is shown in the studies in orthopedics, neurology, vascular surgery and treatment of obesity and osteoporosis, which also used EQ-5D as the primary instrument of quality of life assessment [39–42].

Conclusion

Patients with MV tend to have poorer quality of life especially in three domains presented in the study. The main reasons are the presence of chronic comorbidities in this population which require MV, especially chronic kidney disease and diabetes mellitus type 1 which was of the greatest significance. The influence of individual factors is relatively mild, requiring a holistic approach to quality of life. The determinants of poor quality of life in this population have extended the period of active treatment that requires permanent care, especially bearing in mind the effects of age.

Acknowledgement

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R E F E R E N C E S

1. *Dhar R.* Pneumonia: review of guidelines. J Assoc Physicians India 2012; 60Suppl: 25–8.
2. *Torres A, Peetermans WE, Viegi G, Blasi F.* Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax 2013; 68(11): 1057–65.
3. *Walden AP, Clarke GM, McKechnie S, Hutton P, Gordon AC, Rello J*, et al. Patients with community acquired pneumonia admitted to European intensive care units: an epidemiological survey of the GenOSept cohort. Crit Care 2014; 18(2): R58.
4. *Sinđelić R.* Mechanical ventilation of the lungs. Belgrade: Medicinska knjiga, Medicinske komunikacije; 1998. (Serbian)
5. *Euteneuer S, Windisch W, Suchi S, Köhler D, Jones PW, Schönhofer B.* Health-related quality of life in patients with chronic respiratory failure after long-term mechanical ventilation. Respir Med 2006; 100(3): 477–86.
6. *López-Campos JL, Failde I, Masa JF, Benítez-Moya JM, Barrot E, Ayerbe R,* et al. Factors related to quality of life in patients receiving home mechanical ventilation. Respir Med 2008; 102(4): 605–12.
7. *Khanna D, Tsevat J.* Health-related quality of life--an introduction. Am J Manag Care 2007; 13 Suppl 9: S218–23.
8. *Song JH, Thamlikitkul V, Hsueh PR.* Clinical and economic burden of community-acquired pneumonia amongst adults in the Asia-Pacific region. Int J Antimicrob Agents 2011; 38(2): 108–17.
9. *Welte T, Torres A, Nathwani D.* Clinical and economic burden of community-acquired pneumonia among adults in Europe. Thorax 2012; 67(1): 71–9.
10. *Isturiz RE, Luna CM, Ramirez J.* Clinical and economic burden of pneumonia among adults in Latin America. Int J Infect Dis 2010; 14(10): e852–6.
11. *File TM Jr, Marrie TJ.* Burden of community-acquired pneumonia in North American adults. Postgrad Med 2010; 122(2): 130–41.
12. *Allin D, James I, Zachariah J, Carr W, Cullen S, Middleton A,* et al. Comparison of once- and twice-daily clarithromycin in the treatment of adults with severe acute lower respiratory tract infections. Clin Ther 2001; 23(12): 1958–68.
13. *Wu CL, Chan MC, Chang GC, Lee YL, Chin CS, Chang KM,* et al. Etiology and cytokine expression in patients requiring mechanical ventilation due to severe community-acquired pneumonia. J Formos Med Assoc 2006; 105(1): 49–55.
14. *Meier T, Lange A, Papenberg H, Ziemann M, Fentrop C, Uhlig U,* et al. Pulmonary cytokine responses during mechanical ventilation of noninjured lungs with and without end-expiratory pressure. Anesth Analg 2008; 107(4): 1265–75.
15. *Honselmann KC, Buthut F, Heuwer B, Karadag S, Sayk F, Kurowski V,* et al. Long-term mortality and quality of life in intensive care patients treated for pneumonia and/or sepsis: Predictors of mortality and quality of life in patients with sepsis/pneumonia. J Crit Care 2015; 30(4): 721–6.
16. *Nickler M, Schaffner D, Christ-Crain M, Ottiger M, Thomann R, Hoess C*, et al. Prospective evaluation of biomarkers for prediction of quality of life in community-acquired pneumonia. Clin Chem Lab Med 2016; 54(11): 1831–46.
17. *Sprangers MA, Thong MS, Bartels M, Barsevick A, Ordoñana J, Shi Q,* et al. Biological pathways, candidate genes, and molecular markers associated with quality-of-life domains: an update. Qual Life Res 2014; 23(7): 1997–2013.
18. *Herdman M, Gudex C, Lloyd A, Janssen M, Kind P, Parkin D,* et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res 2011; 20(10): 1727–36.
19. *Szende A, Leidy NK, Ståhl E, Svensson K.* Estimating health utilities in patients with asthma and COPD: evidence on the performance of EQ-5D and SF-6D. Qual Life Res 2009; 18(2): 267–72.
20. *Dupont WD, Plummer WD Jr.* Power and sample size calculations. A review and computer program. Control Clin Trials 1990; 11(2): 116–28.
21. *Hamel MB, Phillips RS, Davis RB, Teno J, Connors AF, Desbiens N,* et al. Outcomes and cost-effectiveness of ventilator support and aggressive care for patients with acute respiratory failure due to pneumonia or acute respiratory distress syndrome. Am J Med 2000; 109(8): 614–20.
22. *Burnham EL, Hyzy RC, Paine R 3rd, Coley C 2nd, Kelly AM, Quint LE,* et al. Chest CT features are associated with poorer quality of life in acute lung injury survivors. Crit Care Med 2013; 41(2): 445–56.
23. *Garcia Gutierrez S, Quintana JM, Baricot M, Bilbao A, Capelastegui A, Cilla Eguiluz CG,* et al. Predictive factors of severe multilobar pneumonia and shock in patients with influenza. Emerg Med J 2014; 31(4): 301–7.
24. *Flamaing J, De Backer W, Van Laethem Y, Heijmans S, Mignon A.* Pneumococcal lower respiratory tract infections in adults: an observational case-control study in primary care in Belgium. BMC Fam Pract 2015; 16: 66.
25. *Bordon J, Aliberti S, Fernandez-Botran R, Uriarte SM, Rane MJ, Duvvuri P,* et al. Understanding the roles of cytokines and neutrophil activity and neutrophil apoptosis in the protective versus deleterious inflammatory response in pneumonia. Int J Infect Dis 2013; 17(2): e76–83.
26. *Tomasi CD, Vuolo F, Generoso J, Soares M, Barichello T, Quevedo J,* et al. Biomarkers of Delirium in a Low-Risk Community-Acquired Pneumonia-Induced Sepsis. Mol Neurobiol 2017; 54(1): 722–6.
27. *Rosengarten B, Krekel D, Kuhnert S, Schulz R.* Early neurovascular uncoupling in the brain during community acquired pneumonia. Crit Care 2012; 16(2): R64.
28. *Busico M, Intile D, Sívori M, Irastorza N, Alvarez AL, Quintana J,* et al. Risk factors for worsened quality of life in patients on mechanical ventilation. A prospective multicenter study. Med Intensiva 2016; 40(7): 422–30.
29. *Alonso J, Ferrer M, Gandek B, Ware JE Jr, Aaronson NK, Mosconi P,* et al. Health-related quality of life associated with chronic conditions in eight countries: results from the International Quality of Life Assessment (IQOLA) Project. Qual Life Res 2004; 13(2): 283–98.
30. *Mo F, Choi BC, Li FC, Merrick J.* Using Health Utility Index (HUI) for measuring the impact on health-related quality of Life (HRQL) among individuals with chronic diseases. ScientificWorldJournal 2004; 4: 746–57.
31. *Kempen GI, Ormel J, Brilman EI, Relyveld J.* Adaptive responses among Dutch elderly: the impact of eight chronic medical conditions on health-related quality of life. Am J Public Health 1997; 87(1): 38–44.
32. *Sabbah I, Drouby N, Sabbah S, Retel-Rude N, Mercier M.* Quality of life in rural and urban populations in Lebanon using SF-36 health survey. Health Qual Life Outcomes 2003; 1: 30.
33. *Lima MG, Barros MB, César CL, Goldbaum M, Carandina L, Ciconelli RM.* Impact of chronic disease on quality of life among the elderly in the state of São Paulo, Brazil: a population-based study. Rev Panam Salud Publica 2009; 25(4): 314–21.
34. Tüzün H, Aycan S, İlhan MN. Impact of comorbidity and socioeconomic status on quality of life in patients with chronic diseases who attend primary health care centers.. Cent Eur J Public Health 2015; 23(3): 188–94.

35. *Zanchetti A, Omboni S, La Commare P, De Cesaris R, Palatini P.* Efficacy, tolerability, and impact on quality of life of long-term treatment with manidipine or amlodipine in patients with essential hypertension. J Cardiovasc Pharmacol 2001; 38(4): 642–50.
36. *Bhargava S, Prakash A, Rehan HS, Gupta LK.* Effect of systemic corticosteroids on serum apoptotic markers and quality of life in patients with asthma. Allergy Asthma Proc 2015; 36(4): 275–82.
37. *Paulsen O, Klepstad P, Rosland JH, Aass N, Albert E, Fayers P,* et al. Efficacy of methylprednisolone on pain, fatigue, and appetite loss in patients with advanced cancer using opioids: a randomized, placebo-controlled, double-blind trial. J Clin Oncol 2014; 32(29): 3221–8.
38. *El Moussaoui R, Opmeer BC, de Borgie CA, Nieuwkerk P, Bossuyt PM, Speelman P,* et al. Long-term symptom recovery and health-related quality of life in patients with mild-to-moderate-severe community-acquired pneumonia. Chest 2006; 130(4): 1165–72.
39. *Yoh K, Hamaya E, Urushihara H, Iikuni N, Yamamoto T, Taketsuna M,* et al. Quality of life in raloxifene-treated Japanese women with postmenopausal osteoporosis: a prospective, postmarketing observational study. Curr Med Res Opin 2012; 28(11): 1757–66.
40. *Date RS, Walton SJ, Ryan N, Rahman SN, Henley NC.* Is selection bias toward super obese patients in the rationing of metabolic surgery justified?-A pilot study from the United Kingdom. Surg Obes Relat Dis 2013; 9(6): 981–6.
41. *Falk Hvidberg M, Brinth LS, Olesen AV, Petersen KD, Ehlers L.* The Health-Related Quality of Life for Patients with Myalgic Encephalomyelitis / Chronic Fatigue Syndrome (ME/CFS). PLoS One 2015; 10(7): e0132421.
42. *Rolfson O, Donahue GS, Hallsten M, Garellick G, Kärrholm J, Nemes S.* Patient-reported outcomes in cemented and uncemented total hip replacements. Hip Int 2016; 26(5): 451–7.

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Physical activity and bone turnover in women with osteopenia

Fizička aktivnost i povećanje volumena kostiju kod žena sa osteopenijom

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Abstract

Background/Aim. Osteoporosis is a systemic disease of the skeleton characterized by a decrease in bone mass and changes in the bone structure. An increased tendency of the bone tissue for fractures occurs as a consequence of these changes. The initial phase of physiological aging of the bones that gradually leads to osteoporosis is osteopenia. This paper tracks the effects of a specific kind of physical exercise program in women with osteopenia. The aim was to quantify the impact of this program on: the concentration of bone metabolism blood markers, muscle strength, aerobic capacity, and physical dimensions. **Methods**. The sample consisted of 26 women in postmenopause (age 46–58) divided into two groups – experimental group (n = 15) and control group (n = 11). A combined program of exercise consisting of aerobic activities and strength training was applied in the experimental group, while the control group did not join in the exercise program. The program lasted for 7 weeks, three times a week with a break day between the trainings. The intensity of the aerobic training was in the span of 60% to 70% of heart rate reserve (HRR), and the intensity of the strength training was in the span of 60% to 85% of one repetitive maximum (1RM). Osteopenia was diagnosed prior to the experiment by applying a dual energy X-ray absorptiometry of the lumbar spine and the hip. The following was measured before and after the experiment: the level of biochemical markers in the serum [Beta-aspartic acid β-cross laps (CTx), total procollagen type 1 N-terminal peptide (tP1NP) and bone isoenzyme of alkaline phosphatase (ALP), 1RM of leg extensors, maximum oxygen consumption (VO_2 max), bodily height and mass, and a calculated Body Mass Index (BMI). **Results.** Significant changes were determined only in the experimental group. During the experimental period, there was a significant increase of muscle strength and VO_2 max, with a decrease of Beta-CTx concentration. No statistically significant changes were recorded in the control group. **Conclusion**. A 7-week period of systematic exercise showed to be sufficient to increase muscle strength and VO_2 max, partially also to decrease bone resorption, but insufficient to alter bone volume, bodily mass, and BMI.

Key words:
bone diseases, metabolic; osteoporosis, postmenopausal; exercise; densitometry; blood chemical analysis; muscle tonus; serbia.

Apstrakt

Uvod/Cilj. Osteoporoza je sistemska bolest koštanog sistema koju karakteriše smanjenje koštane mase i promene u koštanoj strukturi. Povećana sklonost ka prelomima kostiju posledica su gore navedenih promena. Početna faza fiziološkog starenja kostiju je osteopenija, koja postepeno dovodi do osteoporoze. Cilj ovog istraživanja bio je utvrđivanje efekata aerobnih aktivnosti i vežbi snage na volumen kostiju žena sa osteopenijom. Efekat je utvrđivan procenom efekta vežbi na koncentraciju markera koštanog metabolizma u krvi, jačinu mišića, aerobni kapacitet i fizičke parametre. **Metode.** Uzorak je činilo 26 žena u postmenopauzi, koje su podeljene u dve grupe – eksperimentalnu (n = 15) i kontrolnu (n = 11). U eksperimentalnoj grupi primenjen je kombinovani program vežbanja koji se sastojao iz aerobnih aktivnosti i vežbi snage, dok kontrolna grupa nije učestvovala u programu vežbanja. Program je trajao sedam nedelja, tri puta nedeljno sa danom pauze između treninga. Intenzitet aerobnog treninga kretao se između 60% i 70% srčane rezerve, a intenzitet u treningu snage između 60% i 85% jednog repetitivnog maksimuma (IRM). Mineralna gustina kostiju lumbarnog dela kičme i kukova merena je metodom apsorciometrije X zraka (DEXA). Izmereni su nivoi beta-*cross laps* (CTx), ukupnog prokologen tipa 1 N-terminalnog peptida (tP1NP) i koštanog izoenzima alkalne fosfataze (ALP) u serumu. **Rezultati.** Rezultati pokazuju da je eksperimentalni tretman doveo prvenstveno do značajnog povećanja mišićne sposobnosti u eksperimentalnoj grupi. Takođe, utvrđeno je značajno smanjenje koncentracije Beta CTx, uz neznačajne promene nivoa tP1NP i ALP. U kontrolnoj grupi nisu na-

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đene značajne promene ispitivanih parametara. **Zaključak**. Na osnovu rezultata možemo da zaključimo da je period od sedam nedelja dovoljan za smanjenje koštane resorpcije, ali nedovoljan za promenu koštanog volumena, telesne mase, indeksa telesne mase.

Ključne reči:
kosti, metaboličke bolesti; osteoporoza posle menopauze; vežbanje; denzitometrija; krv, hemijske analize; mišići, tonus; srbija.

Introduction

Osteoporosis is a systemic disease of the skeleton characterized by a decrease in mineral bone density (BMD) and by a disruption of microarchitecture of the bone tissue [1, 2]. These changes lead to a lower bone density and increased chance of fracture [3, 4]. Osteoporosis is widespread and is reaching epidemic scales. Currently, there are over 200 million people in the world suffering from osteoporosis, primarily women of older age. After the age of 40, mineral bone density progressively decreases for about 0.5% per year, particularly in women [5]. The prevalence of osteoporosis increases from 4% in women ages 50–59 to 52% in women over the age of 80 [6]. Osteoporotic fractures show a growing trend. The possibility of these fractures occurring during one's lifetime is 50% in women and 25% in men [7].

Osteopenia is the initial phase of physiological aging of the bones that leads to osteoporosis with age. Osteopenia and osteoporosis are diagnosed by applying a dual energy X-ray absorptiometry of the lumbar spine and the hip (DXA) that is used to acquire the values of bone mineral density (BMD) and T-score [8]. T-score is the difference between the current bone mass and the average value of bone mass maximum in young persons [9]. Indication of normal condition is a T-score between -1 and 1. When the values of T-score are between -1 and -2.5 the person suffers from osteopenia, while values lower than -2.5 indicate osteoporosis [9]. Around the age of 25 bones achieve maximum density (peak bone mass). After this, the BMD stagnates and begins to decrease after menopause as the resorption quickens and surpasses bone formation. This leads to osteopenia and it usually occurs in premenopause when BMD decreases for about 2% per year in women. In postmenopause, BMD decreases for about 1%–1.5% a year. In their eight decade, women have about 30% lower BMD than in their third decade of life, which significantly increases the risk of fracture [10, 11]. Osteopenia is a safe indication to start applying therapy treatments [12].

The best way of combating premature osteopenia is prevention [12]. There is a significant link between increased physical activity and BMD. It plays an important role in increasing bone mass during childhood and early adolescence [13]. After the age of 35, dosed physical activity significantly contributes to maintaining bone mass, slows down its loss and decreases the risk of fracture [14] in the elderly. Bone quality gained by exercising cannot be permanent if the exercise is not regular [9]. The evidence of this is the reduction of bone mass even in younger women that occurs as a consequence of immobilization due to injury of movement apparatus [15] (locomotor apparatus). Several scientific studies indicate a positive influence of systematic physical activity on bone mass [16–18]. On the other hand, physical inactivity (hypokinesia) negatively influences bone turnover and increases resorption [19]. Apart from having a direct impact on the bones, hypokinesia decreases muscle strength, which decreases the ability of the locomotor apparatus and increases the risk of falling and fractures [20].

Physical activity shows a significant connection with osteopenia and osteoporosis indirectly through body weight (BW), the amount of fat tissue and body mass index (BMI). BW lower than 63.7 kg and MBI lower than 19 kg/m^2 are believed to be a risk factor for osteoporosis [21]. Some studies also show that obesity is a risk factor considering it is connected with cardiovascular diseases, hypertension and a lowered vitamin D level [22]. Apart from that, fat tissue secretes cytokines, which has an influence on increasing bone resorption and adipokines that change the effect of sympathetic nervous system on the bone tissue [23]. The possibility of using regular aerobic exercise to efficiently influence the decrease of fat tissue and the regulation of BW and MBI is an additional reason for researching the impact of physical activity on osteopenia. The aim of this study was to determine precisely the reaction of the bone system to the 7-weeks exercise program in menopausal women with osteopenia. The exercise program consisted of a combination of aerobic activities and strength exercises with own weight and resistance training.

Methods

Study design

The study was performed in accordance with the Declaration of Helsinki. All examinees were patients of the Department of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Vojvodina in Novi Sad, Serbia and have all signed an agreement to voluntarily participate in the experiment.

The research was realized as an experiment with two parallel groups (experimental and control) and it lasted for 7 weeks. The experiment consisted only of the persons diagnosed with osteopenia by DXA. In the experimental group a special program of exercise adapted to menopausal women of low physical fitness was applied. The program was realized at the Faculty of Sports and Tourism in Novi Sad. Both groups consisted of women whose age, physical fitness and BMD were approximately of the same values. During the experimental period, the examinees of the control group kept to their usual habits and conducted their usual daily activities. Furthermore, examinees of both groups had normal diets (ate in their usual way). None of the examinees took any

special medical therapy that could have potentially disrupted the experimental factor.

Three days before and after the experiment, the examinees of both groups were measured for BW, had their BMI calculated, their maximum oxygen consumption, strength of leg extensors, and had blood sample drawn that resulted in three pieces of data: bone turnover blood markers: β-cross laps (beta-CTx) and total procollagen type 1 N-terminal peptide (tP1NP) and alkaline phosphatase (ALP) bone isoenzyme. Bodily height (BH) was measured only prior to the experiment. Initial values of the tracked variables (pretest) were compared with the corresponding final values (post-test).

Sample

The final sample consisted of 26 able-bodied women, age of 46 and 58 that completed all the necessary criteria. The condition for entering the final sample was that all the examinees are in postmenopause and had low physical activity in the past few years, that they underwent pretest and post-test, that they did not take any medical therapy during the experimental period and that their T-score on DXA analysis was between -1 and -2.5. Additional condition that was given to the examinees of the experimental group was that they participated in every training session. The examinees voluntarily agreed to be divided into the experimental and the control group. The examinees of the experimental group confirmed their willingness to undergo the experimental treatment and to regularly partake in every training session. The examinees of the control group did not wish to undergo systematic exercise, but they have agreed to take all measurements and to continue their usual activities during the experimental period.

The initial sample consisted of 35 examinees (20 in the experimental and 15 in the control group). There was a reduction in the number of subjects during the experimental period. Five women were excluded from the experimental group: 4 that did not participate in all training sessions due to sessional illnesses, and one that was diagnosed with the risk of increased straining (hypertension and tachycardia during the VO_2max test). Four women were excluded from the control group: three who began taking a hormonal treatment during the experiment, and one who did not show up for the final measurements. Therefore, the final number of women in the experimental group was 15, and 11 in the control group.

Instruments and material

Measuring of the bone mass was conducted at the Department of Endocrinology, Diabetes and Metabolic Diseases, Clinical Center of Vojvodina in Novi Sad. A modern device for osteodensitometry of the „Hologic Explorer QDR" type was used. DXA procedure (Dual X-Ray Absorptiometry) was used as a reliable and quick method, as it is considered to be a „golden" standard of measurement for mineral bone density [8]. For the needs of this study, the data regarding BMD recorded on the lumbar spine and the hip were used. Absolute values (g/cm^2) for each examinee were compared with the average values of the female population in menopause and on the basis of individual deviation, the relative values were automatically calculated (T-scores).

To analyze the level of bone resorption, blood marker beta-CTx was used. To analyze the process of bone formation, blood marker tP1NP was also used. As additional biochemical parameter, ALP bone isoenzyme was monitored, the heightened values of which can indicate increased bone turnover. Sampling of the blood and laboratory analysis of both bone markers (beta-CTx and tP1NP) and ALP bone isoenzyme before and after the experiment was conducted in the reference laboratory "Eurolab" in Novi Sad. Sampling of the serum was conducted in early morning hours after a nightly fast. To determine the concentration of bone markers, enzyme-related immunosorbent kit (Roche Diagnostics) was used.

The evaluation of the aerobic capacity was conducted based on VO_2max by applying Bruce submaximal test according to the protocol predicted by the American Collage of Sport Medicine (ACSM) [24]. Running was not applied, only walking on a treadmill of which the grade and speed were progressively increased. The brand of the treadmill was Trackmaster by JAS (model TMX425C). For all women, general indications for stopping an exercise test in low risk adults [24] were excluded. The test protocol consisted of three stages, each lasting 3 minutes. The initial speed was 3 km/h and grade 10%. At second stage speed was 4 km/h with grade of 12% and at the final stage, it was 5.5 km/h and 14%. The examinee's heart rate (HR) was measured every minute. The concept of steady-state HR (HR_{ss}) was applied (within 6 bmp). If the examinee was not at a steady state by the 3rd minute, then they would continue to walk at that same pace for another minute. The examinees should complete all three stages. The first stage was considered to be a warm-up stage. The HR_{ss} should be between 115 and 155 bmp for the last two stages. The maximal aerobic capacity (VO_2max) was calculated according to the ACSM formula [24], in which the HR_{ss} was the main parameter measured during the second and the third stage.

To test the muscle strength of the leg extensors, one repctitive maximum (1RM) testing protocol was used, as suggested by Baechle and Earle [25]. This test protocol was used even in earlier research [26, 27] and was recommended as suitable for mass use since it did not require complex laboratory equipment. The testing session consisted of recording the body weight and age, verification of the equipment adjustments, and performance of about 5–10 repetitions of a slightly heavier weight. Formal strength testing began on the third visit. After a warm-up consisting of 10 repetitions of a light weight, a rest, and 5 repetitions of a medium weight, subjects were tested for the concentric 1RM, the heaviest weight that could be lifted for one repetition. Testing included single attempts at progressively heavier weights until the 1RM was identified.

Experimental program (Combined training program)

The program was performed in 7 weeks with a frequency of 3 nonconsecutive days per week. Aerobic training intensity varied between 60% and 70% heart rate reserve

(HRR, and resistance training intensity varied between 60% and 85% of 1RM. The program consisted of three parts. The first part was performed for 2 weeks, and the aim of this part was to prepare the participants for further loads. In the resistance training section (Table 1) the participants performed exercise which develops muscle endurance of major muscle groups and core exercises. In the aerobic training section, the participants performed exercises such as walking, running, hooping and jumping, with intensity from 60% of HRR. Aerobic exercises were applied during the first and last 10 minutes of each workout. To control the intensity of the activity, meaning HR monitoring, „Polar FT60" Holter pulsemeter monitor was worn by each examinee during the workout.

Table 1

Resistance training program

First part	Second part	Third part
Push-up	Leg press	Leg press
Curl-up	Bench press	Bench press
Trunk extension	Deadlift	Deadlift
Squat	Seated row	Seated row
Core exercise	Abdominal crunch	Abdominal crunch

The percentage of HRR was determined by Karvonen formula [30], the key elements of which were the maximum heart rate (HR_{max}), and resting heart rate (HR_{rest}). HR_{max} was calculated using the formula 220–age [31]. Target heart rate (THR) was calculated using the formula:

$$THR = [(HR_{max} - HR_{rest}) \times \% \text{ intensity}] + HR_{rest}$$

The second part was performed within 3 weeks and in the resistance training section the participants performed resistance exercise with intensity ranging from 60% of 1RM (first week) and in aerobic section from 70% HRR. In the second week, resistance exercise intensity was 65% 1RM and in the third week 70% 1RM. The third part lasted two weeks and in the resistance training section, the participants performed resistance exercises with intensity varying between 75% 1RM (first week) and 85% 1RM (second week) and in the aerobic section with intensity from 70% HRR.

Statistical analysis

For all variables tracked in the research, representative descriptive parameters were calculated (Mean and standard Deviation). *T*-test for independent samples was applied to test the significance of differences between the results of the experimental and the control group on the pre-test and the post-test. To analyze the effects of the experimental treatment, analysis of variance with repeated measures that combined two subjectswas used: the time and the specificity of groups. This statistical procedure is called Mixed between-within subjects ANOVA by Tabacnick [28] and Pallant [29]. Portable IBM SPSS v.19 application was used for the analysis and all the conclusions were drawn based on 0.05 level of significance ($p \leq 0.05$).

Results

DXA analysis showed that all examinees had a minimum of two points with T-score below -1. Far more, such points were registered on the lumbar spine than on the hip, as it was expected and was in accordance with the results of previous studies [10, 32]. Both average T-scores gained from the hip analysis on the level of the total sample were above -1, but all average T-scores gained from the lumbar spine analysis were below -1. This confirmed that both groups (experimental and control) were typical representatives of middle age women with osteopenia. The average values of BMD of the experimental and the control groups did not show a statistically significant difference (Table 2). This is the evidence that the groups were homogenous compared to the bone status. Homogeneity of the groups prior to the experiment was also confirmed by the results of other analyses (Table 3). Arithmetic means of anthropometric variables – body height, weight and mass index (BH, BW and BMI, respectively), biochemical markers (beta-CTx, tP1NP i ALP), VO_2max and 1RM of the experimental and the control group also did not show a statistically significant difference on the pre-test. In this way, numerous factors that could influenced the reliability of the conclusions were eliminated.

Table 2

Dual energy X-ray absorptiometry (DXA) indicators of the bone mass status for two groups of examinees

Variable	Experimental (n = 15)		Control (n = 11)		*t*-test	
	mean	SD	mean	SD	*t*	*p*
L1 BMD (g/cm^2)	0.891	0.063	0.987	0.177	-1.938	0.065
L1 T-score	-1.9	0.5	-1.2	1.4	-3.102	0.005
L2 BMD (g/cm^2)	0.977	0.123	1.001	0.154	-0.435	0.668
L2 T-score	-1.9	1.0	-1.1	1.5	-1.532	0.139
L3 BMD (g/cm^2)	1.020	0.112	1.023	0.1437	-0.055	0.956
L3 T- score	-1.5	0.9	-1.1	1.3	-0.874	0.391
L4 BMD (g/cm^2)	0.993	0.121	1.001	0.183	-0.124	0.903
L4 T- score	-1.3	1.5	-1.2	1.5	-0.143	0.888
L-Total BMD (g/cm^2)	0.973	0.096	1.004	0.159	-0.609	0.549
L-Total T- score	-1.7	0.8	-1.0	1.4	-1.567	0.131
Hip-Neck BMD (g/cm^2)	0.876	0.110	0.830	0.117	0.984	0.335
Hip-Neck T- score	-0.9	0.9	-0.9	0.6	-0.029	0.977
Hip-Total (g/cm^2)	0.924	0.126	0.903	0.087	0.461	0.649
Hip-Total T- score	-0.453	1.138	-0.610	0.604	0.398	0.695

L1-5 – Lumbar vertebral 1-5; BMD – Bone Mineral Density; SD – standard deviation.

Table 3

Descriptive statistics of pretest and post-test for experimental (n = 15) and control group (n = 11)

Variable	Group	Pretest		Post-test	
		mean	SD	mean	SD
Beta-CTx (pg/mL)	Experimental	550.87	164.474	489.09	175.662
	Control	489.09	175.662	438.00	157.425
	t-test	*t* = 0.920	*Sig.* = 0.367	*t* = 0.055	*Sig.* = 0.957
tP1NP (mcg/L)	Experimental	68.27	19.295	64.78	24.1653
	Control	55.12	14.167	52.29	15.716
	t-test	*t* = 1.911	*Sig.* = 0.068	*t* = 1.494	*Sig.* = 0.148
ALP (U/L)	Experimental	77.07	18.425	74.47	19.272
	Control	72.55	26.909	74.73	27.626
	t-test	*t* = 0.509	*Sig.* = 0.615	*t* = -0.028	*Sig.* = 0.978
Leg-Press (kp)	Experimental	118.93	40.006	168.53	40.977
	Control	119.70	40.255	118.10	34.936
	t-test	*t* = -0.063	*Sig.* = 0.951	*t* = 3.333*	*Sig.* = 0.003
VO_2 max (mL/kg/min)	Experimental	29.77	4.831	32.76	4.317
	Control	28.82	3.882	28.61	3.787
	t-test	*t* = 0.530	*Sig.*= 0.601	*t* = 2.514*	*Sig.*= 0.019
Body height (m)	Experimental	1.629	0.060	/	/
	Control	1.649	0.092	/	/
	t-test	*t* = -0.678	*Sig.*= 0.504		
Body weight (kg)	Experimental	71.17	9.482	69.90	8.970
	Control	74.54	14.478	74.33	14.160
	t-test	*t* = -0.720	*Sig.*= 0.479	*t* = -0.974	*Sig.* = 0.340
Body mass index (kg/m^2)	Experimental	26.79	3.082	26.32	2.899
	Control	27.39	4.804	27.31	4.704
	t-test	*t* = -0.385	*Sig.*= 0.704	*t* = -0.663	*Sig.* = 0.513

Beta-CTX – beta-cross Laps; tP1NP – total procollagen type 1N-terminal peptide; ALP – alkaline phosphotase; *Statistically significant difference.

Table 4

Statistics of mixed between-within subjects ANOVA

Variable	Wilks' Lambda	F	*p*	Partial Eta Squared
Beta CTx				
Time^Group impact	0.913	2.280	0.144	0.087
Time impact	0.578	17.553	0.000	0.422*
Group difference		0.226	0.639	0.009
tP1NP				
Time^Group impact	0.999	0.025	0.876	0.001
Time impact	0.915	2.227	0.149	0.085
Group difference		3.035	0.094	0.112
ALP				
Time^Group impact	0.930	1.807	0.191	0.070
Time impact	0.999	0.014	0.907	0.001
Group difference		0.058	0.812	0.002
Leg-Press				
Time^Group impact	0.329	49.028	0.000	0.671*
Time impact	0.357	43.281	0.000	0.643*
Group difference		54.993	0.000	0.817*
VO_2 max				
Time^Group impact	0.478	25.105	0.000	0.522*
Time impact	0.548	18.947	0.000	0.452*
Group difference		10.388	0.013	0.216*
Body weight				
Time^Group impact	0.608	15.501	0.001	0.392*
Time impact	0.428	32.023	0.000	0.572*
Group difference		0.714	0.406	0.029
Body mass index				
Time^Group impact	0.585	17.037	0.000	0.415*
Time impact	0.416	33.761	0.000	0.584*
Group difference		0.272	0.606	0.011

Beta-CTX – beta-cross laps; tP1NP – total procollagen type 1N-terminal peptide; ALP – alkaline phosphatase; VO_2max – maximum oxygen consumption; *Statistically significant difference.

The results of the post-test indicate that after 7 weeks of systematic exercise, statistically significant differences between average values of the experimental and the control group were recorded only on two variables: Leg-Press and VO_2max. However, by inspecting the results of the variance analysis, as many as 5 variables can be noticed that time impact had proven to be statistically significant: beta-CTx, Leg-Press, VO_2max, BW and BMI (Table 4). Group differences were not significant for variables beta-CTx, BW and BMI. Using combined interpretation of values Wilks' Lambda and Partial Eta Squared, it was concluded that no significant change occurred in the control group during the experimental period. At the same time, the exercise program in the experimental group had an influence of significantly decreasing bone resorption, increasing muscle strength, increasing aerobic capacity and decreasing BW and BMI. However, the degree to which bone resorption and bodily dimensions decreased was not sufficiently clear and because of that the post-test missed significant differences between the experimental and the control group. Variance analysis indicated that the 7-weeks physical exercise experimental program was only sufficient for a significant increase of muscle strength and aerobic capacity. Despite a substantial decrease of beta-CTx, BW and MBI in the experimental group, these changes can only be interpreted as the start of the process of positive changes caused by physical activity.

Discussion

Previous research showed that a long-term effect of physical activity on the skeletal system was manifested through increasing of the MBD measured by the DXA method. As the changes of the BMD are very slow, markers of biochemical processes in the bones are used to explain the short-term effects in research. Until now, the most used markers of bone tissue formation have been as follows: ostocalcium, alkaline phosphatase bone isoenzyme, N and C propeptides of procollagen type 1. The most used biomarkers of bone degradation have been: deoxypyridinoline, pyridinoline, N and C telopeptides [33, 34]. The results of most previous research studies indicated that these biochemical markers were sufficiently sensitive to detect bone reaction to training stimuli caused by physical activity. In this research, the effects of the specific 7-week exercise program were quantified by using three parameters taken from the serum of the examinees: beta-CTx (bone resorption marker), tP1NP (bone formation marker) and ALP (metabolism and turnover marker). The results confirmed the findings of previous studies which described a positive influence of dosed physical activity on bone remodeling and metabolism. However, speaking strictly in statistical terms, the recorded changes were not significant, but were only the indications of an initial trend of positive changes. Compared with most of the previous studies where the experimental period lasted much longer (most frequently 6–12 months), the experimental period in this research was much shorter. Even though we were aware beforehand of the risk that shortening the experimental period could cause an absence of significant changes, the advantage was given to consistent control of the activity of all examinees. Such control was possible only with a smaller number of examinees and in a shorter period. Despite the shortened duration, there was a noticeable reduction of subjects in the sample. Some examinees were excluded due to irregular exercise and others for taking hormonal therapy. A small sample and a short duration of the experiment were the basic limiting factors of this study. The lack of a larger number of statistically significant changes was most probably due to the insufficient duration of the training stimuli.

The research registered a significant decrease of bone degradation (decreased concentration of beta-CTx). However, in practice, that piece of data was insufficient to recommend systematic exercise that lasts only 7 weeks as efficient. As expected, this period of systematic training resulted only in a significant increase of muscle strength and aerobic abilities. This data probably proves a positive influence of the applied exercise program on slowing down the process of bone degradation, but not on complete bone remodeling.

Apart from a direct influence on BMD, there is evidence that physical activity also has an indirect effect on the bone metabolism through the fat tissue [22, 23]. Aerobic exercise can be successfully used to regulate the percentage of fat in the organism, due to which BW and BMI were also monitored in this study. Some researches [35, 36] show that thinner women with BMI lower than 19 kg/m^2 are at a higher risk of osteoporosis. Persons with higher BW also have higher BMD and are at a lower risk of osteoporosis [37, 38]. This is explained by increased mechanical load that enables bone formation [39]. Mechanical load stimulates the flow of extracellular fluid through canaliculi and lacunae of the bones that transfer mechano-chemical signals to osteocytes [14]. Yet, excess body weight cannot be interpreted as protection from osteoporosis. On the contrary, newer studies indicate that overweight persons have an increased risk of osteopenia [22, 23]. Osteopenia in overweight persons is primarily connected with hypokinesia and decreased aerobic abilities.

The women that formed the sample in this study had BMI over 25 kg/m^2, but significantly less than 30 kg/m^2, which is the borderline for obesity. According to the World Health Organization (WHO) [40] standards, that puts them into the Overweight group (Pre-obese). Even though heightened BW increases the mechanical force and theoretically spurs bone formation [37–39], all our examinees still had osteopenia. At the same time, they were all measured for low values of VO_2max which indicates weak aerobic capacity. Based on these data, it can be concluded that insufficient physical activity of our examinees was a significant contributing cause for the onset of osteopenia. The level of VO_2max, less than 30 mL/kg/min measured prior to the experiment in both groups indicates low functional ability. This puts our patients in the typical group of sedentary women [41, 42]. Under the influence of applied training program, there was a significant increase in VO_2max in the experimental group. Such a rapid growth of aerobic capacity was not accompanied by the tempo of metabolic changes in the bones. This indicates that a longer application is necessary for a more serious indirect influence of aerobic training.

The influence of the training applied in this study has led to a significant increase of muscle strength in the experimental group. As it was previously proven that the mechanical load leads to increased BMD [39, 43, 44], it was realistic to expect that the increase of strength would be accompanied by changes in the concentration of biochemical markers in the serum. However, the only significant difference in the experimental group was in decreased level of beta-CTx, which indicated a slowing down of bone resorption. At the same time, for tP1NP and ALP, no significant changes were recorded compared to the original values. Based on these data, it was possible to conclude that the experimental program primarily impacted bone resorption, but did not increase the bone mass. The scale of this change was notably lagging behind the degree of which the muscle strength had increased. By comparing the final values of beta-CTx of the experimental and the control group, no statistically significant differences were noticed, which does indicate a small degree of progress. Decreased concentration of beta-CTx in the experimental group was only the initial signal that there were positive changes occurring in the bones. Obviously, for any serious changes to take place, a much longer training program is necessary.

This is also confirmed by the results of previous research in which the training period was much longer. All the experimental treatments lasted for 6 months minimum, which is at least three times longer than the period applied in this research. The results of each study had confirmed significant changes of bone markers in the blood of the examinees. The data were interpreted as a consequence of osteoblast stimulation, which resulted in increased MD. Vincent and Braith [45] applied training similar to that of this research for 6 months, difference being that the sample consisted of male persons older than 60 years old. The examinees were divided into three groups: a control group (without training), a control group with strength exercises of low intensity, and a group with strength exercises of high intensity. The trainings were performed three times per week, and they contained exercises that engage larger muscle groups from the areas most sensitive to changes in BMD. There was an increase in strength, regardless of the applied model of exercise. The results showed that after 6 months of strength training, biochemical markers of the bone formation in both experimental groups increased. As far as other biochemical indicators are concerned, there was a significant increase in osteocalcin (OS) in both groups exposed to strength training. The OS to pyridinoline ratio increased in both experimental groups, while it decreased in the control group. The changes in this ratio were interpreted by the authors as an indicator of the increased bone formation.

Yamazaki et al. [46] pointed out that by applying a 12-month aerobic training (load of about 50% of VO_2max), the concentration of bone resorption markers decreased and it happened only after the third month of exercise. The changes in the mineral bone density during this period were not recorded. These findings strongly correspond with the results of our study. All things considered, if the experiment lasted for several more weeks, the change in bone resorption markers (e.g. beta-CTx) would be statistically significant in our study as well. Here, it only announced positive changes in bone metabolism.

Taking into consideration the results of previous research, strength training combined with aerobic exercise can be considered a very efficient means of improving bone status, as is supported by our study. Until now, the time sufficient for the manifestation of positive effects has not been precisely defined. The different data regarding the period of training probably depend on the specificity of the examinee sample. In some studies focused on older persons, the decrease of bone resorption markers after applying strength training was recorded only after one year, but not after 4 years [47]. All things considered, the decrease in the process of bone resorption can be achieved in the earliest phase of strength training, during the first three months that are seen to be training adaptation. If there is no progression of load, training adaptation will probably not occur nor will the resorption further decrease. This is why it is necessary to conduct permanent control of training and gradually increase the volume and the intensity of training stimuli accordingly, of course, taking into consideration the general health of the person.

Even though there is evidence that aerobic training and strength exercises do have a significant influence on decreasing the consequences of osteoporosis, strength exercises were not proven to be more efficient in previous research studies. Particular attention was dedicated to exercises with one's own weight as they can be applied during the whole lifetime and do not require special equipment (weights and cross-trainers). These exercises include gravity-defying exercises in upright standing position [5]. These activities can be with stronger (high-impact) collisions with solid surfaces (e.g. jumping) and weaker (low-impact) collisions (e.g. walking). Several research studies proved high efficiency of these exercises for maintaining and improving BMD [5, 16, 48, 49]. The other model of exercise training is resistance training. The same authors tested its efficiency and compared it with the effects of exercise where one's own body was used as the resistance. Significant differences were not determined, which is why both models can be equally used when working with persons suffering from osteoporosis and osteopenia.

Conclusion

The results confirmed that regular physical activity has a positive effect on bone tissue. However, in this study, the training led only to a minimal decrease of bone degradation and did not have a significant impact on increasing bone formation. Even though using regular exercise led to a significant decrease in the concentration of beta-CTx markers in the blood, slowing down of bone resorption was not sufficiently manifested. The lack of significant changes can most probably be explained by insufficient duration of the experimental program. At the same time, 7-weeks training program was sufficient to cause a significant increase of muscle strength and aerobic capacity. To induce more serious adaptive processes in the bone metabolism of postmenopausal women, a far longer temporal period is necessary. Further research studies should be designed as experiments

lasting for several months, by varying and progressively increasing the training stimuli.

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R E F E R E N C E S

1. *Jordan KM, Cooper C.* Epidemiology of osteoporosis. Best Pract Res Clin Rheumatol 2002; 16(5): 795–806.
2. *See Tai S, Parsons T, Rutherford O, Lliffe S.* Physical activity for preventing and treating osteoporosis in men. Cochrane Database Syst Rev 2009; 1: 1–5.
3. *National Osteoporosis Foundation,* NOF. Fast Facts. Washington, DC: National Osteoporosis Foundatio; 2012.
4. *Bessette L, Ste-Marie LG, Jean S, Davison KS, Beaulieu M, Baranci M,* et al. The care gap in diagnosis and treatment of women with a fragility fracture. Osteoporosis Int 2008; 19(1): 79–86.
5. *Gomez-Cabello A, Ara I, Gonzalez-Aguero A, Casajus JA, Vicente-Rodriguez G.* Effects of training on bone mass in older adults: A systematic review. Sports Med 2012; 42(4): 301–25.
6. *Looker AC, Wahner HW, Dunn WL, Calvo MS, Harris TB, Heyse SP*, et al. Update data on proximal femur bone mineral levels of US adults. Osteoporosis Int 1998; 18(5): 468–89.
7. National Osteoporosis Foundation, NOF. [cited 2016 Mar 24]. Available from: http://www.nof.org.node/40.Accessed
8. *Kanis JA, McCloskey EV, Johansson H, Cooper C, Rizzolli R, Reginster JY.* European guidance for the diagnosis and management of osteoporosis in postmenopausal women. Osteoporosis Int 2013; 24(1): 23–57.
9. *World Health Organization.* WHO Scientific gropup on the assesment of osteoporosis at the primary health care level. [cited 2016 Mar 24]. Available from: http://www.who.int/chp/topics/Osteoporosis.pdf
10. *Recker R, Davies K, Heaney R. Lappe J,* Characterization of perimenopausal bone loss: A prospective study. J Bone Miner Res 2000; 15(10): 1965–73.
11. *Wayne PM, Kiel DP, Buring JE, Connors EM, Bonato P, Yeh GY*, et al. Impact of Tai chi exercise on multiple fracture-related risk factors in menopausal osteopenic women: A pilot pragmatic, randomized trial. BMC Complement Altern Med 2012; 12: 7.
12. National Osteoporosis Foundation, NOF. Clinician's guide to prevention and tretmant of osteoporosis. Washington DC. [cited 2016 Mar 24]. Available from: http://nof.org/hcp/clinicians-guide
13. *Obradović B, Madić D, Milošević Z, Maksimović N, Mikalački M, Kovačev-Zavišić B.* Body composition and bone mineral density of prebubertal boys involved in different kinesiologic tretmants. Med Pregl 2009; 62(1–2): 23–6. (Serbian)
14. *Compston J, Cooper A, Cooper C, Francis R, Kanis JA, Marsh D,* et al. Guidelines for the diagnosis and management of osteoporosis in postmenopausal women and men from the age of 50 years in the UK. Maturitas 2009; 62(2): 105–8.
15. *Rautava E, Lehtonen-Veromaa M, Kautiainen H, Kajander S, Heinonen OJ, Viikari J*, et al. The reduction of physical activity reflects on the bone mass among young females: A follow-up study of 142 adolescent girls. Osteoporosis Int 2007; 18(7): 915–22.
16. *Kohrt WM, Bloomfield AS, Little KD, Nelson ME, Yngling VR.* ACSM Position stand: Physical activity and bone healt. Medicine and science adults: Cross-sectional data from the Nakanojo study. Osteoporosis Int 2004; 18: 285–93.
17. *Bošković K, Protić-Gava B, Grajić M, Madić D, Obradović B, Tomašević-Todorović S.* Adapted physical activity in prevention and therapy of osteoporosis. Med Pregl 2013; 66(5–6): 221–4. (Serbian)
18. *Roghani T, Torkaman G, Movasseghe S, Hedayati M, Goosheh B, Bayat N.* Effects of short-term aerobic exercise with and without external loading on bone metabolism and balance in postmenopausal women with osteoporosis. Rheumatology Int 2013; 33(2): 291–8.
19. *Gupta S, Ashas I, Mahfooz N, Abdelhamid N, Ramanathan M, Weinstock-Guttman B.* Osteoporosis and multiple sclerosis: Risk factors, pathophysiology and therapeutic interventions. CNS Drugs 2014; 28(8): 731–42.
20. *Ye S, Wu R, Wu J.* Multiple sclerosis and fracture. Int J Neurosci 2013; 123(9): 609–16.
21. *Sedrine WB, Chevallier T, Zegels B, Kvasz A, Micheletti MC, Gelas B*, et al.. Development and assesment of the osteoporosis index of risk (OSIRIS) to facilitate selection of women for bone densitometry. Gynecol Endocrinol 2002; 16(3): 245–50.
22. *Cao JJ.* Effects of obesity on bone metabolism. J Othop Surg Res 2011; 6: 30.
23. *Gudmundsdottir SL, Oskarsdottir D, Indridason OS, Franzson L, Sigurdsson G.* Risk factors for bone loss in the hip of 75-year-old women: A 4-year follow-up study. Maturitas 2010; 67(3): 256–61.
24. *American College of Sports Medicine.* ACSM's health-related physical fitness assessment manual. 2nd ed. Philadelphia: Wolters Kluwer Health; 2008.
25. *Baechle TR, Earle RW.* Essentials of Strength Training and Conditioning. Champaign: Human Kinetics; 2008. p. 394–9.
26. *Hoeger W, Hopkins DB, Barette SL, Hale DF.* Relationship between repetitons and selected percentages of one repetition maximum: A comparasion between untrained and trained males and females. J Appl Sport Sci Res 1990; 4: 47–54.
27. *Ploutz-Snyder LL, Giamis EL.* Orientation and familiarization to 1RM strength testing in old and young women. J Strength Cond Res 2001; 15(4): 519–23.
28. *Tabachnick BG, Fidell LS.* Using multivariate statistics. Boston: Pearson Education; 2007.
29. *Pallant J.* SPSS survival manual. New York: McGrow-Hill Education; 2013.
30. *Karvonen J, Vuorimaa T.* Heart Rate and Exercise Intensity During Sports Activities: Practical application. Sports Med 1988; 5(5): 303–12.
31. *Robergs R, Landwehr R.* The surprising history of the Hrmax=220-age equation. J Exercise Physiol 2002; 5(2): 1–10.
32. *El Maghraoui A, Roux C.* DXA scanning in clinical practice. QJM 2008; 101(8): 605–17.
33. *Maimoun L, Lumbroso S, Manetta J, Paris F, Leroux JL, Sultan C.* Testosterone is significantly reduced in endurance athletes without impact on bone mineral density. Horm Res 2003; 59(6): 285–92.
34. *Kovačev-Zavišić B, Novaković-Paro J, Ćurić N, Ičin T, Todorović-Djilas LJ, Kovačev N.* Biochemical bone markers and biological effects of osteoporosis therapy. Vojnosanit Pregl 2012; 69(5): 432–6.

35. *Kanis JA, Borgstrom F, de Laet C, Johansson H, Johnell O, Jonsson B*, et al. Assesment of fracture risk. Osteoporosis Int 2005; 16(6): 581–9.
36. *Zvekić-Sforcan J, Filipović K, Stanimirov B, Elez I, Repac V*. The importance of body mass index in the development of osteoporosis. Gl Antropol društva Srb 2013; 48: 49–56. (Serbian)
37. *Ho-Pham LT, Nguyen ND, Lai TQ, Nguyen TV*. Contributions of lean mass and fat mass to bone mineral density: A study in postmenopausal women. BMC Musculoskel Dis 2010; 11: 59–67.
38. *Leslie WD, Wieler HA, Nyomba BL*. Ethnic differencies in adiposity and body composition: The first nations bone health study. Appl Physiol Nutr Metab 2007; 32(6): 1065–72.
39. *Duncan RL, Turner CH*. Mechanotransduction and the functional response of bone to mechanical strain. Calcified Tissue Int 1995; 57(5): 344–58.
40. *World Health Organization,* WHO. BMI classification. [cited 2016 Mar 24]. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.ht
41. *Betik CB, Hepple RT*. Determinants of VO2max decline with aging: An integrated perspective. Appl Physiol Nutr Metab 2008; 33(1): 130–40.
42. *Herdy AH, Uhlendorf D*. Reference values for cardiopulmonary exercise testing for sedentary and active men and women. Arq Bras Cardiol 2011; 96(1): 54–9.
43. *Frost HM*. Vital biomechanics: Proposed general concepts for skeletal adaptations to mechanical usage. Calcifi Tissue Int 1988; 42: 145–56.
44. *Frost H*. From Wolff's law to the Utah paradigm: Insights about bone physiology and its clinical applications. Anat Rec 2001; 262(4): 398–419.
45. *Vincent KR, Braith RW*. Resistance exercise and bone turnover in elderly men and women. Med Sci Sport Exerc 2002; 34(1): 17–23.
46. *Yamazaki S, Ichimura S, Iwamoto J, Takeda T, Toyama Y*. Effect of walking exercise on bone metabolism in postmenopausal women with osteopenia/osteoporosis. J Bone Min Metabolism 2004; 22(5): 500–8.
47. *Remes T, Vaisanen SB, Mahonen A, Huuskonen J, Kroger H, Jurvelin JS,* et al. The association of bone metabolism with bone mineral density, serum sex hormone concentrations, and regular exercise in middle-aged men. Bone 2004; 35(2): 439–47.
48. *Guadalupe-Grau A, Fuentes T, Guerra B, Calbet JA*. Exercise and bone mass in adults. Sports Med 2009; 39(6): 439–68.
49. *Ross MD, Denegar CR*. Effect of exercise on bone mineral density in postmenopausal women. Strength Cond J 2001; 23(4): 30–5.

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Reslizumab versus placebo for poorly controlled, severe eosinophilic asthma: meta-analysis

Reslizumab u odnosu na placebo za neadekvatno kontrolisanu, tešku eozinofilnu astmu: meta-analiza

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Abstract

Background/Aim. Reslizumab is humanized monoclonal antibody produced by recombinant DNA technology which binds to circulating interleukin-5 (IL-5) and down-regulates the IL-5 signaling pathway. Reslizumab is indicated for the add-on maintenance treatment of patients 18 years and older with severe eosinophilic asthma phenotype whose symptoms were inadequately controlled with inhaled corticosteroids. The aim of this meta-analysis was to assess the efficacy and safety of reslizumab compared to placebo in patients suffering from inadequately controlled, moderate-to-severe asthma with elevated blood eosinophil counts. **Methods.** Our meta-analysis was based on systematic search of literature and selection of high-quality evidence according to pre-set inclusion and exclusion criteria. The effects of reslizumab and placebo were summarized using Review Manager (RevMan) 5.3.5 and heterogeneity was assessed by the Cochrane Q test and I^2 values. Several types of bias were assessed and publication bias shown by Funnel plot and Egger's regression. **Results.** The meta-analysis included 5 randomized, placebo-controlled clinical trials. Reslizumab 3.0 mg/kg produced substantial improvements in forced expiratory volume in 1. second (FEV 1) (mean difference 0.15 [0.10, 0.21]) and in forced vital capacity (FVC) (mean difference 0.21 [0.09, 0.32]) over the 15 or 16-week treatment period, substantial decrease versus placebo in Asthma Control Questionnaire (ACQ) score (mean difference -0.28 [-0.41, -0.16]), and substantial increase vs. placebo from baseline in Asthma Quality of Life Questionnaire (AQLQ) total score (mean difference 0.24 [0.06, 0.43]). Also, reslizumab 3.0 mg/kg caused less adverse events versus placebo (OR 0.67 [0.51, 0.88]), especially asthma worsening (OR 0.53 [0.36, 0.77]) or bronchitis (OR 0.42 [0.24, 0.74]). **Conclusion.** On the basis of published clinical trials reslizumab could be considered as an effective and safe therapeutic option for severe, poorly controlled eosinophilic asthma for the time being.

Key words:
asthma; eosinophilia; anti-asthmatic agents; reslizumab; treatment outcome; meta-analysis as topic.

Apstrakt

Uvod/Cilj. Reslizumab je humanizovano monoklonsko antitelo, stvoreno rekombinantnom DNK tehnologijom, koje se vezuje za cirkulišući interleukin 5 (IL-5) i dovodi do nishodne regulacije signalnog puta koji pokreće ovaj interleukin. Reslizumab je indikovan kao dodatna terapija održavanja kod bolesnika starijih od 18 godina sa teškim oblikom eozinofilne astme, čiji simptomi nisu dovoljno kontrolisani inhalacionim kortikosteroidima. Cilj ove meta-analize je bio da proceni efikasnost i bezbednost reslizumaba u poređenju sa placebom kod bolesnika sa neadekvatno kontrolisanom, umerenom do teškom astmom, i sa povišenim brojem eozinofila u krvi. **Metode.** Naša meta-analiza je zasnovana na sistematskom pretraživanju literature i selekciji dokaza visokog kvaliteta prema prethodno postavljenim kriterijumima za uključivanje i isključivanje. Efekti reslizumaba i placebo su sumirani pomoću programa *Review Menager* (RevMan) 5.3.5, a hetereogenost studija je procenjena Kohranovim Q testom i vrednošću I^2. Ispitano je nekoliko tipova sklonosti (*bias*), pri čemu je i sklonost za izostavljanje publikacija analizirana pomoću Funnel grafika i Egerove regresije. **Rezultati.** Meta-analiza je uključila pet randomiziranih, placebo-kontrolisanih kliničkih studija. Reslizumab 3.0 mg/kg je doveo do značajnog poboljšanja forsiranog ekspiratornog volumena u 1. sekundi (FEV 1) (srednja razlika 0,15 [0,10, 0,21]) i forsiranog vitalnog kapaciteta (FVC-a) (srednja razlika 0,21 [0,09, 0,32]) posle perioda lečenja od 15 do 16 nedelja, značajnog smanjenja u odnosu na placebo *Ashtma Control Questionnaire* (ACQ) zbira (srednja razlika -0,28 [-0,41, -0,16]) i značajnog povećanja u odnosu na placebo od osnovne vrednosti *Asthma Quality of Life Questionnaire* (AQLQ) ukupnog zbira (srednja razlika 0,24 [0,06, 0,43]). Takođe, reslizumab 3,0 mg/kg je izazvao manje neželjenih dejstava u

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odnosu na placebo (OR 0,67 [0,51, 0,88]), posebno kada je u pitanju pogoršanje astme(OR 0,53 [0,36, 0,77]) ili bronhitis (OR 0,42 [0,24, 0,74]). **Zaključak.** Na osnovu publikovanih kliničkih studija reslizumab se može smatrati efikasnom i bezbednom terapijskom opcijom kod bolesnika sa teškom, neadekvatno kontrolisanom eozinofilnom astmom.

Ključne reči:
astma; eozinofilija; antiastmatici; reslizumab; lečenje, ishod; meta-analiza.

Introduction

There are several phenotypes of bronchial asthma according to specific cellular mechanism and characteristics of patients [1, 2]. Eosinophilic asthma involves subgroup of adult patients with late onset of disease, with hypereosinophilia in blood (> 1,000/mm^3) and in sputum (> 10%), with severe exacerbations which can be prevented by systematic and not by inhaled corticosteroids. Patients with this "endotype" of asthma have decreased level of athopy and their response on bronhodilatators is lower compared to subgroup of patients with allergic asthma [3–5]. This kind of inflammation pathway in asthma where eosinophiles dominate positively correlates with much more severe asthma exacerbations, higher rate of hospitalizations which contribute to increase burden of asthma [6]. Real prevalence of the eosinophilic asthma is not known, but it is estimated that 20% of patients with severe asthma would have this "endotype" of asthma [5]. Since eosinophiles mainly generate interleukin-5 (IL-5), blockade of pathways involving IL-5 can be effective therapeutic approach in patients with eosinophilic asthma [7].

Eosinophils exhibit a substantial role in airway remodeling by promoting and sustaining airway inflammation, airway wall thickening, fibrosis and angiogenesis [8, 9]. Therefore, suppressing the activity and number of eosinophils could be an important biological approach in the management of severe eosinophilic asthma [10]. IL-5 is a key cytokine for production, survival and maturation of eosinophils [11]. Due to a very specific effect on biology of eosinophils, IL-5 is considered to be an ideal molecular target for the treatment of severe eosinophilic asthma [10, 12]. Inhibition of signaling mediated by IL-5 interrupts maturation and survival of eosinophils thus reducing eosinophilic inflammation [12]. Mepolizumab was the first anti-IL-5 antibody that was tested in randomized clinical trials on eosinophilic asthma [13]. Use of mepolizumab decreased exacerbation risk, improved quality of life, lowered eosinophil counts, improved asthma control and lung function in patients with severe eosinophilic asthma in several clinical studies [14].

Reslizumab is humanized monoclonal antibody produced by recombinant DNA technology which binds to circulating IL-5 and down-regulates the IL-5 signaling pathway [12]. Reslizumab is indicated for the add-on maintenance treatment of patients 18 years and older with severe eosinophilic asthma phenotype whose symptoms were inadequately controlled with inhaled corticosteroids, with or without additional asthma controllers [15]. The recommended dosage regimen is 3 mg/kg once every 4 weeks administered by intravenous infusion over 20–50 min [16]. Although common adverse events of reslizumab are mild or moderate, like headache, nasopharyngitis and upper respiratory tract infection, reslizumab can also induce very serious adverse events as anaphylaxis [10]. Reslizumab is contraindicated in patients with known hypersensitivity to reslizumab or accompanying excipients [17].

Although a few clinical trials and one meta-analysis with reslizumab for inadequately controlled asthma with elevated blood eosinophil counts were published, there are still some unresolved issues concerning all possible outcomes of treatment. Summarizing available evidence about all measures of efficacy and safety of reslizumab in this indication tested in clinical trials would be helpful for planning future studies with reslizumab in asthma. The aim of this meta-analysis was to assess the efficacy and safety of reslizumab compared to placebo in patients suffering from inadequately controlled, moderate-to-severe asthma with elevated blood eosinophil counts.

Methods

Our study was registered in the international prospective register of systematic reviews and meta-analyses (PROSPERO) under the number CRD42016041459 prior to commencement of the research.

The following criteria for considering studies for this review were used: 1) types of studies – randomized, double-blind, placebo-controlled clinical trials; 2) types of participants – patients of both sex aged 12–75 years, with at least one blood eosinophil count of 400 cells per μL or higher during a 2–4 week period, or sputum eosinophils of 3% or more, with inadequately controlled asthma (Asthma Control Questionnaire-7 score ≥ 1.5), taking at least a medium dose of inhaled corticosteroids with or without another controller drug (including oral corticosteroids) and with airway reversibility [≥ 12% to short-acting beta-agonist (SABA)]; 3) types of interventions - intravenous infusion of reslizumab 3 mg/kg or of placebo (looking exactly the same as reslizumab) every 4 weeks, for 3 or more doses. Types of outcome measures used in our analysis were: change in forced expiratory volume in 1 second (FEV1) from baseline over 15 or 16 weeks of treatment, change in forced vital capacity (FVC) from baseline, change from baseline in asthma control questionnaire 7-point scale (ACQ-7) score, change from baseline in asthma symptom utility index (ASUI) score, rescue use of blood eosinophil count, asthma quality of life questionnaire (AQLQ) total score, immunogenicity and adverse events types and frequency.

Search methods for identification of studies primarily included electronic databases, and collection of journal articles and books of University Library, University of Kraguje-

vac, Kragujevac, Serbia. Electronic searches of the literature were conducted in Medical Literature Analysis and Retrieval System Online (MEDLINE) (Pub med, coverage from 1966 to present), Scopus/Elsevier (coverage from 1966 to present), Elton B. Stephens Company (EBSCO) (Discovery Service, coverage from 1944 to present), The Cochrane Central Register of Controlled Trials (CENTRAL) (Wiley Online Library, coverage from 1966 to present) and a registry and results database of clinical studies of human participants ClinicalTrials.gov up to July 20, 2016. Additional searches were conducted up to November the 4th, 2016. The search was limited to articles reporting results from patients older than 12 years. Electronic databases were searched independently for relevant studies by three authors: MM, AP and VO. The searching strategies were presented in detail for each of the investigators in the Supplementary file. The most comprehensive strategy was used by the VO for the MEDLINE database, as following: (("reslizumab"[Supplementary Concept] OR "reslizumab"[All Fields]) OR Cinqair[All Fields] OR (SCH[All Fields] AND 55700[All Fields]) OR (DCP[All Fields] AND 835[All Fields])) AND (("asthma"[Medical Subject Heading – MeSH Terms] OR "asthma"[All Fields]) OR ("asthma"[MeSH Terms] OR "asthma"[All Fields] OR ("bronchial"[All Fields] AND "asthma"[All Fields]) OR "bronchial asthma"[All Fields]) OR ("pulmonary eosinophilia"[MeSH Terms] OR ("pulmonary"[All Fields] AND "eosinophilia"[All Fields]) OR "pulmonary eosinophilia"[All Fields] OR ("eosinophilic"[All Fields] AND "asthma"[All Fields]) OR "eosinophilic asthma"[All Fields]) OR (("asthma"[MeSH Terms] OR "asthma"[All Fields]) AND ("eosinophilia"[MeSH Terms] OR "eosinophilia"[All Fields])) OR (("asthma"[MeSH Terms] OR "asthma"[All Fields]) AND elevated[All Fields] AND ("eosinophils"[MeSH Terms] OR "eosinophils"[All Fields])) OR (severe[All Fields] AND ("asthma"[MeSH Terms] OR "asthma"[All Fields])) OR (poorly[All Fields] AND controlled[All Fields] AND ("asthma"[MeSH Terms] OR "asthma"[All Fields])) OR (inadequately[All Fields] AND controlled[All Fields] AND ("asthma"[MeSH Terms] OR "asthma"[All Fields]))). There were no restrictions on publication date, format or language in the search strategy. The references of the retrieved articles were searched for further similar studies ("snowball search"). The collection of journal articles and books of University Library, University of Kragujevac was hand searched for relevant studies by two authors independently (AP and MM).

Data collection and analysis

The data collection sheet was created and the articles included in review were assessed for: 1) study ID; 2) report ID; 3) review author initials; 4) citation and contact details; 5) eligibility for review; 6) study design; 7) total study duration; 8) risk of bias (randomization if any, sequence generation, allocation sequence concealment, blinding, other concerns about bias); 9) total number of patients; 10) age of patients; 11) sex of patients; 12) setting; 13) country; 14) frequency of asthma exacerbations during the last year prior to inclusion in the study; 15) frequency of clinical asthma exacerbations per patient during the study treatment period; 16) mean change in FEV1 from baseline over 16 weeks of treatment; 17) mean change in FVC from baseline; 18) mean change in forced expiratory flow (FEF) at 25% to 75% of FVC (FEF25-75%) from baseline; 19) mean change from baseline in ACQ-7 score; 20) mean change from baseline in ASUI score; 21) frequency of rescue use of short-acting β-agonist per patient; 22) mean blood eosinophil count after 16 weeks of treatment; 23) mean change from baseline in AQLQ total score; 24) percentage of patients developing anti-reslizumab antibodies during the treatment course; 25) number of different intervention groups (reslizumab, placebo); 26) route of administration; 27) dose regimen; 28) duration of administration; 29) incidence of adverse events; 30) treatment discontinuation due to side effects. Values provided as percentages were converted into actual patient numbers for analysis as well as standard errors into standard deviations using number of patients, when reported as such.

Selection of studies

Based on the searching strategy, all titles and abstracts retrieved were independently scanned by five authors (AP, MM, MK, VO and JM). Eligibility of the retrieved articles was assessed at first from the title and the abstract, and if it was not possible, the full text of the articles was retrieved and searched. An article was included for review if all authors (AP, MK, JM, VO and MM) agreed that eligibility criteria had been met. In case that the reviewers had different opinions about eligibility of a study for inclusion, the matter was resolved by the corresponding author (SJ).

Data extraction and management

The data were extracted from eligible studies using the data collection sheet described previously (under the "data collection and analysis" subheading). The data collection sheet was made in electronic form, using an Excel 2007 worksheet. The data were extracted by four investigators independently (AP, MK, VO and MM) and then collating of the four tables was done by another investigator (JM), who produced the final extraction table.

Assessment of risk of bias in included studies

Risk of bias was assessed by two investigators independently (MK and JM), and collating the assessments was done by the corresponding investigator (SJ). The following sources of bias were assessed: 1) randomization if any; 2) sequence generation; 3) allocation sequence concealment; 4) blinding; 5) performance bias; 6) detection bias; 7) attrition bias; and 8) reporting bias. None of the studies had high risk of bias, so none was excluded from further analysis.

Measure of treatment effect

The following outcomes used in the studies were continuous: frequency of clinical asthma exacerbations per patient

during the study treatment period, change in FEV1 from baseline over 16 weeks of treatment, change in FVC from baseline, change in FEF at 25% to 75% of FVC [FEF25-75%]) from baseline, change from baseline in ACQ-7 score, change from baseline in ASUI score, rescue use of short-acting β-agonist, blood eosinophil count and AQLQ total score. For these outcomes the treatment effect was measured by mean difference, since the outcomes were measured on the same scale in all studies. However, two of the continuous outcomes could not be summarized because they were reported in only one of the included studies: frequency of clinical asthma exacerbations per patient during the study treatment period and change in FEF at 25% to 75% of FVC [FEF25-75%]) from baseline. The following outcomes were dichotomous: immunogenicity (whether antibodies are present or not) and adverse events frequency. For these outcomes the treatment effect was measured by odds ratio (OR).

Unit of analysis issues

Unit of analysis in the clinical trials that were included in this meta-analysis were individual patients. Individual participants were randomized to one of two parallel intervention groups, and a single measurement for each outcome from each participant was collected and analyzed.

Dealing with missing data

Missing data were requested directly from the original investigators, however they did not respond to our requests except with courtesy. The missing data were then retrieved from the results presented on ClinicalTrials.gov, when available. Finally, the potential impact of missing data on the findings of the meta-analysis will be addressed in the Discussion section.

Assessment of heterogeneity

Between-study heterogeneity was assessed with the Cochrane Q test using a χ^2 function (p values < 0.10 were considered significant). I^2 values were calculated to quantify inconsistency across studies. I^2 values of 30% or less may represent low heterogeneity, values from 30% to 50% may represent moderate heterogeneity, values from 50% to 90% substantial heterogeneity and values of 90% or more may represent considerably heterogeneity. An I^2 value > 30% was considered significant in this meta-analysis.

Assessment of reporting biases

The possibility of within-study selective outcome reporting was examined for each study included in this meta-analysis. First, by constructing matrix of the outcomes for all studies, we identified studies and specific outcomes that were not reported. Then we searched for published protocols of such studies at ClinicalTrials.gov and other forms of publications of the same studies, in order to find the missing outcomes. Finally, the authors were contacted with a request to provide the missing data, but they did not send us the data. The possibility of between-study publication bias was examined by construction of funnel plots for continuous outcomes and by Egger's regression for discrete outcomes [18]. Klein's number was also calculated for all outcomes [19].

Data synthesis

The random effects model (which includes both within-study and between-study variations in calculation of the weighted average) was used to combine the results from the studies. The Mantel-Haenszel method (fixed effect model) was also used to estimate how our conclusions could be influenced by assumptions about the model and by the study heterogeneity. Since significant heterogeneity of the studies was not found, subgroup analysis was not performed. All calculations were done by Review Manager (RevMan) software version 5.3.5 [20].

Sensitivity analysis

Sensitivity analysis was performed by excluding individual trials one at a time and recalculating the pooled odds ratio and mean difference estimates for the remaining studies. In this way we got insight how each of the included studies influenced our conclusions.

Results

Results of the literature search are shown in Figure 1. Only five clinical trials [21–24] fulfilled all inclusion and missed all exclusion criteria which were set prior the study commencement (two of the trials were published in the same publication. Characteristics of the included studies with risk of bias are shown in detail in Table 1.

Summaries of differences in effects of reslizumab vs. placebo for the main outcomes (using random effects model) were as following: reslizumab 3.0 mg/kg produced substantial improvements in FEV1 (mean difference 0.15) and in FVC (mean difference 0.21) over the 15 or 16-week treatment period; substantial decrease versus placebo in ACQ score (mean difference -0.28), substantial increase vs. placebo from baseline in AQLQ total score (mean difference 0.24) rescue inhaler use (mean difference -0.33) and blood eosinophil count after 15 or 16 weeks of treatment (mean difference -478.17) was observed with reslizumab 3.0 mg/kg; reslizumab 3.0 mg/kg caused less adverse events versus placebo (odds ratio 0.67), especially asthma worsening (odds ratio 0.53) or bronchitis (odds ratio 0.42) while there was no significant difference for nasopharyngitis (odds ratio 0.97) or upper respiratory tract infection (odds ratio 0.86). Details of the summaries of differences in effects are shown in Tables 2 and 3. Sensitivity analysis did not show significant changes with exclusion of single trials.

Table 1

Characteristics of the studies included in the meta-analysis

Study	Bjermer 2016 [24]	Castro 2011 [22]	Castro 2015 [21]	Castro 2015 [21]	Corren 2016 [23]
Methods	Parallel group randomized trial	Parallel group randomized trial	Parallel group randomized trial	Parallel group randomized trial	Parallel group randomized trial
Participants	Patients aged 12-75 years with inadequately controlled asthma, ACQ-7 score ⩾1.5, airway reversibility (⩾12% to SABA), who were receiving treatment with at least a medium-dose inhaled corticosteroids (fluticasone propionate ⩾440 µg/day or equivalent) and had at least one blood eosinophil count of ⩾400 cells/µL during the screening period.	Patients aged 18 to 75 years with asthma (1) confirmed by airway hyperreactivity, (2) treated by high dose inhaled corticosteroids in combination with at least one other agent, (3) poorly controlled and (4) induced sputum eosinophils of 3% or more.	Patients aged 12 to 75 (Child, Adult, Senior) with at least one blood eosinophil count of 400 cells per µL or higher during a 2-4 week screening period and inadequately controlled asthma (ACQ-7 Score ⩾1.5) who were receiving at least a medium dose of inhaled corticosteroids (fluticasone propionate ⩾ 440 mg per day, or equivalent) with or without another controller drug (including oral corticosteroids).	Patients aged 12 to 75 (Child, Adult, Senior) with at least one blood eosinophil count of 400 cells per µL or higher during a 2-4 week screening period and inadequately controlled asthma (ACQ-7 Score ⩾1.5) who were receiving at least a medium dose of inhaled corticosteroids (fluticasone propionate ⩾ 440 mg per day, or equivalent) with or without another controller drug (including oral corticosteroids).	Patients aged 18 to 65 years, with asthma (1), ACQ score of at least 1.5 (2), minimum 12 % airway reversibility to beta-agonist administration (3), treated by fluticasone and stable asthma therapy regimens for 30 days before screening (4), surgically sterile female patients, 2 years postmenopausal, or a negative ßHCG result (5), reproductively potent female patients who agree to use contraception during and 30 days after participation in the study (6).
Interventions	Reslizumab 0.3 or 3.0 mg/kg or placebo	Reslizumab 3 mg/kg vs. placebo	Reslizumab 3 mg/kg vs. placebo	Reslizumab 3 mg/kg vs. placebo	Reslizumab 3 mg/kg vs. placebo
Outcomes	- change in FEV1 from baseline over 16 weeks of treatment - change in FVC from baseline - change in FEF 25-75% from baseline - change from baseline in ACQ-5, ACQ-6 and ACQ-7 scores - change from baseline in AQLQ score - change from baseline in ASUI score - rescue use of short-acting β-agonist - change from baseline in blood eosinophil count - immunogenicity - adverse events types and frequency	- change in FEV1 from baseline over 15 or 16 weeks of treatment - change in FVC from baseline - change from baseline in ACQ-7 score - blood eosinophil count - immunogenicity - adverse events types and frequency	- frequency of asthma exacerbations during the last year prior to inclusion in the study - frequency of clinical asthma exacerbations per patient during the study treatment period - change in FEV1 from baseline over 15 or 16 weeks of treatment - change from baseline in ACQ-7 score - change from baseline in ASUI score - change in frequency of rescue use of short-acting β-agonist per patient (puffs per day) - change from baseline in blood eosinophil count - change from baseline in AQLQ score - immunogenicity - adverse events types and frequency	- frequency of asthma exacerbations during the last year prior to inclusion in the study - frequency of clinical asthma exacerbations per patient during the study treatment period - change in FEV1 from baseline over 15 or 16 weeks of treatment - change from baseline in ACQ-7 score - change from baseline in ASUI score - change in frequency of rescue use of short-acting β-agonist per patient (puffs per day) - change from baseline in blood eosinophil count - change from baseline in AQLQ score - immunogenicity - adverse events types and frequency	- change in FEV1 from baseline at week 16 - change from baseline in ACQ over 16 weeks - change from baseline in FVC at weeks 4, 8, 12, and 16 - change from baseline in average daily use of SABA - change from baseline in blood eosinophil counts

Risk of random sequence generation bias	Low: Randomized study	Low: Randomized study	Low: Randomized study. Randomization was done with use of interactive response technology with computerized central randomization.	Low: Randomized study. Randomization was done with use of interactive response technology with computerized central randomization.	Low: Randomized study. Patients were randomized (4:1) to reslizumab 3.0 mg/kg or placebo given intravenously once every 4 weeks during the treatment period (total of 4 doses), stratified by the occurrence of exacerbations in the 12 months prior to screening (yes/no).
Risk of allocation concealment bias	Low: Randomized study	Low: Randomized study	Low: Randomized study	Low: Randomized study.	Low: Randomized study.
Risk of blinding of patients and personnel bias	Low: Double-blind, placebo-controlled study	High: Patients, investigators, and study personnel were blinded to study treatment group assignment, unlike each site's study pharmacist who were not blinded.	Low: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor), placebo-controlled study	Low: Double Blind (Subject, Caregiver, Investigator, Outcomes Assessor), placebo-controlled study	Low: Double blind (Subject, Caregiver, Investigator, Outcomes Assessor) study
Risk of blinding of outcome assessment bias	Unclear risk: Not described in the report.	Unclear: Not described in the report.	Low: The results of measuring were redacted after initiation of treatment to ensure integrity of masking.	Low: The results of measuring were redacted after initiation of treatment to ensure integrity of masking.	Low: A blind data review meeting was conducted before the database lock to determine the exclusion of affected, individual pulmonary function tests, ACQ, and SABA assessments.
Risk of incomplete outcome data bias	High: Of 315 enrolled patients 265 completed the study. The efficacy analysis set and safety analysis set included 311 of 315 patients.	Low: Only one patient in the reslizumab group and 3 patients in the placebo group were lost to follow-up before the end of the study.	High: Of 244 enrolled patients in placebo group, 215 completed study. Of 245 enrolled patients in reslizumab group, 218 completed study.	High: Of 232 enrolled patients in placebo group, 199 completed study. Of 232 enrolled patients in reslizumab group, 202 completed study.	High: A total of 869 patients were screened. Out of 98 patients who were randomized to placebo, 82 (84%) completed the study and out of 398 who were randomized to reslizumab, 340 (85%) completed the study.
Risk of selective reporting bias	Low: All of the study's pre-specified outcomes were reported in the pre-specified way.	High: Only 3 efficacy outcomes (out of 9 possible) were reported, apart from adverse events.	Low: All of the study's pre-specified outcomes were reported in the pre-specified way.	Low: All of the study's pre-specified outcomes were reported in the pre-specified way.	Low: All of the study's efficacy outcomes were reported.
Risk of other bias	High: Efficacy outcomes were not reported for entire intention-to-treat population.	High: Efficacy outcomes were not reported for entire intention-to-treat population.	Low: Efficacy outcomes were reported for entire intention-to-treat population.	Low: Efficacy outcomes were reported for entire intention-to-treat population.	Low: Efficacy outcomes were reported for entire intention-to-treat population.

ACQ – Asthma Control Questionnaire; SABA – Short acting beta-agonist; β-human chorionic gonadotropin; FEV1 – forced expiratory volume in 1st second; FVC – forced vital capacity;
AQLQ – Asthma Quality of Life Questionnaire; ASVI – Asthma Symptom Utility Index.

Table 2

Summary of continuous outcomes of the studies included in the meta-analysis

Reslizumab compared with placebo for poorly controlled, severe eosinophilic asthma					
Patient or population: patients 12 to 75 years old with poorly controlled, severe eosinophilic asthma Settings: outpatients; Intervention: reslizumab; comparison: placebo					
Outcomes	Comparative effect (95% CI)		Difference between means [95% CI]	Number of participants (Study)	Quality of the evidence (GRADE)
	Mean or mean change Placebo	Mean or mean change Reslizumab 3mg/kg			
Mean change in FEV1 from baseline over 15 or 16 weeks of treatment change expressed in liters 15-16 week of follow up	-0.08 ± 0.11 0.11 ± 0.06 0.09 ± 0.08 0.002 ± 0.238 0.126 ± 0.108	0.18 ± 0.10 0.25 ± 0.06 0.19 ± 0.08 0.272 ± 0.109 0.286 ± 0.107	0.26 ± 0.15 0.14 ± 0.08 0.10 ± 0.11 0.270 ± 0.206 0.160 ± 0.152 Summary difference: 0.15 [0.10, 0.21]	106 (Castro 2011)[22] 489 (Castro 2015)[21] 464 (Castro 2015)[21] 96 (Corren 2016)[23] 211 (Bjermer 2016)[24]	moderate high high low moderate
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 3.89, df = 4 (p = 0.42); I² = 0%, Test for overall effect: Z = 5.35 (p < 0.00001)				
Mean change in forced vital capacity [FVC] from baseline change expressed in liters 15–16 week of follow up	-0.130 ± 0.142 0.055 ± 0.284 0.172 ± 0.120	0.180 ± 0.125 0.230 ± 0.133 0.301 ± 0.120	0.310 ± 0.132 0.175 ± 0.248 0.129 ± 0.170 Summary difference: 0.21 [0.09, 0.32]	104 (Castro 2011)[22] 96 (Corren 2016)[23] 205 (Bjermer 2016)[24]	high low moderate
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 1.99, df = 2 (p = 0.37); I² = 0%, Test for overall effect: Z = 3.43 (p = 0.0006)				
Mean change from baseline in ASUI score Asthma Symptom Utility Index (from 0- worst possible symptoms to 1- no symptoms) 16 weeks of follow up	0.11 ± 0.023 0.08 ± 0.031 0.082 ± 0.042	0.17 ± 0.368 0.12 ± 0.031 0.129 ± 0.042	0.06 ± 0.375 0.04 ± 0.054 0.047 ± 0.059 Summary difference: 0.04 [0.01, 0.08]	476 (Castro 2015)[21] 451 (Castro 2015)[21] 204 (Bjermer 2016)[24]	high high moderate
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 0.04, df = 2 (p = 0.98); I² = 0%, Test for overall effect: Z = 2.34 (p = 0.02)				
Mean change in blood eosinophil count after 15 or 16 weeks of treatment change expressed in cells per µl 16 weeks of follow up	-118 ± 45.47 -76 ± 52.53 -35 ± 53.12	-584 ± 45.08 -555 ± 52.14 -529 ± 52.92	-466 ± 64.03 -479 ± 74.01 -494 ± 75.16 Summary difference: -478.17 [-518.84, -437.49]	484 (Castro 2015)[21] 456 (Castro 2015)[21] 205 (Bjermer 2016)[24]	high high moderate
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 0.31, df = 2 (p = 0.86); I² = 0%, Test for overall effect: Z = 23.04 (p < 0.00001)				
Mean change from baseline in AQLQ total score Asthma Quality of Life Questionnaire - scale range from 1 to 7 16 weeks of follow up	0.695 ± 0.172 0.777 ± 0.226 0.779 ± 0.356	0.933 ± 0.172 0.987 ± 0.227 1.138 ± 0.358	0.238 ± 0.244 0.210 ± 0.320 0.359 ± 0.505 Summary difference: 0.24 [0.06, 0.43]	457 (Castro 2015)[21] 429 (Castro 2015)[21] 200 (Bjermer 2016)[24]	high high moderate

Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 0.24, df = 2 (p = 0.88); I² = 0%, Test for overall effect: Z = 2.65 (p = 0.008)				
Change in frequency of rescue use of short-acting β-agonist per patient puffs per day	-0.360 ± 0.310 -0.440 ± 0.457 -0.100 ± 0.843 -0.300 ± 0.549	-0.640 ± 0.306 -0.500 ± 0.451 -0.800 ± 0.372 -0.900 ± 0.529	-0.280 ± 0.432 -0.060 ± 0.572 -0.700 ± 0.717 -0.600 ± 0.762 Summary difference: -0.33 [-0.64, -0.02]	478 (Castro 2015)[21] 368 (Castro 2015)[21] 96 (Corren 2016)[23] 204 (Bjermer 2016)[24]	moderate high low moderate
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 1.83, df = 3 (p = 0.61); I² = 0%, Test for overall effect: Z = 2.09 (p = 0.04)				
Mean change from baseline in ACQ-7 Asthma Control Questionnaire 7 score (0-totally controlled, 6-severely uncontrolled) 16 weeks of follow-up	-0.300 ± 0.271 -0.68 ± 0.129 -0.66 ± 0.170 -0.368 ± 0.471 -0.494 ± 0.241	-0.7 ± 0.274 -0.94 ± 0.125 -0.86 ± 0.170 -0.858 ± 0.216 -0.853 ± 0.241	0.4 ± 0.386 0.26 ± 0.181 0.20 ± 0.241 0.49 ± 0.408 0.359 ± 0.341 Summary difference: -0.28 [-0.41, -0.16]	106 (Castro 2011)[22] 483 (Castro 2015)[21] 458 (Castro 2015)[21] 96 (Corren 2016)[23] 204 (Bjermer 2016)[24]	moderate high high low moderate
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 1.67, df = 4 (p = 0.80); I² = 0%, Test for overall effect: Z = 4.54 (p < 0.00001)				
GRADE Working Group grades of evidence **High quality:** Further research is very unlikely to change our confidence in the estimate of effect. **Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. **Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. **Very low quality:** We are very uncertain about the estimate.					

Table 3

Summary of adverse effects observed in the studies included in the meta-analysis

Reslizumab compared with placebo for poorly controlled, severe eosinophilic asthma			
Patient or population: patients 12 to 75 years old with poorly controlled, severe eosinophilic asthma Settings: outpatients Intervention: reslizumab Comparison: placebo			
Outcomes	Relative effect: OR [95% CI]	Number of participants (Study)	Quality of the evidence (GRADE)
Incidence of adverse events	0.86 [0.49, 1.50] 0.66 [0.27, 1.62] 0.74 [0.46, 1.18] 0.50 [0.31, 0.81] Summary OR: 0.67 [0.51, 0.88]	208 (Bjermer 2016)[24] 106(Castro 2011)[22] 488 (Castro 2015)[21] 464 (Castro 2015)[21]	moderate moderate high high

Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 2.39, df = 3 (p = 0.50); I² = 0%, Test for overall effect: Z = 2.89 (p = 0.004)		
Number of patients developing anti-reslizumab antibodies during the treatment course	27.91 [1.63, 477.84] 17.50 [1.00, 304.91] 15.47 [0.88, 272.38] Summary OR: 19.66 [3.78, 102.23]	211 (Bjermer 2016)[24] 489 (Castro 2015)[21] 464 (Castro 2015)[21]	moderate high high
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 0.09, df = 2 (p = 0.95); I² = 0%, Test for overall effect: Z = 3.54 (p = 0.0004)		
Asthma worsening	0.78 [0.38, 1.61] 0.60 [0.42, 0.86] 0.39 [0.26, 0.57] Summary OR: 0.53 [0.36, 0.77]	208 (Bjermer 2016)[24] 489 (Castro 2015)[21] 464 (Castro 2015)[21]	moderate high high
Heterogeneity estimate	Heterogeneity: Tau² = 0.06; χ^2 = 4.15, df = 2 (p = 0.13); I² = 52%, Test for overall effect: Z = 3.28 (p = 0.001)		
Nasopharyngitis	1.56 [0.43, 5.71] 2.51 [0.81, 7.83] 0.82 [0.48, 1.41] 0.76 [0.49, 1.18] Summary OR: 0.97 [0.63, 1.51]	208 (Bjermer 2016)[24] 106(Castro 2011)[22] 489 (Castro 2015)[21] 464 (Castro 2015)[21]	moderate moderate high high
Heterogeneity estimate	Heterogeneity: Tau² = 0.07; χ^2 = 4.55, df = 3 (p = 0.21); I² = 34%, Test for overall effect: Z = 0.13 (p = 0.90)		
Upper respiratory tract infection	1.73 [0.40, 7.45] 0.38 [0.07, 2.03] 1.25 [0.75, 2.07] 0.48 [0.20, 1.15] Summary OR: 0.86 [0.45, 1.66]	208 (Bjermer 2016)[24] 106 (Castro 2011)[22] 489 (Castro 2015)[21] 464 (Castro 2015)[21]	moderate moderate high high
Heterogeneity estimate	Heterogeneity: Tau² = 0.19; χ^2 = 5.27, df = 3 (p = 0.15); I² = 43%, Test for overall effect: Z = 0.45 (p = 0.66)		
Bronchitis	0.40 [0.08, 2.09] 0.65 [0.10, 4.08] 0.51 [0.26, 1.03] 0.14 [0.03, 0.60] Summary OR: 0.42 [0.24, 0.74]	208 (Bjermer 2016)[24] 106 Castro 2011[22] 489 (Castro 2015)[21] 464 (Castro 2015)[21]	moderate moderate high high
Heterogeneity estimate	Heterogeneity: Tau² = 0.00; χ^2 = 2.82, df = 3 (p = 0.42); I² = 0%, Test for overall effect: Z = 3.00 (p = 0.003)		
GRADE Working Group grades of evidence **High quality:** Further research is very unlikely to change our confidence in the estimate of effect. **Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. **Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. **Very low quality:** We are very uncertain about the estimate.			

FEV1 – forced expiratory volume in 1st second; CI – confidence interval; FVC – forced vital capacity; ASUI – Asthma Symptom Utility Index.

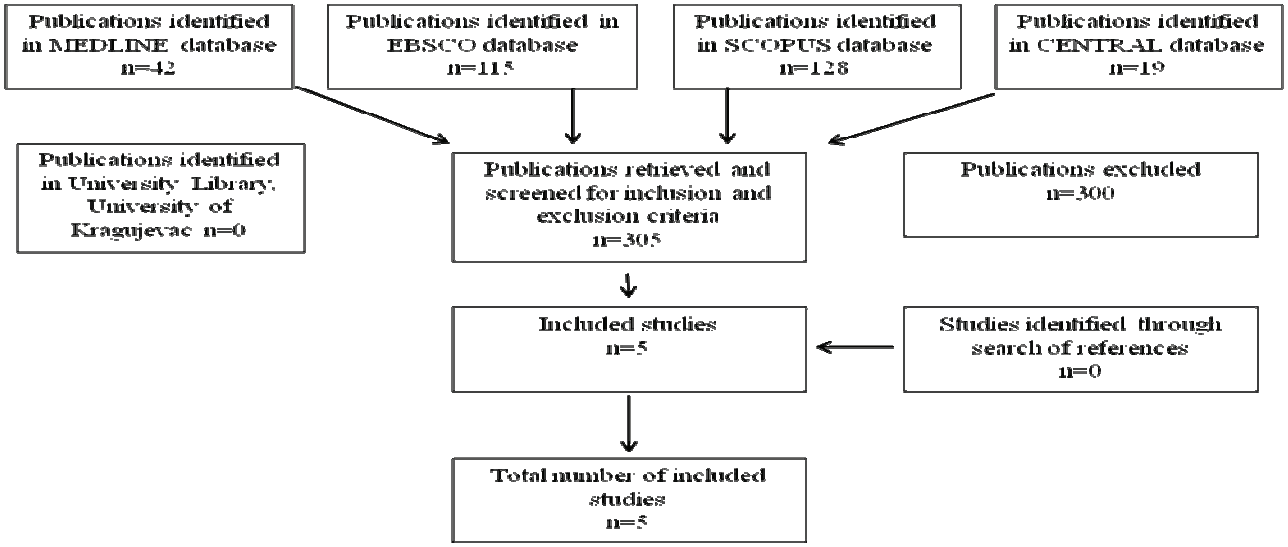

Fig. 1 – Study selection.

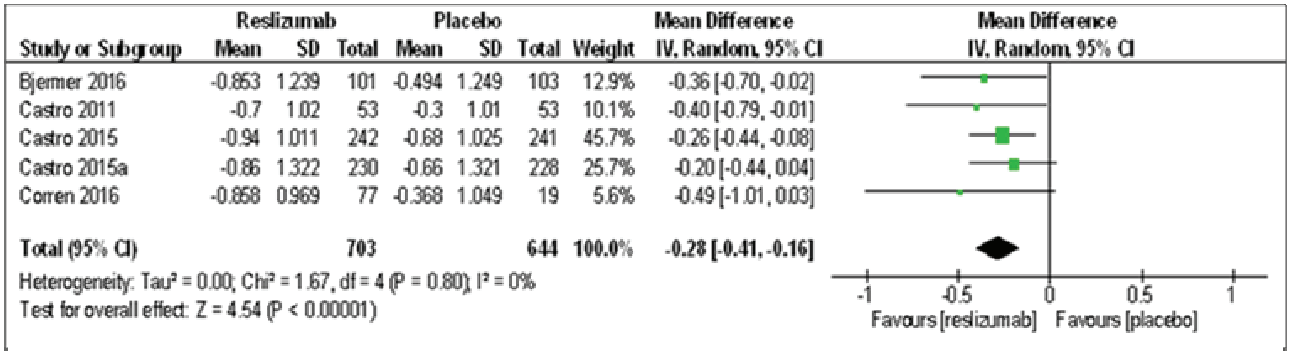

Fig. 2 – Summary of differences in Asthma Control Questionnaire (ACQ) score with reslizumab vs. placebo from baseline over 15-16 weeks of treatment.
SD – standard deviation; CI – confidence interval.

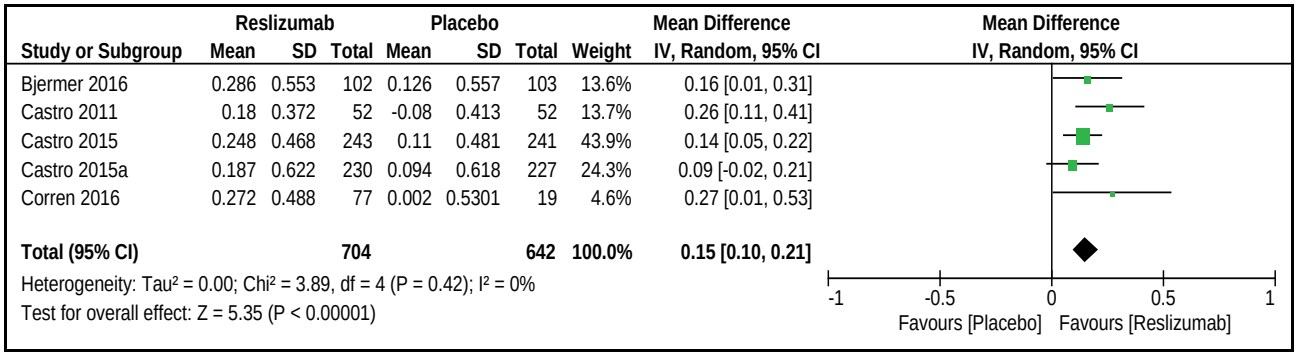

Fig. 3 – Summary of differences in forced expiratory volume in 1 second (FEV1) with reslizumab vs. placebo from baseline over 16 weeks of treatment.
SD – standard deviation; CI – confidence interval.

Summaries of differences in effects of reslizumab and placebo for the most important outcomes (improvements in FEV 1 and decrease in ACQ score) with heterogeneity estimates are shown by Forest plots (Figures 2 and 3).

The reporting bias was assessed by Funnel Plot, using "trim and fill" method for continuous outcomes. The central symmetry axis of Funnel Plots for all tested continuous outcomes did not change place significantly after "trim and fill" exercise. In Figures 4 and 5 Funnel Plots are shown before and after "trim and fill" exercise for two continuous outcomes: improvements in FEV 1 and decrease in ACQ score from baseline over the 15–16 weeks period.

For discrete outcomes (frequencies of adverse effects) the reporting bias was assessed by Klein's number and Egger's regression. Klein's number for total incidence of adverse effects was 4.52 and the Egger's regression showed minimal correction of the summary effect estimate: from OR = 0.67 to OR = 0.42 (Figure 6). For percent of patients developing anti-reslizumab antibodies the Klein's number was 6.79, and OR was corrected by the Egger's regression from 19.66 to 42.23. Klein's number for incidence of asthma worsening was 16.92 and the Egger's regression corrected the summary effect estimate: from OR = 0.53 to OR = 2.25. For incidence of nasopharyngitis, incidence of upper respiratory tract infection and incidence of bronchitis the Klein's numbers were -3.96, -3.45 and 5.48, respectively, and OR was corrected by the Egger's regression from 0.97 to 2.47, from 0.86 to -1.16, and from 0.42 to -0.83, respectively.

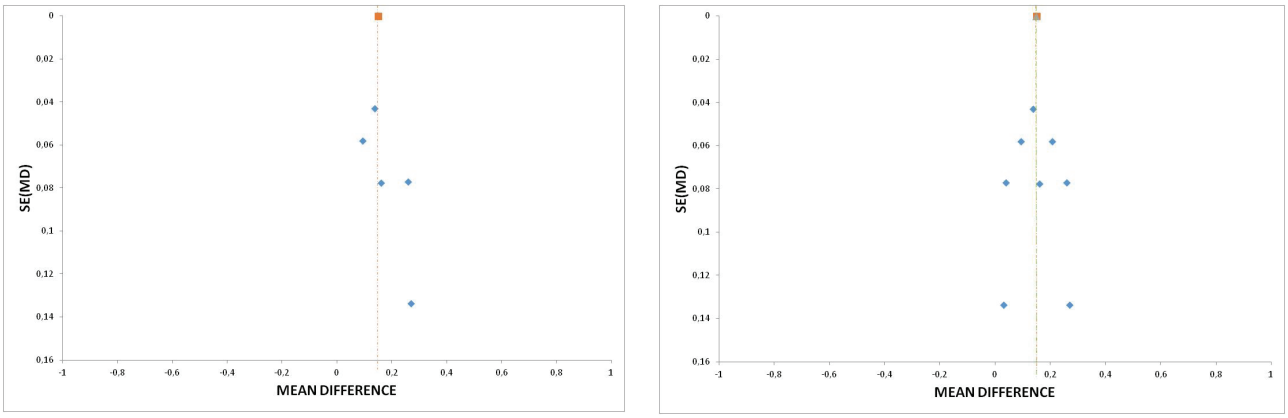

Fig. 4 – Funnel Plots before and after "trim and fill" exercise for improvement in forced expiratory volume in 1 sec (FEV1) from baseline over the 15-16 weeks period.

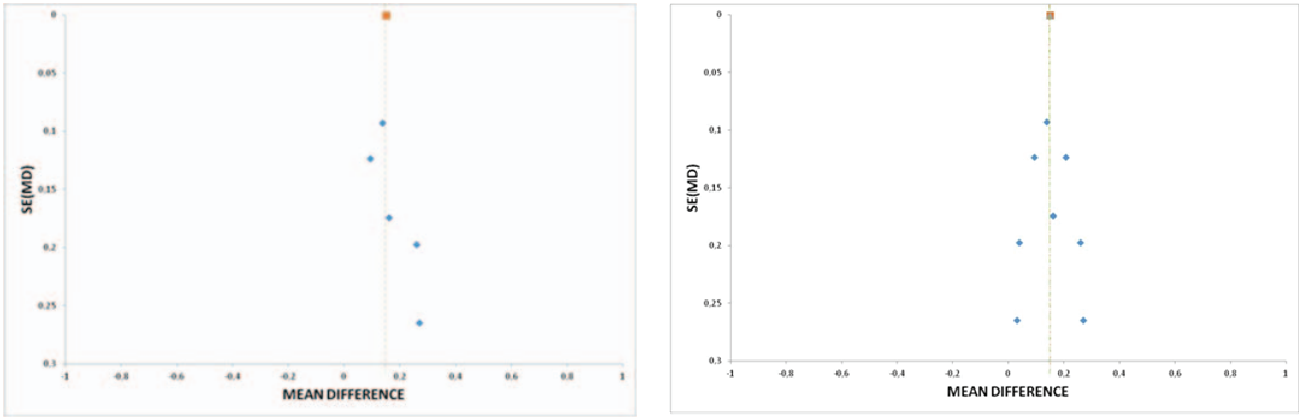

Fig. 5 – Funnel Plots before and after "trim and fill" exercise for decrease in Asthma Control Questionnaire (ACQ) score from baseline over the 15-16 weeks period.

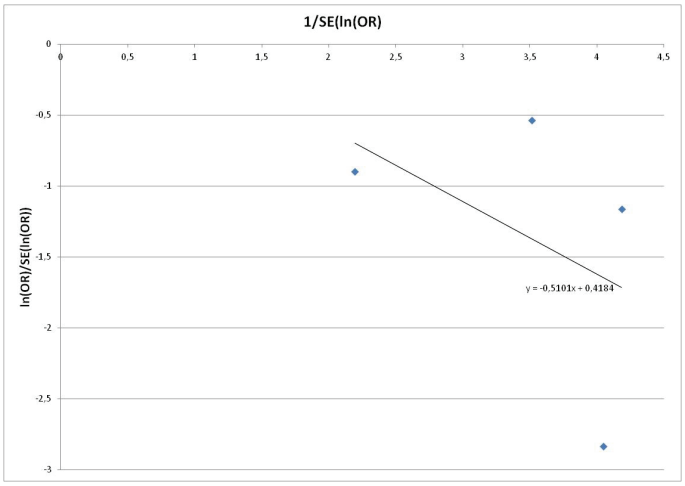

Fig. 6 – Egger's regression for incidence of all adverse effects in the included studie.

Discussion

Results of our study showed that reslizumab is significantly more efficient than placebo in the treatment of severe, poorly controlled eosinophilic asthma. Our results indicated that reslizumab led to significantly greater increase in FEV1 and FVC compared to placebo. On the other hand, reslizumab led to greater reduction of ACQ score and blood eosinophil counts compared to placebo, which also suggested that administration of reslizumab in patients with eosinophilic asthma had significant benefits. These results were in agreement with results of the recently published meta-analysis of reslizumab efficacy and safety [25]. However, in this meta-analysis authors did not analyze impact of reslizumab to AQLQ score, as it was done in another meta-analysis which compared effects of mepolizumab (also monoclonal antibody against IL-5) with placebo in the same clinical entity [26]. After we summarized results of clinical trials with reslizumab in our meta-analysis, it turned out that it improved the AQLQ score much more than placebo. Therefore, reslizumab not only had beneficial effects on clinical outcomes of severe, poorly controlled eosinophilic asthma, but it also markedly improved quality of life of these severely ill patients. Heterogeneity of the included studies was low and publication bias small, so effects were consistent from study to study.

Our meta-analysis indicated that reslizumab use was associated with significantly lower overall incidence of adverse events, asthma worsening and bronchitis compared to placebo. In addition, there was no significant difference in incidence of nasopharyngitis and upper respiratory tract infections in general between reslizumab and placebo. Our results are in agreement with results of the recently published meta-analysis of reslizumab efficacy and safety which has reported that there was no difference in proportion of individuals who withdrew due to adverse events as well as in incidence of upper respiratory adverse events [25]. Although reslizumab use can be associated with development of anti-reslizumab antibodies, they did not appear to have impact on its efficacy and safety and their appearance is transient [21, 27]. All these imply that reslizumab is generally well tolerated, although longer-term safety still needs to be assessed. The risk of anaphylaxis, which was reported in 0.3% of the patients, remains the main reason why reslizumab should be administered in a health care setting where patient can be observed and managed properly in the case of allergy [28].

Our results should be taken with certain reserve, since some of the important clinical outcomes were reported in only one of the included studies [frequency of clinical asthma exacerbations per patient during the study treatment period and change in forced expiratory flow from 25% to 75% of FVC (FEF25-75%) from baseline], and overall number of the included studies was low. Since several clinical trials with reslizumab are ongoing, new meta-analysis should be made in close future to challenge our results.

Conclusion

On the basis of published clinical trials reslizumab could be considered as effective and safe therapeutic option for severe, poorly controlled eosinophilic asthma for the time being. Future studies which would include ongoing clinical trials are necessary to confirm this conclusion.

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REFERENCES

1. *Walford HH, Doherty TA*. Diagnosis and management of eosinophilic asthma: a US perspective. J Asthma Allergy 2014; 7: 53–65.
2. *Durham AL, Caramori G, Chung KF, Adcock IM*. Targeted anti-inflammatory therapeutics in asthma and chronic obstructive lung disease. Transl Res 2016; 167(1): 192-203.
3. *Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C* et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax 2007; 62(12): 1043–9.
4. *Fahy JV*. Eosinophilic and neutrophilic inflammation in asthma: insights from clinical studies. Proc Am Thorac Soc 2009; 6(3): 256–259.
5. *Lötvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A* et al. Asthma endotypes: a new approach toclassification of disease entities within the asthma syndrome. J Allergy Clin Immunol 2011; 127(2): 355–60.
6. *Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P* et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002; 360(9347): 1715–21.
7. *Varricchi G, Bagnasco D, Borriello F, Heffler E, Canonica GW*. Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs. Curr Opin Allergy Clin Immunol 2016; 16(2): 186–200.
8. *Garcia G, Taillé C, Laveneziana P, Bourdin A, Chanez P, Humbert M*. Anti-interleukin-5 therapy in severe asthma. Eur Respir Rev 2013; 22(129): 251–7.
9. *Nissim Ben Efraim AH, Levi-Schaffer F*. Tissue remodeling and angiogenesis in asthma: the role of the eosinophil. Ther Adv Respir Dis 2008; 2(3): 163–71.
10. *Lim HF, Nair P*. Efficacy and safety of reslizumab in patients with moderate to severe eosinophilic asthma. Expert Rev Respir Med 2015; 9(2): 135–42.
11. *Cardet JC, Israel E*. Update on reslizumab for eosinophilic asthma. Expert Opin Biol Ther 2015; 15(10): 1531–9.
12. *Walsh GM*. Profile of reslizumab in eosinophilic disease and its potential in the treatment of poorly controlled eosinophilic asthma. Biologics 2013; 7: 7–11.
13. *Menzella F, Lusuardi M, Montanari G, Galeone C, Facciolongo N, Zucchi L*. Clinical usefulness of mepolizumab in severe eosinophilic asthma. Ther Clin Risk Manag 2016; 12: 907-16.

14. *McIvor RA*. Emerging therapeutic options for the treatment of patients with symptomatic asthma. Ann Allergy Asthma Immunol 2015; 115(4): 265–71.
15. Reslizumab (Cinqair) for severe eosinophilic asthma. Med Lett Drugs Ther 2016; 58(1497): 81–2.
16. *Kaufman MB*. Pharmaceutical Approval Update. P T 2016; 41(6): 355–6.
17. CINQAIR (reslizumab) Label - U.S. Food and Drug Administration. Available from: http://www.accessdata.fda.gov/drugsatfda_docs/label/2016/761033lbl.pdf. Retrieved July 10,2016.
18. *Egger M, Smith GD, Schneider M, Minder C*. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315(7109): 629–34.
19. *Gioacchino L*. Meta-analysis in medical research: the handbook for the understanding and practice of meta-analysis. 1st ed. Malden, USA: Blackwell Publishing Ltd; 2005.
20. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.
21. *Castro M, Zangrilli J, Wechsler ME, Bateman ED, Brusselle GG, Bardin P*, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase, 3 trials. Lancet Respir Med 2015; 3(5): 355–66.
22. *Castro M, Mathur S, Hargreave F, Boulet LP, Xie F, Young J,* et al. Reslizumab for poorly controlled, eosinophilic asthma: a randomized, placebo-controlled study. Am J Respir Crit Care Med 2011; 184(10): 1125–32.
23. *Corren J, Weinstein S, Janka L, Zangrilli J, Garin M*. Phase 3 Study of Reslizumab in Patients With Poorly Controlled Asthma: Effects Across a Broad Range of Eosinophil Counts. Chest 2016; 150(4): 799–810.
24. *Bjermer L, Lemiere C, Maspero J, Weiss S, Zangrilli J, Germinaro M*. Reslizumab for Inadequately Controlled Asthma With Elevated Blood Eosinophil Levels: A Randomized Phase 3 Study. Chest 2016; 150(4): 789–98.
25. *Li J, Lin C, Du J, Xiao B, Du C, Sun J*, et al. The efficacy and safety of Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: a systematic review and meta-analysis. J Asthma 2016; 54(3): 300–7.
26. *Liu Y, Zhang S, Li DW, Jiang SJ*. Efficacy of anti-interleukin-5 therapy with mepolizumab in patients with asthma: a meta-analysis of randomized placebo-controlled trials. PLoS One 2013; 8(3): e59872.
27. *European Medicines Agency, Committee for Medicinal Products for Human Use*. Assessment report for Cinqaero (international non-proprietary name: reslizumab), 2016. [cited 2016 Oct 24]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR
28. *Hussar DA, Dimaculangan CA*. Reslizumab, ixekizumab, and pimavanserin tartrate. J Am Pharm Assoc (2003) 2016; 56(5): 595–8.

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Effects of extremely low frequency pulsed electromagnetic field added to kinesitherapy procedure on quality of life in patients with end stage renal disease on dialysis

Efekti primene elektromagnetnog polja niske frekvencije sa procedurama kineziterapije na kvalitet života bolesnika sa terminalnom bubrežnom slabošću na dijalizi

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Abstract

Background/Aim. Extremely Low Frequency Pulsed Electromagnetic Magnetic Field (ELF-PEMF) has a wide range of therapeutic applications which were expanding during the last decades. ELF-PEMF, as non-invasive, long-term safe method of physical therapy can influence a variety of aspects in chronic diseases including quality of life. Patients with chronic kidney disease (CKD), especially with end stage renal disease (ESRD), treated by dialysis, have lower health-related quality of life and changed normal way of living because of ESRD-related comorbid illnesses, associated conditions and complex dialysis procedures. The objective of this study was to assess the effectiveness of long-term ELF-PEMF in concordance with exercising on quality of life in ESRD patients on dialysis. **Methods.** A total of 124 patients (59 men and 65 women) with ESRD on dialysis program were divided into study group and control group. Patients included in the study group (n = 54) agreed to receive treatment with ELF-PEMF (18 Hz, 2 mT, applied during 40 minutes after ten consecutive dialysis procedures, four times through one year, 120 treatments in total) together with kinesitherapy over three years. The patients in the control group (n = 70) were subjected only to kinesitherapy as a physical therapy procedure. Quality of life was assessed through the Short Form Health Survey, version 2 (SF36v2) and the Functional Assessment of Chronic Illness Therapy, version 4 (FACIT Fatigue v4) questionnaires. **Results.** In the study group, treatment with ELF-PEMF significantly improved FACIT Fatigue v4 scale score as well as physical health, physical functioning, bodily pain and energy/fatigue domains of SF=36v2 scale. There were no effects on mental health domain, limitations due to physical health problems, limitations due to personal or emotional problems, emotional well-being, social functioning, and general health perceptions. In the control group, no beneficial effects on FACIT Fatigue v4 scale and SF36v2 scale item were noticed. **Conclusion**. ELF-PEMF could be a additional and safe strategy for improving quality of life in patients with ESRD on dialysis.

Key words:
electromagnetic fields; magnetic field therapy; kidney failure, chronic; dialysis; quality of life; surveys and questionnaires; treatment outcome.

Apstrakt

Uvod/Cilj. Pulsno elektromagnetno polje ekstremno niske frekvencije (ELF-PEMF) ima široki spektar terapeutske primene koji se povećava poslednjih godina. ELF-PEMF kao neinvazivna, dugoročno bezbedna metoda fizikalne terapije može povoljno uticati na različite aspekte u hroničnim bolestima. Bolesnici sa terminalnom bubrežnom slabošću, posebno oni na dijalizi, imaju nizak kvalitet života uslovljen komorbiditetima, pridruženim stanjima i kom-

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pleksnom procedurom dijalize. Cilj studije bio je ispitivanje efekata dugoročne primene ELF-PEMF uz kineziterapiju na kvalitet života bolesnika sa terminalnom bubrežnom slabošću na dijalizi. **Metode.** U studiju je bilo uključeno 124 bolesnika (59 muškaraca i 65 žena) sa terminalnom bubrežnom slabošću na programu hemodijalize. Formirane su dve grupe ispitanika: studijska grupa (n = 54), bolesnici koji su dobrovoljno pristali da uz kineziterapiju u naredne tri godine dobijaju i ELF-PEMF tretman (18 Hz, 2 mT, tokom 40 minuta posle deset uzastopnih procedura hemodijalize, četiri puta tokom jedne godine, ukupno 120 tretmana) i kontrolna grupa (n = 70) kod koje je od fizikalnih procedura primenjivana samo kineziterapija. Kvalitet života je procenjivan na osnovu *Short Form Health Survay, version 2* (F36v2) i *Functional Assessment of Chronic Illness Therapy, version 4* (FACIT) *Fatigue* v4 upitnika. **Rezultati.** U studijskoj grupi posle primene ELF-PEMF značajno su bili poboljšani skorovi FACIT Fatigue v4 skale, kao i domeni fizičkog zdravlja, fizičkog funkcionisanja, bola i energije SF = 36v2 skale. ELF-PEMF nije imala efekte na domene mentalnog zdravlja, ograničenja zbog fizičkog zdravtsvenog stanja, ograničenja zbog personalnih ili emocionalnih problema, emocionalog stanja, socijalnog funkcionisanja i utiska opšteg zdravlja. U kontrolnoj grupi nije primećeno poboljšanje skora FACIT Fatigue v4 skale ni skora bilo kojeg domena SF36v2 skale. **Zaključak.** ELF-PEMF bi mogla predstavljati dodatnu i bezbednu metodu u pokušaju poboljšanja kvaliteta života bolesnika sa terminalnom bubrežnom slabošću na dijalizi.

Ključne reči:
elektromagnetna polja; magnetoterapija; bubreg, hronična insuficijencija; dijaliza; kvalitet života; ankete i upitnici; lečenje, ishod.

Introduction

Based on experimental trials and early clinical findings, the United States Food and Drug Administration (FDA) approved pulsed electromagnetic fields (PEMF) for the treatment of delayed union or nonunion fractures, failed joint fusions, and congenital pseudarthroses [1, 2]. For therapeutic purposes, PEMF is typically applied at extremely low frequencies between 5 and 300 Hz – Extremely Low Frequency Pulsed Electromagnetic Magnetic Field (ELF-PEMF). ELF-PEMF has a wide range of other therapeutic applications. The scientific evidence for therapeutic effects of PEMF are proven in some indications while data in the others are mostly empiric, observational and insufficient. The review of four meta-analyses of randomized trials investigating the use of ELF-PEMF for fracture healing confirmed clinical validity of this method of physical therapy [3]. Also, in some observation and cross-sectional studies, beneficial effects of ELF-PEMF were found and this procedure is recommended as adjuvant therapy in osteoporosis and other conditions associated with accelerated bone loss or high bone turnover [4]. PEMF treatment was also regarded as a viable alternative for arthritis therapy by virtue of chondroprotective and anti-inflammatory effects [5]. The administration of ELF-PEMF is followed by the high variability in terms of magnetic flux density, signal type, frequency, duration, and number of treatment sessions [2]. Despite the lack of a proven biological mechanism and diversity of applied parameters, a number of indications for ELF-PEMF treatment increased during the last decades. The most frequently mentioned indications are musculoskeletal disorders, but other therapeutic areas include fixation of cementless implants, wound healing, skin ulcers, fibromyalgia, neurological diseases, chronic pain, insomnia, spasticity in multiple sclerosis and even cardiovascular disorders.

It is important that adverse effects of ELF-PEMF as non-invasive, long-term safe method was not reported. There is no discomfort or known risk associated with ELF-PEMF. The method is easy to apply and the cost is low. Some authors assert that ELF-PEMF is important in well-being [2, 6].

Chronic kidney disease (CKD) affects 5%–10% of the world population and is associated with many adverse outcomes. It is progressive and leads to end stage renal disease (ESRD) which is treated mostly by dialysis [7]. According to the United Kingdom renal registry, about 90% of ESRD patients is on the maintenance dialysis program and data show that the incidence of new patients starting on hemodialysis increased by 1.2% in 2013 [8]. Beside the higher mortality rate of ESRD patients, the disease is also associated with greater health expenditures and lower health-related quality of life due to comorbid illnesses and frequent dialysis centers and hospital visits. It implies substantial changes in the patients' normal daily activities and suboptimal quality of life [9]. Also, numerous physical and mental symptoms affect quality of life. Dialysis-dependent patients have numerous physical symptoms, reporting fatigue, pain, cramps, poor nutrition, and inactivity, increased risk of falling and sexual dysfunction due to hypotension, myopathy and peripheral neuropathy [10]. On the other hand, depression, stress, anxiety and sleep disturbances are also very common [11]. Despite the relevance of symptoms, health care providers as well as patients themselves are not adept at recognizing these issues and they are usually underestimated. Additionally, evidence-based dialysis treatment interventions and symptom-targeted pharmaceutical therapies are flawed, except the use of erythropoiesis stimulating agents that can reduce fatigue [12]. Administration of nonpharmacologic steps including exercise and physical therapy that may contribute to improving quality of life is still rare. Physical activity is important aspect for prevention and treatment of chronic diseases, including ESRD. Exercise prescription for the CKD patients is less usual than for other chronic diseases considering that the CKD patients have low aerobic capacity [13]. Taking into account the benefits, in our hemodialysis center, exercise was pointed as an important component of treatment for all patients with ESRD on hemodialysis program.

Results of our previous prospective, controlled study[14], provided evidence for beneficial effect of three-years ELF-PEMF on bone mineral density (BMD) and risk of fracture in the ESRD patients on dialysis, suggesting, for the first time, that this physical procedure was of clinical relevance as a successful adjuvant option in the ESRD patients without reported side-effects. Due to the mentioned pleiotropic effects of ELF-PEMF

and discovering positive effects of this treatment on bones, it can be presumed that this physical procedure may influence overall quality of life. So, the objective of this study was to assess the effectiveness of long-term ELF-PEMF in concordance with exercising on quality of life in the ESRD patients on dialysis.

Methods

Patients

Participants were selected according to the following criteria: diagnosis of ESRD, current hemodialysis treatment and volunteer participation. The patients who met the entry criteria were informed and gave their consent in accordance with ethical standards of the Helsinki Declaration from 1983 and International Conference on Harmonization Good Clinical Practice (ICH-GCP). The study was approved by the Independent Ethics Institutional Review Committee of the University Hospital "Zvezdara" being a part of the Faculty of Medicine, Belgrade University, Serbia on April 19, 2011.

Collection of demographic and case history data was made by reviewing case notes and treatment records. A total of 151 patients with ESRD on dialysis program were divided into a study group and a control group. The study group included the patients who agreed to receive treatment with ELF-PEMF (18 Hz, 2 mT, applied during 40 minutes after ten consecutive dialysis procedures, four times through one year, 120 treatments in total) together with kinesitherapy during three years. The control group involved the patients that were subjected only to kinesitherapy as a physical therapy procedure.

Out of 151 patients who were initially enrolled in the study (64 in the study group and 87 in the control group), a total of 124 patients (54 in the study group and 70 in the control group) completed all treatments and testing after three years. Ten patients in the study group and 17 in the control group were excluded from the study: 2 patients (one from each group) due to a change in concomitant therapy and 25 patients (9 from the study group and 16 from the control group) due to the death related to cardiovascular events. During the follow-up period, not a single patient underwent renal transplantation, nor he/she was transferred to another dialysis center, or changed the dialysis mode. Finally, there were 29 females and 25 males in the study group and 36 females and 34 males in the control group.

All patients had a chronic renal failure of a different origin (primary chronic glomerulonephritis, tubulointerstitial nephritis, nephroangiosclerosis, diabetic nephropathy) and were on dialysis program with hemodialysis product 36, for at least one year. Further inclusion criteria required the patients to be at least 25 years old. All patients have continued with their basic therapeutic regimen (vitamin D, calcium and phosphate binder supplementation) during the observation period. Exclusion criteria were: any relative or absolute contraindication for either ELF-PEMF or kinesitherapy treatment, any disorder affecting the bone metabolism (except renal failure and hyperparathyreoidism) and any medication affecting the bone metabolism (except vitamin D, calcium and heparin during hemodialysis).

Physical therapy procedures

We performed our own treatment protocol based on the fact that the best results are achieved with ELF-PEMF with low frequency (below 60 Hz), induction value between 1pT and 15mT as intermittent use of PEMF stimulation which has been shown to produce superior outcome responses to continuous use [2]. The magnetic field pad (35 × 27 × 13 cm) was a Magomil 2 (Electronic Design Medical, Belgrade, Serbia), with computed device for ELF-PEMF (18 Hz, 2 mT). The therapy was applied during 40 minutes after ten consecutive dialysis procedures, four times through one year (120 treatments in total during three years). The kinesitherapy treatment (active and passive-assisted exercises per segments in two series with ten repeats) was dosed individually, according to general shape during 30 minutes after every dialysis procedure and was carried out by the same physiotherapist who had been trained in the treatment scheme according to the usual program.

Biochemical analyzes were performed routinely using standard certified procedures for measuring the investigated parameters. Serum urea, creatinine, albumin, calcium, phosphate and intact PTH levels were measured and monitored using standard techniques.

Assessment of quality of life

The subjects filled out the following questionnaires at the beginning and once per year: the Short Form Health Survey, version 2 (SF36v2) and Fatigue v4 – the Functional Assessment of Chronic Illness Therapy, version 4 (FACIT) scales [15, 16]. Scores are calculated on line. Because functional capacity is usually impaired in the CKD patients, reaching 60%–65% of the age-predicted value [17], we could not administer some other explicit tests to our patients, except questionnaires.

Statistical analysis

For the statistical analysis, the patients' data were entered into a computer Excel® sheet (Microsoft Office) and subsequently analyzed with the Origin Pro 8.5 statistical software (Stata Corporation, College Station, TX, USA). Group data are expressed as mean ± SD. One-sample Kolmogorov-Smirnov test was used for testing a normal distribution of data. Summary statistics, including mean, standard deviation (SD), range and percentiles were calculated for the demographic data, SF36v2, FACIT Fatigue v4 scales results. One-way ANOVA and *t*-test for depended samples were used to investigate differences between groups for parametric variables and χ^2 test for nonparametric variables. Observations were considered significant if two-tailed *p*-values were below 0.05.

Results

Demographic and clinical data of the patients that completed the study are presented in Table 1 and 2. It is important to note that the patients in finally analyzed groups were comparable in relation to age, duration of dialysis, body mass index (BMI), smoking history, presence of bone frac-

tures, parathyroid hormone (PTH) levels and primary cause of renal failure at the beginning of investigation.

The analyzed groups of the patients were at the beginning of the study also comparable in relation to the values of FACIT Fatigue v4 scale score and SF36v2 scale scores through two domains and eight subdomains (Table 3).

Table 1

Demographic and clinical data of female and male dialysis patients in the study and control groups at the beginning of investigation

Parameter	Female			Male		
	Study group (n = 29)	Control group (n = 36)	*p*	Study group (n = 25)	Control group (n = 34)	*p*
Age (years), mean ± SD	56.9 ± 6.4	61.2 ± 7.6	F = 1.89 *p* = 0.13	63.2 ± 7.4	61.2 ± 13.6	F = 0.55 *p* = 0.85
Duration of dyalisis (years), mean ± SD	9.3 ± 5.6	9.2 ± 6.6	F = 1.64, *p* = 0.17	8.8 ± 3.7	8.7 ± 3.4	F = 1.46 *p* = 0.20
BMI (kg/m^2), mean ± SD	23.7 ± 3.2	24.9 ± 5.4	F = 2.15 *p* = 0.09	25.9 ± 2.8	23.7 ± 3.5	F = 10.9 *p* = 0.08
Duration of menopause (years), mean ± SD	9.0 ± 4.5	10.8 ± 6.2	F=1.72 *p* = 0.15			
Early menopause (%)	20.7	16.7	χ^2 = 0.07 *p* = 0.98			
Ever smoked (%)	44.8	47.2	χ^2 = 0.01 *p* = 0.99	72.0	61.7	χ^2 = 0.13 *p* = 0.87
Present smoking (%)	20.7	19.4	χ^2 = 0.01 *p* = 0.99	40.0	41.1	χ^2 = 0.01 *p* = 0.99
Bone fractures (%)	31.0	22.2	χ^2 = 0.26 *p* = 0.88	24.0	20.5	χ^2 = 0.04 *p* = 0.99
PTH (pg/mL), mean ± SD	761 ± 125	788 ± 147	F=1.08 *p* = 0.61	795 ± 119	774 ± 114	F = 1.18 *p* = 0.55

BMI – body mass index; BMD – bone mineral density; PTH – parathyroid hormone; SD – standard deviation.

Table 2

Frequency of causes of primary diagnosis of renal failure in the study and control groups

Diagnosis	Study group (n = 54)	Control group (n = 70)	*p*
	n (%)	n (%)	
Primary chronic glomerulonephritis	21 (38.9)	29 (41.4)	ns
Tubulointerstitial nephritis	4 (7.4)	6 (8.6)	ns
Nephroangiosclerosis	11 (20.4)	14 (20)	ns
Diabetic nephropathy	16 (29.6)	18 (25.7)	ns
Polycystic renal disease	2 (3.7)	3 (4.3)	ns

Table 3

The Functional Assessment of Chronic Illness Therapy, version 4 (FACIT Fatigue v4) and the Short Form Health Survey, version 2 (SF36v2) scores of patients in the study and the control groups at the beginning of investigation

Parameter	Study group (n = 54)	Control group (n = 70)	*p*
	mean ± SD	mean ± SD	
FACIT Fatigue v4	20.35 ± 9.54	21.36 ± 10.38	0.85
SF36v2 physical health	50.72 ± 10.33	48.75 ± 9.72	0.83
SF36v2 mental health	59.52 ± 17.05	62.58 ± 14.45	0.88
SF36v2 physical functioning	54.38 ± 16.19	52.35 ± 15.23	0.91
SF36v2 bodily pain	50.91 ± 7.55	52.12 ± 10.26	0.92
SF36v2 limitations due to physical health problems	43.61 ± 12.74	44.65 ± 13.24	0.89
SF36v2 limitations due to personal or emotional problems	65.18 ± 23.39	67.58 ± 25.22	0.83
SF36v2 emotional well-being	71.55 ± 19.37	70.25 ± 20.87	0.89
SF36v2 social functioning	49.02 ± 21.70	47.36 ± 22.32	0.87
SF36v2 energy/fatigue	51.55 ± 23.31	50.21 ± 19.27	0.92
SF36v2 general health perceptions	54.05 ± 12.91	55.58 ± 14.35	0.90

SD – standard deviation.

The changes of FACIT Fatigue v4 scale score and SF36v2 scale scores (calculated through physical and mental health domains and all eight subdomains, physical functioning, bodily pain, limitations due to physical health problems, limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue and general health perceptions) after three-years treatment with ELF-PEMF in the study group are presented in Table 4. Treatment with ELF-PEMF significantly improved FACIT Fatigue v4 scale scores as well as physical health, physical functioning, bodily pain and energy/fatigue domains of SF = 36v2 scale. There were no effects on mental health domain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning and general health perceptions.

In the control group, three-years follow-up had no beneficial effects on any FACIT Fatigue v4 scale and SF36v2 scale item (Table 5).

During the study, ELF-PEMF administration was completed without any side-effects.

Table 4

Effects of three-years treatment with Extremely Frequency Pulsed Electromagnetic Filed (ELMF) on values of FACIT Fatigue v4 scale score and SF36v2 scale scores of dialysis patients in the study group (n = 54)

Parameter	Before treatment	After treatment	*p*
	mean ± SD	mean ± SD	
FACIT Fatigue v4	20.35 ± 9.54	41.35 ± 12.35	$p < 0.05$
SF36v2 physical health	50.72 ± 10.33	68.13 ± 11.54	$p < 0.05$
SF36v2 mental health	59.52 ± 17.05	59.33 ± 15.39	$p = 0.98$
SF36v2 physical functioning	54.38 ± 16.19	65.33 ± 16.57	$p < 0.05$
SF36v2 bodily pain	50.91 ± 7.55	69.77 ± 12.87	$p < 0.05$
SF36v2 limitations due to physical health problems	43.61 ± 12.74	51.11 ± 15.86	$p = 0.25$
SF36v2 limitations due to personal or emotional problems	65.18 ± 23.39	63.32 ± 13.41	$p = 0.85$
SF36v2 emotional well-being	71.55 ± 19.37	71.28 ± 18.10	$p = 0.82$
SF36v2 social functioning	49.02 ± 21.70	52.36 ± 19.78	$p = 0.93$
SF36v2 energy/fatigue	51.55 ± 23.31	61.22 ± 21.13	$p < 0.05$
SF36v2 general health perceptions	54.05 ± 12.91	56.05 ± 10.56	$p = 0.89$

FACIT Fatigue v4 – the Functional Assessment of Chronic Illness Therapy, version 4; SF36v2 – The Short Form Health Survey, version 2; SD – standard deviation.

Table 5

Effects of three year treatment with Extremely Low Frequency Pulsed Electromagnetic Filed (ELMF) on values of the Functional Assessment of Chronic Illness Therapy, version 4 (FACIT Fatigue v4) scale score and the Short Form Health Survey, version 2 (SF36v2) scale scores of dialysis patients in the control group (n = 70)

Parameter	Before treatment	After treatment	*p*
	mean ± SD	mean ± SD	
FACIT Fatigue v4	21.36 ± 10.38	22.74 ± 12.54	$p = 0.88$
SF36v2 physical health	48.75 ± 9.72	48.75 ± 15.58	$p = 0.99$
SF36v2 mental health	62.58 ± 14.45	59.14 ± 17.65	$p = 0.78$
SF36v2 physical functioning	52.35 ± 15.23	45.33 ± 20.33	$p = 0.25$
SF36v2 bodily pain	52.12 ± 10.26	59.58 ± 14.53	$p = 0.19$
SF36v2 limitations due to physical health problems	44.65 ± 13.24	41.85 ± 14.20	$p = 0.85$
SF36v2 limitations due to personal or emotional problems	67.58 ± 25.22	65.32 ± 13.96	$p = 0.82$
SF36v2 emotional well-being	70.25 ± 20.87	71.28 ± 14.52	$p = 0.96$
SF36v2 social functioning	47.36 ± 22.32	45.95 ± 15.24	$p = 0.84$
SF36v2 energy/fatigue	50.21 ± 19.27	51.55 ± 19.58	$p = 0.87$
SF36v2 general health perceptions	55.58 ± 14.35	52.25 ± 17.97	$p = 0.78$

SD – standard deviation.

Discussion

In this paper we report the improvement of some aspects of quality of life in the ESRD patients on dialysis subjected to ELF-PEMF in twelve sessions over three years. In our previous article [14], we presented positive effects of this physical procedure on BMD and risk of fracture in the ESRD patients on dialysis without reported side-effects. Also, there was a slight but not significant effect on a patient's overall survival [14]. However, as it was expected, this therapy did not have effects on urea, creatinine and parathormone levels nor on ESRD and dialysis outcome, due to irreversible kidney damage.

In chronic diseases, over the past few decades, quality of life research endpoints developed as valuable research tools in assessing the outcome of therapeutic interventions. Quality of life, as defined by the World Health Organization in 1994, is the individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and

concerns [18]. It comprehends a wide range of indicators covering overall satisfaction with life in areas such as health, housing conditions, employment, safety, education, and leisure [18]. ESRD is a chronic disease causing a high level of disability in different domains of the patients' lives, leading to impaired quality of life. In the ESRD patients, the physical, social and emotional impacts of quality of life are affected by the disease itself and also by its treatment. Dialysis therapy is time intensive and expensive, and requires fluid and dietary restrictions, resulting in a loss of freedom, dependence on caregivers, disruption of marital, family, and social life, and reduced or loss of financial income, and thus compromising quality of life.

Some physical procedures, such as exercises, were shown in CKD patients to improve quality of their lives with regard to positive effects on physical fitness, muscular strength, muscular functioning, walking capacity and cardiovascular function [13]. ELF-PEMF delivered by whole-body mass are promoted in many countries for a wide range of therapeutic applications and for enhancing well-being [6]. The mechanism of biophysical interactions between ELF-PEMF and tissue is still not completely understood. It is suggested that external magnetic stimuli interact with cells either via transmembrane receptors or ion channels, thereby initiating one or more signal transduction cascades or cell functions [19]. ELF-PEM showed that it can increase blood supply [20]. It can mimic and potentiate effects of physical activity on osteogenesis [21]. The application of ELF-PEMF as a physical stress promotes the formation of very small electric currents and piezoelectric potentials. Piezoelectric potentials are primarily due to movement of fluid-containing electrolytes. When these electrolytes move, they generate streaming potentials transforming mechanical stress into an electrical phenomenon capable of stimulating synthesis of tissue components. Time-varying ELF-PEMF also generates changes in metabolic activity. Interaction between cell membrane and ELF-PEMF modulates critical events in signal transduction mechanisms such as Ca^{2+} influx and mobilization, surface receptors redistribution and protein kinase C activity. PEMF can produce a modification of membrane cytoskeleton organization, together with an alteration of protein kinase activity, modify membrane structure and interfere with initiation of signal cascade pathway. Significant reduction of proinflammatory cytokines such as tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) and inflammatory mediators like prostaglandin E2 (PGE2) are noticed. In mice models the expression levels of angiopoietin-2 and fibroblast growth factor-2 are increased and angiogenesis acceleration is suggested as a possible mechanism of the ELF/PEMF action.

In our study, the effects of ELF-PEMF on quality of life of patients with CKD on dialysis were assessed for the first time. We used the SF36v2 scale. The SF 36v2 scale is 36-item generic health-related quality of life questionnaire that consists of 36 questions related to physical and mental health status and an proven objective mean to measure aspects of quality of life such as physical, psychological, social, and cultural conditions from the perspective of patients with chronic diseases. Although some other instruments as self-report measure of quality of life are developed for ESRD patients on dialysis like The Kidney Disease Quality of Life (KDQOL), many investigators are reluctant to use it because of its length (43 kidney-disease targeted items as well as 36 items from SF36v2 scale that provide generic core of instrument) [22].

We found that treatment with ELF-PEMF, combined with exercise, significantly improve FACIT Fatigue v4 scale scores as well as physical health, physical functioning, bodily pain and energy/fatigue domains of SF = 36v2 scale. In the control group, exercise applied as only physical procedure did not show significant effects on these domains, although some slight but not significant effects were reached in physical functioning and bodily pain domains. The effects of exercise on quality of life in ESRD patients on dialysis are often inconsistent. Barcellos et al. [13] analyzed results of 18 studies and in 11 of them an increase of quality of life was found in the exercise group both in aerobic and resistance training. However, 4 of this studies found improvement only in the physical component. The Dialysis Morbidity and Mortality Study, a cohort study, found that dialysis patients engaged in more frequent exercise presented a significantly reduced mortality rate versus less active peers [23].

The findings regarding pain reduction could be an important factor in improving quality of life in the ESRD patients treated with ELF-PEMF in combination with kinesitherapy. ELF-PEMF is a well-known physical agent which can influence chronic pain conditions, especially refractory pain. The investigation of analgesic effectiveness of ELF-PEMF administered twice daily over a 45-day period in 34 subjects with persistent or recurrent pain following back surgery showed that 33% reported a clinically meaningful (≥ 30%) reduction in pain intensity [24]. Improvements in pain intensity were paralleled to improvements in secondary outcomes. Very low-intensity magnetic stimulation may represent a safe and effective treatment for chronic pain and other symptoms associated with conditions without structural damages but with dysfunctional disorders like fibromyalgia [25, 26]. ELF-PEMF can also influence modification of pain in polyneuropathy which is common in the ESRD patients on dialysis [27, 28]. Not only nociception but also transduction, transmission, perception, interpretation and modulation of pain have been reported to be influenced by exposure to electromagnetic fields [29]. The mechanisms by which central nervous exposure to weak electromagnetic fields may have analgesic and antinociceptive effects remain to be explained. There is evidence that endogenous opioid systems are affected by magnetic fields [29].

According to our results there were no effects of ELF-PEMF on mental health domain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning and general health perceptions.

These domains of SF36v2 are narrowly associated with psychological symptoms in patients with ESRD on dialysis. There are many publications related to psychological symptoms in patients with ESRD on dialysis. Previous studies found that the psychological symptoms did affect quality of

life and discussed the association between psychological symptoms with quality of life of dialysis patients [11, 30]. According to some reports, about 27%–36% of dialysis patients express depression, 38%–46% anxiety and about 20% chronic stress [11, 31]. Depression, anxiety and stress significantly contribute to reduced quality of life in dialysis patients' domains of physical health, psychological health, social impact, perceived environment and overall quality of life. Kousolula et al. [9] noticed that overall mood and emotional domains of quality of life correlated with age, higher education, shorter duration of dialysis and better family or social environment. Chronic renal failure affects both patients and their families. Beside socio-demographic variables, many others could be the reason for lower mood and emotional feeling, including health expenditures, frequent dialysis centers visits, ability to travel, financial issues, problems having access to dialysis, comorbid illness, poor nutrition, sexual dysfunction, fluid and dietary restrictions and social support [32]. In our study, ELF-PEMF did not express any significant changes in these domains. In the literature there are a very small number of papers investigating effects of ELF-PEMF on mental health. Martiny et al. [33] published that the transcranial PEMF treatment was superior to sham treatment in patients with ESRD treatment-resistant depression.

The other aspect is effects of ELF-PEMF on fatigue. Regenerative benefits of ELF-PEMF on fatigue in chronic diseases were confirmed in numerous conditions. In a long-term study, a beneficial effect of ELF-PEMF on multiple sclerosis fatigue was demonstrated indicating that it could be a useful therapeutic modality [34]. Evidence from this randomized, double-bind, placebo controlled trial is consistent with results from smaller studies suggesting that exposure to pulsing, weak electromagnetic fields can alleviate symptoms of multiple sclerosis [35].

However, our study had limitations that should be addressed in future research. Some aspects of mental health are assessed by questionnaires but not by mental health professionals. Therefore, the chances of false positive and false negative results are rather big. The other restrictions included the lack of analysis of some socio-demographic and clinical data which might interfere with patient quality of life and a lack of a possibility to study subgroups by energy levels or other parameters of treatment in order to produce recommendations for future studies. Finally, more controlled and double-blind studies, including more patients, might narrow down suspicions and show significant effects with the full support of our findings.

Conclusion

In conclusion, treatment with ELF-PEMF significantly improves physical health, physical functioning, bodily pain and energy/fatigue. Importantly, there have been no reports of side-effects of ELF-PEMF which had a clearly superior safety profile. Our results left enough space for improvement to significant values in forthcoming, larger studies. The time to onset and subsequent longevity of ELF-PEMF effects should be considered in future study design to achieve an accurate measurement. A clearer definition of the mechanisms might also help in choosing patients who are more likely to benefit from such a treatment.

R E F E R E N C E S

1. *Bassett CA.* Fundamental and practical aspects of therapeutic uses of pulsed electromagnetic fields (pEMFs). Crit Rev Biomed Eng 1989; 17: 451–529.
2. *Shupak NM.* Therapeutic uses of pulsed magnetic-field exposure: A review. Radio Science Bulletin 2003; 307: 9–32.
3. *Goldstein C, Sprague S, Petrisor BA.* Electrical stimulation for fracture healing: current evidence. J Orthop Trauma 2010; 24 Suppl 1: S62–5.
4. *Tabrah F, Hoffmeier M, Gilbert F, Batkin S, Bassett CA.* Bone density changes in osteoporosis-prone women exposed to pulsed electromagnetic fields (PEMFs). J Bone Miner Res 1990; 5(5): 437–42.
5. *Negm A, Lorbergs A, Macintyre NJ.* Efficacy of low frequency pulsed subsensory threshold electrical stimulation vs placebo on pain and physical function in people with knee osteoarthritis: Systematic review with meta-analysis. Osteoarthr Cartilage 2013; 21(9): 1281–9.
6. *Hug K, Röösli M.* Therapeutic effects of whole-body devices applying pulsed electromagnetic fields (PEMF): A systematic literature review. Bioelectromagnetics 2012; 33(2): 95–105.
7. *Wagner J, Jhaveri KD, Rosen L, Sunday S, Mathew AT, Fishbane S.* Increased bone fractures among elderly United States hemodialysis patients. Nephrol Dial Transplant 2014; 29(1): 146–51.
8. *Graham-Brown MP, Churchward DR, Smith AC, Baines RJ, Burton JO.* A 4-month programme of in-centre nocturnal haemodialysis was associated with improvements in patient outcomes. Clin Kidney J 2015; 8(6): 789–95.
9. *Kousolula G, Lagou L, Lena M, Alikari V, Theofilou P, Polikandrioti M.* Quality of Life in Hemodialysis Patients. Mater Sociomed 2015; 27(5): 305–9.
10. *Gonçalves FA, Dalosso IF, Borba JM, Bucaneve J, Valerio NM, Okamoto CT,* et al. Quality of life in chronic renal patients on hemodialysis or peritoneal dialysis: A comparative study in a referral service of Curitiba - PR. J Bras Nefrol 2015; 37(4): 467–74.
11. *Bujang MA, Musa R, Liu WJ, Chew TF, Lim CT, Morad Z.* Depression, anxiety and stress among patients with dialysis and the association with quality of life. Asian J Psychiatr 2015; 18: 49–52.
12. *Saad MM, El Douaihy Y, Boumitri C, Rondla C, Moussaly E, Daoud M,* et al. Predictors of quality of life in patients with end-stage renal disease on hemodialysis. Int J Nephrol Renovasc Dis 2015; 8: 119–23.
13. *Barcellos FC, Santos IS, Umpierre D, Bohlke M, Hallal PC.* Effects of exercise in the whole spectrum of chronic kidney disease: A systematic review. Clin Kidney J 2015; 8(6): 753–65.
14. *Rakočević-Hrnjak A, Vuksanović M, Dimković N, Đurović A, Petronijević N, Petronijević M.* The effects of extreme low frequency pulsed electromagnetic field on bone mineral density and incidence of fractures in patients with end stage renal disease on dialysis: Three year follow up study. Vojnosanit Pregl 2017; doi.org/10.2298/VSP160617212R

15. *Ware JE, Kosinski M, Keller SD.* SF-36 physical and mental health summary scales: A user manual. Boston MA: The Health Institute, New England Medical Center; 1995.
16. *Webster K, Cella D, Yost K.* The Functional Assessment of Chronic Illness Therapy (FACIT) Measurement System: Properties, applications, and interpretation. Health Qual Life Outcomes. 2003; 1: 79.
17. *Esteve Simo V, Junqué Jiménez A, Moreno Guzmán F, Carneiro Oliveira J, Fulquet Nicolas M, Pou Potau M,* et al. Benefits of a low intensity exercise programme during haemodialysis sessions in elderly patients. Nefrologia 2015; 35(4): 385–94. (English, Spanish)
18. *Bergner M.* Quality of life, health status, and clinical research. Med Care 1989; 27(3 Suppl): S148–56.
19. *Bachl N, Ruoff G, Wessner B, Tschan H.* Electromagnetic interventions in musculoskeletal disorders. Clin Sports Med 2008; 27(1): 87–105, viii.
20. *Bragin DE, Statom GL, Hagberg S, Nemoto EM.* Increases in microvascular perfusion and tissue oxygenation via pulsed electromagnetic fields in the healthy rat brain. J. Neurosurg 2015; 122(5): 1239–47.
21. *Rajabi AH, Jaffe M, Arinzeh TL.* Piezoelectric materials for tissue regeneration: A review. Acta Biomater 2015; 24: 12–23.
22. *Sathvik BS, Parthasarathi G, Narahari MG, Gurudev KC.* An assessment of the quality of life in hemodialysis patients using the WHOQOL-BREF questionnaire. Indian J Nephrol 2008; 18(4): 141–9.
23. *Stack AG, Molony DA, Rives T, Tyson J, Murthy BV.* Association of physical activity with mortality in the US dialysis population. Am J Kidney Dis 2005; 45(4): 690–701.
24. *Harper WL, Schmidt WK, Kubat NJ, Isenberg RA.* An open-label pilot study of pulsed electromagnetic field therapy in the treatment of failed backsurgery syndrome pain. Int Med Case Rep J 2014; 8: 13–22.
25. *Maestu C, Blanco M, Nevado A, Romero J, Rodriguez-Rubio P, Galindo J,* et al. Reduction of pain thresholds in fibromyalgia after very low-intensity magnetic stimulation: a double-blinded, randomized placebo-controlled clinical trial. Pain Res Manag 2013; 18(6): e101–6.
26. *Sutbeyaz ST, Sezer N, Koseoglu F, Kibar S.* Low-frequency pulsed electromagnetic field therapy in fibromyalgia: A randomized, double-blind, sham-controlled clinical study. Clin J Pain 2009; 25(8): 722–8.
27. *Weintraub MI, Herrmann DN, Smith GA, Backonja MM, Cole SP.* Pulsed electromagnetic fields to reduce diabetic neuropathic pain and stimulate neuronal repair: A randomized controlled trial. Arch Phys Med Rehabil 2009; 90(7): 1102–9.
28. *Wróbel MP, Szymborska-Kajanek A, Wystrychowski G, Biniszkiewicz T, Sieroń-Stołtny K, Sieroń A,* et al. Impact of low frequency pulsed magnetic fields on pain intensity, quality of life and sleep disturbances in patients with painful diabetic polyneuropathy. Diabetes Metab 2008; 34(4 Pt 1): 349–54.
29. *Thomas AW, Graham K, Prato FS, McKay J, Forster PM, Moulin DE,* et al. A randomized, double-blind, placebo-controlled clinical trial using a low-frequency magnetic field in the treatment of musculoskeletal chronic pain. Pain Res Manag 2007; 12(4): 249–58.
30. *Zhang M, Kim JC, Li Y, Shapiro BB, Porszasz J, Bross R,* et al. Relation between anxiety, depression, and physical activity and performance in maintenance hemodialysis patients. J Ren Nutr 2014; 24(4): 252–60.
31. *Anand S, Johansen KL, Grimes B, Kaysen GA, Dalrymple LS, Kutner NG,* et al. Physical activity and self-reported symptoms of insomnia, restless legs syndrome, and depression: the comprehensive dialysis study. Hemodial Int 2013*;* 17(1): 50–8.
32. *Jassal VS, Karaboyas A, Comment LA, Bieber BA, Morgenstern H, Sen A,* Tentori F et al. Functional Dependence and Mortality in the International Dialysis Outcomes and Practice Patterns Study (DOPPS). Am J Kidney Dis 2016; 67(2): 283–92.
33. *Martiny C, Silva ACO, Neto JP, Nardi AE.* Psychiatric disorders in patients with end-stage renal disease. J Ren Care 2012; 38(3): 131–7.
34. *Haase R, Piatkowski J, Ziemssen T.* Long-term effects of Bio-Electromagnetic-Energy Regulation therapy on fatigue in patients with multiple sclerosis. Altern Ther Health Med 2011; 17(6): 22–8.
35. *Lappin MS, Lawrie FW, Richards TL, Kramer ED.* Effects of a pulsed electromagnetic therapy on multiple sclerosis fatigue and quality of life: A double-blind, placebo controlled trial. Altern Ther Health Med 2003; 9(4): 38–48.

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Heroin overdose – suicide or accident?

Predoziranje heroinom – suicid ili zadesno predoziranje?

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Abstract

Background/Aim. Suicide is a public health problem. Due to frequent overdose among drug addicts, there is a question about suicidality in this population. The aim of this study is to determine the specificity and distinctive factors in opiate addicts who have overdosed with an intention to commit suicide compared to addicts who have overdosed accidentally. **Methods.** The survey included 150 heroin addicts who were in the substitution program: 49 subjects who overdosed with a clear suicidal intention and 101 addicts who overdosed without suicidal intention. The subjects filled out the questionnaire about socio-demographic data and data regarding their addiction, the *Minnesota Multiphasic Personality Inventory-2* (MMPI-2) as well as the *Manchester Short Assessment of Quality of Life* (MANSA) questionnaire about the quality of life. For statistical analysis, Pearson's χ^2 test, Student *t*-test and univariate variance analysis were used. **Results.** The addicts who attempted suicide were younger persons (29.7 vs. 36.3 years of age), started to take heroin earlier (17.8 vs. 21.3 years of age; p= 0.013), they use it for a longer period (14.1 vs. 9.2 years; p = 0.00) and take it intravenously for a longer period (11.6 vs. 6.5 years; p = 0.00). The suicide was preceded by a traumatic event (p = 0.015) and there were several attempts of suicide (p = 0.004). The quality of life regarding accommodation, friends and organization of their free time was assessed as not so good (p = 0.03). **Conclusion.** In accordance with the obtained data, it is necessary to design programs for the prevention of suicide among addicts in general and especially programs that would be aimed at younger addicts.

Key words:
heroin dependence; opiate substitution treatment; drug overdose; suicide; risk factors.

Apstrakt

Uvod/Cilj. Samoubistvo je značajan socijalno medicinski problem. Zbog čestih predoziranja među zavisnicima, postavlja se pitanje suicidnosti ove populacije. Cilj rada bio je utvrđivanje specifičnosti i distinktivnih faktora kod zavisnika od opijata koji su se predozirali sa namerom da počine suicid u odnosu na zavisnike kod kojih je predoziranje bilo zadesno. **Metode.** Istraživanjem je obuhvaćeno 150 ispitanika: 49 zavisnika od heroina na programu supstitucije koji su se predozirali sa jasnom suicidnom namerom i 101 zavisnik koji se predozirao bez suicidne namere. U istraživanju je korišćen socio-demografski upitnik i upitnik sa podacima o adikciji, *Minnesota Multiphasic Personality Inventory* (MMPI-2) i *Manchester Short Assessment of Quality of Life* (MANSA) upitnik kvaliteta života. Za statističku analizu su korišćeni Pirsonov χ^2 test, Studentov *t*-test i univarijatna analiza varijanse. **Rezultati.** Zavisnici sa suicidnim pokušajem bili su mlađi (29,7 : 36,3 godine), ranije su počeli da uzimaju heroin (17,8 : 21,3 godine; p = 0,013), duže ga koriste (814,1 : 9,2 meseci; p = 0,00), i duže ga uzimaju intravenski (11,6 : 6,5 godine; p = 0,00). Suicidu je prethodio traumatski događaj (p = 0,015) i imali su više pokušaja suicida (p = 0,004). Kvalitet života su procenili kao manje kvalitetan (p = 0,03) u oblasti zadovoljstva smeštajem, prijateljima i organizacijom slobodnog vremena. **Zaključak**. U skladu sa dobijenim podacima potrebno je koncipirati programe prevencije suicida među zavisnicima uopšte, a posebno programe koji bi bili usmereni ka zavisnicima mlađe životne dobi.

Ključne reči:
zavisnost od heroina; opijati, supstituciona terapija; predoziranost; samoubistvo; faktori rizika.

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Introduction

Everyone who works with drug addicts is concerned with a possibility of overdose. Suicide is a well-known risk for patients with mental health problems, but among addicts, a special attention should be paied to a risk of suicide. Suicide is nearly 6 times more likely to happen among drug and alcohol abusers than in the general population [1]. The rate of suicide among male addicts is 2–3 times higher than among non-addict males [2]. Among women, substance (ab)use increases a risk of suicide from 6.5 to 9 times comparing to non-addict women [3]. Heroin addicts attempt suicide 13 times more frequently than their peers, and suicide attempts among addicts is in a range of 3%–35% [4].

About one-third of persons who commit suicide have been under the influence of drugs, usually opiates or alcohol [5].

Regarding the suicide of addicts, there is a question whether it is more frequently caused by an accidental overdose, or whether it is profiled as a suicide. Overdose could be accidental or intentional. In the United States, in 2014, more than 10,500 people died from heroin overdose [6]. The Centers for Disease Control and Prevention (CDC) found that mortality among women have doubled since 2002. The CDC found that there were 43,982 deaths due to drug poisoning in 2013, out of which 81% were accidental, 12% were intentional suicide while 6% were caused by the unknown motives [7]. According to data from 2013, heroin overdose was fatal for 59% of addicts who, beside heroin, also use another psychoactive substance, usually cocaine or alcohol. There are some key questions to be asked: whether the dependence *per se* bears a high risk of suicide, or, whether some specific subpopulation overdose accidentally, or, whether the addicts overdose with clearly defined suicidal intention.

The aim of this study was to determine the specificity and distinctive factors in opiate addicts who overdosed with an intention to commit suicide and the ones who overdosed accidentally.

Methods

Sample

This cross-sectional study included consecutively recruited 150 opiate addicts who overdosed and were on substitution treatment with methadone [International Classification of Diseases-10 (ICD-10:) F 11.22]. Besides methadone, the patients were taking benzodiazepines, hypnotics and psychostabilizers. Other psychoactive drugs were not prescribed. After overdose, they were divided into two groups: those who had suicidal intentions and ideas, and made a suicidal attempt by overdosing, and those who accidentally overdosed. Suicidal intentions and ideas were assessed by structured interview designed for this research. Thus, the test group included 49 addicts who attempted suicide by overdosing (the first group) and 101 drug addicts who overdosed accidentally (the second group). The study was approved by the Institutional Ethics Committee. All subjects gave written consent and the study was performed in accordance with the Declaration of Helsinki (1965) and later revisions. The survey was conducted from January 2014 to March 2016.

Excluding factors for the study were the following diagnosis (ICD-10): organic, including symptomatic mental disorders, schizophrenia, schizotypal and delusional disorders, mood [affective] disorders, mental retardation [8] and some other addictions.

The average age of the first group was 29.76 years (standard deviation = 9.06) and in the second group it was 36.36 years (standard deviation = 7.96). There were a total of 127 men of whom 38 attempted suicide and 23 females of whom 11 attempted suicide.

Procedure

In this cross-sectional study, the opiate addicts, after the overdose and decision whether it was a suicide attempt or an accidental overdose, completed a set of questionnaires that included: socio-demographic characteristics (gender, age, education level, marital status, children and employment status); family history (family history of psychiatric and suicidal behavior); data regarding dependency (at what age they started using drugs, for how long they used drugs and duration of intravenous drug use) and questions related to whether they were using a psychoactive substance other than regular therapy before overdose, whether it was directly preceded by a traumatic event and whether they previously overdosed and/or had suicide attempts.

The Manchester Short Assessment of Quality of Life [9] focuses on satisfaction with life as a whole and particular life domains (job, financial situation, friends, free time organization, accommodation, personal safety, people who the patient lives with, sexual life, family, health and mental state). MANSA evaluates 12 aspects of quality of life through 7 degrees Likert scale [9] (see Table 4 in the section Results).

The MMPI–2 is used to objectively assess personality structure [10]. The MMPI is the most frequently used standardized psychometric test of adult personality and psychopathology. The test was designed to help identify personal, social and behavioral problems in psychiatric patients. The original MMPI was replaced by the standardized version for adults, 18 years old and over. The new MMPI-2, was released in 1989 and subsequently revised in 2001. The MMPI-2 has 567 items, or questions (true or false format) and comprises 10 clinical scales which assess 10 major categories of abnormal human behavior (hypochondriasis, depression, hysteria, psychopathic deviate, masculinity/femininity, paranoia, psychasthenia, schizophrenia, hypomania and social introversion) and 4 validity scales (L, F, F^b, K), which assess the person's general test-taking attitude and whether the items on the test are answered in an accurate manner [10]. To ensure the anonymity of the subjects, the data were coded and entered into the Excel file.

For statistical analysis, we used Pearson's χ^2 test, Student *t*-test and univariate variance analysis. Odds relations and confidence intervals (CIs) at 95% were also calculated. Due to the large number of variables we mainly showed those which demonstrated a statistically significant difference.

Results

The results of the analysis of sociodemographic factors were shown in Table 1.

In relation to socio-demographic characteristics as well as statistically significant factors, education level and marital status were singled out, and statistical significance of employment status was a borderline. Compared to other variables, a statistically significant difference was reported for the occurrence of traumatic events immediately before the suicide and previous suicide attempts.

The results of the pattern and duration of opiate use analyses were shown in Table 2.

Table 1

The sociodemographic data of the opiate addicts

Variables	Non-suicidal (n %)	Suicidal (n %)	χ^2	*p* value
Employment status				
employed	12 (24.5)	25 (24.7)	5.85	0.953
unemployed	37 (75.5)	76 (75.3)		
disabled	0	0		
Education level				
no primary school	2 (4)	9 (9)		
primary school	8 (16)	21 (21)	77.18	0.026
high school	36 (74)	64 (63)		
graduated college	2 (4)	4 (4)		
graduated faculty	1 (2)	3 (3)		
Marital status				
married	15 (30.6)	18 (17.8)		
unmarried	28 (57.1)	56 (55.5)	10.50	0.014
divorced	5 (10.2)	19 (18.8)		
widowed	1 (2.1)	8 (7.9)		
Children, yes	6 (30)	14 (70)	0.02	0.880
Hereditary, confirmed	22 (34.9)	41 (65.1)	0.69	0.376
Suicide in family, confirmed	36 (45.6)	43 (54.4)	2.87	0.174
Traumatic event, confirmed	23 (31.1)	51 (68.9)	6.236	0.015
Previously overdose, confirmed	26 (35.6)	47 (64.4)	0.024	0.534
Previously suicide, confirmed	1 (14.3)	6 (85.7)	7.956	0.004

Table 2

Characteristics of addiction

Opiate use	Suicidal mean ± SD	Non-suicidal mean ± SD	*t*-value	df	*p*	F ratio	*p*
Start (age), years	17.832 ± 4.947	21.340 ± 5.272	1.520	20	0.051	1.225	0.013
Duration, years	14.138 ± 6.264	9.264 ± 5.635	1.437	20	0.024	1.475	0.000
Duration of i.v. use, years	11.645 ± 5.917	6.573 ± 6.465	1.939	29	0.037	1.187	0.000

i.v. – intravenous; SD – standard deviation; df – degrees of freedom.

Statistically significant suicide occurred to the addicts who started taking opiates early, who (ab)used drugs for a long period and who were long-term intravenous heroin users.

Table 3

Minnesota Multiphasic Personality Inventory 2 (MMPI 2) among opiate addicts

Personality structure	*Suicidal n (%)*	*Non-suicidal n (%)*
Psychopathy	8 (16.3)	11 (10.9)
Hypersensitivity	9 (18.4)	13 (12.9)
Aggravated	3 (6.1)	5 (4.9)
Passive dependent	12 (24.5)	22 (21.8)
Narcissistic	1 (2.0)	10 (9.9)
Borderline	10 (20.4)	23 (22.8)
Passive-agressive	2 (4.1)	8 (7.9)
Schizoid	4 (8.2)	9 (8.9)

The assessment of personal psychopathology was presented in Table 3.

By using the MMPI, we detected personality disorders which were categorized into eight categories: 1) psychopathy, 2) hypersensitivity structure, 3) aggravation of symptoms, 4) passive-dependent structure, 5) narcissistic structure, 6) borderline personality disorders, 7) passive-aggressive-structure, and 8) schizoid structure. In both groups, the most common was a borderline structure. Statistical analysis did not show statistically significant difference between groups in relation to structure of personality: χ^2 – 163.766, df-24, $p = 0.076$.

The results of quality of life were shown in Table 4.

Significantly different variables of the MANSA questionnaire were isolated using univariate analysis, and they were: dissatisfaction with friends, free time organization and conditions of accommodation.

Table 4

Manchester Short Assessment of Quality of Life (MANSA) questionnaire

Univariate analysis results for each different variable sigma-restricted parameterization effective hypothesis decomposition					
	DF	SS	MS	F	*p*
How much are you satisfied in general?	1	0.436	0.436	3.0890	0.081
How much are you satisfied with the job?	1	0.302	0.302	2.1404	0.146
How much are you satisfied with the financial situation?	1	0.129	0.129	0.9157	0.340
How much are you satisfied with your friends?	1	0.547	0.547	3.8729	0.051
Are you satisfied with the organization of your free time?	1	0.918	0.918	6.498	0.012
How much are you satisfied with your accommodation?	1	0.542	0.542	3.838	0.052
How much are you satisfied with your personal safety?	1	0.264	0.264	1.870	0.173
How much are you satisfied with people with whom you live?	1	0.123	0.123	0.876	0.351
How much are you satisfied with your sexual life?	1	0.361	0.361	2.555	0.112
How much are you satisfied with your family?	1	0.023	0.023	0.168	0.682
How much are you satisfied with your health?	1	0.024	0.027	0.170	0.680
How much are you satisfied with your mental state?	1	0.226	0.084	0.050	0.401
Total	150	0.887	0.886	5.648	0.038

DF – degrees of freedom; SS – sum of squares; MS – mean squeare; *p* – probability.

Discussion

Drug dependence does not only increase the risk of suicide, but the drugs are used as a tool for suicide. While (excessive) alcohol consumption leads to behavioral disinhibition and loss of fear making the person susceptible to an easier suicide attempt, heroin users attempt suicide in order to alleviate dissatisfaction, anxiety or other bad feelings [11].

A suicide attempt as a common occurrence in drug and alcohol abusers could be observed in several ways. The first aspect is the comorbidity. Research showed that 44%–86% heroin addicts had some mental disorder in their lifetime [12–14]. According to a research done by Brooner et al. [12], among Axis I disorders, the mood disorders were the first in terms of frequency, with depressive disorder (lifetime prevalence of 15.8%, the current prevalence of 3.2%) occurring more frequently than bipolar affective disorder (lifetime prevalence of 0.4%, the current prevalence of 0.4%). Anxiety disorders also gave high comorbidity with opiate dependence (lifetime prevalence of 8.2%, the current prevalence of 5.0%) [15, 16]. Although various psychotic disorders are relatively frequent cause or consequence of opiates consumption, diagnosis of schizophrenic disorder is, on average 1 per 100 addicts. The risk of suicide was increasing with underlying comorbid psychiatric disorders [17]. In this work, the addicts who had a primary affective disorder were excluded (among others) so that the emotional state was understood as a consequence of their "addictive life".

According to this study, 32% of the opiate addicts who overdosed had suicidal intentions. The sample was dominated by the male subjects 3.5 times more frequently (of 49 subjects, 38 were men). The study did not confirm the information according to which the female addicts were about 4 times more likely to attempt suicide than the male ones [3]. The reason for these inconsistent data is that men are mostly (4/5) users of health services in our community. According to relevant literature, older men who use drugs are at a higher risk of suicide attempts than younger addicts [18, 19]. This study confirmed also that suicidal intentions were more likely to appear in older men. It can also be added that they were persons who had previously started with using heroin and continued to use it intravenously for a long time, [20]. The suicidal addicts brought the decision, earlier and easier, to use intravenous injections of heroin, so heroin administration might be a risk factor for suicidal attempt. The results of our study are in line with the results of this study and other ones [21, 22].

Another problem is that addiction is associated with multiple economic and social consequences, first of all crime, destruction of families, unemployment, homelessness, etc. These conditions would often be the cause of the "balanced" suicide attempts [23]. Life in marriage and/or extramarital community can be singled out from the socio-demographic data. Of the total number of the drug addicts who had attempted suicide, 30% were in the community (married) while in the second group, this percentage was 18%. It can be assumed that the life in marriage and/or extramarital community was an additional requirement that were imposed on addicts, and if they were not able to comply, mental distress was increasing as well as a risk of suicide [24, 25].

Among the addicts in both groups who overdosed, borderline structure of personality dominated. There was the high suicide rate among borderline structure of personality (8%) [26], and suicide was the leading cause of death among heroin users having borderline structure of personality [27, 28]. Our results also suggest that the percentage of borderline structure of personality among the addicts, especially the addicts who had suicidal intentions was as high as 23%. This pattern was highly selective for opiate addicts with clear suicidal intent, and that is why we had so high percentage borderline structure of personality in the sample.

In this research, the drug addicts with suicide intentions frequently experienced some traumatic event before the suicide attempt, which was presented as a statistically significant result, and they had more suicide attempts in the previous period. Similar results was obtained by Maloney et al. [28]. In their research, as many as 19% of the addicts had more suicide attempts. Respondents reported different traumatic

events. These were: arguments with loved ones, loss of income, physical assault, loss of a close person in one case and the weight of a family member's disease in two cases. Earlier research had recognized the tendency for borderline structure of personality and traumatic events, especially posttraumatic stress disorder (PTSD) to co-occur in suicidal addicts [29–31].

A statistically significant difference was obtained in relation to a variable regarding quality of life. Systematic use of life quality indicators to monitor the outcome was poor, despite the wide-ranging effects which substance use disorders had on patients, families, and society [32]. Substance-dependent individuals had lower quality of life [33]. For example, the drug addicts scored significantly lower than the general population as far as physical and mental functioning was concerned, as low as, or lower than patients with lung disease and diabetes and significantly lower than patients awaiting cardiac surgery [34]. The biggest difference in our research appeared in relation to the satisfaction with housing conditions, friends and free time.

By integrating the data obtained, we made two assumptions. One is that a suicide addict is not an uniform phenomenon, but is affected by multiple bio-psycho-social factors. Etio-pathogenic, these are generally addicts who had previously began using drugs, and it lasted for a long time. It can be assumed that the earlier onset partly interplay with borderline personality structure. Combinations of that personality structure and early opiate dependency caused impulsiveness, tendency to risky behavior and a lower quality of life. This aspect will be the research topic in the following works.

There are several limitations of the generalizability of the findings in this study. The cross-sectional design was used on relatively small sample and thus the observed differences in the personality traits do not provide explanation whether they are the causes or consequences of heroine dependence development. Furthermore, the patients in substitute treatment program are likely to have more severe psychopathology and comorbities compared to the general population. Also, patients' personality traits scores were not premorbid and chronic heroin use may modify the assessment of personality traits. The larger prospective study with both gender subjects is needed for further study of complex interplay between heroine addiction, suicide attempts and personality traits. Thus, these findings might inform early interventions and treatments that target heroin addicts at a risk of overdose and suicide attempts during substitute treatment.

Conclusion

The suicide attempts were more frequent among the male opiate users. It was associated with younger age, early onset, intravenous administration of heroine, longer period of taking heroine and risks.

Among the addicts with suicide attempts, borderline personality structure dominated. They reported traumatic events before suicide attempt and more suicide attempts in the previous period.

Addicts with suicide attempts had low quality of life, particularly in the areas of housing satisfaction, friends and the organization of free time.

In accordance with the obtained data, it is necessary to design programs for the prevention of suicide among addicts in general, and especially programs that would be aimed at younger addicts.

REFERENCES

1. *Maloney E, Degenhardt L, Darke S, Mattick RP, Nelson E.* Suicidal behaviour and associated risk factors among opioid-dependent individuals: A case-control study. Addiction 2007; 102(12): 1933–41.
2. *Kwon M, Yang S, Park K, Kim DJ.* Factors that affect substance user's suicidal behavior: A view from the Addiction Severity Index in Korea. Ann Gen Psychiatry 2013; 12(1): 35.
3. *Schneider B.* Substance use disorders and risk for completed suicide. Arch Suicide Res 2009; 13(4): 303–16.
4. *Darke S, Ross J, Lynskey M, Teesson M.* Attempted suicide among entrants to three treatment modalities for heroin dependence in the Australian Treatment Outcome Study (ATOS): Prevalence and risk factors. Drug Alcohol Depend 2004; 73(1): 1–10.
5. *Borges G, Loera CR.* Alcohol and drug use in suicidal behaviour. Curr Opin Psychiatry 2010; 23(3): 195–204.
6. *Centers for Disease Control and Prevention.* Injury prevention and control: Opioid overdose. Available from: www.cdc.gov/drugoverdose/opioids/heroin.html
7. *National Institute on Drug Abuse.* Heroin. 2015. [cited 2015 Sep 15]. Available from: www.drugabuse.gov/drugs-abuse/heroin
8. *International Classification of Diseases.* Tenth Revision (ICD-10). Geneva: World Health Organization; 2010.
9. *Priebe S, Huxley P, Knight S, Evans S.* Application and Results of the Manchester Short Assessment of Quality of Life (Mansa). Int J Soc Psychiatry 1999; 45(1): 7–12.
10. *Butcher JN, Dahlstrom WG, Graham JR, Tellegen A, Kaemmer B.* The Minnesota Multiphasic Personality Inventory-2 (MMPI-2): Manual for Administration and Scoring. Minneapolis, MN: University of Minnesota Press; 1989.
11. *Wilcox HC, Conner KR, Caine ED.* Association of alcohol and drug use disorders and completed suicide: An empirical review of cohort studies. Drug Alcohol Depend 2004; 76(Suppl): S11–9.
12. *Brooner RK, King VL, Kidorf M, Schmidt CW, Bigelow GE.* Psychiatric and substance use comorbidity among treatment-seeking opioid abusers. Arch Gen Psychiatry 1997; 54(1): 71–80.
13. *Schuckit MA, Hesselbrock V.* Alcohol dependence and anxiety disorders: What is the relationship? Am J Psychiatry 1994; 151(12): 1723–34.
14. *Kranzler HR, Liebowitz NR.* Anxiety and depression in substance abuse. Med Clin North Am 1998; 72(4): 867–85.
15. *Charney DA, Paraherakis AM, Negrete JC, Gill KJ.* The impact of depression on the outcome of addictions treatment. J Subst Abuse Treat 1998; 15(2): 123–30.
16. *Montoya AD, Svikis D, Marcus SC, Suarez A, Tanielian T, Pincus HA.* Psychiatric care of patients with depression and comorbid substance use disorders. J Clin Psychiatry 2000; 61(9): 698–705; quiz 706.

17. *Galanter M, Castaneda R, Ferman J.* Substance abuse among general psychiatric patients: Place of presentation, diagnosis and treatment. Am J Drug Alcohol Abuse 1998; 14(2): 211–35.
18. *Sundin M, Spak F, Spak L, Sundh V, Waern M.* Substance use/abuse and suicidal behavior in young adult women: A population-based study. Subst Use Misuse 2011; 46(13): 1690–9.
19. *Haw CM, Hawton K.* Problem drug use, drug misuse and deliberate self-harm: Trends and patient characteristics, with a focus on young people, Oxford, 1993-2006. Soc Psychiatry Psychiatr Epidemiol 2011; 46(2): 85–93.
20. *Srdanović-Maraš J, Kolundžija K, Dukić O, Šobot V, Marković J.* Suicidal behavior in adolescent psychiatric population. Curr Top Neurol Psychiatr Relat Discip 2010; 3(18): 60–4.
21. *Bansal P, Gupta A, Kumar R.* The Psychopathology and the Socio-demographic Determinants of Attempted Suicide Patients. J Clin Diagn Res 2011; 5(5): 917–20.
22. *Nagendra Gouda MR, Rao SM.* Factors related to attempted suicide in Davanagere. Indian J Community Med 2008; 33(1): 15–8.
23. Centers for Disease Control and Prevention. National Vital Statistics System. 2010. Multiple Cause of Death File. Hyattsville, MD: US Department of Health and Human Services; 2012.
24. *Bohnert AS, Roeder K, Ilgen MA.* Unintentional overdose and suicide among substance users: A review of overlap and risk factors. Drug Alcohol Depend 2010; 110(3): 183–92.
25. *Ilgen MA, Burnette ML, Conner KR, Czyz E, Murray R, Chermack S.* The association between violence and lifetime suicidal thoughts and behaviors in individuals treated for substance use disorders. Addict Behav 2010; 35(2): 111–5.
26. *Black DW, Blum N, Pfohl B, Hale N.* Suicidal behavior in borderline personality disorder: Prevalence, risk factors, prediction, and prevention. J Pers Disord 2004; 18(3): 226–39.
27. *Darke S, Degenhardt L, Mattick R.* Mortality among illicit drug users: epidemiology, causes and intervention. Cambridge: CambridgeUniversity Press; 2006.
28. *Maloney E, Degenhardt L, Darke S, Nelson EC.* Impulsivity and borderline personality as risk factors for suicide attempts among opioid-dependent individuals. Psychiatry Res 2009; 169(1): 16–21.
29. *Steil RI, Hinckers A, Bohus M.* Comorbidity of personality disorders and posttraumatic stress disorder. Eur Psychiatry 2007; 22: S17.
30. *Verona E, Patrick CJ, Joiner TE.* Psychopathy, antisocial personality, and suicide risk. J Abnorm Psychol 2001; 110(3): 462–70.
31. *Negredo L, Melis F, Herrero O.* Psychopathy and suicidal behaviour in a sample of mentally disordered offenders. Rev Esp Sanid Penit 2013; 15(1): 3–7. (Spanish)
32. *Dawson DA, Li TK, Chou SP, Grant BF.* Transitions in and out of alcohol use disorders: Their associations with conditional changes in quality of life over a 3-year follow-up interval. Alcohol Alcohol 2009; 44(1): 84–92.
33. *Donovan D, Mattson ME, Cisler RA, Longabaugh R, Zweben A.* Quality of life as an outcome measure in alcoholism treatment research. J Stud Alcohol Suppl 2005; (15): 119–39; discussion 92–3.
34. *Smith KW, Larson MJ.* Quality of life assessments by adult substance abusers receiving publicly funded treatment in Massachusetts. Am J Drug Alcohol Abuse 2003; 29(2): 323–35.

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Serum biomarkers and clinical characteristics of patients with Hodgkin lymphoma

Biomarkeri seruma i kliničke karakteristike bolesnika sa Hočkinovim limfomom

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Abstract

Background/Aim. In classical Hodgkin's lymphoma (cHL) the existing prognostic scoring systems do not include markers that adequately reflect the interaction of malignant Hodgkin and Reed-Sternberg (HRS) cells and tumor environment. The aim of this study was to determine the relationship between serum Galectin-1 (Gal-1) and soluble CD163 (sCD163) and the clinical status of patients with cHL, with special emphasis on the presence of relapse, progression, or resistance to the therapy applied. **Methods**. The research included 79 patients of whom 63 were patients with cHL, and the control group of 16 healthy volunteers. The study group of 63 patients with cHL included a subgroup of newly diagnosed patients without therapy, newly diagnosed patients with therapy, patients with relapse and progression of the disease and primary refractory patients during 2014 and 2015. **Results**. Analysis of the levels of sCD163 and Gal-1 within a group of patients suffering from cHL showed that the values of both molecules were higher in relapsed patients and the subgroup with progressive disease comparing to the subgroup of newly diagnosed patients without therapy or patients with therapy onset. **Conclusion**. Determination of Gal-1 and sCD163 levels is simple and reliable analysis that can contribute to the identification of high-risk patients with cHL and deserves inclusion in current prognostic scoring systems.

Key words:
hodgkin disease; biomarkers, tumor; enzyme-linked immunosorbent assay; treatment outcome; prognosis.

Apstrakt

Uvod/Cilj. Postojeći prognostički skoring sistemi klasičnog Hočkinovog limfoma (cHL)ne obuhvataju markere koji adekvatno reflektuju interakciju malignih Hočkinovih Reed-Sternberg-ovih (HRS) ćelija i tumorskog okruženja. Cilj rada bio je da se utvrdi povezanost nivoa serumskog galektina-1 (Gal-1) i solubilnog CD163 (sCD163) sa kliničkim statusom obolelih od cHL sa posebnim osvrtom na prisustvo relapsa, progresije ili rezistencije na primenjenu terapiju. **Metode.** Istraživanjem je bilo obuhvaćeno 79 ispitanika, od kojih su 63 bili bolesnici sa cHL dok je kontrolnu grupu činilo 16 zdravih dobrovoljaca. Studijska grupa sa 63 bolesnika sa cHL obuhvatala je podgrupe novodijagnostikovanih bolesnika kod kojih nije započeto lečenje, novodijagnostikovane bolesnike kod kojih je započeto lečenje, bolesnike sa relapsom i progresijom bolesti, kao i bolesnika sa primarno refraktornom bolešću tokom 2014. i 2015. godine. **Rezultati.** Analiza nivoa sCD163 i Gal-1 pokazala je da su vrednosti oba molekula kod bolesnika sa cHL bile više kod onih sa relapsom i u podgrupi bolesnika sa progresivnom bolešću, u poređenju sa podgrupom novodijagnostikovanih bolesnika kod kojih nije započeto lečenje ili bolesnika sa započetim lečenjem. **Zaključak.** Određivanje nivoa sCD163 i Gal-1 je jednostavna i pouzdana analiza koja može doprineti identifikaciji bolesnika sa cHL i visokim rizikom i zaslužuje uključivanje u tekući prognostički skoring sistem.

Ključne reči:
hodžkinova bolest; tumorski markeri, biološki; elisa; lečenje, ishod; prognoza.

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Introduction

Hodgkin's lymphoma (HL) is a lymphoid tumor that accounts for less than 1% of all newly diagnosed neoplasms per year in the world [1]. In the case of classical HL (cHL), the proportion of neoplastic cells in the total tumor mass constitutes only 1%, the remainder being composed of surrounding inflammatory cells, lymphocytes, neutrophils, eosinophils, plasma cells, and other cell types [2]. The composition of tumor environment depends on the histologic subtype of cHL, stage of the disease, and the status of anti-tumor immunity in these patients [3]. Although tissue markers such as p53, high Ki-67 proliferative index and bcl-2, correlate with poor response and short survival of patients with HL, they are not suitable for adequate monitoring of therapeutic response and early detection of relapse [4]. Prognostic scoring systems introduced by German Hodgkin Study Group (GHSG) or Hasenclever scoring system International Prognostic Score (IPS) do not include markers that adequately reflect the interaction of malignant Hodgkin and Reed-Sternberg (HRS) cells and tumor microenvironment [5, 6]. Among the major markers that show the level of tumor immunosuppression in cHL and the ability to identify patients with relapsed/refractory (RR) form of the disease, are Galectin-1 (Gal-1) and soluble CD163 (sCD163). We have attempted to analyze the importance of these molecules in the assessment of the clinical status and responsiveness to therapy in patients treated with standard induction chemotherapy ± radiotherapy, and in RR disease, with salvage chemotherapy with the use of high-dose chemotherapy and autologous hematopoietic stem cell transplantation.

The aim of this study was to determine the relationship between serum Gal-1 and sCD163 and the clinical status of patients with cHL, with special emphasis on the presence of relapse, progression, or resistance to the therapy applied. In line with that objective, the focus was on determining the importance of the simultaneous presence of Gal-1 and sCD163 in the prediction of disease progression, relapse, and refractoriness to treatment in patients with cHL.

Methods

This multicentre prospective study included patients treated at the Clinic of Hematology and Clinical Immunology at the Clinical Center in Niš, the Clinic of Oncology in Niš and the Clinic of Hematology at the Clinical Center of Serbia in Belgrade.

The study included 79 patients. Of this number, 63 were patients with cHL and the control group consisted of 16 healthy volunteers. The study group of patients with cHL included 29 (46.03%) female patients and 34 (53.97%) male patients, at the mean age of 34.89 ± 13.79 years.

The study group of 63 patients with cHL included a subgroup of newly diagnosed patients without therapy (before treatment decision), newly diagnosed patients with therapy, patients with relapse and progression of the disease and primary refractory patients during 2014 and 2015. In the follow-up period two patients died.

The control group included 16 healthy volunteers, at the mean age of 39.75 ± 12.81 years.

The study was performed in line with the Declaration of Helsinki. Study design and its realization were approved by local ethical committees of the institutions where it was implemented.

Diagnosis and determining the clinical status of patients with cHL was done in accordance with the Europen Society for Medical Oncology (ESMO) recommendations. Patients were subjected to evaluation of parameter values covered by the Hasenclever's scoring system, with the resulting poor prognosis parameters serum albumin values < 40 g/L, hemoglobin < 105 g/L, male sex, age > 45, clinical stage IV, leukocytes > 15×10^9/L and < 8% of lymphocytes or in absolute values < 0.6×10^9/L. In accordance with the number of negative scoring points, patients were categorized into groups of 0–5 by International Prognostic Score (IPS), 5 being the situation when the patient had 5 or more of these negative points. Within first-line therapy, patients were treated with adramycin, bleomycin, vinblastine, dacarbazine (ABVD) or escalated doxorubicin, etoposide, cyclophosphamide, procarbazine, prednisone, vincristine, bleomycin (eBEACOPP) polychemotherapy protocol, with the use of radiation therapy for consolidation, or in case of RR disease, salvage dexamethasone, high-dose ara-c-cytarabine, platinum (DHAP) chemotherapy protocol with high-dose chemotherapy and autologous hematopoietic stem cell transplantation.

Concentrations of circulating Gal-1 and CD163 were measured using commercial sandwich enzyme-linked immunosorbent assay (ELISA) in the plasma of patients, which is based on competitive binding of polyclonal antibodies specific of Galectin-1, i.e., CD163, according to the manufacturer's instructions (Quantikine ELISA, R&D Systems, Minneapolis, USA). Concentrations of Gal-1 and CD163 were determined by using standard curves and were expressed in ng/mL. Assay values of Gal-1 and sCD163 in the serum were 0.313–20 ng/mL, and 1.60–100 ng/mL, respectively.

According to the manufacturer, neither for Gal-1 and sCD163 nor for CD163 there was significant cross-reactivity or interference with other proteins.

Statistical methods for data processing and analysis

The study used methods of descriptive statistics and normality of distribution of continuous variables, depending on the size of the sample, was analyzed by using Kolmogorov-Smirnov and Shapiro-Wilk's test.

Comparison of values of continuous variables between two independent groups of patients was performed by Student's *t*-test, and in the case of deviation of variable distribution from the norm, Mann-Whitney U test was used. Because the distribution of the most variables was not normal, their values are given as medians (Me) and interquartile ranges (IQR, 25th–75th percentile). As the threshold of statistical significance, standard value – $p < 0.05$ was defined.

Testing the significance of differences in values of continuous variables between several independent groups was performed by Kruskal-Wallis test while testing the connection between continuous variables by Spearman's rank correlation coefficient, given that, in these cases, the distribution of the studied variables deviated from normal.

To assess the classification characteristics of the selected variables, i.e., determine their sensitivity and specificity, receiver operating characteristic (ROC) analysis was conducted. Accuracy is measured by the area under the ROC curve (AUC). Statistical data analysis was done by SPSS 15.0 software package.

Results

Comparison of the levels of sCD163 and Gal-1 between the control group with healthy volunteers and patients suffering from cHL showed that the values of sCD163, 77.30 ± 38.80 ng/mL, were higher in the group of the diseased compared to 66.80 ± 16.80 ng/mL in the control group. Gal-1 level in cHL patients amounted to 28.90 ± 2.80 ng/mL, compared to 27.15 ± 1.33 ng/mL in the control group. Compared to the control group, the values of sCD163 and Gal-1 were higher in the group of patients with cHL ($p < 0.05$ and $p < 0.001$, respectively) (Figure 1).

The sensitivity and specificity of sCD163 and Gal-1 was checked by ROC analysis to classify patients suffering from cHL in relation to healthy volunteers. It turned out that the limit values of 78.45 ng/mL of sCD163 can distinguish patients from healthy volunteers with a sensitivity of 49.2% and specificity of 87.5%, AUC = 0.686, 95% confidence intervals (CI) = 0.56–0.80, $p < 0.05$. The same analysis showed that the value of Gal-1 of 28.45 ng/mL of serum had a sensitivity of 63.5% and specificity of 93.8%, AUC = 0.807, 95% CI = 0.69–0.92, $p < 0.001$. ROC analysis of the sCD163/Gal-1 ratio showed that the value of 2.924 had a sensitivity of 41.3% and specificity of 87.5%, AUC = 0.600, 95% CI = 0.47–0.73, $p > 0.05$. Summarizing the values of sensitivity and specificity for both parameters and their ratio obtained by ROC analysis, it was calculated that the value of Gal-1 higher than 28.45 ng/mL most accurately classified cHL patients, as compared to healthy population.

The clinical status defined the newly diagnosed patients with no treatment started, treated patients as well as the patients in remission, relapse, progression, and resistance.

Accordingly, 4 (6.34%) patients were in relapse, 5 (7.93%) patients showed disease progression and 11 patients had refractoriness to treatment (17.46%). There were 13 (20.6%) newly diagnosed patients, where the analysis of Gal-1 and CD163 was done before deciding on the method of treatment, 12 (19.04%) newly diagnosed ones with the onset of therapy while remission was achieved in 18 (28.57%) patients.

Analysis of the levels of sCD163 and Gal-1 within a group of patients suffering from cHL showed that the values of both molecules were higher in relapsed patients and the subgroup of patients with progressive disease, comparing to the subgroup of patients who were newly diagnosed or with therapy onset (Figure 2).

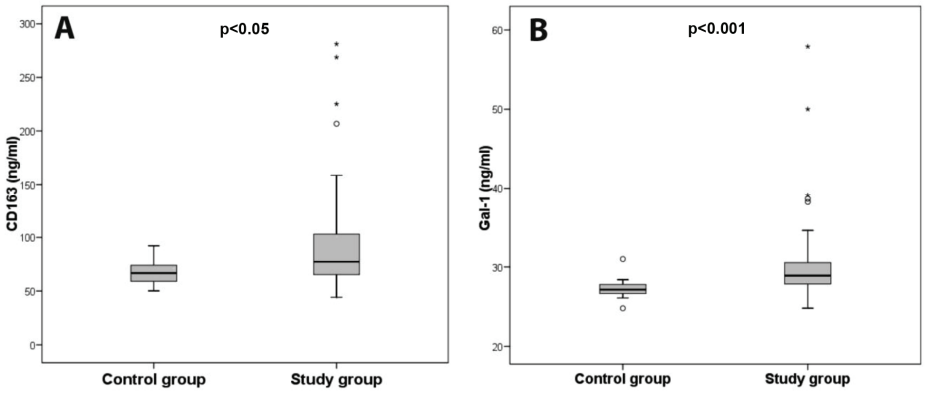

Fig. 1 – A)Values of serum sCD163 in the patients with classical Hodgkin lymphoma (cHL) and the control group; B) Values of Gal-1 in the patients with cHL and the control group. sCD163 – soluble CD163 molecule; Gal-1 – galectin-1.

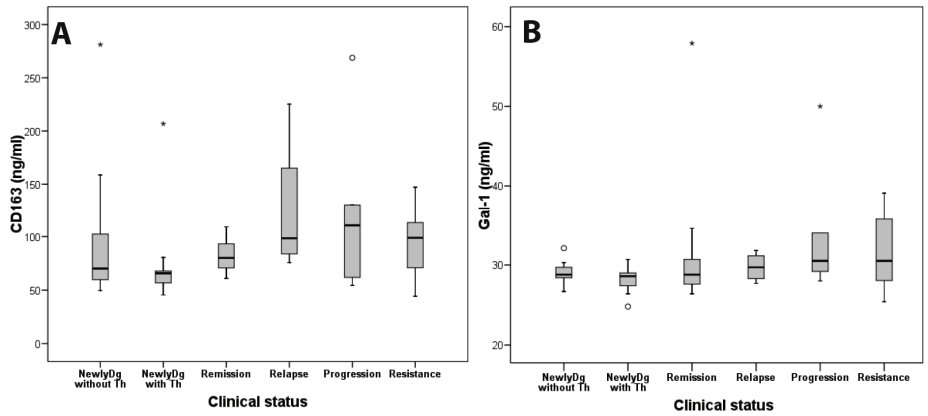

Fig. 2 – A) Values of serum sCD163 in relation to the clinical status of the patients with classical Hodgkin lymphoma (cHL); B) Values of serum Gal-1 in relation to the clinical status of the patients with cHL. sCD163 – soluble CD163 molecule; Gal-1 – galectin-1; Th – therapy.

Thus, in relation to the subgroup of patients with newly diagnosed cHL and therapy onset, the values of sCD163 of 98.50 ± 115.35 ng/mL were significantly higher in the subgroup of patients with relapsed cHL ($p < 0.05$). Comparing to the subgroup of newly diagnosed patients on therapy, sCD163 value was higher in the subgroup of patients who were in remission 80.10 ± 24.10 ng/mL ($p < 0.01$) (Figure 2).

By comparing the value of Gal-1 separately per clinical stages, the highest value of Gal-1 was found in the patients with progression of the disease 30.50 ± 13.40 ng/mL, followed by those with resistance 30.50 ± 10.30 ng/mL] while the lowest values were recorded with the newly diagnosed patients with therapy 28.60 ± 1.70 ng/mL. The values of Gal-1 in the serum were significantly higher in patients with disease progression and resistance ($p < 0.05$), as compared to the newly diagnosed patients with therapy (Figure2).

Although in the disease progression stage the values of sCD163 and Gal-1 were significantly higher than in the newly diagnosed patients with therapy, Kruskal-Wallis test did not show significant differences of their levels between defined clinical statuses. Also, the same test did not show a significant difference of sCD163/Gal-1 ratio between clinical statuses, but by comparing individual values of sCD163/ Gal-1 ratio in the period of treatment (2.32 ± 0.61) in relation to the phase of relapse (3.31 ± 3.98) and remission (2.63 ± 0.83), statistically significant differences were found ($p < 0.05$).

ROC analysis conducted between subgroups of patients according to clinical status showed very good sensitivity and specificity of parameter sCD163 and ratio of sCD163/Gal-1 between the subgroups of the newly diagnosed patients with therapy and the patients in remission as well as between the newly diagnosed patients with therapy and the patients in relapse.

It turned out that the limit values of 68.55 ng/mL of sCD163 can distinguish the patients in remission from the newly diagnosed patients with therapy with a sensitivity of 88.9% and specificity of 83.3%, AUC = 0.843, 95% CI = 0.67–1.03, $p < 0.01$. The same analysis showed that the value of sCD163/Gal-1 ratio of 2.36 had a sensitivity of 77.8% and specificity of 75.0%, AUC = 0.772, 95% CI = 0.52–0.93, $p < 0.05$. It turned out that the limit values of 72.00 ng/mL of sCD163 can distinguish the patients in relapse from the newly diagnosed patients with therapy with a sensitivity of 100.0% and specificity of 83.3%, AUC = 0.917, 95% CI = 0.77–1.06, $p < 0.05$. The same analysis showed that the value of sCD163/Gal-1ratio of 2.42 had a sensitivity of 100.0% and specificity of 75.0%, AUC = 0.896, 95% CI = 0.73–1.06, $p < 0.05$.

ROC analysis showed very good sensitivity and specificity of Gal-1 parameter between subgroups of newly diagnosed patients with therapy and patients with disease progression.

It turned out that the limit values of 29.15 ng/mL of Gal-1 can distinguish the patients with cHL proregression from the newly diagnosed patients with therapy with a sensitivity of 80.0% and specificity of 83.3%, AUC = 0.825, 95% CI = 0.60–1.05, $p < 0.05$. Analysis of levels of Gal-1 and sCD163 depending on IPS in patients with cHL showed that their values were highest in cases where IPS = 3. Values of sCD163 at IPS = 3 were statistically significantly higher than IPS = 0, 1, and 4 ($p < 0.05$) as well as than IPS = 2 ($p < 0.01$). Values of Gal-1 at IPS = 3 were statistically significantly higher compared to IPS = 0 ($p < 0.01$) (Table 1).

It should be pointed out that score IPS = 5 was registered only in one patient that was also a newly diagnosed under therapy at the time of taking the biomarker sample which is why a comparison with other groups did not make sense (Table 1).

In order to highlight the difference among the patients based on Hasenclever and Diehl's scoring system, the subgroup with 37 low-risk patients with IPS 3 or less was formed, while 8 patients with IPS 4 and 5 scores were combined into a high-risk subgroup. Level of sCD163 in the group with IPS 0–3 amounted to 75.80 ± 48.70 ng/mL and in the group with IPS 4–5 to 69.60 ± 56.40 ng/mL. Values of Gal-1 in the group with IPS 0-3 amounted to 28.80 ± 2.70 ng/mL and in the group with IPS 4–5 to 29.35 ± 4.90 ng/mL. Comparing the value of sCD163 and Gal-1 among the combined groups did not point to statistically significant difference, although it was evident that the value of CD163 was higher in the patients with IPS up to 3. The finding that the values of Gal-1 and sCD163 were the highest at IPS = 3, and then fell, can be explained by the fact that in all patients with IPS 4 and 5 therapy was applied.

Table 1

Values of serum sCD163 and Gal-1 in the patients diagnosed with classical Hodgkin lymphoma (cHL) compared to the value of International Prognostic Score (IPS)[6]

IPS score	Patients (number)	sCD163 (ng/mL)	Gal-1 (ng/mL)
0	8	67.25	28.05
		25.90 (57.53–83.43)	2.18 (26.82–29.00)
1	5	75.80	28.80
		37.10 (62.70–99.80)	4.75 (26.75–31.50)
2	17	66.50	28.70
		51.00 (61.90–112.90)	2.45 (27.95–30.40)
3	7	158.70 [abd*c**]	30.50[a**]
		163.70(105.10–268.80)	2.70(29.40–32.10)
4	7	77.30	29.50
		65.70 (45.50–111.20)	6.00(27.30–33.30)
5	1	61.50	28.90

Values are given as medians (Me) and interquartile ranges (IQR, 25th–75th percentile);
sCD163 – soluble CD163 molecule; Gal-1 – golectin-1; * – p < 0.05, ** – p < 0.001; a – vs IPS = 0, b – vs IPS = 1, c – vs IPS = 2, d – vs IPS = 4.

Table 2

Values of serum sCD163 and Gal-1 in the classical Hodgkin lymphoma (cHL) patients in relation to applied therapeutic modalities

Therapy (Th) modalities	n	sCD163 (ng/mL)	Gal-1 (ng/mL)
One Th line	40	70.60	28.80
(ABVD or eBEACOPP as first line)		31.45(61.60–93.05)	2.75(27.52–30.27)
Two and more Th lines	23	91.90	29.50
(DHAP, ESHAP, ICE, Brentuximab)		41.00(70.20–111.20)	6.00(28.00–34.00)
Salvage Th, HDT and ASCT	15	100.70**	31.80**
		43.00 (80.80 – 123.80)	10.00(28.20–38.20)
Salvage Th	48	70.60	28.75
		31.45 (61.60–93.05)	2.67(27.60–30.27)

Values are given as medians (Me) and interquartile ranges (IQR, 25th–75th percentile).
**** – $p < 0.01$ *vs* salvage Th.**
sCD163 – soluble CD163 molecule; Gal-1 – galectin-1.
HDT– high dose chemotherapy; ASCT– autologous stem cell transplantation.
ABVD – adriamycin, bleomycin, vinblastine, dacarbazine; eBEACOPP – escalated doxorubicin, etoposide, cyclophosphamide, procarbazine, prednisone, vincristine, bleomycin; DHAP – dexamethasone, high-dose ara-c-cytarabine, platinum; ESHAP – etoposide, methylprednisolone, cytarabine & cisplatin.

Spearman's correlation coefficient for IPS and sCD163 amounted to 0.20, and for IPS and Gal-1 0.35, which shows that both markers correlate positively with IPS, but that only IPS and Gal-1 ($\rho = 0.35$, $p < 0.05$) had a statistically significant correlation of an average intensity. On the basis of the earlier identified elevated levels of sCD163 and Gal-1 in the patients with cHL and disease progression, further analysis focused on the relationship between their values based on the applied therapy. After the initial application of ABVD protocol in the treatment, relapse occurred in 4 patients, progression during treatment in 5 patients and primary refractory disease in 7 patients. Primary refractoriness to eBEACOPP was recorded only in two cases. DHAP protocol was administered as a second-line therapy in 13 patients, and most often continued after ABVD in 4 patients in relapse and two with progression during ABVD treatment. Etoposide, methylprednisolone, cytarabine & cisplatin (ESHAP) protocol was in the second line of treatment given to one patient. The largest number of transplant patients was from the group of 9 primarily refractory patients, 3 patients were in relapse, and only one patient had disease progression.

It was observed that in the patients who received two or more therapeutic modalities, values of sCD163 and Gal-1 were elevated, but not statistically significantly, compared to the patients with one line of treatment (Table 2). Values of sCD163 and Gal-1 were higher in the patients who underwent transplantation than in those who did not, at the level of statistical significance of $p < 0.05$ (Table 2). The value of Gal-1 and sCD163 was measured after transplantation.

Transplantation was not applied in all patients because of the lack of complete and/or partial remission (CR/PR), chemosensitive relapse (CSR), and chemoresistant disease (HD).

Discussion

The basis of this study is the importance of Gal-1 and sCD163 to identify patients with cHL compared to reactive states as well as their significance in the assessment of the disease phase of patients. There is unequivocal evidence of the links of both Gal-1 and sCD163 with the processes of immune suppression in cHL.

M2a-type macrophages are the most common in advanced tumor stages, which is associated with the progression of malignant disease and correlates with primary and secondary failure of therapy [7, 8].

Presence of M2 macrophages correlates with levels of sCD163, making this protein considered to be their reliable serum marker [9]. Antiinflammatory function of M2 macrophages is reflected in the secretion of immunosuppressive cytokines (transforming growth factor-β), transforming growth factor beta (TGF-β1), and IL-10, which further induce T helper cells type 2 (Th2) differentiation, favoring the development of T-regulatory (Treg) lymphocytes promoting the growth of tumors by inhibition of anti-tumor immune response [10].

The findings of high levels of sCD163 primarily in the patients with disease progression as well as in relapse phase, where these levels were statistically significantly higher compared to patients whose treatment has just begun is in line with the information referred to above. These results support the impact of immunosuppression on the deterioration of the clinical course of the disease in cHL patients, which can be observed through a high level of sCD163 in the patients who were treated with a large number of chemotherapy lines and in the subgroup of patients undergoing transplantation.

The lowest values of sCD163 among the investigated cases were recorded in the subgroup of newly diagnosed patients who started chemotherapy. A possible explanation of these findings lies in the fact that chemotherapy, together with the elimination of malignant cells, modifies the population of infiltrating cells in HL by killing Treg lymphocytes and monocytes of type M2, thus reducing tumor immunosuppression [9, 11].

Galectin-1 belongs to a group of proteins with an affinity for binding carbohydrates which is in large quantities produced by HRS cells. Immunosuppressive function of Gal-

1 is reflected in the inhibition of secretion of IL-2, interferon-γ [IFN-γ and tumor necrosis factor α (TNFα)] with the induction of the immunosuppressive IL-10 creation. Furthermore, it selectively kills Th17 cells, influences the polarization of Th1 response in the direction of Th2 response, by initiating Th1 cell apoptosis [10]. Therefore, Gal-1 is considered to be an indicator of immunosuppression, caused by the malignant HRS cells and the indicator of absence of Th1 anti-tumor immune response [12].

Clinical studies have shown that elevated Gal-1 is associated with a shorter period and leads to progression in patients with cHL [13].

Findings regarding our patients are in line with literature data, because the values of Gal-1 were significantly higher in subgroups of relapsing, refractory patients who had to receive more therapeutic lines and undergo autologous hematopoietic stem cell transplantation.

Low levels of Gal-1 in the patients with chemotherapy in our study are most likely the result of reduction in the HRS cells under the influence of therapy. Such a drop in the level of Gal-1 during treatment compared to the level before therapy is recorded in the work of Plattel et al. [14].

However, the values of Gal-1 and sCD163 can also be elevated to normal cells and grow with age [15]. These findings may partly explain the high levels of these molecules in healthy volunteers who made up the control group in this study. Our study has for the first time identified limit values by which it is possible to reliably distinguish reactive states from the active form of cHL.

Over the past decade, a large number of prognostic systems have been developed in order to identify high-risk patients with HL. Despite some shortcomings, IPS is today still used in risk stratification at the initial presentation of advanced stages of HL [6]. However, this system does not contain markers that accurately reflect the state of immunosuppression and tumor microenvironment that play a significant role in the evolution and prognosis of HL. By comparing the levels of sCD163 and Gal-1 with the IPS system scores in our study, two important findings were obtained. In spite of the influence of the administered therapy, it was found that the levels of sCD163 and Gal-1 correlated with the IPS score, but that only IPS and Gal-1, had statistically significant correlation of an average intensity.

These findings open up the possibility of correction of IPS system by incorporating these simple serum molecules, which could eliminate its shortcomings, especially in the domain of R/R forms of cHL.

Conclusion

The values of Gal-1 > 28.45 ng/mL and levels of sCD163 > 78.45 ng/mL exclude reactive states and they can be considered as reliable values found in variants of cHL.

High values of sCD163 and Gal-1 are characteristics of the patients with relapsing and/or refractory variant of cHL, requiring the use of salvage chemotherapy with high-dose chemotherapy and hematopoietic stem cell transplantation, while serum concentrations of these proteins were the lowest during chemotherapy in the newly diagnosed patients.

Applied therapy in the patients with cHL affects the values of Gal-1 and CD163, but research has pointed to a positive correlation among IPS score and values of CD163 and Gal-1 which is statistically significant in the case of Gal-1 and IPS score relationship.

Determination of Gal-1 and sCD163 levels is simple and reliable analysis that can contribute to the identification of high-risk patients with cHL and deserves inclusion in current prognostic scoring systems.

REFERENCES

1. *Pileri SA, Ascani S, Leoncini L, Sabattini E, Zinzani PL, Piccaluga PP,* et al. Hodgkin's lymphoma the pathologist's viewpoint. J Clin Pathol 2002; 55(3): 161–76.
2. *Hnátková M, Mociková H, Trnený M, Zivný J*. The biological environment of Hodgkin's lymphoma and the role of the chemokine CCL17/TARC. Prague Med Rep 2009; 110(1): 35–41.
3. *de la Cruz-Merino L, Lejeune M, Nogales Fernández E, Henao Carrasco F, Grueso López A, Illescas Vacas A,* et al. Role of immune escape mechanisms in Hodgkin's lymphoma development and progression: a whole new world with therapeutic implications. Clin Dev Immunol 2012; 2012: 756353.
4. *Smolewski P, Robak T, Krykowski E, Blasińska-Morawiec M, Nieviadomska H, Pluzanska A,* et al. Prognostic factors in Hodgkin's disease: multivariate analysis of 327 patients from a single institution. Clin Cancer Res 2000; 6(3): 1150–60.
5. *Josting A, Franklin J, May M, Koch P, Beykirch MK, Heinz J*, et al. New prognostic score based on treatment outcome of patients with relapsed Hodgkin's lymphoma registered in the database of the German Hodgkin's lymphoma study group. J Clin Oncol 2002; 20(1): 221–30.
6. *Hasenclever D, Diehl V*. A prognostic score for advanced Hodgkin's disease. International Prognostic Factors Project on Advanced Hodgkin's Disease. N Engl J Med 1998; 339(21): 1506–14.
7. *Zhang B, Yao G, Zhang Y, Gao J, Yang B, Rao Z,* et al. M2-polarized tumor-associated macrophages are associated with poor prognoses resulting from accelerated lymphangiogenesis in lung adenocarcinoma. Clinics (Sao Paulo) 2011; 66(11): 1879–86.
8. *Mantovani A, Sozzani S, Locati M, Allavena P, Sica A*. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002; 23(11): 549–55.
9. *Jones K, Vari F, Keane C, Crooks P, Nourse JP, Seymour LA*, et al. Serum CD163 and TARC as disease response biomarkers in classical Hodgkin lymphoma. Clin Cancer Res 2013; 19(3): 731–42.
10. *Tsai C, Chiu Y, Hsu T, Lin-Ying I, Hsieh S, Lin KI*. Galectin-1 promotes immunoglobulin production during plasma cell differentiation. J Immunol 2008; 181(7): 4570–9.
11. *Maeda K, Hazama S, Tokuno K, Kan S, Maeda Y, Watanabe Y*, et al. Impact of chemotherapy for colorectal cancer on regulatory T-cells and tumor immunity. Anticancer Res 2011; 31(12): 4569–74.

12. *Ouyang J, Plütschow A, Pogge von Strandmann E, Reiners KS, Ponader S, Rabinovich GA*, et al. Galectin-1 serum levels reflect tumor burden and adverse clinical features in classical Hodgkin lymphoma. Blood 2013; 121(17): 3431–3.
13. *Kamper P, Ludvigsen M, Bendix K, Hamilton-Dutoit S, Rabinovich GA, Møller MB,* et al. Proteomic analysis identifies galectin-1 as a predictive biomarker for relapsed/refractory disease in classical Hodgkin lymphoma. Blood 2011; 117(24): 6638–49.
14. *Plattel WJ, Alsada ZN, Imhoff GW, Diepstra A, Berg A, Visser L.* Biomarkers for evaluation of treatment response in classical Hodgkin lymphoma: Comparison of sGalectin-1, sCD163 and sCD30 with TARC. Br J Haematol 2016; 175(5): 868–75.
15. *Verschuere T, van Woensel M, Fieuws S, Lefranc F, Mathieu V, Kiss R*, et al. Altered galectin-1 serum levels in patients diagnosed with high-grade glioma. J. Neurooncol 2013; 115(1): 9–17.

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The impact of gender differences on mortality in elderly patients after hip fracture

Uticaj polnih razlika na mortalitet starijih bolesnika nakon preloma kuka

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Abstract

Background/Aim. Hip fracture is one of the leading causes of death in elderly population. We evaluated the impact of gender differences on mortality rate in elderly patients with hip fracture. **Methods.** The study included all hospitalized elderly patients (aged over 65 years) with hip fracture during 2013. The patients were classified into four risk groups in accordance with institutional Şişli Etfal risk factor assessment scale ISERFAS to estimate postoperative mortality. Clinical, laboratory and risk score results were gender matched between survived and deceased patients. Hospital mortality was monitored as well as mortality at intervals of three and six months. The prediction effect of gender and overall risk variables on mortality rate were determined by univariate and multivariate logistic regression analyses. **Results.** The complete sample included 434 female and 163 male patients. Average age of men was 77.95 years and 79.18 years for women. Femoral neck fracture was more often seen in women (44.5%), but with no statistically significant difference (p = 0.57). Significant difference between the genders in relation to the risk score values was determined (p = 0.024). It was observed that the values of risk score was lower in the female patients and higher in the male patients. Cumulative mortality was 6% during hospital stay, 17.8% after three months and 25% after six months, respectively. In-hospital and six months after the hip fracture, the mortality rates were similar in both genders. The mortality rate was significantly higher in male patients (p = 0.035) three months after the hip fracture. The overall risk observed at all mortality intervals was a significant predictor by itself (p = 0.000). Independent gender prediction effect disappeared in joint effects of patients' overall risk. **Conclusion.** Gender can be defined as a significant mortality predictor in patients with hip fracture. A risk assessment system to estimate postoperative mortality for hip fractures would be helpful in planning treatment for each patient.

Key words:
hip fractures; aged; risk factors; sex; mortality; comorbidity; serbia.

Apstrakt

Uvod/Cilj. Prelom kuka je jedan od vodećih uzroka smrti u starijoj populaciji. Analiziran je uticaj polnih razlika na stope mortaliteta starijih bolesnika sa prelomom kuka. **Metode.** U studiji su bili uključeni svi hospitalizovani bolesnici stariji od 65 godina sa prelomom kuka tokom 2013. godine. Bolesnici su bili podeljeni u četiri grupe rizika, mali, srednji, visok i veoma visok, u skladu sa institucionalnom Sisli Etfal skalom za procenu faktora rizika postoperativnog mortaliteta (institutional Şişli Etfal risk factor assessment scale – ISERFAS). Klinički, laboratorijski i rizik rezultati upoređeni su prema polu između preživelih i umrlih bolesnika. Praćen je bolnički, tromesečni i šestomesečni mortalitet bolesnika. Prediktivni uticaj pola i ukupnog rizika na stope mortaliteta testirane su univarijantnom i multivarijantnom regresionom analizom dobijenih rezultata. **Rezultati**. Kompletni uzorak činilo je 434 ženskih i 163 muških bolesnika. Prosečna starost muškaraca je bila 77,95, a žena 79,18 godina. Prelom vrata butne kosti bio je češći kod žena (44,5%), bez statističkI značajnosti (p = 0.57). Pokazana je značajne razlike u odnosu na muškarce razlika između polova u odnosu na rizik skor (p = 0,024). Kod ženskih bolesnika primećen je niži stepen rizika, dok je kod muškaraca

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primećen viši. Kumulativni mortalitet bio je 6% tokom hospitalizacije, 17,8% nakon tri, odnosno 25% nakon šest meseci. Stope bolničkog i šestomesečnog mortaliteta bile su slične kada je u pitanju pol, jedino su muškarci značajno češće umirali (p = 0.035) tri meseca nakon povrede. Ukupan rizik je na svim posmatranim tačkama mortaliteta (p= 0.000) bio značajan prediktor sam po sebi. Samostalni prediktorni uticaj pola se gubio u sadejstvu sa ukupnim rizikom. **Zaključak.** Pol se može definisati kao značajan prediktor mortaliteta kod bolesnika sa prelomom kuka. Sistem ocenjivanja rizika u proceni postoperativne smrtnosti usled prelom kuka mogao bi biti od pomoći u planiranju tretmana za svakog bolesnika.

Ključne reči:
kuk, prelomi; stare osobe; faktori rizika; pol; mortalitet; komorbiditet; srbija.

Introduction

Hip fracture is one of the leading causes of death in elderly population. Mortality rates have not changed in the last four decades and ranges from 2% to 8% during hospitalization, 8%–10.5% within thirty days after the fracture, about 17% after three months, 11%–28% after six months and 22% –36% after a year [1–3].

As for gender structure of the world's population, female population dominates over male population in all elderly groups [4, 5]. Almost all the publications confirmed the hip fractures were more common in women than in men in relation between 1.7:1 [6] and even 4.5:1 [7]. Predominance of hip fractures in the female population is a universal phenomenon. In China (Shenyang province) and in Turkey, where men work hard physical labor, the female / male ratio of hip fracture is reversed [8, 9].

Chronic, multi-systemic and more or less controlled diseases are characteristical for a person with the hip fracture (on average, 3.7 comorbidities). Such person can have cognitive impairment in high percentage and is given multiple medications [10].

A lot of variables affecting the treatment outcomes in the patients with fracture were described and include: age, gender [11], interval between admission and the surgery, the level of surgery risk, functional and mental status before the fracture [12, 13], cognitive impairment [14], presence of multiple diseases before the fracture, American Society of Anesthesiologists (ASA) score [15], hemodynamic disorders [16], type of treatment (surgical or non-surgical) and type of surgical technique [17].

Many studies have indicated gender as an important factor that affects mortality after the hip fracture. This gender conversion is interesting and can be seen in higher incidence rates in women and in higher mortality rates in men [18, 19].

Statistically significant difference in mortality rate in men in comparison to women is even 2.38 times higher [20]. Generally, mortality rate is higher in men and is in the range between 32% and 62% annually, and in women it ranges between 17% and 29% [21, 22].

Methods

The study enrolled all the patients with the proximal femoral fracture over the age of 65 years, hospitalized at the Orthopaedic and Traumatology Clinic of the Clinical Center Niš and Kragujevac, Serbia. All the data, including anamnestic (gender, age), clinical (type of fracture, type of treatment, type of surgery treatment and implant type), laboratory and radiography data were collected using hospital discharge database for the patients hospitalized in both clinical centers.

In accordance with the institutional Şişli Etfal risk factor assessment scale [23] (Table 1), comprising the data on patients' age, daily activities, osteoporosis, dementia, cardiac diseases, etc., the patients were classified into low, moderate, high and very high risk groups.

Individual data were also obtained from the Institute of Statistics Death Registry – Republic of Serbia, six month after the injury, to assess survival rate, obtain the diagnosis by identifying the immediate cause of death and determine factors responsible for mortality in patients with the hip fracture. Mortality rate in elderly patients can be monitored at different intervals. Generally speaking, there are two main periods: in-hospital mortality occurring during the hospital stay and post-discharge mortality occurring after the discharge from hospital. These data are important for survival rate analysis after the fracture (three, six and twelve months). In our study, in-hospital mortality as well as three-month and six-month mortalities were evaluated.

Clinical findings (general health status evaluation, the presence of associated diseases, quantity and type of medication, mobility assessment, type of fracture and injury location), laboratory findings and risk score were gender compared between survived and dead patients.

Complete statistical analysis of the data was done with the statistical software package, SPSS Statistics 17 (Chicago, IL, USA). Most of the variables were presented as the frequency of certain categories, while statistical significance of differences was tested with the χ^2 test.

In case of continuous data, the variables were presented as the mean value ± standard deviation (SD) and the statistical significance of differences was tested by *t*-test.

Calculations of odds ratios (OD) and their 95% confidence intervals (CI) were done to determine the association between risk factors and outcomes (survival). For that purpose, the most promising independent variables as a single risk factor were incorporated into binary logistic regression analyses. All the analyses were estimated at $p < 0.05$ level of statistical significance.

The prediction effect of gender and overall risk variables on mortality rate were determined by logistic regression analysis; univariate analysis was performed as the first and then multivariate analysis as the second one.

Table 1

Şişli Etfal Research and Training Hospital Risk Scoring System before hip fracture surgery [23]

Patient's characteristics	Score		
Age (years)		Diabetes Mellitus	1
< 70	0	Vascular occlusion	1
70–79	1	Gastrointestinal disease	1
80–89	2		
> 90	3		
Daily activity degree before fracture		Lung pathologies	
free	0	asthma	1
one crutch	1	infection	1
walker	2	Chronic obstructivc lung disease	
bedridden	3	tumor	1
		tuberculosis	1
Osteoporosis (Singh)		Electrocardiogram	
0–3	0	normal	0
4–5	1	aritmia	1
6	2	infarction sign	2
Dementia (Hagerawa criteria's)		ST-T changes, AV block	3
normal	0	Blood tests	
borderline	1	Hb (g/dL) 11<	1
predemantia	2	Hb (g/dL) 11>	0
dementia	3	Total protein (< 6 g)	1
Heart Pathologies		Total protein (> 6 g)	0
myocardial infartion	1	Neurological disease	
angina pectoris	1	hemiplegia	1
right heart failure	1	parkinson	1
ventricular extrasistol	1	Genitourinary disease	1
cardiac aritmia	1	Obesity	1
hypertension	1	Cancer	1

Total risk score: 0–5 – Low risk; 6–10 – Moderate; 11–15 – High; > 15 – Very high.

Results

The complete sample consisted of 597 patients, 434 (72.7%) female and 163 (27.3%) male patients. Average age of women was 79.18 years (age ranges from 65 to 101 years) and 77.95 years for men (age ranges from 65 to 92 years). Intertrochanteric fractures were present in 241 (55.5%) female patients and in 95 (58.3%) male patients. Fractures of the femoral neck were present in 193 (44.5%) females and 68 (41.7%) males. The difference was not statistically significant ($p = 0.57$). As for the injury location, there was a statistically significant difference between male and female patients, namely, 380 (87.6%) women got fractures indoors in comparison to 41 (25.2%) men who got injured outdoors ($p = 0.000$).

Before the hip fracture, 234 (53.9%) women were able to walk independently, 199 (45.9%) required some kind of assisting device and one (0.2%) patient walked with the help of another person before the fracture. On the other hand, 112 (68.7%) males walked independently, 50 (30.7%) required some kind of assisting aid and one (0.6%) male patient walked with the help of another person. The χ^2 test showed significant difference between the genders and mobility before the fracture ($p = 0.003$).

The level of creatinine ($p = 0.001$) was significantly more increased in 61 (37.4%) males in comparison to 100 (23%) females. The level of hemoglobin was lower in 207 f (47.7%) emales in comparison to 64 (39.3%) males.

Of 23 most frequently occurring morbidities among the elderly with hip fracture who were followed in our study, there were only 7 morbidities with a significant difference between men and women (Table 2). Out of 21 groups of medications, the gender difference in medication consumption was registered only in 4.

The χ^2 test also showed significant difference between the genders in the risk score values ($p = 0.024$). In the female patients, the values of low risk score (13.8%) and moderate risk score (46.1%) were observed while in the male patients the risk score was high (49.1%) and very high (1.8%), (Tables 3). Tables 4, 5 and 6 show distribution values of in-hospital, three-month and six-month mortality according to the gender and risk score.

Table 7 shows in-hospital, three-month and six-month mortality rates. In-hospital and six-month mortality rates were similar in both genders. Only three-month mortality rate after the hip fracture was significantly higher in male patients ($p = 0.035$).

Univariate analysis indicated that individual gender prediction effect was defined as a significant mortality predictor only three months after the fracture ($p = 0.032$) while its significance was not registered for in-hospital and six-month mortality rate. Overall risk for patients (ISERFAS) at all mortality intervals is by itself a significant predictor ($p = 0.000$) (Table 8).

Table 2

Groups of morbidities and medications with significantly evident gender differences

Morbidity	Women, n (%)	Men, n (%)	*p*
Anemia	211 (48.6)	65 (39.9)	0.056
Epilepsy	4 (0.9)	7 (4.3)	0.006
Kidney disease (chronic renal insufficiency, nephropathy, azotemia, etc.)	98 (22.6)	55 (33.7)	0.005
Diseases of the genitourinary tract	4 (0.9)	6 (3.7)	0.019
Hearing problems	6 (1.4)	8 (4.9)	0.011
Thyroid disease	14 (3.2)	0 (0.0)	0.020
Gastritis, ulcus of the stomach /duodenum	14 (3.2)	15 (9.2)	0.002
Medications			
antiepileptics	4 (0.9)	6 (3.7)	0.019
bronchodilators	42 (9.7)	26 (16.0)	0.032
thyroid hormones	10 (2.3)	0 (0.0)	0.051
stomach protector	125 (28.8)	64 (39.3)	0.014

Table 3

Difference between the gender according to the risk score values

Gender	Risk score, n (%)				Total
	low	moderate	high	very high	
Women	60 (13.8)	200 (46.1)	168 (38.7)	6 (1.4)	434 (100.0)
Men*	10 (6.1)	70 (42.9)	80 (49.1)	3 (1.8)	163 (100.0)
Total	70 (11.7)	270 (45.2)	248 (41.5)	9 (1.5)	597 (100.0)

*** *p* = 0.024 vs. women.**

Table 4

Distribution values of in-hospital mortality according to the gender and risk score

Gender	Risk score, n (%)				Total
	low	moderate	high	very high	
Survivors					
women	60 (14.7)	198 (48.5)	148 (36.3)	2 (.5)	408 (100.0)
men	10 (6.5)	70 (45.8)	71 (46.4)	2 (1.3)	153 (100.0)
total	70 (12.5)	268 (47.8)	219 (39.0)	4 (0.7)	561 (100.0)
Non-survivors					
women		2 (7.7)	20 (76.9)	4 (15.4)	26 (100.0)
men		0 (.0)	9 (90.0)	1 (10.0)	10 (100.0)
total		2 (5.6)	29 (80.6)	5 (13.9)	36 (100.0)

Table 5

Distribution values of three-month mortality according to the gender and risk score

Gender	Risk score, n (%)				Total
	low	moderate	high	very high	
Survivors					
women	58 (16.9)	178 (51.7)	106 (30.8)	2 (.6)	344 (100.0)
men	10 (8.5)	62 (53.0)	45 (38.5)	0 (.0)	117 (100.0)
total	68 (14.8)	240 (52.1)	151 (32.8)	2 (.4)	461 (100.0)
Non-survivors					
women	2 (3.1)	20 (31.3)	42 (65.6)	0 (.0)	64 (100.0)
men	0 (.0)	8 (22.2)	26 (72.2)	2 (5.6)	36 (100.0)
total	2 (2.0)	28 (28.0)	68 (68.0)	2 (2.0)	100 (100.0)

Table 6

Distribution values of six-month mortality according to the gender and risk score

Gender	Risk score, n (%)				Total
	low	moderate	high	very high	
Survivors					
women	58 (18.3)	166 (52.4)	92 (29.0)	1 (.3)	317 (100.0)
men	10 (9.0)	60 (54.1)	41 (36.9)	0 (.0)	111 (100.0)
total	68 (15.9)	226 (52.8)	133 (31.1)	1 (.2)	428 (100.0)
Non-survivors					
women		12 (44.4)	14 (51.9)	1 (3.7)	27 (100.0)
men		2 (33.3)	4 (66.7)	0 (.0)	6 (100.0)
total		14 (42.4)	18 (54.5)	1 (3.0)	33 (100.0)

Table 7

In-hospital, three-month and six-month mortality rates for men and women

Mortality rate	Total		Women		Men	
	n	%	n	%	n	%
In-hospital	36	6	26	6	10	6.1
three-month	100	17.8	64	15.7	36	23.5*
three- to six-month	33	7.2	27	7.8	6	5.1
six-month	133	25	91	23.5	42	28.6

[1] **six-month mortality rate is sum of the mortality rates obtained after three and between three to six months after the injury.**

***p* = 0.035 vs. women**

Table 8

Univariate analysis of the gender and overall risk as predictors of mortality includely patients with hip fractures

Mortality rate	Wald	Sig.	Odds ratio (OR)	95% CI for OR lower	upper
Hospital					
gender	0.004	0.947	0.975	0.459	2.069
risk	32.203	0.000	13.293	5.439	32.487
Three-month					
gender	4.614	0.032	0.605	0.382	0.957
risk	41.362	0.000	3.754	2.508	5.618
Six-month					
gender	0.958	0.328	1.576	0.634	3.917
risk	12.621	0.000	3.029	1.643	5.581

CI – confidence interval; * *p* = 0.000

Multivariate regression analysis that the total joint effect of gender [OR (95% CI) 1.279] and risk score (ISAR-FAS) in in-hospital mortality defined the multiple risk levels as significant predictors ($p < 0.001$). A three-month mortality rate risk score also proved to be a significant predictor and in the low risk patients as well ($p = 0.006$). A six-month mortality rate showed that joint effects of gender and risk score were disappeared, so neither gender, nor the lowest risk score in the patients were no longer predictors, but something else was.

The independent gender prediction effect disappeared in joint effects with a total risk score in the patients and gender was not a responsible mortality predictor according to multivariate regression model.

Discussion

A typical image of a person with proximal femoral fracture can be compared to an old lady in the eight decade of life living alone, with decreased motility and problems in daily activities and health problems typical of her age. The image accounts for the male patients as well, but the number of male population of the same age is lower in comparison to females [4].

Autier et al. [24] estimated that by 2031 there would be about 600,000 hip fractures in women and about 150,000 in men, unless effective prevention measures in the European Union (EU) were applied. It was estimated that one in three women and one in nine men over the age of 80 years would experience osteoporotic hip fracture.

Hip fracture incidence doubles for each decade of life after the age of 50, so 93% of women who live up to 80 years of age have at least one fracture, accounting for 33% of hip fracture [25].

One of the leading etiological factors is osteoporosis that is most common in women since the menopause induces hormonal deficit that causes osteoporosis. Besides, incidence rates of hip fractures are caused by multiple falls in women due to less muscle mass [6]. An important factor is also the longer life expectancy for women in comparison to men [4, 6].

However, it is well known that the countries promoting prevention mostly in female population (bisphosphonates, calcium and vitamin D) faced less dramatic incidence of hip fractures in women and the fracture rate was steady in men, as reported for Scandinavian countries [26]. In another study (USA) [27], the efficacy of prevention measures were also pointed out as well as higher incidence of fractures in Caucasian female immigrants not involved in prevention strategies.

Identification of patients at a risk and determination of treatment options can be facilitated by understanding predictors of mortality [28].

In their study, Hu et al. [28] identified 12 preoperative predictors for post-operative mortality in patients with hip

fracture. They included advanced age, male gender, nursing home or facility residence, poor preoperative walking capacity, poor activities of daily living, higher ASA grading, poor mental state, multiple comorbidities, dementia or cognitive impairment, diabetes, cancer and cardiac disease.

During the performance of our study we observed that gender variable had prognostic value on incidence and mortality rates, but in different ways. This gender conversion showed higher incidence rates in women and higher mortality rates in men. It is clear that female gender is considered a risk factor for hip fracture. So, the risk of hip fracture in women aged 50 years was estimated to be 14%, while in men the risk was only 3% [29].

This gender difference cannot be clearly explained. One explanation can be that higher morbidity rate of 32% is registered in males one year after the hip fracture unlike the female population with morbidity rate of 18% [21].

The significance of these data lies in the fact that although men are relatively young when sustain a hip fracture, they obviously have poorer general health than women which affects the final outcome of hip fracture [30]. In a study made by Carpintero et al. [31,] it was found that increased mortality rate in men was due to poor nutritional status, multiple comorbidities, habitual cigarette smoking and excessive use of alcohol. However, Allegri-Lopez et al. [32] reported that besides decreased functional activities prior the fracture, female gender was proved to be a predictor of increased mortality.

Mobility degree before the injury was significant in patients with hip fracture in our study as well, since its consequences affect general health status in these patients. Women were less motile in comparison to men, which implies that an indoor or outdoor fracture shows patients' physical and mental status prior to injury. About a half of hip fractures in female population was caused by experiencing a loss of confidence in walking, so they restricted their daily activities and became unable, or, unwilling to leave their homes which was increasing the risk of further fractures [33]. Moreover, in a very old woman and men, the risk of hip fracture was equal [34, 35], suggesting that women and men susceptibility to the occurrence of hip fractures was increasing with age.

The health status before the fracture is the best predictor of recovery after fractures. Up to three quarters of patients had the following diseases on admission (hypertension: 20%–40%; ischemic heart disease: 8%–40%; anemia: 25%–35%; dementia: 10%–35%; chronice obstructive pulmology disease (COPD): 10%–35%; fibrillation: 9%–20%; diabetes mellitus: 7%–20% [36]. Comorbidities proved to be significant prognostic factors in our study as well as in some others. Analyzing association between preoperative comorbidity and the risk of postoperative complications and mortality, it was found that in the elderly with hip fracture, the presence of three or more preoperative comorbidities represented the strongest risk factors while respiratory infections and heart failure were the most common post-operative complications and proven lead to increased mortality [37].

An association between preoperative abnormal values of creatinine and postoperative mortality was established in a study with smaller number of patients [38]. Increased values of creatinine in male gender were also found in our study. Decompensated chronic renal dysfunction in elderly patients, resulting from intraoperative or postoperative complications, oliguria and hyperkalemia additionally worsen kidney function. However, preoperative and postoperative preventive measures for reducing renal function may be effective in reducing mortality rate. Chronic renal dysfunction in elderly patients is decompensated due to intraoperative or postoperative complications, the function of the kidneys is additionally worsen by oliguria and hyperkalemia. However, preoperative and postoperative prevention measures against reducing renal function may be effective in mortality decrease.

The studies on mortality rate after discharge from hospital (most commonly monitored three, six and twelve months) [39] showed that the advances in surgery and anesthesiology did not significantly reduce mortality rate [22]. According to literature data, mortality was mostly registered three to six months following the fracture [19, 40, 41]. Mortality rate declined after that, although never decreased to the level of mortality rate in general population [22]. The dominance of male gender as a mortality predictor was also registered in our study.

So, the elderly men, who suffer from more chronic diseases (heart failure, COPD hypertension, diabetes), those who live in nursing homes and those with a higher degree of dependence in daily activities are at the greatest risk of dying during the first year after hip fracture [13].

Conclusion

The most common result of all the studies is that preoperative health status is the most efficient criterion for postoperative mortality prediction. Short-term mortality is explained by a combination of comorbidity and acute effects of trauma or a combination thereof. However there is an increase in the rate of early mortality even in patients with hip fracture without evident comorbidities, suggesting that at least a certain percentage of mortality was caused by immediate consequences of fractures or surgical intervention. We used the risk assessment scale in our study because its broad content facilitates overall assessment. Mortality rates and scored results were statistically significant and correlated with each other.

This study showed evident gender differences: female gender as a risk factor for hip fracture and male gender as a risk factor for lethal outcome. In-hospital and six-month mortality rates were similar regarding the gender, but three-month mortality, after sustaining the injury, showed that men had higher mortality rate. Thus, gender can be defined as a significant mortality predictor in patients with hip fracture.

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REFERENCES

1. *Giversen IM.* Time trends of mortality after first hip fractures. Osteoporos Int 2007; 18(6): 721–32.
2. *Haleem S, Lutchman L, Mayahi R, Grice JE, Parker MJ.* Mortality following hip fracture: Trends and geographical variations over the last 40 years. Injury 2008; 39(10): 1157–63.
3. *Panula J, Pihlajamäki H, Mattila VM, Jaatinen P, Vahlberg T, Aarnio P,* et al. Mortality and cause of death in hip fracture patients aged 65 or older-a population-based study. BMC Musculoskelet Disord 2011; 12(1): 105.
4. *Kammerlander C, Gosch M, Kammerlander-Knauer U, Luger TJ, Blauth M, Roth T.* Long-term functional outcome in geriatric hip fracture patients. Arch Orthop Trauma Surg 2011; 131(10): 1435–44.
5. *Odumala AO, Iqbal MR, Middleton RG.* Failure of closed reduction after dislocation of Austin Moore hemiarthroplasty: an analysis of risk factors a 6-year follow-up study. J Arthroplasty 2010; 25(5): 781–4.
6. *Ferrández L.* Epidemiology of osteoporotic fractures. In: *Fernandez L, Herrera A*, editors. Osteoporotic fractures. Group Osteoporosis Study and Research (GENIE) of the Spanish Society of Orthopaedic Surgery and Traumatology (SECOT). S. A. Madrid: Medical and Marketing Communications; 2006. p. 75–86.
7. *Rodriguez PFJ.* Comparative study of morbidity and mortality in patients with hip fracture in relation to the time of treatment [dissertation]. Cordoba: University of Cordoba; 2006. (Spanish)
8. *Yan L, Zhou B, Prentice A, Wang X, Golden MH.* Epidemiological study of hip fracture in Shenyang, People's Republic of China. Bone 1999; 24(2): 151–5.
9. *Lyritis GP.* Epidemiology of hip fracture: The MEDOS study. Osteoporos Int 1996; 6(3): S11–5.
10. *Tirado PA.* Evolución of the elderly with hip fracture: experience in the Eastern vallés. [dissertation]. Madrid: University of Madrid; 2013. (Spanish)
11. *Hershkovitz A, Polatov I, Beloosesky Y, Brill S.* Factors affecting mortality of frail hip-fractured elderly patients. Arch Gerontol Geriatr 2010; 51(2): 113–6.
12. *Alarcón AT, González-Montalvo JI.* Osteoporotic hip fracture. Predictive factors of short-and long-term functional recovery. An Med Interna 2004; 21(2): 87–96.
13. *Hannan EL, Magaziner J, Wang JJ, Eastwood EA, Silberzweig SB, Gilbert M,* et al. Mortality and locomotion 6 months after hospitalization for hip fracture: Risk factors and risk-adjusted hospital outcomes. JAMA 2001; 285(21): 2736–42.
14. *Ignjatovic Ristic ID, Ristic B, Obradovic Z.* Cognitive impairment, hip fractures and risk of mortality in elderly. Psichogeriatria Polska 2005; 2(2): 81–8.
15. *Donegan DJ, Gay NA, Baldwin K, Morales EE, Esterhai JL, Mehta S.* Use of medical comorbidities to predict complications after hip fracture surgery in the elderly. J Bone Joint Surg Am 2010; 92(4): 807–13.
16. *Bhaskar D, Parker MJ.* Haematological indices as surrogate markers of factors affecting mortality after hip fracture. Injury 2011; 42(2): 178–82.
17. *Bogosavljević M, Stokić D, Friscić Z, Ristić BM.* Unstable intertrochanteric fractures: How to prevent uncontrolled impaction and shortening of the femur. Vojnosanit Pregl 2011; 68(5): 399–404.
18. *Fransen M, Woodward M, Norton R, Robinson E, Butler M, Campbell JA.* Excess mortality or institutionalization after hip fracture: Men are at greater risk than women. J Am Geriatr Soc 2002; 50(4): 685–90.
19. *Forsén L, Sogaard AJ, Meyer HE, Edna T, Kopjar B.* Survival after hip fracture: Short- and long-term excess mortality according to age and gender. Osteoporos Int 1999; 10(1): 73–8.
20. *Lawrence VA, Hilsenbeck SG, Noveck H, Poses RM, Carson JL.* Medical complications and outcomes after hip fracture repair. Arch Intern Med 2002; 162(18): 2053–7.
21. *Bass E, French DD, Bradham DD, Rubenstein LZ.* Risk-adjusted mortality rates of elderly veterans with hip fractures. Ann Epidemiol 2007; 17(7): 514–9.
22. *Brauer CA, Coca-Perraillon M, Cutler DM, Rosen AB.* Incidence and mortality of hip fractures in the United States. JAMA 2009; 302(14): 1573–9.
23. *Öztürk I, Toker S, Ertürer E, Aksoy B, Seçkin F.* Analysis of risk factors affecting mortality in elderly patients (aged over 65 years). Acta Orthop Traumatol Turc 2008; 42(1): 16–21.
24. *Autier P, Haentjens P, Bentin J, Baillon JM, Grivegnée AR, Closon MC,* et al. Costs induced by hip fractures: a prospective controlled study in Belgium. Belgian Hip Fracture Study Group. Osteoporos Int 2000; 11(5): 373–80.
25. *Papadimitropoulos EA, Coyte PC, Josse RG, Greenwood CE.* Current and projected rates of hip fracture in Canada. CMAJ 1997; 157(10): 1357–63.
26. *Löfman O, Berglund K, Larsson L, Toss G.* Changes in hip fracture epidemiology: Redistribution between ages, genders and fracture types. Osteoporos Int 2002; 13(1): 18–25.
27. *Zingmond DS, Melton LJ 3rd, Silverman SL.* Increasing hip fracture incidence in California Hispanics, 1983 to 2000. Osteoporos Int 2004; 15(8): 603–10.
28. *Hu F, Jiang C, Shen J, Tang P, Wang Y.* Preoperative predictors for mortality following hip fracture surgery: A systematic review and meta-analysis. Injury 2012; 43(6): 676–85.
29. *Manzarbeitia J.* Hip fractures in the elderly. Rev Esp Econ Salud 2005; 4(4): 209–10. (Spanish)
30. *Hawkes WG, Wehren L, Orwig D, Hebel RJ, Magaziner J.* Gender differences in functioning after hip fracture. J Gerontol A Biol Sci Med Sci 2006; 61(5): 495–9.
31. *Carpintero P, Lopez P, Leon F, Lluch M, Montero M, Aguilera C.* Men with hip fractures have poorer nutritional status and survival than women: A prospective study of 165 patients. Acta Orthop 2005; 76(3): 331–5.
32. *Alegre-López J, Cordero-Guevara J, Alonso-Valdivielso JL, Fernández-Melón J.* Factors associated with mortality and functional disability after hip fracture: An inception cohort study. Osteoporos Int 2005; 16(7): 729–36.
33. *Pasco JA, Sanders KM, Hoekstra FM, Henry MJ, Nicholson GC, Kotowicz MA.* The human cost of fracture. Osteoporos Int 2005; 16(12): 2046–52.
34. *Lauritzen JB.* Hip fractures. Epidemiology, risk factors, falls, energy absorption, hip protectors, and prevention. Dan Med Bull 1997; 44(2): 155–68.
35. *Marks R.* Physical activity and hip fracture disability: A review. J Aging Res 2011; 2011: 741918.
36. *González-Montalvo JI, Alarcón T, Hormigo Sanchez AI.* Why do hip fracture patients die. Med Clin (Barc) 2011; 137(8): 355–60.
37. *Roche JJ, Wenn RT, Sahota O, Moran CG.* Effect of comorbidities and postoperative complications on mortality after hip fracture in elderly people: prospective observational cohort study. BMJ 2005; 331(7529): 1374–9.
38. *Bombaci H, Erdogan Ö, Durakbafla O, Ermifl N, Kuyumcu M.* Postoperative mortality rates in elderly patients with hip fractures: which factors are effective? 12th National Orthopaedics and Traumatology Congress; Oct 31 - Nov 5 2011; Antalya,

Turkey. Acta Orthop Traumatol Turc 2011; 45(Suppl 1):111-112. (Turkish)

39. *Cumming RG, Nevitt MC, Cummings SR.* Epidemiology of hip fractures. Epidemiol Rev 1997; 19(2): 244–57.
40. *Melton LJ, Therneau TM, Larson DR.* Long-term trends in hip fracture prevalence: The influence of hip fracture incidence and survival. Osteoporos Int 1998; 8(1): 68–74.
41. *Johnell O.* The socioeconomic burden of fractures: today and in the 21st century. Am J Med 1997; 103(2A): 20S–5S; discussion 25S–6S.

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Socioeconomic inequalities and non-communicable diseases in Serbia: national health survey

Socijalno-ekonomske nejednakosti i hronične nezarazne bolesti u Srbiji: nacionalno istraživanje zdravlja

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Abstract

Background/Aim. Non-communicable diseases (NCDs) are a major public health challenge worldwide. Although they are preventable, NCDs are the major global causes of morbidity and mortality, absenteeism, disability and premature death. The aim of this study was to examine socioeconomic inequalities in the prevalence of non-communicable diseases in Serbia. **Methods.** Data from the 2013 National Health Survey of the population of Serbia was used in this study. There were 13,765 adults interviewed, aged ≥ 20 years. We used multivariate logistic regression analyses with demographic and socioeconomic determinants of health as independent variables and prevalence of non-communicable diseases as a dependent variable. The minimum level of significance was $p < 0.05$. **Results.** Hypertension was the most prevalent NCDs (36.1%). The prevalence of multimorbidity was 47.1%. Multivariate logistic regression analysis showed that gender, age, place of residence, employment status and education were associated with the presence of NCDs. The odds ratio (OR) for age was 1.074 [95% confidence interval (CI) : 1.070–1.077). Women were at a higher risk of NCDs by 58.9% when compared to men (OR = 1.589; 95% 95% CI : 1.467–1.726). Respondents who lived in the rural areas were at a higher risk for NCDs by 14.1% compared to those who lived in urban areas (OR = 1.141; 95% CI : 1.047–1.244). Odds ratio for unemployment was 1.227 (95% CI: 1.118–1.346). Respondents with primary education were at a higher risk for chronic diseases by 47.1% (OR = 1.471; 95% CI : 1.281–1.687) while those with secondary school were at a higher risk by 27.7% (OR = 1.277; 95% CI : 1.142–1.428) compared to respondents who had higher education. When it comes to Wealth Index, univariate logistic regression analysis showed that respondents who belonged to the poor and middle classes were at a higher risk for NCDs (OR = 2.031; 95% CI : 1.819–2.267; OR = 1.473; 95% CI : 1.343–1.615) compared to respondents who belonged to the rich class. Multivariate logistic regression analysis did not show statistically significant correlations between the Wealth index and NCDs. **Conclusion.** Socioeconomic inequalities in health status are the major challenge and should be a target of national health policy in Serbia, not only because they represent social injustice but also because solving the health problems of underprivileged groups of the population can influence improvement of health status of the population as a whole.

Key words:
chronic disease; prevalence; risk factors; sociological factors; economics; serbia.

Apstrakt

Uvod/Cilj. Opterećenje društva nezaraznim bolestima predstavlja veliki javno-zdravstveni izazov širom sveta. Iako preventabilne, nezarazne bolesti su danas vodeći uzroci obolevanja, apsentizma, invalidnosti i prevremenog umiranja. Cilj ovog rada bio je da ispita socijalno-ekonomske nejednakosti u prevalenci nezaraznih bolesti u Srbiji. **Metode.** U studiji su korišćeni podaci nacionalnog istraživanja zdravlja stanovnika Republike Srbije koje je obavljeno 2013. godine. Broj anketiranih osoba starijih od 20 godina bio je 13 765. Povezanost demografskih i socijalno-ekonomskih determinanti zdravlja (nezavisnih varijabli) i prisustva nezaraznih bolesti (zavisne varijable) ispitivana je bivarijantnom i multivarijantnom logističkom regresijom. Statistički značajnim smatrale su se vrednosti $p < 0.05$. **Rezultati.** Hipertenzija je najučestalija nezarazna bolest (36.1%). Prevalencija multimorbiditeta bila je 47.1%. Multivarijantna

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logistička regresija pokazala je da su pol, godine starosti, tip naselja, radni status i obrazovanje povezani sa prisustvom nezaraznih bolesti. Unakrsni odnos (OR) za godine starosti bio je 1.074 [95% interval poverenja (IP) : 1.070–1.077). Kod žena je uočen veći rizik od oboljevanja od nezaraznih bolesti (58.9%) u odnosu na muškarce (OR = 1.589; 95% IP : 1.467–1.726). Ispitanici koji žive na selu su za 14.1% bili pod većim rizikom od oboljevanja od nezaraznih bolesti u odnosu na one koji žive u gradu (OR = 1.141; 95% IP: 1.047–1.244). Unakrsni odnos za nezaposlene bio je 1.227 (95% IP : 1.118–1.346). Ispitanici sa osnovnim obrazovanjem su za 47.1% bili pod većim rizikom od oboljevanja od hronične bolesti (OR = 1.471; 95% IP : 1.281–1.687), dok su oni sa srednjom školom bili pod većim rizikom za 27.7% (OR = 1.277; 95% IP : 1.142–1.428) u odnosu na ispitanike koji imaju visoko obrazovanje. Kada je u pitanju Indeks blagostanja, univarijantna logistička regresija pokazala je da ispitanici koji pripadaju siromašnoj i srednjoj klasi su pod veći rizikom od oboljevanja (OR = 2.031, 95% IP : 1.819–2.267; OR = 1.473, 95% IP : 1.343-1.615) u poređenju sa onima koji pripadaju bogatoj klasi. Multivarijantna logistička regresija nije pokazala statistički značajnu povezanost između Indeksa blagostanja i nezaraznih bolesti. **Zaključak:** Socijalno-ekonomske nejednakosti u zdravlju veliki su izazov za zdravstvenu politiku, ne samo zato što predstavljaju socijalnu nepravdu nego i zbog toga što se rešavanjem zdravstvenih problema neprivilegovanih grupa stanovništva može uticati na poboljšanje zdravstvenog stanja stanovništva u celini.

Ključne reči:
hronična bolest; prevalenca; faktori rizika; socijalni faktori; ekonomski faktori; srbija.

Introduction

Non-communicable diseases (NCDs) are a major public health challenge worldwide [1]. Although they are preventable, NCDs (mainly cardiovascular diseases, neoplasms, chronic respiratory diseases and diabetes) are the major global causes of morbidity and mortality, absenteeism, disability and premature death [2]. According to the World Health Organization (WHO) report, more than 36 million people die from NCDs annually which is about 63% of the 57 million global deaths [3]. About 16 million people die prematurely, before the age of 70, during the most productive period of life. The prevalence and impact of NCDs continues to rise, especially in countries with low and middle income that already participate in premature deaths with 86% [4]. NCDs have serious socioeconomic consequences, ranging from increasing individual and household impoverishment to high cost of healthcare which hinder social and economic development [5]. The rapidly growing number of NCDs is under the influence of many factors including population aging, negative effects of globalization, rapid and unplanned urbanization and unhealthy behaviours [6].

Individual characteristics and socioeconomic status are important determinants of health inequalities [7]. The impact of these determinants on the morbidity has been studied in many countries, and the results showed clear correlations between socioeconomic determinants and health status of respondents [1, 8, 9]. In all countries, there are significant differences in health between socioeconomic groups. People with lower socioeconomic status (SES) are associated with higher prevalence of chronic diseases and injuries, unhealthy behaviors such as smoking, inadequate diet, alcohol use, and lack of physical exercise [10–12]. People with lower level of education, lower occupational class, or lower income live shorter in good health, have higher rates of mortality and die at younger age [10, 13]. There is substantial evidence that those with lower socioeconomic status are at increased risk of adverse health outcomes, including cardiovascular disease, neoplasms and mental health problems [14–17].

The presence of diseases and their symptoms are indicators of health status of the population. In health research, self-reporting of diseases is widely used. Information on the prevalence of NCDs in the population is most commonly obtained through questionnaires [3].

The aim of this study was to examine socioeconomic inequalities in the prevalence of NCDs in Serbia, using the data from the 2013 National Health Survey.

Methods

Study population and sample

This study used the data from the 2013 National Health Surveys for Serbia. The study was designed as a cross-sectional study on a representative probability sample of the population aged 15 years and above. The survey was conducted in accordance with the methodology and instruments of the European Health Interview Survey wave 2 (EHIS-wave 2). It was implemented by the Ministry of Health of the Republic of Serbia.

The sample consisted of all households listed by all enumeration areas of Census 2013. The mechanism used to generate a random sample of households and respondents is a combination of two sampling techniques: stratification and multi-stage sampling. Two-stage stratified sample of the population of the Republic of Serbia was selected in order to obtain a statistically reliable estimation of indicators which affect the health of the population at the national level and at the levels of four geographical regions of Serbia (Vojvodina, Belgrade, Šumadija, Western Serbia and South-Eastern Serbia) [18].

Of the total number of 10,089 households contacted, 6,500 of them agreed to participate in the survey, so that the response rate of households was 64.4%. Of the total number of 16,474 registered household members aged 15 years and more, 14,623 of them agreed to be interviewed, giving a response rate of 88.9%. For the purposes of this study, we analyzed data related to respondents aged 20 years and older (13,765 interviewed respondents).

Instruments

Data on demographic and socioeconomic characteristics of the respondents and their own health assessment was ob-

tained through a face-to-face interview carried out at home, while information about the wealth level of the household was obtained by means of a household questionnaire. The questions were validated instruments and based on the standard questionnaires from similar types of surveys. Ethical Standards in this study are in compliance with the international ethical standards (the World Medical Association Declaration of Helsinki) and the specific legislation of our country.

As independent variables, we used demographic characteristics (age, gender, type of settlement and marital status) and socioeconomic status (education, employment status and Wealth index). The age of participants was categorized into eight age groups (≤ 24 years, 25–34 years, 35–44 years, 45–54 years, 55–64 years, 65–74 years, 75–84 years and 85 years or more). Gender is coded as male and female, place of residence as urban and rural while the marital status was categorized as married/living with a partner and not married, divorced/widowed. Education was defined as high level (university degree), medium level (three of four years of secondary school), or low level (no education, incomplete primary school, or primary school). Employment status was categorized as employed and unemployed (including economically inactive people: pensioners, people attending some form of education, housewives who are inactive due to family reasons, people who are ill, unable to work or elderly and other inactive categories). The Wealth Index was based on household assets and housing characteristics, such as (the number of bedrooms per household member, materials used in the construction of a floor, roof, and walls, type of drinking water source and sanitation facilities, fuel used for heating, color TV set, mobile phone, refrigerator, washing machine, dish washer, personal computer, air conditioning, central heating, car and access to the Internet). According to the Wealth Index, households were divided into five equal-sized groups (quintiles): the poorest (Q1), poorer (Q2), middle (Q3), richer (Q4) and the richest (Q5). For the purposes of this paper, the respondents were classified into three socioeconomic categories: poor class, middle class and rich class.

Self-reported diagnosis of NCDs was taken as a dependent variable. The respondents were asked: "Did you have some of the following diseases or conditions in the last twelve months: hypertension, deformity of lower spine or other chronic back problems, hyperlipidemia, neck deformity or another chronic problem with cervical spine, coronary heart disease or angina pectoris, arthrosis, allergies (excluding allergic asthma), diabetes and depression? " Of the total seventeen NCDs reported in the National Health Survey, we considered nine to be major NCDs. All diseases were coded as dummy variables (yes/no). Multimorbidity was used to refer to those who had two or more morbidities.

Statistical analysis

All data of interest were presented and analyzed by adequate mathematical-statistical methods appropriate for the data type. The χ^2 test was used to compare proportions between the groups. The *t*-test was used to compare continuous variables between the groups. The relations between the presence of NCDs, as a dependent variable and a set of independent variables was examined by univariate and multivariate logistic regression. Univariate logistic regression models were used to examine the associations between potential factors and the presence of NCDs. The variables that were statistically significant ($p < 0.05$) were further examined in multivariable logistic regression. The unadjusted odds ratios (ORs) with their corresponding 95% confidence intervals (95% CI) were also obtained. All statistical calculations were performed using the commercial, standard software package SPSS, version 18.0. [The Statistical Package for Social Sciences software (SPSS Inc, version 18.0, Chicago, IL)].

Results

The study included 13,765 respondents, where 46% were men and 54% were women. The demographic and socioeconomic characteristics of the sample of respondents are presented in Table 1. The mean age of the respondents was 51.78 (SD = 17.467); there were 20.9% in the 55–64 age group. The majority of respondents resided in urban areas (56.4%), 65.4% of them were married or lived with a partner, 54.2% had a middle level of education, 67.2% were unemployed (and belonged to the middle class (60.1%) according to the Wealth Index.

In the present study, 60% reported at least one of nine analyzed chronic diseases. Table 2 shows the prevalence of NCDs by demographic and socioeconomic variables. The most prevalent self-reported NCDs across the sample were hypertension (36.1%) followed by deformity of the lower spine or other chronic problems with the back (21.5%) and hyperlipidemia (14.7%). Allergy (8.9%) and depression (7.2%) were the least reported NCDs in the study sample. All diseases were more prevalent among women than among men. Significant differences by age were found for all diseases. The difference in mean values of years of life between respondents with chronic diseases and respondents without them is statistically significant (t = -69,635, $p < 0.0005$). The respondents with a chronic disease were 59.45, (SD = 14.94) years on average and those without chronic diseases were on average at the age of 41.26 (SD = 15.01) years.

Two fifths (40%) of the respondents reported that they did not have any NCDs, while 12.9% had one NCDs. More than two NCDs was reported by 47.1% of the respondents (Table 3). The median number of diseases among persons with multimorbidity was 3, ranging from 2 to 10. The number of NCDs differed significantly by gender, age groups, marital status, education, employment status and Wealth index ($p < 0.005$). The number of NCDs did not differ significantly by type of settlement ($p = 0.412$). Women, elderly people, those with low educational level, unemployed, inactive and respondents who belonged to the poor class were sensitive to multimorbidity.

Results of the univariate and multivariate logistic regression analysis are presented in Table 4. Univariate logistic regression analysis showed that the prevalence of NCDs differed significantly across gender, age, marital status, education, place of residence, employment status, education and the Wealth Index. Multivariate logistic regression analysis showed that gender, age, place of residence, employment

status and education can be associated with the presence of NCDs. The prevalence of NCDs increased with age. Number of chronic diseases was positively correlated with age (r = 0.557; $p < 0.0005$). The OR for age was 1.074 (95% CI : 1.070–1.077), which means that each year the risk of a chronic disease was increasing by 7.4%. Women were under the increased risk of a chronic disease by 58.9% compared to men (OR = 1.589; 95% CI : 1.467 to 1.726). Respondents who lived in rural areas were under the higher risk of chronic non-communicable diseases by 14.1% compared to those who lived in urban areas (OR = 1.141; 95% CI : 1.047–1.244). Odds ratio for unemployment was 1.227 (95% CI : 1.118–1.346). Unemployed and inactive people were under the increased risk of a chronic disease by 22.7% in relation to the employed. (OR = 1.227; 95% CI: 1.118–1.346). The prevalence of a chronic diseasewas inversely proportional to the level of education. Respondents with primary education were under the higher risk for NCDs by 47.1% (OR = 1.471; 95% CI : 1.281–1.687) while those with secondary school were under the higher risk by 27.7% of (OR = 1.277, 95% CI : 1.142–1.428) compared to the respondents who had higher education. When it comes to the Wealth index, univariate logistic regression analysis showed that the respondents who belonged to the poor and middle class were in a higher risk for NCDs (OR = 2.031; 95% CI : 1.819–2.267; OR = 1.473; 95% CI : 1.343–1.615) compared to the respondents who belonged to the rich class. Multivariate logistic regression analysis did not show a statistically significant impact of the Wealth Index on the prevalence of NCDs.

Discussion

The results showed that there were significant differences in the prevalence of NCDs, depending on the demographic and socioeconomic variables.

This study presented a high prevalence of NCDs. Similar results were found in a population survey conducted in eight countries in Europe where 55.1% of the adult population had at least one chronic disease [19]. National Population Health Survey in Canada found that more than a half of adults aged 25 years and over had a chronic condition [20].

The prevalence of multimorbidity was 47.1% which is similar to that found in the other studies (Banglades – 53.7%, Germany – 58.6%, Sweden – 55%) [21–23]. Opposite to our data some studies found a smaller proportion of multimorbidity (Canada – 24%, South Africa – 22.5%) [24, 25]. Many studies reported variable prevalence of multimorbidity, which is likely due to a sample type, source of data, data collection method, observed age groups, diagnoses that were considered and study population [26].

Table 1

Demographic and socioeconomic characteristics of study population

Variables	Men		Women		All	
	n	%	n	%	n	%
Total	6,328	46.0	7,437	54.0	13765	100
Men age (years), mean ± SD	51.02 ± 17.236		52.43 ± 17.636		51.78 ± 17.467	
Age (years)						
20–24	412	6.5	452	6.1	864	6.3
25–34	937	14.8	1,024	13.8	1,961	14.2
35–44	1,068	16.9	1,123	15.1	2,191	15.9
45–54	1,055	16.7	1,273	17.1	2,328	16.9
55–64	1,328	21.0	1,553	20.9	2,881	20.9
65–74	875	13.8	1,080	14.5	1,955	14.2
75–84	589	9.3	796	10.7	1,385	10.1
≥ 85	64	1.0	136	1.8	200	1.5
Marital status						
married or living with a partner	4,384	69.3	4,617	62.1	9,001	65.4
not married, divorced, widowed	1,944	30.7	2,820	37.9	4,764	34.6
Emplyment status						
employed	2,570	40.6	1,951	26.2	4,521	32.8
unemployed	3,758	59.4	5,486	73.8	9,244	67.2
Type of settlement						
urban	2,497	55.3	4,263	57.3	7,760	56.4
rural	2,831	44.7	3,174	42.7	6,005	43.7
Education						
low	1,368	21.6	2,644	35.6	4,012	29.1
middle	3,846	60.8	3,611	48.6	7,457	54.2
high	1,114	17.6	1,182	15.9	2,296	16.7
Wealth Index						
poor class	1,440	22.8	1,658	22.3	3,098	22.5
middle class	3,785	59.8	4,482	60.3	8,267	60.1
rich class	1,103	17.4	1,297	17.4	2,400	17.4

SD – standard deviation.

Table 2

Prevalence of self reported non-communicable diseases (NCDs)

Variables	Coronary heart disease or Angina pectoris	Hypertension	Arthrosis	Deformity of lower spine	Neck deformity	Diabetes	Allergies	Depression	Hyperlipidemia
Total	1687 (12.3)	4965 (36.1)	1518 (11.0)	2954 (21.5)	1906 (13.8)	1129 (9,1)	1230 (8.9)	989 (7.2)	2030 (14.7)
Gender									
Men	635 (37.6)	1977 (39.8)	416 (27.3)	1065 (36.1)	547 (28.7)	535 (43.5)	396 (32.2)	310 (31.3)	761 (37.5)
Women	1052 (62.4)	2988 (60.2)	1102 (72.6)	1889 (63.9)	1359 (71.3)	694 (56.5)	834 (67.8)	679 (68.7)	1269 (62.5)
Age (years)									
20–24	2 (0.1)	14 (0.3)	3 (0.2)	28 (0.9)	11 (0.6)	6 (0.5)	63 (5.1)	9 (0.9)	8 (0.4)
25–34	26 (1.5)	89 (1.8)	16 (1.1)	113 (3.8)	53 (2.8)	25 (2.0)	157 (12.8)	48 (4.9)	38 (1.9)
35–44	48 (2.8)	309 (6.2)	68 (4.5)	309 (10.5)	175 (9.2)	54 (4.4)	186 (15.1)	106 (10.7)	177 (8.7)
45–54	165 (9.8)	741 (14.9)	169 (11.1)	463 (15.7)	331 (17.4)	144 (11.7)	206 (16.7)	174 (17.6)	372 (18.3)
55–64	434 (25.7)	1508 (30.4)	418 (27.5)	801 (27.1)	555 (29.1)	361 (29.4)	289 (23.5)	273 (27.6)	684 (33.7)
65–74	506 (30.0)	1256 (25.3)	418 (27.5)	640 (21.7)	406 (21.3)	368 (29.9)	191 (15.5)	221 (22.3)	473 (23.3)
75–84	452 (26.8)	929 (18.7)	368 (24.2)	509 (17.2)	317 (16.6)	257 (20.9)	121 (9.8)	141 (14.3)	258 (12.7)
⩾85	54 (3.2)	119 (2.4)	58 (3.8)	91 (3.1)	58 (3.0)	14 (1.1)	17 (1.4)	17 (1.7)	20 (1.0)
Marital status									
married or living with a partner	1047 (62.1)	3291 (66.3)	928 (61.1)	1932 (65.4)	1234 (64.7)	801 (65.2)	759 (61.7)	585 (59.2)	1428 (70.3)
not married, divorced, widowed	640 (37.9)	1674 (33.7)	590 (38.9)	1022 (34.6)	672 (35.3)	428 (34.8)	471 (38.3)	404 (40.8)	602 (29.7)
Type of settlement									
urban	928 (55.0)	2722 (54.8)	770 (50.7)	1604 (54.3)	1045 (54.8)	673 (54.8)	795 (64.6)	518 (52.4)	1193 (58.8)
rural	759 (45.0)	2243 (45.2)	748 (49.3)	1350 (45.7)	861 (45.2)	556 (45.2)	435 (35.4)	471 (47.6)	837 (41.2)
Education									
low	866 (51.3)	2130 (42.9)	804 (53.0)	1273 (43.1)	812 (42.6)	538 (43.8)	348 (28.3)	424 (42.9)	734 (36.2)
middle	637 (37.8)	2195 (44.2)	565 (37.2)	1325 (44.9)	866 (45.4)	528 (43.0)	634 (51.5)	479 (48.4)	979 (48.2)
high	187 (10.9)	640 (12.9)	149 (9.8)	356 (12.1)	228 (12.0)	163 (13.3)	248 (20.2)	86 (8.7)	317 (15.6)
Emplyment status									
employed	156 (9.2)	883 (17.8)	149 (9.8)	593 (20.1)	373 (19.6)	156 (12.7)	374 (30.4)	137 (13.9)	433 (21.3)
unemployed	1531 (90.8)	4082 (82.2)	1369 (90.2)	2361 (79.9)	1533 (80.4)	1073 (87.3)	856 (69.6)	852 (86.1)	1597 (78.7)
Wealth Index									
poor class	504 (29.9)	1339 (27.0)	467 (30.8)	768 (26.0)	461 (24.2)	340 (27.7)	207 (16.8)	300 (30.3)	408 (20.1)
middle class	1006 (59.6)	3007 (60.6)	898 (59.2)	1787 (60.5)	1178 (61.8)	729 (59.3)	771 (62.7)	588 (59.5)	1304 (64.2)
rich class	177 (10.5)	619 (12.5)	153 (10.1)	399 (13.5)	267 (14.0)	160 (13.0)	252 (20.5)	101 (10.2)	318 (15.7)

Table 3

Associations between the number of non-communicable diseases (NCDs) and demographic and socioeconomic characteristics

Variables	Number of NCDs			
	0	1	≥ 2	*p*
Total number	5,505 (40)	1,786 (13)	6,474 (47.0)	
Gender				
men	2,879 (45.5)	872 (13.8)	2,577 (40.7)	< 0.0005
women	2,626 (35.5)	914 (12.3)	3,897 (52.4)	
Age (years)				
20–24	735 (85.1)	94 (10.9)	35 (4.1)	
25–34	1,495 (76.2)	286 (14.6)	180 (9.2)	
35–44	1,301 (59.4)	353 (16.1)	537 (24.5)	
45–54	920 (39.5)	370 (15.9)	1,038 (44.6)	< 0.0005
55–64	652 (22.6)	344 (11.9)	1,885 (65.4)	
65–74	248 (12.7)	201 (10.3)	1,506 (77.0)	
75–84	130 (9.4)	113 (8.2)	1,142 (82.5)	
≥ 85	24 (12.0)	25 (12.5)	151 (75.5)	
Marital status				
married or living with a partner	3,472 (38.6)	1,208 (13.4)	4,321 (48.0)	< 0.0005
not married, divorced, widowed	2,033 (42.7)	578 (12.1)	2,153 (45.2)	
Type of settlement				
urban	3,117 (40.2)	1,027 (13.2)	3,616 (46.6)	0.412
rural	2,388 (39.8)	759 (12.6)	2,858 (47.6)	
Education				
low	940 (23.4)	446 (11.1)	2,626 (65.6)	
middle	3,485 (46.7)	999 (13.4)	2,973 (39.9)	< 0.0005
high	1,080 (47.0)	341 (14.9)	875 (38.1)	
Emplyment status				
employed	2,583 (57.1)	671 (14.8)	1,267 (28.0)	< 0.0005
unemployed	2,922 (31.6)	1,115 (12.1)	5,207 (56.3)	
Wealth Index				
poor class	1,033 (33.8)	395 (12.8)	1,670 (53.9)	
middle class	3,298 (39.9)	1,047 (12.7)	3,922 (47.4)	< 0.0005
rich class	1,174 (48.9)	344 (14.3)	882 (36.8)	

Data are present as number (%) of study population; *χ^2 test.

Table 4

Odds ratios (OR) and 95% confidence intervals (CI) for the presence of non-communicable diseases (NCDs) depending on demographics and socioeconomic characteristics

Variables	n (%)	Binary logistic regression [OR (95% CI)]		*p*
		Univariate	Multivariate	
Age	13,765 (100)	1.077 (1.074–1.080)	1.074 (1.070–1.077)	< 0.0005
Gender				
men	6,328 (46.0)	1.00	1.00	
women	7,437 (54.0)	1.556 (1.452–1.667)	1.589 (1.467–1.726)	< 0.0005
Marital status				
has a partner	4,764 (34.6)	1.00	1.00	
has no partner	9,001 (65.4)	0.872 (0.812–0.937)	1.001 (0.914–1.096	0.985
Type of settlement				
urban	7,760 (56.4)	1.00	1.00	
rural	6,005 (43.6)	1.046 (0.977–1.212)	1.141 (1.047–1.244)	0.003
Emplyment status				
employed	9,244 (67.2)	1.00	1.00	
unemployed	4,521 (32.8)	3.722 (3.457–4.008)	1.227 (1.118–1.346)	< 0.0005
Education				
high	2,296 (29.1)	1.00	1.00	
low	4,012 (29.1)	3.062 (2.774–3.418	1.471 (1.281–1.687)	< 0.0005
middle	7,457 (54.2)	1.046 (0.952–1.150)	1.277 (1.142–1.428)	< 0.0005
Wealth Index				
rich class	2,400 (17.4)	1.00	1.00	
poor class	3,098 (22.5)	2.031 (1.819–2.267)	0.993 (0.844–1.167	0.929
middle class	8,267 (60.1)	1.473 (1.343–1.615)	1.074 (0.955–1.207)	0.235

Low SES means greater prevalence of almost all diseases. Systematic reviews by Sommer et al. [27] showed that low SES increased the risk of cardiovascular diseases, lung and gastric cancer, type 2 diabetes and chronic obstructive pulmonary disease, etc. The famous Whitehall study [28] conducted among state officials in the UK showed that socioeconomic inequalities were reflected in various diseases such as heart diseases, some forms of malignant diseases, chronic lung disease, gastrointestinal disease, depression, suicide and back pain. Socioeconomic inequalities in NCDs were associated with the unequal distribution of behavioural risk factors, particularly smoking, alcohol use, an unhealthy diet and a sedentary lifestyle [29].

Similar to other studies, increasing age, being female, having low educational level, being unemployed, inactive and belonging to the poor class were characteristics associated with presence of chronic diseases [21, 25, 30].

Demographic characteristics of the respondents such as gender and age are important predictors of health. There is a positive correlation between them and the morbidity. Our analysis revealed that the prevalence of NCDs was increasing with age. In other words, morbidity prevalence increased with age [31]. Most NCDs were found to be prevalent among women compared to men [32, 33]. These findings repeated in many studies focusing on poorer health of women and the elderly [34–37], indicating that special attention should be focused on the care of these vulnerable groups, as they were more likely to develop morbidity.

Education is a very important socioeconomic determinant of health. Men and women with lower levels of education had higher morbidity rates and premature mortality from all causes than their higher-educated counterparts [33]. People with higher levels of education were more likely to be employed, to have a higher social status and more stable income and they have more skills to cope with and overcome everyday life difficulties which could negatively affect their health[37].

In this study we found that unemployed people had poorer health status compared to those who were employed. This could be explained by the fact that a large part of the unemployed were economically inactive, the largest number of them being pensioners who were more frequently physically inactive and more often suffered from hypertension. The second large group of economically inactive persons were housewives. It is generally assumed that female workers had better health than full-time housewives [35].

The studies in several countries showed that the unemployed and their families had poorer health and were under the significantly higher risk of premature death than the employed [36]. The health consequences of unemployment were associated with both psychological and financial consequences of unemployment, such as the inability to satisfy everyday needs, insecurity and lack of self-esteem. The unemployed used health services to a lesser extent, and rarely visited a doctor of general medicine/occupational medicine and got hospital treatment [37].

In the current study, univariate logistic regression analysis showed that the respondents who belonged to the poor and middle class were in a higher risk for NCDs compared to the respondents who belonged to the rich class. Multivariate logistic regression analysis did not show a statistically significant impact of the Wealth Index on the prevalence of NCDs. We found that many NCDs were mostly concentrated among the poor and middle class, and were inversely associated with decreasing wealth level. Our findings were in accordance with previous reports about the presence of angina, arthritis, asthma, depression, gastritis and migraine. However, cancer, allergy and diabetes mellitus were slightly more concentrated among wealthy individuals [30]. This results could be explained by the fact that people who lived in poverty may experience material deprivation and high stress levels, which may lead to constrained choices and a higher likelihood of engaging in risky health behaviours, increasing the risk of disease, following disease onset and reduced access to healthcare hindered opportunities to prevent complications [38].

Unmarried, divorced or widowed people had higher values of the morbidity index and often assessed their health worse than those who were married [39].

Strengths and limitations

The main strength of this study is the large sample which is representative of the adult population of the Republic of Serbia (aged ≥ 20 years). However, this study has several limitations. First, the cross-sectional design of the study makes it difficult to judge causal relations. This limitation can be overcome with the use of longitudinal data, which might better explain the changes in socioeconomic status and their impact on NCDs. Another limitation of the study is the problem of the accuracy of self-reports for chronic diseases, which may be subject to bias. Differences in prevalence rates between self-reported diagnoses and standardized measure may vary. The accuracy of self-reports for NCDs depends on different factors such as knowledge of the health problem, consequences on everyday life, willingness to report the problem and frequency of visits to healthcare services. According to the current literature, individuals with lower socioeconomic status tend to under-report symptoms which might result in an underestimation of the presence of NCDs.

Regardless of the above mentioned shortcomings of the methodical approach and a lack of a better, but much more expensive study design, this study, if repeated with the same methodical approach in a period of several years, could make us able to estimate the trends of diseases and their relationships to health determinants. That is why it is important to promote the repetition of similar studies on the health of the population in Serbia.

Conclusion

The elderly, females, those with lower levels of education and unemployed people have a greater prevalence of chronic diseases. Socioeconomic inequalities in health are the major challenge for health policy, not only because they represent social injustice but also because solving the health problems of underprivileged groups of the population can influence the improvement of the health status of the population as a whole.

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R E F E R E N C E S

1. *World Health Organization*. Gaining health, The European Strategy for the Prevention and Control of Noncommunicable Diseases. Copenhagen: WHO Regional Office for Europe; 2006.
2. World Health Organization. Global action plan for the prevention and control of noncommunicable diseases 2013-2020. Geneva: World Health Organization; 2013.
3. *World Health Organization*. A Comprehensive Global Monitoring Framework, Including Indicators, and a Set of Voluntary Global Targets for the Prevention and Control of Noncommunicabale Diseases. In: Revised WHO Discussion Paper. 2012. Available from: http://www.who.int/nmh/ events/2012/discussion_paper3 .pdf
4. *World Health Organization*. Global status report on noncommunicable diseases 2010. Geneva: World Health Organization; 2011.
5. *World Economic Forum*. Working towards wellness. The business rationale. Geneva: World Economic Forum; 2008.
6. *Hosseinpoor AR, Bergen N, Kunst A, Harper S, Guthold R, Rekve D,* et al. Socioeconomic inequalities in risk factors for non communicable diseases in low-income and middle-income countries: results from the World Health Survey. BMC Public Health 2012; 12: 912.
7. *Paul P, Valtonen H*. Inequalities in perceived health in the Russian Federation, 1994-2012. BMC Public Health 2016; 16: 165.
8. *Siegrist J, Marmot M*. Social inequalities in health. New evidence and policy implications. Oxford: University Press; 2006.
9. *Kaikkonen R, Rahkonen O, Lallukka T, Lahelma E*. Physical and psychosocial working conditions as explanations for occupational class inequalities in self-rated health. Eur J Public Health 2009; 19(5): 458-63.
10. *Mackenbach JP, Meerding WJ, Kunst AE*. Economic implications of socio-economic inequalities in health in the European Union. Luxembourg: European Commission; 2007.
11. *Dorjdagva J, Batbaatar E, Dorjsuren B, Kauhanen J*. Explaining differences in education-related inequalities in health between urban and rural areas in Mongolia. Int J Equity Health 2015; 14(1): 154.
12. *De Looper M, Lafortune G*. Measuring Disparities in Health Status and in Access and Use of Health Care in OECD Countries. Paris: OECD OECD Health Working Paper, No. 43; 2009.
13. *Mackenbach JP*. The persistence of health inequalities in modern welfare states: The explanation of a paradox. Soc Sci Med 2012; 75(4): 761-9.
14. *Forrest LF, Adams J, Wareham H, Rubin G, White M*. Socioeconomic inequalities in lung cancer treatment: Systematic review and meta-analysis. PLoS Med 2013; 10(2): e1001376.
15. *Fryers T, Melzer D, Jenkins R*. Social inequalities and the common mental disorders: a systematic review of the evidence. Soc Psychiatry Psychiatr Epidemiol 2003; 38(5): 229–37.
16. *Jokela M, Batty GD, Vahtera J, Elovainio M, Kivimaki M*. Socioeconomic inequalities in common mental disorders and psychotherapy treatment in the UK between 1991 and 2009. Br J Psychiatry 2013; 202: 115–20.
17. *Mackenbach JP, Bos V, Andersen O, Cardano M, Costa G, Harding S,* et al. Widening socioeconomic inequalities in mortality in six Western European countries. Int J Epidemiol 2003; 32(5): 830–7.
18. *Institute of Public Health of the Republic of Serbia "Dr Milan Jovanović Batut"*. Results of the national health survey of Serbia. 2013. Belgrade: Institute of Public Health of the Republic of Serbia "Dr Milan Jovanović Batut"; 2014.
19. *Alonso J, Ferrer M, Gandek B, Ware JE, Aaronson NK, Mosconi P,* et al. Health-related quality of life associated with chronic conditions in eight countries: Results from the International Quality of Life Assessment (IQOLA) Project. Qual Life Res 2004; 13(2): 283–98.
20. *Iron KS, Manuel DG, Williams J*. Using a linked data set to determine the factors associated with utilization and costs of family physician services in Ontario: effects of self-reported chronic conditions. Chronic Dis Can 2003; 24(4): 124–32.
21. *Khanam MA, Streatfield PK, Kabir ZN, Qiu C, Cornelius C, Wahlin Å*. Prevalence and patterns of multimorbidity among elderly people in rural Bangladesh: A cross-sectional study. J Health Popul Nutr 2011; 29(4): 406–14.
22. *Kirchberger I, Meisinger C, Heier M, Zimmermann AK, Thorand B, Autenrieth CS*, et al. Patterns of multimorbidity in the aged population. Results from the KORA-Age Study. PLoS One 2012; 7(1): e30556.
23. *Marengoni A, Winblad B, Karp A, Fratiglioni L*. Prevalence of chronic diseases and multimorbidity among the elderly population in Sweden. Am J Public Health 2008; 98(7): 1198–200.
24. *Ward BW, Schiller JS*. Prevalence of multiple chronic conditions among US adults: Estimates from the National Health Interview Survey, 2010. Prev Chronic Dis 2013; 10: E65.
25. *Phaswana-Mafuya N, Peltzer K, Chirinda W, Musekiwa A, Kose Z, Hoosain E,* et al. Self-reported prevalence of chronic noncommunicable diseases and associated factors among older adults in South Africa. Glob Health Action 2013; 6: 20936.
26. *Diederichs C, Berger K, Bartels DB*. The Measurement of Multiple Chronic Diseases-A Systematic Review on Existing Multimorbidity Indices. J Gerontol A Biol Sci Med Sci 2011; 66(3): 301–11.
27. *Sommer I, Griebler U, Mahlknecht P, Thaler K, Bouskill K, Gartlehner G,* et al. Socioeconomic inequalities in noncommunicable diseases and their risk factors: an overview of systematic reviews. BMC Public Health 2015; 15(1): 914.
28. *Ferrie JE*. Work, stress and health: The Whitehall II study. London: Council of Civil Cervice Union; 2004.
29. *Adler NE, Newman K*. Socioeconomic disparities in health: pathways and policies. Health Aff (Millwood) 2002; 21(2): 60–76.
30. *Sozmen K, Unal B*. Socioeconomic Inequalities in Non-Communicable Diseases and Self Assessed Health in Turkey. Iran J Public Health 2014; 43(6): 736–48.
31. *Saarni SI, Suvisaari J, Sintonen H, Koskinen S, Härkänen T, Lönnqvist J*. The health-related quality-of-life impact of chronic conditions varied with age in general population. J Clin Epidemiol 2007; 60(12): 1288–97.
32. *Vellakkal S, Millett C, Basu S, Khan Z, Aitsi-Selmi A, Stuckler D,* et al. Are estimates of socioeconomic inequalities in chronic disease artefactually narrowed by self-reported measures of prevalence in low-income and middle-income countries? Findings from the WHO-SAGE survey. J Epidemiol Community Health 2015; 69(3): 218–25.
33. *Arroyave I, Burdorf A, Cardona D, Avendano M*. Socioeconomic inequalities in premature mortality in Colombia, 1998-2007: The double burden of non-communicable diseases and injuries. Prev Med 2014; 64: 41–7.

34. *Szwarcwald CL, Souza-Júnior PR, Esteves MA, Damacena GN, Viacava F.* Sociodemographic determinants of self-rated health in Brazil. Cad Saude Publica 2005; 21 Suppl: 54–64.
35. *Artazcoz L, Borrell C, Benach J, Cortès I, Rohlfs I.* Women, family demands and health: The importance of employment status and socio-economic position. Soc Sci Med 2004; 59(2): 263–74.
36. *Espelt A, Borrell C, Rodríguez-Sanz M, Muntaner C, Pasarín IM, Benach J,* et al. Inequalities in health by social class dimensions in European countries of different political traditions. Int J Epidemiol 2008; 37(5): 1095–105.
37. *Pappa E, Kontodimopoulos N, Papadopoulos AA, Niakas D.* Assessing the socio-economic and demographic impact on health-related quality of life: Evidence from Greece. Int J Public Health 2009; 54(4): 241–9.
38. *Hosseinpoor AR, Bergen N, Mendis S, Harper S, Verdes E, Kunst A,* et al. Socioeconomic inequality in the prevalence of noncommunicable diseases in low- and middle-income countries: Results from the World Health Survey. BMC Public Health 2012; 12(1): 474.
39. *Schütte S, Chastang JF, Parent-Thirion A, Vermeylen G, Niedhammer I.* Association between socio-demographic, psychosocial, material and occupational factors and self-reported health among workers in Europe. J Public Health 2014; 36(2): 194–204.

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Prognostic value of EEG in West syndrome

Prognostički značaj elektroencefalografskog nalaza u West-ovom sindromu

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Abstract

Background/Aim. West syndrome (WS) is an epileptic encephalopathy which is characterized by the trias: infantile spasms, psychomotor delay and specific electroencephalography (EEG) pattern. The aim of this study was to determine the prognostic value of EEG in the therapy of West syndrome. **Methods.** This study group comprised 68 patients (40 boys and 28 girls) with the diagnosis of WS. Criteria for inclusion of patients in this study were the disease onset in the first or the second year of life, specific seizure type and a characteristic EEG pattern. All patients were divided into 2 groups: symptomatic (37 patients) and cryptogenic (31 patients) WS. The outcome was assessed through the response to the therapy (seizure control and EEG findings). Follow-up was at 3, 6, 12 and 24 months after the diagnosis was established. **Results.** Three months after starting the treatment 80.6% of patients with improved EEG were seizure free ($p < 0.01$); 85.7% of patients with EEG improvement at 3 months check-up were seizure free after 6 months ($p < 0.01$); 82.8% of patients with better EEG findings after 3 months had no seizures after 12 months ($p < 0.05$). Also, the majority of patients with improvement in EEG at 6 month follow-up (95.8%) had no seizures at one year follow-up ($p < 0.01$). The presence of seizures during this period did not depend on EEG after 6 months of treatment ($p > 0.05$). Most of the patients with improved (89.7%) and unchanged (70.6%) EEG after 12 months had no seizures after two years, whereas the patients with worsened EEG were with seizures. **Conclusion.** Seizure control after 6, 12 and 24 months depended on EEG finding at 3 months follow up. Seizure control after 12 months correlated with EEG after 6 months. The correlation between EEG after 12 months and seizure control after 24 months was not clear. EEG at 6 months follow-up did not affect seizure control after 2 years.

Key words:
electroencephalography; prognosis; treatment outcome; spasm; infant, newborn; epilepsy.

Apstrakt

Uvod/Cilj. West-ov sindrom (WS) je epileptička encefalopatija koja se karakteriše trijasom: infantilni spazmi, zaostajanje u psihomotornom razvoju i karakterističan elektroencefalografski (EEG) nalaz (hipsaritmija). Cilj istraživanja bio je da se utvrdi prognostički značaj EEG nalaza u terapiji WS. **Metode.** Istraživanjem je obuhvaćeno 68 bolesnika (40 dečaka i 28 devojčica) sa dijagnozom WS. Kriterijumi za uključivanje u istraživanje bili su pojava simptoma u prvoj ili drugoj godini života, specifičan obrazac napada i karakterističan EEG zapis. Bolesnici su bili podeljeni u dve grupe: simptomatski (37 bolesnika) i kriptogeni (31 bolesnik) WS. Ishod je procenjivan kao odgovor na terapiju (kontrola napada i EEG nalaz). Bolesnici su praćeni u periodima 3, 6, 12 i 24 meseca od postavljanja dijagnoze. **Rezultati.** Posle tri meseca od početka terapije 80.6% bolesnika sa poboljšanjem u EEG nalazu bilo je bez napada ($p < 0,01$). Ukupno, 85,7% bolesnika sa poboljšanjem EEG nalaza posle tri meseca nije imalo napade ni posle šest meseci ($p < 0,01$). 82.8% bolesnika sa poboljšanjem EEG nalaza posle tri meseca nije imalo napade ni posle 12 meseci ($p < 0,05$). Takođe, najveći deo bolesnika koji su imali poboljšanje EEG nalaza posle šest meseci (95,8%) nije imao napade ni posle jedne godine ($p < 0,01$). Prisustvo napada u ovom periodu nije zavisilo od karakteristika EEG posle 6 meseci od početka lečenja ($p > 0,05$). Najveći deo bolesnika sa poboljšanjem (89,7%) ili nepromenjenim EEG (70,6%) posle 12 meseci nije imao napade ni posle dve godine, dok su bolesnici sa pogoršanjem u EEG nalazu imali napade. **Zaključak.** Kontrola napada posle 6, 12 i 24 meseca zavisila je od nalaza na EEG posle tri meseca. Kontrola napada posle 12 meseci bila je u korelaciji sa karakterom EEG posle šest meseci. Korelacija između EEG nalaza posle 12 meseci

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i kontrole napada posle 12 i 24 meseca nije bila jasna. Nalaz EEG-a posle šest meseci nije imao uticaj na kontrolu napada posle 24 meseca.

Ključne reči:
elektroencefalografija; prognoza; lečenje, ishod; spazam; novorođenče; epilepsija.

Introduction

West syndrome (WS) is an age-dependent epileptic encephalopathy characterized by a triad of symptoms: infantile spasms (IS), hypsarrhythmia and delay and/or regresion in psychomotor development (PMD). Although this is a well-defined syndrome, variations within all three components of the syndrome are well recognized. It is a multietiological disorder with special emphasis on perinatal factors due to their relative prevalence and opportunities for prevention. Etiologically, WS occurs in three forms: symptomatic, cryptogenic and idiopathic [1]. According to the latest International League Against Epilepsy (ILAE) proposed diagnostic scheme, WS is classified as: structural/metabolic, of unknown cause and genetic (or probably genetic) [2]. Although WS is considered to be one of the intractable epilepsies, the prognosis differs widely and depends primarily on the etiology and the degree of structural damage of the central nervous system (CNS) [3, 4].

The aim of this study was to determine the significance of electroencephalography (EEG) for long-term prognosis of WS in treated children.

Methods

Our study group comprised 68 patients (40 boys and 28 girls) with the diagnosis of WS, aged 2 to 17 months (mean 6.5 ± 2.7 months), who were examined and treated at the University Children's Hospital in Belgrade from 1987 to 2008.

Inclusion criteria for this study were the disease onset in the first or the second year of life, specific seizure type and a characteristic EEG pattern.

Bearing in mind the purpose of the research and for practical reasons, all patients were divided according to the old classification and terminology [5] into two groups: symptomatic (37 patients) and cryptogenic (31 patients) WS. Idiopathic forms of WS were excluded from our study group due to the small percentage of these patients as well as the fact that the prognosis for these children is generally better than for those with cryptogenic or symptomatic forms [6–11]. All patients underwent a standard EEG examination while awake and during sleep, where the existence of the characteristic EEG pattern – hypsarrhythmia was noted. After making the diagnosis of WS, therapy was introduced to all patients according to the current treatment protocols [12]. Control EEG examination was performed after 3, 6, 12 and 24 months and was defined as: improved (in the case of a better organization of the background activity and the partial or complete disappearance of specific graphoelements), unchanged or worsened.

The outcome was assessed through the response to therapy (seizure control and EEG findings) at each follow-up. The absence of seizures and improvement of EEG were considered as a good response to therapy, while the incomplete seizure control and unchanged EEG were regarded as partial response to therapy. Poor response to the treatment implied the continous presence of seizures and further aggravation of EEG. Response to therapy after two-years follow-up was considered equivalent to the outcome.

Statistical analysis was performed using Chi squared test and Spearman's rank correlation. All data were anlyzed using SPSS for Windows version 16.0 (SPSS Inc, Chicago, IL, USA).

Results

At 3 months follow-up, 34 (50%) patients were seizure-free, while 34 (50%) still presented with seizures. EEG improvement after 3 months was observed in 36 (52.9%) patients, the findings were unchanged in 25 (36.8%) while EEG deterioration was present in 7 (10.3%) patients.

Three months after starting the treatment 29 (80.6%) patients with improved EEG were seizure free, while seizure control was obtained in a small number of patients with unchanged (3, 12%) and worsened (2, 28.6%) EEG. Thus, there was a clear and statistically significant correlation between EEG findings and presence of seizures at 3 months follow-up ($p = 0.0001$, $p < 0.01$).

At 6 months follow-up, 20 (47.6%) patients were seizure free while 22 (52.4%) patients still had seizures. EEG showed improvement in 18 (42.9%) patients, 21 (50%) patients had unchanged EEG and worsened findings were recorded in only 3 (7.1%) patients.

Seizure control at 6 months follow-up clearly correlated with EEG findings at 3 months follow-up. Twelve (85.7%) patients with EEG improvement at 3 months check-up were seizure free after 6 months. On the other hand, the majority of patients with unchanged (13, 68.4%) and worsened (6, 85.7%) EEG continued to have seizures ($p = 0.0001$, $p < 0.01$) (Table 1).

Six months after starting the treatment, EEG was in correlation with the presence of seizures in the same period ($p = 0.0001$, $p < 0.01$). Sixteen (88.9%) patients with improved EEG had no seizures, while the majority of patients with unchanged (17, 81%) or worsened EEG (3, 100%) still had seizures.

Evaluation after 12 months showed that the large number of patients (40, 65.6%) was seizure free, while 21 (34.4%) patients continued to have seizures. EEG after 12 months in comparison to previous findings was improved in 34 (55.8%), unchanged in 21 (34.4%) and worsened in only 6 (9.8%) patients.

The presence of seizures 12 months after starting the treatment clearly depended on the EEG at 3 months evaluation, with 24 (82.8%) patients with better EEG findings after 3 months having no seizures after 12 months ($p = 0.019$, $p < 0.05$) (Table 1).

Table 1
Correlation between presence of seizures and electroencephalography (EEG) findings at 3, 6 and 12 months after starting the treatment follow-up period

EEG follow-up (findings)	Seizures		Total	*p*
	no	yes		
After 3 months				
	6 months			
improved	12 (85.7)	2 (14.3)	14	0.0001
unchanged	6 (31.6)	13 (68.4)	19	
worsened	1 (14.3)	6 (85.7)	7	
total	19	21	40	
	12 months			
improved	24 (82.8)	5 (17.2)	29	0.019
unchanged	10 (45.5)	12 (54.5)	22	
worsened	4 (57.1)	3 (42.9)	7	
total	38	20	58	
	24 months			
improved	31 (96.9)	1 (3.1)	32	0.0001
unchanged	10 (52.6)	9 (47.4)	19	
worsened	3 (100)	0	3	
total	44	10	54	
After 6 months				
	12 months			
improved	23 (95.8)	1 (4.2)	24	0.001
unchanged	10 (47.6)	11 (52.4)	21	
worsened	2 (50)	2 (50)	4	
total	35	14	49	
After 12 months				
	24 months			
improved	26 (89.7)	3 (10.3)	29	0.087
unchanged	12 (70.6)	5 (29.4)	17	
worsened	2 (50)	2 (50)	4	0.031*
total	40	10	50	

All data are given as number or (percentage) of patients.
***Spearman's rank correlation.**

Also, the majority of patients with improvement in EEG at 6 months follow-up (23, 95.8%) had no seizures at one year follow-up. This correlation was highly statistically significant ($p = 0.001$, $p < 0.01$) (Table 1).

There was no correlation between the one year evaluation EEG and the presence of seizures in the same period ($p = 0.323$, $p > 0.05$).

Two years after starting the treatment, epileptic seizures were still present in 10 (20.4%) patients, whereas 39 (79.6%) patients were seizure free. On the 24 months check-up, EEG improvement was recorded in 15 (30.6%), it was unchanged in 29 (59.2%), while it was worsened in 5 (10.2%) patients.

After a two-year period, the presence of seizures was clearly dependent on the EEG findings at 3 months follow-up ($p = 0.0001$, $p < 0.01$). Hence, the majority of patients who still had seizures 24 months after starting the treatment (10, 52.6%) had no change in EEG on the first evaluation while the majority of patients without seizures after two years (31, 96.9%) had improved EEG 3 months after initiation of the treatment (Table 1).

The presence of seizures during this period did not depend on EEG after 6 months of the treatment ($p = 0.331$, $p > 0.05$).

EEG findings after 12 months correlated with presence of seizures after two years: most of the patients with improved (26, 89.7%) and unchanged EEG (12, 70.6%) had no seizures after two years, whereas patients with worsened EEG were with and without seizures, 2 (50%) respectively. The χ^2 distribution was not statistically significant ($p = 0.087$, $p > 0.05$), but there was a positive Spearman's rank correlation ($p = 0.031$, $p < 0.05$) (Table 1).

There was a statistically significant correlation between EEG findings after 24 months and the presence of seizures in the same period ($p = 0.012$, $p < 0.05$). The absence of seizures was registered in all 15 (100%) patients with EEG improvement and in 22 (75.9%) patients without changes in EEG while the majority of patients with worsened EEG (3.6%) continued to have epileptic seizures.

Discussion

Great heterogeneity of WS, in terms of numerous etiological factors, considerable variability in clinical presentation and different neurophysiological findings, precludes giving the accurate prognosis. In order to provide more adequate diagnostic procedures, treatment and further follow-up, we

considered it would be useful to find out predictiveness of EEG during the course of the disease.

In our study group, there was a predominance of symptomatic and cryptogenic forms of WS. Idiopathic WS was rarely recorded and therefore excluded from our research. However, it should be taken into consideration that some of our patients were diagnosed more than 20 years ago and nowdays sophisticated functional and anatomic neuroimaging have resulted in a shift towards symptomatic WS. Both seizure control and results of the control EEG 3 months after initiating treatment showed improvement which is certainly consistent with already known fact that EEG is an important indicator of the CNS maturation [6, 11, 13].

The trend of EEG improving under treatment continued after 6 months so that 88.9% of patients with improved EEG were seizure-free, while the majority of patients with no change or worsening of the EEG continued to have seizures which was in correlation with literature data [6, 12–15].

EEG improvement continued on the evaluation 12 months after initiating the treatment. This result correlates with the findings of Kotagal [13] who observed that chaotic hypsarrhythmic pattern in patients with WS gradually became better organized, fragmented and disappeared with time and, in clinical terms, disease may go into remission or evolve to the other form of epilepsy syndrome. However, clinical practice and various studies so far, have shown that the one year follow-up period is insufficient to make adequate conclusions and long-term prognosis [6, 16, 17].

At two-year follow-up, EEG was unchanged in most of our patients (59.2%), improvement was registered in 30.6%, whereas worsening was present in 10.2% of patients, which is consistent with the fact that WS generally has a poor prognosis [8, 10].

In our study, we tried to determine whether there were specific clinical and electrophysiological parameters based on which we could, in an early stage, provide parents with a certain prediction of seizure control, as an indicator of subsequent disease outcome.

In that way, we found that the EEG improvement after 3 months of the treatment was a good prognostic indicator in terms of seizure control after 6, 12 and 24 months of therapy. Similar observation was confirmed on 12 months follow-up, when a certain connection between EEG in this period and seizure control after 24 months of the treatment, which is consistent with the findings of Mackay et al. [18].

Response to therapy after 12 months depended on the EEG after 6 months of therapy, thus the majority of patients with better EEG in this period had a good outcome while the majority of patients with dysrrythmic EEG had partial or bad response after one year of treatment.

Our results suggest the existence of a clear correlation between the presence of seizures on two-year follow-up and EEG findings after one and two-year treatment. So, all of these parameters represent predictors of outcome after two years of treatment. That was in correlation with findings of Kotagal [13].

Conclusion

EEG improvement after 3 months was a predictor of better seizure control at 6, 12 and 24 months follow-up. EEG improvement after 6 months of treatment was associated with good seizure control and good response to therapy after one year. On the other hand, EEG after 6 months was not predictive of disease outcome after 2 years, while EEG improvement after 12 months of therapy correlated with good seizure control at two-year evaluation, but could not be used as a predictor.

REFERENCES

1. *Johnston M.* Generalized seizures. In: *Behrman RE, Kliegman R, Jenson J*, editors. Nelson textbook of pediatrics. Philadelphia: W. B. Saunders; 2007. p. 2462–4.
2. *Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross HJ, Emde BW,* et al. Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 2010; 51(4): 676–85.
3. *Arzimanoglou A, Guerrini R, Aicardi J.* Aicardi's epilepsy in children. 3rd ed. USA: Philadephia: Lippincot Wiliams & Wilkins; 2004.
4. *Panayiotopoulos CP.* The Epilepsies. Seizures, Syndromes and Management. Oxfordshire (UK): Bladon Medical Publishing; 2005.
5. *Engel J Jr.* ILAE classification of epilepsy syndromes. Epilepsy Res 2006; 70 Suppl 1: S5–10.
6. *Dulac O, Plouin P, Jambaque I.* Predicting Favorable Outcome in Idiopathic West Syndrome. Epilepsia 1993; 34(4): 747–56.
7. *Cvitanović-Sojat L, Gjergja R, Sabol Z, Hajnzić TF, Sojat T.* Treatment of West syndrome. Acta Med Croatica 2005; 59(1): 19–29. (Croatian)
8. *Shields WD.* Infantile Spasms: Little Seizures, BIG Consequences. Epilepsy Curr 2006; 6(3): 63–9.
9. *Cohen-Sadan S, Kramer U, Ben-Zeev B, Lahat E, Sahar E, Nevo Y,* et al. Multicenter long-term follow-up of children with idiopathic West syndrome: ACTH versus vigabatrin. Eur J Neurol 2009; 16(4): 482–7.
10. *Fois A.* Infantile spasms: Review of the literature and personal experience. Italian J Pediatr 2010; 36: 1–10.
11. *Go CY, Mackay MT, Weiss SK, Stephens D, Adams-Webber T, Ashwal S*, et al. Evidence-based guideline update: Medical treatment of infantile spasms: Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Committee of the Child Neurology Society. Neurology 2012; 78(24): 1974–80.
12. *Hrachovy RA, Frost JD.* Severe Encephalopathic Epilepsy in Infants: Infantile Spasms (West Syndrome). In: *Pellock JM, Bourgeois B, Dodson WE*, editors. Pediatric Epilepsy - Diagnosis and treatment. 3rd ed. New York: Demos; 2008. p. 249–69.
13. *Kotagal P.* Multifocal independent Spike syndrome: Relationship to hypsarrhythmia and the slow spike-wave (Lennox-Gastaut) syndrome. Clin Electroencephalogr 1995; 26(1): 23–9.
14. *Riikonen R.* Infantile spasms: Modern practical aspects. Acta Paediatr Scand 1984; 73(1): 1–12.

15. *Vigevano F, Cilio MR*. Vigabatrin versus ACTH as first-line treatment for infantile spasms: A randomized, prospective study. Epilepsia 1997; 38(12): 1270−4.
16. *Jeavons PM*. West syndrome: Infantile spasms. In: *Roger J, Dravet C, Bureau M, Dreifuss FE, Wolf P*, editors. Epileptic syndromes in infancy, Childhood and Adolescence. London: John Libbey; 1985. p. 42−50.
17. *Riikonen R*. Infantile spasms: Therapy and outcome. J Child Neurol 2004; 19(6): 401−4.
18. *Mackay MT, Weiss SK, Adams-Webber T, Ashwal S, Stephens D, Ballaban-Gill K*, et al. Practice parameter: medical treatment of infantile spasms: report of the American Academy of Neurology and the Child Neurology Society. Neurology 2004; 62(10): 1668−81.

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CASE REPORTS

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Bilateral carotid-cavernous fistula presented with unilateral symptomatology

Bilateralna karotidno-kavernozna fistula sa unilateralnom simptomatologijom

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Abstract

Introduction. Carotid-cavernous fistula presents an abnormal communication between carotid arterial system and the venous cavernous sinus. Due to anatomical characteristics of the sinus, the clinical picture is first manifested as ophthalmic problem. These fistulas are either of spontaneous or traumatic origin with different hemodynamics. They are usually on one side. However, bilateral carotid-cavernous fistulas are rare and deserve special interest. **Case report.** We presented a 76-years-old female patient, who complained on diplopia and right eye protrusion and esotropia. Ophthalmological findings aroused suspicion of a right-side spontaneous carotid-cavernous fistula. Further non-invasive and invasive investigations confirmed our initial diagnosis. Unexpectedly, digital subtraction angiography revealed another fistula, on her left side. Both fistulas were of low flow and did not need therapy. **Conclusion.** Carotid-cavernous fistula represent certain diagnostic and therapeutic challenge. Although they are not indicated in a clinical picture, sometimes bilateral fistulas can be found during appropriate diagnostic procedures.

Key words:
carotid-cavernous sinus fistula; diagnosis; diagnostic techniques and procedures; angiography, digital subtraction.

Apstrakt

Uvod. Karotidno-kavernozna fistula je patološka komunikacija između karotidnog arterijskog sistema i kavernoznog sinusa. Zbog anatomskih karakteristika kavernoznog sinusa, kliničke manifestacije su najpre oftalmološke. Ove fistule mogu biti spontane ili traumatske, sa različitom hemodinamikom. Obično su unilateralne. Bilateralne fistule su retke i zaslužuju poseban interes. **Prikaz bolesnika.** Prikazali smo bolesnicu staru 76 godina sa inicijalnim tegobama u vidu dvoslika, protruzija i ezotropijom na desnom oku. Oftalmološki nalazi pobudili su sumnju na desnostranu karotidno-kavernoznu fistulu. Dalje neinvazivne i invazivne dijagnostičke procedure potvrdile su našu incijalnu sumnju. Neočekivano, digitalna subtrakciona angiografija otkrila je i drugu fistulu, na levoj strani. Obe fistule su imale nizak protok i nisu morale biti lečene. **Zaključak.** Karotidno-kavernozna fistula je dijagnostički i terapijski izazov. Iako klinička slika ne ukazuje na to, ponekad se može otkriti i bilateralan proces, tokom odgovarajućih dijagnostičkih procedura.

Ključne reči:
karotidno-kavernozna fistula; dijagnoza; dijagnostičke tehnike i procedure; angiografija, digitalna suptrakcijska.

Introduction

Carotid-cavernous fistula (CCF) is an abnormal communication between the carotid arterial system and the venous cavernous sinus. Fistulas are broadly classified as direct or indirect (dural) in relation to angiographic findings, and according to hemodynamics they are of high-flow or low-flow nature. Based on etiology, CCFs are traumatic or spontaneous. Depending on arterial supply presented on angiography, they are classified into 4 subtypes [1].

Direct fistulas are most often of traumatic origin (basal skull fracture) and have high flow. Rarely, they result from spontaneous rupture of an existing aneurysm or atherosclerotic artery, usually in postmenopausal, hypertensive females [2]. Their symptomatology is usually pronounced. Small meningeal arteries supplying dural wall of cavernous sinus can rup-

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ture spontaneously while internal carotid artery itself may remain intact. Clinical features are usually mild in such cases.

Many patients initially consult an ophthalmologist. Signs of the disease can originate from damage of some of the cranial nerves that pass through the sinus (III, IV, V1 and VI), due to the increase of the orbital volume and compression of the orbital structure and/or because of hemodynamic changes.

Patients with CCF usually have more than one clinical sign or symptom: decreased vision, proptosis, arterialization of conjunctival vessels, conjunctival edema-chemosis, cranial nerve palsy, elevated intraocular pressure (IOP) and optic neuropathy [2–4].

Indirect CCF may be presented with mild or nonspecific features like red eye, atypical glaucoma, diplopia, pulsatile tinnitus, temporal headache and ptosis. Direct CCFs have far more pronounced symptomatology, including characteristic pulsatile bruit.

Imaging techniques as orbital ultrasound and color doppler imaging (CDI) are very useful in these cases both for diagnosis and monitoring, while some other radiological methods are often essential in complete examination [5].

CSFs are usually on one side. However, bilateral CCFs are rare and deserve special interest.

Case report

A 75-year-old female patient visited an ophthalmologist complaining to double vision, protrusion and inward right eye deviation. Visual acuity of the right eye (RE) was reduced, where the best corrected visual acuity (BCVA) was 20/50; on her left eye (LE) BCVA was 20/20. Intraocular pressure was 34 mmHg on RE and 18 on LE. Obvious protrusion of the RE was measured by Hertel exophtalmometer and showed difference of 5 mm: right – 21 mm; left – 16mm; base 106 mm. RE was inward in primary position and there was a limitation of right abduction. On a slit lamp RE had a mild epibulbar injection (venous congestion). Indirect fundus examination showed on her RE: optic nerve head (ONH) normal, venous dilatation, druse retinae. Our diagnoses were: RE *Protrusio bulbi*, *Paresis n. abducens*, *Glaucoma secundare*. In the patient's history, there were no data about trauma, prodromes or any other disease, except arterial hypertension.

Orbital echography showed extended right superior ophthalmic vein (SOV): right SOV diameter was 5.03 and left 2.26 mm (Figure 1).

Retrobulbar blood flow was examined by CDI, (Aloka Prosound Alfa 5 SV, Aloka, Tokyo, Japan) with 7.5 MHz linear probe. Color Doppler sonogram showed an enlarged SOV with reversed blood flow on the right side (Figure 2). Spectral Doppler analysis confirmed the arterialized flow with a peak systolic velocity (PSV) of 16.2 cm/s and diastolic velocity (EDV) of 8.7 cm/s while resistance index (RI) was 0.46.

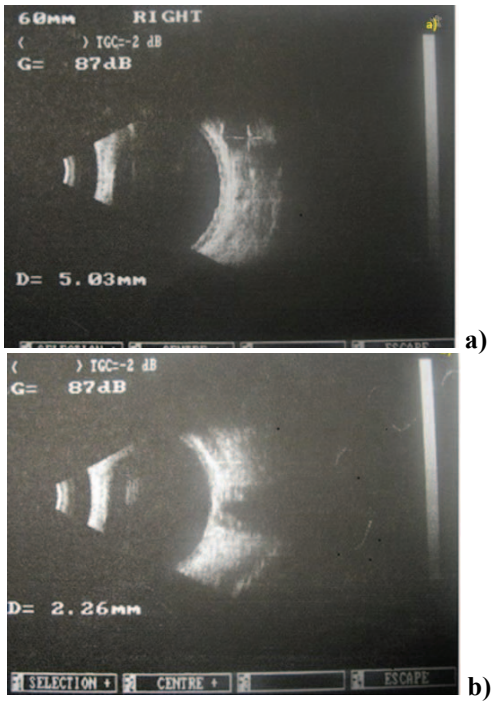


Fig. 1 – a) Orbital echography with extended right ophthalmic vein diameter (5.03 mm); b) ophthalmic vein diameter within normal limits on the left eye.

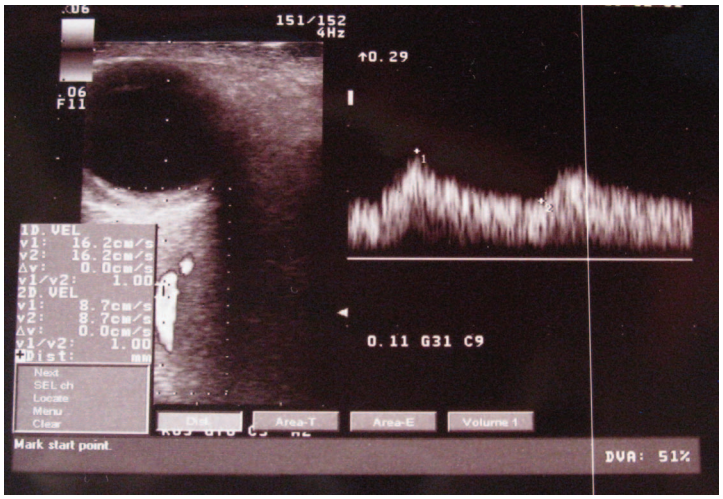
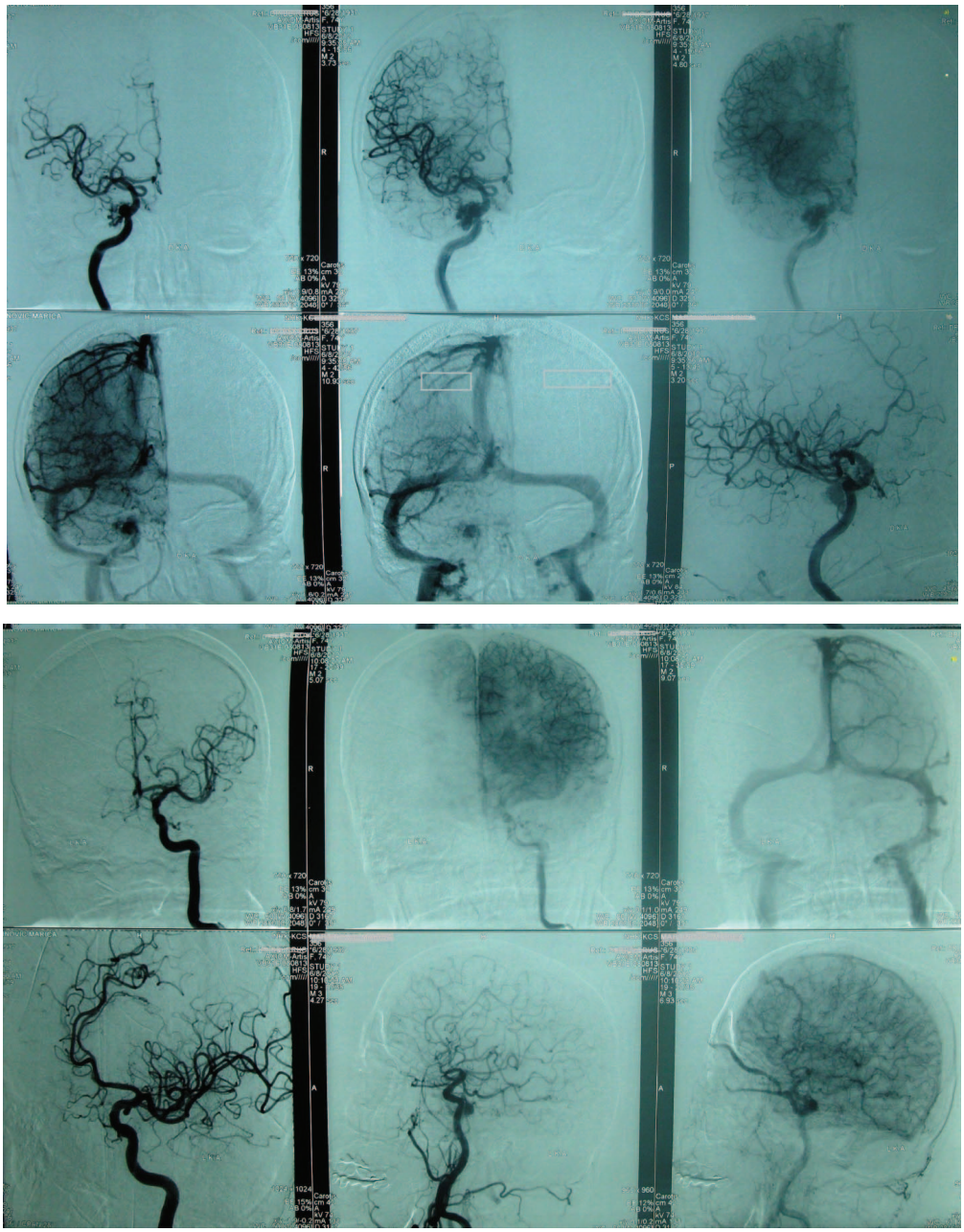


Fig. 2 – Color Doppler imaging shows enlarged superior ophthalmic vein (SOV) on the right side with reversed flow. Spectral Doppler analysis confirms the arterialized flow with a peak systolic velocity (PSV) of 16.2 cm/s.

The next step was to consult neurologist and neurosurgeon. They referred the patient to the computed tomography (CT) and magnetic resonance imaging (MRI) scan, where only slight thickening of the right lower and medial rectus muscle on the right side were obvious. Local absence of a perceptible bruit almost certainly excluded CCF of large flow but not the one of low flow. Examination by an endocrinologist excluded thyroid disease of ophthalmological origin.

Digital subtraction angiography (DSA) revealed an indirect (dural) CCF on both sides, with predominance on the right one. CCF on the right side was irrigated from the terminal branches of maxillary artery branches, meningeal and dural branches of the carotid siphon. Drainage was carried out through the superior and inferior ophthalmic veins, as well as cortical vein that drained to the upper sagittal sinus. CCF on the left was irrigated by the terminal branches of the left maxillary artery and accessory meningeal artery and drained into the right cavernous sinus and cortical drainage veins frontopolary and frontobasilary (Figure 3).

Endovascular treatment was not advised. Some 16 months later, on a checkup, the results for both eyes were as follows: BCVA was 20/25, IOP RE 14 and LE 16 mmHg, with RE antiglaucomatous therapy. Right esotropia and diplopia were less pronounced as well as conjunctival redness.



a)

b)

Fig. 3 – Digital subtraction angiography (DSA) shows indirect (dural) carotid-caveronous fistula (CCF) on both sides, predominantly on the right one. CCF on the right side (a) is irrigated from the terminal branches of maxillary artery branches, meningeal and dural branches of the carotid siphon and drains through superior and inferior ophthalmic veins and cortical vein. CCF on the left side (b) is irrigated from the terminal branches of the left maxillary artery and accessory meningeal artery and drains into the right cavernous sinus and cortical drainage veins.

Discussion

Imaging techniques as orbital ultrasound, CDI, MRI and CT usually presents enlarged superior ophthalmic vein, which can only arouse suspicion of CCF, and they can confirm foresight based on ophthalmologists examination results. Serial dynamic enhanced CT (serial DE-CT) as a diagnostic tool for CCF was found to be useful for the initial diagnosis of both high-low and low-flow CCFs. MR angiogram may demonstrate some of the higher-flow fistulas but does not provide the details necessary for complete evaluation and treatment [4–7].

Color Doppler images may demonstrate arterialized blood flow in dilated SOV and return of normal venous flow after successful treatment or spontaneous withdrawal. CDI of retrobulbar vessels represents a non-invasive and painless method which may be used both for diagnosis and monitoring patients with indirect CCF [6].

Bilateral selective arteriography of both internal and external carotid arteries may be necessary to completely characterize the blood supply and drainage of a dural cavernous sinus arteriovenous fistula [2, 4, 7].

Unilateral CCF can cause bilateral eye symptoms [8], via interacavernous plexus. However, a bilateral CCF can present with unilateral eye symptoms. Bilateral CCF cases comprise 12%–15% of all and are usually indirect in variety [9, 10].

Drainage pattern and low flow from left CCF of our patient explains absence of symptoms on that side as well as that clinical features in CCFs correlate with venous drainage. The disease may be of self-limiting pathology (spontaneous venous thrombosis), not always requiring surgery.

Conclusion

Our finding suggests that in every patient with CCF both sinuses should be examined. In diagnosis and classification of cases like this one, DSA is still a good standard, although findings from non-invasive imaging techniques are helpful.

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R E F E R E N C E S

1. *Barrow DL, Spector RH, Braun IF, Landman JA, Tindall SC, Tindall GT.* Classification and treatment of spontaneous carotid-cavernous sinus fistulas. J Neurosurg 1985; 62(2): 248–56.
2. *Chaudhry IA, Elkhamry SM, Al-Rashed W, Bosley TM.* Carotid cavernous fistula: Ophthalmological implications. Middle East Afr J Ophthalmol 2009; 16(2): 57–63.
3. *Vilela MA.* Carotid-cavernous fistula. Rev Bras Oftalmol 2013; 72(1): 70–5.
4. *Preechawat P, Narmkerd P, Jiarakongmun P, Poonyathalang A, Pongpech SM.* Dural carotid cavernous sinus fistula: Ocular characteristics, endovascular management and clinical outcome. J Med Assoc Thai 2008; 91(6): 852–8.
5. *Tranquart F, Bergès O, Koskas P, Arsene S, Rossazza C, Pisella P,* et al. Color Doppler imaging of orbital vessels: Personal experience and literature review. J Clin Ultrasound 2003; 31(5): 258–73.
6. *Duan Y, Liu X, Zhou X, Cao T, Ruan L, Zhao Y.* Diagnosis and follow-up study of carotid cavernous fistulas with color Doppler ultrasonography: Analysis of 33 cases. J Ultrasound Med 2005; 24(6): 739–45.
7. *Miller NR.* Diagnosis and management of dural carotid-cavernous sinus fistulas. Neurosurg Focus 2007; 23(5): E13.
8. *Karadag R, Bayraktar N, Kirbas I, Durmus M.* Unilateral, indirect spontaneous caroticocavernous fistula with bilateral abduction palsy. Indian J Ophthalmol 2011; 59(4): 336–7.
9. *Aralasmak A, Karaali K, Cevikol C, Senol U, Sindel T, Toprak H,* et al. Venous drainage patterns in carotid cavernous fistulas. ISRN Radiology 2014; 2014: 760267.
10. *Fattahi TT, Brandt MT, Jenkins WS, Steinberg B.* Traumatic carotid-cavernous fistula: Pathophysiology and treatment. J Craniofac Surg 2003; 14(2): 240–6.

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CASE REPORT

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Coronary artery spasm – one medical entity with different treatment options

Spazam koronarne arterije – jedan medicinski entitet sa različitim terapijskim opcijama

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Abstract

Introduction. Myocardial infarction (MI) is characterized by a critical myocardial ischemia followed by an irreversible myocardial cell damage or death. **Case report.** We presented two similar clinical cases with the ST segment elevation myocardial infarction (STEMI) diagnosis due to a prolonged coronary artery spasm, but with different treatment options due to different basic patophysiological supstrates and clinical presentations. **Conclusion.** Coronary artery spasm is a very complex pathophysiological entity with a different medication therapy management. Although the percutaneous coronary intervention can be the first choice, the treatment strategy should be carefully planned.

Key words:
myocardial infarction; coronary vasospasm; therapeutics; verapamil; angioplasty, transluminal, percutaneous, coronary.

Apstrakt

Uvod. Infarkt miokarda se karakteriše kritičnom ishemijom miokarda praćenom ireverzibilnim oštećenjem ili smrću ćelija. **Prikazi bolesnika.** Prikazali smo dva slična klinička slučaja bolesnika sa dijagnozom infarkta miokarda sa elevacijom ST segmenta (STEMI), oba sa vazospazmom koronarnih arterija, ali sa različitim terapijskim pristupom, zbog različitih osnovnih patofizioloških supstrata i kliničkih prezentacija. **Zaključak.** Spazam koronarne arterije je veoma kompleksan patofiziološki entitet, sa različitim terapijskim opcijama. Iako perkutana koronarna intervencija može biti prvi izbor, strategija lečenja ipak treba da bude pažljivo isplanirana.

Ključne reči:
infarkt miokarda; aa.coronariae, spazam; lečenje; verapamil; angioplastika, tanslumenska, perkutana, koronarna.

Introduction

Myocardial infarction (MI) occurs when myocardial ischemia exceeds a critical threshold and overwhelms myocardial cellular repair mechanisms designed to maintain a normal operating function and homeostasis. Critical myocardial ischemia results in an irreversible myocardial cell damage or death. It can occur as a result of increased myocardial metabolic demand, decreased delivery of oxygen and nutrients to the myocardium via coronary circulation, or both. MI can be subcategorized on the basis of anatomic, morphologic, and diagnostic clinical information. A new definition can classify acute MI by a clinical scenario into the various subtypes (1–5 types). Type 2 is secondary to the ischemia from a supply-and-demand mismatch. Coronary artery spasm is one possibility and sometimes is an unrecognized and undiagnosed cause of MI [1]. Coronary angiogram shows the state of vasculature at the

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time of the exam, and the stenosis absence does not necessarily mean the disease absence.

We presented two similar clinical scenarios in which both patients suffered from the myocardial necrosis due to a prolonged coronary artery spasm, but with the completely different treatment options.

Case report 1

A 39-year-old male was admitted to the regional hospital with ECG abnormalities, which included ST segment elevation in precordial leads [ST segment elevation myocardial infarction (STEMI) of anterior wall]. Therefore, thrombolytic therapy was prescribed resulting in the clinical, laboratory and ECG signs of reperfusion. However, five days later, he experienced the chest pain followed by the ST segment elevation in precordial leads (reSTEMI of anterior wall) (Figure 1A). Taking everything into account, the patient was immediately transferred to our hospital due to the necessity of primary percutaneous coronary intervention (PCI).

At the admission he breathed normally, with the rhythmic heart beats and normal blood pressure (130/80 mmHg). Coronary angiography was performed and a subocclusive (99%) lesion in proximal left anterior descending (LAD) artery was visualized (Figures 1B and C). Circumflex branch of the left coronary artery (LCA) and right coronary artery (RCA) were without a significant stenosis. After intracoronary (i.c.) administration of nitroglycerin (NTG), the subocclusive stenosis in LAD turned into 30%, which indicated a spasm (Figure 1D). At this point, the ECG changes were in regression. However, five minutes later, angiogram showed the progression of the stenosis up to 80% (Figure 1E) with the chest discomfort and the ST reelevation in the precordial leads. I.C. administration of NTG was repeated, as well as verapamil i.c. application, but the same thing happened again. Therefore, we decided to stent the lesion. Resolute Integrity 3.5 × 15 mm was implanted up to 16 atm from the ostium of LAD (Figure 1F). After the procedure, the patient was asymptomatic and ECG showed absence of ST segment elevations with the T waves inversion in precordial leads (Figure 1G).

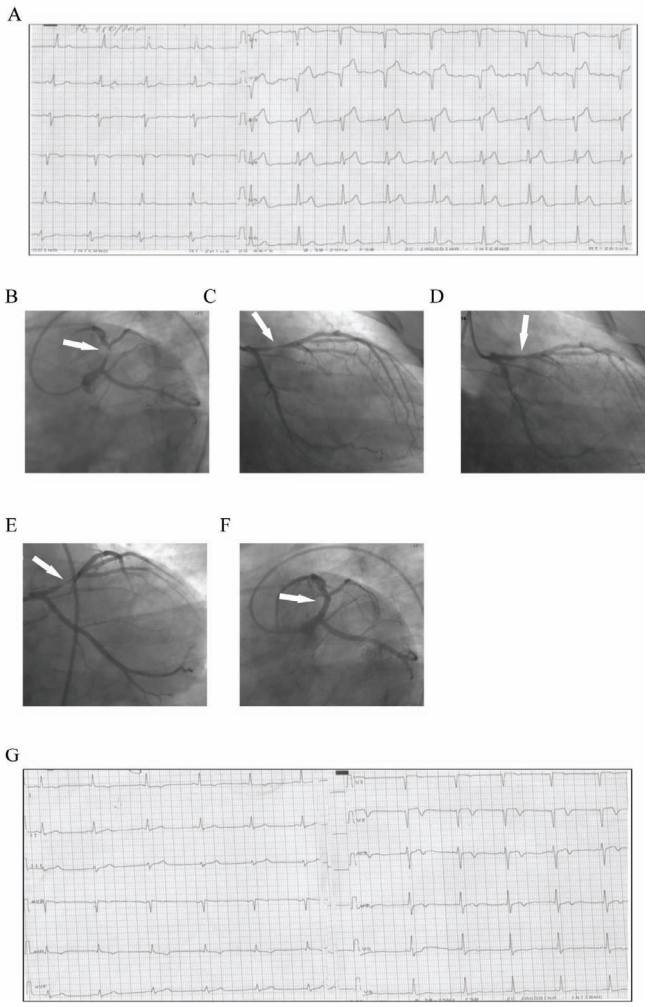

Fig. 1 – A) ECG: ST elevation in precordial leads (V1-V5); B) Left anterior oblique (LAO) caudal: subocclusive proximal left anterior descending (LAD) lesion; C) Right anterior oblique (RAO) caudal: subocclusive proximal LAD lesion; D) After intracoronary (i.c.) nitroglicerin (NTG) bolus, only 30% of residual stenosis present; E) Few minutes after NTG was given, severe stenosis in LAD occurred again; F) Final result, after stent implantation in LAD; G) ECG: T waves inversion in precordial leads (V1-V5).

Case report 2

A 49-year-old male was admitted to the Cardiology Department due to MI, presented with the chest pain and nausea two hours before the admission. His previous medical records indicated the coronary artery disease diagnosed by coronary angiography a few months before the admission (borderline stenosis in LAD). At the admission, the patient was pale, with slow heart rate and low blood pressure (95/60 mmHg). ECG showed the ST segment elevations in the inferior leads (STEMI of the inferior wall) and the sinus rhythm but along with an occasional occurrence of the third-degree AV block (Figure 2A). The coronary angiography showed LCA with no significant stenosis as well as the RCA with a significant spasm in the proximal and medial part along with the occlusion of the posterolateral branch (PL) (Figure 2B). After the intracoronary administration of NTG, the spasm in the RCA disappeared (Figure 2C) which resulted in a significant hemodynamic improvement of the patient. However, the patient still had a serious chest pain and closed PL branch (Figure 2C). Our decision was to repeat i.c. administration of the NTG, but nothing has changed. The PL branch was still occluded and it seemed that we needed to continue with the PCI. However, we decided to inject verapamil i.c. and after the second bolus the PL branch appeared all the way (Figure 2D). The angiogram performed 20 minutes later revealed a normal RCA system, while the ECG showed the absence of the ST elevations in the inferior leads with the T waves inversion in lateral leads.

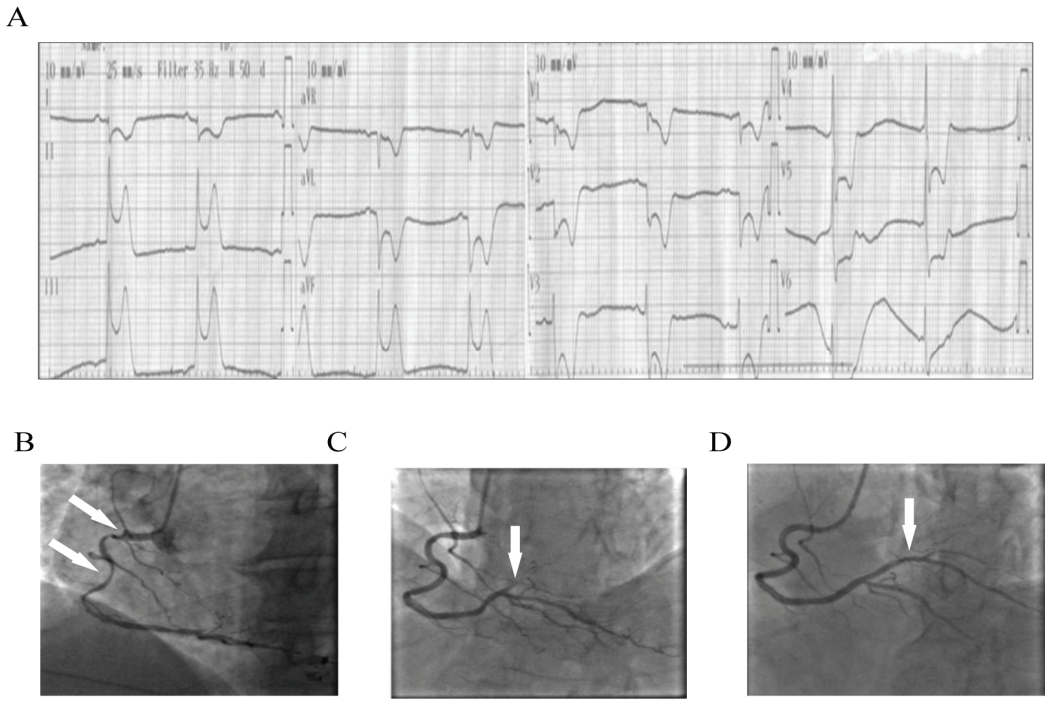

Fig. 2 – A) ECG: ST elevation in inferior leads (II, III, aVF) with ST depression in anterolateral leads (V1-V6, I, aVL); B) Right coronary artery (RCA) with spasm in proximal and medial part; occlusion of the posterolateral branch (PL); C) After nitroglycerin (NTG) administration, normal luminogram of RCA, PL branch occluded; D) After verapamil administration, normal luminogram of RCA, PL branch appeared.

Discussion

MI is characterized by an interruption in the supply of the myocardial oxygen and nutrients. Mostly, it occurs when a thrombus is superimposed over an ulcerated or unstable atherosclerotic plaque, thus resulting in a high grade (> 75%) fixed coronary artery stenosis or coronary occlusion. But, the limited supply of oxygen and nutrients in MI can also be caused by a dynamic stenosis associated with the prolonged coronary vasospasm. In both situations, there will be clinical, electrocardiographical and laboratory abnormalities indicating acute MI, but the pathophysiological mechanisms are different [1].

As it was revealed in the CASPAR study, most patients (98.5%) with the STEMI had the culprit lesions as an underlying cause of STEMI, but only 41.3% of the patients suffering from an unstable angina had culprit lesions [2]. In the first case, pathophysiologic mechanisms on the atherosclerotic plaque surface, including the release of local vasoconstrictors, triggered the acute coronary syndrome (ACS). A small erosion or fissure plaque surface releases the potent thrombogenic material and vasoconstrictors as well as platelet-derived factors and thrombin [3]. Clinical presentation is ACS (STEMI in case of coronary artery occlusion) followed by the ECG and laboratory abnormalities. It requires a careful decision in terms of treatment. Lesions, presumably responsible for the acute ischemic event, have only mild to moderate stenosis [4]. Plaque rupture with its consequent thrombus formation and local vasospasm should be taken in-

to consideration for the PCI. In our first case, intracoronary administration of verapamil and NTG stenosis turned to 30%, but not for a long time, because locally released vasoconstrictors from the ruptured plaque seriously endangered the patient. Our patient had clinical presentation of STEMI five days before the admission, and was treated with thrombolytic therapy. The patient responded well and the ST elevation vanished. But five days after that, reSTEMI occurred and the coronary angiography was performed. Since there was no response to the intracoronary NTG and verapamil, we treated the culprit lesion with the PCI, risking the spam occurrence below the stent. Three months later, we repeated the coronary angiography and the spasm occurred in the long segment of the radial artery (Figure 1A). It was resolved with the NTG. The coronary angiogram was normal, without restenosis in stent or in segment (Figures 1B and C), and also without any presence of spasm in the coronary arteries. The patient had no chest pain or ECG abnormalities.

The long term treatment included cardioselective beta adrenergic blockers, whose benefit is well established, although mostly from the trials pre-dating the advent of the modern reperfusion therapy and pharmacotherapy [5, 6].

In the second case, prolonged vasospasm was the key role abnormality responsible for ACS. The awareness and knowledge of coronary artery spasm and its pathophysiology has a great importance for a cardiologist who deals with the ACS patients. Angiographically normal coronary arteries occur in 25% of the patients with the ACS [2]. Related to that, as it was revealed in the CASPAR study, only 1.5% of the patients with STEMI have no culprit lesions (e.g. ruptured plaque) as an underlying cause of STEMI [2]. There are varieties of mechanisms responsible for the coronary spasm: endothelial dysfunction, primary hyperreactivity of vascular smooth muscle cells (VSMCs), and other factors (clinical risk factors, inflammation, ethnic influences) [3]. Endothelial dysfunction might impair the endothelium-mediated vasodilatation (mostly, higher prevalence of mutations of NO synthase gene) and can favor coronary artery spasm in response to the vasoconstrictors at the site of the predisposed segments [7]. In patients with the variant angina, coronary artery spasm can be elicited by the several stimuli that act through different receptors and cellular mechanisms, responsible for the hyperreactivity. One of the mechanisms of VSMC hyperreactivity is represented by an increase in Rho-kinase activity [8]. Mast cells, accumulated predominantly in the adventitia, can activate matrix metalloproteinase and release vasoconstrictors (especially histamine), causing ACS [9, 10]. Among the potential triggers of coronary artery spasm, the autonomic nervous system (i.e. an increase in sympathetic and parasympathetic tone) has received a great deal of attention. But, the trigger could also be an abnormal platelet activation [releasing large amounts of vasoconstrictor substances, including thromboxane A_2 (TXA2) and serotonin], an increased release of the powerful vasoconstrictor endothelin-1 (ET-1) by endothelial cells, or hyperventilation (increasing arterial pH, which leads to an increased intracellular calcium influx) [3]. Coronary vasospasm form – variant angina, mostly occurring in the early morning. There is an association with the circardian variation of an increased tonus of the epicardial coronary artery in the early morning and the decreased one in the afternoon [4]. In this case, the prolonged variant angina caused the necrosis of the myocardial cells. The medicament treatment should be the key for these patients, as it was in our second case. Calcium-channel blockers seem to be the established therapy for the coronary artery spasm, and the decrease in the frequency of the variant angina is attributed to the widespread use of these drugs. Long-acting nitrates were also found to be efficient, and their vasodilatatory effect may be the additive to the calcium antagonists. Magnesium deficiency is a possible factor contributing to the coronary artery spasm, and its long-term supplementation might also have a preventive effect [11].

In our case, the coronary angiography showed no stenosis or lesions in the RCA after the i.c. administration of verapamil. We decided to perform an optimal medicament treatment.

Six months later, the patient had no chest pain or ECG abnormalities.

Conclusion

These two cases illustrate that there is no unique solution for resolving the coronary vasospasm, thus requiring additional caution in practice. The unstable atherosclerotic plaque associated with STEMI is more frequent, thus the invasive strategy with stent implantation certainly has the priority. The above mentioned also includes the additional invasive diagnostic (e.g. intravascular ultrasound, optical coherence tomography) whenever possible. Anyway, the operator has to be patient and tactful and sometimes should try the less invasive treatment which has significantly fewer complications.

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REFERENCES

1. *Thygesen K, Alpert JS, White HD.* Universal Definition of Myocardial Infarction. J Am Coll Cardiol 2007; 50(22): 2173–95.
2. *Ong P, Athanasiadis A, Hill S, Vogelsberg H, Voehringer M, Sechtem U.* Coronary Artery Spasm as a Frequent Cause of Acute Coronary Syndrome. The CASPAR (Coronary Artery Spasm in Patients With Acute Coronary Syndrome) Study. J Am Coll Cardiol 2008; 52(7): 523–7.
3. *Lanza GA, Carreri G, Crea F.* Mechanisms of Coronary Artery Spasm. Circulation 2011; 124(16): 1774–82.

4. *Wang LX, Lü SZ, Zhang WJ, Song XT, Chen H, Zhang LJ*. Coronary spasm, a pathogenic trigger of vulnerable plaque rupture. Chin Med J 2011; 124(23): 4071–8.
5. *Steg G, James SK, Atar D, Badano LP, Blomstrom-Lundqvist C, Borger MA*, et al. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J 2012; 33(20): 2569–619.
6. *Giacoppo D, Baber U, Mehran R*. Current developments in dual antiplatelet therapy after stenting. Minerva Cardioangiologica 2014; 62(3): 261–76.
7. *Kaneda H, Taguchi J, Kuwada Y, Hangaishi, Aizawa T, Yamakado M*, et al. Coronary artery spasm and the polymorphisms of the endothelial nitric oxide synthase gene. Circ J 2006; 70(4): 409–13.
8. *Kandabashi T, Shimokawa H, Miyata K, Kunihiro I, Kawano Y, Fukata Y*, et al. Inhibition of myosin phosphatase by upregulated Rho-kinase plays a key role for coronary artery spasm in a porcine model with interleukin-1beta. Circulation 2000; 101(11): 1319–23.
9. *Otsuka F, Sakakura S, Virmani R*. Are mast cells the real culprit in atherosclerosis? Eur Heart J 2013; 34(48): 3681–3.
10. *Zdravkovic V, Pantovic S, Rosic G, Tomic Lucic A, Zdravkovic N, Colic M,* et al. Histamine Blood Concentration in Ischemic Heart Disease Patients. J Biomed Biotechnol 2011; 2011: 315709.
11. *Teragawa H, Kato M, Yamagata T, Matsuura H, Kajiyama G*. The preventive effect of magnesium on coronary spasm in patients with vasospastic angina. Chest 2000; 118(6): 1690–5.

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CASE REPORT

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Emerging pathology: pulmonary disease caused by *Mycobacterium xenopi* – a challenge in clinical practice

Urgentna patologija: bolest pluća prouzrokovana *Mycobacterium xenopi* – izazov u kliničkoj praksi

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Abstract

Introduction. Human nontuberculous *mycobacteria* (NTM) or environmental *mycobacteria* related disease is on increase. Risk factors are unclear and associations are observed in relation to climate differences, population density, or host susceptibility. With availability of molecular techniques for NTM identification, we faced emergence of NTM pulmonary cases. The work is an invitation more to colleagues to enroll the rare NTM cases into large study group. **Case report.** During an episode of productive cough and fever in a 73-year-old HIV-negative man smoker with minimal sequellae of pulmonary tuberculosis, sputum smears were acid fast bacilli positive on direct microscopy. The Löwenstein-Jensen culture results were positive with 20, 30 and 50 colonies, and molecular identification confirmed *Mycobacterium xenopi* (*M. xenopi*). Standard chest radiography showed no signs of active lesions. Examination was completed with bronchoscopy and thorax multi-slice computed tomography (MSCT). Cavitary lesions in the apico-posterior part of the left upper lobe (LUL) were detected. Under treatment (rifampicin, ethambutol, clarithromycin) sputum conversion was achieved, but irregular cavitation in the LUL remained at MSCT after 6 and after 12 months with signs of minimal regression. Patient's general condition only mildly improved and asthenia remained. Observed risk factors were previous pulmonary disease, tobacco smoking, malnutrition and prolonged emotional stress. **Conclusion.** *M. xenopi* related pulmonary disease, difficult to cure and with uncertain prognosis, is a challenge in clinical practice. Since treatment is still controversial, more randomized clinical trials are needed. Current international multicentre approach might be a good option for a larger sample size and development of new guide.

Key words:
lung diseases; mycobacterium xenopi; risk factors; diagnosis; drug therapy, combination; antibiotics; treatment outcome.

Apstrakt

Uvod. Broj obolelih od bolesti izazvanih netuberkuloznim mikobakterijama (NTM) – mikobakterijama iz okoline, je u porastu. Faktori rizika za pojavu ovih bolesti nisu jasni, a uočene su razlike zavisno od klime, gustine naseljenosti i predispozicije ljudskog organizma. Većoj izolaciji uzročnika doprinosi i dostupnost molekularnih tehnika za njihovu identifikaciju. Ovaj rad je poziv kolegama da svoje retke pojedinačne slučajeve priključe u veliku studijsku grupu. **Prikaz bolesnika.** Povodom epizode produktivnog kašlja i febrilnosti, u tri uzorka sputuma 73-godišnjeg HIV-negativnog bolesnika, dugogodišnjeg pušača sa minimalnim sekvelama plućne tuberkuloze, nađeni su acido-alkoholorezistentni bacili direktnom mikroskopijom. Rezultati kulture Löwenstein-Jensen bili su pozitivni sa 20, 30 i 50 kolonija, a molekularna identifikacija je potvrdila *Mycobacterium xenopi* (*M. xenopi*). Kako je standardni radiogram grudnog koša bio bez znakova aktivnih lezija, ispitivanje je dopunjeno bronhoskopijom i multi-slajsnom kompjuterizovanom tomografijom (MSCT) toraksa. Otkrivene su ekskavirane promene u apikoposteriornom delu levog gornjeg režnja pluća. Pod terapijom (rifampicin, etambutol i klaritromicin) postignuta je konverzija sputuma, ali se nepravilna ekskavacija održavala na MSCT toraksa posle šest i posle 12 meseci uz znake minimalne regresije. Opšte stanje bolesnika je bilo

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nepromenjeno, uključujući i asteniju. Faktori rizika u ovom slučaju su prethodna plućna bolest, pušenje, pothranjenost i produženi emotivni stres. **Zaključak.** Plućna bolest izazvana *M. xenopi* predstavlja izazov u kliničkoj praksi. Teško se leči i ima neizvesnu prognozu. Kako optimalni terapijski pristup još nije poznat, potrebna su dobro kontrolisana klinička ispitivanja. Uključivanje retkih pojedinačnih slučajeva u tekuću međunarodnu multicentričnu studiju dovelo bi do velikog uzorka bolesnika i donošenja novih smernica korisnih za praksu.

Ključne reči:
pluća, bolesti; mycobacterium xenopi; faktori rizika; dijagnoza; lečenje kombinovanjem lekova; antibiotici; lečenje, ishod.

Introduction

The nontuberculous *mycobacteria* (NTM) comprise all *mycobacteria* which do not belong to the *Mycobacterium tuberculosis* complex. They are also known as environmental *mycobacteria* and *mycobacteria* other than tuberculosis (MOTT) as well as atypical, opportunistic or anonymous *mycobacteria*. Disease caused by NTM has gained increased attention, in part due to an assumed increase in its incidence worldwide since 2000 [1–3]. Human NTM disease has typically pulmonary, skin and soft tissue, lymphatic and/or disseminated presentation. Having molecular techniques for NTM identification available in Serbia since the end of 2008, we also have observed such increase in our pulmonary clinical practice [4].

In 1954, Timpe and Runyon [5] demonstrated that NTM were pathogenic and five years later, Runyon [6] established the first classification based on colony pigmentation and the growth rate. Many NTM species have been subsequently described and new *mycobacteria* are discovered regularly. Nowadays, the NTM are a group of more than 170 species of bacteria. Twenty-five species are found to be strongly associated with disease in humans [7]. They are ubiquitous in soil and water, and they exhibit varied pathogenicity. Factors associated with human NTM infection like climate differences, population density, or host factors are still unclear. Thoracic skeletal abnormalities, rheumatoid arthritis, and treatment with immunomodulatory drugs have been recently highlighted as possible host factors [1]. Only a few of the NTM (*Mycobacterium avium complex* – MAC, *Mycobacterium kansasii – M. kansasii*, *Mycobacterium xenopi – M. xenopi*, *Mycobacterium abscessus – M. abscessus* and *Mycobacterium malmoense – M. malmoense*) have a demonstrated pulmonary pathogenicity [8].

The distribution of NTM species that are isolated from clinical samples differs strongly by region [3, 9]. Worldwide distribution of different NTM from pulmonary samples in 2008 showed that *M. xenopi* comprises some 8% of the respiratory NTM isolates on average, being 14% in Europe, and similarly, 12% in North America while no isolates were found in South America, Africa, Asia and Australia (Queensland) [9]. Increased rate of *M. xenopi* clinical isolation is reported with predominant respiratory specimens as the source [10].

Diagnostic criteria for NTM disease have been established for few decades. Since the presence of NTM in one specimen is not sufficient to confirm lung infection, a crucial part of diagnostic procedure is to distinguish between colonization and infection/disease, which is not always easy.

In 1974, the American Thoracic Society (ATS) issued the first diagnostic criteria for NTM disease [11], and in 1990 these became more restrictive [12]. Adapted later, they include clinical, radiographic, and bacteriologic criteria [13]. Only repeated isolation of the same NTM and the combination with particular clinical and radiological findings can provide sufficient support to diagnose true NTM disease.

Clinical picture of *M. xenopi* pulmonary disease is characterized by presence of respiratory symptoms in the vast majority of subjects at the time of diagnosis [14, 15]. In a half of the patients, the disease has an onset of a subacute illness developing during 2 to 4 months, usually with increasing cough and sputum production, weight loss, and malaise. Radiographic changes may vary and were believed to be predominantly cavitary lesions [15]. The majority of patients with *M. xenopi* in a recent study had computed tomography (CT) patterns of random nodules or consolidation and/or ground-glass opacities rather than classically described findings [16].

The treatment of pulmonary disease caused by NTM is controversial [15]. It is still uncertain whether *in vitro* drug sensitivity testing predicts clinical response in the way it does for *M. tuberculosis*. The results of susceptibility tests performed by the modal resistance method did not correlate with the patient's response to chemotherapy. Some literature data suggested that the combination of rifampicin and ethambutol was important whereas isoniazid might not be. In 1999, German authors reported a case of *M. xenopi* related pulmonary disease with sputum conversion achieved by the combination of clarithromycin, rifabutin and sparfloxacin [17]. Sparfloxacin is highly active against *mycobacteria*, but the use in clinical practice is restricted by its side effects. The British Thoracic Society has conducted the first trial, a randomized study of two regimens in HIV- negative patients with pulmonary disease caused by *M. avium intracellulare*, *M malmoense*, and *M xenopi* [18]. Contrary to previous suggestions that pulmonary disease with *M. xenopi* was relatively easy to treat and that it carried a good prognosis, nowadays it is considered as an illness difficult to cure and with uncertain prognosis [8, 19]. The first results with *in vitro* susceptibility patterns to rifampicin, isoniazid, and streptomycin varied and were inconsistent, although the organisms were sensitive to cycloserine and ethionamide whenever tested [8]. Clarithromycin and moxifloxacin may have similar activity within treatment regimens for *M. xenopi* disease [20]. Future studies *in vitro* and *in vivo* are needed. Failure to treatment in many cases made it necessary to perform new prospective comparative randomized study [21].

The authors aimed to highlight the emerging pathology, describe and discuss an example of *M. xenopi* caused pulmonary disease from current clinical practice.

Case report

A 73-year-old HIV-negative man smoker without history of previous pulmonary disease, asked for medical care due to productive cough and fever of three-week duration. History taking revealed a loss of appetite and weight during previous several months together with prolonged emotional stress. His general condition has markedly worsened with malaise.

The patient's vital signs on admission were as follows: blood pressure 110/70 mmHg, body temperature 36.4°C, respiratory rate 18 breaths·min^{-1}, pulse and heart rates: 88 beats·min^{-1}, and oxygen saturation 95%, while breathing room air. The thorax physical examination showed findings that fitted an asthenic subject. Laboratory results of the peripheral blood and urine were within normal limits, except for mild thrombocytopenia [119 × 10^9/L (normal lower value 140 × 10^9/L)]. White blood cells (WBC) range was normal: 6.23 × 10^9/L with 64.8% of neutrophils and 25.13% of lymphocytes.

Minimal sequellae of pulmonary tuberculosis were found on chest x-ray, and sputum smears were found to be acid-fast bacilli positive on direct microscopy. The Löwenstein-Jensen culture results were positive with 20, 30 and 50 colonies, and molecular identification confirmed *M. xenopi* [GenoType® *MTBC* (Hain)]. Since standard chest posteroanterior and left lateral projection radiographies showed no signs of active pulmonary disease, examination was completed with bronchoscopy and thorax multi-slice computed tomography (MSCT). Cavitary lesions in the apico-posterior part of the left upper lobe (LUL) were detected (Figure 1).

Under treatment (rifampicin, ethambutol, clarithromycin), sputum conversion was achieved within a month, but irregular fibrocavitary opacity in the LUL remained at MSCT after 6 and after 12 months with signs of minimal regression (Figure 2). The patient's general condition was mildly improved. The 18-month treatment was interrupted after 12 months following the patient's decision. Observed risk factors were previous pulmonary disease, tobacco smoking, malnutrition and prolonged emotional stress.

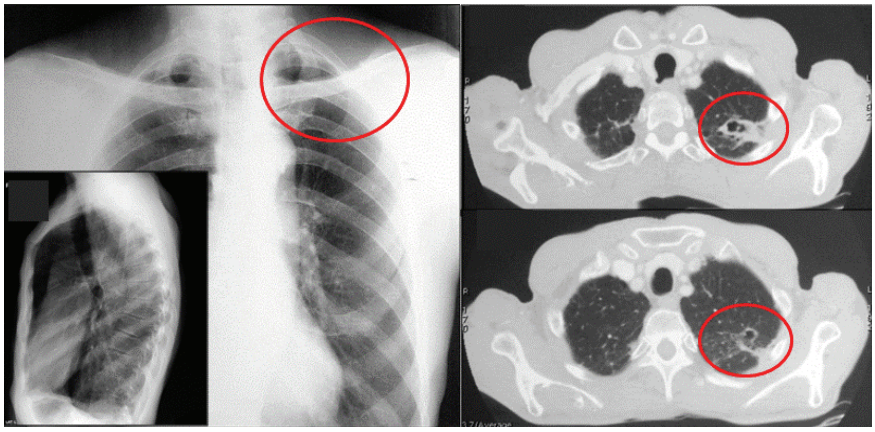

Fig. 1 – Computed tomography scan (right) on admission shows irregular fibrocavitary opacity of the left upper lobe apicoposterior part in the presence of normal chest radiographies in frontal and lateral position (left).

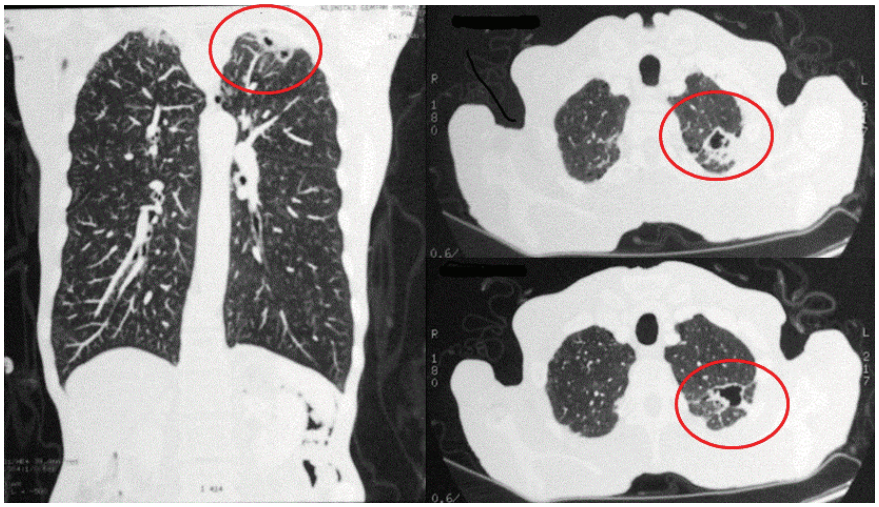

Fig. 2 – Chest computed tomography, coronal (left) and axial (right) scans, after six-month treatment – still existing irregular fibrocavitary opacities with thinner cavity wall.

Discussion

We presented a rare case of *M. xenopi* caused pulmonary disease in a HIV-negative patient hospitalized in our tertiary level health care facility. After MAC and *Mycobacterium gordonae, M. xenopi* was the third most frequently isolated species in a recent worldwide survey, though its isolation was limited to distinct geographical regions, mainly in Europe and Ontario, Canada [9]. *M. xenopi* was more frequently isolated in Southern (21% of isolates) compared to Northern Europe (6% of isolates) partly due to substantial contribution of *M. xenopi* isolated in a single country. Interestingly, in Hungary, the country which is a north neighborough of Serbia, *M. xenopi* is the predominant NTM isolate comprising 49% of all the NTM [9]. In the same study, *M. xenopi* was the second most frequently isolated NTM, after *M. gordonae*, in neighbouring Croatia. We lack a report on such NTM distribution analysis in Serbia. Although the distribution may very within the same country, based on the data on high proportion of *M. xenopi* isolates in two neighborough countries, we assume that *M. xenopi* might present an important emerging pulmonary pathogen in Serbia as well. The recent cases from our clinical practice speak in favour of it.

Diagnostic criteria, which are necessary to distinguish *M. xenopi* active disease from a casual contaminant without a clinical significance were fulfilled in the presented case [13]. We obtained multiple isolations of *M. xenopi* from the sputum samples. Sputum smears were acid-fast bacilli positive on direct microscopy, three cultures positive, and *M. xenopi* proved by molecular technique in the absence of concurrent isolates of other pathogens, including *M. tuberculosis*. Finally, the symptomatic patient had abnormal chest MSCT consistent with mycobacterial disease. In a half of the patients with *M. xenopi* pulmonary disease, initial pulmonary radiographic changes are unilateral and cavitary changes are present in 96% [15]. While our finding is in concordance with this classical presentation of fibrocavitary disease, a recent study highlighted marked proportion of nodulary pattern changes, which were also present in the latest Japanese case report, suggesting dissemination of the disease in an immunocompromised patient under steroid treatment [16, 22].

In 1994, Terashima et al. [23] reported two cases of pulmonary disease caused by *M. xenopi* – one occurred after gastrectomy and the other synchronously with *M. tuberculosis* infection, both presented with features of an infectious disease and unilateral cavitary lesion at chest x-rays [23]. Several cases of Japanese authors have been reported thereafter [22, 24]. While all the firstly mentioned isolates were susceptible to streptomycin and kanamycin, the later results highlighted importance of drug susceptibility testing on disease outcome and levofloxacin as potential antibiotic with proven *M. xenopi* susceptibility in the presented cases [24]. Due to many challenges in therapy of *M. xenopi* pulmonary disease in recent years, an international study has been launched to assess the validity of two currently suggested three-drug therapy regimens: rifampicin + ethambutol and moxifloxacin or clarithromycin as the third one (CaMoMy study) [21]. We started therapy with available combined clarithromycin regimen and achieved sputum conversion in a month together with marked clinical improvement, but only mild radiographic regression over 12-month period.

Clinical picture of *M. xenopi* pulmonary disease in our patient was characterized by usual presence of general (weight loss, malaise) and respiratory symptoms (cough with sputum production) at the time of diagnosis. Sometimes, longer histories of respiratory symptoms, often present for several years, associated with slowly progressive changes on chest radiographs are seen. Hemoptysis is not a common sign. Only rarely, the disease appears as an acute illness characterized by repeated hemoptysis and weight loss occurring over a few weeks or as an asymptomatic disease discovered on regular physical examination or after mass miniature radiography [8].

Since the prognosis in *M. xenopi* pulmonary disease is unpredictable with drug treatment alone, there would seem to be a good argument for lung resection in some patients, particularly in those who fail to respond initially to chemotherapy or who relapse [8]. For the same reason, prevention measures of NTM disease are under further investigation. Thus, the factors implicated in increased susceptibility to NTM pulmonary disease are also in the focus of current research. They may be local or systemic, congenital or acquired [25]. Our patient had a history of tobacco smoking, prolonged emotional stress, malnutrition, and sequellae tuberculosis as underlying condition. In many of the patients with NTM pulmonary disease in Europe, no underlying lung disease or immunodeficiency condition have been detected [26].

NTM usually require abnormal airway mucosa to initiate bronchopulmonary infection. Local factors that exacerbate damage to the mucosal surface, or that increase the tissue burden of NTM, may promote disease. These include airway inflammation, ciliary dysfunction, abnormal sputum composition, mucus plugging of large or small airways, and the elongated bronchi, the majority present in our patient, a long-term heavy smoker. Apart from local irritation and inflammation, tobacco smoking contents are proved to decrease both cellular and humoral immunity in humans [27]. If it seems acceptable and evidence based that strengthening measures of tobacco control may have an additional and beneficial impact on tuberculosis elimination [28], they might have an impact on the prevention of NTM pulmonary disease as well.

Conclusion

Clinicians' awareness should be kept on the NTM related disease as an emerging pathology increasingly recognized in subjects with and without immunodeficiency. Increased detection rates of both slowly and rapidly growing NTM in pulmonary samples require careful estimation of diagnostic criteria for the NTM caused pulmonary disease and implementation of preventive measures. Treatment options need further estimation through new clinical trials aimed to develop a useful guide in current clinical practice.

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R E F E R E N C E S

1. *Prevots RD, Marras TK.* Epidemiology of human pulmonary infection with nontuberculous mycobacteria: A review. Clin Chest Med 2015; 36(1): 13–34.
2. *Thomson RM.* Changing epidemiology of pulmonary nontuberculous mycobacteria infections. Emerg Infect Dis 2010; 16(10): 1576–83.
3. *Marras TK, Daley CL.* Epidemiology of human pulmonary infection with nontuberculous mycobacteria. Clin Chest Med 2002; 23(3): 553–67.
4. *Stjepanović MI, Pešut DP, Lešić AR, Stević RS.* Pulmonary and Vertebral Mycobacterium avium Disease in a HIV-negative 71-year-old Man A Case Report. Infez Med 2016; 24(4): 345–8.
5. *Timpe A, Runyon EH.* The relationship of atypical acid-fast bacteria to human disease: A preliminary report. J Lab Clin Med 1954; 44(2): 202–9.
6. *Runyon EH.* Anonymous mycobacteria in pulmonary disease. Med Clin North Am 1959; 43(1): 273–90.
7. *van Ingen J.* Diagnosis of nontuberculous mycobacterial infections. Semin Respir Crit Care Med 2013; 34(1): 103–9.
8. *Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F,* et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007; 175(4): 367–416.
9. *Hoefsloot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P,* et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. Eur Respir J 2013; 42(6): 1604–13.
10. *Bluth MH, Vera R, Razeq J, Kramer M, Abu-Lawi KI.* Mycobacterium xenopi: Evidence for Increased Rate of Clinical Isolation. Int J Biomed Sci 2009; 5(2): 96–100.
11. *American Thoracic Society.* Diagnostic standards and classification of tuberculosis and other mycobacterial diseases. New York: American Lung Association; 1974.
12. *Wallace RJ Jr, O'Brien R, Glassroth J, Raleigh J, Dutt A,* American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. Am Rev Respir Dis 1990; 142(4): 940–53.
13. *Medical Section of the American Lung Association.* Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. Am JRespir Crit Care Med 1997; 156(2 Pt 2): S1–25.
14. *van Ingen J, Bendien SA, de Lange WCM, Hoefsloot W, Dekhuijzen PNR, Boeree MJ,* et al. Clinical relevance of non-tuberculous mycobacteria isolated in the Nijmegen-Arnhem region, The Netherlands. Thorax 2009; 64(6): 502–6.
15. *Banks J, Hunter AM, Campbell IA, Jenkins PA, Smith AP.* Pulmonary infection with Mycobacterium xenopi: Review of treatment and response. Thorax 1984; 39(5): 376–82.
16. *Carrillo MC, Patsios D, Wagnetz U, Jamieson F, Marras TK.* Comparison of the spectrum of radiologic and clinical manifestations of pulmonary disease caused by Mycobacterium avium complex and Mycobacterium xenopi. Can Assoc Radiol J 2014; 65(3): 207–13.
17. *Schmitt H, Schnitzler N, Riehl J, Adam G, Sieberth HG, Haase G.* Successful treatment of pulmonary Mycobacterium xenopi infection in a natural killer cell-deficient patient with clarithromycin, rifabutin, and sparfloxacin. Clin Infect Dis 1999; 29(1): 120–4.
18. *Research Committee of the British Thoracic Society.* First randomised trial of treatments for pulmonary disease caused by M avium intracellulare, M malmoense, and M. xenopi in HIV negative patients: Rifampicin, ethambutol and isoniazid versus rifampicin and ethambutol. Thorax 2001; 56(3): 167–72.
19. *Dauendorffer JN, Laurain C, Weber M, Dailloux M.* In vitro sensitivity of Mycobacterium xenopi to five antibiotics. Pathol Biol (Paris) 2002; 50(10): 591–4.
20. *Ferro BE, van Ingen J, Wattenberg M, van Soolingen D, Mouton JW.* Time-kill kinetics of slowly growing mycobacteria common in pulmonary disease. J Antimicrob Chemother 2015; 70(10): 2838–43.
21. *Andrejak C.* (PI) Treatment of M. xenopi infections (CaMoMy Trial) TBnet #34 / NTM-NET #5. [cited 2015 Dec 15]. Available from: http//:www.tb-net.org/index.php/tbnet-research/ongoing-projects
22. *Okano Y, Shinohara T, Imanishi S, Takahashi N, Naito N, Taoka T,* et al. Miliary pulmonary nodules due to Mycobacterium xenopi in a steroid-induced immunocompromised patient successfully treated with chemotherapy: A case report. BMC Pulm Med 2016; 16(1): 92.
23. *Terashima T, Sakamaki F, Hasegawa N, Kanazawa M, Kawashiro T.* Pulmonary Infection due to Mycobacterium xenopi. Int Med 1994; 33(9): 536–9.
24. *Matsui Y, Tamura A, Nagayama N, Akashi S, Araki K, Kimura H,* et al. Review of pulmonary Mycobacterium xenopi infection cases: 11 cases of our own and 18 other cases reported in Japan. Kekkaku 2010; 85(8): 647–53. (Japanese)
25. *Sexton P, Harrison AC.* Susceptibility to nontuberculous mycobacterial lung disease. Eur Respir J 2008; 31(6): 1322–33.
26. *Wassilew N, Hoffmann H, Andrejak C, Lange C.* Pulmonary Disease Caused by Non-Tuberculous Mycobacteria. Respiration 2016; 91(5): 386–402.
27. *Sopori M.* Science and society: Effects of cigarette smoke on the immune system. Nat Rev Immunol 2002; 2(5): 372–7.
28. *Zellweger JP, Cattamanchi A, Sotgiu G.* Tobacco and tuberculosis: Could we improve tuberculosis outcomes by helping patients to stop smoking? Eur Respir J 2015; 45(3): 583–5.

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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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