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Josef Brudzinski (1874–1917), poljski pedijatar, poznat je po svojim istraživanjima neuroloških znakova udruženih sa meningitisom. Oni još uvek imaju veliki značaj za rano otkrivanje bolesti i pravovremeno započinjanje terapije (vidi str. 598-604).

ORIGINAL ARTICLES

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Risk factors associated with early childhood caries in autonomous province of Vojvodina, Republic of Serbia

Faktori rizika od nastanka karijesa u ranom detinjstvu u autonomnoj pokrajini Vojvodini, Republika Srbija

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Abstract

Background/Aim. Early childhood caries (ECC) is still unexplored in Vojvodina an autonomous province of the Republic of Serbia. The aim of this study was to determine its prevalence in preschoolers and to define the risk factors that affect the prevalence of this disease. **Methods.** The survey was designed as a cross-sectional analytical study of preschool children in the region of Vojvodina, the Republic of Serbia. Sample type has been projected as a systematic sample and contained both parents and their children from 13 to 71 months of age. The study was designed with a dental examination of children and self-administered questionnaire which included: gender of children, self-referred socioeconomic status, parental education, oral status and health information level about parents and their child, child oral hygiene habits, type of feeding during infancy, consumption of sweets, and use of medical syrups. The data was further analyzed using the SPSS for Windows Microsoft Excel, version 21. The percentage of caries-free children was compared using χ^2 test; one way ANOVA was used to compare the mean disease indices at the 5% level of significance. Categorical variables were compared for statistical difference across groups using contingency χ^2 tests together with multinomial logistic regression modeling regarding the predictive model for ECC prevention. **Results.** The case group involved 452 (52.44%) males and 410 (47.56%) females. The prevalence of ECC in children 13-71 months old was 46.64%. Logistic regression model showed that those children who used sweets between meals, were more likely to have ECC (OR = 181.16; 95%, CI = 84.29–389.34), as well as those who used medical syrups more than five times a year in comparison to those who never used medicines (OR = 8.08; 95% CI = 3.78–17.27), among parents with poor oral status (OR = 3.09; 95% CI = 1.65–5.79) and low health informed parents (OR = 217.57; 95% CI = 84.46–560.50). **Conclusion.** This study suggests an association between the examined risk factors and high ECC prevalence in preschool children in Vojvodina.

Keywords:
dental caries; prevalence; risk factors; child, preschool; parents; surveys and questionnaires; food habits; oral hygiene.

Apstrakt

Uvod/Cilj: Karijes u ranom detinjstvu (KRD) još uvek nije istražen u Vojvodini autonomnoj pokrajini Republike Srbije. Cilj našeg istraživanja bio je da se odredi prevalencija KRD kod predškolske dece i definišu faktori rizika koji utiču na rasprostranjenost ovog oboljenja. **Metode.** Istraživanje je sprovedeno kao analitička studija preseka kod predškolske dece u Vojvodini. Projektovan je sistematski uzorak, koji je obuhvatio roditelje i njihovu decu uzrasta od 13 do 71 mesec. Istraživanje je dizajnirano kao stomatološki pregled deteta, tokom kojeg je sproveden intervju sa roditeljima. Anketni upitnik sadržao je varijable: pol deteta, socioekonomski status porodice, nivo obrazovanja i zdravstvene obaveštenosti roditelja, oralni status roditelja i deteta, oralno-higijenske navike kod dece, način ishrane, učestalost konzumacije slatke hrane i upotreba medicinskih sirupa u toku ranog detinjstva. Statistička obrada podataka je sprovedena primenom statističkog paketa SPSS *for Windows Microsoft Excel,* verzija 21. Proveravana je značajnost utvrđenih razlika između frekvencija nezavisnih varijabli kod dece bez karijesa putem χ^2 testa, a jednosmerna analiza varijanse (ANOVA) korišćena je radi utvrđivanja značajnosti razlika parametrijskih obeležja (za oba testa nivo značajnosti $p < 0,05$ smatran je statistički značajnim). Proveravana je statistička značajnost razlika između proporcija različitih kategorija opisnih obeležja primenom neparametrijskog, univarijantnog testa (χ^2 test), a potom su putem multivarijantne logističke regresije generisani statistički modeli predikcije faktora koji mogu doprineti prevenciji KRD. **Rezultati.** Uzorak je

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obuhvatio 452 (52,44%) dečaka i 410 (47,56%) devojčica. Prevalencija KRD iznosila je 46,64%. Logističkim regresionim modelom neđeno je da su češće obolevala deca koja su uzimala slatkiše između obroka (OR = 181,16; 95%, CI = 84,29–389,34), koristila medicinske sirupe više od pet puta godišnje (OR = 8,08; 95% CI = 3,78–17,27), kao i deca roditelja lošeg oralnog statusa (OR = 3,09; 95% CI = 1,65–5,79) i niske zdravstvene obaveštenosti (OR = 217,57; 95% CI = 84,46–560,50). **Zaključak.** Ovom studijom je utvrđena povezanost ispitivanih faktora rizika i visoke prevalencije KRD kod predškolske dece u Vojvodini.

Ključne reči:
zub, karijes; prevalenca; faktori rizika; deca, predškolska; roditelji; ankete i upitinici; ishrana, navike; usta, higijena.

Introduction

Epidemiological studies in the world [1] and in Serbia as well, clearly indicate high trend rate of the early childhood caries (ECC), especially in socially disadvantaged, economically challenged society with poorly health informed parents and their children mainly from the rural environments, who did not understand the official language in the country where they live [2]. Moreover, it is very important to point out that ECC has with no doubt, multifactor etiology but it is still very difficult to present with assurance the reason for its appearance. The American Academy of Paediatric Dentists (AAPD) defined ECC as "the presence of one or more decayed, missing or filled tooth surfaces in any primary tooth in children 71 months of age and younger" [3]. This is a special form of caries of the primary dentition that affects the teeth after the eruption, and it has a rapid progression resulting in a number of symptoms and complications. ECC begins with white-spot lesions in the upper primary incisors along the margin of the gums. If the disease continues, caries can progress, leading to complete destruction of the crown [4]. The upper incisors are most vulnerable, while the mandible incisors are protected by the tongue and by saliva from submandibular and sublingual glands. ECC affects three aspects of daily living, namely systemic health, body weight and growth and quality of life [5]. The specific problem which needs to be especially emphasized is the early age of its appearance and significant negative influence on children's overall health. If ECC is left untreated it can lead to pain, acute infections, nutritional insufficiencies, speech problems and affect the growth and maturation of the permanent dentition. Infants with ECC grow at a slower pace than caries-free infants. Some young children with ECC may be severely underweight because of associated great degree of pain and suffering which is proven to have an impact on general health factors such as child weight, together with their obvious disinclination to eat [6]. Published studies showed higher ECC prevalence figures for 3-year-olds that ranged from 36% to 85% in the Far East Asia region [7], whereas that figure was 45.33% in the East Indian studies [8]. In England, the United States of America and Canada's North, the prevalence of ECC has been documented to vary between 7.0%, 12.0% and from 28% to 98.9%, respectively [9]. Researchers have attempted to expand basic microbiological models for ECC development and to include various social, demographic and behavioral factors such as ethnicity, family income, maternal education level, family status and parental knowledge [10]. Current evidence suggests that use of a sugar-containing liquid in a bottle at night may be an important but not necessarily the only etiological factor. Consequently, today is still a very complex task to determine the precise risk factors for ECC and the unique model for its prevention in regard to the numerous predisposed biological, socioeconomic, cultural, psychosocial and other factors that dictate and interlace with each other. There is a need for serious medical and social researching including sophistically software systems for data evaluation – Synthetic Minority Over-Sampling Technique (SMOTE) classification algorithm [11], support vector machines (SVM), logistic model tree (LMT), Data Mining classification and regression tree (CART) analysis [12], to find out which etiological factors are associated with ECC and its different clinical manifestation. Caries prediction has always been a challenge for both clinicians and researchers. The multifactor nature of the disease necessitates the evaluation and combination of multiple factors 13. ECC remains of particular scientific concern because of its devastating nature, rapid progression and mainly missed public health opportunities for its successful prevention, especially in some socially closed and disadvantaged groups in both developed and developing countries, with Serbia being no exception. In Vojvodina as part of the Republic of Serbia, ECC still has an increasing prevalence rate. The reason for that we can, unfortunately, find firstly, in poverty progression trend of the population, especially in some minority groups than in therapeutic approach to disease treatment and furthermore in specific presence of diverse ethnicities, languages, cultures and social structures, that may be risk factors that are unique to the youngest population in this region [2]. The aim of this study was to estimate the prevalence of ECC in preschool children living in the region of the Republic of Serbia, Vojvodina, to evaluate the correlation of several social and behavioral factors associated with ECC prevalence, and to define a predictive model for ECC.

Methods

Study sample

Vojvodina is an autonomous province of Serbia, located in the northern part of the country, and has a population of approximately 2 million. It has a multiethnic and multicultural identity. In addition to the multiethnic and multicultural characteristics of this region, there are also differences in the education, personal income and unemployment rates in this population. Assessing the presence of these variables is important in determining the correlation between the outlined

demographic, educational and socioeconomic factors and the prevalence of ECC in toddler and preschool children. The fluoride concentration in drinking water is generally low (< 0.3 ppm). The study was conducted from November 2014 to May 2015. The survey was designed as a cross-sectional analytical study of preschool children in Vojvodina. Sample type has been projected as a systematic sample and contained both parents and their children from 13 to 71 months old, of different gender, social status, and nationality (Serbian, Hungarian, Slovak, Russian, Roma etc.). The estimated age of a child was the age at the time of the examination. The dental survey comprised, and recorded within the case file, 862 children and the questionnaire has comprised 1,724 of their parents. The multi-grade type sample (systematic sample) was elected in survey unit definition, which comprised 10% of children of the target population and, as a step of choice the following formula:

k = N/n (k = step of choice; n = number of units within sample; N = number of units in the basic set).

The number of units in the basic set (N) was determined by records of preschool institutions within certain municipalities of the Vojvodina on the number of enrolled children in the 2014/15 school year; a share of 10% of children was established which reflected the number of units within the sample (n). By implementing the step of choice (k), based on the records of the preschool institutions, units for the sample were defined. Parents of all eligible children were informed in writing about the study objectives and invited to participate. The duration of the study was 5 months. In the first stage, the parents were given specially designed questionnaire written in official (Serbian) and minority languages, with the personal data of parents and each child separately. The questionnaire was made up of 64 closed types of questions concerning ethnicity, demographic features and socioeconomic status of the family, knowledge, attitudes and habits of parents concerning diet, oral hygiene, fluoride prophylaxis, behavior towards the oral as well as general health. It also included questions about the importance of preventive measures concerning the parents' health values, as well as questions about the sources of information and their indices on the oral health of children and their parents. The health information level of parents was evaluated with the answers connected with: child's diet, the oral hygiene and fluoride prophylaxis, dental visits, the use of medical and vitamin syrups, diet, oral hygiene and fluoride use during pregnancy, the attitudes concerning oral health, the source of information concerning oral health etc. According to the number of the correct answers the estimation was done about the health information of parents to the following groups: not informed, average informed and well informed. After getting the parents' consent, the second phase was performed, i.e. dental check-ups of children and evidence of the prevalence of ECC. The dental examinations were conducted by a single well-trained and calibrated dentist. The youngest group of children, 13–24 months old, were examined by a visual, non-tactile method, referred to as a "lift the lip" technique. The examiner lifted the upper lip of every child to check up the four maxillary primary incisors and two canines for presence and severity of ECC. All other children were examined with plane dental mirror and probe, using natural light, without previous brushing and drying teeth. Exams were performed in the kindergarten nursing room, except for children that lived in disadvantaged settlements, and who were examined "in the open air". All primary teeth were examined and caries was recorded using World Health Organization, recognized indices of decayed, missing, and filled teeth and surfaces, decayed, missing, and filled teeth (DMFT) and decayed, missing, filled tooth surfaces (DMFS) respectively. Regarding the numerous divisions, the authors decided to use Wine modification by Drury classification, which included: caries lesions on the maxillary incisors and canines with molars being present or not and the lower incisors appearing healthy [14]. The teeth that did not fully erupt or congenitally missing teeth were excluded from the dmft, and dmfs scores. Incomplete data from the parents` questionnaire and/or data on children which could not have been examined were excluded from the following evaluation and statistic analysis.

Pilot Study

The questionnaire, the study design, and the obtained data were initially tested in a pilot study. It included by random selection the preschool children from the kindergarten "Little Bee" in Novi Sad and their parents from different socioeconomic background and nationality. ECC was found in 28 (26.92%) of 104 examined preschool children. The highest disease frequency was found in male children, who didn't speak the Serbian language, in children of part-time employed parents, who had secondary education and were poorly informed about oral health.

This study was approved by Committee on Human Research of the Medical Faculty of Novi Sad, process number: 1206/07. Children were examined after a written consent signed by their parents. All identifiable personal information was adequately disguised in the data in order to preserve the anonymity of the individuals involved.

Statistical analysis

The obtained clinical and questionnaire data were further analyzed using the SPSS for Windows Microsoft Excel, version 21. Descriptive statistics were calculated to determine the percentage caries-free children, mean caries disease severity indices (dmft, dmfs) and the standard deviations of the mean for each variable investigated. The percentage of caries-free children were compared using χ^2 test, and one-way ANOVA was used to compare the mean disease indices at the 5% level of significance. Categorical variables were compared for statistical difference across groups using contingency χ^2 tests together with multinomial logistic regression modeling regarding the predictive model for ECC prevention.

Results

In total 862 children, 13-71 months old were examined, 452 (52.44%) boys and 410 (47.56%) girls, mean age 3.41

year (95% CI = 3.34–3.48 year) and prevalence of ECC was 46.64%. Out of those examined 460 (53.36%) were caries-free. In the maxillary inter-canine section 3,614 deciduous teeth were healthy, 1,514 were decayed, 7 extracted and 32 were filled. Every child had on average 1.81 decayed teeth (dmft) and 3.68 decayed tooth surfaces (dmfs). The basic demographic factors that are linking the social environment and caries prevalence in the early childhood (ECC) are presenting in Table 1. There is statistically significant difference (χ^2 test) in ECC prevalence between male children (56.5%) in relation to female ones (43.5%, $p = 0.027$). The boys had higher ECC frequency comparing to the girls. The third and next born child in the family had a higher probability for ECC (17.7%, $p = 0.023$) in relation to the first and second born child. The statistically significant higher ECC frequency was found in children who were not breastfed or in children who were breastfed more than 12 months (47.3%, $p = 0.000$), or in children who have used a baby bottle with pacifier from birth (51.2%, $p = 0.009$). Our study also showed the significant association between child's dietary habits and ECC prevalence. The low ECC prevalence was observed in children who use food that was not additionally sweetened (25.6%, $p = 0.000$), and in children who did not take sweets (8.0%, $p = 0.000$). The influence of the parent's socioeconomic factors and their oral health status on the ECC prevalence was presented in Table 2. The highest prevalence of ECC was found in children of unemployed parents (29.1%, $p = 0.004$), who had only elementary education (9.7%, $p = 0.025$), and in parents who had an income less than 300.00 € *per* month (29.6%, $p = 0.001$), and parents

Table 1

Influence of child's socioeconomic factors and dietary habits on early childhood caries (ECC) prevalence

Parameter	Children with ECC n (%)	Caries free children n (%)	Subtotal n (%)	Total n (%)	*p*
Sex of the child					
male	227 (50.2)	225 (49.8)	452 (52.4)	862 (100.0)	0.027
female	175 (42.7)	235 (57.3)	410 (47.6)		
Child order in family					
first, second	331 (45.0)	404 (55.0)	735 (85.3)	862 (100.0)	0.023
all others	71 (55.9)	56 (44.1)	127 (14.7)		
History of breastfeeding					
never breastfed or breast feeding after 12 months	190 (55.2)	154 (44.8)	344 (39.9)	862 (100.0)	0.000
6–12 months breastfeeding	212 (40.9)	306 (59.1)	518 (60.1)		
Bottle nursing					
never use of bottle	196 (42.5)	265 (57.5)	461 (53.5)	862 (100.0)	0.009
bottle feeding from birth	206 (51.4)	195 (48.6)	401 (46.5)		
Additional sweeten of food					
never	103 (25.1)	308 (74.9)	411 (47.7)	862 (100.0)	0.000
every day	299 (66.3)	152 (33.7)	451 (52.3)		
Use of sweets					
never use sweets or with meals	32 (8.2)	356 (91.8)	388 (45.0)	862 (100.0)	0.000
use of sweets between meals	370 (78.1)	104 (21.9)	474 (55.0)		

***χ^2 – test with $p < 0.05$ as a level of statistical significance value.**

Table 2

The influence of parent's socioeconomic factors and oral status on the early childhood caries (ECC) prevalence

Parameter	Children with ECC n (%)	Caries free children n (%)	Subtotal n (%)	Total n (%)	*p*
Parents working status					
employed	285 (43.8)	365 (56.2)	650 (75.4)	862 (100)	0.004
unemployed	117 (55.2)	95 (44.8)	212 (24.6)		
Parents education level					
illiterate or elementary school	39 (60.0)	26 (40.0)	65 (7.5)	862 (100)	0.025
middle or high school	363 (45.5)	434 (54.5)	797 (92.5)		
Family income *per* month					
≤ 300.00	119 (56.7)	91 (43.3)	210 (24.4)	862 (100)	0.001
> 300.00	283 (43.4)	369 (56.6)	652 (75.6)		
Parents health information level					
uninformed (low)	388 (64.9)	210 (35.1)	598 (69.4)	862 (100)	0.000
well informed (high)	14 (5.3)	250 (94.7)	264 (30.6)		
Parents oral status					
well	119 (26.9)	323 (73.1)	442 (51.3)	862 (100)	0.000
poor or don't	283 (67.4)	137 (32.6)	420 (48.7)		

***χ^2 – test with $p < 0.05$ as a level of statistical significance value.**

who were uninformed about oral health (96.5%, $p = 0.000$). Moreover, the children of the parents who had poor oral health were also at higher risk for ECC (70.4%, $p = 0.000$). The higher disease frequency (Table 3) was noticed in children with poor oral hygiene ($p = 0.012$) who did not use fluoride toothpaste ($p = 0.000$) and fluoride tablets (p=0.000). Children with oral bad habits (mouth breathing, sucking thumb) or children who were used sweet medical syrups more than 5 times *per* year, had also higher ECC prevalence ($p = 0.000$). Logistic regression model (Forward Stepwise Method) showed (Table 4) that those children who use sweets between meals, in comparison to those who did not, were more likely to have ECC (OR = 181.16; 95% CI = 84.29–389.34), as well as those who use medical syrups more than five times a year in comparison to those who never use medicines (OR = 8.08; 95% CI = 3.78–17.27), among parents with poor oral status (OR = 3.09; 95% CI = 1.65–5.79) and low health informed parents (OR = 217.57; 95% CI = 84.46–560.50).

Discussion

This study analyzed the prevalence of ECC and its relationship with socio-behavioural factors as the risk factors for disease presence. ECC prevalence in Vojvodina of 46.64%, was in the range of moderate values of prevalence compared to Sweden [10]; low prevalence-11.4%) and the recorded higher prevalence in children from Southwest China [8], (85%), and in Canada [15] (high prevalence -98%). Studies from neighborhood countries [16] stated that 40.29% of the children 25–71 months old had ECC and those values were similar with our study. Our research showed that every child had on average 1.81 (dmft) deciduous teeth with caries [(i.e. 3.68 decayed tooth surfaces (dmfs)] and it was in agreement with results reported by Al-Mendalawi and Karan [17] (2.03 ± 1.39) and Borges et al. [18]. Our findings also correlate with those of Anitha et al. [19] who revealed DMFS ≥ 5 in three-year-old children in India.

Table 3

Early childhood caries (ECC) prevalence according to child's oral hygiene habits and use of medical syrups

Parameter	Children with ECC n (%)	Caries free children n (%)	Subtotal n (%)	Total n (%)	*p**
Oral hygiene					
yes from birth	377 (45.8)	447 (54.2)	824 (95.6)	862 (100)	0.012
no	25 (65.8)	13 (34.2)	38 (4.4)		
Use of fluoride toothpaste					
yes	257 (40.5)	377 (59.5)	634 (73.5)	862 (100)	0.000
no	145 (63.6)	83 (36.4)	228 (26.5)		
Use of fluoride supplements					
yes	19 (16.4)	97 (83.6)	116 (13.5)	862 (100)	0.000
no	383 (51.3)	363 (48.7)	746 (86.5)		
Oral bad habits☻					
no	213 (40.2)	317 (59.8)	530 (61.5)	862 (100)	0.000
yes	189 (56.9)	143 (43.1)	332 (38.5)		
Use of medical syrup					
never	214 (34.2)	411 (65.8)	625 (72.5)	862 (100)	0.000
more than five times a year	188 (79.3)	49 (20.7)	237 (27.5)		

***χ^2 – test with $p < 0.05$ as a level of statistical significance value.**

Table 4

Risk factors for early childhood caries (ECC)

Risk factors for ECC	S.E.	Exp(B)	95% CI for Exp(B)		*p*
			lower	upper	
Step 1					
use of sweets between meals	0.215	39.258	25.738	59.881	0.000
Step 2					
use of sweets between meals	0.372	223.817	107.996	463.852	0.000
health information level	0.428	241.585	104.466	558.679	0.000
Step 3					
use of sweets between meals	0.379	172.872	82.273	363.237	0.000
use of medical syrup*	0.371	7.790	3.767	16.110	0.000
health information level	0.465	293.828	118.054	731.317	0.000
Step 4					
use of sweets between meals*	0.390	181.156	84.289	389.344	0.000
use of medical syrup	0.388	8.076	3.776	17.273	0.000
parents oral status	0.320	3.092	1.652	5.788	0.000
parents health information level	0.483	217.573	84.457	560.500	0.000

Ordered logistic regression (n = 862).

***use of medical syrups more than five times a year.**

ECC appeared to be more frequent in male children and this is similar to the results of Abu Hamila [20]. The rationale for the gender difference is unclear but it has been reported that male children have 13 times greater risk of caries development [21] and the possible earlier "vertical" and "horizontal" transmission of mutant streptococci (MS) from mothers to male children [22]. This gender gap might result from genetic, hormonal and cultural influences [23]. Moreover, in our study, the third and every next born child in a family have a higher risk for ECC as confirmed by Prakash et al. [24] study. It is an interesting assumption that was pointed out in Congiu et al. [25] study concerning the families with more children that the "available" free time that parents have to spend with each child was reduced. This may be especially true for working mothers and it may play a role in the caries development in children with siblings.

AAPD declared that breastfeeding and bottle-feeding are a potentially risk factors for ECC [3]. In our study caries prevalence was significantly lower in toddlers who was exclusively breastfed 6–12 months ($p = 0.000$). The children who were bottle-fed from birth or who were breastfed after 12 months of life had more frequently ECC. Recent research by Bahuguna et al. [26] confirmed significantly higher percentage of children developed ECC on having prolonged breastfeeding, bottle feeding, nocturnal bottle feeding containing sweet drink and milk and higher frequency of consumption of sweets. A systematic review of the epidemiological evidence suggests that prolonged breastfeeding after the first birthday may be associated with an increased prevalence of caries. When breast milk is consumed several times during the day and at night it can be linked with ECC [27]. It can be explained by the accumulation of milk residues contained lactose that is a perfect substrate for MS, especially during the night, when the salivary flow rate is reduced. Infant feeding practices greatly influence a child's risk of developing ECC.

In our study, we found that additional sweeten of food and use of sweets between meals can be the significant factors for ECC development and that was also confirmed by Majorana et al. [27]. It is notable that sugary foods and beverages in early childhood are known to lead to the establishment of a habit that persists for a long time [28]. The most important fact is a high-frequency intake of sugary food and drinks, as well as sweetened feeding bottles, particularly at night time instead of quantity of sugar intake [18]. In our study, we noticed that children who used additionally sweetened food had two times more caries compared to other children. Children who used sweets between meals had four times frequently caries in relation to the children who never used them or took sweets with meals.

The evaluation of the results of our study (Table 2) clearly indicates that there was a direct correlation among parents education level, their working status, family income *per* month and ECC prevalence. Those results were also confirmed in other studies [6–8, 10, 18, 20]. ECC frequency in the children of highly educated parents was considerably reduced compared to the children whose parents had elementary or no education at all, as confirmed by Schroth et al. [15] and Borges et al. [18]. Children from working parents had less ECC comparing to the children from unemployed parents. This can be explained by higher education level and better health information level of the employed parents and with the fact that their children more often stay in kindergarten were less ECC prevalence was recorded [2]. Considering the fact that the existing network of educational institutions in Vojvodina is not adjusted to the population demographic, socioeconomic or educational needs, it is quite possible to expect a continued increase of ECC. The influence of parent's socioeconomic status and their oral health on the ECC prevalence was also notable in our study. The children had more frequently ECC if their parents had less monthly income, which is in accordance with the study of Prakash et al. [24] and Borges et al. [18]. They found the linear increase of ECC prevalence and severity with decreasing of parents' annual income. The wealthy parents have more ability to pay for qualitative and quantitative balanced food, fluoride supplements and better oral hygiene for their children who then less socioeconomic status. The parents of low socioeconomic status gave their children the poor quality of food rich in carbohydrates and they didn't have enough money to pay for quality oral hygiene devices and because of the limited income, they couldn't reach adequate dental service. In the Western world, the ECC prevalence at 3 years of age was 19.9%, and strong associations were found with low socioeconomic status and ethnicity [29]. In contrast, a study involving Chinese children reports an association between dental caries and a higher monthly income [30]. Low socioeconomic status of the parents was often connected with their low health information level and that was significant risk factor for ECC in our study. Parents of young children receive very little oral health preventive information from non-dental health care providers. Advising first-time pregnant mothers on the prevention of ECC, decreased disease prevalence at 20 months of child's age 5-fold [31]. In the present study, it was found that low education level of parents was significantly related to the occurrence of caries, which is consistent with other studies [32]. Epidemiological research in Iraq also pointed out that parental education level was found to be a risk factor significantly associated with ECC ($p < 0.01$). Educated parents have better health knowledge and positive attitudes toward oral health, including ECC. Hence, they have children's sound dentition. Oral hygiene habits and dietary habits established during preschool days and parents, particularly the mothers, can function as role models for their children [17]. It can be concluded that low socioeconomic status and poor parental knowledge of how diet affects their children's teeth also contribute to this growing problem [3].

The possible correlation between parental education level and their annual income in relation to ECC occurrence in Vojvodina will probably provide some further investigation especially because the level of education and the working status of the young parents in Vojvodina are not always in direct correlation with their monthly income. Parent's behavior is correlated with children's oral health. The toothbrushing and dietary habits of the mother are associated directly with those of her child. Children's dietary habits vary

according to their mothers' educational level, resulting in low-income families consuming diets higher in added sugars than diets of higher income families [32]. Cariogenic bacteria, such as MS and *Lactobacilli* (LB), are typically transmitted from the mother to her child by behaviors that directly pass saliva, such as sharing a spoon when tasting a baby food, cleaning a dropped pacifier by mouth, or wiping the baby's mouth with saliva. Our study showed two times more ECC prevalence in children from parents who had bad oral health. Children with a history of dental caries, whose primary caregiver or siblings have severe dental caries, are regarded as being at increased risk for the disease [33]. Vertical transmission of MS from caregiver to a child has been reported [34]. The major reservoir of MS is the mother, from whom the child acquires it during a window period of around 2 years of age. At this time, the child is probably most susceptible to acquiring MS. Successful infant colonization of maternally transmitted MS may be related to several factors, which include the magnitude of the inoculum, the frequency of small-dose inoculations, and the minimum infective dose. Mothers with dense salivary reservoirs of MS are at high risk of infecting their infants very early in life [35]. Thus, poor maternal oral hygiene and higher daily frequencies of snacking and sugar exposure increase the likelihood of transmission of the infection from mother to child. In addition to maternal transmission of MS, the father-to-child transmission has been studied. Horizontal transmission was also examined; transmission of microbes may occur between members of a group (e.g., siblings, toddlers at a nursery).

Many studies have indicated that ECC is largely preventable by good oral hygiene of parents and children and proper eating habits instituted by parents early on [31]. It is better if prevention of ECC begins in the prenatal and after birth periods and addresses the health of both the mother and the infant, so the mother's or caregiver's teeth should be examined. Infants whose mothers have high levels of MS due to untreated dental decay are at greater risk of acquiring cariogenic microorganisms. Better oral health education of the mother can delay infant inoculation [36]. The strategy to battle the early MS transmission from parents to their child is often named primary-primary prevention. The preventive intervention is most often directed to pregnant women and/or mothers of newborn babies. The goal is to prevent or delay children as long as possible from acquiring the bacteria that cause tooth decay. Reduce the bacteria in the mouth of the mother could be possible by use of chlorhexidine digluconate in the form of mouth rinses, gels, and dentifrices [37]. Early screening for signs of dental caries development, starting from about 7–8 months of age, could identify infants who are at risk of developing ECC, assist in providing information for parents about how to promote oral health and prevent the development of tooth caries. High-risk infants include those with early signs of ECC, poor oral hygiene, limited exposure to fluoride, and frequent exposure to sweet beverages. These infants should be targeted with a professional preventive program that includes oral hygiene instructions for the parents and child, fluoride use, and diet counseling [36].

Our study showed three times higher frequency of ECC in children who did not maintain the proper oral hygiene and two times higher ECC prevalence in children who never used fluoride toothpaste as well as other fluoride supplements. On oral hygiene significance as the risk factor for ECC development was also pointed out in the research of Al-Mendalawi and Karam [17], who emphasized that there was a significantly higher statistical correlation between tooth brushing frequency and ECC. There was a significantly higher statistical correlation between tooth brushing frequency and ECC ($p < 0.001$). This augments the notion that low frequency and improper tooth brushing methods are closely associated with ECC [17].

Development of oral hygiene habits may be sensitive to the economic environment in which children live. Such environmental factors include caregivers' social status, poverty, ethnicity, deprivation, the number of years of education, and dental insurance coverage. There are numerous studies that support the benefits associated community water fluoridation [38]. Research from Kavvadia et al. [13] of the children from 2–6 years old revealed the high ECC prevalence as the result of use the cariogenic diet (83%) and the fact that 17% of the children did not use any form of fluoride. Moreover, less than satisfactory oral hygiene was recorded in 67% of all children and 23% displayed poor oral hygiene. Thus, emphasizing daily supervised toothbrushing with fluoridated toothpaste is of great importance for the youngest individuals residing in a non-fluoridated area and without access to regular dental care. The exposure to fluoride provides an important protective factor against dental caries. It inhibits demineralization and drives remineralization by incorporating into the enamel crystals at the tooth surface as fluoride apatite. Fluoride enhances remineralization of enamel by attaching to the surface and absorbing calcium and phosphate ions from the saliva. In high concentration, it also inhibits the plaque bacteria's metabolism, therefore decreasing acid production [39]. Fluoride varnish works by increasing the concentration of fluoride in the outer surface of teeth, thereby enhancing fluoride uptake during early stages of demineralization [37].

Sweetened medicine usage is another important risk factor in the ECC development. It was noted the statistically significant difference in ECC frequency in children who took medical syrups in relation to the others who never used them. Our research showed more than two times higher ECC prevalence in children who took five times *per* year sweet medical syrups ($p < 0.01$). In relation to this, Ölmezz and Uzamris [40] pointed out that irregular and self-initiative usage of medicine can be the significant predictor of ECC. This paradoxical situation where medical syrups treated well some illness and at the same time increase the risk for ECC, impose at the first place need for further better cooperation between physicians and dentists. Furthermore, the whole community together with all health care providers will be needed to try to reach somehow policy makers and to provide the regulations of the medical syrups manufacture by the law. In relation to this, it will be of great importance that pharmaceutical industries in their production assortment of all medical syrups substitute sucrose with the sanitary safe sugar substitutes which will not have at the same time the si-

de effects. The Recent publication of Alaki et al. [41] noted that children who used systemic antibiotics during the first year of life had a significantly greater risk of ECC compared with children who did not use antibiotics.

Logistic regression model in our study showed that those who use sweets between meals, in comparison to those who did not, are more likely to have ECC (OR = 181,16; 95% CI = 84.29–389.34), as well as those who use medical syrups more than five times a year in comparison to those who never use that (OR = 8.08; 95% CI = 3.78–17.27), among parents with poor oral status (OR = 3.09; 95% CI = 1.65–5.79) and low health informed parents (OR = 217.57; 95% CI = 84.46–560.50), which is in accordance with the similar research [18, 33, 36]. Sex and age of the child, age of the parents, child order in family, parents education level and working status, family income *per* month, child weight at birth, nursing and bottle feeding, additional sweeten of food, use of fluoride toothpaste and tablets and oral bad habits etc. did not show statistical significance in applied statistical model. High confidence level values in statistical model used, refer on two among four variables (in the model which compared 20 variables) – "regular use of sweets between meals" and "low health informed parents"– can be explained with a probability of unexplored confounders. Those "hidden factors" should be more investigated in further research.

Conclusion

ECC is a serious widespread health problem in Vojvodina with increasing trend rate. We found a strong association between certain sociobehavioural risk factors like low parental health information level and their poor oral health status on ECC prevalence. The use of sweets between meals as a common bad habit in general population has also a significant influence on disease development along with the use of sweet medical syrups more than five times *per* year. There is an urgent need for changes in primary health care activities particularly in parental prenatal and postnatal counseling, addressing at the first place mothers awareness that their better oral hygiene, knowledge and positive attitudes toward oral health could significantly improve child oral health and decrease ECC prevalence.

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Patients' general satisfaction with the appearance of anterior maxillary teeth

Zadovoljstvo pacijenata izgledom prednjih gornjih zuba

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Abstract

Background/Aim. Dental appearance plays an important role in practically all personal social interactions. The main factors that define the dental appearance are tooth colour, shape and position, quality of restoration, and the general position of the teeth in arch, especially in the anterior region. The aim of this study was to evaluate the impact of dental status (tooth shape, fracture, dental and prosthetic restorations and presence of plaque) on patient's satisfaction with the dental appearance, controlling for the age and gender. **Methods**. A total of 700 Caucasian subjects (439 women) aged 18–86 (median 45 years) participated in the cross-sectional study. Study included clinical examination and self-administrated questionnaire based on self-perceived aesthetics and satisfaction with the appearance of their maxillary anterior teeth. **Results.** A regression analysis demonstrated that presence of dental plaque, tooth fracture, composite fillings and crowns had significant independent contribution and were negative predictors of satisfaction with teeth appearance. Participants with presence of plaque on upper teeth ($p < 0.001$), fractures ($p = 0.005$), composite fillings ($p < 0.001$) and crowns ($p = 0.032$) were less satisfied than those without it. Model explains 12% or variance of general satisfaction with the appearance of maxillary frontal teeth ($p < 0.001$) and the major contributors are composite fillings (5.3%) and plaque (3.2%). Tooth shape, age and gender were not significant predictors of satisfaction. **Conclusion.** Satisfaction with the teeth appearance is under the influence of many factors with significant negative influence of presence of dental plaque, fractures, composite restorations, and crowns.

Key words:
esthetics, dental; patient satisfaction; surveys and questionnaires; age factors; dental plaque; dental restoration, permanent.

Apstrakt

Uvod/Cilj. Izgled zuba igra važnu ulogu u praktički svim kontaktima jedne osobe. Glavni faktori koji definišu izgled zuba su boja, oblik i pozicija zuba, kvalitet restauracije i položaj zuba u zubnom luku, posebno u prednjem delu. Cilj ovog rada bio je da se utvrdi uticaj životnog doba, pola, frakture zuba i plak indeksa na zadovoljstvo pacijenta izgledom zuba. **Metode.** U istraživanju je učestvovalo 700 ispitanika u životnom dobu 18–86 godina (medijana 45 godina). Istraživanje je bilo bazirano na kliničkom pregledu i ispunjavanju upitnika koji je uključivao pitanja bazirana na samoproceni zadovoljstva izgledom gornjih prednjih zuba. **Rezultati.** Regresijska analiza je pokazala da dentalni plak, frakture zuba, kompozitni ispuni i krunice imaju statistički značajan uticaj i da su negativni prediktori zadovoljstva izgledom zuba. Ispitanici s plakom na gornjim prednjim zubima ($p < 0,001$), frakturom ($p = 0,005$), kompozitnim ispunima ($p < 0,001$) i krunicama ($p = 0,032$) bili su manje zadovoljni od ispitanika bez njih. Model objašnjava 12% varijanse generalnog zadovoljstva izgledom gornjih prednjih zuba ($p < 0,001$) kao i najveći učinak imaju kompozitni ispuni (5,3%) i plak (3,2%). Oblik zuba, životno doba i pol nisu bili značajni prediktori zadovoljstva. **Zaključak.** Zadovoljstvo izgledom zuba je pod uticajem mnogih faktora, a negativan uticaj imaju prisustvo zubnog plaka, fraktura, kompozitnih ispuna i krunica.

Ključne reči:
estetika, stomatološka; bolesnik, zadovoljstvo; ankete i upitnici; životno doba, faktor; zub, plak; zub, trajni ispuni.

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Introduction

In the past, restorative dentistry considered mostly functional demands, but with the decrease in caries prevalence, interest in dental aesthetics has increased rapidly among both patients and dentists [1–4]. Nowadays, public appearance plays an extremely important role in both advertising industry and media in general since it affects other people's perception in numerous daily situations [1, 5]. Therefore in the last two decades esthetics in dental practice has become just as important as functional, structural and biological characteristics.

Dental appearance is a leading feature in determining the overall attractiveness of one's face, thus playing an important role in practically all personal social interactions. Principal factors which define the dental appearance are tooth colour, its shape and position, quality of restoration, and the general positioning of the teeth in arch (crowding, diastemas), which is especially important in the anterior region [6, 7]. The overall appearance of the dentition may be influenced by gender, age and education level. Moreover, gender-related differences play quite a significant role in aesthetic dentistry, since it has been demonstrated that women and men seem to have different approaches and needs in their pursuit of a more favorable dental appearance [8, 9]. Consequently, it is very difficult to address individual needs with specific guidelines or a unique systematic approach that will undoubtedly lead to consistent results [10, 11].

In general, patients want white Hollywood teeth. Thus, tooth colour is absolutely one of the most important factors determining patient satisfaction with their smile [1, 6, 7, 12, 13]. In fact, bright teeth have been related to high social skills, intelligence, prestige, ability to balance conflicting needs, and relationship status [14]. Alternatively, untreated dental caries, discoloured front teeth restorations and missing teeth in the anterior region are sources of displeasure and lack of satisfaction [1, 15–17].

Malocclusion is a common oral finding. Regardless of its high frequency, treatment needs and demands vary depending on cultural and personal differences. In some populations, tooth misalignments are not regarded as serious defects which would necessitate treatment, either orthodontic or prosthetic [18] while, in other populations, with high standards of dental appearance the need for orthodontic treatment may similarly be quite pronounced [19]. There is a general agreement in the literature that people who are motivated to seek orthodontic treatment of malocclusion do so prompted by its negative physical, psychological and social impacts. However, the studies focusing on the effects of malocclusion and consequences of its treatment on people's lives have offered inconsistent and confusing results [20].

As mentioned before, the harmonious smile is defined not only by the dental esthetic elements – shape, position, and color of the teeth – but also by the gingival (soft) tissues. Gingival health defined by colour, margins and visibility is the essential component of an attractive smile [21].

Since the aesthetics is an important dimension in dental practice, and it is a result of a pleasing composition of many elements modified by individual preferences, cultural influences, sociodemographic factors and self-perceived need for dental treatments, the aim of this study was to determine the predictors of patients' satisfaction with esthetic appearance of their maxillary anterior teeth.

Methods

A total of 700 Caucasian subjects from Rijeka region, Croatia, (aged 18-86; median 45 years) participated in the cross-sectional study (439 women). Sampling procedure included convenient sample – consecutive voluntary blood donors at the Department of Transfusion Medicine University Hospital Rijeka, subjects at regular annual check-ups at the Institute for Public Health Rijeka, and patients seeking treatment at the University Dental Clinic Rijeka. All the participants included in the study gave written informed consent to the survey procedures, which were approved by the Ethical Committee of the Rijeka University Faculty of Medicine.

The inclusion criteria were individuals with all six anterior teeth present in the upper jaw; while exclusion criteria were: evidence of gingival inflammation or hyperplasia, observable gingival recession, observable occlusal wear, active orthodontic therapy by edgewise appliances, temporary crowns in prosthetic rehabilitation, progressive endodontic therapy, usage of splints for the treatment of temporomandibular disorders and participants with craniofacial syndromes.

Study included clinical examination and Aesthetic Questionnaire. The Aesthetic Questionnaire was self-administrated and comprised five questions related to satisfaction with dental appearance in general (tooth colour, shape, position in a dental arch and appearance of gingiva of maxillary anterior teeth). Assessments were made using a three-point scale with possible answers 'dissatisfied' = 1, 'moderately satisfied' = 2, or 'completely satisfied' = 3.

To test psychometric properties of this five-item Aesthetic Questionnaire the preliminary investigation was conducted which included 70 individuals who answered the 5 questions from the Questionnaire. Plaque index was determined using the method described by O'Leary et al. [22] in 1972. Plaque levels were assessed on four tooth surfaces. Presence or absence of plaque was noted with „+“ and „- “.

In univariate analyses subjects were divided into 3 age groups: young age < 35 years; middle aged, 35–54 and old, ≥ 55 years. To examine the differences in several aspects of dental satisfaction in respect to gender a series of χ^2-tests were performed within each age group ($p < 0.05$). The multiple linear regression analysis was made to evaluate influence of presence of plaque, tooth shape (1 = ovoid; 2 = triangle and 3 = quadratic), fracture (0 = absent; 1 = present), composite fillings and crowns on maxillary anterior teeth on satisfaction with dental appearance, while controlling for the gender and age. For this purpose the variable plaque was introduced as a dichotomous variable: 0 = subject with no plaque or 1 = subject with plaque present on at least one tooth. Similar was done for presence of fractures, fillings and crowns.

The data were analyzed using a statistical software package SPSS 10.0 (SPSS 10.0; SPSS Inc., Chicago, IL, USA).

Results

In the young age group most of the participants were moderately satisfied with the appearance and most of them were completely satisfied with colour, shape, position and gingiva of their teeth. As presented in the Table 1, there were no significant differences in gender in observed variables of dental satisfaction.

Participants from the middle age group were mostly completely satisfied with the appearance, colour, shape, position and gingiva. However, several gender differences in satisfaction were obtained. Men and women from middle age group had a significantly different appraisal of satisfaction with the shape ($\chi^2 = 10.175$, $p = 0.006$) of their teeth with 61.4% of women being completely satisfied with the shape compared to 39.7% of men, and with 28.6% men dissatisfied with the shape compared to 12.6% of women (Table 2.) Also, there was a significant difference in satisfaction with the position of teeth between men and women in the middle age group ($\chi^2 = 5.961$, $p = 0.05$).

Table 1

Results of χ^2-test for satisfaction with different dental aspects between men (n = 79) and women (n = 180) in the young group (< 35 years)

Variable	Dissatisfied	Moderately	Completely satisfied	χ^2	df	*p*
	n (%)	n (%)	n (%)			
Appearance				0.963	2	0.618
male	17 (33.3)	31 (39.2)	31 (39.2)			
female	34 (19)	83 (45.8)	63 (35.2)			
total	51 (19.8)	114 (43.8)	94 (36.4)			
Colour				1.373	2	0.503
male	12 (15.2)	29 (36.7)	38 (48.1)			
female	37 (20.6)	68 (37.8)	75 (41.7)			
total	49 (18.9)	97 (37.5)	113 (43.6)			
Shape				1.552	2	0.460
male	11 (13.9)	24 (30.4)	38 (48.1)			
female	19 (10.6)	68 (37.8)	75 (41.7)			
total	30 (11.6)	92 (35.5)	113 (43.6)			
Position				2.247	2	0.325
male	16 (20.3)	19 (24.1)	44 (55.7)			
female	31 (17.2)	60 (33.3)	89 (49.4)			
total	47 (18.1)	79 (30.5)	133 (51.4)			
Gingiva				1.096	2	0.578
male	8 (10.1)	19 (24.1)	19 (24.1)			
female	26 (14.4)	37 (20.6)	3 (20.6)			
total	34 (13.1)	56 (21.6)	56 (21.6)			

Note: % within the gender.

Table 2

Results of χ^2-test for satisfaction with different dental aspects between men (n = 63) and women (n = 127) in the middle age group (36–54 years)

Variable	Dissatisfied	Moderately	Completely satisfied	χ^2	df	*p*
	n (%)	n (%)	n (%)			
Appearance				5.602	2	0.061
male	19 (30.2)	26 (41.3)	18 (28.6)			
female	28 (22)	40 (31.5)	59 (46.5)			
total	47 (24.7)	66 (34.7)	77 (40.5)			
Colour				5.543	2	0.063
male	15 (23.8)	28 (44.4)	20 (31.7)			
female	24 (18.9)	40 (31.5)	63 (49.6)			
total	68 (35.8)	68 (35.8)	83 (43.7)			
Shape				10.175	2	0.006
male	18 (28.6)	20 (31.7)	25 (39.7)			
female	16 (12.6)	33 (26)	78 (61.4)			
total	34 (17.9)	53 (27.9)	103 (54.2)			
Position				5.961	2	0.051
male	18 (28.6)	19 (30.2)	26 (41.3)			
female	23 (18.1)	28 (22)	76 (59.8)			
total	41 (21.6)	47 (24.7)	102 (53.7)			
Gingiva				3.564	2	0.168
male	14 (22.2)	15 (23.8)	34 (54)			
female	18 (14.2)	23 (18.1)	86 (67.7)			
total	32 (16.8)	38 (20)	120 (63.2)			

Note: % within the gender.

Again, women were more frequently completely satisfied with the position of their teeth compared to men, while men were more frequently dissatisfied with the position compared to women (Table 2).

As presented in Table 3, participants from the old age group were mostly completely satisfied with the appearance, shape, position, and gingiva and moderately satisfied with colour of their teeth. There were no significant gender differences in dental satisfaction in this age group (Table 3).

Table 3

Results of χ^2-test for satisfaction with different dental aspects between men (n = 119) and women (n = 132) in the old age group (> 55 years)

Variable	Dissatisfied	Moderately	Completely satisfied	χ^2	df	*p*
	n (%)	n (%)	n (%)			
Appearance				1.024	2	0.599
male	27 (22.7)	41 (34.5)	51 (42.9)			
female	25 (18.9)	53 (40.2)	54 (40.9)			
total	52 (20.7)	94 (37.5)	105 (41.8)			
Colour				.378	2	0.828
male	31 (26.1)	46 (38.7)	42 (35.3)			
female	30 (22.7)	53 (40.2)	49 (37.1)			
total	61 (24.3)	99 (39.4)	91 (36.3)			
Shape				4.076	2	0.130
male	12 (10.1)	48 (40.3)	59 (49.6)			
female	25 (18.9)	45 (34.1)	62 (47)			
total	37 (14.7)	93 (37.1)	121 (48.2)			
Position				3.800	2	0.150
male	15 (12.6)	44 (37)	60 (50.4)			
female	29 (22)	44 (33.3)	59 (44.7)			
total	44 (17.5)	88 (35.1)	119 (47.4)			
Gingiva				.136	2	0.934
male	21 (17.6)	29 (24.4)	69 (58)			
female	21 (15.9)	33 (25)	78 (59.1)			
total	42 (16.7)	62 (24.7)	147 (58.6)			

Note: % within the gender

In order to further examine dental satisfaction, a regression analyses were performed with dominant shape, dominant plaque, at least one fracture on upper incisors, at least one composite fillings on upper incisors, at least one crowns on upper incisors, age, and gender while dependent variable was satisfaction with dental appearance expressed as the average score on the Aesthetic Questionnaire (Table 4). The results showed that the model explains 12% of variance of dental satisfaction. Dental plaque, tooth fracture, composite fillings and crowns have significant independent contribution and are negative predictors. Participants with presence of plaque on upper teeth, fractures, composite fillings and crowns were less satisfied than those without it. The major contributors were composite fillings (5.3%) and plaque index on upper incisors (3.2%).

Discussion

Numerous factors are influencing satisfaction with its-own dental esthetics and this study may help dentists to pay increased attention to the factors of patients' concern. Some factors are to some extent interrelated, complemented, share the same variance, or reduce the effects of the other factor. Therefore they should be explored simultaneously in a broader model. Multiple regression provide us this opportunity. This is demonstrated in present study. Although there is a different satisfaction with teeth appearance between genders in some age groups in univariate models, in general gender and age are not principal, significant or even highly influential factors of satisfaction in multiple model. Dental appearance is adversely affected by abnormalities and deviations in the oral region [12]. In this study we investigated satisfaction with dental appearance in relation to age, gender, presence/absence of composite fillings and crowns, plaque index and tooth fracture.

Table 4

Regression analyses for dental satisfaction

Parameter	Beta	*p*
Dominant shape	0.046	0.197
Dominant plaque	-0.183	0.000
Fracture	-0.101	0.005
Composite filling	-0.247	0.000
Crowns	-0.083	0.032
Age	0.257	0.797
Gender	0.489	0.625
	$R^2 = 0.122$	
	F = 13.747	
	p = 0.000	

Limited number of documented literature was present on influence of different factors on satisfaction in Croatian population. This study would help subjects to show their satisfaction with dental appearance and it may help dentists to pay increased attention to the factors of patient concern.

Gender and age

Contrary to our findings some evidence exist that apart from visible dental features, perception of dental appearance

is modified by cultural factors and individual preferences, varying between individuals and cultures and changing over time and with age [5, 6, 23–25]. It appears that men regard dental appearance as more important than women [24], but females tend to be more satisfied with the general appearance of their teeth [1]. Results of our study showed that in the age group between 36 and 54 years, men are significantly less satisfied with the dental appearance, tooth shape and tooth position and achieved a significantly lower score on the average satisfaction of teeth in relation to women. This is likely due to strong impact of the media which portray men and women of all ages as needing to look younger and more attractive. Indeed, a study of 160 people of six different age strata ranging from 13 to 64 years showed that personal satisfaction with tooth colour was age-independent [12]. Although younger usually have healthier teeth, and less frontal restoration and discolorations they may be more concerned with slight imperfections. On contrary older people, in general, are more likely to be satisfied with their dental appearance [12, 15], suggesting that the appearance of their teeth is not as important to older than to younger individuals [5].

Visible dental features

Dental appearance is adversely affected by abnormalities and deviations in the oral region [12] so those visible traits must be primarily sources of dissatisfaction. Our model included and investigated several dominant dental traits – presence/absence of composite fillings and crowns, tooth shape, presence of plaque and tooth fracture. As it is demonstrated the presence of composite fillings and plaque on maxillary anterior teeth are major factors influencing dissatisfaction with dental aesthetics, but also not to a large extent.

Restorations – fillings and crowns

Composite fillings are the most common restorations in maxillary anterior teeth because of their low price in comparison to the prosthetic restorations. Although composite restorations can be completed in a single treatment session with no added laboratory cost, this material is presently limited by several restrictions – inability to completely replicate natural tooth in colour and changes induced during time due to polymerization-induced shrinkage, low wear resistance and surface porosity, which may influence the patient's level of satisfaction [26]. Those limitations of composite materials may contribute to poorer aesthetics in time and induce decrease of satisfaction with dental appearance. Therefore these materials need to improve their performances.

Younger people and women tend to expose more maxillary teeth than older and men [24, 25]. The shortcoming of present study was that it did not assess the quality of restorations as an element that could influence satisfaction. Still, probably people sometimes think that when they have some dental restoration their dental appearance is altered or less natural and they are to some extent dissatisfied. Dental appearance may influence social interactions and contribute to social selection [25]. It also may reflect economic status [27]. Wealthier people, even with worse oral health, are likely to have better frontal restoration, brighter and straighter teeth and a higher red-white esthetics. Perhaps that is why the presence of composite restorations, even well-made, may be a higher source of dissatisfaction than presence of fixed prosthetic restorations.

Plaque

Satisfaction with teeth appearance may reflect general attitude on health, particularly oral health. That explains why presence of plaque is regarded second-order factor in dissatisfaction. The presence of gingivitis was not assessed in this study, but plaque index highly positively correlates with gingivitis [28]. Therefore it can be an indicator of periodontal health and oral health care. Quite expectedly, the increase of the degree of plaque index reduces the assessment of satisfaction with dental appearance. The participants who had a higher degree of plaque index reported a lower level of satisfaction with dental appearance. The plaque index was a statistically significant negative predictor of general satisfaction with their teeth ($p < 0.001$).

Shape

Tooth shape does not significantly influence satisfaction with teeth appearance. Still, it appears that incisor shape may be the key determinant of their esthetic preferences with round incisors perceived as the most esthetic [29]. So to improve smile esthetics some mildly rounding the mesial and distal corners of square incisors can be done [30, 31].

Many authors agree that the upper central incisors in particular are the key determinants in evaluating anterior dental aesthetics [24, 26, 27, 31]. This can be taken as implying that these teeth probably play the subconsciously important role in people's judgements concerning dental aesthetics. Maxillary teeth are often the most visible during smile, although this feature is age- and gender related.

Conclusion

This study revealed that composite fillings and a presence of dental plaque in the anterior maxillary teeth are negative predictors to a self-perceived dental appearance.

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Complex modulation of fingertip forces during precision grasp and lift after theta burst stimulation over the dorsal premotor cortex

Kompleksna modulacija sila tokom preciznog hvata šake primenom ponavljane transkranijalne magnetne stimulacije pražnjenjima u teta frekvenciji iznad dorzalnog premotornog korteksa

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Abstract

Background/Aim. Adaptive control and fingertip force synchronization of precise grasp stability during unimanual manipulation of small objects represents an illustrative example of highly fractionated movements that are foundation of fine motor control. It is assumed that this process is controlled by several motor areas of the frontal lobe, particularly applicable to the primary motor (M-1) and dorsal premotor cortex (PMd). Aiming to examine the role of PMd during fine coordination of fingertip forces we applied theta burst repetitive magnetic stimulation (TBS) to disrupt neural processing in that cortical area. **Methods.** Using a single-blind, randomized, crossover design, 10 healthy subjects (29 ± 3.9 years) received single sessions of continuous TBS (cTBS600), intermittent TBS (iTBS600), or sham stimulation, separate from one another at least one week, over the PMd region of dominant hemisphere. Precision grasp and lift were assessed by instrumented device, recording grip (G) and load (L) forces, during three manipulation tasks (ramp-and-hold, oscillation force producing and simple lifting tasks), with each hand separately, before and after interventions. **Results.** We observed the improvement of task performance related to constant error (CE) in oscillation task with the dominant hand (DH) after the iTBS ($p = 0.009$). On the contrary, the cTBS reduced variable error (VE) for non-dominant hand (NH), $p = 0.005$. Considering force coordination we found that iTBS worsened variables for NH (G/L ratio, $p = 0.017$; cross-correlation of the G and L, $p = 0.047$; Gain, $p = 0.047$). **Conclusion.** These results demonstrate the ability of TBS to modulate fingertip forces during precision grasping and lifting, when applied over PMd. These findings support the role of PMd in human motor control and forces generation required to hold small objects stable in our hands.

Key words:
motor cortex; transcranial magnetic stimulation; hand strength.

Apstrakt

Uvod/Cilj. Adaptivna kontrola i sinhronizacija sila prstiju šake tokom preciznog hvata pri manipulisanju malim predmetima jednom rukom predstavlja ilustrativni primer visoko frakcionisanih pokreta koji predstavljaju temelj motorne kontrole preciznih pokreta. Pretpostavlja se da ovim procesom upravlja nekoliko motornih oblasti frontalnog režnja, i to prvenstveno primarni motorni (M-1) i dorzalni premotorni korteks (PMd). Cilj istraživanja bio je ispitivanje uloge PMd-a tokom vršenja pokreta koji zahtevaju finu koordinaciju sila prstiju šake. U istraživanju smo primenili ponavljanu magnetnu stimulaciju pražnjenjima u teta frekvenciji, kako bi ometali neuralno procesiranje u toj oblasti moždane kore. **Metode.** Primenom jednostrano slepe studije, uz nasumičnu raspodelu i ukršteni dizajn, 10 zdravih ispitanika (29 ± 3,9 godina) bilo je izloženo pojedinačnim sesijama kontinuirane magnetne stimulacije (cTBS600), ili intermitentne ponavljane magnetne stimulacije (iTBS600), pražnjenjima u teta frekvenciji kao i prividnoj stimulaciji iznad PMd regiona dominantne hemisfere, odvojenih međusobno, najkraće nedelju dana. Precizanost hvata šake i podizanja procenjivani su uređajem koji je registrovao silu stiska (G) i silu podizanja (L) prilikom izvođenja tri zadatka (zadatak sa zadatim profilom L, zadatak sa oscilatornim variranjem nivoa L i zadatak sa podizanjem), koji su izvođeni sa obe ruke odvojeno, i to pre i nakon svake intervencije. **Rezultati.** Nakon primene iTBS protokola zabeleženo je poboljšanje izvođenja iskazano konstantnom greškom (CE) u zadatku sa oscilatornim variranjem nivoa L, kada je izvođen dominantnom rukom (DH), $p = 0.009$. Suprotno to-

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me, primena cTBS protokola dovela je do smanjenja promenjive greške (VE) za nedominantnu ruku (NH), $p = 0.005$. Sa aspekta koordinacije sila utvrđeno je da je iTBS protokol doveo do pogoršanja rezultata praćenih pokazatelja za nedominantnu ruku (G/L odnos, $p = 0.017$; korelacija G i L, $p = 0.047$; prirast sile $p = 0.047$). **Zaključak.** Rezultati našeg istraživanja ukazuju na mogućnost modulacije sila prstiju šake tokom preciznog hvata i podizanja, ukoliko se TBS primeni iznad PMd-a. Dobijeni nalazi podržavaju ulogu PMd u motornoj kontroli i generisanju sila neophodnih za stabilno držanje malih predmeta kod ljudi.

Ključne reči:
motorna kora; transkranijalna magnetna stimulacija; ruka, snaga.

Introduction

The development of a skilled and sophisticated grasping technique represents one of the key evolutionary advantages of human beings comparing to subhuman primates [1]. Therefore, grasping is a subject of interest of many researchers, given the importance of precision grasping in the activities of daily life [2].

In order to evaluate these functions, different manipulandums have been developed, that serve to evaluate complex control over precision grip and coordination of grip and load forces applied to the object [3, 4].

Hand grip force and their coordination are controlled by the nervous system, so that a number of receptors (visual, mechanoreceptors, tactile receptors) passed through somatosensory afferents information about the mechanical characteristics of cases [3, 5] as well as change the path of movement, and through feedback [6, 7] and feed-forward mechanisms [8, 9] which regulate the process. However, in addition to afferent mechanisms of motor control, the precise modulation of grip and load force is provided by the activation of primary and non-primary motor areas. Despite the fact that the primary motor cortex (M-1) and its main output projection, the corticospinal tract, are considered as neural basis of hand dexterity, there are several non-primary motor areas (premotor, supplementary motor, and cingulate motor areas). These parts of the frontal lobe play a role in modulation of the output signal at the levels of the M-1 and spinal cord [10]. Most of the findings related to the role of non-primary motor areas are collected on the basis of cell recordings on monkeys [11]. However, the trains of magnetic pulses, repetitive transcranial magnetic stimulation (rTMS) applied over intact scalp, provide the new tool to investigate modulation of motor output with humans awake, on safe and painless way. Because the effects of rTMS extend beyond the period of stimulation, there is a possibility to modulate cortical plasticity. In the case of creation of so-called virtual lesions of restricted brain areas, trains of TMS pulses temporarily interfere with neural processing while the subject is performing behavioral tasks. Through rTMS, there are different possibilities of modulation functions at the very site of stimulation, but also on other distal sites producing a disinhibition through the synaptic connections [12].

Contrary, to extensively study the role of M-1 and the corticospinal projection in control of skilled hand movements, the role of premotor cortex in this function is less known. The success of the skilled manipulation of objects with hand depends on setting hand grip before the object is reached, requiring cooperation of visuo-motor and sensory-motor loops, the kind of transformation that takes place within the parieto-frontal connections, including the M-1, but not least ventral premotor cortex (PMv) [13, 14]. It has been shown that the function of premotor dorsal cortex (PMd) in monkeys refers to the planning and execution of reaching movements. However, in humans, the contribution of PMd in the execution of complex hand grip is reflected through connecting sensory information with motor actions [15], as well as visually guided activities [16], although many aspects remain essentially unknown.

Transcranial magnetic stimulation represents non-invasive, safe and painless method aimed to activate restricted neuronal population at target point, with purpose of modulating activity of certain cortical area. Depending on the stimulation intensity, the cortical interneurons are commonly activated, and only at higher intensities the pyramidal cells could discharge, too. However, in this way, the excitatory and inhibitory neurons are activated at the same time, and related to the stimulation pattern, the net effect of repetitive TMS could be either inhibitory or facilitatory. However, there is an additional differences between M-1 and PMd, because functional imaging studies have revealed the activation of premotor regions in both hemispheres, contrary to primarily M-1 activation on the contralateral side during a variety of motor tasks, including isolated movements of the distal arm (e.g. opening a drawer and retrieving food with the same hand) [17, 18].

The aim of this study was to determine the role and contribution of PMd during precision grip in healthy subjects assessed by kinetic analysis of various static and dynamic manipulation tasks with both hands after rTMS intervention over dominant PMd.

Methods

Subjects

Ten healthy volunteers (6 males) aged 29 ± 3.9 years, without history of any neurological and psychiatric conditions, neurosurgery, or metal or electronic implants participated in the study. Subjects were screened for potential risk of adverse reactions to TMS by using the adult safety screen questionnaire for transcranial magnetic stimulation [19]. None of the subjects did take any CNS-acting medications.

Nine subjects were right-handed and one was left-handed according to the Edinburgh handedness inventory [20]. Considering hand motor with manipulandum applied in the study, none of the subjects had previous experience.

The experimental protocol was approved and monitored by the local ethics committee according to the Declaration of Helsinki (www.wma.net/en/30publications). After an explanation of the treatment procedures, all subjects signed a written informed consent.

Grip-lift tasks

Subjects were seated in a comfortable chair in front of the manipulandum which consisted of the single handle in the form of lever with the grasping surfaces covered by rubber (Figure 1A) and steel stand fixed to the table. A single-axis force transducer (SW-20L, CAS Cor., NY, USA; range 200 N; linearity 0.03%; hysteresis 0.03%) located inside the handle recorded the grip force (G) of the finger and the thumb applied perpendicularly against the opposing grasping surface. Another single-axis transducer (LCM300 FUTEK Advanced Sensor Technology, Inc, CA, USA; range 450 N; non-linearity 0.5%; hysteresis 0.5%), located at the bottom of the handle, recorded the load force (L) exerted tangentially to the grasping surfaces. With its lower part load force transducer is attached to the spherical joint so that the force that transmits to the fixed transducer L when pulling the handle upwards is always projected in the ideal vertical position. By the spherical joint, the handle could be either externally fixed to the steel stand, or attached to additional weights and be free to move. Additional weights in steps of 100 and 200 g of mass served to adjust the total weight of device to the prescribed L_{max}.

Within a single session subjects were tested on three manipulation tasks – two "static" (ramp-and-hold and oscillatory task) and one "dynamic" (lifting task) [21]. Each experimental task was well explained and demonstrated by experimenter. Thereafter, subjects were submitted to a familiarization procedure practicing manipulation tasks unimanualy, with three practice trials performed by each hand. After practicing subjects performed four experimental trials and the last three trials were taken for further analysis. The sequence of tasks, as well as the sequence of hands within each task was pseudo-randomized. During testing subjects were focused on the movement task based on L exertion, since G was never mentioned throughout the entire experiment. All measurements conducted by same experienced investigator. Figure 1B illustrate horizontal projection of subjects' body while performing the task [21].

While performing ramp-and-hold task (R&H-T), manipulandum was externally fixed to the steel stand. Subjects were asked to match a prescribed L_{max} profile by pulling up device corresponding to a gradual increase and, thereafter, a steady L exertion against an externally fixed device. Both, the prescribed L_{max}, as well as the current value of L were displayed on a computer monitor placed in front of the seated subject. The profile had the following three phases: zero L (duration 1 s), gradually increasing L (3 s), and constant L (3 s) (Figure 2A). The initiation of each phase and the termination of the last one were indicated by four consecutive computer-generated auditory beeps.

In the oscillatory task (Osc-T) of subjects were expected to correspond to a rapidly changing L against externally fixed device. They were instructed to exert a sinusoidal L on the computer monitor, by pulling the device vertically (upward-downward) in a way that L minima and maxima corresponded to 0 N and the individually prescribed L_{max} (Figure 2B). The computer monitor displayed those horizontal lines depicting the prescribed minima and the maxima, as well as the current value of L. Frequency of oscillatory variations (1.33 Hz) was set by a metronome, while duration of the trials was 8 s.

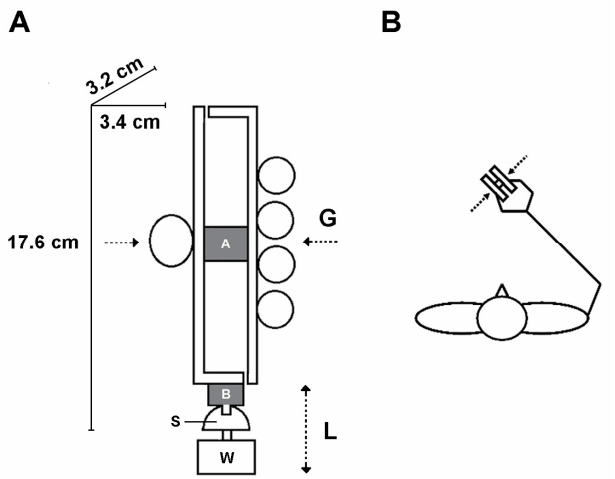

Fig. 1 – (A) Schematic illustration of experimental manipulandum for the assessment of grip and load performance and force coordination. The circles illustrate the position of the tips of the fingers and the thumb of subject's hand applying a precise grasp against the manipulandum. Letters A and B denote grip and load force transducers – load force transducers records grip force (G) and load force (L), respectively. S indices spherical joint, and W – additional weight or, alternatively, fixation of the manipulandum to the steel stand; (B) Position of subject during the task performance - top view.

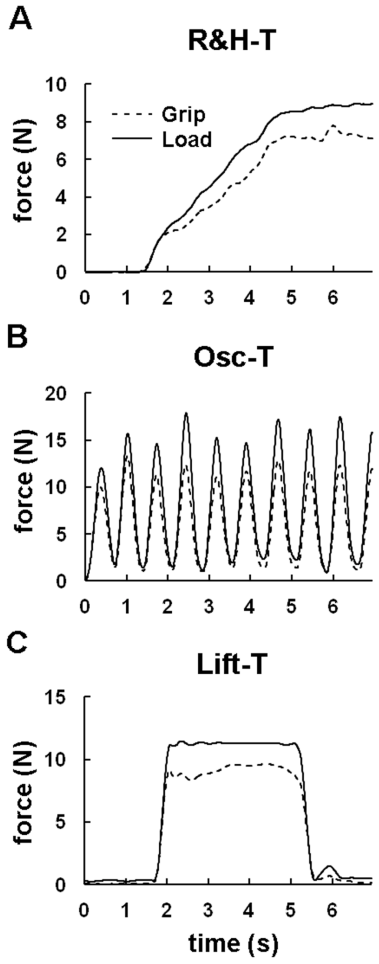

Fig. 2 – Grip and load force exerted against the manipulandum in the: A) ramp-and-hold task (R&H-T), oscillation task (Osc-T) and lifting task (Lift-T) obtained from a healthy subject.

In the lifting task (Lift-T), based on individually prescribed L_{max}, manipulandum was attached with additional weights served to adjust the total weight of device to the prescribed L_{max}. The subjects were instructed to prepare their hand for grasping the device by opening their fingers near the grasping area without touching it. Upon the first computer-generated beep, subject grasped the device, lifted it approximately 3 cm above the table, and held steady until the second beep (3 s later) and, thereafter, place it back on the table and release (Figure 2C).

Transcranial magnetic stimulation

The subjects were seated in a reclining chair that allowed them to keep their arms and hands relaxed during TMS and recording of motor evoked potentials (MEPs).

Single Pulse TMS

Magnetic stimulation was delivered by a 70-mm figure-eight coil and a Magstim Rapid^2 (Magstim Co., Whitland, UK) stimulator for rTMS and a Bistim module (Magstim) for single pulse TMS. MEPs were recorded from the thenar muscle (*abductor pollicis brevis* – APB) using surface electrodes and (Medelec Synergy, VIASYS Healthcare, UK) with a band pass of 20 to 2,000 Hz. Resting motor threshold (RMT) was determined in the contralateral APB muscle, determined with TMS delivered to the optimal scalp site for induction of MEPs in target muscle, according to international standards [22]. The coil was placed tangentially to the scalp, with the handle pointing 45° posterolaterally.

Thirty magnetic pulses were delivered successively (inter-trial interval of 5 ± 1.2 sec), at the intensity optimal to evoke MEPs of 1 mV amplitude (measured from peak to peak). The intensity was approximately between 120-130 % RMT. The time points of MEP measurements were immediately before (PRE) and after (POST) intervention.

Repetitive TMS

Theta burst stimulation (TBS) was performed according to current safety recommendations [23], using original protocols with triplets of very short bursts at 50 Hz repeated at 0.2 s (5 Hz – the range of EEG theta frequency band) for a total of 600 pulses. Therefore, $cTBS_{600}$ protocol lasted for 40 s, while $iTBS_{600}$ protocol includes 10 burst of triplets who were applied every 10 seconds (with pause of 8 s) causing the delivery of 600 pulse over a period of 190 s. Sham TBS was delivered using a matching coil produced by Magstim that delivers only 5% of the stimulator output, but with similar clicking sound produced mechanically by the sham coil with each TMS pulse.

TBS was applied at subthreshold level (80% of RMT), over the PMd of the dominant hemisphere. The stimulation point on the scalp was determined in accordance with the PMd localization at the specific location situated about 2 cm rostral to the representation of hand muscles in the primary motor cortex (half of the distance between Cz and Fz and 15% of the distance from tragus to tragus to the left) [24].

Experimental design

All recordings were conducted in the Laboratory for non-invasive brain stimulation, Military Medical Academy (MMA) in Belgrade. Experiment was carried out in three individual sessions for each individual subject, each separated not shorter than one week. After an evaluation of the excitability of the motor cortex, surface electromiography (EMG) electrodes were removed, and the place where they had been placed was labeled by the marker. Subjects then carried out the hands manipulative tasks (Figure 3).

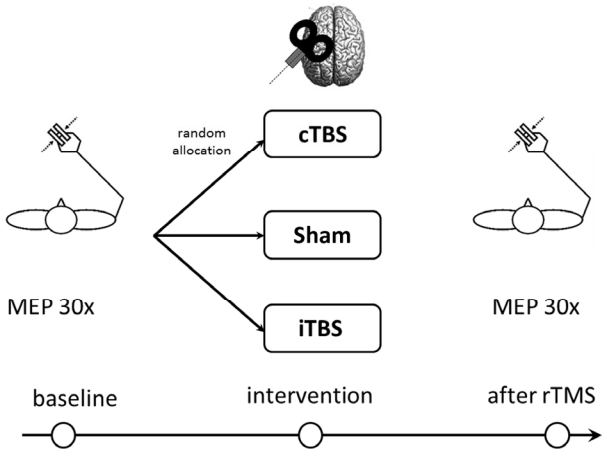

Fig. 3 – Overview of the experimental design. All subjects underwent three different interventional protocols (intermittent theta burst stimulation – iTBS, continous theta burst stimulation – cTBS and Sham), in a crossover study design. At the baseline, immediately before intervention, motor evoked potentials (MEP) amplitudes were collected, and grip performance and force coupling were evaluated by unimanual task on three different tasks: ramp and hold task (R&H-T), oscillation task (Osc-T) and lifting task (Lift-T). The same procedure was performed immediately after intervention, aiming to evaluate each single-session effects.

Before starting the test fingertips were cleaned with alcohol. Since previous results of Jaric et al. [25] have shown that prolonged tasks require L below 15% of the maximum G to avoid fatigue, maximum G exerted by tips of all 5 fingers of each hand was recorded separately. Ten percent of the maximum G of the weaker hand was prescribed as the maximum L (L_{max}) [21] in each of the experimental tasks and was participant specific (range 5-17 N).

After the baseline evaluation, TBS protocols or sham were applied in pseudo-randomized order. Following interventions, all baseline procedure were repeated immediately in the same way.

Data processing

A custom made LabView application (National Instruments, Austin, TX, USA) was used for the data acquisition and processing of data obtained from the grip-and-lift tasks. The signals from both transducers were A/D converted and recorded at the sampling rate of 200 Hz. The raw force data were low-pass filtered at 10 Hz with a fourth order (zero-phase lag) Butterworth filter [21]. In the R&H-T the ramp

phase and the hold phase were separately analyzed [21]. To exclude the initial and final adjustments, in the Osc-T, only the middle 5 s were analyzed [26]. The lift phase (the initiation of lifting, starts when L reaches 8 % of L_{max} and ends with reaching L_{max}) and the hold phase (interval of 2 s, after the period of 0.25 s when L reaches L_{max}) in the Lift-T, were also analyzed separately [21].

Based on directly measured variables (G and L) obtained using LabView application, derived variables were calculated. To assess hand function, two groups of dependent variables were selected. The ability of subject to exert the required pattern of L was assessed by task performance variables (describing how successful subject were regarding performing the instructed task), while the ability of subject to exert the required pattern of L was assessed by root mean square error (RMSE) of L in the R&H-T and coefficient of variation (CV) of L in the Lift-T. Constant error (CE), calculated as a difference of peaks of L and required level of force, and variable error (VE), assessed by standard peak deviations of L, were selected as an indices of task performance in the Osc-T [21].

Force coordination variables describing to what extent G and L were coordinating and assessed the relationship between the temporal profiles of G and L. G-L scaling, assessed by grip-to-load ratio (G/L ratio) evaluated the magnitude of G with respect to the magnitude of L assuming that lower ratio was index of better coordination [27]. It was calculated from the steady holding phases of the R&H-T and the Lift-T, as well as from the averaged G and L of the Osc-T [21]. As an index of G-L coupling, the cross-correlation of the G and L (r) of the R&H-T and the Osc-T were used [25]. Note that maximum correlation coefficient, based on previous studies, should indicate higher force coordination [28,29]. G-L modulation was assessed from G–L diagrams (the slope and intercept were interpreted as Gain and Offset, respectively) of the Osc-T [26,29,30]. Higher force coordination was expected to be revealed by high Gain and low Offset of G [31].

Statistical analysis

For the assessment of normality of distribution the Shapiro-Wilk test was used. To assess the effects of intervention protocols (Sham *vs* iTBS *vs* iTBS) on the global excitability of the motor cortex, the results obtained before (PRE) and after intervention (POST) are normalized (POST/PRE) and ANOVA for repeated measures and *post-hoc* test with Bonferroni correction were used. To assess effects of TMS intervention on hand function, non-parametric statistics was applied. Potential differences between TMS protocols PRE, for the dominant (DH) and the non-dominant hand (NH), separately, were assessed by Friedman's test. Differences between DH and NH PRE and POST, separately, as well as for the results obtained PRE and POST for each of the three interventions, for each hand separately, were assessed by Wilcoxon's signed-rank test. To assess the differences between the interventions, the results obtained PRE and POST were normalized (POST/PRE) and the Friedman's test was applied. When significant differences were found, additional Wilcoxon's signed-rank tests with Bonferroni correction were performed. The level of statistical significance was set to $p < 0.05$. Statistical analysis was performed in SPSS v 20.0 (SPSS Inc., Chicago, USA).

Results

Table 1 shows baseline data of kinetic analysis of static and dynamic manipulation tasks with an instrumented device that recorded the grip and load force, before interventions.

The effects of interventions on motor cortex excitability

The evaluation of motor cortex excitability was performed through comparison of resting motor threshold ($F_{(2,14)} = 0.41$, $p = 0.575$) and MEP modulation (starting from baseline value of 1 mV). Normalized data for MEP modulation have shown significant differences between $iTBS_{600}$ (137.28 ± 27.76) *vs* Sham (99.58 ± 7.79) and $cTBS_{600}$ (87.34 ± 19.54) stimulation ($F_{(2,14)} = 10.80$, $p < 0.05$) (Figure 4). Statistical significance was achieved only for iTBS intervention, that MEP amplitude was increased.

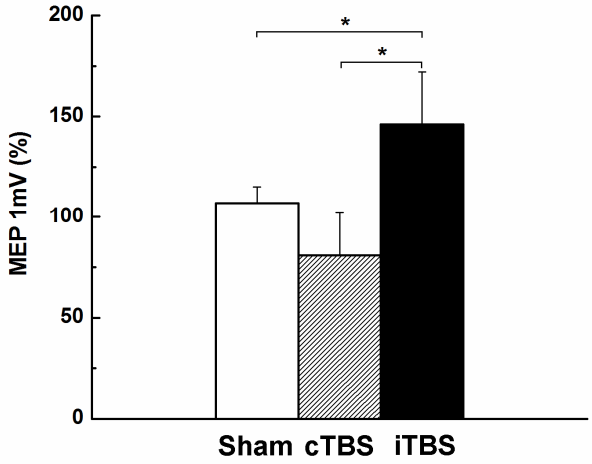

Fig. 4 – Histogram showing normalized data (mean and standard deviation) for three experimental protocols obtained from changes of motor evoked potentials (MEP) amplitudes, after motor cortex stimulation. Data are averaged across the subjects. *$p < 0.05$; cTBS – continous theta burst; iTBS – intermittent theta burst magnetic stimulation.

The effects of interventions on the task performance variables

Our results do not reveal any effects of the interventions on modulation of grip performance and force coordination in the R&H-T and the Lift-T tasks. The performance of R&H-T, assessed by RMSE, was not affected by the intervention, both for DH [Sham (Mdn = 1.05) *vs* cTBS (Mdn = 1.15) *vs* iTBS (1.02), $x^2_{(2)} = 0.60$, $p = 0.741$] and NH [Sham (Mdn = 1.17) *vs* cTBS (Mdn = 1.07) *vs* iTBS (0.96), $x^2_{(2)} = 0.60$, $p = 0.741$]. Coefficient of variation of L, indicator of task performance in the Lift-T, also remained unchanged [DH, Sham (Mdn = 1.00) *vs* cTBS (Mdn = 0.65) *vs* iTBS (Mdn = 0.69), $x^2_{(2)} = 0.20$, $p = 0.905$; NH, Sham (Mdn = 0.87) *vs* cTBS (Mdn = 0.94) *vs* iTBS (Mdn = 0.66), $x^2_{(2)} = 0.60$, $p = 0.670$].

Table 1

Baseline characteristics of healthy subjects pre - interventional

Task	Task permormance		SHAM	cTBS	iTBS	
			Mdn (int)	Mdn (int)	Mdn (int)	*p*
R&H-T	RMSE	DH	2.3 (1.2–3.8)	3.3 (1.4–4.8)	2.9 (1.5–4.6)	0.202
		NH	2.6 (1.7–4.2)	2.8 (2.1–3.5)	2.7 (1.85–3.4)	0.273
Osc-T	CE	DH	2.01 (0.82–6.32)	2.70 (0.34–6.24)	2.22 (0.39–9.57)	0.497
		NH	2.40 (1.16–6.02)	3.56 (0.80–10.69)	2.62 (0.53–11.97)	0.273
	VE	DH	6.72 (5.03–11.38)	6.30 (5.03–8.33)	6.80 (4.78–9.70)	0.326
		NH	5.76 (4.12–11.66)	7.44 (5.13–9.90)	7.14 (3.36–11.88)	0.497
Lift-T	CV	DH	0.81 (0.33–2.24)	0.89 (0.53–3.83)	1.01 (0.22–1.62)	0.905
		NH	1.01 (0.26–1.66)	0.91 (0.60–1.80)	0.96 (0.28–2.09)	0.670
Force coordination						
R&H-T	G/L ratio	DH	1.03 (0.77–1.14)	0.95 (0.59–1.21)	1.01 (0.72–1.24)	0.407
		NH	0.93 (0.68–1.42)	0.89 (0.44–1.73)	0.93 (0.39–1.42)	0.905
	r	DH	0.995 (0.992–0.998)	0.995 (0.992–0.997)	0.996 (0.986–0.997)	0.497
		NH	0.996 (0.985–0.998)	0.995 (0.991–0.998)	0.996 (0.964–0.998)	0.905
Osc-T	G/L ratio	DH	1.18 (0.78–1.29)	1.00 (0.60–1.35)	0.96 (0.73–1.42)	0.497
		NH	1.01 (0.74–1.27)	1.01 (0.56–1.49)	0.95 (0.46–1.38)	0.670
	r	DH	0.980 (0.967–0.993)	0.981 (0.966–0.992)	0.982 (0.972–0.991)	0.741
		NH	0.986 (0.963–0.994)	0.986 (0.963–0.992)	0.986 (0.974–0.993)	0.407
	Gain	DH	0.96 (0.82–1.26)	1.05 (0.87–1.36)	1.07 (0.69–1.41)	0.497
		NH	1.08 (0.79–1.37)	1.09 (0.86–1.82)	1.07 (0.82–2.28)	0.407
	Offset	DH	-0.46 (-1.89–0.36)	-0.56 (-2.12–0.72)	-0.21 (-1.38–0.64)	0.067
		NH	-0.20 (-1.54–0.64)	-0.18 (-1.29–0.68)	-0.28 (-0.79–0.39)	0.895
Lift-T	G/L ratio	DH	1.03 (0.94–1.15)	1.02 (0.77–1.35)	0.94 (0.79–1.23)	0.082
		NH	1.05 (0.76–1.19)	1.05 (0.63–1.52)	0.96 (0.53–1.25)	0.497

SHAM – sham stimulation; R&H-T – ramp and hold task; Osc-T – oscillation task; Lift-T – lifting task; RMSE – root mean square error; CE – constant error; VE – variable error; CV – coefficient of variation; G/L ratio – grip-to-load ratio; DH – dominant hand; NH – non-dominant hand; r – cross correlation of the G and L; *p* – probability; Mdn – median; CTBS – continous theta burst stimulation; iTBS – intermittent theta burst stimulation.

In the Osc-T the ability of subjects to exert required pattern of L was assessed by absolute CE and VE (Figure 5A–B). Our results have shown better task performance regarding to CE for DH *vs* NH after iTBS protocol (z = -2.60, $p = 0.009$) with a large difference between hands (r = 0.58). Median of the results for both hands decreased after the iTBS, but note that differences between PRE and POST was larger for DH (from Mdn = -2.22 pre-intervention, to Mdn = 1.28 after intervention) relative to NH (Mdn = 2.61, PRE *vs* Mdn = 2.23, POST). Between different TBS protocols were no significance differences either for DH [Sham (Mdn = 1.08) *vs* cTBS (Mdn = 0.85) *vs* iTBS (Mdn = 0.95), $\chi^2_{(2)} = 1.40$, $p = 0.497$], as well as for NH [Sham (Mdn = 1.03) *vs* cTBS (Mdn = 0.95) *vs* iTBS (Mdn = 0.98), $\chi^2_{(2)} = 0.60$, $p = 0.741$].

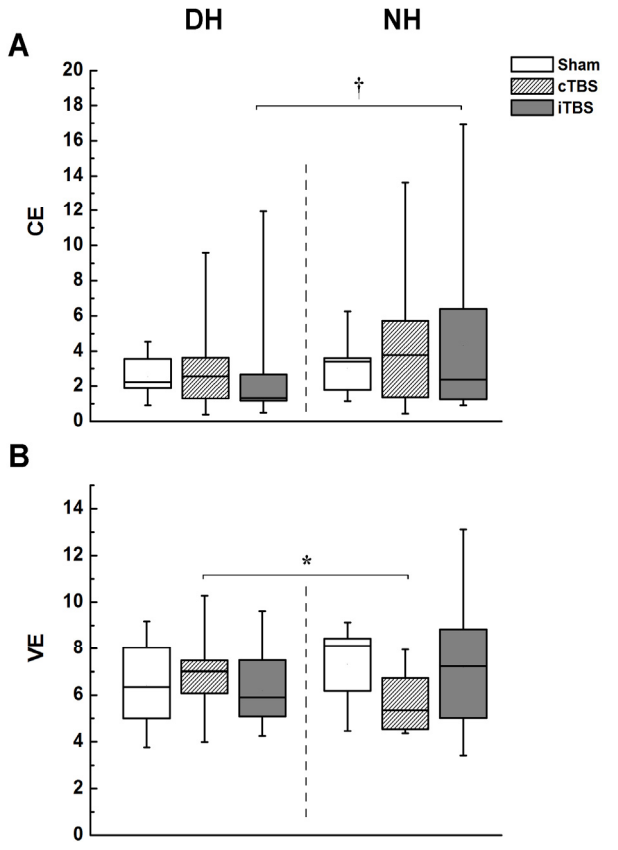

Fig. 5 – Task performance variables in oscillation task. A) Constant error (CE) and variable error (VE) values for dominant (DH) and B) non-dominant hand (NH) are averaged across the subjects for each of three experimental protocols (Sham, continous theta burst stimulation – cTBS and intermittent theta burst stimulation – iTBS). The box plots represent the 25th and 75th percentile of the distribution and the middle line represents the median. †$p < 0.01$ and *$p < 0.05$ between the groups (DH *vs* NH). Note that iTBS improved the CE when the task was performed with DH, while cTBS improved the VE if the task was performed with NH.

Results of Wilxocon's test for VE (the standard deviations of peaks of L) revealed better task performance for NH after the cTBS (z = -2.80, $p = 0.005$) with a large effect size (r = 0.63). Contrary, for DH there were no differences between values of VE pre- and post- the cTBS intervention (z = -0.56, $p = 0.575$). The only significant differences have shown for better task performances for NH relative to DH (z = -2.09, $p = 0.037$, r = 0.47). Using the Friedman's test on normalized set of data aiming to detect potential differences between protocols, we found no significant differences between Sham, cTBS and iTBS for DH ($\chi^2_{(2)} = 4.20$, $p = 0.122$), while results for NH revealed different effects of intervention [Sham (Mdn = 0.81) *vs* cTBS (Mdn = 0.81) *vs* iTBS (Mdn = 0.97), $\chi^2_{(2)} = 6.20$, $p = 0.045$]. Additional Wilcoxon's tests with Bonferroni correction for multiple comparisons revealed significant differences between cTBS i iTBS (z = -2.40, $p < 0.05$) with a large effect size (r = 0.54) in a form of worse task performance for NH using iTBS protocol.

The effects of interventions on the force coordination variables

TBS interventions did not affect the coordination of G and L in the R&H-T and the Lift-T. The adjustment of G and L forces during precision grip (assessed by G/L ratio) was not affected in the R&H-T [DH, Sham (Mdn = 0.93) *vs* cTBS (Mdn = 0.96) *vs* iTBS (Mdn = 1.00), $\chi^2_{(2)} = 0.20$, $p = 0.905$; NH, Sham (Mdn = 1.03) *vs* cTBS (Mdn = 1.14) *vs* iTBS (Mdn = 1.05), $\chi^2_{(2)} = 0.20$, $p = 0.905$], nor in the Lift-T [DH, Sham (Mdn = 0.94) *vs* cTBS (Mdn = 0.99) *vs* iTBS (Mdn = 1.09), $\chi^2_{(2)} = 4.20$, $p = 0.122$; NH, Sham (Mdn = 1.00) *vs* cTBS (Mdn = 1.01) *vs* iTBS (Mdn = 1.10), $\chi^2_{(2)} = 2.60$, $p = 0.273$]. The force coupling between G and L (assessed by *r*) in the R&H-T [DH, Sham (Mdn = 1.000) *vs* cTBS (Mdn = 1.000) *vs* iTBS (Mdn = 1.001), $\chi^2_{(2)} = 0.60$, $p = 0.741$; NH, Sham (Mdn = 1.000) *vs* cTBS (Mdn = 1.000) *vs* iTBS (Mdn = 1.000), $\chi^2_{(2)} = 4.20$, $p = 122$] was unchanged, too.

The results obtained by assessing force coordination variables in the Osc-T (Figure 6A-C) showed impairment of G-L scaling for NH at iTBS protocol, comparing results before (Mdn = 0.95) and after (Mdn = 1.02), z = -2.40, $p = 0.017$; see (Figure 6A), with a large effect of intervention (r = 0.54), while differences were not revealed for the Sham (z = -1.27, $p = 0.203$) neither for cTBS protocol (z = -0-56, $p = 0.57$). Contrary to those findings, no differences were shown, when task was performed with DH [Sham (z = -0.25, $p = 0.799$), cTBS (z = -0.50, $p = 0.646$) i iTBS (z = -1.82, $p = 0.69$).

Friedman's test for repeated measure did not reveal different effects of interventions on a G-L scaling either for DH or for NH (DH, $\chi^2_{(2)} = 0.80$, $p = 0.670$; NH, $\chi^2_{(2)} = 2.40$, $p = 0.301$).

Analyzing the data about G and L coordination through so called force coupling we found the similar impairment performing oscillatory task with NH after iTBS (z = -1.99, $p = 0.047$) (Figure 6B), while at the other two protocols (Sham and cTBS), as well as for DH at all three experimental protocols (Sham, cTBS and iTBS) were not significantly changed by interventions. However, between-group comparison of post-interventional data has shown significant differences for NH ($\chi^2_{(2)} = 13.40$, $p = 0.001$). Additional *post-hoc* tests with Bonferroni correction showed differences between cTBS and iTBS (z = -2.80, $p < 0.05$), with a large effect of intervention (r = 0.63).

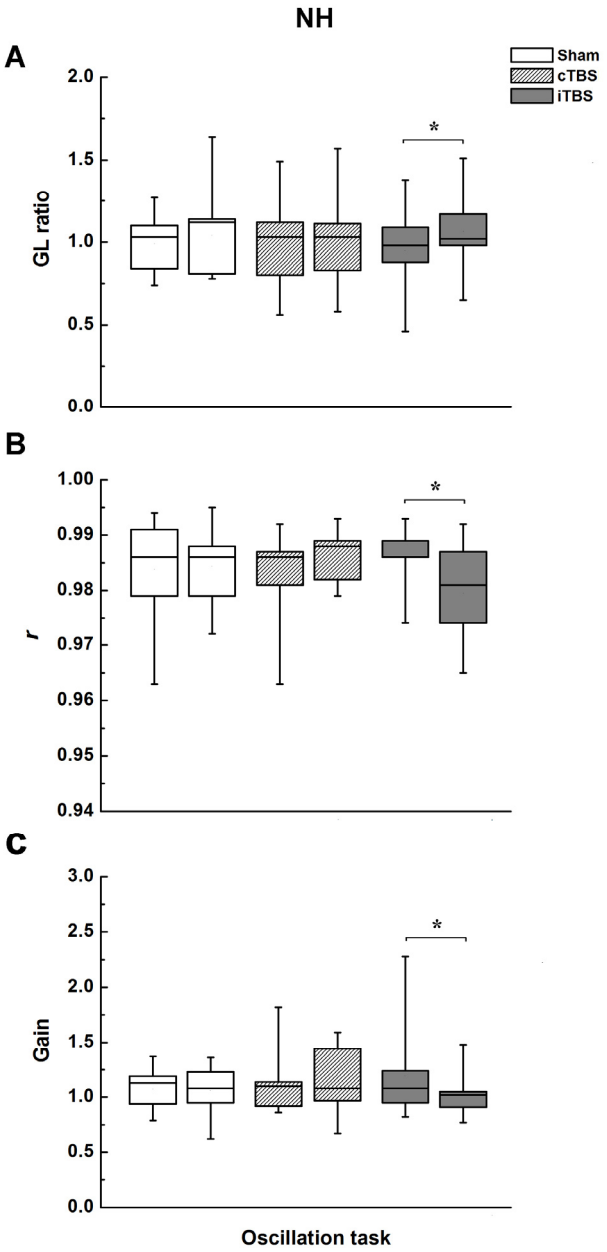

Fig. 6 – Force coordination variables, for non-dominant hand (NH), in oscillation task, before and after interventions. A) Grip-to-load ratio (G/L ratio), B) cross-correlation of the G and L (*r*), and C) gain of G, data are averaged across the subjects for each of three experimental protocols (Sham, continous theta burst stimulation – cTBS and intermittent theta burst stimulation – iTBS). The box plots represent the 25th and 75th percentile of the distribution and the middle line represents the median. Note that iTBS worsened most of the coordination variables for NH.
***p* < 0.05 within the group (PRE *vs* POST).**

Examination of median results for *r*, showed lower G-L coupling POST for NH at iTBS (Mdn = 0.980), relative to cTBS (Mdn = 0.988). Friedman's test used on relativized set of data (POST/PRE) revealed absence of the effects of intervention for DH ($\chi^2_{(2)}$ = 0.60, p = 0.741). Contrary, for NH significant differences between experimental protocols were found ($\chi^2_{(2)}$ = 12.80, p = 0.002) between Sham and iTBS (z = -2.29, p < 0.05, r = 0.51) and cTBS and iTBS (z = -2.80, p < 0.05, r = 0.63) in the form of lower G-L coupling after iTBS (Mdn = 0.997) relative to the Sham (Mdn = 1.000) and cTBS (Mdn = 1.004). The effect of intervention (POST/PRE) was revealed for DH *vs* NH, also at iTBS protocol [DH (Mdn = 1.000) *vs* NH (Mdn = 0.997), z = -1.99, p = 0.47, r = 0.44], while at Sham [DH (Mdn = 1.000) *vs* NH (Mdn = 1.000), z = -0.56, p = 0.575] and cTBS [DH (Mdn = 1.001) *vs* NH (Mdn = 1.004), z = -0.51, p = 0.959] differences as an effect of intervention were not found.

The G-L modulation in the Osc-T was assessed by Gain and Offset, disclosing the presence of significant differences, exclusively for Gain (Figure 6C). Wilcoxon's test revealed difference between PRE and POST at iTBS protocol, as the impairment hand function for NH after intervention (Mdn = 1.07, PRE; Mdn = 1.01, POST), z = -1.99, p = 0.047, r = 0.44. Comparing the results obtained from all three interventional protocols, for the same parameter, we did not find differences between-group for after-intervention effect (DH, $\chi^2_{(2)}$ = 0.60, p = 0.741; NH, $\chi^2_{(2)}$ = 5.00, p = 0.082), as well as within-group effects [POST/PRE; DH ($\chi^2_{(2)}$ = 1.40, p = 0.497), NH – $\chi^2_{(2)}$ = 2.60, p = 0.273].

Considering the Offset, we did not find any within-group, either between-group differences (DH, $\chi^2_{(2)}$ = 0.67, p = 0.717; NH, $\chi^2_{(2)}$ = 0.67, p = 0.717).

Discussion

In the present study we demonstrate that application of facilitatory and inhibitory TBS protocols over the dominant PMdn lead to bi-directional and complex modulation of grip performance and coordination when unimanual tasks were performed in healthy individuals. To our knowledge, this is the first experiment designed specifically to address effects of rTMS intervention on precision grasp including both hemispheres. This is especially important if one bears in mind that previous study has revealed the inhibitory effects of low-frequency rTMS (1 Hz) on MEP amplitudes, as well as differences in cerebral blood flow in multiple brain regions, including motor regions in the frontal cortex as well as more associational regions in the parietal and prefrontal cortices, when it applied over the PMd [32]. However, beyond these basic parameters of cortical excitability, virtual lesions produced by low frequency rTMS over M-1 and PMd of the dominant hemisphere lead to disturbances of anticipatory scaling of force for pinch grip [33]. Results of that study have shown that virtual lesion of M-1 causes disruption of scaling force based on information from a previous attempt, while lesion of PMd disturbs scaling based on arbitrary visual cues. These findings, actually confirm the prominent role of PMd in coupling arbitrary sensory cues to motor acts [34, 35].

In our study, we were using unimanual tasks that were primarily focused on scaling grip and load forces, but also included a visuomotor coordination. However, changes after TBS intervention were detected in only one of three manipulative tasks, the oscillatory task, interfering with the ability of subject to reach the required L peaks (task performance

variables), but also with the ability of grip and load forces coupling (force coordination variables).

Namely, the application of $iTBS_{600}$ or $cTBS_{600}$ over the M-1 in healthy individuals produced a relatively simple effect in terms of increased or decreased global motor system output, respectively [36]. In our study, however, most of the changes are registered after the $iTBS_{600}$ protocol and, as already indicated. As regards the task performance variables, during the oscillatory task, it was shown that application of $iTBS_{600}$ over dominant PMd induce the significant increase of tracking accuracy task, expressed as reduction of CE when task was performed with DH, while precision to follow prescribed peaks was disturbed for the NH performance. The effects of $cTBS_{600}$ protocols were significant only as improved task performance with NH.

In accordance with the contemporary viewpoint, cortical activity which reflects the performance of unimanual voluntary movements (or bimanual with a pronounced asymmetry), is distributed across both hemisphere [37]. Furthermore, communication between the hemispheres is carried out through transcallosal fibers, which transmit both, inhibitory and excitatory signals, although the prevailing opinion is that the inhibitory effects are stronger [38]. However, it is important to note that in addition to the most important interhemispheric communication between two homologous M-1 areas, a couple of non-primary motor areas are also included in the interhemispheric inhibitory network, but with a significantly less impact [39, 40].

Pronounced indirect changes, as we noted in our experiment, can be attributed to changes in the level of interhemispheric inhibition. Namely, according to the hypothesis of interhemispheric competition, two hemispheres behave as opposing systems, so that modulation of cortical excitability can change tonic transcallosal inhibition that is present under normal circumstances [41, 42].

If we apply this model of hemispheric rivalry to our experiment, it would mean that the facilitatory rTMS protocol (iTBS) over dominant PMd, in addition to increase of cortical excitability at the site of stimulation, leads to strengthening of interhemispheric inhibition directed against the homologous area of non-dominant hemisphere which is not under stimulation. By contrast, the use of inhibitory protocol (cTBS) over the dominant hemisphere should result in the weakening of interhemispheric inhibition transmitted *via* transcallosal fibers, so this would facilitate and improve precise grasping and object lifting in oscillatory task force.

Previous studies have shown that the application of iTBS in healthy subjects, leads to post-interventional reduction of MEP amplitudes over contralateral hemisphere [41]. In this case, it is assumed that the iTBS changes transcallosal input and amplifies interhemispheric tonic inhibition, leading to reduced excitability of non-stimulated hemisphere. In contrast, the study in which cTBS was applied over the M-1, showed the weakening of tonic interhemispheric inhibition and subsequent increase of cortical excitability over the hemisphere that was not stimulated [43]. This sequence of events might suggest that amplification of the motor output of the ipsilateral hand could interfere with the precise force gradation or magnification of the error.

Conclusion

This study further explores the relevant parameters involved in precise hand grip, mediated by PMd, including the effects on contralateral and ipsilateral hand. In this way, these results expand the knowledge arising from animal experiments and neuroimaging studies in humans, confirming the pivotal role of the PMd activation for the scaling of forces.

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The value of transbronchial needle aspiration cytology in the diagnosis of stage I and II sarcoidosis

Vrednost transbronhijalne aspiracione citologije u dijagnozi sarkoidoze stadijuma I i II

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Abstract

Background/Aim. Sarcoidosis is a multisystem infmammatory disease of unknown etiology, with the lungs and intrathoracic lymph nodes the most commonly involved. The aim of this study was to assess the contribution of conventional transbronchial needle aspiration (TBNA) cytology in the diagnosis of sarcoidosis presenting as mediastinal/hilar lymphadenopathy. **Methods.** In this retrospective study, 58 patients with suspicion of stage I and II sarcoidosis underwent first flexibile, and then, a rigid bronhoscopy, during which TBNA of mediastinal or hilar lumph node with a 19 gauge (G) needle was done. Material from the needle was put on glass slide and prepared for the cytological and histopathological examination. **Results.** Out of 58 patients submitted to TBNA of mediastinal or hilar lymph nodes, adequate material for cytological diagnostics was obtained in 53 (91.37%). Out of 53 adequate cytological samples, in 38 (71.69%) noncaseous granulomatous inflammation (NGI) was found, while in corresponding histopathological samples, NGI was found in 48 (90.56%), which was significantly higher ($p < 0.05$). Of cytological smears, out of the cell types typical for granulomatous inflammation, in 26 (63.15%) patients the clusters of the epitheloid cells were found, in 8 (21.05%) there were both, clusters of epitheloid cells and giant multinuclear hystiocytes, and in 6 (15.76%) only single scattered epitheloid cells or small clusters of several epitheloid cells were found. The sensitivity of TBNA cytology in our group of patients with sarcoidosis was 76%, specificity 100% and accuracy 77.34%. **Conclusion.** TBNA is an efficient and safe procedure in the diagnosis of sarcoidosis, minimally invasive and with a little risk of complications. Using 19 G needle enables obtaining material for histological and cytological analyses, as well which contribute to the sensitivity of diagnosting sarcoidosis. The value of this type of diagnostics depends on qualification and experience both of bronchoscopist and cytologist/pathologist, as well, of the interpreter of such a material.

Key words:
sarcoidosis; diagnosis; diagnosis, differential; lymph nodes; mediastinum; biopsy, fine-needle; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Sarkoidoza je multisustemsko oboljenje nepoznate etiologije koje najčešće zahvata pluća i intratorakalne limfne čvorove. Cilj ovog rada bio je da se proceni doprinos konvencionalne transbronhijalne aspiracione (TBNA) citologije u dijagnozi sarkoidoze prikazane kao medijastinalna/hilarna adenopatija. **Metode.** U ovoj retrospektivnoj studiji, na 58 bolesnika sa sumnjom na stadijum I i II sarkoidoze urađena je prvo fleksibilna bronhoskopija, a potom, u toku rigidne bronhoskopije, TBNA medijastinalnih ili hilarnih limfnih čvorova iglom od 19 gejdža (G). Materijal iz igle je istisnut na predmetno staklo i pripremljen za citološku i patohistolosku dijagnostiku. **Rezultati.** Od 58 bolesnika kojima je urađena TBNA medijastinalnih ili hilarnih limfnih čvorova, kod 53 (91,37%) dobijen je adekvatan material za citološku dijagnostiku. Od 53 adekvatna citološka uzorka, kod 38 (71,69%) nađena je nekazeozna granulomatozna inflamacija (NGI), dok je u odgovarajućim patohistološkim uzorcima NGI nađena kod 48 (90,56%), što je bio statistički značajno veći broj ($p < 0,05$). U citološkim uzorcima, od ćelija tipičnih za granulomatoznu inflamaciju, u uzorcima 26 (63,15%) bolesnika nađene su nakupine epiteloidnih ćelija, kod 8 (21,05%) uz nakupine epiteloidnih ćelija nađeni su i džinovski multinuklearni histiociti, a kod 6 (15,76%) samo pojedinačne epiteloidne ćelije ili male grupe od po nekoliko epiteloidnih

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ćelija. Senzitivnost TBNA citologije u našoj grupi bolesnika sa sarkoidozom bila je 76%, specifičnost 100% i tačnost 77,34%. **Zaključak.** Metoda TBNA je efikasna i sigurna za dijagnozu sarkoidoze, minimalno invazivna i s malim rizikom od komplikacija. Upotrebom igle od 19 G dobija se materijal i za citološku i za histološku analizu. Vrednost ovog tipa dijagnostike zavisi od obučenosti i iskustva kako pulmologa koji uzima materijal, tako i od citologa i patologa koji taj materijal interpretitraju.

Ključne reči:
sarkoidoza; dijagnoza; dijagnoza, diferencijalna; limfni čvorovi; medijastinum; biopsija tankom iglom; senzitivnost i specifičnost.

Introduction

Sarcoidosis is multisystemic inflammatory disease of unknown etiology and pathomechanism. It is characterized by noncaseating granulomas which may be found in virtually all organs. The granulomatous inflammation in sarcoidosis may result from a prolonged immunogenic response to a persistent, yet unknown antigen, which leads to immune system exhaustion [1]. There is no gold standard diagnostic test for sarcoidosis, although a recent investigation has shown that identification of serum amyloid A may be a specific marker for sarcoidosis-related granulomatous inflammation [2].

The lungs and intrathoracic lymph nodes are the most commonly involved organs in sarcoidosis. When clinical and radiological picture is suspicious of sarcoidosis, a histologic diagnosis of nocaseating granulomatous inflammation (NGI) is required to exclude lymphomas and lung cancer, but also the other diseases with similar histology like mycobacteria and fungal infections or chronic beryllium disease [3, 4].

Previously applied surgical procedures for obtaining tissue samples such are thoracotomy, thoracoscopy and mediastinoscopy are now being replaced by less invasive ones bronhoscopic techniques such as endobronchial (EBB), or transbronchial lung biopsy (TBLB), or minimally invasive ones like conventional transbronchial needle aspiration (TBNA), or endobronchial ultrasound-guided TBNA (EBUS-TBNA), and endoscopic ultrasound-guided fine needle aspiration (EUS-FNA).

TBNA is mostly used for the diagnosis and staging of bronchogenic carcinoma, using rigid or flexible bronchoscopy, with histology, 18, 19 gunge (G) or cytology (21, 22, 26 G) needles. Thin, cytology needles are more easy for use, and excellent for samplings, but advantage of histology needle is obtaining the material for both cytology and histology which increases the possibility of diagnosing, especially in benign lung disease such as sarcoidosis. In a recently published systematic review and meta-analysis of efficacy and safety of conventional TBNA in sarcoidosis, a good diagnostic yield (62%) with practically no complications in more than 900 patients was found [5].

The aim of this study was to assess the contribution of conventional TBNA cytology by a 19 G needle in the diagnosis of sarcoidosis presenting as mediastinal/ hilar lymphadenopathy.

Methods

Design of study

In this retrospective study, 58 patients with suspicion of stage I and II sarcoidosis according to clinical and radiological findings, in the period between January 2013 and June 2015, were included. To establish the definitive diagnosis of sarcoidosis, at the Department of Interventive Pulmology at the Clinic for Pulmonary Disease, Military Medical Academy, Belgrade, Serbia, the patients underwent TBNA of hilar or mediastinal lymph nodes to obtain material for cytological and pathohystological diagnostics. Before the bronhoscopic procedures, along with laboratory and functional evaluation, patients were submitted to multisliced thoracic scan for precise localization and dimension of hilar and mediastinal lymph nodes which were found in all the examined patients, with the diameter of 15−45 mm. Cytological and pathohystological analyses were done in the Institute for Pathology and Forensic Medicine, Military Medical Academy, Belgrade, Serbia.

The procedure of sampling

In short analgosedation, video bronhoscopy (Olympus BF260) with bronchoalveolar lavage (with infusion of 150 mL saline in the middle lobe) was first done. After that, rigid bronhoscopy (Karl Storz, GmbH&Co.KG, Tuttlingen, Germany) and TBNA of mediastinal or hilar lyumph nodes using a 19 G needle was performed, with taking samples for cytological and pathohistological evaluation.

In 49 patients and 9 patients TBNA of subcarinal (number 7) and hilar lymph nodes (number 10), respectively, were done. There were no immediate no late complications. Material from the needle was expelled on glass slide. The compact particle of material was thoroughly removed from slide by scalpel and put into bottle with formaline for histological diagnostics. The rest of material was directly smeared on slides for cytological analysis. Bronchoscopy and sampling for both diagnostics were done by the pulmologist with the assistence of a medical technician. There were no cytotechnologist or cytologist at that time, so evaluation of the cytological material was not performed at the same time ("rapid *on site* cytopathologic examination − ROSE").

Material processing for cytological and pathohistological analysis

Samples for cytological analysis were air-dried and stained with May-Grünwald-Giemsa, and for histopathological analysis samples were placed in 4% formalin, being classically processed in continuity by the standard protocol for fixation, parafin tissue embedded samples, slicing, and stained with hematoxyllin-eosin (HE) and Ziehl-Nilseen.

During TBNA every patient had one sample of material for histology, and all smears maid from the rest of the mate-

rial were considered as one sample for cytology, so in that way we had the equal number of patients and samples. Samples were considered inadequate for cytological evaluation if there were only bronchial epithelial cells and macrophages without lymphocytes, or rare lymphocytes (if it were less than 30% of all cells on smears) [6].

Cytological diagnosis of NGI which corresponds to sarcoidosis was established only if there were epithelioid cells amongst lymph node cells, solely or in clusters, with or without giant, multinuclear hystiocytes, without the presence of caseous necrosis.

Stastistical evaluation

Beside the usual parameters of descriptive statistics for the age of patients (mean value ± SD), the standard definitions of TBNA cytology sensitivity (TP/TP + FN), specificity (TN/TN + FP) and accuracy (TP+ TN/TP+TN + FP + FN) were used (TP – true positive; TN – true negative; FP − false positive; FN – false negative). The unit of analysis was a patient. The true positive were considered all cytological findings with the picture of NGI corresponding to sarcoidosis, being confirmed with histopathological finding. False negative were considered all adequate cytological samples without elements of granulomatous inflammation and histopathologically verified NGI as Ziehl -Nilseen negative, corresponding to sarcoidosis. True negative were considered adequate cytological samples without elements for granulomatous inflammation, confirmed by histology. There were no false positive cytological findings.

For the evaluation of statistical significance of certain parameters (at the level of $p < 0.05$), χ^2 test, *t*-test and Wilcoxon's test were used. Analyses were performed with the computer program IBM SPSS 20 and Microsoft Office Excel 200.

Results

During the period of two and a half years, in 58 patients with suspicion of I and II stage of sarcoidosis, based on clinical and radiological findings, the diagnosis was confirmed histopathologically and/or cytologically on the material taken by bronchoscopic procedures. The mean age of patients (± SD) was 40.83 ± 11.43 (23−65) years. There were 36 (62%) men, mean age 37.42 ± 8.84 years and 22 (38%) women, mean age 46.41 ± 13.09 years. There were more men than women, while women were older, but these differences in the numbers ($p = 0.066$; $p > 0.05$) and age ($p = 0.281$; $p > 0.05$) were not statistically significant.

Out of 58 patients submitted to TBNA of mediastinal or hilar lymph nodes, adequate material for cytological diagnostics was taken from 53 (91.37%) and inadequate from 5 (8.62%) of the patients, therefore the comparison of cytological *versus* histological findings was done for 53 patients, from whom only two (3.45%) of the samples for histological diagnostics were inadequate.

Out of 53 adequate cytological samples, in 38 (71.69%) NGI was found, while out of 53 corresponding histopathological samples, NGI was found in 48 (90.56%). This difference in the number of positive histological *versus* cytological findings reached a statistical significance ($p = 0.002$; $p < 0.05$) (Table 1).

Out of 5 negative histological samples, 2 of them were inadequate (there were no cells from lymph node or there were so little of them). In cytological smears, out of the cell types typical for granulomatous inflammation, the clusters of the epitheloid cells were found in 26 (63.15%) of the patients which was the most frequent finding (Figure 1). In the smears of 8 (21.05%) of the patients there were both, clusters of the epitheloid cells and giant multinuclear hystiocytes (Figure 2), and in 6 (15.76%) only scattered or small clusters of several epitheloid cells were found (Figure 3). Beside elements of granulomatous

Table 1

The results of cytological and histopathological analysis of samples obtained by transbronchial needle aspiration (TBNA) of mediastinal or hilar lymph nodes

Parameter	Cytology, n (%)	Histopathology, n (%)
Granulomatous inflammation	38 (71.69)	48 (90.56)*
Without elements for granulomatous inflammation	15 (28.31)	5 (9.44)
Total number	53 (100)	53 (100)

***Significantly higher number of histological findings with granulomatous inflammation *versus* cytological findings ($p < 0.05$)**

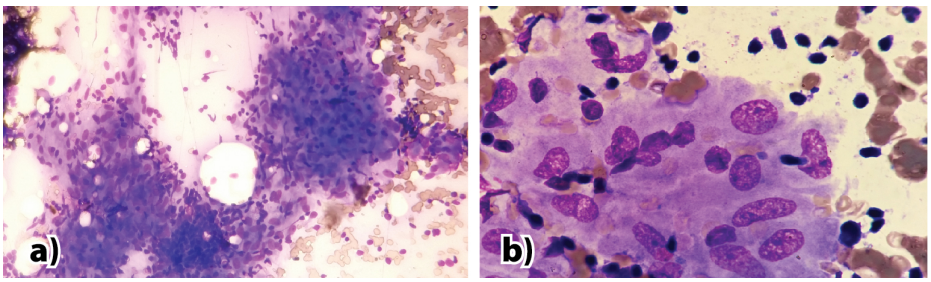

Fig. 1 – Transbranchial needle aspiration (TBNA) smear obtained from mediastinal/hilar lymph nodes: a) Large clusters of epitheloid cells and scattered small lymphocytes (May-Grünwald-Giemsa, ×200); b) A group of epitheloid cells, some of them with typical elongated nucleus (May-Grünwald-Giemsa, ×1,000).

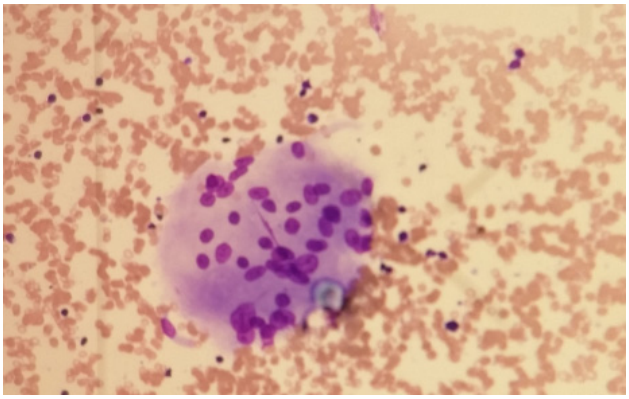

Fig. 2 – Transbranchial needle aspiration (TBNA) smear obtained from mediastinal/hilar lymph nodes. Multinucleated giant cell and scattered lymphocytes (May-Grünwald-Giemsa, ×100).

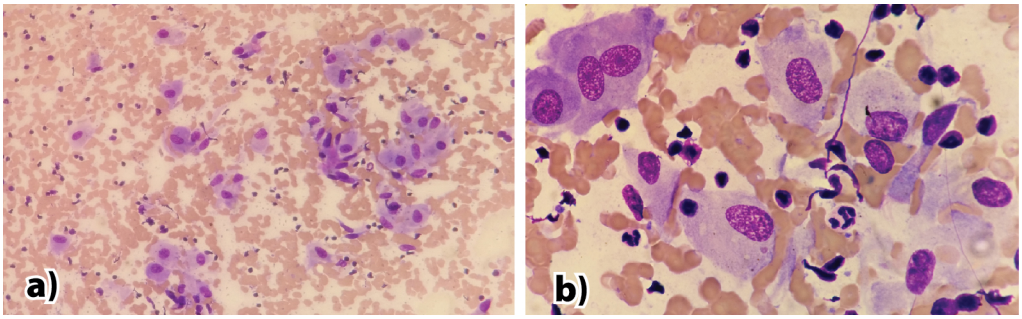

Fig. 3 – Transbranchial needle aspiration (TBNA) smear obtained from mediastinal/hilar lymph nodes: a) Scattered small groups and single epitheloid cells, lymphocytes and rare cylindrical cells (May-Grünwald-Giemsa, ×100); b) Single epiteloid cells, lymphocytes and one cylindrical cell (May-Grünwald-Giemsa, ×1,000).

inflammation, small matured lymphocytes or polymorphous picture of lymph node with lymphohystiocytic aggregates were the most frequently present.

In 15 (28.30%) adequate cytological samples there were no elements of granulomatous inflammation. Out of that, 12 findings were false negative, 3 were true negative. There were no false positive cytological findings. The sensitivity of TBNA cytology in our group of patients with sarcoidosis presenting as mediastinal/hilar lymphadenopathy was 76%, specificity 100%, and accuracy 77.34%.

Discussion

Sarcoidosis is a multisystemic disease which might represent a serious diagnostic problem. Though it can affect any of the organs, the most predilected are lungs, and it usually requires rulling out other diseases which could give the similar clinical and radiological simptomatology. The necessity for less invasive, yet sufficiently effective technics for obtaining diagnostic material have put the emphasis on TBNA, whether it is done "on blind" during conventional flexibile bronchoscopy, or ultrasound guided (EBUS-TBNA, EUS-FNA).

Recent investigations have proved a significantly higher sensitivity and accuracy of ultrasound−guided TBNA whether from esophagus or great airways *versus* conventional one, in the diagnosis of pulmonary sarcoidosis which is manifested with hilar/mediastinal lymphadenopathy. In that way, Annema et al. [7] suggest EUS-FNA as the next diagnostic procedure after nondiagnostic bronchoscopy and Gnass et al. [8], on the basis of the results obtained by comparison of conventional and US needle biopsy technics, suggest EUS-FNA as the method of choice for patients with suspicion of sarcoidosis. Ribeiro et al. [9] suggest EBUS-TBNA as the first diagnostic line, while Navani et al. [10] suggest EBUS-TBNA in combination with the standard, initial bronchoscopic technics (EBB and TBB) as the first line of examination for patients with suspicion of sarcoidosis and intrathoracic lymphadenopathy. However, technology of US-guided TBNA is still expensive in order to be used as the first diagnostic line, especially for poor countries.

On the basis of the results of meta-analysis of efficacy and security of conventional TBNA, Agarwal et al. [5] suggest that in order to diagnose sarcoidosis, TBNA must be included in combination with TBLB as the routine method there, where it is not possible to introduce EBUS-TBNA.

Though TBNA was introduced over 60 years ago, first during rigid [11], later during flexible bronchoscopy [12], lots of authors consider it still as underused technics of obtaining material especially for the diagnosis of benign diseases such as sarcoidsis [5, 13−15].

It has been shown that the value of TBNA in the diagnosis of sarcoidosis could be influenced by various factors such as the stage of disease, needle type (whether it is cytological or histological), the number of patients, trained staff and experience both of bronchoscopist and cytologist/pathologist [5, 16−19].

In the last 10 years TBNA has been performed routinly in the Bronchoscopic Department of our hospital for staging of lung cancer, the diagnosis of hilar and mediastinal lymphadenopathy, as well as in the diagnosis of sarcoidosis.

In this retrospective study we presented our experience with this type of diagnostics for patients suspicion of sarcoidosis I and II stage, based on clinical and radiological data, with emphasis on estimation of value of cytological diagnostics.

Various age groups could he affected by sarcoidosis, but mostly persons younger than 40 years old. The average age of our patients was 40. However, though women got sick of sarcoidosis more frequently than men, in our group there were more men than women, while women were of older age, but those differences were of no statistical significance. The fact of not being in agreement with the literature regarding sex distibution was most probably due to relatively small number of analyzed patients.

Important fact in the morphological confirmation of sarcoidosis is the adequacy of material obtained with this precedure (TBNA). In our tested group, the adequate cytological material was obtained in 91.37% of the patients which is very close to the percentage (94%) of adequate cytological samples of lymph nodes in the results of the study by Trisolini et al. [16], in the group of the equal number of patients (53) with sarcoidosis, submitted to TBNA by 19 G needle.

Such good results for TBNA in both of papers were most probably associated with the highly skilled bronchoscopist, as well as the fact that samples were obtained mostly from subcarineal (number 7) and (in our group, less frequently) paratracheal nodes (number 10), and it is well-known that both localizations give best results with conventional TBNA [16, 20, 21]. However, it should be underlined here that these authors had adequate samples for histopathological analysis only in 51% of the patients, while we obtained it from 95.5% of the patients, for this type of analysis.

Considering that for both investigations samples were obtained from the same localised nodes, with the same needle type (19 G), it is the experience of pulmologist that affected the quality of the obtained material for this procedure.

Trisolini et al. [16] explain the smaller contribution of histological TBNA samples to the diagnosis of sarcoidosis by frequent use of thin cytological needles in the recent years, which could lessen the manipulative skill using the histological needles that were used previously for such investigations.

In our study the contribution of cytological TBNA material to the diagnosis of sarcoidosis is significantly smaller than histopathological one (71.69% positive cytological findings *versus* 90.56% positive histological findings).

If we include two of the patients diagnosed with NGI only cytologically to the number of the patients diagnosed with NGI histopathologically, then we confirmed sarcoidosis in 94.5% of the patients (in 50 out of 53 patients), by the method of TBNA.

Such a good result obtained by TBNA in our tested group, beside the manipulative skill and great experience of the bronchoscopist, is surely the consequence of the very design of this retrospective study to choose only patients with a high clinical suspicion of sarcoidosis, confirmed cytologically and/or histologically, since the purpose of this study was to estimate cytology in the diagnosis of sarcoidosis using histological needle.

On the contrary to our results, Trisolini et al. [16] had much higher contribution of cytological TBNA material than histological one in their group: in 79% (42 out of 53) of the patients sarcoidosis was confirmed cytologically, and in only 30% (16 out of 53) histopathologically.

Total diagnostic value of histopathological and cytological diagnosis of sarcoidosis in that study was 79% (42 out of 53 patients).

In our group of patients, we had more inadequate cytological samples as well as significantly less cytological samples with the elements of NGI, due to the fact that the best quality material, the compact part of material obtained with TBNA was separated for histopathology, and the rest, usually with the tinge of blood, was left and used for cytology.

Cetinkaya et al. [22] made the diagnosis of sarcoidosis in 87.5% of the patients (7 out of 8 patients) based on material obtained from TBNA during flexible bronchoscopy, also with the 19 G needle, but they did not declare the exact part of it regarding cytological and histopathological diagnostics. Having chosen a 22 G needle, they made the diagnosis of sarcoidosis in 76% of the patients (16 out of 20 patients) [23].

In our group of patients, out of 5 histopathological samples with no granulomatous inflammation found, two were inadequate (but the cytological findings for these patients were positive), while the three of samples were adequate but without signs of NGI, and for these patients cytology was negative as well, so they were true negative cytological findings (in all of these three patients the diagnosis of sarcoidosis was proven, later).

We had no false positive cytological findings, and the cause for false negative findings was not the interpretative fault but the insufficiency of elements for the granulomatous inflammation.

The sensitivity of cytological diagnostics in our group of patients was 76%, and accuracy 77.34%. In the paper by Smojver-Jezek et al. [24] the sensitivity of TBNA cytology in the diagnostics of sarcoidosis manifested with mediastinal/hilar lymphadenopathy was 78.7%, the accuracy 86.2%, and in 63.6% of the patients the only morphological diagnosis of sarcoidosis was a cytological one. However, TBNA was performed by the 26 G needle, which is used for obtaining samples only for cytological diagnostics.

Agarwal et al. [5] within the scope of systematic check-ups and meta-analyses estimated the diagnostic value and security of TBNA for sarcoidosis. On the basis of analysis of 12 studies that used 19 G needle and 9 studies that used cytological needles, they concluded that it was better to use 19 G needle for TBNA diagnostics of sarcoidosis. This type of needle gives the possibility to obtain histological and cytological material as well, therefore to contribute to sensitivity of TBNA.

In our cytological material, granulomatous inflammation was presented most frequently with dense aggregates of epitheloid cells (epitheloid hystiocytes), less frequently with

multinuclear, giant hystiocytes. Least frequent, in 15.76% of positive samples, we found epitheloid cells, single or in very small clusters out of few, while Smojver-Jezek et al. [24] found such a cytological picture in 25% of positive findings, considering it to be a specific finding consistent with sarcoidosis. Though such finding alone is not sufficient for the diagnosis of granuloma, we assumed that these authors interpreted findings along with the clinical and radiological data, as well. By all means, adding such samples into positive ones, contributes to the overall sensitivity and accuracy of cytological diagnostics for both studies.

To interprete cytological findings it is essential for the personnel to be qualified and experienced. Insufficient experience in the identification of granuloma in cytological specimens out of interthoracic lymphadenopathy could negatively influence the value of TBNA diagnostics, whether it is conventional one or EBUS-TBNA. Therefore Tremblay et al. [18] in their study compared conventional *versus* EBUS-TBNA on the patients with the suspicion of sarcoidosis, and showed that identification of granuloma in the same cytological material was significantly different between two of the pathologists involved in the study, and Chee et al. [19], in cytological estimation of EBUS-TBNA in sarcoidosis, found good interobserver agreement between cytopathologists, but even better results after the revision of slides by the pulmonary cytopathologist.

Cancellieri et al. [25] analyzed sarcoid granulomas in cytological specimens from interthoracic lymph nodes, and stated that two of the major mistakes in the diagnosis of granuloma on TBNA within cytological specimens are: a simple aggregate of epitheloid histiocytes – exhibiting a photo of a lymphohistiocytic aggregate (which is the common picture within the reactive hyperplasia of lymph node), and cohesive clusters of cylindrical epithelial cells.

We consider less likely for such mistakes to happen in the interpretation if it is done by the qualified and experienced pathologist/cytologist.

Histopathological verification of granulomatous lymphadenitis, as well as the biopsy of granulomatous changes within the lungs, could present itself as a huge differential and diagnostic issue, especially in the absence of significant clinical data.

The finding of granulomatous inflammation within interthoracic lymph nodes, require the exclusion from berylliosis, sarcoidosis-like lymphadenitis and also reaction on malignancy and anthracotic pigment [26, 27]. In our analyzed group of patients with sarcoidosis, histopathological confirmation of granulomatous disease was missing for 5 (8.62%) patients. For two patients it was due to inadequate material, and for three other, the material was adequate but without signs of NGI. The reasons for negative finding could be various.

In the early stage of the disease microscopic changes within lymph node could exhibit all of the characteristics of nonspecified lymphadenitis.

Epitheloid nodulus could be discrete and localized cortically. Multinuclear cells need not to be within each of the granulomas, not even in the whole histopathologically processed specimen of a lymph node. Sometimes, in the late phase of sarcoid lymphadenitis, the multiplication of the collagen fibers with hyalinization could be seen instead of granuloma [26, 27]. To differentiate the type of necrosis it usually requires obligatory use of histochemical staining method like Ziehl-Neelsen which we used for our patients to differentiate the nature of suspicious zones within granulomas looking like caseous necrosis, in order to exclude the presence of acidsfast bacilli.

Conclusion

TBNA is an efficient and secure procedure in the diagnosis of sarcoidosis, minimally invasive and with a little risk of complications.

With this procedure, using a thin 19 G needle, the material for histological and cytological analyses is obtained.

Though we found out cytological diagnostics of sarcoidosis significantly less sensitive than histological one, its advanage is in the fact that it is fast, as well as that it could substitute histological one, in cases with no adequate material for histological analysis, along with the positive clinical and radiological findings for stage I and II sarcoidosis.

It was found that significantly less sensitivity of cytological diagnosis in our research was the consequence of the fact that the compact, the best quality material was separated for histological, and only the rest of material was submitted for cytological analysis.

The value of this type of diagnostics depends much on qualification and experience of the bronchoscopist and cytologist/pathologist, as well as on interpretation of such a material.

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Total knee arthroplasty in patients with rheumatoid arthritis – midterm results

Totalna artroplastika kolena kod bolesnika sa reumatoidnim artritisom – srednjoročni rezultati

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Abstract

Background/Aim. Total knee arthroplasty in patients with rheumatoid arthritis is an effective method of eliminating pain and improving functional status, but it is associated with a number of unique challenges. The aim of this study was to evaluate the clinical and radiographic outcomes of total knee arthroplasty, as well as patient satisfaction, in this series of patients with rheumatoid arthritis. **Methods**. Between January 2001 and 2012, 108 total knee arthroplasties in 78 patients with rheumatoid arthritis were performed, utilizing a posterior-stabilized prostheses. The average age of the patients was 58 ± 12.4 years, and 88% were females. Median follow-up of patients was 80 months with interquartile range of 34 months (min-max: 36-132 months). **Results**. Average Knee Society score improved from preoperative 18 ± 11.4 to postoperative 83 ± 3.5, and Functional Knee Society score from 21 ± 9.9 to 50 ± 5.9. Western Ontario and McMaster Universities Arthritis (WOMAC) knee injury and osteopaedic outcome scores improved from 23 ± 6.4 to 69 ± 4.6 postoperatively. In 25 (23.14%) knees radiolucent lines of less than 2 mm were found and they were not progressive. Survival rate excluding deep infection was 99.1%. **Conclusion**. Total knee arthroplasty is an effective procedure of treatment of damaged knee joint in patients with rheumatoid arthritis.

Key words:
arthritis, rheumatoid; knee; arthroplasty; patient satisfaction; treatment outcome.

Apstrakt

Uvod/Cilj. Totalna artroplastika kolena kod bolesnika sa reumatoidnim artritisom je uspešna metoda u otklanjanju bola i poboljšanja funkcionalnog statusa, ali je povezana sa nekoliko jedinstvenih izazova. Cilj ove studije bio je procena kliničkih i radiografskih rezultata totalne artroplastike kolena, kao i zadovoljstvo bolesnika, kod ove serije ispitanika sa reumatoidnim artritisom. **Metode**. U periodu od januara 2001 g. do 2012 g. uradjeno je 108 totalnih artroplastika kolena kod 78 bolesnika sa reumatoidnim artritisom, korišćenjem endoproteze sa zadnjom stabilizacijom. Prosečna starost bolesnika iznosila je 58 ± 12, 4 godine, a 88% su bile osobe ženskog pola. Medijana vremena praćenja bolesnika bila je 80 meseci sa interkvartilnim opsegom od 34 meseca (min–max: 36–132 meseca). **Rezultati**. Prosečan *Knee Society score* (KSS) je bio poboljšan sa preoperativnih 18 ± 11,4 na postoperativnih 83 ± 3, 5, a *Functional Knee Society score* (FKSS) sa 21 ± 9, 9 na 50 ± 5, 9. *Western Ontario and McMaster Universities Arthritis* (WOMAC) skor bio je poboljšan sa 23 ± 6, 4 na 69 ± 4, 6 postoperativno. Kod 25 (23, 14%) kolena konstatovane su radiolucentne linije manje od 2 mm koje nisu bile progresivne. Stepen preživljavanja isključujući duboku infekciju je bio 99,1% u prosečnom vremenskom praćenju od 80 meseci. **Zaključak**. Totalna artroplastika je efikasna procedura lečenja oštećenog zgloba kolena kod bolesnika sa reumatoidnim artritisom.

Ključne reči:
artritis, reumatoidni; koleno; artroplastika; bolesnik, zadovoljstvo; lečenje, ishod.

Introduction

The knee joint is one of the most commonly affected joints in rheumatoid arthritis (RA) and one or both knees are affected in 90% of the cases of long-term rheumatoid arthritis. Despite of highly effective biological therapies, in approximately 20% to 25% of patients with rheumatoid arthritis, an advanced form of arthritis with joint destruction develops and the knee joint is the one most commonly affected [1]. Patients with rheumatoid arthritis have poor quality of bones and soft tissue due to the inflammatory disease itself, inactivity and application of corticosteroid therapy. In these

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patients, there are problems with regard to wound healing and the development of systemic infections due to existing immunosuppression [2]. In order to improve total knee arthroplasty (TKA) in these patients, significant considerations in terms of preoperative evaluation and surgical techniques are required [3]. The aim of this study is to evaluate the clinical and radiographic outcomes of total knee arthroplasty, as well as satisfaction in this series of patients with rheumatoid arthritis.

Methods

In the period from January 2002 to January 2012, at the III Female Department of the Institute for Orthopedic Surgery "Banjica" in Belgrade, Serbia, 108 posterior stabilized total knee arthroplasties were performed in 78 patients with rheumatoid arthritis. The average age of the patients was 58 ± 12.4 years, with an average body mass index of 26.0 ± 4.4 kg/m^2 and 88.0% were females. All patients had been previously treated for RA for at least 5 years. All total arthroplasties were performed with a standard medial parapatellar arthrotomy and resection of the posterior cruciate ligament, as well as complete synovectomy. Distal femoral cut was made with intramedullary guide and proximal tibial cut with extramedullary alignment guide. Knee balance was done by combined techniques. In varus and valgus deformities gradual medial and lateral release was done. Patella was replaced in all cases. Median follow-up time was 80 months with interquartile range of 34 months (min-max: 36-132 months). Patient evaluation was done preoperatively and postoperatively in a time period of 3, 6 and 12 months, and annually thereafter. Patients were clinically assessed preoperatively and postoperatively applying the clinical grading system of the Knee Society (Knee Society Clinical Rating System) and Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) [4, 5]. In order to evaluate the pain located in the knee after TKA, we used a visual analogue scale (VAS). The degree of patient satisfaction after total knee arthroplasty was measured by the visual analogue scale of satisfaction. Radiographic evaluation of the knee was performed preoperatively and postoperatively after 3,6 and 12 months and then annually. Radiological assessment was carried out according to the radiographic evaluation system of the Knee Society (Knee Society Roentgenographic Evaluation System) by making an anteroposterior and lateral knee X-ray in standing position and tangential X-ray of the patella (Merchant's view). Composite event, which was considered as the outcome of this study, included deep infection of wound and revision surgery.

Following a test of statistical normality, continuous variables are presented as mean ± standard deviation (SD) or median (interquartile range). Categorical variables are reported as counts with percentages. Continuous variables were compared using Student's *t*-test for paired groups. Differences in categorical variables were tested by χ^2-test or Fisher's test, as appropriate. Kaplan-Meier survival curves were used to show overall surviving during follow-up period and the difference between groups according to value of VAS of pain and VAS of satisfaction. Statistical significance was estimated by the Log-rank test. The receiver operating characteristic (ROC) analysis was used for assessing the accuracy of diagnostic tests. Correlation between the scores was assessed by Pearson's correlation test.

A $p < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS, version 18.0 (SPSS Inc) software package.

Results

Average preoperative Knee Society Score (KSS) increased from 18 ± 11.4 to 83 ± 3.5 postoperatively ($p < 0.05$); preoperative functional KSS from 21 ± 9.9 to 50 ± 5.9 ($p < 0.05$); and WOMAC score from preoperatively 23 ± 6.4 to postoperatively 69 ± 4.6 ($p < 0.05$) (Figure 1).

The average VAS score of pain < 20 was present in 77 (71.3%) of the patients. Average VAS score of satisfaction > 80 was present in 90 (83.3%) of TKAs.

Scaled outcomes of clinical scores are shown in Table 1. An excellent score is 80 to 100 points; very good, 60 to 79 points; good, 40 to 59 points; satisfactory, 20 to 39 points; and unsatisfactory less than 20 points.

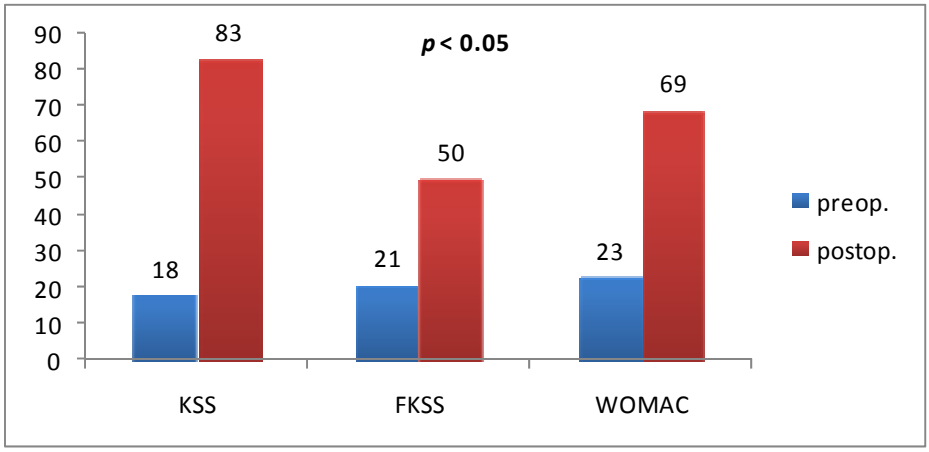

Fig. 1 – Comparison of clinical scores pre- and postoperatively.
KSS – Knee Society Score; FKSS – Functional Knee Society Score; WOMAC – Western Ontario and McMaster University Osteoarthritis Index.

Table 1

The results of clinical scores in knees with rheumatoid arthritis (RA)

Scores	Excellent	Very good	Good	Satisfactory	Unsatisfactory
Preop FKSS, n (%)	0 (0)	0 (0)	0 (0)	56 (51.9)	52 (48.1)
Preop FKSS, n (%)	0 (0)	0 (0)	0 (0)	53 (49.1)	55 (50.9)
Preop WOMAC, n (%)	0 (0)	0 (0)	0 (0)	72 (66.7)	36 (33.3)
Postop KSS, n (%)	90(88.3)	18 (11,7)	0 (0)	0 (0)	0 (0)
Postop FKSS, n (%)	0 (0)	4 (3.7)	96 (88,9)	8 (7.4)	0 (0)
Postop WOMAC, n (%)	4 (3.7)	103 (95.4)	1 (0.9)	0 (0)	0 (0)

KSS – Knee Society Score; FKSS – Functional Knee Society Score; WOMAC – Western Ontario and McMaster University Osteoarthritis Index.

In early postoperative radiographs and subsequent and final examinations on anteroposterior (AP) and lateral radiographs in standing position signs of loosening of endoprosthetic system were found in 3 patients and they underwent revisional surgery. Radiolucent lines of less than 2 mm were found in 25 (23.14%) knees which were not progressive. Average postoperative femoro-tibial angle was 6.2° (range 3.5 to 12.0°). In radiographic analysis of component position the average α (alpha angle) was 95° (range 84° to 101°), average tibial angle β was 89.1° (range 81° to 94°). The flexion of the femoral component, γ angle was 2.7° on average (range 0° to 14°), and the slope of the tibial component, angle δ was 88.4° on average (range 82° to 95°) (Figure 2).

In two (1.9%) patients intraoperative fracture of proximal tibia occurred, which was treated by closed reduction and osteosynthesis. Loosening occurred in one patient 72 months after the periprosthetic fracture and revision surgery was perfomed, while another patient's implant remained stable at follow-up. In two more cases there was loosening of the endoprosthetic system, one after 108 months and the other after 36 months, and revision surgery was done in both cases. One (0.9%) patient developed an infection 36 months after primary surgery. The treatment was carried out in two stages. In 2 (1.9%) patients aseptic loosening occurred, after which the treatment was carried out by applying the revision implant. In 2 (1.9%) patients there was a patellar luxation which was treated non-operatively. In one patient peroneal nerve palsy appeared, that only partially withdrew after the treatment. One patient developed deep vein thrombosis in the lower leg but it was cured without any consequences; in another case pulmonary embolism developed, but the patient recovered after the adequate treatment. Survival rate, excluding deep infection, in an average period of 80 months, was 99.1%. Regarding the survival rate with revision surgery as the final outcome, after 5 years it was 98.2%, and after 10 years the percentage decreased to 96.4%. In 4 (12.9%) patients who had VAS score of pain > 20 revision knee arthroplasty was executed during the monitoring period, while in patients with VAS of pain ≤ 20 there was no revision, which was a significant difference ($p = 0.001$) (Figure 3). VAS score of pain itself showed high discriminatory ability [area under curve (AUC) 0.931, $p = 0.003$] for prediction of revision surgery (Figure 4).

Out of 18 patients with VAS score satisfaction < 80, 3 (16.7%) of them had revision, i.e. out of 90 cases with VAS satisfaction ≥ 80, only 1 (1.1%) had revision which was a significant difference ($p = 0.001$) (Figure 5). VAS score of satisfaction itself showed high discriminatory ability (AUC 0.933, $p = 0.003$) for prediction of revision surgery (Figure 6).

Limiting values of VAS score of pain > 20 and VAS of satisfaction < 80 showed a high degree of sensitivity and specificity for predicting revision surgery (Table 2) in patients with RA. Sensitivity and specificity of VAS score of pain > 20 was 100% or 74.0%, respectively, and for VAS score

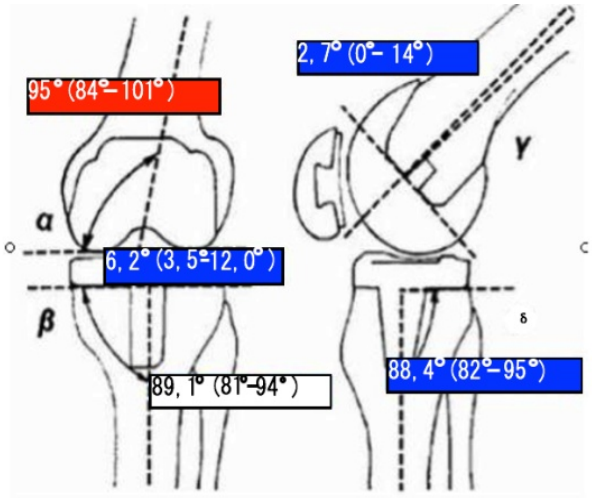

Fig. 2 – Radiographic analysis - the average angles components positions.

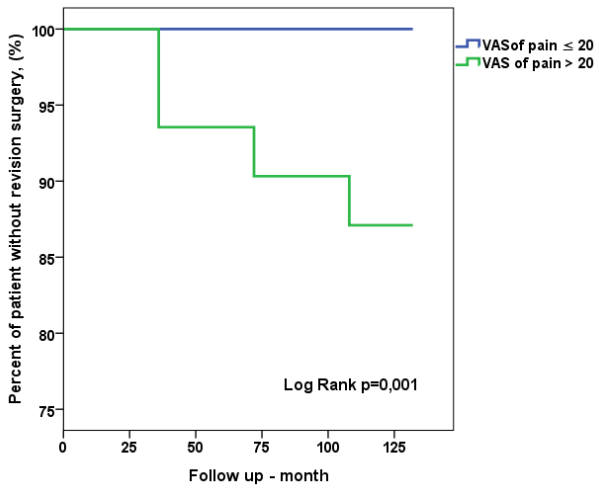

Fig. 3 – Kaplan Meier survival curve of patients with rheumatoid arthritis (RA) without revision during the follow-up depending on the perception of pain. VAS – Visual analogue scale.

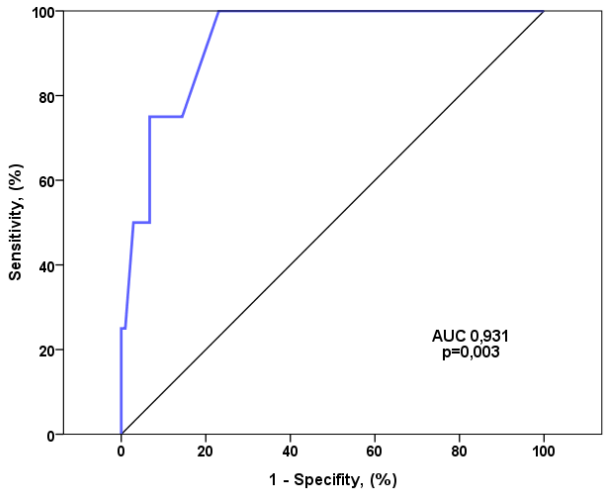

Fig. 4 – Receiver operating characteristic (ROC) curve of visual analogue scale (VAS) of pain for the revision surgery event occurrence.
AUC – area under curve.

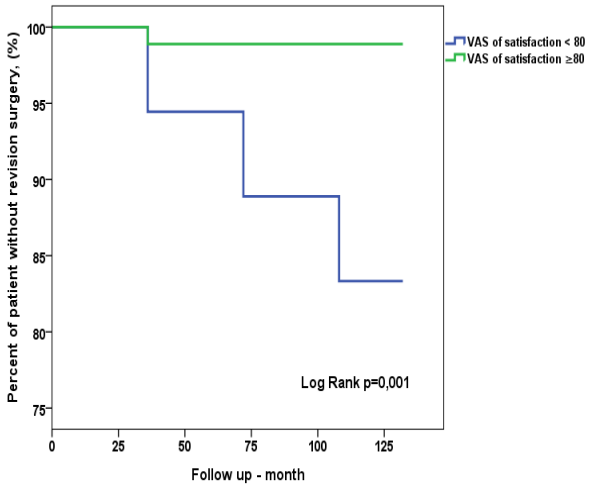

Fig. 5 – Kaplan Meier survival curve of patients with rheumatoid arthritis without revision during the follow-up depending on the perception of satisfaction.

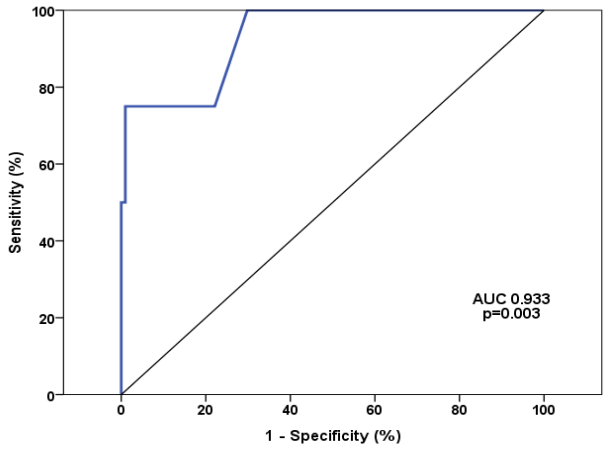

Fig. 6 – Receiver operating characteristic (ROC) curve of visual analogue scale (VAS) of satisfaction for the revision surgery event occurrence.
AUC – area under curve.

Table 2

Comparison of the representation of after surgery revision, depending on the "critical values" of visual analogue scale (VAS) of pain and VAS of satisfaction in a population of patients with rheumatoid arthritis

VAS score	With revision, n = 104	Without revision, n = 4	*p*
of pain > 20, n (%)	27 (26.0)	4 (100)	0,006
of satisfaction < 80, n (%)	15 (14.4)	3 (75.0)	0,014

of satisfaction sensitivity was 75.0% and specificity 85.6%.

Clinical score analysis showed that there was a significant correlation between VAS of satisfaction and VAS of pain with postoperative KSS, and somewhat lower correlation between VAS of satisfaction with postoperative FKSS and WOMAC score. Other correlations were not distinct (Table 3).

Disscusion

Total knee arthroplasty in patients with rheumatoid arthritis is a reliable procedure that reduces pain, increases range of motion and functional status of patients. Regarding the clinical outcomes of total knee arthroplasty in patients with RA and osteoarthritis (OA) there are well-documented studies, with a significantly smaller number of studies regarding the outcomes in patients with RA [6–8]. In our study, all patients had excellent outcomes in terms of reduction of pain and stability, but the functional scores were slightly lower. There are several reasons for that, but probably the main reason is that patients also had significantly lower preoperative scores, which is linked to socioeconomic factors, due to which patients came with established deformities and extremely poor function. In addition, these patients had other joints affected as well and therefore worse functional outcomes. Evaluating the outcome of total knee arthroplasty, it cannot be said that the KSS is a sensitive parameter because almost in majority

Table 3

Correlation of clinical scores

Variables	VAS score of pain		VAS score of satisfaction	
	r	*p*	r	*p*
Preop KSS	= 0.063	= 0.489	= 0.099	= 0.306
Preop FKSS	= 0.033	= 0.736	= 0.045	= 0.644
Preop WOMAC	= 0.026	= 0.789	= 0.037	= 0.707
Postop KSS	= 0.411	< 0.001	= 0.579	< 0.001
Postop FKSS	= 0.293	= 0.002	= 0.346	< 0.001
Postop WOMAC	= 0.321	= 0.002	= 0.349	< 0.001

VAS – visual analogue scale; KSS – Knee Society Scale; FKSS – Functional Knee Society Score; WOMAC – Western Ontario and MC Master Universities Osteoarthritis Index.

of the presented studies it was higher than 80. Also, the radiographic findings themselves are not specific in terms of predicting the outcomes of TKA, because of the lack of correlation between the occurrence of thin radiolucent lines and clinical outcomes. Therefore, the introduction of additional scores for assessing the outcomes, WOMAC score, would probably result in a more comprehensive picture of the outcome of TKA. In the opinion of the orthopedic community, however, although using more than one scoring system produces a reliable result in terms of the efficiency of the procedure, all of them are mainly the result of patients'response to the level of pain, function, return to specific activities and the medical objective measurement of motion range and stability. However, in addition to the above, the outcomes of total knee arthroplasty may also be affected by patients' expectations [9].

Patients' satisfaction is an important parameter of successfully performed total arthroplasty [10]. There is a well-known discrepancy between rating the success of TKA by the operator and by the patient, i.e. there are certain discrepancies with regard to improving the quality of life of patients with total knee arthroplasty. Numerous studies show that the degree of patients' satisfaction after total arthroplasty is only 82% to 89% [11, 12].

In our study, the degree of satisfaction of patients with RA was exceptional, only 16.7% had < 80. One major reason was probably patients' expectations regarding TKA, in addition to other factors such as low preoperative scores and complications. In order to find an adequate explanation for this degree of satisfaction with total knee arthroplasty, we tried to determine the connection between VAS score of satisfaction and scores applied. We found a significant correlation between VAS score of satisfaction and postoperative KSS, FKSS and less strong correlation with postoperative WOMAC score. In addition, we found a significant correlation between VAS score of pain and VAS score of satisfaction and marked VAS score of pain and VAS score of satisfaction as significant predictors of revision surgery. Based on this, we can say that the elimination of pain, along with well performed total knee arthroplasty, correlates well with quality of life and patients' satisfaction, with the additional observation that VAS score of pain and satisfaction, as independent predictors of TKA revision surgery, represent an important parameter for predicting success of total knee arthroplasty.

Survival rate of total arthroplasty ranges from 81% to 98.2% depending on the presented studies and depending on the set parameters concerning the index event, where revision was most often cited as the final event [13]. In our study the survival rate depending on the selected scenario was: excluding deep infection, survival rate in patients with RA in an average period of 80 months was 99.1% and in patients with RA within the same monitoring time 97.5% (best scenario). The survival rate with revision surgery as the final outcome in patients with RA was 98.2% after 5 years, and after 10 years the percentage decreased to 96.4%, while in patients with primary osteoarthritis, survival rate showed a smaller rate of decline, from 95% to 94%. During the average follow-up time of 80 months, survival rate of TKA in patients with RA was 97.1%, and in patients with OA 95%.

By introducing another parameter associated with TKA into consideration, VAS score of pain, the survival rate in total population of patients with VAS of pain ≤ 20 was 100%, while in patients with VAS score of pain > 20 during the average period of 80 months it was 84%, which clearly indicates a high discriminatory ability of VAS score of pain to predict revision surgery. In the same specified conditions, survival rate in patients with RA in cases where VAS score of pain was ≤ 20 was 100%, and when it was > 20, survival rate was 90% during the average monitoring period. The survival rate of patients with OA and VAS score of pain ≤ 20 was 100%, while with VAS score of pain > 20 it was 78%. In both groups VAS score of pain showed high discriminatory ability for revision surgery of TKA.

Analyzing the survival rate with revision surgery as the final outcome and adding the parameter of patients' satisfaction with their TKA, within the average follow-up period, the survival rate of patients in the total population of patients with VAS of satisfaction ≥ 80 was 99%, and whenVAS of satisfaction was < 80, the survival rate was 90%. In the same conditions, the survival rate in patients with RA was: VAS of satisfaction ≥ 80 – survival rate of 98%, and VAS of satisfaction < 80 – survival rate 88%. In patients with PA in the same specified conditions, with VAS of satisfaction ≥ 80, the survival rate was 100%, and with VAS of satisfaction < 80, the survival rate was 91%.

Survival rate of total arthroplasty in our study is consistent with other studies known to us.

In terms of complication rate of total knee arthroplasty in patients with RA, there are several studies that indicate the existence of a higher complication rate compared to TKA in primary knee arthrosis [14, 15], as well as others which do not mention a greater complication rate [6, 16]. There are many rea-

sons why patients with RA, compared to those with OA, may be at greater risk of early complications after total knee arthroplasty. Firstly, it is the effect of drugs that modify the disease, systemic corticosteroid application, use of biological drugs, all of which act through immuno modulation and are associated with delayed wound healing and increased risk of postoperative infection [17, 18]; furthermore, systemic nature of RA contributes to the occurrence of multiple comorbiditities [19]. A particular problem is synovitis itself, so that patients with RA compared to those with primary osteoarthritis have more tender soft tissues, weakened tendons, ligaments and enthuses [20]. Patients with RA often have osteopenia or osteoporosis and altered periarticular bone anatomy [21]. These anatomical changes represent an additional problem during the treatment of soft tissue, establishment of joint shaft and balance, component fixation and establishment of good articular mechanics after TKA. In our study, the infection rate was 0.9% and a deep infection was formed in only one patient. This rate of post-operative infection is consistent with some studies, while being significantly lower than in others. The probable reason for this could be a comprehensive and thorough preoperative preparation, the involvement of other specialists, balanced use of drugs in order to avoid infection and worsening of RA, strict application of the basic principles of total arthroplasty, prophylactic use of antibiotics, and an experienced surgical team which, in addition to a well-performed total arthroplasty, reduced the time of the surgery to the minimum. The rate of other complications in this study was consistent with the studies known to us.

Conclusion

Total knee arthroplasty is one of the most successful surgical procedures performed with the aim of reducing pain and improving functional status of patients with rheumatoid arthritis. It is necessary to fully understand the systemic nature of rheumatoid arthritis for a successful total knee arthroplasty, because these patients often have accompanying diseases, anesthesiological and multiple musculoskeletal problems with considerable potential for complications. Despite all the complex conditions that are often encountered in patients with rheumatoid arthritis, a timely, well performed total knee arthroplasty becomes an effective procedure with a moderate degree of complication and can lead to improvements in global function and quality of life of patients with rheumatoid arthritis.

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Blood concentrations of B-type natriuretic peptide and N-terminal prohormone B-type natriuretic peptide as markers of left ventricle diastolic function in patients with chronic renal failure

Koncentracije B-tipa natriuretskog peptida i N-terminalnog prohormon B-tipa natriuretskog peptida u krvi kao pokazatelji dijastolne funkcije leve komore kod bolesnika sa hroničnom bubrežnom insuficijencijom

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Abstract

Background/Aim. Patients with chronic renal failure (CRF) have increased cardiovascular morbidity and mortality. It is unknown which biomarkers best describe the degree of diastolic dysfunction in patients with CRF. The aim of this study was to determine the correlation between B-type natriuretic peptide (BNP), N-terminal prohormone brain natriuretic peptide (NTproBNP) and left ventricular diastolic dysfunction (DD-LV) with the degree of CRF. **Methods.** The study included 100 adult patients with CRF without major cardiac and cerebral incidents who did not start actively treating CRF. According to the degree of CRF, the patients were divided into two groups: G1 (moderate degree), glomerular filtration rate (GFR) ≥ 30 mL/min/1.73 m², and G2 (more severe degree), GFR < 30 mL/min/1.73 m². Blood concentrations of BNP and NTproBNP were measured and Doppler echocardiographic measurement performed to estimate diastolic dysfunction (DD-LV). According to the degree of DD-LV, all the patients were divided into two groups: DD-LV1 (mild diastolic dysfunction) and DD-LV2 (severe diastolic dysfunction). According to the degree of CRF and DD-LV, the patients were divided into four groups: I (G1, DD-LV1), II (G1, DD-LV2), III (G2, DD-LV1) and IV (G2, DD-LV2). **Results.** There was a highly significant statistical correlation between BNP and NTproBNP with GFR ($p < 0.001$), and DD-LV with BNP ($p < 0.023$) and NTproBNP ($p = 0.035$). In patients with DD-LV2, a statistically significantly higher BNP concentrations were registered in patients with G2 ($p < 0.001$). Unlike BNP in the patients with diastolic dysfunction DD-LV1 and those with diastolic dysfunction DD-LV2, significantly higher concentrations of NTproBNP were registered in the patients with G2 (DD- LV1: $p = 0.006$; DD-LV2: $p < 0.001$). **Conclusion.** Biomarkers BNP and NTproBNP are not the best predictors in the assessment of diastolic dysfunction because they are correlated with the degree of renal insufficiency.

Key words:
kidney failure, chronic; glomerular filtration rate; ventricular function, left; natriuretic peptides; biological markers; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Bolesnici sa hroničnom bubrežnom insuficijencijom (HBI) imaju povećan kardiovaskularni morbiditet i mortalitet. Nije poznato koji biomarkeri najbolje opisuju stepen dijastolne disfunkcije kod bolesnika sa HBI. Cilj ove studije bio je da se utvrdi povezanost koncentracije B-tipa natriuretskog peptida (BNP), N-terminalnog prohormon moždanog natriuretskog peptida (NTproBNP) i dijastolne disfunkcije leve komore (DD-LV) sa stepenom HBI. **Metode.** U studiju je bilo uključeno 100 odraslih bolesnika sa hroničnom bubrežnom bolesti koji nisu imali srčane i cerebralne incidente i nisu započeli akti-

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vno lečenje HBI. Prema stepenu HBI bolesnike smo podelili u dve grupe: G1 (umereni stepen), glomerularna filtracija (GFR) ≥ 30 mL/min/1,73 m², i G2 (teži stepen), GFR < 30 mL/min/1,73 m². U krvi su određene koncentracije BNP i NTproBNP, a dopler ehokardiografskim merenjem procenjena je dijastolna disfunkcija leve komore (DD-LV). Prema stepenu DD-LV bolesnici su podeljeni u dve grupe: DD-LV1 (blaga dijastolna disfunkcija) i DD-LV2 (teška dijastolna disfunkcija). Prema stepenu HBI i DD-LV bolesnici su podeljeni u četiri grupe: I (G1, DD-LV1), II (G1, DD-LV2), III (G2, DD-LV1) i IV (G2, DD-LV2). **Rezultati.** Utvrđena je visokoznačajna statistička korelacija BNP i NTproBNP sa GFR ($p < 0,001$), a DD-LV sa BNP ($p < 0,023$), odnosno NTproBNP ($p = 0,035$). Registrovane su statistički značajno više koncentracije BNP kod bolesnika sa DD LV2, nego kod bolesnika sa G2 ($p < 0,001$). Za razliku od BNP, kod bolesnika sa disfunkcijom DD-LV1, ali i onih sa disfunkcijom DD-LV2, registrovane su statistički značajno veće koncentracije NTproBNP kod bolesnika sa G2 (DD-LV1: $p = 0,006$; DD-LV2: $p < 0,001$). **Zaključak.** Biomarkeri BNP i NTproBNP nisu najbolji prediktori u proceni dijastolne disfunkcije jer su u korelaciji sa stepenom hronične bubrežne insuficijencije.

Ključne reči:
bubreg, hronična insuficijencija; glomerulska filtracija; srce, funkcija leve komore; natriuretski peptidi; biološki pokazatelji; senzitivnost i specifičnost.

Introduction

Patients with chronic renal failure (CRF) have increased cardiovascular risk for development of cardiovascular morbidity and mortality [1–4]. In these patients more than 50% of death outcomes is due to cardiovascular diseases [5], while before dialysis 40% of patients have cardiovascular diseases [6]. Until now, heart failure has been most frequently accounted to the patients with end-stage renal disease (ESRD) who are already on dialysis. There are only a few studies which have examined the correlation between mild decrease of renal function and cardiac function [7–9]. It has been proved than even the mildest damage of renal function (GFR < 90 mL/min/1.73 m^2) significantly increases cardiovascular risk [10–12].

Determination of non-traditional cardiovascular risk factors and cardiac biomarkers in patients with CRF revealed increased cardiovascular morbidity and mortality in the second and third stage of chronic renal disease [13]. Diastolic dysfunction (DD) usually precedes the left ventricular (LV) systolic dysfuntion [14] and it is related with morbidity and mortality in patients with ESRD on dialysis [15]. Diastolic dysfunction in patients with preserved systolic function and ESRD is also a significant risk factor for cardiovascular morbidity and mortality [16, 17]. The European Society of Cardiology suggests that natriuretic peptide (BNP) or N-terminal pro b-type natriuretic peptide (NTproBNP) may serve as markers of heart failure because they point to the heart failure in patients with preserved left ventricular ejection fraction [18]. NTproBNP is also a useful non-invasive marker of atherosclerosis in the renal disease [13].

The aim of our study was to determine if BNP, NTproBNP and the left ventricular diastolic dysfunction may serve as predictors of cardiovascular risk in patients with chronic renal failure at the pre-dialysis stage.

Methods

After obtaining Institutional Ethical Committee's approval, 100 adult patients with CRF were included in the prospective sectional study. All the patients were examined and recruited in our Outpatient Department. Study inclusion criteria comprised CRF patients without previous cerebral or heart complications or current active treatment for CRF. According to the stage of the renal function, glomerular filtration rate (GFR), clearens of creatinine was determined using the formula CKD-EPI ($GFR = 141 \times \min(S_{cr}/\kappa, 1)^{\alpha} \times \max(S_{cr}/\kappa, 1)^{-1.209} \times 0.993^{age} \times 1.018$ [if a female]) and expressed as mL/min/1.73 m^2 [19] divided initially into five groups: (GI) GFR 90−120 mL/min; (GII) GFR 60−89 mL/min; (GIII) GFR 30−59 mL/min; (GIV) GFR 15−29 mL/min i (GV) GFR ≤ 15 mL/min. Afterwards, all the patients were divided into two groups. The first group, G1 with GFR ≥ 30 mL/min/1.73m^2, comprised of totally 60 patients with milder renal insufficiency (GFR I-III). The second group G2 with GFR < 30 mL/min, comprised of totally 40 patients with more severe degree of the renal insufficiency (GFR IV−V). Study exclusion criteria comprised of patients without previous cardiovacular and cerebrovascular diasease, malignancies, autoimmune disorders and other chronic diseases which might have an impact on results.

Blood samples for determination of BNP and NTproBNP concentration were harvested after 12 h of starvation. Analysis were performed using a SIMENS/ADVIA Centaur XP : principle CMIA (direct chemiluminescence, Munich-Germany, 2012), and the levels of NT-proBNP in plasma were determined by electroluminescence assay Roche Cobas e 601: principle "ECLIA„ (Basel, Switzerland, 2004).

Echocardiographic study was performed on a Simens 141148, type Acuson SC 2000, with a matric probe 4D for 2D live heart recording 4 z1c (Munich, Germany, 2012). Diastolic function was determined by measuring the following echocardiographic parameters in M-mode: E and A wave velocity, E/A ratio, isovolumetric relaxation time (IVRT), Doppler tissue echocardiography (DTE) as well as by tissue Doppler imaging measurement of E/Ea ware velocities.

According to the degree of DD-LV all the patients were divided into two groups: DD-LV1 – mild diastolic dysfunction (DD-N, DD-I), and DD-LV2, severe diastolic dysfunction (DD-II, DD-III, DD-IV).

According to the degree of CRF and DD-LV all the patients were divided into four gropus: I (G1, DD-LV1), mild chronic renal disease and mild diastolic dysfunction (n = 11), II (G1, DD-LV2), mild chronic renal failure and severe diastolic dysfunction (n = 48), III (G2, DD-LV1), severe chronic

renal failure and mild diastolic dysfunction (n = 5); and IV (G2, DD- LV2), severe chronic renal failure and severe diastolic dysfunction of the left ventricle (n = 36).

Results

Within a period from June 1, 2014 to March 31, 2015 we recruited and analyzed 100 patients of whom 61 were men and 39 women. The demographic data were as follows: mean age 56.75 ± 10.60, average body mass index (BMI) 27.25 ± 4.02 kg/m^2, active smokers 20%, former smokers 29% and non-smokers 51%. Arterial hypertension (HTA) was noted in 93% of the patients. Mean sistolic blood pressure was 132.30 ± 17.98 mmHg and mean diastolic blood pressure was 81.60 ± 10.02 mmHg (Table 1).

The most frequent causes of renal disease were chonic glomerulonephritis (20), kidney stones (19), arterial hypertension (18), kidney cysts (13), diabetes mellitus (13), adult policystic kidney disease (12), nephritis tubulointerstitialis (3) and isolated erythrocyturia (2).

The distribution of basic causes of renal disease for which patients required physician's attention and the most frequent disorder that caused ESRD was not equal (Table 1).

Comparison of the concentrations of BNP and NTproBNP in the group of patients with different degree of CRF (G1 and G2) revealed a highly significant statistical corellation $p < 0.001$. In the patients with different degree of diastolic dysfunction (DD-LV1 and DD-LV2) a statistically significant difference was oberved for BNP ($p = 0.023$ and for NTproBNP $p = 0.035$) (Table 2).

Table 1

Baseline characteristics of the patients in relation to glomerular filtration rate (GFR) groups

Parameters	Total	Glomerular filtration rate groups		Probability
		G1	G2	(*p*)
Age (years), mean	56.75	55.95	57.28	0.54
BMI (kg/m^2), mean	27.25	26.69	27.63	0.25
Gender (%)				
male	61.0	60.0	61.7	0.97
female	39.0	40.0	38.3	
Smoking (%)				
nonsmoker	51.0	52.5	50.0	
former smoker	29.0	27.5	30.0	0.96
current smoker	20.0	20.0	20.0	
Arterial hypertension (%)	93.0	97.5	90.0	0.29
Basic disorder, % (n)				n.s.
chronic glomerulonephritis	20	27.5	15.0	
nephrolithiasis	19	5.0	28.3	
arterial hypertension	18	25.0	13.3	
diabetes mellitus	13	15.0	11.7	n.s.
cystic kidney disease	13	0.0	21.7	
adult polycystic renal disease	12	20.0	6.7	
tubulo-interstitial nephritis	3	7.5	0.0	
isolated erithrocyturia	2	0.0	3.3	
Total	100	100	100	

BMI − body mass index; G1 − GFR ≥ 30 mL/min/1.73 m^2 (modest degree of the chronic renal failure); G2 − GFR < 30 mL/min/1.73 m^2 (more severe degree of the chronic renal failure).

Table 2

Clinical and biochemical parameters in relation to glomerular filtration rate (GFR) and LVDD groups

Parameters	Median (interquartile range)		Mann-Whitney
	G1	G2	test
BNP (pg/mL)	20.2 (7.5−44.1)	54.9 (22.7−135.5)	z = 3.846 $p < 0.001$
NTproBNP (pmol/L)	8.7 (4.3−20.5)	73.6 (29.4−210.0)	z = 6.116 $p < 0.001$
	DD–LV1	DD–LV2	
BNP (pg/mL)	15.7 (6.8−50.8)	29.0 (14.5−88.4)	z = 2.26 $p = 0.023$
NTproBNP (pmol/L)	9.7 (5.2−35.3)	24.5 (7.6−90.6)	z = 2.11 $p = 0.035$

BNP − B-type natriuretic peptide; NTproBNP − N-terminal pro-brain natriuretic peptide; DD-LV1 − milder degree of the left ventricular diastolic dysfunction (LVDD); DD-LV2 − more severe degree of LVDD; G1 − GFR ≥ 30 mL/min/1.73 m^2 (modest degree of the chronic renal failure); G2 − GFR < 30 mL/min/1.73 m^2 (more severe degree of the chronic renal failure).

Comparison of the concentration of BNP with the group of patients with the same degree of GFR impairment but with the different degree of left ventricular diastolic dysfunction (Table 3) did not reveal a statistically significant difference (G1: $p = 0.124$; G2: $p = 0.281$). In the patients with diastolic dysfunction DD-LV2, higher statistically significant concentration of BNP were registered in the patients with simultaneous dysfunction G2 ($z = 3.498$; $p < 0.001$) (Table 3).

in the group G V (50.8 years). The specificity of comparison existed between the second and the fifth group of GFR, as well as with the fourth and the fifth group of GFR ($p < 0.01$). Comparison of GFR and BMI did not shows statistically significant difference ($p > 0.188$). Comparison of age and diastolic dysfunction revealed that patients below 50 years (mean age 48.27) had normal left ventricular systolic function, but the patients from 50 to 70 years had diastolic

Table 3

Concentration of BNP and NTproBNP regarding the corresponding clinical groups

Parameter	Groups	Number of patients, median (interquartile range)		Probability
		DD-LV1	DD-LV2	
BNP (pg/mL)	G1	n = 11 15.2 (1.7−37.2)	n = 48 22.0 (9.6−44.1)	z = 1.538 $p < 0.124$
	G2	n = 5 44.7 (15.8−56.6)	n = 36 57.3 (23.4−142.0)	z = 1.127 $p = 0.281$
Probability		z = 1.567 $p = 0.138$	z = 3.498 $p < 0.001$	
NTproBNP (pmol/L)	G1	n = 11 6.6 (3.8−11.7)	n = 48 9.2 (4.3−23.6)	z = 0.983 $p = 0.326$
	G2	n = 5 60.6 (24.8−69.3)	n = 36 80.0 (29.7−219.5)	z = 1.037 $p = 0.324$
Probability		z = 2.61 $p = 0.006$	z = 5.595 $p < 0.001$	

BNP − B-type natriuretic peptide; NTproBNP – N-terminal pro-brain natriuretic peptide; DD-LV1 − milder degree of the left ventricular diastolic dysfunction; DD-LV2 − more severe degree of the left ventricular diastolic dysfunction; G1 − glomerular filtration rate (GFR) ≥ 30 mL/min/1.73 m^2 (modest degree of the chronic renal failure); G2 − GFR < 30 mL/min/1.73 m^2 (more severe degree of the chronic renal failure).

Comparison of the concentration of NTproBNP with the group of patients with the same degree of GFR impairment but with the different degree of left ventricular diastolic dysfunction (Table 3) did not reveal a statistically significant difference (G1: $p = 0.326$; G2: $p = 0.324$). Contrary to BNP, in the patients with left ventricular diastolic dysfunction DD-LV1, as well as those with DD-LV2, a statistically significantly higher concentration of NTproBNP was registered in the patients with simultaneous diastolic dysfunction G2 (DD- LV1: $p = 0.006$; DD-LV2: $p < 0.001$) (Table 3).

Normal diastolic function (DD-N) was observed in 14% of the patients while diastolic dysfunction (DDI, II, III) was observed in 86% of the patients. The most severe degree of diastolic dysfunction (DD-IV) was not observed in any single patient. However, DD-III was observed in the patients with GFR ≤ 60 mL/min/1.73 m^2.

The impaired values of diastolic dysfunction, BNP and NTproBNP were more prevalent among men.

The distribution of impaired values according to gender (men-women) was as follows: DD 54 (90.1%) : 31 (79.5%), BNP 22 (55%) : 18 (45%), NTproBNP (52.5%) : 19 (47.5%), respectively.

Comparison of age in respect to GFR showed a statistically significant difference ($p < 0.001$). In the groups G II and G IV were the oldest patients (61.80 *vs* 61.10 years, respectively). On the other hand, the youngest patients were dysfunction (DDI, DDII and DDIII degree). A correlation of diastolic dysfunction and age revealed a statistically significant difference ($p < 0.001$).

According to the BMI all the patients were classified as moderately obese (BMI: 25−29.9 kg/m^2). Comparison of diastolic dysfunction and BMI did not reveal a statistically significant difference ($p > 0.220$).

Discussion

The values of BNP, NTproBNP and left ventricular diastolic dysfunction were corellated with the stage of chronic renal failure in hundred patients, divided in two groups, G1 and G2. The group G1 comprised of 60 patients with mild degree of CRF (GFR > 30 mL/min), while the group G2 included 40 patients with more severe degree of CRF (GFR < 30 mL/min).

Comparison of concentration of BNP and NTproBNP with the stage of chronic renal disease (G1 and G2) showed a highly statistical significant difference ($p \leq 0.001$). Comparison of the concentration of BNP and NTproBNP with the degree of left ventricular diastolic dysfunction revealed a statistically significant difference for BNP ($p = 0.023$), and for NTproBNP ($p = 0.035$), respectively. In the patients with diastolic dysfunction DD-LV2 a statistically higher concentration of BNP was registered simultaneously with diastolic dysfunction G2 ($z = 3.498$; $p < 0.001$). In contrary to BNP,

in the patients with diastolic dysfunction DD-LV1 as well as in those with simultaneous diastolic dysfunction DD-LV2, a statistically higher concentration of NTproBNP was registered in the patients with simultaneous diastolic dysfunction G2 (DD-LV1: $p = 0.006$; DD-LV2: $p < 0.001$). A positive correlation was obtained by comparing BNP, NTproBNP [19–21] and diastolic dysfunction with the stage of chronic renal disease [22, 23]. Statistical analysis by comparison data for BNP, NTproBNO, left ventricular diastolic dysfunction, age and stage of chronic renal disease revealed a statistically significant difference ($p < 0.001$).

The most signifficant finding was obtained by correlation of diastolic dysfunction with the stage of chronic renal disaease where we noticed impaired diastolic function of the left ventricle (DDI, II, III degrees) in 86% of the patients. Impaired diastolic dysfunction of the left ventricle was present already in the group G-I in 60% of the patients, in G-II, G-III and G-IV in 90% of the patients, and in G-V in terminal stage of the chronic renal disease in 95% of the patients. The most frequent was DD-II in 57% of the patients, thereafter DD-I in 24% of the patients, while the most rare was DD-III in 5% of the patients. DDIII of severe degree was not present in the groups G-I and G-II, however it was present in the groups GIII, GIV and GV in the patients whose GFR was ≤ 60 mL/min/1.73 m^2. The most severe degree of diastolic dysfunction (DD-IV) was not observed in any single patient.

Our results show, as in some other studies, that even the smallest impairment of renal function GFR < 90 mL/min/1.73 m^2 is related to the signifficant increase of cardiovascular risk [11, 24–26].

A higher increase in the concentration of NTproBNP observed in the third stage of chronic renal disease was in accordance with the previous study [13] which allows us to explain why this is a risk group with an increased risk for cardiovascular morbidity and mortality. Concentration of BNP was in positive correlation with the degree of GFR, too. In the group G-I an increased concentration of BNP was immanent in 5% the patients, and in the group G-V in 65% of the patients, respectively.

Comparison of diastolic dysfunction with the concentrations of BNP and NTproBNP showed that an increased concentration of BNP and NTproBNP was followed by the degree of diastolic dysfunction up to the moderate level (DD-II). Noteworthy, DD-III and increased concentration BNP were frequently observed in women while NTproBNO in men.

A correlation of gender, BMI, and smoking status with GFR, diastolic dysfunction and an increased concentration of BNP and NTproBNP, did not show a signifficant difference. Comparison of left ventricular function with age showed that patients over 48 had impaired diastolic function.

The results shown in our group of patients might have greater importance because they point out to the signifficant impairment of diastolic function with only moderate impairment of glomerular filtration G-II (GFR < 90 mL/min/1.73 m^2). An increased concentration of BNP and NTproBNP are in correlation with GFR. Diastolic dysfunction of the left ventricle is in correlation with the stage of chronic renal disease, arterial hypertension and its evolution as well as with age. Non-conventional cardiovascular risk factors and cardiac biomarkers during the early stage of chronic renal failure may serve as predictors of heart failure.

Conclusion

The values of BNP, NTproBNP and diastolic dysfunction are in correlation with the degree of renal failure. The impaired values of these parameters are also present in the early stages of renal failure, so they may serve for estimation of cardiovascular risk in patients with chronic renal failure at the predialysis stage in whom high cardiac morbidity and mortality can be expected.

We think that the simultaneous follow-up of renal function and cardiovascular risk is neccessary in patients with chronic renal failure at the predialysis level.

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The influence of various risk factors on the strength of pelvic floor muscle in women

Uticaj različitih faktora rizika na jačinu mišića poda karlice kod žene

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Abstract

Background/Aim. Damage of any element of pelvic floor leads to its functional damages, reflected in the occurrence of urinary incontinence, prolapse of pelvic organs, fecal incontinence and sexual dysfunction. Basic aim of our paper was to investigate the influence of various risk factors on pelvic floor muscle strength in women. **Methods.** The study included 90 female patients and examined how age, job, body weight and height, number of deliveries, sports activities, incontinence occurrence, previous prolapse-caused gynecological surgeries, other gynecological surgeries and other conservatively treated gynecological diseases influence the value of pelvic floor muscle strength. Pelvic floor muscle strength was measured using vaginal dynamometer. **Results.** Univariate regression analysis showed that parameters such as age, demanding job, body height, number of deliveries, sports activities, prolapse-caused gynecological surgeries, other gynecological surgeries and other gynecological diseases were in positive correlation with the values of pelvic floor muscle strength. In multivariate regression model, incontinence and gynecological operation of prolapse were singled out as independent risk factors. **Conclusion.** If risk factors that cause damage to pelvic floor muscle are known, it is possible to prevent the damages and improve the quality of women's life.

Key words:
pelvic floor; muscle tonus; women; risk factors; pelvic organ prolapse; pelvic inflammatory disease; urinary incontinence; physical and rehabilitation medicine.

Apstrakt

Uvod/Cilj. Slabost mišića poda karlice dovodi do funkcionalnih oštećenja, uključujući urinarnu i fekalnu inkontinenciju, prolaps organa karlice i seksualnu disfunkciju. Cilj našeg istraživanja bio je ispitivanje uticaja različitih faktora rizika na slabost mišića poda karlice kod žena. **Metode.** U studiju je bilo uključeno 90 žena. Ispitivana je kako starosna dob, težina posla kojim se bave, telesna masa i visina, broj porođaja, bavljenje sportskim aktivnostima, prisutnost inkontinencije, prethodne ginekološke intervencije i lečenje prolapse organa male karlice, kao i druge bolesti, utiču na snagu mišića poda karlice. Snaga mišića poda karlice merena je vaginalnim dinamometrom. **Rezultati.** Univarijantnom regresionom analizom pokazano je da su faktori kao što su starost, težina posla, telesna visina, broj porođaja, bavljenje sportskim aktivnostima, prethodno ginekološko lečenje uzrokovano prolapsom organa male karlice i druge ginekološke intervencije i bolesti, u pozitivnoj korelaciji sa slabošću mišića poda karlice. Multivarijantnom regresionom analizom pokazano je da su ginekološke operacije prolapsa organa karlice i inkontinencija nezavisni faktori rizika. **Zaključak.** Poznavanjem faktora rizika koji dovode do slabosti mišića poda karlice mogu se prevenirati oštećenja i poboljšati kvalitet života žena.

Ključne reči:
karlica, pod; mišići, tonus; žene; faktori rizika; karlični organi, prolaps; karličlni organi, zapaljenske bolesti; inkontinencija, urinarna; medicina, fizikalna i rehabilitacija.

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Introduction

One in four American women has moderate to severe symptoms of at least one pelvic floor disorder [1]. Up to one in seven undergoes surgery for pelvic organ prolapse and/or urinary incontinence in her lifetime [2–4]. Advancing age, childbirth, obesity and race are associated with both pelvic organ prolapse and urinary incontinence [5–10]. Other risk factors, such as hysterectomy, hormone therapy and family history, have been less well explored. The rate of pelvic floor disorders is expected to increase dramatically with the aging of the American population [11]. Understanding modifiable risk factors is crucial. Preventing 25% of women from developing pelvic floor disorders would save 90 000 women *per* year from experiencing prolapse or incontinence. "Pelvic floor disorders", a term used to describe conditions related to changes in anatomy or functioning of the pelvic organs, include urinary incontinence, pelvic organ prolapse (when one or more of the pelvic organs fall into the vagina) and fecal incontinence. Pelvic floor in women consists of muscles, ligaments, fascia and nerves that are mutually connected into a complex, dynamic and coordinated system. The protection of integrity of this complex system provides performance of basic functions of pelvic floor such as supportive function for the organs of pelvic floor, function of urination, defecation as well as sexual function [1]. The damage of any segment of pelvic floor will lead to the damage of its functioning, which is manifested in occurrence of urinary incontinence, vaginal prolapse, fecal incontinence and sexual dysfunction [2]. The damage of connective-muscular fibers *musculus levator ani*, i.e. *pubis-rectal* muscle is considered the first and most significant event in the occurrence of pelvic floor dysfunction due to weakening of its strength and opening urinary-genital hiatus and lowering the uterus and vaginal walls. The causes can be very different [3, 4]. However, there may be a threshold for the pelvic floor at which physical activity's benefit is negated. Mild or moderate activity and strenuous activity may impact pelvic floor disorders differently, and in a bidirectional manner. Regular low impact activity, like walking, is associated with a lower prevalence of stress incontinence [12–14]. However, many young women report stress urinary incontinence during high-impact, vigorous intensity activities: 28% of college varsity athletes, 41% of elite athletes and 43% of women participating in club sports [15–17]. Some particularly strenuous activities may damage the pelvic floor. Nulliparous military women that completed paratrooper training were more likely to have stage II pelvic organ prolapse than women undergoing regular summer training [18]. Women may stop exercising because of urinary incontinence or fear that such activity will promote pelvic floor disorders [19, 20]. Of 60% of women that are employed, 9.8% are engaged in repeated strenuous physical activity for four or more hours each day [21]. Some data indicate that women with prolapse or incontinence are more likely to report strenuous jobs than women without such disorders [22–26]. However, these studies are variably limited by poorly defined occupational and activity histories, non-standardized outcome assessment, and lack of consideration for confounders.

Various methods, both subjective and indirect, such as the method of digital palpation [5], perineometer measurements [6, 7], the application of perineal ultrasound [8, 9], vaginal balloon [10] and surface electromyography [11] can be used for measuring the strength of pelvic floor in women as well as the estimation of therapy effects. Direct and precise measurement of pelvic floor muscle strength is possible using vaginal dynamometer a newly designed device for measuring and monitoring the pelvic floor muscle strength in women. This modern device works on the principle of measurement ribbons and Winston bridge. Physical effect of the pelvic floor muscle is transmitted to the dynamometer where it is transformed into an electric signal proportional to the magnitude of strengths [12, 13].

The aim of this study was to examine the effect of various risk factors on the strength of pelvic floor muscle in women.

Methods

Prospective clinical study was carried out at Clinic of Gynecology and Obstetrics, Clinical Center Kragujevac, in 2012/2013 after Ethic Committee of Clinical Centre Kragujevac approved the study and oral and written approval of the patients were obtained. The study included 90 women aged 20–58 (on average 41.53 ± 10.35) years. The pelvic floor muscle strength of all examinees was measured using vaginal dynamometer, a device for measuring and monitoring the pelvic floor muscle in women, presented in the previous paper [12, 13]. The influence of age, physically exhausting work, body weight and height, number of vaginal deliveries, sports activities (at least twice weekly one-hour exercise), occurrence of urinary incontinence with or without disturbances of static of genital organs, previous gynecological surgeries of prolapse, other gynecological surgeries, such as abdominal hysterectomy, and other conservatively treated gynecological diseases, such as urinary incontinence, on the strength of pelvic floor muscle was examined. The patients with caesarean section, and those who suffered from cardio-vascular, endocrine, neurological, malignant and other acute gynecological diseases (pelvic inflammation, bleeding) were not included in the study.

Results

The characteristics of the examined group are presented in the Table 1. In this group of patients, the greatest number had two deliveries, 35.56% (χ^2 test: $p = 0.001$). According to physically exhausting work, 27.78% of patients worked hardly, 24.44% worked with medium efforts, while 47.78% had physically easy work. About two thirds of the patients did not have any sports activities (66.89%) which is statistically significantly different in comparison to those who did some kind of recreation (χ^2 test: $p = 0.000$). Problems of urinary incontinence were found in 55.56% of the patients, while 44.44% of examinees did not reveal any data about the occurrence of incontinence. The frequency of patients with urinary incontinence was not statistically significantly different in comparison to the number of patients without these problems (χ^2 test: $p = 0.292$). Only 16% of the patients had previous surgery due to prolapse of uterus, which was sig-

Table 1

General characteristics of the examinees (n = 90)

Observed parameters	Obtained values
Age (years), $\bar{x} \pm SD$ (median; min-max)	41.53 ± 10.35 (44; 20–58)
Work, n (%)	
exhausting	25 (27.8)
medium effort	22 (24.4)
easy	43 (47.8)
Body height (cm), $\bar{x} \pm SD$ (median; min-max)	165.23 ± 5.53 (165.5; 155–175)
Body mass (kg), $\bar{x} \pm SD$ (median; min-max)	64.04 ± 8.88 (63.5; 47–92)
Delivery, n (%)	
yes	59 (65.6)
no	31 (34.4)
Number of deliveries, n (%)	
0	31 (34.4)
1	8 (8.9)
2	32 (35.6)
3	19 (21.1)
Sport activities, n (%)	
yes	28 (31.1)
no	62 (68.9)
Urinary incontinence, n(%)	
yes	40 (44.4)
no	50 (55.6)
Gynecological surgeries – prolapse, n (%)	
yes	15 (16.7)
no	75 (83.3)
Gynecological surgeries – others, n (%)	
yes	13 (14,4)
no	77 (85.6)
Other gynecological diseases, n (%)	
yes	18 (20)
no	72 (80)
Values of muscle strength (daN), $\bar{x} \pm SD$ (median, min–max)	1.14 ± 0.48 (1.10; 0.33–2.30)

$\bar{x}$ – mean; SD – standard deviation; daN – decaNewton.

nificantly lower when compared to the number of patients who did not have this kind of surgery (χ^2 test: $p = 0.000$). Other gynecological surgeries were in the history of 14.44% of patients (χ^2 test: $p = 0.000$). About 20% of patients were previously treated using conservative methods for various gynecological diseases, which is significantly lower in comparison to the number of patients without any treatment for gynecological diseases (χ^2 test: $p = 0.000$).

Mean values of muscle strength were measured by vaginal dynamometer and analyzed in relation to the examined parameters (Table 2).

The average value of muscle strength in the examined patients, measured using vaginal dynamometer was 1.14 daN (decaNewton). The lowest measured value was 0.33 daN, while the highest was 2.30 daN. The mean value of muscle force measured using vaginal dynamometer in the patients who had deliveries was 0.959 daN, while in those who did not have any deliveries it amounted 1.477 daN, which was statistically significant ($p = 0.000$).

Independent *t*-test showed that the difference in the values of muscle strength between the patients who had and did not have any deliveries was statistically significant ($p = 0.000$). The patients who had a delivery had on average weaker muscles. In relation to the number of deliveries, the strongest muscle strength was found in women who had only one delivery, followed by those with two deliveries, while the weakest pelvic floor muscle was found in the women who had three deliveries. Bonfferoni test for multiple comparison showed that the difference in muscle strength was statistically signi`ficant different between the patients who did not have deliveries and those with two deliveries ($p = 0.000$), as well as between the patients who had no deliveries and those with three deliveries ($p = 0.000$), while being not proved between those without deliveries and those with one vaginal delivery. According to variant analysis, differences in values of muscle strength among the patients with physically exhausting jobs and those whose jobs required moderate or no physical efforts, were statistically significant ($p = 0.029$) (Table 2). The women who did physically hard work had, on average, the weakest muscle, followed by women who made medium efforts, while those who had physically easy job had the strongest muscle strength. Bonfferoni test for multiple comparison showed that only the difference between physically hard and easy work was statistically significant ($p = 0.029$).

Independent *t*-test showed that the difference in muscle strength values between the patients who did sports and who did not was statistically significant ($p = 0.000$). The women who did some sport had, on average, stronger muscle strength (Table 2).

Table 2

Mean values of muscle strength measured by vaginal dynamometer and analyzed in relation to the examined parameters (n = 90)

Risk factor	Values of muscle strength (daN), $\bar{x}$ ± SD	*p*
Delivery		
yes	0.959 ± 0.432	0.000
no	1.477 ± 0.385	
Physical effort at work		
hard	0.956 ± 0.384	0.029
medium	1.085 ± 0.434	
easy	1.269 ± 0.527	
Sports activities		
yes	1.495 ± 0.401	0.000
no	0.976 ± 0.429	
Urinary incontinence		
yes	0.780 ± 0.318	0.000
no	1.422 ± 0.396	
Vaginal prolapse surgery		
yes	0.558 ± 0.152	0.000
no	1.253 ± 0.441	
Gynecological surgeries		
yes	0.762 ± 0.260	0.000
no	1.201 ± 0.484	
Gynecological diseases		
yes	0.916 ± 0.277	0.003
no	1.193 ± 0.509	

$\bar{x}$ – mean; SD – standard deviation; daN – decaNewton.

Independent *t*-test showed that the difference in the values of muscle strength in the patients with and without urinary incontinence was statistically significant ($p = 0.000$). The women with incontinence had, on average, weaker muscle.

Statistically significant difference ($p = 0.000$) in the values of muscle strength in the patients who previously had gynecological surgery of prolapse of uterus and/or vagina and those who did not have the surgeries and without vaginal prolapse are presented in Table 2. The patients who had the surgery for genital prolapse had, on average, weaker muscle (0.558 ± 0.152 daN).

Independent *t*-test showed that the difference in muscle strength values between the patients who previously had other gynecological surgeries and those who did not have any ones was statistically significant ($p = 0.000$). The patients who had other gynecological surgeries had, on average, weaker muscle (0.762 ± 0.26 daN) (Table 2). Independent *t*-test showed that the difference in muscle strength values between the patients who previously had other gynecological diseases and those who did not have any such diseases was statistically significant ($p = 0.000$). The patients who had other gynecological diseases had, on average, weaker muscle (0.916 ± 0.277 daN) (Table 2).

Univariate regression analysis (Table 3) showed that parameters such as age, physically exhausting work, body height, vaginal delivery, number of deliveries, sports activities, gynecological surgeries of prolapse, other gynecological surgeries and other gynecological diseases significantly correlated with the values of pelvic floor muscle strength. All these parameters were included in multivariate regression model. In multivariate regression model, urinary incontinence and gynecological surgery of prolapse singled out as independent risk (Table 4) for lower strenght of pelvic floor muscle factors.

Table 3

Results of univariate regression used in the analysis of influence of the observed factors on the strength of pelvic floor muscle

Observed parameters	Regression coefficient	Non-standardized coefficient B	*p*
Age	0.538	-0.025	0.000
Body height	0.271	0.024	0.010
Body weight	0.064	-0.003	0.549
Delivery	0.512	0.518	0.000
Number of deliveries	0.531	-0.219	0.000
Exhausting work	0.279	0.158	0.008
Sport activities	0.500	-0.519	0.000
Gyn. surgeries of prolapse	0.539	0.695	0.000
Other gynecological surgeries	0.321	0.438	0.002
Other gynecological diseases	0.231	0.277	0.029

Table 4

Results of multivariate regression used in the analysis of influence of the observed factors on the strength of pelvic floor muscle in women

Observed parameters	Non-standardized	*p*
Age	-1.558	0.070
Exhausting work	-0.008	0.494
Body height	0.007	0.279
Vaginal delivery	0.332	0.055
Number of deliveries	0.130	0.087
Sport activities	-0.164	0.078
Urinary incontinence	0.420	0.000
Gynecological surgery of prolapse	0.309	0.005
Other gynecological surgeries	0.115	0.298
Other gynecological diseases	0.082	0.396

Discussion

The use of vaginal dynamometers to measure pelvic floor muscle function is recent and there are only few studies, including our studies comparing the muscle strength in women with or without urinary incontinence [12–17]. Pelvic organ prolapse is a common condition characterized by descent of the vaginal wall or vault, and the uterus [18]. Data show that 75% of women aged 45–85 years had some degree of prolapse [19]. The prevalence of typical signs, vaginal bulge, reported to be about 3–12% [19, 20]. Moreover, other symptoms, such as pelvic pressure/heaviness or pelvic pain and urinary or bowel symptoms may occur [21].

The most important factors that lead to pelvic organ prolapse, primarily vagine, are aging and the forthcoming menopause which is followed by the changes in hormonal status of a woman [22]. Although the damages of connective and muscular structures of pelvic floor and their denervation occur during vaginal delivery, symptomatic vaginal prolapse does not appear immediately after the delivery, but usually after a few decades [22]. On the other side, it could be explained by the fact the damaged muscular and connective tissue of the pelvic floor (during reparation of connective tissue the predominant collagen of the type I is replaced with less valuable collagen of the type III) additionally weakens (decompensates) after menopause due to the lack of estrogen stimulation [19]. On the other hand, during the physiological process of ageing after the menopause, the contents of collagen are reduced in the connective tissue [20]. In menopause, the lack of estrogen stimulation leads to reduced synthesis of the collagen type I, which results in the reduction of its content in the connective tissue, thus causing the decrease of the connective tissue strength and occurrence of clinically manifested vaginal prolapse [22].

The damage of cross-striated pelvic floor fibers leads to urinary and fecal incontinence, vaginal prolapse and sexual dysfunction [2]. The patients with such disorders have hygienic and social problems and feel humiliated [23]. One of the problems which are found in practice is measuring the pelvic floor muscle strength aimed at selection of the mode of treatment, either surgical or kinesthetic as well as objective measuring of the effects of the application of pelvic floor muscle exercise program [13]. The number of studies regarding the evaluation of pelvic floor muscle function is growing [5–11]. With this paper, we tried to make contribution to better knowledge and understanding of causes that led to dysfunction of pelvic floor muscle.

Our study showed that the strength of pelvic floor muscle declines with the number of deliveries. The difference in the pelvic floor strength was statistically significantly between the women who did not have any deliveries and those with two deliveries, as well as between the women who did not have any deliveries and those with three deliveries. The difference was not proved for the women with one delivery and those without any deliveries.

Numerous papers show that vaginal delivery leads to weakening of cross-striated pelvic floor muscle structures in women due to the trauma of the muscles and/or their denervation. Sampselle et al. [24] also showed that vaginal delivery had effect on muscle strength. They measured the strength of pelvic floor muscle in 77 women using the palpation method in the 32nd and 35th weeks of pregnancy, as well as after the delivery. Considerable decrease of muscle force was found after the delivery. In the other article Sampselle [25] also concluded that the strength of the pelvic floor muscle declined with the number of deliveries. The women with greater number of deliveries had weaker muscle strength in comparison to those with one delivery only.

Exhausting physical work, hence the increased stress of pelvic floor muscles can also lead to decrease of pelvic floor muscle strength. In Denmark, the study was conducted on 28,000 nurses aged 20–69 who, due to their profession, were exposed to physically exhausting work, such as lifting the patients, the study showed that the risk of their prospective gynecological prolapse surgeries as a result of weakened pelvic floor increased for 1.6 in comparison to other population [26]. According to variant analysis, our results show statistically significant differences in the values of pelvic floor muscle strength ($p = 0.000$) between the women with physically exhausting jobs and those whose jobs require moderate or no physical efforts. The women who perform physically difficult jobs had, on average, the weakest muscle strength, while those with an easy job had the strongest muscles. Bonfferoni test for multiple comparison showed that only the difference between the physically most exhausting and the easiest jobs was statistically significant. It was also found that the difference in the values of muscle strength between the patients who had sports activities and those who did not

was statistically significant. The women who did sports activities had, on average, stronger muscle force.

One of clinical manifestations of the weakness of connective-muscular structures of pelvic floor is also the presence of urinary incontinence and vaginal prolapse, i.e. previous treatments using conservation method or surgery due to the stated troubles. In this paper, the mean value of muscle strength in the patients with urinary incontinence, measured with vaginal dynamometer was 0.780 daN, in comparison to 1.422 daN in those without incontinence (p = 0.000), which proved that the women with urinary incontinence showed statistically significant weakness of pelvic floor muscle. FitzGerald et al. [27] also showed the connection between the weakness of pelvic floor muscle and urinary incontinence. The weakness of pelvic floor muscle can also be influenced by previous gynecological surgery of prolapse. The mean value of muscle strength in these patients was 0.558 daN, while those who did not have this surgery it amounted 1.253 daN (P =0.000). In other study, Uma et al. [28] investigated the values of pelvic floor strength after the surgical treatment of prolapse and concluded that pelvic floor muscle was weak even after the surgery; hence, the conducting of kinesis therapeutic program was necessary. We also proved that the occurrence of other gynecological diseases and surgeries in the pelvis has statistically significant effect on weakening of connective-muscular structures of pelvic floor. This is explained with the fact that in the process of wound healing, after trauma (delivery) or surgery (hysterectomy) a minor quality collagen type III is formed, which leads to decrease of total strength and resistance of pelvic floor [24].

Multivariate regression analysis showed that only the urinary incontinence and previous gynecological surgeries of prolapse were independent statistically significant risk factors connected with the decreased strength of connective-muscular pelvic floor structures. This is in accordance with the current knowledge of pathological physiology of vaginal prolapse and urinary incontinence as clinical manifestations of pelvic floor dysfunction, i.e. their occurrence as a result of weak peritoneal muscles. Namely, in the patients in these conditions, the values of measured muscle strength were considerably lower in comparison to those without these kinds of problems. For these patients, the application of physical therapy aimed to improvement of muscle strength values is necessary, as well as the permanent follow-up in order to prevent the occurrence of possible, even more serious aggravation of the current condition [12]. The device is quite simple for application, and the muscle strength is read as a digital record. For that reason, it is most favorable in comparison to all other methods.

Conclusion

The strength of pelvic floor muscle is best to determine by an objective method such as vaginal dynamometer. Using vaginal dynamometer, it is possible to read on display the muscle strength which is weaker in elderly female patients due to several deliveries, physically exhausting work, lack of sports activities or previous surgeries for prolapse of uterus and/or vagina, or other gynecological surgeries. High-risk groups of women are women after abdominal hysterectomy and operation of uterine prolapse, and in these patients the implementation of prevention is recommended.

The knowledge of risk factors that lead to damage of pelvic floor muscle and newly designed dynamometer for objective measuring the muscle strength make it possible to prevent further impair of pelvic floor muscle by applying kinesis therapeutic exercises and thus improve patients' life quality.

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Insulin resistance in drug naive patients with multiple sclerosis

Insulinska rezistencija kod nelečenih bolesnika sa multiplom sklerozom

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Abstract

Background/Aim. Due to the fact that there is a relatively small number of data related to systemic insulin abnormalities in the multiple sclerosis (MS), the main objective of our study was to determine whether a dysbalance of glucose and insulin metabolism exist in patients with natural course of MS. Our hypothesis was that the metabolic disorder that characterizes state of the insulin resistance (IR) and reduced insulin sensitivity (IS) in untreated patients with MS could play a role in disease progression and degree of functional disability. **Methods.** The study included 31 patients with relapsing-remitting (RR) MS and 14 healthy controls from the same geographic area matched by age, ethnicity and number of smokers. The glucose tolerance, IS, and IR were examined using an oral glucose tolerance test (OGTT) and using basal plasma glucose and insulin levels. The functional disability and disease progression were evaluated by the Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Severity Score (MSSS). **Results.** The MS patients tolerated glucose equally well as the healthy controls. Basal concentrations of insulin were significantly higher in the MS group ($p < 0.05$), as well as insulin plasma level 30 min after oral glucose load ($p < 0.01$). The patients with MS had significantly higher values of homeostasis model assessment indexes of IR (HOMA-IR) ($p = 0.027$; $p = 0.028$). The percentage of IS (HOMA2 %S) and whole body IS index (ISI Matsuda) showed significantly lower values in the MS patients than in the controls ($p = 0.005$; $p = 0.001$). The insulinogenic index in the first 30 min of OGTT was significantly higher in MS patients ($p = 0.005$). The measures of functional disability and MS progression did not correlate significantly with the investigated parameters of IR and IS indexes. **Conclusion.** This study demonstrates for the first time the existence of hyperinsulinemia, reduced insulin sensitivity and normal glucose tolerance that indicate the initial phase of IR in the natural course of MS. Additional research is necessary in order to define the mechanisms of occurrence and the impact of IR on the complex pathophysiological processes in MS.

Key words:
multiple sclerosis; insulin resistance; glucose tolerance test; surveys and questionnaires.

Apstrakt

Uvod/Cilj. S obzirom na činjenicu da ima relativno malo podataka koji se odnose na sistemske insulinske poremećaje u multiploj sklerozi (MS), osnovni cilj našeg istraživanja bio je da utvrdimo da li kod obolelih od MS sa prirodnim tokom bolesti postoji disbalans u metabolizmu glukoze i insulina. Naša hipoteza je da metabolički poremećaj koji karakteriše stanje insulinske rezistencije (IR) i snižene insulinske senzitivnosti (IS) kod nelečenih bolesnika sa MS može imati ulogu u progresiji bolesti i stepenu funcionalne onesposobljenosti. **Metode.** U studiju je bio uključen 31 bolesnik sa dijagnozom relapsno remitentne (RR) MS i 14 zdravih kontrolnih ispitanika sa istog geografskog područja usaglašenih po starosti, etničkoj pripadnosti, i zastupljenosti pušača. Tolerancija na glukozu, IS i IR procenjivani su na osnovu oralnog testa opretećenja glukozom (OGTT) i bazalnih vrednosti insulina i glukoze u serumu. Za kliničku procenu funkcionalne onesposobljenosti i progresije bolesti korišćeni su *Expanded Disability Status Scale (*EDSS) i *Multiple Sclerosis Severity Score* (MSSS) skorovi. **Rezultati.** Bolesnici sa MS jednako su dobro tolerisali glukozu kao i kontrolna grupa zdravih. U grupi MS bolesnika bazalne koncentracije insulina u plazmi bile su statistički značajno veće ($p < 0,05$) kao i koncentarcije insulina 30 min nakog opterećenja glukozom ($p < 0,01$). Značajno veće vrednosti indeksa IR, i Homeostasis Model Assessment (HOMA-IR), imali su bolesnici sa MS nego zdrava kontrola. Pokazane su značajno niže vrednosti procenta IS (HOMA 2%S) i indeksa insulinske senzitivnosti celog tela (ISI Matsuda) kod MS

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bolesnika nego kod kontrolne grupe (p = 0,005; p = 0,001). Insulinogeni indeks u prvih 30 min OGTT-a bio je značajno veći kod bolesnika sa MS. Ispitivani parametri IR i indeksi IS nisu pokazali značajnu povezanost sa stepenom funkcionalne onesposobljenosti i progresijom MS. **Zaključak.** U ovoj studiji je po prvi put pokazano prisustvo hiperinsulinemije, smanjene IS uz očuvanu toleranciju na glukozu, što sve zajedno ukazuje na postojanje inicijalne faze sindroma IR kod bolesnika sa prirodnim tokom MS. Neophodna su dodatna istraživanja za definisanje mehanizama nastanka i uticaja IR na kompleksne patofiziološke procese kod MS.

Ključne reči:
multipla skleroza; insulin, rezistencija; glukoza, test tolerancije; ankete i upitnici.

Introduction

Multiple sclerosis (MS) is a disease in which the inflammatory and degenerative processes coexist from the beginning with the different contribution in the pathogenesis, depending on the type and the course of the disease [1].

In recent years there have been more and more evidence suggesting the importance of insulin in many physiological and pathophysiological functions in the central nervous system (CNS), exceeding its exclusive role as a peripheral hormone. The presence of the insulin receptor as well as the insulin-sensitive glucose transporter was identified in the brain of humans and animals [2].

Insulin reactive areas of the brain are involved in the regulation of peripheral metabolism, food intake, body weight, behavior control and cognition with primary effect on memory [3]. Peripheral insulin crosses the blood-brain barrier by insulin-mediated transport mechanism. So far there is not enough evidence which would confirm its local synthesis in the human brain [4, 5]. On the other hand metabolic requirements of the brain are obtained by constant influx of glucose from blood. It is considered that the cerebral glucose transport and metabolism are meinly insulin independent and that the role of insulin in brain glucose metabolism is controversial [6].

The mechanisms by which insulin achieves its central effects are associated with insulin receptor expression, insulin signaling cascade, the neurotransmitter expression and modulation of several aspects of neuroinflammatory response [7, 8].

Transport of insulin in CNS is compromised in conditions that are characterized by a reduced insulin sensitivity (IS) and insulin resistance (IR) in the periphery which is associated with the concept of brain insulin resistance [5, 9].

Research in the past decade indicated a high degree of correlation between IR and peripheral hyperinsulinemia with neurodegenerative diseases such as Alzheimer's dementia, Huntington's disease and Parkinson's disease [10–12].

A small number of recent studies conducted in different groups of MS patients suggest the presence of abnormalities in glucose and insulin metabolism.

People with MS share equal risk of developing diabetes mellitus type 2 compared to the general population, but are more prone to develop other autoimmune diseases, including diabetes mellitus type 1 [7]. Immunomodulatory therapy with interferon beta (IFNβ) further contributes to the risk of developing glucose dysregulation [13–15].

Studies have indicated the prevalence of IR higher than 40% in MS patients compared to the healthy subjects, *ie* 21% [16]. Reduced IS and postprandial hyperinsulinemia was observed in the group of newly diagnosed MS patients [17].

Due to the fact that there is a relatively small number of data related to IR in MS, especially in the group of patients with the natural course of the disease, the aim of this study was to examine glucose tolerance, insulin sensitivity and insulin resistance from an oral glucose tolerance test (OGTT) as well as from basal plasma glucose and insulin levels in the group of untreated patients with MS compared with healthy controls. The aim of this study was also to determine the relation between the level of functional disability expressed as Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Severity Score (MSSS) with IR markers, in patients with MS.

Our hypothesis is that the metabolic disorder that characterizes state of IR and reduced IS in patients with the natural course of MS could play a role in disease progression and degree of functional disability.

Methods

This clinical observational cross sectional study was conducted in the Clinic of Neurology of Military Medical Academy in Belgrade. All procedures were conducted in accordance with regulations of Good Clinical Practice and were approved by the Ethics Committee of the Military Medical Academy in Belgrade. Before enroled in the study the patients had to sign a informed consent for participation in the study.

The patients suffering from MS were recruited from the register of patients of the Clinic of Neurology, Military Medical Academy in Belgrade. The study included 31 patients diagnosed with relapsing-remitting MS (RRMS) according to the revised Mac Donald criteria from 2010, who did not receive treatment that modifies the natural course of the disease [18].

All the RRMS patients were in a state of clinical remission and did not receive corticosteroid therapy three months before joining the study.

The control group consisted of 14 patients selected from a population of healthy veterans from the same geographic area.

A control group of healthy subjects and MS patients were matched by age, ethnicity and the number of smokers.

The MS patients and healthy controls who had in their medical history any disease or medical condition that might be associated with brain pathology, diabetes mellitus, hepatic, renal insufficiency, severe psychiatric or organic disease were excluded from the study. In addition, the patients, as well as the control group of healthy subjects were not taking

any anti-inflammatory and hormone therapy or therapy which may affect the metabolic status. The subjects did not abuse alcohol or psychoactive substances nor were on the specific dietary regimen.

Upon the arrival all the subjects were interviewed in order to collect demographic data, check inclusion and exclusion criteria and information about the history of MS. Physical examination, as well as anthropometric measurements (body weight, body height), were carried out. Body mass index (BMI) was obtained from the ratio of body weight (kg) and square of height (m^2). In the patients with MS EDSS and MSSS were used for clinical evaluation of functional disability and disease progression.

Blood samples were collected early in the morning after 12 hours of fasting and venous cannula was placed. First off all, the basal values of glucose and insulin were determined in plasma and after that the subjects were asked to drink a solution containing 75 g of glucose in 250 mL water over 3 minutes. Blood sampling for analysis of glycemia and insulinemia was then carried out 30 min and 120 min after the OGTT test.

Plasma glucose concentrations were determined by enzymatic method using autoanalyzer (Dimesion RxL Siemens Health Care Diagnostics, Erlangen, Germany). Plasma insulin concentrations were determined by chemiluminescence immunoassay (Architect, Abbott Diagnostics Dicision, Wiesbaden, Germany).

The parameters determined on the levels of fasting insulin and glucose were: homeostasis model assessment (HOMA), a widely accepted, surrogate, instrument for evaluation of IR in clinical and epidemiological studies which correlates well with the glucose clamp, and minimal model as direct and precise methods for the evaluation of IR and IS [19–21]; [HOMA-IR index, the value obtained from the product of basal insulin levels (I_0, mU/mL) and basal glycemia (G_0, mmol/L) divided by the constant 22.5 [22]. Insulin resistance in the general population determined by the value of HOMA-IR $\geq$ 2.5]; updated HOMA 2 computer model also used for the assessment of IR (HOMA2-IR) and for determining IS (HOMA2-% S) and beta-cell function (HOMA2 -%β) from basal glucose and insulin values (this model output was calibrated so that normal β-cell function is 100%, and normal IR $\leq$ 1; http://www.dtu7.ox.ac.uk/Homacalculator/download.php) [23].

The parameters obtained from the OGTT test were: glucose tolerance, with impaired glucose tolerance (IGT) defined by values G_0 (5.6−6.9 mmol/L) and/or glycemia in 2 h test G_{120} (7.8−11.0 mmol/L) [19]; insulinogenic index in the first 30 min of the test ($IGI_{30'}$), as a measure of the first phase of insulin response to glucose, is the parameter for estimation of β-cell activity, (the early phase of insulin secretion is calculated as the quotient of the incremental insulin and glucose concentrations in the first 30 minutes [21]); indices of insulin sensitivity (Cederholm index (ISI_{CED}) is a measure of peripheral insulin sensitivity and Matsuda index (ISI_{MAT}) is the index of insulin sensitivity of the whole body which implies a composite assessment of hepatic and muscle insulin sensitivity [24–26].

Statistical analysis

Differences in frequencies of categorical variables between the patients and the controls were calculated by χ^2. Distribution of continuous variables was tested by Kolmogorov-Smirnov test with Lillieform's correction. The influence of categorical variable on the variability of continuous parameters was analyzed using ANOVA or Kruskal Waliss ANOVA. The correlations were investigated by Spearman's rank correlation test. The results are presented as mean ± standard deviation (SD). Statistical analysis was performed using Statistica Version 8, software package (StatSoft Inc, 2008). In all tests, the differences with two-tailed alpha–probability $p < 0.05$ were considered significant.

Results

The main characteristics of the analyzed groups, the patients with MS and the controls, are presented in Table 1. There were no significant difference in age, percentage of smokers, prevalence of glucose intolerance and insulin resistance between the patients and the controls. The patients with

Table 1

Main characteristics of controls and patients with multiple sclerosis

Characteristics	Controls n = 14	Patients n = 31	*p*
Age (years)	35.07 ± 6.41	35.20 ± 9.92	0.52
BMI (kg/m^2)	26.33 ± 2.54	23.70 ± 3.61	0.02
Smoking, n (%)	7 (50.00)	11 (35.5)	0.36*
HOMA-IR1, n (%)	0 (0.0)	6 (19.35)	0.07*
HOMA-IR2, n (%)	4 (28.57)	12 (38.71)	0.51*
Imp gluc tol, n (%)	5 (35.71)	12 (38.71)	0.85*
EDSS		2.56 ± 1.09	N/A
MSSS		4.48 ± 2.13	N/A

Values are mean ± standard deviation for age, BMI, EDSS, MSSS; One-way ANOVA was used for comparison of continuous variables with normal distribution; *χ^2-test was used for categorical variables; *p* values < 0.05 were considered statistically significant; N/A- not applicable; BMI – body mass index; HOMA-IR – homeostasis model assessment index of insulin resistance; Imp gluc tol – impared glucose tolerance; EDSS – expanded disability status scale; MSSS – multiple sclerosis severity score.

MS had significantly lower BMI compared to the control subjects ($p = 0.02$).

The values of glucose and insulin during 2 h OGTT, measured at the time points of 0, 30 and 120 minutes, are presented in Figure 1. Plasma glucose values changed significantly during OGTT (increased and than decreased) in both, patients and controls (both $p < 0.001$, effect of test), but there was no difference between the patients and the controls ($p > 0.05$, effect of disease). On the other hand, the basal concentrations of insulin were significantly higher in the MS patients than in the controls ($p < 0.05$). Oral administration of glucose induced the significant increase of insulin after 30 minutes ($p < 0.05$, effect of test) as well as between patients with the MS and controls ($p < 0.01$, effect of disease) (Figure 1a and b).

The parameters of insulin resistance, sensitivity and β cell function derived from fasting and OGTT measurements of glucose and insulin are presented in Table 2. We detected the significant difference in indexes of insulin resistance HOMA1-IR and HOMA2-IR between the patients and controls. The patients with MS had significantly higher values of HOMA, defined by both formulas for HOMA calculation. The percentage of insulin sensitivity, HOMA 2% S, was also significantly different, with lower values detected in the MS patients (Figure 2). The whole body insulin sensitivity index (ISI Matsuda) was significantly lower in the patients than in the controls, while peripheral index of insulin sensitivity (ISI Cederholm) was not.

Also, the insulinogenic index in the first 30 min of OGTT ($IGI_{30'}$), the parameter that estimates β–cell function, was significantly higher in the patients with MS than in the controls (Figure 3). The insulin area under the curve (AUC) was significantly higher in the patients with MS, while the glucose AUC was comparable in both groups (Table 2).

The measures of functional disability and MS progression (EDSS and MSSS) did not correlate significantly with the investigated parameters of insulin resistance (HOMA1-IR, HOMA2-IR, INS_0, INS_{30}, INS_{120}) as well as insulin

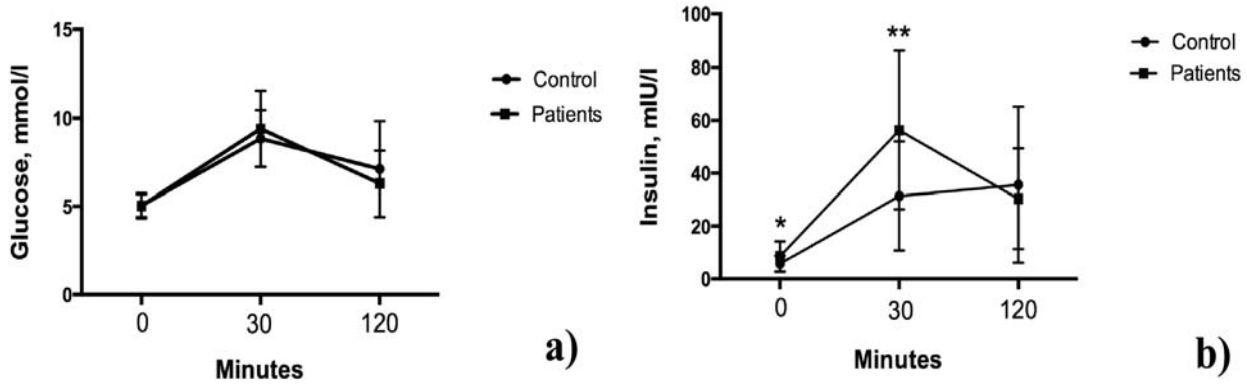
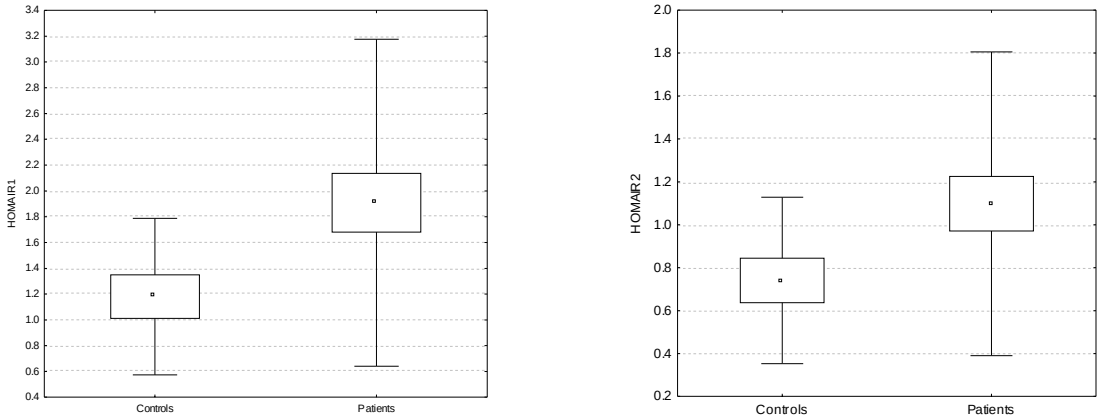


Fig. 1 − Glucose and insulin plasma levels during OGTT in the patients with multiple sclerosis (MS) and the controls; a) Glucose during 2 h OGTT at the time points 0, 30 and 120 minutes in the controls and the patients with MS; b) Insulin during 2 h OGTT at the time points 0, 30 and 120 minutes in controls and patients with MS. (controls, n = 14; patients, n = 31); All values are expressed as means, with their standard deviations represented by vertical bars; Kruskal-Wallis ANOVA,*$p < 0.05$, **$p < 0.01$.
OGTT – oral glucose tolerance test.

Table 2
Parameters of insulin metabolism in controls and patients with multiple sclerosis (MS)

Parameters	Controls n=14	Patients n=31	*p*
HOMA-IR1	1.27 ± 0.67	1.91 ± 1.27	0.027*
HOMA-IR2	0.74 ± 0.39	1.10 ± 0.71	0.028*
HOMA2 %β	77.09 ± 31.63	102.65 ± 49.18	0.06*
HOMA2 %S	9.26 ± 4.47	5.80 ± 2.22	0.005
ISI_{MAT}	166.69 ± 80.40	104.32 ± 40.05	0.001
ISI_{CED}	57.42 ± 22.27	51.12 ± 11.33	0.21
AUC INS	59.5 ± 31.95	80.11 ± 33.81	0.023
AUC GLUC	15.44 ± 2.83	15.42 ± 3.11	0.98
$IGI_{30'}$ (mU/mmoL)	6.30 ± 4.09	17.37 ± 22.65	0.005*

Values are presented as mean ± standard deviation; One-way ANOVA was used for comparison of continuous variables with normal distribution; *Kruskal-Wallis ANOVA test was used to compare values between controls and patients with MS for continuous variables that had skewed distribution; *p* values < 0.05 were considered statistically significant.
HOMA-IR – homeostasis model assessment index of insulin resistance; ISI_{MAT} − insulin sensitivity index; ISI_{CED} − peripheral insulin sensitivity index; AUC − area under the curve; INS − insulin; GLUC – glucose; $IGI_{30'}$ − insulinogenic index during the first 30 min. of oral glucose tolerance test.



a) **b)**

Fig. 2 – HOMA-IR in the patients with multiple sclerosis (MS) and the controls: a) HOMA1-IR in the controls and the patients with MS; b) HOMA2-IR in the controls and the patients with MS (controls, n = 14; patients, n = 31).
All values are expressed as means, with their standard deviations represented by vertical bars; Kruskal-Wallis ANOVA,*$p < 0.05$. HOMA-IR – Homeostasis model assessment-insulin resistance.

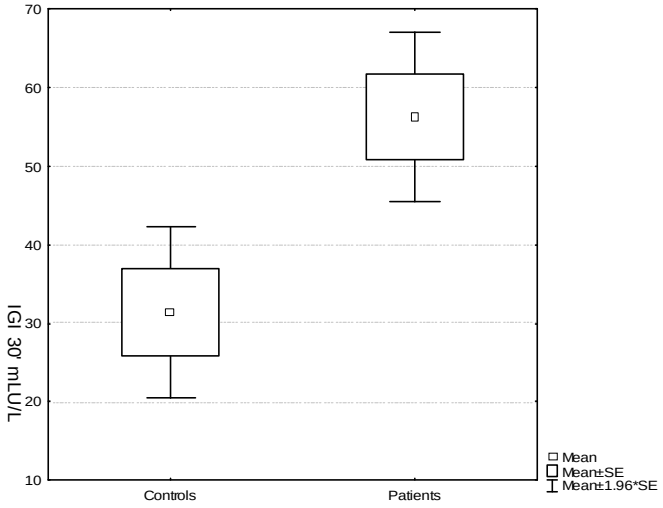


Fig. 3 − Insulinogenic index (IGI) during the first 30 min of OGTT (IGI 30′) in the controls and the patients with multiple sclerosis (controls, n = 14; patients, n = 31); Kruskal-Wallis ANOVA, **$p < 0.01$).
OGTT – oral glucose tolerance test.

sensitivity indexes (ISI $_{MAT}$ and ISI $_{CED}$) or insulin response to glucose (IGI $_{30'}$).

Discussion

Our study indicate the presence of abnormal insulin and glucose metabolism in the patients with MS compared to the healthy control subjects matched in age, ethnicity and percentage of smokers.

Insulin resistance means a reduced capacity of endogenous as well as exogenous insulin to stimulate tissue glucose utilization. Decreased tissue reactivity to insulin starts maladaptive mechanisms with the aim to maintain optimal levels of glucose in blood and preserve homeostasis [7, 8]. Pancreatic β-cells secrete more insulin and hyperinsulinemia temporarily maintains euglycemia. Investigation of IR progressed from its primary role in the pathogenesis of diabetes and latterly it is the area of interest in a wide range of diseases. IR could represent a kind of common pathway through which negative environmental factors in interaction with genetic predisposition lead to metabolic, hemodynamic and inflammatory disorders.

The main objective of our study was to demonstrate whether a dysbalance in the metabolism of glucose and insulin exist in patients with the natural course of MS, based on the parameters obtained from the OGTT.

In the group of the patients with MS the prevalence of IR showed a trend towards significance compared to the healthy control subjects. Population studies in different geographic areas indicated the existence of large variability in cut-off HOMA-IR values that define IR [27, 28]. Unfortunately, studies have not been conducted yet in our

population, so commonly accepted cut-off value for IR > 2.5 was used [22].

Among this study subjects, who shared the same geographical and cultural characteristics and a similar genetic background, comparison of the HOMA-IR index values showed that the patients with MS, as a group, had significantly higher values compared to the healthy controls.

Unlike the previous studies that examined the glucose and insulin metabolism in patients with MS, our study selected a group of RR MS patients with natural course of the disease with the average disease duration of 6 years. The study of Penesova et al. [17] included a group of untreated RR MS patients with a short duration of the disease, diagnosed immediately after the first episode of the symptoms, and a lower degree of functional disability where no statistically significant differences in the values of HOMA-IR indexes were found between the group of MS patients and the healthy subjects.

Oliveira et al. [16] found a higher prevalence of IR based on HOMA-IR index in the group of MS patients with a similar duration of the disease as in our patients, but they included patients with RR MS and secundary progressive (SP) MS who were receiving immunomodulatory therapy. It has been shown that treatment with interferon induces IR and increases an incidence of diabetes mellitus [14, 29].

A recent study has indicated that IFN β leads to IR by activation of signal transducer and activation of the transcription (STAT1)/suppressor of cytokine signaling (SOCS). Increased expression of SOCS in liver and adipose tissue results in suppression of insulin signaling and reduction of glucose uptake and it also leads to suppression of insulin-induced phosphorylation of insulin receptor substrate (IRS)-1 protein [14].

Significantly higher values of HOMA-IR index in the group of our MS patients are consistent with the results of Oliveira et al. [16] although our patients were younger and were not treated with immunomodulatory therapy. Hence it can be suggested that insulin resistance is associated with MS independently on the therapy and a higher prevalence of IR in MS patients, 41% in their study compared to our group of subjects, 19.3%, could partly be explained by the effect of the therapy and older age.

Wanting to explore the mechanism of glucose metabolism disorders in MS better, we opted for the use of OGTT by which we could gain an insight into glucose tolerance, insulin response to glucose stimulation as well as the sensitivity of peripheral tissues to insulin.

Our results show that the MS patients tolerated glucose equally well as the healthy controls. There was no difference in individual glycemic time points, the form of curve and the area under the curve. However, the MS patients had significantly higher basal insulin concentrations and a significant increase in insulin after oral administration of glucose. The values of the area under the curve for insulin were significantly higher in MS patients than in the healthy controls. Increased insulin response suggests a reduced IS which confirms a significantly lower IS index, HOMA 2%S, obtained in our work in the group of MS patients based on the calculations from basal levels of insulin and glucose. Moreover, we established a significantly lower insulin sensitivity index of the whole body ISI_{MAT}, obtained on the basis of OGTT. These results are in agreement with the findings of Penesova et al. [17] who pointed out that the reduced IS is a probable cause for hyperinsulinemia in the newly diagnosed and untreated MS patients. The difference in the value of the peripheral insulin sensitivity index ISI_{CED} did not express a statistical significance between our patients and the healthy controls, in contrast to the results of Penesova et al. [17]. A possible explanation is that the formula for calculating the ISI_{CED} contains value of the body weight included in the calculation of BMI, which was significantly lower in the group of our patients.

With the aim to get an indirect insight into the activity of pancreatic β-cells in the group of MS patients, we used a surrogate marker for evaluation of the first-phase insulin response to glucose, $IGI_{30'}$, with a significantly higher value compared to the healthy control group.

Low insulinogenic index proved to be a predictor of the development of DM type 2. A decrease in this parameter value accompanies the development of glucose intolerance when an impaired β-cell function does not provide a sufficient increase in insulin secretion in relation to the requirements, which results in inadequate hyperinsulinemia [10]. Considering that our patients tolerated glucose as well as the healthy subjects, an increased value of this index would speak in favor of preserved β-cell function compared to the increased demands dictated by reduced IS.

Common findings for hyperinsulinemia, reduced IS and normal glucose tolerance could indicate the existence of the initial phase of IR syndrome when there has still not occurred a collapse of glucoregulation and when the pancreatic β-cells respond to IR with a significantly increased insulin secretion in a group of patients suffering from MS with the natural course of the disease and low levels of functional disability.

The results of our study do not indicate any association between IR and IS with the level of functional disability and the disease progression. A low average EDSS score in our group of patients does not significantly limit their physical activity which annuls the potential impact of reduced physical activity in IR. On the other hand, given the low EDSS score and a shorter disease duration, 6 years in average, it is possible that it would be necessary to check data on the absence of the connection of IR with the severity of functional disability and the rate of disease progression, obtained in this study, in a group of patients with a longer duration of the disease or in longitudinal studies which track the temporal and causal link.

Data from the previous studies indicate that IR accompanies other autoimmune diseases as well [30, 31]. In patients with systemic lupus the connection with adipocytokines adiponectin, leptin, and visfatin in pathophysiology of IR and their possible role were indicated, while in rheumatoid arthritis (RA) the connection between seropositivity for rheumatoid factor (RF) and anti-citrullinated antibodies to citrullinated protein antigens (ACPA) with IR was observed [31, 32].

In patients with RA an increased production of insulin was detected. The conclusion was that a common trigger of autoimmunity could contribute to the production of antibodies directed to the insulin receptor, leading to IR, rather than the destruction of β cells [31].

Inflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin(IL)-6, IL-17, (IFN)–γ, which are elevated in patients with MS, are implicated in negative interactions at the level of insulin receptors, signaling pathways and regulation of the genes responsible for normal insulin activity, as well as at the level of mechanisms involved in the processes of reaction to insulin and peripheral glucose uptake. On the other hand, insulin abnormalities are associated with increased oxidative stress and inflammatory response [33, 34].

The drugs from the thiazolidinedione group, agonists of peroxisome proliferator-activated receptor-γ (PPAR γ), which are widely used in the therapy of diabetes, because they lead to a decrease in the IR, expressed the ability to prevent the occurrence of clinical signs and reduce the severity of the disease in the model of experimental autoimmune encephalomyelitis EAE. PPARγ receptor is expressed in the inflammatory infiltrates of the spinal cord in EAE [35].

Considering that the activation of PPARγ has shown beneficial effects in the treatment of inflammatory diseases their potential benefit in the treatment of MS was suggested [36]. These data suggest that chronic systemic inflammation and oxidative stress may have a prominent role in the development of IR and thus there is an assumption of a possible connection between IR with chronic inflammation in MS.

Moreover, neurodegenerative diseases, particularly Alzheimer's dementia (AD), are associated with the existence of IR [7, 37, 38]. A concept was recently introduced that considers AD a metabolic disease with impaired energy metabolism and glucose utilization in the brain, based on the brain resistance to insulin and insulin-like growth factor [39].

Cerebral IR is associated with peripheral IR and chronic hyperinsulinemia to which aging, obesity and dyslipidemia contribute. On the other hand, it could be determined as soon as in the fetal period by maternal metabolic disorders which interact with the fetal brain as well as the genetic background [4, 7, 31]. The influence of cerebral IR to neurodegeneration is carried out through a variety of mechanisms which include enhanced activation of kinases that pathologically phosphorylate the Tau protein, increase in the expression of amyloid-β precursor protein (AβPP) and the accumulation of amyloid, as well as an increase of oxidative stress, mitochondrial dysfunction and the activation of pro-inflammatory and pro-apoptotic cascades which represent a part of the pathogenetic process in MS [37, 38].

A parallel degenerative process which takes place in the natural course of MS suggests a possible analogy in pathophysiological processes with other neurodegenerative diseases of the CNS, especially since MS is largely characterized by the cognitive impairment [39–41].

Limitation of the above research, a small number of subjects in the control group, is justified by the relatively difficult and invasive procedure that had to be carried out in the healthy subjects. All control subjects were males. This, however, did not affect the accuracy of the results given the fact that statistical comparative analysis of all relevant parameters of the assessment within the group of patients with MS did not indicate a significance in relation to gender [42]. Considering a relatively small number of MS patients in this preliminary study, the results require further confirmation in a study with a larger number of drug naive patients with MS.

Conclusion

This study demonstrates for the first time the existence of IR in the natural course of RRMS, in patients not treated with any type of therapy that alters the natural course of the disease. Given the previously demonstrated presence of IR in the patients with MS who received immunomodulatory therapy, as well as a reduced insulin sensitivity in the newly diagnosed, untreated patients with MS, our opinion is that there is a dysregulation of glucose and insulin metabolism in MS and that additional research is necessary in order to define the mechanisms of occurrence and the impact on the complex pathophysiological processes in the CNS of the patients with MS.

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GENERAL REVIEW

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The ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography in breast cancer

Pozitronska emisiona tomografija/kompjuterizovana tomografija primenom ^{18}F-fluorodeoksiglukoze kod karcinoma dojke

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Key words:
breast neoplasms; tomography, emission-computed; tomography, x-ray computed; radiopharmaceuticals; sensitivity and specificity.

Ključne reči:
dojka, neoplazme; tomografija, kompjuterizovana, emisiona; tomografija, kompjuterizovana, rendgenska; radiofarmaci; osetljivost i specifičnost.

Introduction

Breast cancer is the most common malignancy diagnosed in women. A number of 231,840 new cases of breast cancer was expected in 2015, and 40,290 women were estimated to die from breast cancer in 2015 [1]. In 2012 in Central Serbia, 3,186 new cases of breast cancer in women were registered and 1,175 cause-related deaths [2, 3]. There is an increasing incidence and mortality of breast cancer in Vojvodina, a northern part of Serbia (Figure 1) [3].

Positron emission tomography (PET) is a modern imaging method which plays an important role in oncology. ^{18}F-fluorodeoxyglucose (^{18}F-FDG) is a radiolabelled glucose analogue presenting a glucose metabolism marker. Since glucose uptake is increased in malignant tumors, ^{18}F-FDG PET has a major performance in oncology. A quantitative measurement of FDG uptake is expressed by the standardized uptake value (SUV) and is used mostly for diagnosis and response to treatment assessment. In fact, the SUV represents a relative measure of FDG uptake in tissue and is automatically calculated by PET and PET/computed tomography (CT) scanners as follows: SUV= r/(a/w), where *r* is the radioactivity measured within the region of interest (ROI) in kilobecquerels *per* millimeter (kBq/mm), *a* is the decay-corrected amount of injected radiolabeled FDG (kBq), and *w* is the weight of the patient (g). There are several factors that affect the SUV, such as plasma glucose concentration, the amount of injected FDG, the patient size and, the time from injection to imaging which is perhaps one of the most important factor [4, 5].

^{18}F-FDG PET imaging is a so-called metabolic imaging because of the ability to detect malignant metabolism changes. These changes in fact, precede morphologic changes which are visualized by conventional anatomic imaging such as CT and magnetic resonance (MR).

In the last decade, PET and combined PET/CT were introduced in imaging of breast cancer. The CT part provides exact anatomic information and is used for attenuation correction of PET images. Comparing these two imaging modalities, PET/CT has been accepted to have better diagnostic accuracy than PET itself [6–8].

General considerations

Several authors have studied intensity of FDG uptake in different types of breast cancer. In comparison to ductal carcinoma, the lower FDG uptake was detected in infiltrating lobular carcinoma [9–13]. This phenomenon might be explained by several reasons: lower tumor cell density, a diffuse infiltration of surrounding tissue, a low level of glucose transporter 1 (GLUT1) expression and a decreased proliferation rate in infiltrating lobular carcinoma [10, 14, 15].

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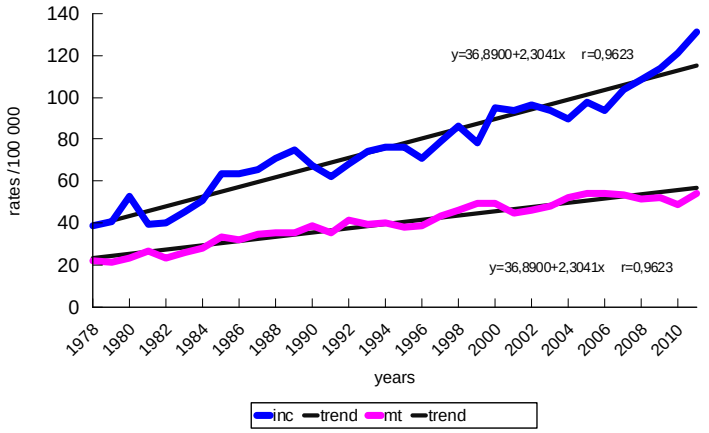

Fig. 1 – The crude incidence and mortality rates for breast cancer in females in Vojvodina in a period from 1985 to 2011 (inc – incidence; mt – mortality).

It has been reported that FDG uptake strongly correlates with high tumor proliferation index (Ki67 expression measured by immunohistochemical analysis) [9, 13, 14, 16] and p53 factor status [9, 12]. The relation between ^{18}F-DG uptake and steroid hormone receptor status is still controversial. Some authors reported no correlation between hormone receptor status and SUV values [11–13, 16]. Several studies detected a higher SUV in estrogen receptor negative (ER−) than in estrogen receptor positive (ER+) tumors [9, 17–19]. In contrast to Osborne et al. [19] who detected no correlation between the SUV and progesterone receptor status, Groheux et al. [20] documented a significant difference in ^{18}F-FDG uptake in progesterone receptor negative (PR−) tumors *vs* progesterone receptor positive (PR+) tumors ($p = 0.003$). Triple-negative breast tumors (negative for estrogen and progesterone receptors, and no human epidermal growth factor receptor-HER 2/neu overexpression) present a great subject to investigate because of their aggressiveness, poor prognosis and lack of targeted regimens. They are characterized with significantly higher SUV values than non-triple negative tumors [21].

Indications

Primary tumor

^{18}F-FDG PET or ^{18}F-FDG PET/CT plays an important role in the diagnostic workup of breast cancer. However, it has no role in breast cancer screening due to limited spatial resolution (disability to detect tumors less than 10 mm) and the low sensitivity in less FDG-avid low-grade breast tumors. In a comparison study, Kumar et al. [22] concluded that tumor size and tumor grade are independent factors associated with false negative results. The eight times higher chances of obtaining false negative results were detected in smaller (< 10 mm) tumors *vs* larger (>10 mm) tumors. Results from another study showed that breast carcinomas were identified with an overall sensitivity of 64.4% and 80.3%, respectively. ^{18}FDG-PET detected only 68.2% breast cancer at stage T1, compared to 91.9% of breast malignancy stage T2 [10]. Analyzing 13 different studies, Samson et al. [23] reported that ^{18}FDG PET was 88% sensitive and 80% specific for detection of primary breast cancer showing false negative results in 12% cases. In another PET study done by Danforth et al. [24], the primary breast cancer was accurately imaged with 90% sensitivity in early staged breast cancer (stage I, II). Moreover, ^{18}FDG-PET is able to image locally advanced skin changes in locally advanced tumors (stage III, IV). In the same study, ^{18}FDG-PET sensitivity for detection of the primary tumor, skin, and axillary lymph node metastases was 96%, 77%, and 83%, respectively. However, in comparison to MR, ^{18}FDG-PET is less sensitive and accurate in the assessment of the primary tumor and screening for tumor multifocality (54% *vs* 77%, respectively) [25].

With the aim to overcome the low spatial resolution of ^{18}FDG-PET, a high-resolution PET scanners dedicated to breast imaging, so-called "positron emission mammography (PEM)" has been recently introduced. There are several publications based on clinical performance of PEM. In comparison to conventional whole-body PET, PEM is favorable in detection of ductal carcinoma *in situ* and lesions ≤ 1.0 cm. The advantages of ^{18}FDG-PEM include: reduced attenuation, improved geometric sensitivity, higher spatial resolution, shorter acquisition time (4−10 min), easy feasibility, device mobility, gentle breast immobilization, possible PEM-guided biopsy. The limitation of the study includes imaging of posterior lesions and variable ^{18}FDG uptake in small tumors [26–30]. In a recent study done by Berg et al. [31], the efficacy of PEM was compared to MR imaging. At the lesion-level, PEM was more specific than MR (79.9% *vs* 65.6%) which helps in avoiding unnecessary biopsies. However, in detection of additional malignant lesions MR imaging was more sensitive than PEM which results in better assessment of disease extent and less frequent mastectomy (53% *vs* 41%) [31].

As generally accepted, ^{18}FDG-PET has no clinical role in diagnostic algorithm of suspicious breast lesions. How-

ever, if there is inconclusive or suspicious mammography, ^{18}FDG PET may be useful. If ^{18}FDG PET unexpectedly detects FDG avid breast foci, patient needs additional conventional imaging and biopsy [32].

Axillary staging

The status of axillary lymph nodes remains one of the most important prognostic indicators in patients with breast cancer. Sentinel lymph node biopsy (SLNB) and axillary lymph node dissection (ALND) are standard procedures that are used for axillary staging. If axillary metastases are identified on SLNB, ALND is necessary. However, patients with negative SNB results may avoid ALND [33].

Despite high diagnostic accuracy, both SLNB and ALND are invasive procedures associated with morbidity, including lymphedema. Therefore, a non-invasive ^{18}FDG-PET imaging has been introduced for axillary staging in breast cancer. It is a metabolic radionuclide imaging technique that detects higher glycolytic rate of cancer cells in comparison to normal cells. The axillary lymph node involvement is shown at ^{18}F-FDG PET/CT (Figure 2).

Veronesi et al. [34] compared SLNB and ^{18}FDG-PET imaging, in detection of occult axillary metastases. Sensitivity of ^{18}FDG-PET scan was low (37%). However, specificity and positive predictive values were high (96% and 88%, respectively). The high specificity of PET imaging indicates that patients with a PET-positive axilla should have an ALND without SLNB for axillary staging. On the contrary, poor sensitivity of PET scan suggests the need for SLNB in patients with a PET-negative axilla.

A recent meta-analysis reported about lower sensitivity and specificity of PET in comparison to SLNB. Analysis of 7 PET/CT studies on 862 patients showed the mean sensitivity and specificity of 56% and 96%, respectively. Across 19 PET studies on 1,729 patients the mean sensitivity was 66% and the mean specificity 93%. In terms of evaluation of axillary extension, ^{18}FDG-PET cannot replace SLNB [35]. In another study, Gil-Rendo et al. [36] reported about 84.5% sensitivity and 98.5% specificity of FDG-PET in detecting axillary involvement. Avril et al. [37] performed FDG PET in women with suspected breast cancer in preoperative staging. They reported the sensitivity of 79% and specificity of 96% for detection of axillary lymph node metastases. Sensitivity increased to 94% in patients with primary breast tumors sized more than 2 cm. Similarly, Danforth et al. [24] suggested that sensitivity of PET in detection of axillary metastases increases with the stage of the disease. He reported sensitivity of 43% for stage I/II and 83% in stage III/IV.

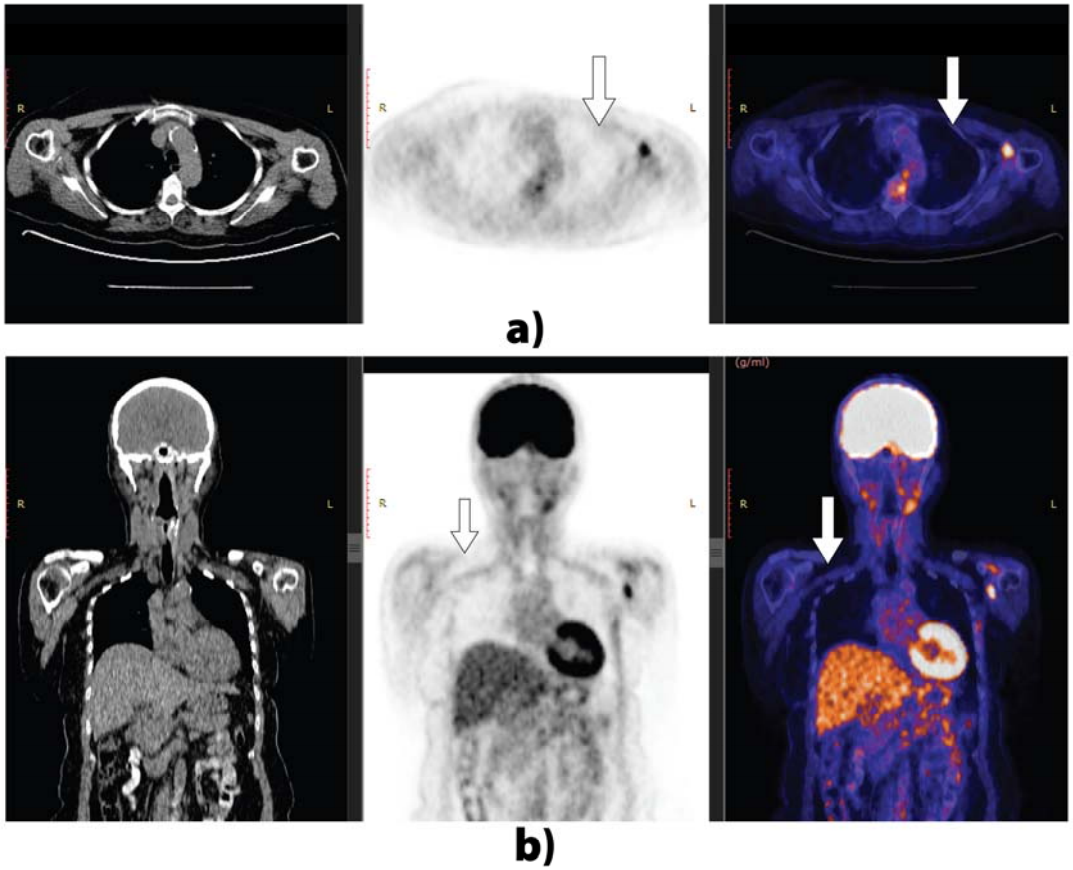

Fig. 2 – a) A 61-year-old woman with right invasive ductal breast cancer, stage pT1, underwent mastectomy, chemotherapy and radiation followed by tamoxifen and trastuzumab (Herceptin®). Transaxial sections (a) and coronal sections (b) detect hypermetabolic lymph node in the left axilla 1.7 cm in size, SUVmax = 10, consistent with axillar involvement.
SUV – standardized uptake value.

A review by Rosen et al. [30], suggests that ^{18}FGD-PET has no clinical role in a routine axillary staging of early stage breast cancer. However, preoperative ^{18}FDG-PET may be worthwhile in locally advanced and inflammatory breast cancers. In a multicentric study, detection of axillary nodal metastases by ^{18}FDG-PET was evaluated in 360 patients with newly diagnosed invasive breast cancer. The reported sensitivity and specificity were 61% and 80%, respectively [38].

Distant metastases

^{18}FDG-PET is also important for detection of occult distant metastases. In high stage breast cancer, Alberini et al. [39] discovered more distant lesions by PET/CT than by conventional diagnostic procedures (31% *vs* 10%). Some authors reported advantage of PET/CT over conventional staging and detecting metastatic involvement of internal mammary chain nodes in patients with stage II and stage III breast cancer [40, 41]. In a work by Carkaci et al. [42], out of 41 studied patients with inflammatory breast cancer, 24% of mediastinal nodal metastases and 15% of liver metastases were correctly identified by PET/CT. Figure 3 shows a patient with breast cancer and liver metastasis.

In the evaluation of metastatic bone involvement, PET is complementary to bone scintigraphy which remains the standard imaging procedure [30]. PET is superior for the detection of osteolytic and mixed bone metastases, but often fails to demonstrate blastic lesions. In contrast, bone scintigraphy is better for depicting sclerotic (blastic) lesions [43]. In a comparison study of bone scintigraphy *versus* PET/CT, Nakai et al. [44] reported on different detection rate for blastic, mixed and lytic type of lesions (100% *vs* 56% ; 84% *vs* 95% ; and 70% *vs* 100%, respectively).

In terms of accurate detection of bone metastases, ^{18}F sodium fluoride PET seems to be better than bone scintigraphy and ^{18}F-FDG PET/CT [45].

Recurrent cancer and restaging

Current diagnostic strategy for the detection of recurrent disease in patients with breast cancer includes physical examination and imaging tests such as mammography, ultrasonography (US), CT, MR and bone scintigraphy. These diagnostic tests are part of routine clinical monitoring during the course of breast cancer. In patients without clinical symptoms and with rising levels of tumors markers PET imaging alone or combined with CT (PET/CT) is useful in detection of recurrent disease. Additionally, in proven recurrent disease or in suspicious recurrence by using conventional imaging, PET/CT helps to distinguish between isolated and multiple metastatic disease.

In a recent study by Aukema et al. [46], additional lesions not visible at conventional imaging were detected by PET/CT in 45% cases. Results of one meta-analysis indicated that MR and PET (including PET and PET/CT) had higher sensitivity than US or CT, which resulted in higher detection rate of recurrent breast cancer. However, there was no difference in sensitivity between PET and MR [47]. Accross 28 studies included in the review Pennant et al. [48] found that PET had a significantly higher sensitivity (89% *vs* 79%) and significantly higher specificity (93% *vs* 83%) compared with conventional imaging tests. In addition, PET/CT had a significantly higher sensitivity compared with CT (95% *vs* 80%) but without a significant increase in specificity (89% *vs* 77%). Furthermore, PET/CT had a significantly higher sensitivity compared with PET (96% *vs* 85%) but no significant increase in specificity (89% *vs* 82%). There were no significant differences in the sensitivity or specificity of PET *versus* MRI, and PET/CT *vs* MRI, respectively.

In another study by Piperkova et al. [49] PET/CT and contrast enhanced CT were compared for initial staging in patients with breast cancer. They reported better diagnostic accuracy for PET/CT than contrast-enhanced CT (CE-CT): the sensitivity, specificity, accuracy, positive productive value, and negative productive value for PET/CT were 97.8%, 93.5%, 97.3%, 99.1%, and 85%, respectively, and for CE-CT were 87.6%, 42%, 82.1%, 91.6%, and 31.7%, respectively. The staging of the disease was changed in 65% of cases: 36% of patients were down-staged and 64% of patients were upstaged. PET imaging is important in restaging of breast cancer, because it might affect treatment management. An example of patient upstaging after ^{18}FDG PET/CT is shown in Figure 4.

In a study by Eubank et al. [50] PET revealed more lesions than CT and consequently altered therapeutic manage-

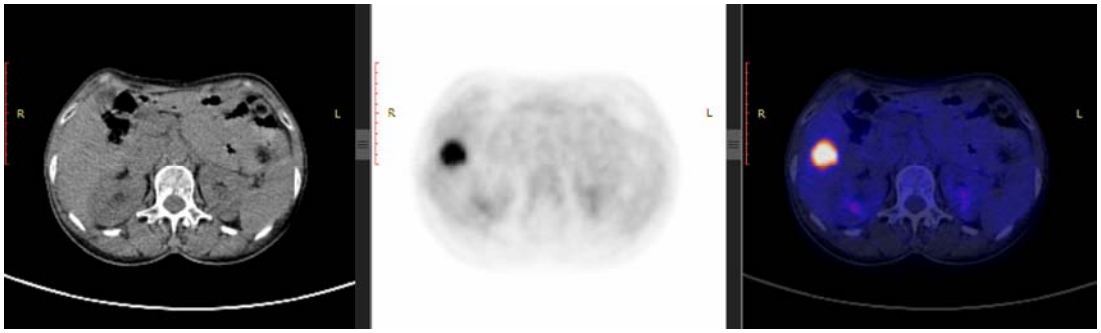

Fig. 3 – A 57-year-old woman with invasive ductal carcinoma of the left breast. The patient underwent mastectomy followed by adjuvant chemotherapy, paclitaxel (Taxol®) and trastuzumab (Herceptin®). Transaxial images show a hypermetabolic liver mass in S6 segment, 3.1cm in size, SUVmax = 12.5 corresponding to hepatic involvement. SUV – standardized uptake value.

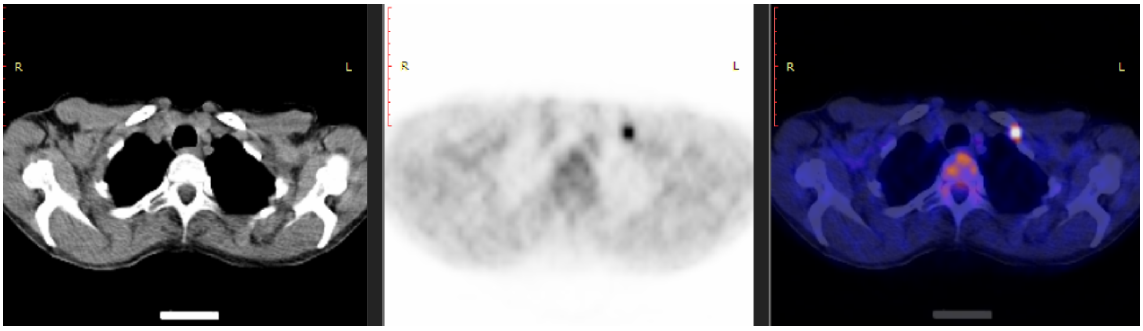

Fig. 4 − A 58-year-old female patient in the postsurgery status and post-adjuvant chemotherapy due to invasive ductal carcinoma of the left breast. The patient presents for restaging after completion of chemotherapy. Transaxial images show left retroclavicular lymph node 1.5 cm in size, SUVmax = 5.64 consistent with metastatic involvement.
FDG – ^{18}F-fluorodeoxyglucose; SUV – standardized uptake value.

ment in up to 44% of patients with suspected locoregional recurrent disease. ^{18}F-FDG PET/CT may have a potential role in clinically asymptomatic patients with rising markers and negative conventional imaging (US, X-mammography, CT and MR). Radan et al. [51] reported about 90% sensitivity of ^{18}FDG-PET in detection of recurrent disease. Consequently, treatment management was changed in 51% of patients. Additionally, if compared to contrast-enhanced CT, PET/CT showed better sensitivity (85% *vs* 70%), specificity (76% *vs* 47%), and accuracy (81% *vs* 59%). Similar results were obtained in a recent study by Dirisamer et al. [52]. They studied 52 patients and detected suspicious recurrence and rising tumor markers levels in 62%. PET/CT had better patient-based accuracy than CE-CT (96% *vs* 73%, respectively), and lesion-based sensitivity and specificity (93% *vs* 66% and 100% *vs* 92%, respectively). Grasseto et al. [53] studied patients with post-treatment rising tumor serum levels of Ca 15−3 but negative clinical examination and conventional imaging. ^{18}F-FDG PET/CT was able to detect cancer lesions in 45% of cases. Finally, there are reports that ^{18}F-FDG PET/CT imaging in patients with rising CA15-3 levels alters treatment management in up to 50% [54–56].

Monitoring of the treatment response

Neoadjuvant or so-called preoperative chemotherapy is the initial standard treatment for patients with locally advanced breast cancer. This treatment results in a reduction of the tumor volume and is followed by conservative surgery and radiotherapy. The assessment of treatment response includes conventional methods such as physical examination, radiography, ultrasound and mammography. However, these methods are usually evaluated after completion of three cycles of chemotherapy. In addition, clinical response does not necessarily reflect the pathological response [57]. Due to the fact that changes in tumor metabolism precede the tumor shrinkage, ^{18}FDG PET is able to detect tumor response at an earlier stage than conventional imaging methods [58, 59].

Evaluation of changes in FDG uptake at different time points of the systemic treatment is based on comparison between the baseline (pretherapy) PET scan and postherapy PET scan. Some authors performed PET imaging early, after only 1 or 2 cycles, or during midtherapy, or at treatment completion. In the settings of early stage at chemotherapy, PET imaging is capable of predicting the pathologic response. In addition, PET is possible to distinguish between patients who respond to treatment (responders) and those who do not (non-responders) [30]. Approximately 70% of patients demonstrate clinical response to neoadjuvant chemotherapy, but only 20% achieve pathological complete response. Since the SUV decline early in the course of chemotherapy predicts a treatment failure, the regimen should altered with aim to avoid unnecessary toxic side effects [60, 61]. Jung et al. [62] suggested that the reduction rate of SUV has a prognostic value after the completion of the fourth cycle of chemotherapy before surgery. They detected 70% of sensitivity and specificity when 84.8% SUV reduction was used as a cutoff value for the pathologic complete response.

In another study, Schelling et al. [63] studied the role of ^{18}F-FDG PET in the assessment of early response to neoadjuvant chemotherapy in locally advanced and inflammatory breast cancer. Decline in SUV values by more than 55% after one cycle, was predictive of a good response with sensitivity of 100% and specificity of 85%. In addition, after one and two cycles pathologic response was predicted with accuracy of 88% and 91%, respectively.

In nonmetastatic, non-inflammatory breast cancer, Kolesnikov-Gouthier et al. [64] detected < 15% of SUV decline after the first chemotherapy course which was used as a strong predictor for inefficient neoadjuvant chemotherapy. Additionally, a 4-year recurrence free survival rate was significantly longer in metabolic responders than non-responders (85% *vs* 44%, respectively).

Park et al. [65] used diffusion weighted imaging (DWI) MR and PET/CT to predict pathologic complete response to preoperative neoadjuvant chemotherapy in patients with invasive breast cancer. PET/CT showed the same sensitivity of 100% as DWI MR, but better specificity (77.8% *vs* 70.4%). A study by Andrade et al. [66] indicated that decrease of SUV values after the second course of neoadjuvant chemotherapy (NAC) can predict pathological response in ductal breast carcinomas, and potentially identify a subgroup of non-responding patients. Keam et al. [67] analyzed the relation between changes in ^{18}FDG uptake and different molecular phenotype of breast cancer treated with neoadjuvant chemotherapy. During the early metabolic response, they detected that the estrogen receptor negative phenotype showed

a higher pre-chemotherapy SUV (8.6 *vs* 6.4) and reduction rate of SUV (48% *vs* 30%) than estrogen receptor positive phenotype. In triple negative breast cancer, the pre-chemotherapy SUV was higher than in not triple-negative breast cancer (9.8% *vs* 6.4%).

In another study, Rousseau et al. [68] demonstrated the efficacy of ^{18}F-FDG PET in the assessment of early response to neoadjuvant chemotherapy in I/II staged breast cancer. They also analyzed the variation of SUV values after the first, second, third and six chemotherapeutic cycles. After 1 cycle of chemotherapy, using a 60% decline in baseline SUV as their threshold for response, PET was 61% sensitive and 96% specific with 68% predictive negative value. After 2 cycles, PET showed better sensitivity, specificity and negative predictive value (89%, 95%, 85%, respectively). After 3 courses of chemotherapy, if compared to values obtained after the second cycle, lower sensitivity, specificity and negative predictive value of PET were detected (88%, 73%, 83%, respectively). These results may suggest possible prediction of final response to treatment.

However, if ^{18}F-FDG PET is performed after the completion of chemotherapy residual FDG uptake may predict residual disease. In contrast, the absence of FDG uptake does not exclude residual microscopic malignancy and may not indicate pathologic response [69, 70]. In patients with large residual disease, ^{18}F-FDG PET is complementary to MR to define the degree of residual mass [71]. Moreover, if ^{18}F-FDG PET is performed after the chemotherapy it has a prognostic value. Cachin et al. [72] showed that negative PET scan was an indicator for a significantly better survival than PET positive scan. Additionally, ^{18}F-FDG PET scan was the most powerful and independent predictor of survival. Patients with negative post-treatment ^{18}F-FDG PET had a longer median survival than patients with positive ^{18}F-FDG PET (24 months *vs* 10 months).

The examples of PET/CT imaging in the assessment of the treatment response are shown in Figures 5a and b, and Figures 6a and b.

New positron emission tomography/ computed tomography tracers in breast cancer imaging

FDG is specific for increased metabolism of glucose and ^{18}F- FDG-PET/CT is able to detect the presence of viable tumor tissue in the human body. However, the new agents that are able to target the cellular processes have been recently developed. These agents are still under investigation and are not available in the routine clinical practice. The recent development of radiolabeled-thymidine compounds allows measurement of the exact tumor proliferation. According to some authors, the ^{18}F-fluoro-thymidine PET (^{18}FLT-PET) imaging has a role in the assessment of therapeutic response and prediction of response to therapy [73–76]. Regarding recently published data, PET is also able to evaluate estrogen (ER) expression by using estrogen receptor ligand, 16α-[18F]-fluoro-17β-estradiol (^{18}F-FES). While increased up-

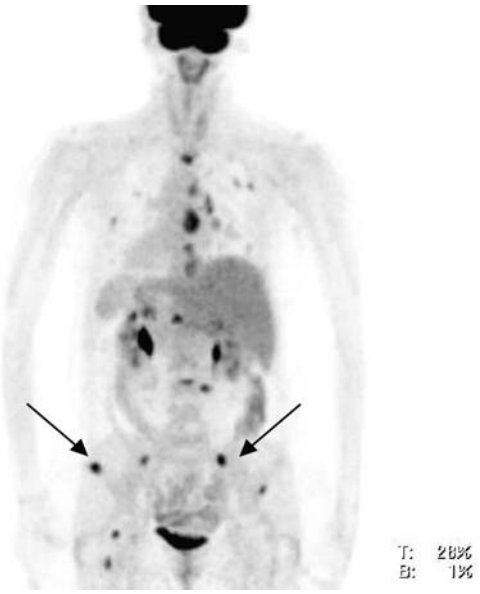

Fig. 5a – A patient presents after mastectomy, before chemotherapy. Maximal Intensity Projection (MIP) image shows multiple mediastinal FDG avid foci (pre- and paratrachealis, aortopulmonalis, subcarinealis, esophageal and hilar) on the right with multiple lung hypermetabolic foci bilaterally. There are multiple FDG avid foci in the skeleton: spine (thoracic V2 and V12, and lumbar V3), iliac bones (SUVmax = 9.47 on the left, and SUVmax = 10.10 on the right) and proximal left femur.
FDG – ^{18}F-fluorodeoxyglucose; SUV – standardized uptake value.

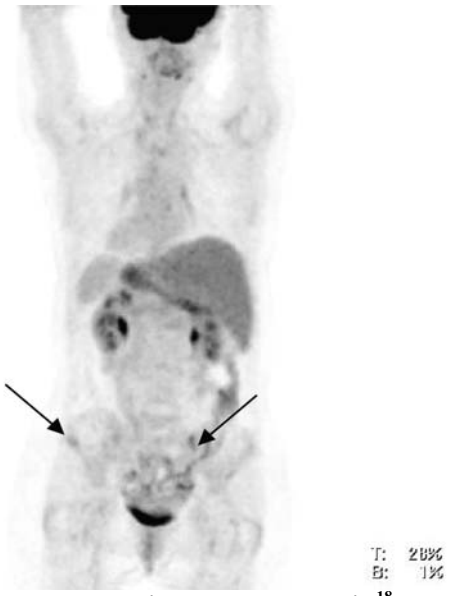

Fig. 5b – The same patient, posttherapeutic ^{18}F-FDG PET/CT scan. MIP image shows restitution of most of the hypermetabolic foci previously seen. There are only two FDG avid foci in the right iliac bone, SUVmax = 4.05 and in the left iliac bone, SUVmax = 2.84. This is an example of partial response to treatment and partial remission.
PET/CT – positron emission tomography/ computed tomography.
FDG – ^{18}F-fluorodeoxyglucose; SUV – standardized uptake value.

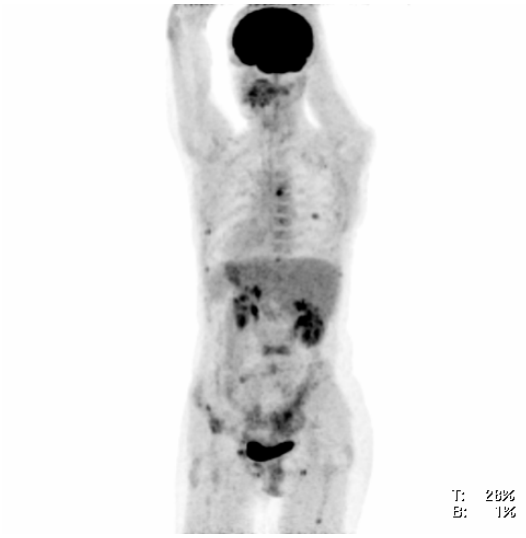

Fig. 6a − A 54-year-old woman with right invasive ductal breast cancer.
Hormone receptor ER, PR positive; HER2 negative. She underwent breast quadrectomy, chemotherapy and external radiation therapy. This is the pretherapeutic PET/CT scan. The MIP image shows multiple hypermetabolic foci in the skeleton located in the spine (cervical level 2, thoracic level 6, 8 and 10, lumbal level 1, 4 and 5), 8th rib on the right and 8th rib and 10th on the left, both iliac and ischiadic bones bilaterally, left pubic bone, and right femoral diaphysis).
MIP – maximal intensity projection; PET/CT – positron emission tomography/computed tomography .

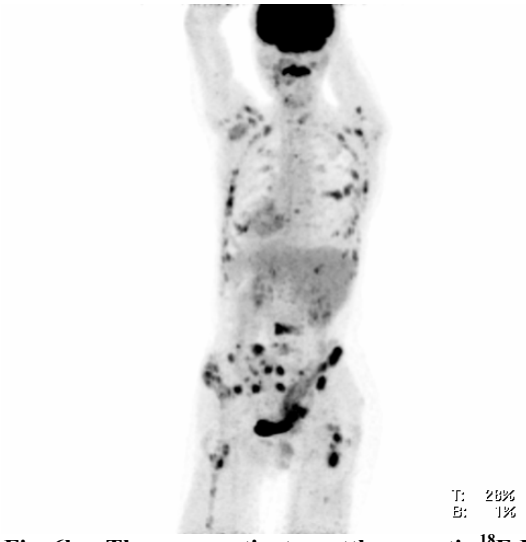

Fig. 6b − The same patient, posttherapeutic ^{18}F-FDG PET/CT scan patient.
After the completion of chemotherapy, MIP image shows persistent FDG avid foci and new hypermetabolic foci spread all over the skeleton wich indicates progressive disease. This is an example of patient, who is non-responder or shows no response to the treatment.
^{18}F-FDG − ^{18}F-fluorodeoxyglucose. PET/CT – positron emission tomography/computed tomography;
MIP – maximal intensity projection.

take of ^{18}F-FES can reliably detect ER-positive lesions, its low uptake seems to be a strong predictor for failure of antihormonal therapy [77–81]. Another important feature of malignant disease is hypoxia. Numerous studies have been done on malignant tumors, mostly head and neck and lung cancers, but less in breast cancer. The results of these studies indicate that tumor hypoxia is important prognostic factor that influences the response to therapy and overall survival. In addition, hypoxia increases the risk of invasion and metastasis, as well as the resistance to chemo- and radio-therapy. Hussain et al. [82] correlated the hypoxia-regulated carbonic anhydrase (CA) IX expression with the outcome in patients with invasive breast cancer. They indicated that CA IX expression is a predictor of poor survival which may subsequently lead to better patient selection for adjuvant treatment. Additionally, hypoxia-related gene expression may present a basis for novel targeted therapies. In head and neck cancers, [^{18}F]fluoromisonidazole (^{18}FMISO-PET) is proven to be a promising agent for detection and localization of significant hypoxia, delineation for external radiation, and for selecting treatment strategy [83]. In another study, Rajendran et al. [84] compared ^{18}FDG-PET to the ^{18}FMISO-PET in different malignant tumors, including breast cancer. They found that despite the fact that hypoxia influences glucose metabolism, some highly metabolic tumors are not hypoxic. They suggested that different tracer uptake in examined tumors can be tumor type-specific. The future will bring the results of currently ongoing studies with new, ^{18}FDG-PET, PET tracers ^{18}FMISO-PET evaluating tumor angiogenesis, chemo resistance and metastatic potential of malignant tumors.

Conclusion

^{18}F-FDG PET/CT is new non-invasive whole-body imaging of breast cancer. In particular, it helps in staging of recurrent or metastatic cancer and in evaluating the treatment response in patients with locally advanced and metastatic disease. Besides evaluation of increased glucose metabolism by FDG-PET, recently developed radiotracers have the ability to assess receptor expression, tumor cell proliferation and tumor viability in patients with breast tumors. However, future molecular imaging studies are necessary for better understanding of tumor biology and behavior. This is directly connected with the development of new PET agents and their introduction in clinical practice.

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CASE REPORTS

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Eosinophilia as a first sign of Hodgkin's lymphoma – A case report

Eozinofilija kao prvi znak Hodžkinove bolesti

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Abstract

Introduction. It is well known that eosinophilia appears in a malignant disease. Frequency of all Hodgkin`s lymphoma patients is estimated to about 15%. Prognostic importance of this phenomenon is not completely investigated. Therefore we decided to present a female patient with eosinophilia, six months before lymphoma appearance. **Case report.** We presented a 51-years old female, from Serbia, who had eosinophilia (1,530–2,040 eosinophils *per* μL of blood), six months before Hodgkin's lymphoma appearance. Eosinophilic granuloma was confirmed by tumor's biopsy and histopathologic examination, from the right femoral region. As eosinophilia was increasing, lymph nodes became enlarged (120 × 65 mm diameter), in the right parailiac region. All infectious and allergic examinations did not reveal eosinophilia's cause. Histopathologic revision was made with added immunohistochemical stains 17 months after tumor's biopsy. The diagnosis was changed from eosinophilic granuloma to mixed cellularity Hodgkin's lymphoma. After conducted Ann Arbor staging classification, II B clinical stage was established. The treatment was done by chemotherapy according to adriamycin, bleomycin, vinblastine, dacarbarine (ABVD) protocol, with 6 courses. Complete remission of the disease was achieved after 4 courses. Eosinophils number dropped to 640 *per* μl blood. **Conclusion.** Eosinophilia without revealed cause can precede Hodgkin's lymphoma. We suggest careful search for enlarged lymph nodes, anywhere in the patients' body who suffer from eosinophilia. Timely and accurate histopathologic diagnostic is a right way to resolve such conditions.

Key words:
eosinophilia; hodgkin disease; signs and symptoms; diagnosis; abvd protocol; treatment outcome.

Apstrakt

Uvod. Dobro je poznato da se eozinofilija može javiti u okviru malignih bolesti. Učestalost je procenjena na oko 15% svih obolelih od Hodžkinovog limfoma. Prognostički značaj ovog fenomena nije u potpunosti razjašnjen. Smatrali smo korisnim da prikažemo bolesnicu sa eozinofilijom koja se javila nekoliko meseci pre pojave Hodžkinovog limfoma. **Prikaz bolesnice.** Prikazana je bolesnica, stara 51 godinu, iz Srbije, koja je 6 meseci pre pojave Hodžkinovog limfoma imala eozonofiliju (1 530–2 040 eozinofila u μL krvi). Biopsijom i histopatološkim pregledom tumorske promene na desnoj butini postavljena je inicijalna dijagnoza eozinofilnog granuloma. Kako je eozinofiija rasla, došlo je do pojave uvećanih limfnih čvorova, promera 120 × 65 mm u parailijačnom prostoru, desno. Sva infektološka i alergološka ispitivanja nisu ukazala na uzrok eozinofilije. Posle 17 meseci učinjena je revizija histopatološkog nalaza biopsije tumora uz dodatna imunohistohemijska ispitivanja. Dijagnoza je promenjena od eozinofilnog granuloma u Hodžkinov limfom, tipa mešovite celularnosti. Posle sprovedene Ann Arbor "stejdžing" procedure zaključeno je da se radi o II B kliničkom stadijumu Hodžkinovog limfoma. Lečenje je sprovedeno hemioterapijom po protokolu adriamicin, bleomicin, vinblastin, dekarbazin (ABVD) sa 6 ciklusa. Uspostavljena je kompletna remisija bolesti već posle 4 ciklusa. Broj eozinofila se sveo na 640/μL krvi. **Zaključak.** U retkim slučajevima, eozinofilija bez otkrivenog uzroka, može prethoditi Hodžkinovom limfomu. Kod takvih bolesnika trebalo bi pažljivo ispitati moguće prisustvo uvećanih žlezda bilo gde u organizmu. Pravovremena i tačna histopatološka dijagnostika se pokazala kao pravi put u rešavanju ovakvih stanja.

Ključne reči:
eozinofilija; hodžkinova bolest; znaci i simptomi; dijagnoza; abvd protokol; lečenje, ishod.

Introduction

Eosinophilia becomes a diagnostic challenge for haematologists. It is defined as as the presence of > 500 eosinophils per μL of blood are eosinophilic leukocytes [1]. Precise characterization of eosinophilia is very important because successful treatment relies on the underlying disease aetiology [2]. Currently, eosinophilia can be divided into three types: secondary, clonal and idiopathic. Secondary (reactive) eosinophilia is a consequence of a cytokine-induced phenomenon that most often results from an upsurge in interleukin-5 secretion, leading to the proliferation of eosinophils and

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their precursors [3]. The main causes include parasitic infections, allergic conditions or vasculitis, drug reactions and non-myeloid malignancies. It is well known that reactive eosinophilia appears in lymphoid malignancies. Its frequency is estimated to be about 15% of all Hodgkin`s lymphoma (HL) patients [4]. Reactive eosinophilia is based on treating the underlying condition.

We here presented a diagnostic challenge in a female patient with persistent eosinophilia just six months before lymphoma appearance.

Case report

A female patient aged 51, from Serbia had leukocytosis of 12,000/ μL of blood, with eosinophilia 17% (2,040/ μl in absolute number) first time recorded in May 2012. She also had pain and swellings in hand joints, spine and hips. The first visit was at general practitioner in Kovačica city.

General symptoms were not present besides periodic malaise. A rheumatologist prescribed prednisone 5 mg daily, orally, mostly because of the left forearm swelling. The treatment lasted 10 days.

In November 2012, for the first time, a wen appeared in the right femoral region, upper third of thighs. Therefore, further investigation and treatment were continued in Pančevo General Hospital.

The first ultrasound examination showed the soft tissues tumor, hypoechogenic, not homogenous, 60 × 35 mm in size, in the frontal region of right thighs. Enlarged lymph nodes were not seen in the right hurdle and in abdomen, too. The finding was from November 20, 2012.

Computed tomography (CT) examination confirmed a limited tumour, pseudolobulated, expansive, 50 × 80 × 100 mm in size, without infiltrative features. The normal finding was shown on other organs, without enlarged lymph nodes in abdomen.

The surgical removal and histopathologic examination were indicated.

During the preoperative preparation, leukocytosis (16,5/μL of blood), with eosinophilia of 23% (absolute number 8,745/μL) were registered. Middle thrombocytopenia degree, with 82,000 of platelets *per* μL of blood, was found, as well.

Routine biochemical analyses were within normal range.

Tumor was surgically removed. Histopathological result showed eosinophilic granuloma.

Thereafter, gynecologic examination was performed showing normal finding. The breast and armpits ultrasound examination revealed cystic formations in glandular tissue. Lymph nodes were not enlarged.

The patient was sent to detailed bronchological and infectological examinations for persistent eosinophilia.

Chest roentgenogram and spirometry showed normal results. Oxygen saturation was 98%, partial oxygen pressure was 10,7 kPa. Skin allergic tests and methacholine test were negative. Stool tests for parasites and helminth eggs were negative for three times. Bronchial asthma and hypereosinophilic syndrome were excluded. Besides a haematologist and other specialist's efforts from Pančevo General Hospital, clear cause of eosinophilia was not found and the patient was sent to the Clinic for Haematology of the Clinical Centre of Serbia, Belgrade, for further examinations.

We saw the patient for the first time on March 23, 2014 and she complained of itchy skin. Physical finding was without signs of anaemia, hemorrhagic syndrome and enlarged lymph nodes in typical sites (neck, armpits and groin).

Blood parameters were: hemoglobin (HB) 140 g/L, hematocrit (HT) 0.40, mean corpuscular volume (MCV) 93 *f*L, platelet count (PLT) 137 × 10^9/L, white blood count (WBC) 20.4 × 10^9/L, with 54% (11,016/μL) absolute eosinophils number, segmented neutrophils 24.7%, lymphocytes 17.3 % and monocytes 4.0 %.

Myelogram analysis from March 25, 2014 showed reactive, normal bone marrow, with slight eosinophilia in hypocellular specimen (Figure 1).

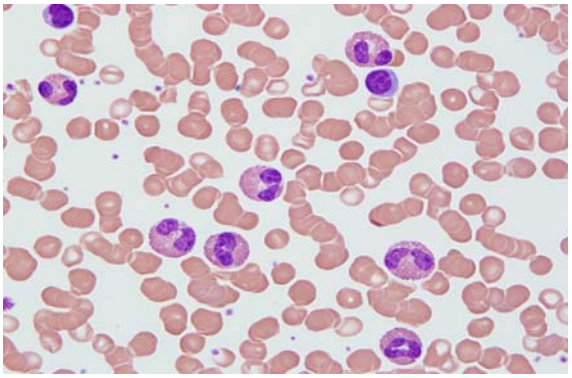

Fig. 1 – Blood eosinophilia.

Another ultrasound of abdomen was undertaken. Normal finding on parenchymal organs was found again, but for the first time, enlarged lymph nodes in conglomerate, size 70 mm, were found. They were right sided in the parailiac region.

On May 7, 2014 CT examination was also performed. It revealed conglomerate of enlarged lymphnodes, now 120 × 65 mm in size, in the parailiac right region, imprinting right bladder contour (Figure 2). Enlarged lymph nodes were found too in left-sided inguinal region, 15 mm in diameter.

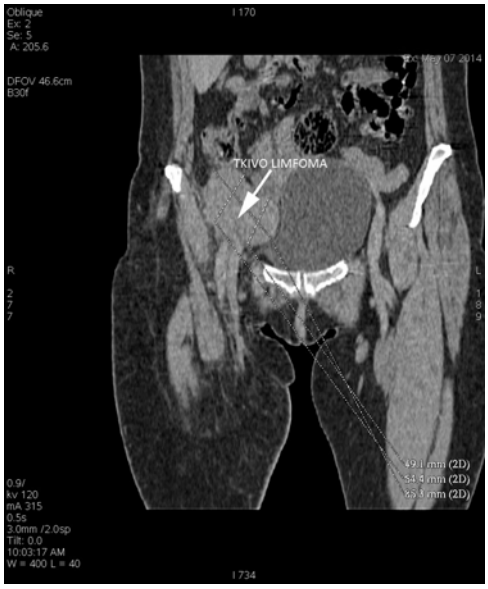

Fig. 2 – Lymph nodes conglomerate (parailiac right region).

It was necessary to perform revision of patient's whole condition. The patient was hospitalized at the Clinic for Allergology and Immunology, Clinical Centre of Serbia, Belgrade, Serbia, from June 12 to July 7, 2014 when some of diagnostic procedures were repeated and extended.

Biochemical analysis in the serum showed: glucose level 4,19 mmol/L, urea 5,4 mmol/L, creatinine 67 μmol/L, cholesterol 5,16 mmol/L, triglycerides 1,12 mmol/L, C reactive protein (CRP) 4,14 mg/mL, fibrinogen 4,22 g/L, bilirubin 98,9 μmol/L, total proteins 75 g/L, aspartate-aminotransferase (AST) 15 U/L, alanine aminotransferase (ALT) 17 U/L, alkaline phosphatase (AF) 96 U/L, gamma-glutamyl transferase (γGT) 31 U/L, lactate dehydrogenase (LDH) 423 U/L, β-2 microglobulin 2,49 mg/mL.

Protein immunoelectrophoresis showed suspicious M paraprotein finding but below detectability limit. Thereby M paraprotein was not defined clearly.

There were no parasites and protozoa in stool, 5 times determinated.

Toxocara canis IgG antibodies were positive twice [19,6 novaghost units (NU) first, and 15,1 NU second time]. IgM class titer was normal. This indicated former infection time not the acute disease.

Lung X-ray examination showed no pathologic changes in pulmonary tissue and mediastinum.

Spirometry showed slightly decreased lung diffusion capacity with the negative metacholine test. Lung capacity was as shown: vital capacity (VC) 100%, forced vital capacity (FVC) 103%, forced expiratory volume in the first second (FEV)-1 100%, peak expiratory flow (PEF) 122%, maximum expiratory flows $(MEF)_{50\%FVC}$, 115%, $MEF_{25\%FVC}$, 67%.

Ultrasound heart examination revealed mitral and tricuspid regurgitation +1. Small interventricular aneurism was observed, too.

Infectologist prescribed albendazole, 400 mg *per os* twice daily for two weeks.

There were no systemic connective tissue disease and no signs of vasculitis according to final allergist`s conclusion.

On September 16, 2014 the patient was presented to the oncology team of the Hematology Clinic, Clinical Centre of Serbia. Pathohistological (PH) finding revision of the removed tumor from 2013 was suggested.

PH revision finding, from September 29, 2014, showed: Morphologic and immunophenotype finding of classic Hodgkin lymphoma, mixed cellularity type.

Immunophenotype was: CD 20-, CD 3-, CD 15+, CD 30+, MUM 1+, CD 68-, EBV -, Ki 67+ in most tumour cells (Figure 3).

After such conclusion, staging of lymphoma was performed. According to Ann Arbor classification staging (CS) the patient was classified into II B CS, International Prognostic Score (IPS) 2, infradiaphragmal localisation.

As the patient had $37,9 \times 10^3$ the leukocytes/μL (eosinophils 59% or 22,361/μL) we started corticosteroid therapy with prednisone orally in a total daily dose of 70 mg, (1mg/kg/body mass). Treatment was continued with chemotherapy: adriamycin, bleomycin, vinblastme, decarbarine (ABVD) regimen protocol. The patient received on the first and fifteenth day doxorubicine 45 mg i.v; vinblastine 10 mg i.v; bleomycine 15 mg i.v; and dacarbazine 700 mg i.v.

After fourth ABVD course, abdomen and pelvis control CT examination was done. The finding was normal, without lymph nodes enlargement and tumour mass. The complete remission (CR) was achieved after four courses of chemotherapy. Treatment was discontinued in March 2015, after six courses of ABVD protocol.

After that, the blood analysis showed normal findings except for slight eosinophilia – 640/μL.

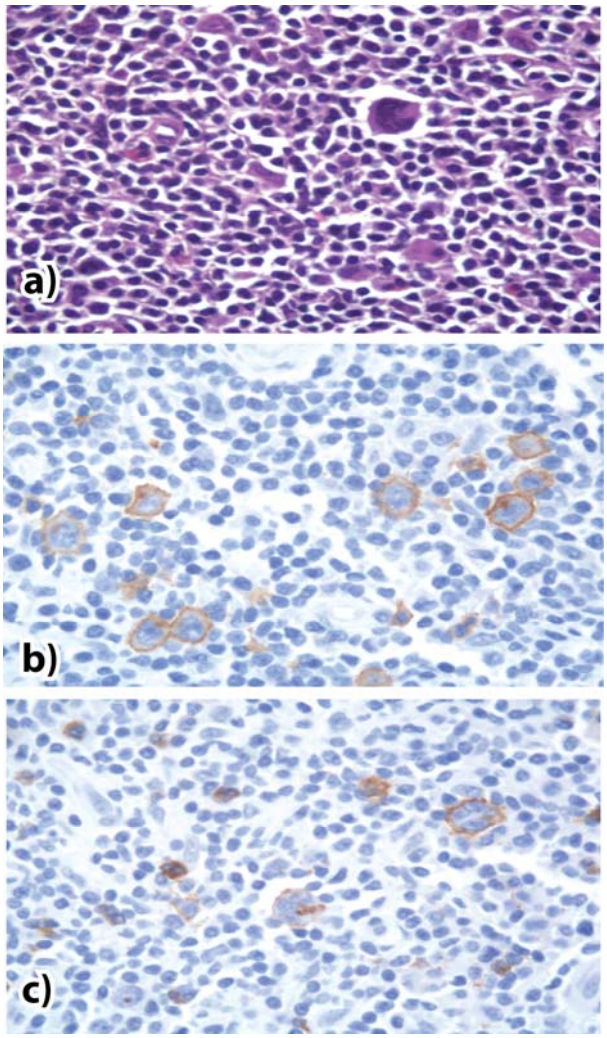

Fig. 3 – Classic Hodgkin lymphoma, mixed cellularity type: a) HE, ×400; b) CD 30+; c) CD 15+.

Discussion

The female patient was presented with blood eosinophilia (1,530–2,040/μL blood) 6 months before HL diagnosis. Despite of many examinations made by several physicians (a general practitioner, surgeon, radiologist, infectologist, alergologist and haematologist) eosinophilia cause was not defined.

Key point was histopathological revision of the removed tumour, unfortunately primarily classified as eosinophilic granuloma. These two diseases are not easy to differ and mistakes are possible. In German publication from 2002 there was a suspicion that two patients had lymphoma and eosinophilic granu-

loma at the same time [5]. 18 fluoro-2-deoxy-D-glucose (FDG) elevated uptake with mediastinal positive positron emission tomography (PET) lesions were found in the second case. Further specimen histopathological revision confirmed that eosinophilic granuloma was a crucial diagnosis while lymphoma was not.

Eosinophilia and HL association is estimated to be present in 15–38% of all lymphoma patients [4, 6]. Its prognostic significance is not entirely clear.

In the article published in 2000 [6] authors analysed 1,511 specimens of HL patients. Significant tissue eosinophilia was found in 38% of the patients. The finding negatively affected patients with nodular sclerosis type. Comparing to the other histopathologic types shorter survival and disease free survival were recorded.

There are few cases referring to association between eosinophilia and HL. One of them is almost identical to our case. This case described by Ayyub et al. [7] from 2003 had hypereosinophillic syndrome that preceded HL even four years. In the other one, reported by Chinese authors, Hodgkin's lymphoma with multifocal spine involvement, was observed with eosinophilia that preceded 6 months before [8].

Eosinophils role in lymphoma pathogenesis is yet unequivocally. Eosinophils provide ligands for receptors originated from tumor necrosis factor (TNF), CR 30, CD 40, and CD 95 Fas; also they can stimulate growth and send anti-apoptotic signals on Reed Sternberg cells [9].

Therefore it should be kept in mind that eosinophilia, without obvious reason, can precede HL.

Conclusion

Eosinophilia without revealed cause can precede HL. We suggest carefull searching for enlarged lymph nodes, anywhere in the body of patients with eosinophilia. Timely and accurate histopathologic diagnostic is a right way to resolve such conditions.

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CASE REPORT

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Erdheim-Chester disease – A case report

Oboljenje Erdhajm-Čester

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Abstract

Introduction. Erdheim-Chester disease (ECD) is a rare non-Langerhans-cell histiocytosis. About 500 cases are published so far. It is multisystemic disease characterised by bilateral symmetric long bones sclerosis. Main histopathological finding is accumulation of big foamy histiocytes, immunohistochemically positive to CD68, and negative to S-100 and CD1a. There are no guidelines that reliably identify population that requires therapy, but symptomatic ECD, organ failure and central nervous system involvement require treatment. **Case report.** We described a patient with a multisystemic form of ECD affecting long bones, the hypophysis, abdomen, and the peripheral nerves. Five years after initial symptoms ECD was suspected. Prednison was initiated, 60 mg once a day. After obtaining the diagnosis of ECD, interferon alpha 2A was introduced, but soon after stopped due to severe side effects. Considering that histiocytes were positive to platelet derived growth factor receptor alpha (PDGFR alpha) imatinib mesylate was started, but after two months stopped due to no clinical and radiological improvement. The disease was worsening and the patient died. **Conclusion.** We described the patient with intraperitoneal form of ECD, without cardiac and pulmonary involvement. There are several important issues: the diagnosis of ECD could be difficult to make, three treatment regimens were included and the patient died nine years after the initial symptoms due to indolent course of the disease and unsuccessful treatment.

Key words:
erdheim-chester disease; diagnostic techniques and procedures; immunohistochemistry; diagnosis, differential; drug therapy.

Apstrakt

Uvod. Oboljenje Erdhajm-Čester (Erdheim-Chester, ECD) je redak oblik ne-Langerhansove histiocitoze sa oko 500 bolesnika prikazanih do sada. To je multisistemska bolest koju karakteriše bilateralna simetrična kortikalna skleroza dugih kostiju. Patohistološki, karakteriše se nakupljanjem histiocita imunohistohemijski pozitivnih na CD68 i negativnih na S-100 i CD1a. Nema jasnih preporuka za lečenje, ali bolesnici sa simptomima, disfunkcijom organa ili zahvatanjem centralnog nervnog sistema zahtevaju lečenje. **Prikaz bolesnika.** Prikazali smo bolesnika sa multisistemskim oblikom ECD, sa zahvatanjem dugih kostiju, hipofize, trbušne duplje i perifernih nerava. Pet godina posle prvih simptoma posumnjalo se da se radi o ECD. Lečenje je započeto prednisonom, 60 mg jednom dnevno. Kada je potvrđena dijagnoza ECD uveden je interferon alfa 2A, ali je ubrzo ukinut zbog neželjenih efekata. S obzirom na to da su histiociti bili pozitivni na trombocitni faktor rasta alfa (PDGFR alfa) u terapiju je uveden imatinib mesilat. Nakon dva meseca terapije, imatinib je ukinut zbog nedostatka terapijskog efekta. Zbog pogoršanja bolesti, nastupio je smrtni ishod. **Zaključak.** Prikazali smo bolesnika sa intraperitonealnom lokalizacijom bolesti, bez zahvatanja srca i pluća. Postoji nekoliko ključnih stavki: dijagnoza ECD može biti otežana, bila su uključena tri režima terapije i bolesnik je preminuo devet godina od početnih simptoma zbog sporog toka bolesti i neuspešnog lečenja.

Ključne reči:
erdheim-chesterova bolest; dijagnostičke tehnike i procedure; imunohistohemija; dijagnoza, diferencijalna; lečenje lekovima.

Introduction

Erdheim-Chester disease (ECD) is a rare non-Langerhans-cell histiocytosis of unknown etiology. Around 500 cases have been published so far, especially in the last 10 years due to increased awereness of this disease. The disease was named after William Chester and Jakob Erdheim who first described two cases with "lipoid granulomatosis", in 1930 [1]. It affects adults,

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both sexes equally, rarely affects children. The pathogenesis of ECD is not known. Th1 immune response is dominant, thus suggesting it as inflammatory disease, but a recent finding of BRAF V600E mutation suggests it as a clonal disease, dependent on impaired RAS/RAF/MEK/ERK signalling [2]. Activating mutation of NRAS gene is found in 3.7% of patients [3]. Pathogenic BRAF V600E mutation is found in 54% of patients [4]. The disease is characterized by extensive proliferation and accumulation of histiocytes in tissue, particularly connective and fat ones. It primarily affects long bones leading to bilateral, symmetric cortical sclerosis, sparing epiphysis. Bone lesions could be confirmed on magnetic resonance imaging (MRI), technetium-99m bone scintigraphy, ^{18}fluoro-2-deoxy-d-glucose (^{18}FDG) positron emission tomography/computed tomography (PET)/CT scanning, but they are often missed on plain radiographs. FDG PET/CT is also usefull for extraskeletal disease and in surveillance [5]. The diagnosis of ECD relies on two criteria, proposed by Veyssier-Belot et al. [6] and they are typical histological and skeletal findings. Almost 50% of the patients have extraskeletal manifestations, including hypophysis, orbit, heart, lungs, kidneys, retroperitoneum, central nervous system (CNS) and skin. The histology is characterized by the accumulation of big foamy histiocytes with lipid-rich eosinophilic cytoplasm. Histocytes are immunohistochemically positive to CD68, negative to S-100 and CD1a, lacking Birbeck granules on electronic microscopy. The prognosis depends upon visceral manifestations. Approximately 50–60% of the patients die after 3 years of disease, most often due to cardiac and pulmonary involvment. Treatment regimens include: glucocorticoids, cyclophosphamide, vincristine, cladribine, methotrexate, lenalidomide, imatinib mesylate, anakinra, interferon alpha, sirolimus, infliximab, vemurafenib, canakinumab, autologous hematopoietic-stem cell transplantation, radiotherapy, and surgery [7–33].

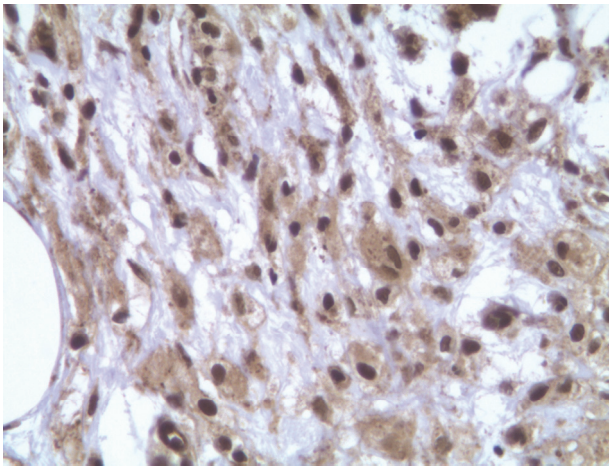
Case report

A male patient, 58 years old, was diagnosed with central diabetes insipidus of unknown etiology. He was treated with substitution therapy – desmopressin (Minirin® spray). Two years after, surgical ablation of the tumor, affecting soft tissue of the right lower extremity, was done. There was neither bone infiltration, nor distant metastasis. The final histopathological finding showed dedifferentiated liposarcoma or dedifferentiated schwannoma. Following the surgery, the patient was treated with radiotherapy and chemotherapy and scheduled for follow-up. Two years later, due to nausea, abdominal pain and distension, multislice computed tomography (MSCT) of the abdomen was performed. It showed ascites and the thickened peritoneum and mesenteric structures. After that, explorative laparoscopy and biopsy of the omentum, lymph nodes and small bowel mesentery were done. The final histopathological finding confirmed *inflamatio chronica xantogranulomatosa textus adiposus.* A year later, ECD was suspected based on anamnesis, clinical features and examinations performed (increased serum markers of inflammation, plain radiography survey showing bilateral simmetric cortical sclerosis of the lower extremities sparing epiphysis), wihout cardiac and pulmonary involvement. Prednisone was started, 60 mg once a day, tapered to 15 mg once a day which he was taking for the next three years.

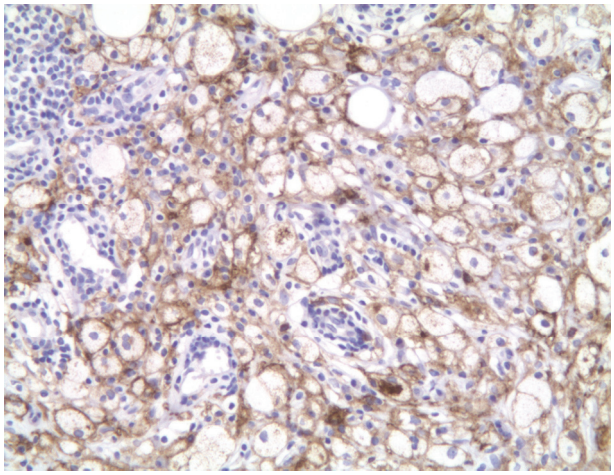
Due to paresthesia and weakness of the lower extremities we performed electromyoneurography (EMNG), showing sensory polyneuropathy. Alpha-lipoic acid was introduced, resulting in partial improvement. Few months later, he developed gangrene of the right lower extremity, thus above knee amputation was performed. Eight months later, we performed revision of the histopathological finding of biopsies of the peritoneum, omentum and lymph nodes, done 4 years ago, with immunihystochemical staining that confirmed the ECD (CD68+, CD1a-, S100-, CD14+, CD163+). After evaluation [upper and lower endoscopy, CT of the abdomen, bone scintigraphy, magnetic resonance imaginig of the lumbosacral spine (MRLS), bone marrow biopsy, peripheral blood and bone marrow samples testing for clonal rearrangments Ig (immunoglobulin)/TCR (T cell receptor) genes, dual-energy x-ray absorptiometry], progressive disease (PD) was confirmed, along with osteoporosis as sequelae of prednison therapy (Figures 1 and 2). Interferon alpha 2A was introduced, 3,000,000 IJ 3 times weekly subcutaneously. Osteoporosis was treated with bisphosphonates. Three months later interferon alpha 2A was discontinued due to severe side effects (nausea, loss of appetite, fever, abdominal pain, diarrhea, depression), and prednisone was restarted, 20 mg once a day.

Additional immunihystochemical staining confirmed that histiocytes were positive to PDGFR alpha, so imatinib mesylate was introduced, 400 mg once a day, with tapering off prednison till discontinuation. After two months of the treatment with imatinib mesylate there was no clinical improvement. Laboratory analysis showed increased serum markers of inflammation, low albumin (21 g/L), so imatinib mesylate was discontinued and prednisone restarted, 20 mg once a day. The introduction of prednisone led to a short-term clinical improvement. The patient's general condition got worse, swelling of the legs was pronounced, he had syncope. The patient was not motivated for hospitalization, and died.

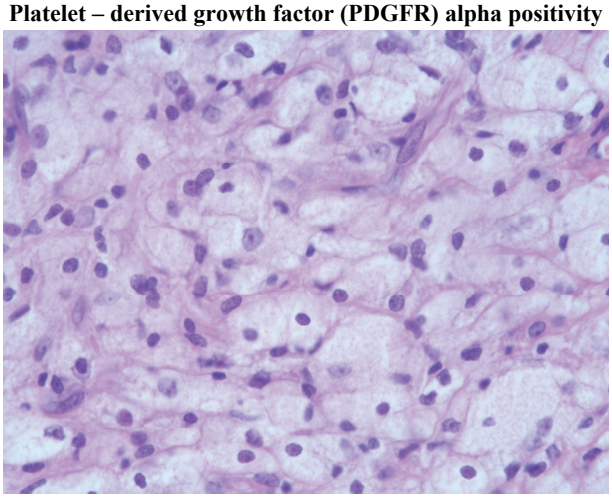
Fig. 1 – Bone scyntigraphy – increased accumulation of radiopharmaceutical along the diaphysis of radius, ulna and humerus bilaterally, in the lower part of the left femur and along the left tibia.



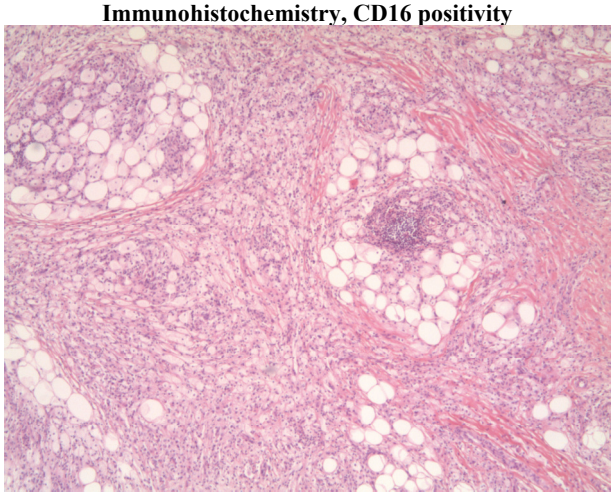
Platelet – derived growth factor (PDGFR) alpha positivity



Immunohistochemistry, CD16 positivity



Hematoxylin – eosin (HE, × 20)



Hematoxylin – eosin (HE, × 5)

Fig. 2 – Patohistological findings

Discussion

ECD is a rare disease. Considering different clinical features and disease activity, varying from asymptomatic to fulminant organ failure, the diagnosis is usually delayed. Patients without cardiac and pulmonary involvement have longer life expectancy. Intraperitoneal localization of ECD is very rare. That is exactly what we want to emphasize with this case report – the diagnosis is delayed, almost every organ can be affected, intraperitoneal localisation is very rare, there are no established guidelines for treatment. Regarding the abdominal form, the most affected is the retroperitoneum [33]. There are only few cases of ECD with intraperitoneal involvement published [16–19], one case describes histiocytes in ascites fluid [15]. The gastrointestinal tract is rarely affected [20, 21]. Only one case published in the literature describes mucosal findings with histiocyte infiltration found on histology – nodular gastritis of the entire stomach and small hyperpigmented lesions on colonic mucosa [22].

Treatment regimen depends on the localization of the disease. Cytotoxic therapy is commonly used, but interferon alpha 2A, 3–9 million units 3 times *per* week, is considered as the first-line therapy [23]. The mechanism of action of interferon alpha 2A in ECD is not fully understood: it influences maturing and activation of dendritic cells, activates the natural killer cells which leads to the destruction of histiocyte and acts cytotoxicly directly to histiocyte [23].

As salvage therapy in six patients with severe multisystemic ECD, refractory to other therapies, and histiocytes positive to PDGFR beta, Haroche et al. [24] used imatinib mesylate, 100–800 mg once a day. Autologous hematopoietic-stem cell transplantation in a young patient, after disease progression following a few lines of therapy, has also been described [25]. In case of bone involvement, zoledronic acid showed promising results [26]. Mammalian target of rapamycin (mTOR) could be a new target in ECD. Gianfreda et al. [29] treated 10 patients with prednison and sirolimus, and achieved stable disease in 8 patients. Tumor necrosis factor alpha (TNF α) is a key regulator of inflammation in ECD. Infliximab was safely used in treating two patients, with clinical improvement [30]. BRAF inhibitor vemurafenib could be used to treat patients with BRAF mutation [31]. Recent data suggest that canakinumab is a potential new drug for ECD [32]. Systematic review of 448 patients, published recently, provides detailed clinical features, prognostic and predictive factors. INF α based therapy is a reliable option, but a new therapy is emerging: infliximab, BRAF inhibitors, and mTOR inhibitors [33].

We described a case with the multisystemic form of ECD, affecting long bones, the hypophysis, abdominal cavity, and peripheral nerves. The disease presented with diabetes *insipidus*, then manifested as soft-tissue tumor of the right lower extremity. It was probably histiocytoma, unrecognized histopathologically. Xanthogranulomatous inflammation of fat tissue of the omentum was confirmed with laparoscopic biopsy, while the diagnosis of ECD was made based on clinical features, pathological/immunohistochemical findings and radiological criteria. The differential diagnosis included: Langerhans cell histiocytosis, Rosai-Dorfman disease, multiple myeloma, Whipple's disease, hemophagocytic syndromes. Taking into consideration that the patient was not motivated for chemotherapy, treatment included: prednisone, interferon alpha 2A, imatinib mesylate. Due to disease progression the patient died, nine years after the initial symptoms (five years after the diagnosis was made).

Conclusion

ECD is a rare form of a non-Langerhans-cell histiocytosis of unknown etiology. It affects elderly, usually is unrecognized in the beginning. It is mainly multisystemic, thus it is necessary to start therapy as soon as possible. The prognosis depends on the extraskeletal manifestations of the disease, especially cardiac and pulmonary involvement. There are no guidelines for treatment of ECD. Interferon alpha is thought to be the first-line therapy. New therapy appears on horizon: infliximab, BRAF inhibitors, mTOR inhibitors.

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Facing dengue fever – our first experience

Suočavanje s dengom: naše prvo iskustvo

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Abstract

Introduction. Dengue fever is a mosquito-borne disease caused by dengue virus, endemic in tropical and subtropical regions, where it is mostly imported from. The most common clinical form is classic dengue fever. We presented the first dengue case microbiologically confirmed in Serbia. **Case report**. A 34-year-old male got classic dengue fever after arrival from Cuba. The disease occurred suddenly with fever, myalgias, skin rash, hepatosplenomegaly, cytopenia, abnormal aminotransferase and creatine kinase levels. The diagnosis was confirmed with virological diagnostic methods. Significant leukopenia and thrombocytopenia as well as elevation of serum creatine kinase activity were recorded from the very beginning of hospitalization, but were gradually normalized. The whole duration of hospitalization was accompanied by laboratory signs of liver lesion. The disease had favourable outcome. At hospital discharge, the patient was afebrile, asymptomatic, with discrete erythematous rash on torso and arms, normal hemathological values and creatine kinase level and moderately elevated alanine-aminotransferase level. **Conclusion**. Considering global climate changes and growing international traffic, our health care service needs to be ready for possible massive outbreaks of dengue and other tropical infectious diseases in forthcoming years.

Key words:
dengue; diagnosis; signs and symptoms; leukopenia; thrombocytopenia; creatine kinase.

Apstrakt

Uvod. Denga je oboljenje izazvano virusom denge, endemsko u tropskom i suptropskom pojasu. Autohtoni slučajevi retki su u Evropi. Oboljenje je najčešće uvezeno iz endemskih regija sveta. Prikazan je prvi slučaj denge koji je mikrobiološki potvrđen u Srbiji. **Prikaz bolesnika**. Po povratku s Kube, 34-godišnji muškarac se razboleo naglo, s febrilnošću, mialgijama, kožnim osipom, hepatosplenomegalijom, citopenijom, patološkim nalazima aminotransferaza i kreatin kinaze. Dijagnoza denge potvrđena je metodama virusološke dijagnostike. Značajna leukopenija i trombocitopenija bile su glavne karakteristike krvne slike. Laboratorijski znaci lezije jetre registrovani su tokom cele hospitalizacije. Aktivnost kreatin-kinaze u serumu bila je povišena na prijemu u bolnicu. Uz simptomatsko lečenje, bolest je imala povoljnu evoluciju, bez komplikacija. Bolesnik je otpušten iz bolnice afebrilan, bez tegoba, s diskretnim eritemom kože trupa i ruku. Vrednosti hematoloških parametara i kreatin kinaze na otpustu bile su uredne, a aktivnost alanin aminotransferaze umereno povišena. **Zaključak**. Zdravstvena služba naše zemlje trebalo bi da, u uslovima globalnih klimatskih promena i sve razvijenijeg međunarodnog transporta, bude spremna, u organizacionom, kadrovskom i materijalno-tehničkom pogledu, da odgovori na epidemijsku pojavu denge i drugih tropskih infektivnih bolesti u predstojećem periodu.

Ključne reči:
denga; dijagnoza; znaci i simptomi; leukopenija; trombocitopenija; kreatin kinaza.

Introduction

Dengue fever is an infectious disease caused by dengue virus, Flaviviridae family. There are 4 subtypes of the virus (DEV 1-4). The disease is worldwide distributed. It is endemic in many countries throughout Africa, Asia and South America. A half of million people throughout the world is being diagnosed with dengue *per* year [1]. Most dengue cases in Europe are imported from endemic regions. A total of 1,118 confirmed cases were reported in 15/24 countries of Europian Union in 2012; 78% of them were imported from Asia, and 10% originated from South America [2]. Autochtonous dengue cases were described in Europe as well, like in Croatia [3] and France [4]. A dengue outbreak was reported in

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Madeira (Portugal) with more than 2,000 cases [5]. In urban areas, the virus maintains in life cycle between human and *Aedes aegypti* mosquito. Mosquito gets infected by feeding on viremic person and transmits the infection to a healthy person during repeated blood feeding. Viremia lasts for 4–7 days. The virus then replicates in reticuloendothelial cells [6]. Dengue is sometimes difficult to differentiate from the other vector-borne diseases: malaria, chikungunya, yellow fever, tick-born encephalitis. In these cases, virological diagnostics is necessary, like virus isolation on cell culture, viral antigen NS1 serologic tests like enzyme immunoassay (ELISA) and rapid tests [7]. Polymerase chain reaction (PCR) is used for detection of viral ribonucleic acid (RNA). Treatment of dengue is symptomatic. The vaccine for human use for protection against dengue is not licenced so far. However, ongoing phase III clinical studies of attenuated tetravalent vaccine are promising [8]. We presented the first microbiologically confirmed dengue case in Serbia.

Case report

A 34-year-old male from Novi Sad surroundings was hospitalized at the Clinic for Infectious Diseases, Clinical Center of Vojvodina, Novi Sad, Serbia on 5 October, with the diagnosis of dengue. He has been living in Havana, Cuba, for the last three years. The disease started abruptly on 30 September; he complained of fever raising up to 39.7°C, chills, headache and myalgias. The temperature was dropping temporarily with abundant sweating and then raising again. On the third day of the disease, his general condition improved, the body temperature was normal, but he started caughing dry. From day 4 after onset of the disease, the temperature reached 39^0 C again, followed by constitutional symptoms. Laboratory findings taken just before hospital admission (Table 1) showed marked leukopenia (1.6×10^9/L) and thrombocytopenia (74×10^9/L normal range 140–400 × 10^9/L) mild elevation of creatine kinase-352 U/L (normal range 24–195 U/L), two-fold elevation of alanine aminotransferase (ALT) and gamma glutamyl transferase (GGT) level, one-third-fold elevation of alanine aminotransferase (AST) level, while the other laboratory findings were within normal limits. On hospital admission (day 6 after onset of the disease), the patient had discrete maculopapular skin rash on torso and forearms and on palpation. The serum sample was sent to Department for Virology, Institute for Public Health of Vojvodina, Novi Sad, Serbia. Real-time polymerase chain reaction (PCR) test (using reagents and protocols obtained from the Centers for Disease Control and Prevention (CDC) to dengue virus 1-4 was done; it was positive to type 3 dengue virus on day 7. In adition, indirect immunofluorescence test (IIFT) immunoglobuline (IG) M/IgG (manufacturer Euroimmun, Lübeck, Germany) against dengue virus 1-4, West Nile virus, Japanese encephalitis virus, yellow fever virus and tick-borne encephalitis virus were applied. The only positive finding was related to IgM antibodies to subtype 3 dengue virus on day 7 of the disease. The patient was isolated and rehydrated. He was permanently afebrile during the stay at hospital. His only complaint was mild dry cough with normal chest radiogram finding. The peak serum creatine kinase activity was reached on the first day of hospitalization – 975 U/L and gradually normalized to the sixth day. Moderately elevated ALT level of 221 U/L was recorded at hospital discharge. Both the white blood count and platelets were normaly on discharge (Figures 1 and 2). The patient was dismissed from the hospital on 9th day after admission afebrile, in a good general condition, with slight diffuse erythematous rash on the trunk and arms.

Discussion

Dengue is the most common arthropod-borne infection in the world. According to World Health Organisation (WHO), since seventh decade of the former century, its incidence has raised up to 50–100 millions of cases *per* year and has inc-

Table 1

Blood laboratory findings just before hospital admission

Laboratory finding	Actual values	Reference values
Erythrocyte sedimentation rate (mm/h)	5	2–6
White blood count (× 10^9/L)	1.58	4.0–10.0
neutrophils (%)	64.3	40.0–74.0
lymphocytes (%)	23.0	19.0–50.0
monocytes (%)	4.5	2.0–10.0
eosinophils (%)	2.4	0.0–7.0
Platelets (× 10^9/L)	74	140–400
Creatine kinase (U/L)	352	24–195
Total bilirubin (μmol/L)	9.8	2.0–21.0
Direct bilirubin (μmol/L)	4.2	0.0–3.4
Alanine-aminotransferase (U/L)	104	0–50
Aspartate-aminotransferase (U/L)	100	10–75
Alkaline phosphatase (U/L)	95	60–142
Gamma glutamyl transferase (U/L)	148	0–73
Urea (mmol/L)	4.3	3.2–8.2
Glucose (mmol/L)	6.7	4.1–5.9
Lactat dehydrogenase (U/L)	586.0	0.0–1327.0
C-reactive proteine (mg/L)	3.3	0.0–5.0
Potassium (mmol/L)	4.0	3.5–5.5
Sodium (mmol/L)	137	132–146

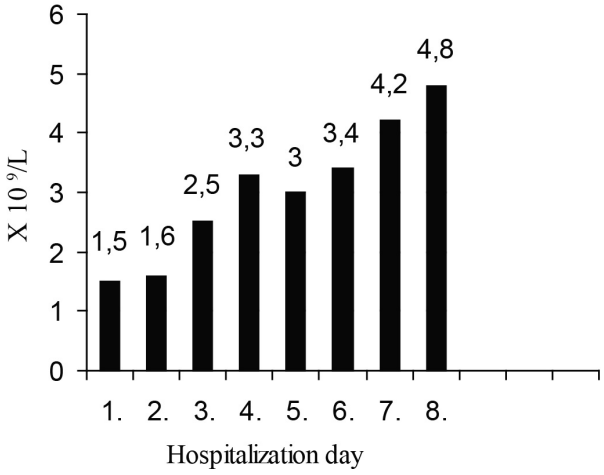

Fig. 1 – Peripheral blood leukocytes during hospitalization.

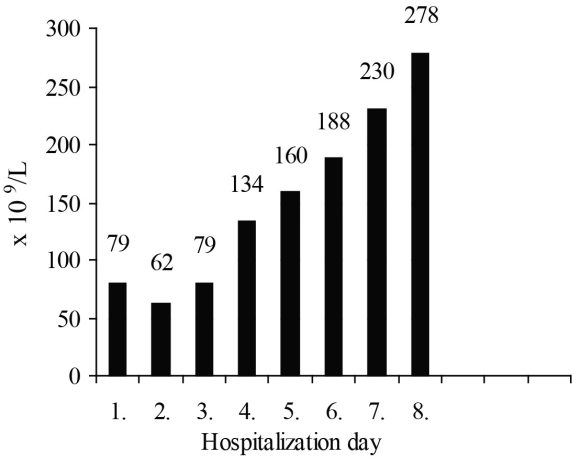

Fig. 2 – Peripheral blood platelets during hospitalization.

reased 30-fold. Great public importance of dengue is also highlighted by the fact that half of the world population lives in the areas where dengue is endemic disease. During last several years, Serbia has been affected by drastic migrations of its own people, motivated by the search for higher life standard. At the same time, our country is exposed to the flows of refugees from the Middle East, who are trying to escape the war in their own countries. This is the reason why our health system must be capable to handle the threat of tropical infectious diseases such as dengue and other haemorrhagic fevers, not only in terms of technological and material resources but in terms of medical knowledge as well. Naturally, we suppose that dengue in our patient would be recognized faster and easier in a country which is endemic for dengue.

Since 1970, the Caribbean (where Cuba is located) have been hit by frequent dengue outbreaks. Our patient aquired dengue virus infection in Cuba. In our patient IgM antibodies against dengue virus type 3 were found out by serologic method (IIFT). These antibodies are detectable in 99% cases of dengue until 10th day of disease. IgG antibodies can be detected in most cases over the first week of disease and their titer raises slowly [9]. Type 3 dengue virus infection was confirmed by real time PCR test. Mild form of disease can be explained by patient´s younger age (severe haemorrhagic form hits children in more than 90% of cases), lack of comorbidity as well as lack of secondary infections with other types of virus which predispone to severe forms of the disease. Viremic phase in dengue matches clinical presentation of the disease, lasting for 4–7 days, so in that period molecular diagnostics remains a good diagnostic tool [9]. Literature data describe "flu-like syndrome" in clinical presentation of dengue; our patient presented with the same clinical features. "Flu-like syndrome" is the consequence of inflammatory response with cytokine production. Maculopapular rash on the trunk and arms went away soon after admission, so from the second day of hospitalization only a diffuse non-itchy rash could be seen on those parts of the body. According to literature data, rash in dengue most commonly appears as morbilliform or maculopapular. Moderate splenomegaly indicated a generalised disease of viral origin. On the third day of hospitalization a short-term improvement occurred, followed by a drop in temperature to its normal values. Biphasic course of the disease is being mentioned in clinical studies of dengue as a common feature [9, 10].

Cytopenia can be expected in dengue patients because of a potential direct infection of chematopoetic cells of bone marrow in the course of early viremia [11]. Our patient had a significant leukopenia followed by a moderate thrombocytopenia which did not require substitutional therapy. During the hospitalization, gradual and complete spontaneous normalisation of white blood count and platelets was registered. Blood leukocytes tended to rise from the admission (1.5×10^9/L) to the discharge from the hospital (4.8×10^9/L). Platelets varied from 74×10^9/L on the admission to 278×10^9/L at the discharge from hospital. Normal blood coagulation tests during the whole course of hospitalization were responsible for the lack of haemorrhagic syndrome. High serum creatine kinase level was recorded several times during the hospitalization. It might be the consequence of perivascular mononuclear cell infiltration of muscles. Data describe dengue cases with myositis and rhabdomyolysis [9, 12]. Fortunately, deadly outcome due to hepatic failure is rare. AST is somewhat higher than ALT at early stage of the disease, which is supported by muscle damage due to infection. In our patient, ALT level was higher than AST level, probably because of late blood testing in relation to the beginning of the disease [9, 13].

Among the surrounding countries, the autochtonous transmission of dengue was recorded only in Croatia [14]. In this country, the presence of mosquito *Aedes albopictus* may be responsible for autochtonous appearance of dengue [15]. In Serbia there have been no conditions for autochtonous occurrence of dengue so far, because neither the suitable vector has been registered nor continuous appearance of imported

dengue cases has been occurring. Epidemiology of dengue in Serbia appeared to be slightly different in comparison to the sixties of the former century. At that time, the initial investigation on seroprevalence to dengue virus 1 and 2 has been carried out and exposure of Serbian human population to those subtypes of dengue virus has not been confirmed [16]. The presence of viremic patient and suitable vector are necessary conditions for local outbreak appearance [17]. If those conditions were fulfilled in the future, Serbia could face autochtonous dengue in the same way Croatia is facing it now.

Conclusion

Considering global climate changes and growing international traffic, our health service needs to be ready for possible massive outbreaks of dengue and other tropical infectious diseases in forthcoming years.

Patients with history of previous stay in tropical regions known for dengue appearance who have high fever, "flu-like" syndrome followed by a skin rash, hepatosplenomegaly and cytopenia, optionally, and followed by haemorrhagic syndrome should arouse the suspicion on dengue fever.

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CASE REPORT

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First patient in Serbia with biochemically and genetically diagnosed pyridoxine-dependent epilepsy

Prvi bolesnik u Srbiji sa biohemijski i genetički dijagnostikovanom piridoksin zavisnom epilepsijom

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Abstract

Introduction. Pyridoxine-dependent epilepsy (PDE) is a rare autosomal recessive inborn error of metabolism present with early-onset seizures resistant to common anticonvulsants. PDE has been shown to be caused by a defect of a α-aminoadipic semialdehyde dehydrogenase (also known as ALDH7A1 or antiquitin) in the cerebral lysine degradation pathway. Its deficiency results in accumulation of α-aminoadipic semialdehyde (α-AASA), piperideine -6-carboxylate and pipecolic acid, which serve as diagnostic markers in urine, plasma and cerebrospinal fluid of the disease. α-Aminoadipic semialdehyde dehydrogenase is encoded by the ALDH7A1 or antiquitin gene and definite confirmation of diagnosis of PDE is made by genetic analysis. **Case report**. We present a first patient in Serbia who was diagnosed clinically, biochemically and genetically. We suspected PDE due to drug-resistant seizures in the seventh day of life when we attempted with pyridoxine. Since that time the patient has taken pyridoxine and the seizures have not recured. Our patient had markedly elevated α-AASA in urine while on treatment with individual dosages of pyridoxine. Molecular-genetical analysis identified mutations of the ALDH7A1 (antiquitin) gene. **Conclusion**. α-AASA is reliable marker to select PDF patient for molecular analysis of the ALDH7A1(antiquitin) gene. Diagnosis is confirmed by molecular-genetical analysis and pyridoxine withdrawal is no longer needed to establish the diagnosis of „definite" PDE.

Key words:
epilepsy; infant, newborn; vitamin B6; genetic diseases, inborn; diagnosis, differential; drug therapy; serbia.

Apstrakt

Uvod. Piridoksin zavisna epilepsija (PZE) je redak, urođeni autozomno-recesivan poremećaj metabolizma sa ranom pojavom konvulzija rezistentnih na uobičajene antikonvulzivne lekove. Utvrđeno je da je PZE posledica poremećaja α-aminoadipin semialdehid dehidrogenaze (poznate i kao ALDH7A1 ili antikvitin) na putu degradacije cerebralnog lizina. Njegov nedostatak dovodi do nakupljanja α-aminoadipin semialdehida (α-AASA), piperidin-6-karboksilata i pipekolične kiseline u urinu, plazmi i cerebrospinalnoj tečnosti, i oni se koriste kao dijagnostički markeri oboljenja. α-Aminoadipin semialdehid dehidrogenaza je kodirana ALDH7A1 ili antikvitin genom i definitivna dijagnoza PZE se utvrđuje genetičkom analizom. **Prikaz bolesnika**. Ovo je prikaz prvog bolesnika u Srbiji čije je oboljenje dijagnostikovano klinički, biohemijski i genetički. Na PZE smo posumnjali zbog konvulzija rezistentnih na lekove koje su se javile sedmog dana života kada smo kod bolesnika pokušali lečenje primenom piridoksina. Od početka primene pirodiksina bolesnik više nije imao ponavljane konvulzije. Bolesnik je imao značajno povišen α-AASA u urinu tokom lečenja pojedinačnim dozama piridoksina. Analizom na molekularno-genetičkom nivou identifikovane su mutacije ALDH7A1 ili antikvitin gena. **Zaključak**. α-AASA je pouzdan marker za selekciju bolesnika sa PZE radi molekularne analize ALDH7A1 gena. Dijagnoza našeg bolesnika potvrđena je analizom na molekularno-genetičkom nivou i nije bilo potrebno prekidati terapiju piridoksinom radi potvrđivanja dijagnoze PZE.

Ključne reči:
epilepsija; novorođenče; vitamin B6; genetičke bolesti, urođene; dijagnoza, diferencijalna; lečenje lekovima; srbija.

Introduction

Pyridoxine-dependent epilepsy (PDE) is an autosomal recessive disease which occurs in 1 in 100,000 to 700,000 individuals. At least 100 cases have been reported worldwide [1]. Typically, patients present with neonatal seizures, but atypical manifestations up to 3 years of age, as well as transient response to common anticonvulsants or poor initial response to pyridoxine have been reported [2–4]. Until recently, a definite diagnosis of PDE had been established

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upon a successful therapeutic trial with pyridoxine and further proof of pyridoxine after a controlled withdrawal with recurrence of seizures [5, 6]. Recently, PDE has been shown to be caused by a defect of α-aminoadipic semialdehyde dehydrogenase (also known as ALDH7A1or antiquitin, ATQ), in the cerebral lysine degradation pathway and to catalyze the conversion of α-aminoadipic semialdehyde (α-AASA) to α-aminoadipic acid. α-AASA is in chemical equilibrium with piperideine-6-carboxylic acid (P6C). P6C has been shown to inactivate pyridoxal phosphate (PLP), the active vitamer of pyridoxine, by a Knoevenagel condesation reaction, leading to a severe secondary PLP deficiency. As PLP is a cofactor of various enzymes in the central nervous system, seizures in PDE are more probably due to a pertubation in metabolism of cerebral amino acids and neurotransmitters [3].

Biochemically, ALDH7A1or ATQ deficiency is characterizied by the accumulation of α-AASA and P6C and by the accumulation of pipecolic acid (PA), which is formed proximally to the primary enzyme defect. Screening for ATQ deficiency is possible *via* determination of urinary or plasma α-AASA and P6C, and of plasma PA. The diagnosis is confirmed by mutation analysis [3].

Molecular analysis has revealed mutations of the ALDH7A1or ATQ gene in all individuals with PDE and increased PA and/or α-AASA in plasma or urine. Pyridoxine therapy is life-long, but despite treatment many patients with PDE have a disorder of psychomotor development. Gathering evidence on the usefulness of lysine restricted diet is in progress [2, 7].

We report the first patient in Serbia with biochemical and genetically diagnosed classic form of PDE.

Case report

A 6-day old boy was referred to our Neonatal Unit from a maternity hospital due to recurrent episodes of seizures with shrieking, starting within the first days of life. The patient was born to a 23-year-old mother by spontaneous vaginal delivery after an uneventful pregnancy. A full term male neonate had 3,900 g, a length of 57 cm and head circumference 37 cm (97th percentile). At birth, his Apgar scores were 9 and 9, at 1 and 5 min, respectively. He was the first child of healthy parents without known consanguinity. In addition, there was no family history of epilepsy or neurological disorders.

The baby was incapable of tolerating oral feedings. The baby seizures were complex: flexion of the limbs, twisting and jerking of the body and limbs, jerks of facial muscles with blinking, and all accompanied by occasional screams. When applied anticonvulsants (phenobarbital and/or midazolam), the response was short-lived and seizures were repeated. The first electroencephalography (EEG) performed after a seizure that lasted 6 minutes showed a discharge of high voltage bizarre spike-wave complexes and multiple spikes. His lactate level was elevated (up to 12.7 mmol/L, normal < 2 mmol/L) during seizures and normal in the periods without seizures. His ammonia level was 28 μmol/L (normal 42–144 μmol/L), while urine ketones were negative. All other biochemical analyses (glycemia, calcium, magnesium, sodium, potassium) and ultrasound examinations did not indicate the cause of drug-resistant seizures. After about 24 hours, an attempt was made to stop drug-resistant seizures with intravenous *iv* pyridoxine at a dose of 100 mg. Apnea did not occur during the administration of pyridoxine. EEG monitoring was not performed simultaneously due to technical reasons. He became seizure-free after the first dose. We continued with the administration of *iv* pyridoxine, 15 mg/kg/day over the next 7 days, and then moved on to oral administration. By day 10 cranial magnetic resonance imaging was performed. The significance of this finding was mild cerebellar hypoplasia and small subdural hemorrhage posteriorly.

Measurement of urinary α-AASA by electrospray ionisation tandem mass spectrometry under pyridoxine treatment was performed at the Biochemistry Department, Institute of Child Health in London. Urinary α-AASA level was markedly elevated (206.46 mmol/mol creatinine; normal at age < 6 months: < 2.0 mmol/mol creatinine). To make gene analysis and establish the cause of seizures we used the patient's DNA. The result of genetic analysis was performed at the Regional Molecular Genetic Laboratory at Great Ormond Street Hospital for Children in London. Sequence analysis for mutations associated with PDE identified that the patient was a heterozygous for the c.328 C > T mutation in exon 4 of the ALDH7A1 gene resulting in the substitution of arginine with stop codon at amino acid position 110 (p.Arg110*) and a heterozygous single transversion of G > T at a highly conserved donor splice site in intron 17 (c.1566-1 G > T). Both mutations have been reported previously in patients with PDE [3, 4].

After 3 weeks, the baby was discharged from the hospital in a good clinical condition with oral pyridoxine (50 mg/day). Five months after starting pyridoxine, the patient's EEG was normal in drowsy and sleep states. His neurological examination showed normal head growth, truncal hypotonia, and mildly decreased peripheral tone in the extremities. At 12 months of age the patient's neuropsychological testing was done. According to Brunet Lézin-scale development, the overall index was 83 units, i.e. at the level of a 10-month-old infant.

Discussion

Clinical course of events in our patient suggests a classic form of PDE. Seizures occurred in the first days of life. After admission at our Neonatal Unit anticonvulsants were administered, but the attacks were repeated. After 24 hours, pyridoxine was applied, and the seizures ceased completely. During the 12 months of life on pyridoxine treatment, neither seizures have been reported nor was EEG recorded. To make sure that late and masked response is not missed, treatment with oral/enteral pyridoxine should be continued until ALDH7A1 or ATQ deficiency is excluded by negative biochemical and/or genetic testing.

For diagnostic workup of neonates and infants with therapy-resistant seizures, many previous studies recommended serum or/and urine samples for the determination of α-AASA and PA regardless of a therapeutic trial with pyridoxine [3, 5, 7, 8]. While α-AASA is a pathognomonic marker of defects in the ALDH7A1 or ATQ gene, as illustrated by our pa-

tient, PA has the disadvantage of also being elevated secondarily in liver disease and peroxisomal defects like Zellweger syndrome [8, 9]. In contrast, α-AASA is the primary substrate of the affected enzyme, ALDH7A1 or ATQ, and remains more markedly elevated than PA in plasma and urine in all patients while on pyridoxine. Levels of urinary α-AASA in normal individuals decline during the first year of life: < 6 months: < 2.0 mmol/moL creatinine; 6–12 months: < 1.0 mmol/mol creatinine, > 1 year: < 0.5 mmol/mol creatinine. Pathological values for α-AASA are several folds above the upper limit of the appropriate reference range and seem to depend on the nature of the mutation, and the child's age, treatment with pyridoxine, and possibly on nutritional lysine intake. In our patient urinary α-AASA level was markedly elevated. It is important to note that PA levels in urine normalize under treatment, and patients with mild missense mutations may have near normal PA levels in plasma while on pyridoxine [3, 4].

Mutation analysis of the ALDH7A or ATQ gene is recommended to confirm the diagnosis. α-AASA and/or PA are reliable markers to select PDE patients for molecular analysis of the ALDH7A or ATQ gene. To date more than 60 different mutations within the 18 exons of the ALDH7A or ATQ gene at chromosome 5q31 have been published [10–12]. Of these, 50–60% are missense mutations resulting in an altered amino acid in the protein sequence. A sequence analysis has shown that our patient is an apparent compound heterozygote for two mutations both of which are highly likely to be pathogenic. Furthermore, an intronic mutation, c.1566-1G > T, as that of our patient, may have an increased frequency among European patients [3]. Prenatal diagnosis by molecular analysis is feasible in forthcoming pregnancies [3, 13].

If seizures subside after the administration of pyridoxine, instead of PDE, the patients may have: folinic acid-responsive seizures (genetically identical to ATQ deficiency but not biochemically), pyridox(am)ine phosphate oxidase deficiency, neonatal/infantile hypophosphatasia (tissue-nonspecific alkaline phosphatase deficiency), familial hyperphosphatasia (phosphatidylinositol glycan anchor biosynthesis type V deficiency) and hyperprolinemia type II [2, 3]. Importantly, these affected patients with pyridoxine responsive seizures haven't elevations in α-AASA in plasma and/or urine. Detection of elevated levels of this organic acid is a sensitive marker for PDE. While α-AASA was first thought to be a specific biomarker for PDE, recent research has demonstrated that α-AASA is also elevated in patients with molybdenum cofactor deficiency (MoCoFa) and isolated sulfite oxidase deficiency (SOX) [14]. In patients with elevated levels of α-AASA, these latter two conditions may be differentiated from PDE by measuring urinary sulfite/sulfocysteine levels. MoCoFa and SOX deficiency are serious and often fatal diseases for wich no effective therapy has been available until cyclic pyranopterin was used successfully in a patient with MoCoFa [14, 15].

Conclusion

The diagnosis of PDE is not routine and requires significant involvement of medical personnel, so that the patient could receive appropriate treatment. Clinical diagnostic approach to PDE can now be replaced by measurement of reliable diagnostic marker. Our patient with possible PDE was diagnosed according to the elevated concentrations of urinary α-AASA and did not undergo the risk of a diagnostic pyridoxine withdrawal. So far all published PDE patients with mutations of *ATQ* gene have had neonatal onset of seizures. Future studies are needed to investigate genotype-phenotype correlation of PDE.

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History of development of inflammatory diseases of the nervous system – meningitis and encephalitis

Istorijat razvoja zapaljenjskih bolesti nervnog sistema – meningtis i encefalitis

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Ključne reči:
istorija medicine; mozak; zapaljenje; meningitis; encefalitis; lečenje.

Introduction

A look back through history reveals that a man has always been exposed to diseases**,** injuries and accidents. He has used different ways and methods of treatment, and at the same time investigated the causation of disease outbreaks. Centuries ago, infectious diseases were a great unknown to mankind, primarily due to the inability of early detection of pathogens, but also the attribution of supernatural origin of many diseases of epidemic proportions.

Although described through the history of medicine as a segment of other infectious diseases, most commonly fever or sepsis, with present headache as a symptom, meningitis has long remained unrecognized as a distinct clinical entity. Epidemic forms of meningitis occurrence, history recorded in the beginning of 19th century and the most important discoveries related to inflammation of the central nervous system, meningitis and encephalitis, were published by the end of the 19th century.

History of meningitis evolution

Infections of the central nervous system, which can be of infectious and non-infectious genesis, are inflammatory process of the brain and the brain membranes. These, otherwise serious infections that can endanger human life, take on even greater weight when, not so rare, complicate with neurological manifestations. Bacterial meningitis means inflammation of the meninges, that different types of bacteria cause. Viral meningitis presents a serous inflammation of leptomeninges [1].

First data connected with inflammatory diseases of nervous system were noted by Thomas Willis (1621–1675), English physician, who described patients suffering from "inflammation of the meninges and present fever," and it can be said that he spoke of meningitis, even in 1661 [2]. The first time the epidemic meningitis was noted in 1805 in Geneva, then 1814 in Grenoble and Genova. After that epidemic appeared in France in 1822 then in 1823 in Germany, and after 7 years at Sunderland [3]. An epidemic of meningococcal meningitis had been described by Swiss physicians, Gaspard Vieusseux (1746–1814), and Andre Matthey (1778–1842) in Geneva. First epidemic in Africa was described in 1840. They had become more common in the 20th century, so the first bigger epidemic was noted in Nigeria and Ghana (1905–1908), when a large number of people had suffered [4]. The origin of the disease, and even the name of it, stayed unknown. This disease was identified by doctors as "sinking typhoid", or "spotted fever" [5]. Although still Herophilos (335–280 BC), an ancient Greek physician, first explained the anatomical structure of the brain and brain membrane, describing fourth ventricle, representing it as a place of the soul, it was not known for the occurrence of inflammation of the meninges. Herodotus (c.484–425 BC), an ancient Greek physician, spoke of "doctors for the head" in Egypt, who dealt with both physical and mental causes of the disease [5].

Certainly based on historical medical data, as well as other diseases in prehistoric times, supernatural causes were attributed to the occurrence of inflammatory processes in the brain tissue, also. One particularly popular method of diagnosis, and treatment, linked to brain disease, known in time of prehistory, was a trepanation of the skull or "šaronjanje", name used in some parts of our country as intervention on bone parts of the skull. It was used in the occasion of the existence of neurological diseases, and psychiatric disorders, but most commonly in post-traumatic headaches. While the prehistoric "surgeons", thought that opening the skull can rid

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the patient of evil thoughts and demons, the latter in some parts of our country, during the 19th century, the whole process had an element of vendetta. Particularly interesting is the fact that a high percentage of these patients, underwent surgery, and survived without the inflammatory process of the brain and meninges. A staggering figure when one considers that the intervention was performed by opening the skull, with unsterilized instruments, on which occasion would perpetrator watch brain membrane and thus establish "if it is dropped blood to the brain" (Figure 1) [5, 6].

Fig. 1 – Trepanation – The Extraction of the Stone of Madness (a painting by Hieronymus Bosch).

Nature cause of a disease was first described by Galen (c.130 AD–c.210/c.216AD), a Roman physician and philosopher of Greek origin, who was stating that the challengers were in the water, air and rotting substances, calling them "miasmas". Since the natural cause of the disease was first mentioned in paper "De contagione et contagiosis morbis" by an Italian physician, Girolamo Fracastoro (1478–1553), having started involvement in determining the etiology of the disease and finding cause of the infectious diseases including inflammatory diseases of the nervous system [3] .

For the occurence of meningitis various agents have been blamed through the history. Meningitis was commonly called "brain fever" or "brain inflammation" in the past, and it was believed that potential triggers for its appearance were: the sun, the temperature change, mental disorders, stress, and numerous other factors. In the early 20th century, these beliefs were discarded in the United States, as in the most Western European countries. Nerve disturbances and mental disorders, were also blamed, as shown in the literature of Russian writers [3, 7].

As a very common complication of inflammatory diseases of the nervous system was hydrocephalus, which was represented as an enlargement of brain ventricles due to increased amounts of cerebrospinal fluid in the brain chambers. Hydrocephalus was described by Hippocrates (c. 460–c.370 BC), Galen and Arabian doctors, who believed it was a disease caused by extracerebral accumulation of water [3].

Concept of cerebrospinal fluid (CSF) is closely linked to historical and medical development of meningitis. It is now known that the CSF is fluid that fills the subarachnoid space of the brain and spinal cord, and brain ventricles [1]. It is believed that Imhotep, an Egyptian doctor, and the notary, was the author of papyrus from 3000 BC, and the first one who discovered intracranial CSF. Name of cerebrospinal fluid was introduced by Francois Magendie (1783–1855), French physiologist, in the first half of the 19th century. Nicolo Massa (1485–1569), an Italian anatomist, in his book“ Liber Introductorius Anatómiae“ in 1536 was the first who described the CSF with cerebral chambers, based on autopsies. Previously, it was thought, by the beliefs of Hippocrates and Galen, that the brain ventricles contained "spirit of animals". It is generally accepted in medical history that the CSF was discovered by Domenico Felice Cotugno an Italian physician (1736–1822) (Figure 2). This famous doctor who lived in Naples in the 18th century published a paper, when he was 25, about the anatomical structures of the ear. He stated that not only were brain ventricle but the subarachnoid spaces filled by CSF, which was called "liquor cotunii" [8, 9].

Fig. 2 – Domenico Cotugno (1736–1822).

A very important moment in the history of development of meningitis, was the introduction of lumbar puncture. Lumbar puncture includes diagnostic procedure used to obtain cerebrospinal fluid, but in rare cases, therapeutic method for reducing increased intracranial pressure [1]. In the beginning, the purpose was purely therapeutic. But soon, lumbar puncture was introduced as a diagnostic procedure [10]. In 1881 Wernicke performed sterile ventricular puncture and external drainage of cerebrospinal fluid. These were followed-up in 1891 with serial lumbar punctures by Quincke (Figure 3) [11].

The first technique was described by an English physician Walter Essex Wynter, in 1889 in patients with tuberculous meningitis, when he used cannulation, which then had more therapeutic than diagnostic significance [12]. Definitive lumbar puncture technique was introduced by a German

Fig. 3 – Heinrich Irenaeus Quincke (1842–1922).

physician Heinrich Irenaeus Quincke (1842–1922) who presented his experience at the Conference of Internal Medicine in Wiesbaden, Germany. In the United States, the procedure was implemented in 1891 by Arthur H.Wentworth in the children's hospital [12]. Winter's technique was involved in making incisions in 2nd lumbar region, such as major cut and placing a tube with a rubber drainage and so knocked infected content and reduced the pressure [13] (Figure 4). Also, he measured the level of sugar and protein in the cerebrospinal fluid, describing low sugar in purulent meningitis. He found of the tuberculosis *Mycobacterium tuberculosis* in the CSF and thus diagnosed tuberculous meningitis [14].

Fig. 4 – Lumbar puncture, at the beginning of the 20th century.

Neurotuberculosis is a chronic inflammation of the nervous system, which is primarily caused by *Mycobacterium tuberculosis*, accounting for about 5–10% of all forms of extrapulmonary tuberculosis [15]. Tuberculous meningitis was first described by an Edinburgh physician Sir Robert Whytt (1714–1766), but his work was published posthumously in 1768. However, the link between tuberculous bacillus and meningitis was discovered even after 100 years [4].

In the years after the World War 2nd, at the Clinic of Infectious Disease in Belgrade, a large number of patients with tuberculous meningitis, mostly children, a population that had been the most frequent and the most vunerable were hospitalized and treated. Great merit in the fight against this disease, which mortality rate was 88.7% during the period from 1947 to 1949 belonged to Professor Dr. Kosta Todorović, a famous infectologist and scientist, who invested great efforts in his work. According to the data from the Clinic of Infectious Diseases, ten years later children were the most numerous patients still, but the mortality rate was 9.5%. The statistics speak in favor of a successful fight of infectology team and associates in post-war years against the serious and deadly disease, and certanly immeasurable satisfaction in recovering patients [16].

In the historical development of meningitis special place had meningeal signs, and the credit for that had scientists Kernig and Brudziński. Meningeal signs are in fact reflections that occur in response to the increase of elevated intracranial pressure, which can occur when performing certain movements at the examination of patients [1]. Disclosed are: stiff neck; sign of Brudzinski that is positive when in passive flexion of the head and neck, the response is flexion of the ankle of hip and knee; the character of the lower Brudzinski is positive, when during flexion of legs in the ankle of hip and knee, due to the stretching of the nerve ischiadic and distal spinal cord, the response is flexion of the other leg in the same joint; a positive sign of Kernig means that in the course of moving the patient from a lying to an upright position, the patient is unable to take a right angle between torso and legs and also when lifting the leg, they can not take the right angle in relation to the body [1].

Vladimir M. Kernig (1840–1917), was a neurologist of Russian-Baltic origin. Kernig's sign was described in 1882 in the article of the Weekly Medical Journal, where was stated that during the years of test cases of meningitis he noticed a sign that had practical value. He stated that "if one attempts to extend the patients knees they will succed only to an angle of approximately 135°. In cases which the phenomenon is very pronounced the angle may even remain 90° (Figure 5) [17].

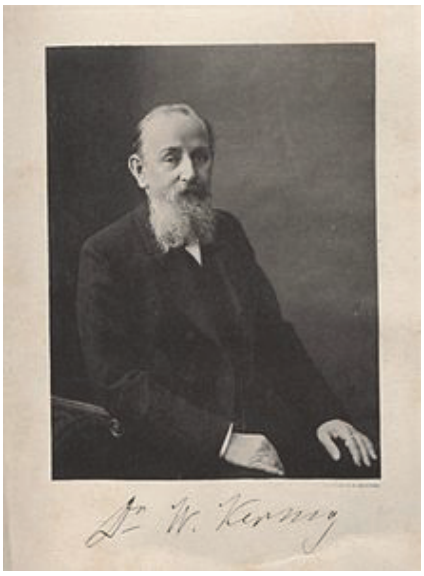

Fig. 5 – Vladimir Kernig (1840–1917).

Józéf Brudziński (1874–1917) (Figure 6) was a Polish doctor, a pediatrician. After World War I, he renewed the Polish University in Warsaw, and was appointed as a director of the same. In that time, Brudziński released four maneu

Fig. 6 – Józéf Brudziński (1874–1917).

vers in detection of meningitis. First it was cheek positive sign and that means when putting pressure on both cheeks under zygomatic arch can cause flexion of the forearm and arm. Also, symphyseal positive sign shows that pressure on the pubic symphysis causes flexion of the hip and knee, abduction of the leg. When passive flexion of the knee causes flexion of oposite knee, than it is named Brudziński's reflex. And Brudziński's sign is described as positive, when the passive flexion of the neck causes flexion of the hips and knees [17]. The majority of the patients were children, the population most affected by tuberculosus meningitis. Brudziński recorded 21 cases of tuberculous meningitis out of 42 investigated cases of meningitis [17].

Contemporary literature registers that bacterial meningitis is caused in 80% of cases mostly by *Hemophylus influenzae, Neisseria meningitidis,* and *Streptococcus pneumonia* [1]. These organisms that caused meningitis were discovered in the late 19th century. Serum therapy against meningococcal meningitis was introduced by a physician George Joachmann (1874–1915) in Germany and Simon Flexner (1863–1946) in America. Antibiotic therapy began in the 20th century with the use of sulfonamides by Francois Schwentker (1904–1954) and penicillin by Chester Keefer (1897–1972), an American physician. The vaccination began in early 20th century [18].

In 1881, two microbiologists, George M.Stenberg (1898–1915) in the United States, and Louis Pasteur (1822–1895) in France, independently described a couple of bacteria in human saliva. Both of them found *diplococcus* in the blood of rabits. Paster called it „Microbe septicemique du saliva", and Sternberg „*Micrococcus pasteuri*". In 1886 it was named *Pnemococcus* by Frenkel, due to the possibility to cause pneumonia [19]. However, the full name of *Streptococcus pneumoniae* was obtained in 1974 according to the growth of bacteria in chains. The role of germs in lobar pneumonia was proven in 80's of the 19th century and later in the same decade, *pneumococcus* was presented as the cause of meningitis and middle ear infections [19].

An Austrian pathologist and bacteriologist Anton Weichselbaum (1845–1920) in 1887 was the first who showed that there was a connection between *N. meningitidis* (then known as *Diplococcus intracellularis meningitidis*) and epidemic cerebrospinal meningitis. An Italian scientist, Vieusseux (1779–1863), was the first who described meningococcal disease, in Geneva 1805 and he was stating that the infection was being spread through "bad air". Simon Flexner succeeded in producing meningococcal serum in 1906 of equine origin, which reduced mortality of meningococcal disease from 75%–80% to 30%. The disease was fatal to the military forces during World War II, but thanks to the antiserum and antimicrobial treatment has become a curable disease. One of the biggest epidemic of meningococcal meningitis in the history of medicine, was in Brazil in 1974 before a vaccine was used. The World Health Organization annually records at least 500 000 new cases of meningococcal disease, resulting in more than 50 000 deaths annually [20].

Haemophilus influenzae was described in 1892 by Richard Pfeiffer, during an influenza pandemic, and for a long time, until 1933 was considered as a challenger of influenza, so the infection by this bacteria was often referred as "bacterial flu". Later was discovered that it caused bacteremia, pneumonia and acute bacterial meningitis in infants and children. From 1990 a conjugated Hib vaccine that reduces the incidence of meningitis has been used in the United States [21].

In the prevention of bacterial meningitis at the present time, in addition to maintaining a healthy life style and sanitary habits, avoiding close contact with carriers of a disease, chemoprophilaxis and immunoprophilaxis are used. Rifampicin, ceftriaxone and ciprofloxacin are suggested in case of meningococcal disease. Immunoprophilaxis covers a monovalent vaccine antigen of specific meningococcal serogroup and quadrivalent, and does not offer protection against the B serogroup. Chemoprophilaxis of pneumococcal infections is being implemented in the form of rifampicin, and immunoprofilaxis includes 23-valent pneumococcal vaccine. *Haemophilus influenzae* B meningitis includes rifampicin chemoprophilaxis and immunoprophilaxis implementation of conjugated vaccines with polyribose-ribitol phosphate *Haemophilus influenza* B with the carrier protein. Also hyperimmune globulin can be used according to indications [1, 22]. Before using specific antiserums, the odds of one who suffered from bacterial meningitis were bad. In 1920 a total of 77 of the 78 children at Boston Children's Hospital with *Haemophilus influenzae* meningitis died. A similar was the prognosis for untreated pneumococcal meningitis when from 300 observed patients all died. Meningococcal meningitis in the beginning of the 20th century had a mortality rate of 75–80%. Simon Flexner succeeded in use of intrathecal equine meningococcal antiserum in 1,300 patients in 1913, when he reduced mortality to 31%. Unfortunately, forecasts with pneumococcal meningitis stayed bad and after using specific antiserum. In the thirties of the last century, mortality rate in meningococcal meningits decreased introducing sulfonamides. In the early 50's of the last century, the use of chloramphenicol and

sulfadiazine reduced the number of fatal cases, and thus ruled out the use of antiserum. Penicillin therapy of pneumococcal meningitis began in the mid-40's of the last century. In the second half of the 20th century, in the treatment of bacterial meningitis were used: intravenous penicillin, ampicillin and cephalosporins of 3rd generation [18]. It is important to begin empirical treatment, and than to identify the causative agent and use the target – specific therapy. Penicillin, chloramphenicol and ampicillin, ceftriaxone, amikacin, meropenem, ciprofloxacin, cephalosporins of the 4th generation parenterally are used [1, 22]. In patients with *Haemophilus* meningitis dexamethasone was introduced in 1990 too, which has not changed mortality, but reduced the existence of neurological sequelae, especially hearing loss. A particular problem in the antibiotics era makes the emergence of antimicrobial resistance. In future, more emphasis will be placed on preventive, rather than therapeutic measures [1, 22].

Complications of bacterial meningitis may be systemic, related to distant organs or neurological nature. Although much less common, neurological complications can be very serious. The most common neurological complications include: hearing loss, motor deficits, cognitive defects and problems with speech, sensory deafness, followed by seizures and motor deficit, which is more common in children. Patients with bacterial meningitis are admitted to the intensive care unit if they are in a coma or with complications of the disease. Very common, serious and complex complications are reported during viral encephalitis, due to the possibility of being threatened as emergencies. Neurological sequelae are related to nerve damage, seizures, hemiparesis, as well as damage of intelligence [1].

History of viral meningitis and encephalitis

It is known, today, that the causes of acute viral meningitis make a wide range of viruses among which the most common are: enteroviruses, mumps virus, arboviruses, lymphocytic choriomeningitis virus (LCM), and herpes viruses (HSV) [23]. Acute viral encephalitis is difficult infectious disease with abrupt beginning, progredient flow and carries a high risk of complications. It presents the inflammation of the brain parenchyma that occurs due to direct cytopathic effect of the virus or during viremia [23]. Although almost all of the viruses can cause encephalitis, usually a tropism in brain tissue expresses herpes viruses, influenza viruses, smallpox viruses and Human immunodeficiency virus (HIV), and also rabies, arbo and adenoviruses [24].

In the past, it was not known very much about encephalitis, and the clinical picture was described mainly as a severe form of meningitis. Virchow Rudolf, a German scientist and pathologist described encephalitis in his work [3]. Aulus Cornelius Celsus (c. 25 BC–c. 50 AD), a scientist and doctor, was the first who described ear disease, and three kinds of "madness" of which one was called encephalitis "letargos", during which he proposed binding people who have seizures [3]. Since Dmitry Ivanovsky (1864–1920), a Russian virologist, in 1892 in his article described non-bacterial causes of tobacco mosaic disease, which infects tobacco plants passing through a porcelain filter, as well as the discovery by Martinus Beijerinck's (1851–1931), a Dutch microbiologist, who in 1898 repeated experiment of Ivanovsky and discovered the infectious agent who called virus, until today more than 5,000 species of virus have been described, but there are millions of different types Sve pobrkano ne mogu da znam sta je trebalo reci. With regard that encephalitis can cause any virus, the historical development of encephalitis is directly related to the discovery of the virus that has been found to cause it [25]. The first discovered viral agent in humans was virus of yellow fever in 1901 discovered by Walter Reed. It was followed by discovery of Rabies virus (Remlinger, Riffat-Baz) and in 1906 smallpox viruses. Then polio virus, chicken leukemia virus were discovered in 1908, as well as in 1911 Rous sarcoma virus. Swine flu was revealed in 1931, and human influenza virus in 1933. There are a number of undiscovered species of human viruses. More than two-thirds of the human viruses can infect human hosts, first mammals, sometimes birds. But not all newly discovered viruses are new because many of them have been present in humans for a long time, but they have been found soon [26].

Discovery of encephalitis contracted through ticks (arboviral encephalitis) occurred in the Far East in 1937 and the first vaccine was discovered a year later [27]. St. Louis encephalitis was noted in 1933, and within 5 weeks in the fall of the epidemic of encephalitis in St. Louis, the Missouri, over 1,000 cases were reported. The virus was isolated in monkeys and white mice. Japanese encephalitis had origin in 1500's in Indonesia, the Malaysia region, but in the thirties of the last century was revealed as forms of arboviral encephalitis. From 1950 to 1970 a great interest was shown in the "slow viral infections", considering them as a potential model for chronic diseases of the nervous system. Owing to diagnostic methods and advances in molecular medicine, modern era of neurovirology has begun. Lately, new viruses have been detected: Nipah, HIV, West Nile, which influence the occurrence of encephalitis [25].

The infections caused by Nipah virus were noted as illness of pigs and humans during September 1998 to April 1999 which resulted in death of 105 people. The dominant clinical syndrome in people was encephalitis, which often ended with coma during 48 h. Nipah virus was firstly characterized as a cause of Japanese encephalitis, but later was determined that the cause was a virus of the *Paramyxoviridae* family. Friut bats were pronounced as the primary natural reservoir of the virus [28].

West Nile virus was isolated in 1937 from febrile patients in areas of West Nile in North Uganda. During the observation of clinical picture, the neurotrophic effect of the virus was discovered. The first outbreak occurred in Israel in 1951 during which the majority of the diseased were children. Through the outbreaks in Egypt, the manner of transmission by vectors was found. Than the outbreak appeared in an urban environment in 1996 by vectors in Romania, followed by Italy, Israel, and as meningoencephalitis in 1999 in Russia and New York City. From then, the occurrence of epidemic and sporadic cases, with clinical manifestations of encephalitis was recorded [29]. Human immunodeficiency vi-

rus (HIV) was detected for the first time in 1981 in the United States, primarily among addicts of psychoactive substances and homosexuals, who had symptoms of pneumonia with *Pseudocystis carinii*. In 1983 Robert Gallo and Luc Montagnier claimed independently one of another, that new retrovirus was cause of acquired immunodeficiency syndrome (AIDS) in patients and published their observations calling them Human T-lymphotropic viruses (HTLV), and Lymphadenopathy virus – associated (LAV). In 1986 both viruses were called HIV [30].

Limbic encephalitis was mentioned in 1968 for the first time. It was associated with cancer, in context of paraneoplastic syndrome. Today, one can specify a viral origin of the disease, autoantibodies mediated encephalitis and encephalitis as part of an autoimmune disease [31].

Herpes simplex virus (HSV) is responsible for 5–10% of cases of encephalitis around the world, and this virus as a trigger of encephalitis has been given special attention [1]. HSV infections have been known since ancient Greece. Hippocrates described the spread of HSV lesions, calling them herpes. Even Shakespeare described it in his works. But in 1893 Vidal recognized human transmission of HSV infection from person to person, and in 1919 Lowenstein confirmed experimentally the infectious nature of HSV. During the 1920's and 1930's the nature of diseases such as meningitis challengers were discovered. During 40's and 50's was found that virus caused other diseases. *Varicella zoster* virus had had a long history, and it took a long time to be separated from other kinds of smallpox virus. Ebstein-Barr virus was isolated as a viral particle from limfoblastoid cells from Burkitt lymphoma. *Cytomegalovirus* has been detected recently, found in patients with citomeglovirus inclusion disease. In 1888 the same origin of these viruses was proposed [32].

Through the history of medicine, it has been excelled a special relationship between influenza virus with pandemic proportions and appearance of encephalitis as a complication of primary infection. In 1933 English doctors isolated the influenza virus type A, while in 1940 and in 1950 type B and type C virus were isolated. Outbreaks of flu were recorded in Europe as early as 1510 and in 1557. In 1580 the flu was expanding in Africa and Asia, reported as the first known pandemic flu. Thereafter, three pandemics during the 18th century were noted, as well as three pandemics during 19th century. During the 20th century, they were noted in 1918 "Spanish fever", in 1957 "Asian flu" and in 1968 "Hong Kong flu". At the end of the World War I "Spanish flu" was one of the deadliest pandemics in history of mankind, which claimed about 50 million people, in fact 5 times more lives than the World War I [33]. Many people who recovered from influenza, felt for a long term consequences, which appeared many years after epidemic. Phenomenon was described by dr Aleksandar Radosavljevic: "one of the main symptoms was a lethargic condition with a numb facial expression, so that the face of the diseased had the expression of a statue or a mask. The disease was called " encephalitis lethargica" [34]. Vladimir F. Vujić (1894–1953), a famous neurologist and psychiatrist, described the new phenomenon for Kaligaris syndrome, a particular kind of disease of the brain and also meningeal sign, later named "Sign of Vujić". In the 1939 Vujić was collaborating with a physician Kurt Levy, and formulated entity of encephalitis larvata in a book "Die Patologie des optischen Nachbilder". He studied and analyzed the flu epidemic in Belgrade in 1948 and published a monography "Encephalitis larvata" [35].

Current treatment of viral meningitis rarely involves administration of antiviral therapy and treatment is based on symptomatic and substitution therapy and rest. Antiedematous treatment is required in severe cases [1, 24]. In addition to hygiene and sanitation measures and protection from respiratory infections, as well as insects and veterinary measures, immunization is very important, also. The immunization is now conducted in order to prevent poliomyelitis, mumps and arbo-virus infection in endemic areas, and immunization against flu is important. First vaccines were produced in 1941. It was important to prevent the mass spread of flu among soldiers during the World War II. Passive immunoprophilaxis is conducted using hyperimmune immunoglobulin [1, 36]. Severity flow of encephalitis requires constant monitoring of vital functions in intensive care units. It is compulsory to perform antiedematous therapy. It is very important to use an antiviral treatment, corticosteroids, proton pump blockers, sedatives, and antibiotics, if necessary. In addition to basic hygienic and sanitation measures, prevention of encephalitis is reflected in active immunization against possible pathogens. Immunoglobulins may also be used in cases of immunodeficiency [1, 37].

Conclusion

Although unrecognized as an inflammatory process of the nervous system in the past, meningitis and encephalitis, by their difficult course, uncertain outcome, complications, and possibility of epidemic occurence left a big mark on the history of medicine. Today, owing to implementation of an adequate therapy these diseases have a better prognosis. But the story does not end here, because there must always be a willingness to discover new potential causes of meningitis and encephalitis, and therefore a timely response.

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I N M E M O R I A M

Prim. dr DUŠAN MILIĆ
pukovnik u penziji
(1935–2016)

Dvadeset četvrtog januara prošle godine preminuo je naš uvaženi anesteziolog, cenjeni kolega, saradnik i prijatelj, pukovnik u penziji, primarijus dr Dušan Milić, čovek koji je nosio u sebi najbolje i vojničke i lekarske osobine i spajao ih u jedinstvenu ličnost koja je plenila stručnošću i znanjem, impresionirala disciplinom i odgovornošću, željom i istrajnošću da unapredi vojni sanitet i medicinsku struku, uopšte.

Rođen je na Božić 1935. godine u užičkom selu Drežnik, u čestitoj, zemljoradničkoj, srpskoj porodici. Završio je čuvenu Užičku gimnaziju, odakle se 1954. godine otisnuo u Beograd, na studije medicine, u vreme kada je školovanje na fakultetu bilo pravi podvig i za roditelje sa sela i za njihovu decu. Roditelji su se odricali svega, a deca opet nisu imala dovoljno. Istrajan, savestan i disciplinovan, s visokim prosekom, u roku je završio studije medicine. Na studijama smo se i upoznali, sprijateljili i do poslednjeg njegovog dana delili sve što nam se u životu i dobro i loše dešavalo.

Kao vojni stipendista (od 1957. godine) završio je Sanitetsku oficirsku školu i u činu poručnika, kao lekar opšte prakse, poslat je 1963. godine u Mali Lošinj da u trupi odsluži obavezni lekarski staž. Specijalizaciju iz anesteziologije, tada mlade medicinske grane, započeo je 1968. godine na Vojnomedicinskoj akademiji (VMA) u Beogradu, u kojoj je potom proveo ceo svoj radni vek.

Specijalistički ispit položio je 1971. godine, a zvanje primarijusa stekao je 11. januara 1978. godine. Učestvovao je u pionirskim nastojanjima da se unapređenjem anestezioloske službe omogući bolje lečenje bolesnika operisanih od najtežih bolesti, kao što su operacije na otvorenom srcu. Voleo je da istakne da je radio s akademikom, generalom Isidorom Papom, za koga je stalno govorio da je velikan naše hirurgije. To je iziskivalo celog čoveka, dvadesetčetvoročasovnu posvećenost pacijentu, pa se dešavalo da u toku nedelje više noći probdimo s bolesnicima nego što prespavamo kod kuće.

Po prelasku u novu zgradu VMA Dušan Milić bio je zadužen za organizaciju rada u novoformiranom Centru za hitnu pomoć, za čijeg je načelnika postavljen 7. februara 1984. godine. Njegove izuzetne organizacione sposobnosti, visoka medicinska stručnost, vojnička odlučnost i disciplina došli su do punog izražaja na ovom mestu. Pod njegovim rukovodstvom Centar za hitnu pomoć funkcionisao je kao jedna od najbolje organizovanih celina VMA, gde su mnogi životi spaseni blagovremenom intervencijom koju su to znanje i organizacija omogućavali. Iskustva i znanja stečena u anesteziološkoj službi i tesna saradnja s Klinikom za anesteziju i reanimaciju, bili su dobar temelj za osmišljavanje i uspostavljanje sistema hitne vazdušne pomoći. U saradnji sa Auto-moto savezom tadašnje Jugoslavije i uz pomoć Auto-moto saveza Nemačke, organizovan je sistem hitne vazdušne medicinske pomoći, a najvažniji zadatak bio je osposobljavanje medicinskog osoblja i izrada medicinskih indikacija i kriterijuma za upotrebu helikoptera u prevoženju povređenih i obolelih. Uputstva, preporuke lekarima i shemu organizacione

strukture hitne vazdušne medicinske pomoći sabrali smo na jedno mesto i zajedno objavili u knjizi *Hitna vazdušna medicinska pomoć*. Od 1106 intervencija detaljno je analizirano 508 slučajeva, koji su potvrdili opravdanost hitne evakuacije povređenih i obolelih helikopterom. Ovakvu službu danas nemamo, a Dušan Milić je otišao s ovog sveta žaleći što niko od nadležnih u Ministarstvu odbrane nije našao vremena da ga primi i sasluša, da im pokloni knjigu i kaže da ne moraju osmišljavati nešto što je davno osmišljeno i besprekorno funkcionisalo.

Prim. dr Dušan Milić je imao izuzetnu sklonost ka naučno-istraživačkom radu, istoriji medicine i istoriji uopšte, a posebno se bavio istorijom srpskog naroda i vojske u balkanskim ratovima i u Prvom svetskom ratu. Jedan je od autora monografije o našem čuvenom kardiohirurgu Isidoru Papi čiji je dugogodišnji saradnik bio [Isidor Papo (1913–1996) - Život i delo", urednik Vladimir T. Jokanović, Beograd: Akademijska medicinskih nauka Srpskog lekarskog društva i Vojniomedicinska akademija, Beograd, 2013]. Pored toga, doprineo je da brojna dostignuća anesteziologa VMA budu opisana i objavljena u tada najprestižnijim medicinskim časopisima, a iz tih radova učili su lekari iz cele tadašnje Jugoslavije. To je Dušana Milića preporučilo za mesto načelnika Instituta za naučne informacije VMA. Na tu dužnost stupa krajem 1995. godine i s tog mesta odlazi u zasluženu penziju početkom 2000. godine. U tom periodu (1995–2000), kao načelnik Instituta za naučne informacije VMA bio je glavni i odgovorni urednik časopisa "Vojnosanitetski pregled".

Za svoj rad, pukovnik prim. dr Dušan Milić dobio je Orden JNA sa zlatnom zvezdom, Orden rada sa zlatnim vencom, Orden JNA sa srebrnom zvezdom, Orden JNA za vojne zasluge sa zlatnim mačevima, Orden JNA za vojne zasluge u oblasti odbrane i bezbednosti, Orden JNA za vojne zasluge sa srebrnim mačevima i brojna druga priznanja.

Dušan Milić je bio čovek od svakog reda, precizan i disciplinovan, vaspitan u najboljim tradicijama srpskog domaćina, oficir koji je izučavao i upijao moral srpskog vojnika iz oslobodilačkih ratova, lekar izuzetnog stručnog znanja i humanih načela koja su njegovi kolege i pacijenti umeli da prepoznaju.

Ostaće u trajnom sećanju svih nas koji smo imali privilegiju i čast da radimo sa njim i učimo od njega. Neka mu je večna slava i hvala!

Prof. dr Tomislav Marenović
pukovnik u penziji

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

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

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

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

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

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

3. ***Tekst članka***

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

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

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

Literatura

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

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Tabele

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

Ilustracije

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

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

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

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

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

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