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PROGNOSTIC SIGNIFICANCE OF ESTROGEN RECEPTOR, PROGESTERONE RECEPTOR AND HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2 IN PATIENTS WITH BREAST CANCER

PROGNOSTIČKI ZNAČAJ ESTROGENIH, PROGESTERONSKIH I RECEPTORA FAKTORA RASTA 2 KOD BOLESNICA SA KARCINOMOM DOJKE

Kosta Z. ZDRAVKOVIĆ[1], Jelena ZDRAVKOVIĆ[2], Momčilo STOŠIĆ[3], Igor STOJANOVIĆ[3], Marina RAŠIĆ POPOVIĆ[4] and Marina ZLATKOVIĆ[4]

Summary
Introduction. The importance of understanding the biology of breast cancer is increasing and the determination of certain phenotypic characteristics of malignant cells, especially estrogen, progesterone and human epidermal growth factor receptor 2 expression, became a standard evaluation procedure in breast cancer patients, in order to provide prognostic information and best therapeutic options. **Material and methods.** This study included a total of 206 patients, all treated and followed up in the *Daily Chemotherapy Hospital* of the *Health Center Vranje*. Estrogen, progesterone and human epidermal growth factor receptor 2 statuses were evaluated in all patients to assess their potential impact on the progression-free and overall survival. **Results.** Two-thirds of patients were diagnosed at the early stage of the disease. Ductal carcinoma was the most common histological type. Patients with early stage breast cancer, with hormone-receptor positive and human epidermal growth factor receptor 2 negative tumors, had a significantly longer progression-free survival. **Conclusion.** Hormone-receptor and human epidermal growth factor receptor 2 status evaluation is still of great clinical importance with a reliable prognostic value in breast cancer patients.
Key words: Breast Neoplasms; Receptors, Estrogen; Receptors, Progesterone; Receptor, Epidermal Growth Factor; Prognosis; Immunohistochemistry; Biomarkers, Tumor

Sažetak
Uvod. Značaj razumevanja biologije raka dojke sve je veći i određivanje pojedinih fenotipskih odlika malignih ćelija, pre svega ekspresije receptora za estrogen, progesteron i humani epidermalni faktor rasta 2, postali su standard celokupnog sagledavanja stanja obolelih, a sa ciljem postavljanja što preciznije prognoze, uspešnijeg lečenja i odgovora na sprovedeni tretman. **Materijal i metode.** Ukupno je retrospektivno analizirano 206 pacijenata sa rakom dojke, lečenih u Dnevnoj bolnici za hemioterapiju, Zdravstvenog centra u Vranju. Svima je u okviru histopatološke dijagnoze određivan status receptora za estrogen, progesteron i humani epidermalni faktor rasta 2, a nakon toga analiziran uticaj njihove ekspresije na dužinu perioda bez progresije bolesti i ukupnog preživljavanja obolelih. **Rezultati.** Kod dve trećine pacijenata dijagnostika je urađena u ranom kliničkom stadijumu. Duktalni karcinom je bio najčešće zastupljen histopatološki tip. Pacijenti kojima je karcinom dijagnostikovan u ranoj fazi sa tumorom pozitivnim na hormonski receptor i tumorom negativnim na humani epidermalni hornom rasta receptor 2, imali su statistički značajno duži period preživljavanja bez progresije bolesti. **Zaključak.** Određivanje ekspresije hormonskog receptora i receptora za humani epidermalni faktor rasta 2 na malignim ćelijama raka dojke sa pravom je i dalje standard u onkološkoj kliničkoj praksi jer pouzdano ukazuje na prognozu obolelih.
Ključne reči: karcinom dojke; estrogenski receptori; progesteronski receptori; receptori epidermalnog faktora rasta; prognoza; imunohistohemija; tumorski biomarkeri

Introduction

According to the *National Cancer Registry of Serbia*, breast cancer accounts for 20.2% of all diagnosed and additionally for 18.2% of all registered cancer deaths in women of central Serbia [1]. Factors that are the cornerstone for the tumor, nodes, and metastasis (TNM) staging system (size of primary tumor, involvement of regional lymph nodes, and presence of distant metastases) are prognostic factors used to describe the anatomic extent of the disease and stratify all patients with breast cancer with comparable outcomes [2]. Over the past half century, our understanding of breast cancer has improved and the focus has shifted from radical mastec-

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Abbreviations

ER – estrogen receptor
PR – progesterone receptor
HER2 – human epidermal growth factor receptor 2
PFS – progression-free survival
OS – overall survival
TNM – tumor, nodes and metastasis

tomy to breast-conserving surgery and personalized systemic therapy, based mainly on prognostic and predictive biological factors of the primary tumor. The relevance of the TNM staging system in the era of biomarkers, genomic analysis, and personalized medicine is becoming limited, but at the same time, parameters of tumor biology represent essential factors for therapeutic decision making. That is also the reason why patients staged similarly using the TNM staging system, have significantly different outcomes [2, 3]. Factors, such as histological grade, invasion of lymphovascular elements and biomarker status, have become very important in identifying patients with biologically aggressive cancers who may not show a good response to adj- uvant systemic regimens. Breast cancer is a biologically heterogeneous disease with many different subtypes, which are differentiated using immuno-histochemical and molecular analyses [4].

Molecular classification has become a standard procedure in the diagnosis of breast cancer, based on gene profiling, with the aim to predict the disease outcome [5, 6]. Despite all the remarkable achievements of molecular biology, clinicians still rely on traditional clinical pathological features and rapid test markers such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). The determination of these markers is routinely done by immuno-histochemistry in breast cancer tissue samples and the results have not only a prognostic value, but also play a crucial role in therapeutic decision making. This method is still a reasonable substitute for expensive molecular technologies [7].

The aim of this study was to evaluate the prognostic significance of the estrogen and progesterone receptors and human epidermal growth factor receptor 2 status in patients with breast cancer.

Material and Methods

This study included a total of 206 patients, all treated and followed up in the *Daily Chemotherapy Hospital* of the *Health Center Vranje*, in the period 2009–2014. The retrospective analysis included parameters obtained from patients' medical records. We evaluated the impact of primarily phenotypic

Table 1. Clinical stage and tumor characteristics
Tabela 1. *Klinički stadijum bolesti i karakteristike tumora*

Clinical stage/*Klinički stadijum*	N	%
Early breast cancer/*Rani karcinom dojke*	140	68
Advanced breast cancer/*Uznapredovali karcinom dojke*	66	32
Total/*Ukupno*	206	100
Histological types/*Histološki tipovi*		
Ductal carcinoma/*Duktalni karcinom*	162	78.6
Lobular carcinoma/*Lobularni karcinom*	38	18.4
Other subtypes/*Ostali suptipovi*	6	3
Total/*Ukupno*	206	100
Estrogen receptor/*Estrogenski receptor*		
ER- + status/*ER pozitivni status*	158	76.7
ER- – status/*ER negativni status*	48	23.3
Total/*Ukupno*	206	100
Progesterone receptor/*Progesteronski receptor*		
PR + status/*PR pozitivni status*	126	61.2
PR – status/*PR negativni status*	80	38.8
Total/*Ukupno*	206	100
Hormone receptor status/*Hormon-receptorski status*		
Hormone receptor-positive tumor/*Tumor pozitivan na hormonski receptor*	122	59.2
Partially hormone receptor-positive tumor*/*Tumor parcijalno pozitivan na hormonski receptor**	40	19.4
Hormone receptor-negative tumor/*Tumor negativan na hormonski receptor*	44	21.4
Total/*Ukupno*	206	100
HER2 status/*HER2 status*		
HER2- + status/*HER2 pozitivni status*	36	17.5
HER2- – status/*HER2 negativni status*	170	82.5
Total/*Ukupno*	206	100

ER – *estrogeni receptor,* PR – *progesteronski receptor,* HER2 – *receptor za humani epidermalni faktor rasta 2;* *ER+ or PR+/*ER+ ili PR+

tumor characteristics on the progression-free survival (PFS) and overall survival (OS) of patients.

Statistical analysis was performed using SPSS 17.0 statistical package. All results were expressed as mean values ± standard deviation (SD). The statistical significance of differences between mean values was analyzed using the t-test. Correlations between the investigated variables were studied by a Pearson's test. A p-value < 0.05 was considered statistically significant.

Results

This retrospective study included a total of 206 patients. Early stage breast cancer was diagnosed in 140 patients, while one-third of patients (32%) presented with an advanced stage **(Table 1).** Histopathological analysis confirmed ductal carcinoma in 162 patients (78.6% of the total number of patients), and lobular carcinoma in 18.4% **(Table 1)**. Out of 206 patients, only 6 had a diagnosis of another histological type of breast cancer (papillary, tubulopapillary medullary, all in two patients), which makes a total of 3%.

Immunohistochemical phenotype expression of the steroid receptor as well as the HER2 protein status were determined in all breast cancer tissue samples. The estrogen receptor expression was confirmed in 158 (76.7%) patients, i.e. in the majority of the investigated group. The progesterone receptor positivity was found in 126 patients or 61.2% **(Table 1)**. The assessment of steroid receptor status showed that 59.2% (122) of patients had both estrogen and progesterone receptor positive phenotypes, and additionally 19.4% (40) of patients had ER or PR positive steroid receptor status. Therefore, hormone-sensitive tumors accounted for 78.6% of patients **(Tables 1 and 2)**. Overexpression of HER2 protein was detected in 17.5% of cases (36 patients).

The main objective of this study was to analyze the impact of the clinical stage of the disease, as well as of some characteristics of the tumor tissue (histopat-

Table 2. Hormone receptor and HER2 status
Tabela 2. *Hormon-receptorski i HER2 status tumora*

Parameters *Parametri*	Hormone receptor positive tumor† *Hormon receptor pozitivni tumor†*	Hormone receptor negative tumor *Hormon receptor negativni tumor*	Total *Ukupno*
HER2- – status *HER2 negativni status*	140 (68%)	30 (14.5%)	170 (82.5%)
HER2- + status *HER2 pozitivni status*	22 (10.6%)	14 (6.9%)	36 (17.5%)
Total/*Ukupno*	162 (78.6%)	44 (21.4%)	206 (100%)

HER2 - receptor za humani epidermalni faktor rasta 2; † ER+ and/or PR+/† ER+ i/ili PR+

Table 3. Differences in progression-free survival related to clinical parameters and different receptor status
Tabela 3. *Razlike u dužini preživljavanja do progresije bolesti u odnosu na kliničke parametre i različiti status receptora*

Histological types *Histološki tipovi*	N	%	PFS (mean value in months) *PFS (srednja vrednost u mesecima)*	SD	p value *p vrednost*
Ductal carcinoma/*Duktalni karcinom*	162	78.6	49,62	21,14	p = 0,104
Lobular carcinoma/*Lobularni karcinom*	38	18.4	43,16	25,18	
Clinical stage of disease/*Klinički stadijum bolesti*					
Early breast cancer/ *Rani karcinom dojke*	140	68	56,99	14,88	p = ,001*
Advanced breast cancer/*Uznapredovali karcinom dojke*	66	32	30,36	23,39	
Estrogen receptor/*Estrogeni receptor*					
ER- + status/*ER pozitivni status*	158	76.7	49,73	21,0	p = 0,129
ER- – status/*ER negativni status*	48	23.3	44,25	24,29	
Progesterone receptor/*Progesteronski receptor*					
PR- + status/*PR pozitivni status*	126	61.2	53,62	19,1	p = 0,001*
PR- – status/*PR negativni status*	80	38.8	40,33	23,56	
Hormone receptor status/*Hormon-receptorski status*					
Receptor positive tumor †/*Receptor pozivni status†*	162	78.6	50,11	20,89	p = 0,03*
Receptor negative tumor/*Receptor negativni status*	44	21.4	42,36	24,48	
HER2 status/*HER2 status*					
HER2- + status/*HER2 pozitivni status*	36	17.5	40,11	25,43	p = 0,011*
HER2- – status/*HER2 negativni status*	170	82.5	50,22	20,7	

*HER2 - receptor za humani epidermalni faktor rasta 2; * p<0,05*

Table 4. Correlation analysis of the examined parameters
Tabela 4. *Korelacijska analiza ispitivanih parametara*

Parameters *Parametri*		Clinical stage *Klinički stadijum*	Histological types *Histološki tipovi*	ER status *ER status*	PR status *PR status*	HR status *HR status*	HER2 status *HER2 status*
Clinical stage *Klinički stadijum*	Cor.coefficient	1	0,184	-0,34	0,179	0,089	-0,287
	p value		0,008*	0,628	0,01*	0,202	0,000*
	N	206	200	206	206	166	206
Histological types *Histološki tipovi*	Cor.coefficient	0,184	1	-0,166	0,093	-0,013	-0,033
	p value	0,008*	200	0,017*	0,185	0,853	0,640
	N	200		200	200	166	200
ER status *ER status*	Cor.coefficient	-0,34	-0,166 0,017*	1	0,597	0,877	-0,170
	p value	0,628	200	206	0,000*	0,001*	0,015*
	N	206			206	166	206
PR status *PR status*	Cor.coefficient	0,179	0,093	0,597	1	0,909	-0,368
	p value	0,01	0,185	0,000*	206	0,000*	0,001*
	N	206	200	206		166	206
HR status *HR status*	Cor.coefficient	0,089	-0,013	0,877	0,909	1	-0,308
	p value	0,202	0,853	0,001*	0,000*		0,000*
	N	166	166	166	166	166	166
HER2 status *HER2 status*	Cor.coefficient	-0,287	-0,033	-0,170	-0,368	-0,308	1
	p value	0,000*	0,640	0,015*	0,001*	0,000*	
	N	206	200	206	206	166	206

* p<0,05; *HER2 - receptor za humani epidermalni faktor rasta 2; ER status – estrogen-receptorski status; PR status – progesteron-receptorski status; HR status – hormon-receptorski status*

hological type, ER, PR, HER2 status) on the PFS and OS.

The majority of studied patients, nearly 70%, diagnosed with early breast cancer, had a clinically and statistically longer PFS compared with patients with advanced breast cancer, 26 months on average (56.99 vs. 30.36 months, respectively) **(Table 3)**. This indicates and confirms that lower stage of disease at the time of diagnosis, is a very important factor for a longer PFS. **Table 3** shows no evidence of a statistically significant

Table 5. Differences in OS related to clinical parameters and different receptor status
Tabela 5. *Razlike u dužini ukupnog preživljavanja (UP) u odnosu na kliničke parametre i različiti status receptora*

Histological types/*Histološki tipovi*	N	%	OS (mean value in months) *UP (srednja vrednost u mesecima)*	SD	p value *p vrednost*
Ductal carcinoma/*Duktalni karcinom*	42	77.78	31,38	16,53	p=0,624
Lobular carcinoma/*Lobularni karcinom*	12	22.22	33,83	8,6	
Clinical stage of disease/*Klinički stadijum bolesti*					
Early breast cancer/*Rani karcinom dojke*	18	32.14	42	9,22	p=0,000*
Advanced breast cancer/ *Uznapredovali karcinom dojke*	38	67.86	27	14,61	
Estrogen receptor/*Estrogenski receptor*					
ER- + status/*ER pozitivni status*	40	71.42	32,85	14	p=0,417
ER- – status/*ER negativni status*	16	28.58	29,25	16,91	
Progesterone receptor/*Progesteronski receptor*					
PR- + status/*PR pozitivni status*	22	39.28	30,63	15,72	p=0,635
PR- – status/*PR negativni status*	34	60.72	32,58	14,41	
Hormon receptor status/*Hormon-receptorski status*					
Receptor positive tumor/*Receptor pozitivni status*†	40	71.42	32,85	14	p=0,417
Receptor negative tumor/*Receptor negativni status*	16	28.58	29,25	16,91	
HER2 status/HER2 status					
HER2-+ status/*HER2 pozitivni status*	14	25	27,43	18,18	p=0,203
HER2-– status/*HER2 negativni status*	42	75	33,29	13,46	

HER2 - receptor za humani epidermalni faktor rasta 2; * p<0,05; † ER+ and/or PR+/† *ER+ i/ili PR+*

prolongation of PFS in the group of patients with ductal breast cancer compared to patients with lobular carcinoma, although the first group had a longer PFS, 6 months on average (49.62 vs. 43.16 months, respectively).

Patients with steroid receptor-positive tumors had almost 8 months longer PFS compared to patients with hormone-independent tumors (50.11 vs. 42.36 months) presenting a statistically significant difference **(Table 3)**. This difference in duration of PFS cannot be associated with the fact that the group of patients with hormone receptor-positive tumors were diagnosed at an earlier stage of the disease, because a statistically significant correlation between these two parameters was not observed **(Table 4)**. A highly statistically significant difference in PFS was found in the group of patients with PR positive status in comparison with the PR negative group, over a year longer in favor of patients from the first group (53.62 vs. 40.33 months) **(Table 3)**. A statistically significantly longer PFS was also recorded in the group of patients whith HER2-negative tumor status. On average, they had 10 months longer PFS compared with the group of patients with overexpression of HER2 protein (50.22 vs. 40.11 months).

Just like in case of PFS, a statistically signifi- cantly longer OS was found in patients with early breast cancer (15 months longer OS) compared with patients with advanced breast cancer (42 vs. 27 months, respectively) **(Table 5)**. In contrast to the stage of the disease at the time of diagnosis, histopathological type had no significant impact on the OS. There was no statistically significant difference in OS between patients with ductal and lobular breast cancer (31.38 vs. 33.83 months). The impact of steroid-receptor and HER2 tumor phenotype on overall survival is also shown in **Table 5.** In evaluating the impact of all receptor parameters on OS, a statistically significant difference within individual groups was not observed. There was a difference between OS of patients with HER2-negative status compared to the group with HER2 overexpression (almost 6 month longer survival), but probably due to high standard deviation, because statistical significance was not achieved.

The above analysis examined individual groups to establish differences in PFS and OS in relation to the clinical stage of breast cancer and specific phenotypic characteristics of tumors. We also performed a correlation analysis of all the parameters **(Table 6)** in regard to living status (alive vs deceased) at the last follow-up examination. The correlation between the clinical stage of the disease at the time of diagnosis with actual life status, showed that there was a statistically significant positive correlation between advanced stage and lethal outcome. Out of 56 deceased patients, 38 had advanced, and 18 early breast cancer. There was also a statistically significant positive correlation between steroid receptor-positive breast cancer and longer survival, while in the group of patients with hormone-independent disease, at the time of analysis 36% were deceased (16 of 44 patients). The correlation anal- ysis between expression of HER2 protein and actu- al li-

Table 6. Correlation between the life status (alive/deceased) with clinical stage of the disease and some phenotypic tumor features
Tabela 6. *Korelacija životnog statusa pacijenata (živi/umrli) sa kliničkim stadijumom bolesti i pojedinim fenotipskim karakteristikama tumora*

Parameters *Parametri*		Life status/*Životni status*		Total	p value
		alive/*živi*	deceased/*umrli*	*Ukupno*	*p vrednost*
Clinical stage *Klinički stadijum*	Early breast cancer/*Rani klinički stadijum*	122	18	140	p=0,001*
	Advanced breast cancer *Uznapredovali klinički stadijum*	28	38	66	
	Total/*Ukupno*	150	56	206	
Histological types *Histološki tipovi*	Ductal carcinoma/*Duktalni karcinom*	120	42	162	p=0,735
	Lobular carcinoma/*Lobularni karcinom*	26	12	38	
	Total/*Ukupno*	146	54	200	
Estrogen receptor *Estrogeni receptor*	ER-+ status/*ER pozitivni status*	118	40	158	p=0,274
	ER- – status/*ER negativni status*	32	16	48	
	Total/*Ukupno*	150	56	206	
Progesterone receptor *Progesteronski receptor*	PR-+ status/*PR pozitivni status*	104	22	126	p=0,002*
	PR- – status/*PR negativni status*	46	34	80	
	Total/*Ukupno*	150	56	206	
Hormon receptor status *Hormon receptorski status*	Receptor positive tumor † *Hormon pozitivni tumor†*	122	40	162	p=0,001*
	Receptor negative tumor *Receptor negativni tumor*	28	16	44	
	Total/*Ukupno*	150	56	166	
HER2 status/*HER2 status*	HER2-+ status/*HER2 pozitivni status*	22	14	36	p=0,082
	HER2- – status/*HER2 negativni status*	128	42	170	
	Total/*Ukupno*	150	56	206	

HER2 - receptor za humani epidermalni faktor rasta 2; * p<0,05; † ER+ and/or PR+/† *ER+ i/iliPR+*

ving status showed a statistically significant association, although 24.7% of patients with HER2 negative status (42 of 170) and 38.8% with HER2 positive status (14 of 36) were deceased **(Table 6)**.

All the analyzed parameters were correlated with each other, and the results are shown in **Table 4.** A statistically significant positive correlation was established between early breast cancer and ductal histological type, while lobular carcinoma was more often diagnosed at an advanced stage. Patients with early stage breast cancer were mostly PR-positive and at the same time had a HER2-negative receptor status. An interesting fact is that the ductal carcinoma, which is commonly diagnosed at an early stage, was ER-negative in most cases. There was a statistically significant correlation between positive ER status and simultaneous positive PR, but a negative correlation with HER2 overexpression; so, ER positive tumors were mostly HER2 negative. If HER2 status is analyzed as a variable, we can conclude that HER2 overexpression was statistically significantly positively correlated with advanced stage and steroid receptor-negative breast cancer.

Discussion

In the examined patients, almost two thirds were diagnosed with an early stage, and one third with advanced breast cancer. Despite improved survival for all stages of the disease, survival remains poor in patients with a widespread disease. The group of patients with early breast cancer had 26 months longer PFS, and 15 months longer OS than the group of patients with advanced cancer **(Tables 3 and 5)**. Improvments in survival for specific stages of breast cancer in the last 30 years are considered to be the result of better treatment regimens (neoand adjuvant chemotherapy, radiotherapy, hormone and target therapy), followed by a better characterization of prognostic factors, as well as the progress in terms of increasing the number of diagnosed patients with small size tumors [8, 9].

For a long time it has been known that without adjuvant systemic therapy ER-negative breast cancer is associated with poor outcome compared with ER-positive breast cancer. Estrogen receptor is also a very reliable factor in predicting response to endocrine therapy; higher expression in tumor cells is associated with a higher response rate to hormone therapy [10]. In our group, two-thirds of patients (76.7%) were with ER-positive status, which is in agreement with a series of patients that have already been tested in our country and in the region [11, 12]. The rate of PR-positive status was slightly lower (61.2% of patients), which is also in accordance with literature data.

In general, all phenotypic features of tumor tissues determined by immunohistochemical analysis are very important and necessary in order to obtain significant prognostic and predictive information for better classification and differentiation of various types of breast cancer. In patients included in this study, 40 patients (almost 20%), had a positive ER or PR status. If we add 122 patients who had both ER positive and PR positive status, we can see that the majority of patients (78.6%) had a steroid-receptor positive breast cancer. Concordant expression of steroid-receptor phenotypes (PR and ER positive) with a negative HER2 protein is a characteristic of tumors mainly found in elderly/postmenopausal women [13]. In our study, 140 patients had this type of phenotype, which makes 68% of the total number of examined patients. According to clinical and epidemiological studies, this immunohistochemical phenotype of steroid receptors in breast cancer is the most favorable in terms of prognosis and therapy [14]. Therefore, the presence of ER in the primary tumor remains the most important predictive factor predicting response to hormonal therapy. Positive estrogen and progesterone receptors can determine even with greater accuracy the probability of response to hormone therapy, so that patients with ER and PR positive tumors respond to this therapy in 75% of cases, while less than 10% of patients with negative steroid-receptor phenotype benefit from hormone therapy [15, 16].

Steroid receptors are not strong independent prognostic factors, but in combination with other factors and HER2 status, they are used for classification of patients into prognostic subgroups.

The triple positive subtype, namely: ER+ and/or PR+ with HER2+ status was found in 10.6% of cases, while triple negative (TN) breast cancer was found in 14.5%; it means that 30 patients were in the phenotypic group, which, according to the literature data, is most common in younger/premenopausal women. Recent studies reported that the incidence of triple negative breast cancer accounts for 12 - 25% of all invasive carcinomas [17, 18]. This entity, triple negative breast cancer is characterized by aggressive biological behavior and lack of response to the currently available systemic therapy.

In the literature, the percentage of HER2- overexpressed/amplified breast carcinomas range from 3% to 30% [19, 20]. In our group of patients, HER2 status was determined in all patients, and overexpression was found in 36 (17.5%), which is in agreement with data published in extensive breast cancer histologic assessment in our country, and in our region as well [11, 12]. It is well known that anthracyclines have inhibitory effects on topoisomerase II alpha and a few recent studies have shown that amplification of the gene for this enzyme may be a better predictor of response to anthracycline drugs than the gene encoding the HER2 protein (c-erbB-2) gene [15]. Although these two genes are close together, the amplification of c-erbB-2 gene is not always associated with an amplification of the gene for the enzyme to- poisomerase II alpha. In this case the status of HER2 protein in breast cancer tissue would not be needed for a therapeutic decision with anthracycline containing chemotherapy, but should be decided on the basis of the status of gene for topoisomerase II alpha enzyme. However, HER2 status assessment remains a mandatory parameter in order to assess the best further treatment, in terms of using target therapies directed at this protein receptor.

The proliferation marker Ki67 has become a standard predictive factor in histopathological assessments

of breast cancer. This antigen is expressed in the nucleus of neoplastic cells through all phases of the cell cycle and for that reason it is a useful marker for assessing the proliferative potential of malignant cells. Detection of changes in the expression of Ki-67 after neoadjuvant, chemo- and endocrine treatment is a useful predictor of long term outcome in some cases. So, if "something" inhibits new cell growth, Ki67 expression decreases, which is also a good predictor of response to applied therapy [21, 22]. Considering the fact that in the group of patients included in this study, Ki-67 was determined just sporadically (our Center had not established a routine testing of Ki67 in these patients at that time), so it could not have been systematically analyzed as a predictor of prognosis.

Conclusion

The results of this study suggest that determination of estrogen receptor, progesteron receptor, and human epidermal growth factor receptor 2 status is of great clinical importance for all breast cancer patients, not only to predict prognosis, but also for the prediction of response to therapy, which is applied after complete insight into the status of the tumor phenotype. This study has confirmed the previosly reported facts that patients with a hormone-dependent and human epidermal growth factor receptor 2 negative breast cancer have a better prognosis and therapeutic options than patients with other breast cancer immunophenotypes.

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VALIDATION OF THE *CHILD ORAL IMPACT ON DAILY PERFORMANCE* INDEX AMONG SCHOOLCHILDREN IN BOSNIA AND HERZEGOVINA: A PILOT STUDY

PRIMENA VERZIJE UPITNIKA CHILD ORAL IMPACT ON DAILY PERFORMANCE *ZA PROCENU KVALITETA ŽIVOTA DECE NA PODRUČJU BOSNE I HERCEGOVINE: PILOT STUDIJA*

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Summary

Introduction. The aim of this study was to translate the *Child Oral Impact on Daily Performance* index into Serbian language and to evaluate its reliability in practice. **Material and Methods.** Following internationally established methods, adaptation of the *Child Oral Impact on Daily Performance* index for children and adolescents in the Serbian speaking areas consisted of three steps: forward translation of the *Child Oral Impact on Daily Performance* index, backward translation, and a pilot study. **Results.** A pilot study included 42 participants (21 males and 21 females), mean age of 12.0 ± 1.01 years. All items showed a corrected item-total correlation coefficient above >0.20 and the standardized Cronbach's alpha coefficient was 0.80. All correlation coefficient values were positive. The prevalence of oral health impact measured by the *Child Oral Impact on Daily Performance* index was relatively high and 54.8% of the participants reported at least one oral impact on the daily performance in the last 3 months. The most common activities affected were eating (38.1%) and cleaning the teeth (16.7%). **Conclusion.** The Serbian version of the *Child Oral Impact on Daily Performance* index is suitable for use in everyday practice and it provides useful information in the evaluation of oral health-related quality of life. The prevalence of oral impacts was high with the most prevalent impact referring to eating. Further clinical research, including larger number of participants, is required for exact verification of the *Child Oral Impact on Daily Performance* index.

Key words: Oral Health; Quality of Life; Surveys and Questionnaires; Child; Adolescent; Dental Health Surveys; Oral Hygiene; Health Behavior

Sažetak

Uvod. Cilj ovog rada bio je da se upitnik *Child Oral Impact on Daily Performance* prevede na srpski jezik i proveri njegova pouzdanost u praksi. **Materijal i metode.** Prateći internacionalno ustanovljen metod, proces adaptiranja upitnika za decu i adolescente na srpskom govornom području uključivao je tri koraka: lingvistički prevod upitnika sa engleskog na srpski jezik, povratni prevod i pilot-studiju. **Rezultati.** U pilot-studiji učestvovala su 42 ispitanika (21 ispitanik muškog i 21 ispitanik ženskog pola), prosečne starosti 12 ± 1,01 godina. Sva pitanja su imala *corrected item-total correlation coefficient* > 0,20, dok *je* Kronbahov alfa koeficijent iznosio 0,80. Sve vrednosti korelacionog matriksa bile su pozitivne. Prevalencija oralnog uticaja, merena indeksom *Child Oral Impact on Daily Performance* upitnika, bila je relativno visoka, pri čemu je 54,8% ispitanika imalo najmanje jedan oralni uticaj tokom poslednja tri meseca. Tegobe su najviše bile izražene tokom jela (38,1%) i čišćenja zuba (16,7%). **Zaključak.** Srpska verzija *Child Oral Impact on Daily Performance* upitnika je pogodna za korišćenje u svakodnevnoj praksi i može da pruži korisne informacije pri proceni kvaliteta života. Prevalencija oralnog uticaja je bila visoka, pri čemu su tegobe najviše bile izražene tokom jela. Kliničko istraživanje koje će uključiti veći broj ispitanika je potrebno radi definitivne verifikacije upitnika za srpsko govorno područje.

Ključne reči: oralno zdravlje; kvalitet života; ankete i upitnici; dete; adolescent; ispitivanje stanja zdravlja zuba; oralna higijena; briga o zdravlju

Introduction

The World Health Organization (WHO) defines health as a "state of complete physical, mental and social wellbeing, and not merely the absence of disease or infirmity" [1]. According to the concept proposed by Terris [2], health is a condition of adequate functioning of the organism in the given genetic and environmental conditions. Thus, the measurement of health must encompass much more than clinical indicators [3]; the clinical measures are insufficient to assess health because they fail to consider functional and psychosocial aspects of health and do not adequately reflect the health status, functioning, concerns and perceived needs of individuals [4, 5]. As a result, an increasing interest in the concepts of health-related

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Abbreviations

WHO – Wordl Health Organization
HRQOL – health-related quality of life
OHRQOL– oral health-related quality of life
C-OIDP – Child Oral Impact on Daily Performance
ICIDH – International Classification of Impairment, Disabilities and Handicaps
CPQ – Child Perception Questionnaire
COHIP – Child Oral Health Impact Profile
OIDP – Oral Impact on Daily Performance

quality of life (HRQoL) and oral health-related quality of life (OHRQoL) has been seen in the literature in recent years [6–12]. OHRQoL has become an integral part of numerous clinical oral health studies both in the world [8–10] and in our country [11, 12] with the previous monitoring of clinical indicators of oral diseases.

HRQoL is "a multidimensional concept that reflects how long people live and how well people live at the same time". Similarly, OHRQoL is "a multidimensional complex of inter-related domains such as the absence of a potentially lethal disease and pathological changes, absence of pain and other symptoms, the presence of adequate masticatory function, adequate emotional and social function and overall oral health satisfaction" [13].

Oral health-related quality of life is based on the conceptual framework derived from the WHO - International Classification of Impairments, Disabilities and Handicaps (ICIDH) [14], which was modified for dentistry by Locker [15]. It provides a theoretical basis for the empirical exploration of the relationships between various dimensions of general and oral health and consists of the following key concepts: impairments, functional limitations, pain and discomforts, disabilities and handicaps. Impairments refer to the immediate biophysical outcomes of a disease, commonly assessed by clinical indicators. Functional limitations are concerned with functioning of body parts; whereas, pain and discomforts refer to the experiential aspects of oral conditions in terms of symptoms. Finally, the ultimate outcomes of disability and handicap refer to any difficulties in performing activities of daily living, and also to broader social disadvantages.

Numerous OHRQoL indices have been developed to assess the oral impacts on quality of life in adult population. However, only few OHRQoL are specifically directed at the child-adolescent population: the Child Perception Questionnaire (CPQ11-14) [16], the Michigan OHRQoL [17], the Child-Oral Impact on Daily Performance (C-OIDP) [8], the Child Oral Health Impact Profile (COHIP) [18], the Early Child Oral Health Impact Scale (ECOHIS) [19], and the Scale of Oral Health Outcomes (SOHO-5) [20]. These indices are designed to cover a variety of oral conditions such as dental caries, malocclusion and craniofacial anomalies. The most frequently used measures in children and adolescents are the CPQ, the C-OIDP and COHIP. They include a wide age range and a variety of conditions and therefore most likely to be of use in a range of studies.

The C-OIDP index is a well known OHRQoL index specifically directed at the child-adolescent population. In comparison with other indices, it is theoretically based on the ICIDH framework but focuses only on the third level [14] and demonstrates a strong theoretical coherence and reduces the possibility of double scoring the same oral impacts on different levels [21]. To date, the C-OIDP index has been validated for psychometric properties in Thailand [8], France [9], United Kingdom [10], Peru [22], Brazil [23], Italy [24], and Spain [25]. Also, OIDP index for adults was translated to Serbian and its validity, reliability and responsiveness was tested in the elderly population of Bosnia and Herzegovina and it can be used in further researches [11, 12].

Considering the fact that the OIDP index has been internationally accepted and availability of the Serbian version of OIDP for adults, the aim of this study was to translate the C-OIDP index into Serbian and to assess its reliability in practice.

Material and Methods

The C-OIDP questionnaire was originally constructed in English at the *University College London*. It was translated and adapted into Serbian language following the published guidelines [26]. The process of cross-culture adaptation involved three steps: translation of the original C-OIDP into Serbian language, backward translation into English, and a pilot study. The C-OIDP was translated from English to Serbian language by two native Serbian-speaking professional translators familiar with dental terminology in English. Each translator worked independently, without consulting the other, or the research team. The two translations were compared and a consensus forward version was made. It was used in a study with a convenience sample of ten 11 - 14 year-olds to test the clarity of questions. Subsequently, the Serbian version of the questionnaire was translated into English by another professional translator with excellent knowledge of English who had no prior knowledge of the original version. The differences between the backward translation and the original English version were analyzed and discussed by the research team and the translators. The next step was to carry out a pilot study, to test the clarity, appropriateness and cultural relevance of the Serbian language version of the C-OIDP (**Table 1**). During this process, close contact and discussions were maintained with the original research team of the *University College London* who created the C-OIDP index.

The C-OIDP index was specifically developed for assessing oral well-being and oral impacts on daily activities in the child/adolescent population. It focuses on eight basic daily life activities and behaviours such as: eating, speaking, cleaning the teeth, sleeping, emotional state, smiling, studying and social contacts. The impact on each activity was assessed in terms of frequency (from 0 to 3) and severity (from 0 to 3) (**Table 2**). Single performance score was calculated by multiplying the frequency and severity scores, with zero score if no impact was reported. To obtain the C-OIDP score for an individual the sum of eight scores was divided by the possible maximum performance scores (eight performances X maximum frequency score (3)

Table 1. Serbian version of Child-Oral Impact on Daily Performances (C-OIDP)
Tabela 1. *Srpska verzija Upitnika za određivanje uticaja oralnih tegoba na svakodnevne aktivnosti kod dece*

Q1: In the past 3 months, have you had any difficulty …..ACTIVITY/BEHAVIOUR… due to problems with your mouth or teeth?
P1: ***Da li ste poslednja 3 meseca imali ikakve poteškoće tokom .....aktivnosti/ponašanja ..... zbog problema sa ustima ili zubima?***
* If the answer is **"no",** it will be coded as "0" and you must assess the presence of difficulty with the following ACTIVITY/BEHAVIOUR.
* *Ako je odgovor* ***„ne"*** *vi ćete označiti „0" i oceniti prisustvo teškoća tokom sledeće aktivnosti/ponašanja!*
*If the answer is **"yes",** ask questions from Q2 to Q5
* *Ako je odgovor* ***„da"****,odgovorite na preostala pitanja* ***(2−5).***

Q2: **Have you had this difficulty on a regular basis over the past three months or only for part of this period?**
P2: ***Da li ste imali ove poteškoće prilikom jela redovno/povremeno ili kroz određeno razdoblje/kraće vreme?***
*If the answer is: **"on a regular basis"** ask Q3
Ukoliko su se poteškoće javljale **redovno/povremeno, zabeležite učestalost teškoće u pitanju br. 3.*
*If the answer is: **"only for part of this period"** ask Q4
***Ukoliko su se poteškoće javljale **kroz određeno razdoblje/kraće vreme**, zabeležite trajanje teškoće u pitanju br. 4.*

Q3: **During the past three months, how often have you had this difficulty?**
P3: ***Koliko često ste u poslednja 3 meseca imali ovakve poteškoće?***

About 3 - 4 times a week or nearly every day	
Oko 3-4 puta sedmično ili gotovo svaki dan	3
About 1 - 2 times a week/*Jednom/dvaput sedmično*	2
About 1 - 2 times a month or less often than once a month	
Jednom/dvaput mesečno ili ređe.	1

Q4: **For how much of the past three months have you had this difficulty?**
P4: Koliko dugo ste u poslednja 3 meseca imali ovakve poteškoće?

For more than 3 months/*Preko 3 meseca ukupno*	3
For more than 1, up to 3 months/*Od 1 do 3 meseca*	2
For one, two days up to a month/*Dan-dva, do mesec dana*	1

Q5: **Using a scale from 0 to 3, where 0 is no effect and 3 a very severe effect, how much effect would you say has the difficulty had on your everyday life?**
P5: ***Koristeći skalu 0−3, gde 0 označava mali uticaj, a 3 označava vrlo ozbiljan uticaj, koji broj bi najbolje odražavao uticaj poteškoća na svakodnevni život?***

No effect/*Bez uticaja*	0
A fairly minor effect/*Mali uticaj*	1
A moderate effect/*Umeren uticaj*	2
A fairly severe effect/*Velik uticaj*	3

X maximum severity score (3) = 72), and multiplied by 100 to provide a percentage score.

The Serbian version of C-OIDP index was validated in a pilot study. All parents of included children were

Table 2. Scoring of oral impacts on daily performance of schoolchildren
Tabela 2. *Način bodovanja uticaja oralnih tegoba na svakodnevne aktivnosti dece školskog uzrasta*

Activities/Behaviours *Aktivnosti/Ponašanje*	Q1: Presence of Difficulty P1:*Prisustvo teškoće*	Q2: Period *P2: Period*		Q5: Effect *P5: Efekat*
	No: 0/Yes:1 *Ne:0/Da:1*	Frequency/*Učestalost* Duration/*Trajanje*		
		Q3/P3	Q4/P4	
Eating/*Jelo*	0 / 1	1-3	1-3	0-3
Speaking/*Govor*	0 / 1	1-3	1-3	0-3
Cleaning the teeth/*Pranje zuba*	0 / 1	1-3	1-3	0-3
Sleeping/*Spavanje*	0 / 1	1-3	1-3	0-3
Emotion/*Emocionalno stanje*	0 / 1	1-3	1-3	0-3
Smiling/*Smejanje*	0 / 1	1-3	1-3	0-3
Studying/*Učenje*	0 / 1	1-3	1-3	0-3
Social contacts/*Socijalni kontakti*	0 / 1	1-3	1-3	0-3

fully informed regarding the nature of the study before giving consent. The study included 42 children between the ages of 11 and 14 attending two public schools in Foča, Bosnia and Herzegovina. Two trained dentists conducted the interviews. A face-to-face interview was performed with each child individually, after a short discussion with regard to his/her level of understanding the questions. All words which might have been confusing or possibly misunderstood were replaced by alternative words proposed by children themselves. This testing was conducted in order to make slight changes to the wording of the version adopted.

All statistical tests were done using the SPSS software package, version 20.0 (IBM Corp., Armonk, NY, USA). Internal consistency was evaluated using the corrected item-total correlations, Cronbach's alpha coefficient and alpha if item deleted.

Results

The mean age of the study sample was 12.0±1.01 with equal distribution of males and females. Most participants (88.1%) lived in the urban area, and only 11.9% in the rural area.

The internal consistency analysis of the C-OIDP resulted in a corrected item total values above 0.20 and standardized Cronbach's alpha coefficient of 0.80 (**Table 3**). The correlations matrix showed no negative correlation, with values between 0.10 and 0.73 (**Table 4**).

Overall, 54.8% children reported that they had at least one oral impact in the last three months. The most common affected performances in the total sample were: eating (38.1%) and cleaning the teeth (16.7%) (**Table 5**). The mean overall C-OIDP was 3.9%. Eating and cleaning the teeth were the performances with the highest intensity (56.25% of subjects with impacts on eating and 42.9% with impacts on cleaning the teeth reported severe intensity); on the other hand, studying was the performance with the lowest intensity.

Discussion

There were no great problems with the translation of C-OIDP index and comparison of the original version with the backward translation showed no content or conceptual differences. However, during the pilot study, some problems with understanding the

Table 3. Cronbach's alpha, item-total correlation and alpha if item deleted for C-OIDP index
Tabela 3. *Kronbahov alfa, korigovani koeficijent korelacije i alfa ako je neko pitanje izbrisano za C-OIDP upitnik*

Performance *Dnevne aktivnosti*	Corrected item-total correlation *Korigovani koeficijent korelacije*	Alpha if item deleted *Alfa ukoliko je pitanje izbrisano*
Eating/*Jelo*	0.72	0.73
Speaking/*Govor*	0.63	0.76
Cleaning the teeth/*Pranje zuba*	0.62	0.76
Sleeping/*Spavanje*	0.45	0.78
Emotional state/*Emocionalno stanje*	0.44	0.78
Smiling/*Smejanje*	0.44	0.78
Studying/*Učenje*	0.47	0.78
Social contacts/*Socijalni kontakti*	0.35	0.80
Cronbach's alpha coefficient *Kronbahov alfa koeficijent*	=0.80	

Table 4. Psychometric properties of the Serbian C-OIDP. Inter-item correlations matrix
Tabela 4. *Prikaz pouzdanosti C-OIDP upitnika: Korelacioni matriks za sva C-OIDP pitanja*

Performance *Aktivnost*	Eating *Jelo*	Speaking *Govor*	Cleaning the teeth *Pranje zuba*	Sleeping *Spavanje*	Emotional status *Emocionalno stanje*	Smiling *Smejanje*	Studying *Učenje*	Social contacts *Socijalni kontakti*
Eating/*Jelo*	1.00							
Speaking/*Govor*	0.41	1.00						
Cleaning the teeth/*Pranje zuba*	0.57	0.73	1.00					
Sleeping/*Spavanje*	0.35	0.54	0.37	1.00				
Emotional status *Emocionalno stanje*	0.52	0.10	0.18	0.15	1.00			
Smiling/*Smejanje*	0.52	0.10	0.18	0.15	1.00	1.00		
Studying/*Učenje*	0.29	0.69	0.50	0.37	0.37	0.37	1.00	
Social contacts *Socijalni kontakti*	0.41	0.45	0.29	0.23	0.23	0.23	0.69	1.00

Table 5. Percentage of children with oral impacts on daily performance
Tabela 5. *Učestalost oralnih tegoba na uobičajne dnevne aktivnosti kod dece u %*

Performance/*Aktivnost*	%
Eating/*Jelo*	38.1%
Speaking/*Govor*	9.5%
Cleaning the teeth/*Pranje zuba*	16.7%
Sleeping/*Spavanje*	7.1%
Emotional state/*Emocionalno stanje*	14.3%
Smiling/*Smejanje*	14.3%
Studying/*Učenje*	4.8%
Social contacts/*Socijalni kontakti*	9.5%

questions emerged. For this reason, the order of questions was changed, as recommended by the author [10]. Namely, there were problems about understanding the first question: "whether or not problems with mouth and teeth have caused any difficulty with …(performance)…?" Wording modifications did not significantly reduce this problem. Thus, the order of questions was changed and children were first asked the fourth question of the original version of the C-OIDP index. They were given a list of common oral problems occurring in this age group. So, the use of the questionnaire was much easier. Also, it has been recommended to show pictures with child's emotions during daily performance. In this way, the completion time was shortened from 20 to 10 minutes, and the children answered questions much easier.

In terms of reliability, inter-item correlation, corrected item-total correlation and Cronbach's alpha, indicated that C-OIDP index had excellent internal consistency. All inter-item correlations were positive, and all item-total correlations were above the minimum recommended level of 0.20 [27], showing homogeneity of the index. Furthermore, Cronbach's alpha was significantly higher than the recommended level [26] and higher than in some previous studies carried out in Italy (0.57), [24], Spain (0.68) [25] and Chile (0.72) [28]. The prevalence of oral impacts on daily performances was high, because 54.8% of participants reported at least one daily activity affected in the last 3 months. The prevalence of oral impacts was higher than in most studies [29, 30] except in studies carried out in Brazil (32.0%) [31], Tanzania (28.6%) [32], and England (40.4%) [10].

The most common oral impact was difficulty eating (38.1%) in accordance with results of other studies using C-OIDP [8, 24]. The prevalence of impacts on the emotional status and smiling were also relatively high. These results were consistent with findings in Italian [24] and Thai schoolchildren [8]. It is apparent that an important reason for the high prevalence of oral impacts in children are natural processes such as exfoliation of primary teeth or spaces due to absence of permanent teeth.

This paper presents only results of a pilot study, while clinical research including larger number of participants (>200) is required for the definitive verification of C-OIDP index in Bosnia and Herzegovina. It is necessary to examine the validity of C-OIDP through the assessment of several subjective health status variables. Also, responsiveness to change should be tested in participants in need for dental treatment. Thus, after validation of C-OIDP, it will be used for the assessment of OHRQoL in children and adolescents in Serbian-speaking areas.

Conclusion

The Serbian version of the Child-Oral Impact on Daily Performance index is suitable for use in everyday practice in Serbian- speaking areas and it can provide useful information for assessing oral health-related quality of life. The prevalence of oral impacts was high, and the most prevalent impact was difficulty eating. Clinical researches including larger number of participants are necessary for definitive verification of the Child-Oral Impact on Daily Performance index.

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SOCIO-DEMOGRAPHIC CHARACTERISTICS OF PERSONS WITH PSYCHIATRIC DISORDERS WHO COMMITTED SUICIDE

SOCIODEMOGRAFSKE KARAKTERISTIKE OSOBA SA MENTALNIM OBOLJENJIMA KOJE SU POČINILE SAMOUBISTVO

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Summary

Introduction. Psychiatric disorders represent an important risk factor for death by suicide. The aim of this study was to assess the incidence of psychiatric disorders among the persons who committed suicide in the territory of the City of Niš in the period 2001 - 2010. Additionally, this study aimed to compare the socio-demographic characteristics between persons with and without psychiatric disorders. **Material and methods**. This retrospective study included 524 persons who committed suicide (330 with and 194 without psychiatric disorders) in the period 2001 - 2010. Data on socio-demographic characteristics, previous suicide attempts, and methods of suicide were obtained from medical and police records (Police Directorate for the City of Niš, and Statistical Office of the Republic of Serbia). **Results**. We studied the incidence of suicides among the persons with psychiatric disorders compared to persons without any medical condition in the studied period. Depression (104, 31.5%) and personality disorders (103, 31.2%) were the most common psychiatric disorders among the persons who committed suicide, whereas only 21 persons (6.36%) had schizophrenia. Persons with psychiatric disorders had a higher level of education, more of them were divorced, had a private source of income and more frequently attempted suicide compared to persons without any diseases ($p<0.001$). The most common methods of suicide were hanging and poisoning in both investigated groups. **Conclusion**. Depression was the most common disorder registered among the persons with psychiatric disorders who committed suicide. It is necessary to develop a national strategy for suicide prevention for groups at high risk of suicide.

Key words: Mental Disorders; Suicide; Suicide, Attempted; Demography; Population Characteristics; Risk factors; Depression; Asphyxia

Sažetak

Uvod. Mentalna oboljenja predstavljaju značajan faktor rizika za nastanak samoubistva. Cilj istraživanja bio je da se utvrdi učestalost pojedinih mentalnih oboljenja kod osoba koje su počinile samoubistvo na teritoriji Grada Niša u periodu između 2001. i 2010. godine. Takođe, istraživanje je imalo za cilj da uporedi sociodemografske karakteristike osoba koje su počinile samoubistvo, a koje su bile i mentalno obolele sa osobama koje nisu bile mentalno obolele. **Materijal i metode**. Retrospektivno istraživanje obuhvatilo je 524 osobe koje su počinile samoubistvo (330 sa mentalnim oboljenjima i 194 bez registrovanog oboljenja) u periodu između 2001. i 2010. godine. Podaci o sociodemografskim karakteristikama, istoriji prethodnih pokušaja samoubistva i načinu izvršenja samoubistva prikupljeni su iz medicinskih i policijskih izveštaja baze Policijske uprave Grada Niša i Zavoda za statistiku Republike Srbije. **Rezultati**. Uočen je trend povećanja broja samoubistava među osobama sa mentalnim oboljenjima u odnosu na osobe bez registrovanog oboljenja u analiziranom periodu. Najveći broj osoba koje su počinile samoubistvo imale su dijagnozu depresije (104, 31,5%) i poremećaja ličnosti (103, 31,2%), a 21 osoba (6,36%) imala je shizofreniju. Osobe sa mentalnim oboljenjima imale su veći stepen obrazovanja, u većem procentu vlastiti izvor prihoda, ali i češći pokušaj samoubistva i razvoda u odnosu na osobe bez registrovanog oboljenja ($p < 0,001$). Najčešći način izvršenja samoubistva u obe analizirane grupe bilo je vešanje i trovanje. **Zaključak**. Depresija je najčešće oboljenje dijagnostikovano kod mentalno obolelih osoba koje su počinile samoubistvo. Neophodno je razviti nacionalnu strategiju za prevenciju samoubistava među grupama sa rizikom.

Ključne reči: mentalni poremećaji; samoubistvo; pokušaj samoubistva; demografija; karakteristike stanovništva; faktori rizika; depresija; asfiksija

Introduction

Suicide is an act with a fatal outcome which the deceased, knowing or expecting a fatal outcome, had initiated and carried out with the purpose of provoking the changes that he desired [1]. It is a complex, dynamic and heterogeneous social phenomenon that affects the whole society, not only the people who commit suicide. A single explanation why people commit suicide does not exist. Basi-

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cally, it is a consequence of several interacting factors, such as personal, social, psychological, cultural, biological and environmental [2, 3].

Compared with the data from 2000, the number of suicides has decreased by 9%. However, suicide still represents a major public health problem worldwide. The World Health Organization (WHO) estimated that 804.000 suicide deaths occurred worldwide in 2012, with a suicide rate of 11.4 per 100.000 population (15.0 for males and 8.0 for females). In 2012, suicide was the fifteenth cause of death in the general population and the second leading cause of death in 15 - 29-year-olds [4, 5].

Previous studies showed that the prevalence, characteristics and methods of suicide vary widely in regard to geographic regions, different communities and ethnic origin, sex, age and time [6, 7]. Although the age-standardized suicide rate is slightly higher in high-income countries (HICs) than in low- and middle-income countries (LMICs) (12.7 versus 11.2 per 100.000 population), 75.5% of all global suicides occur in LMICs. Globally, men commit suicide more frequently than women. Suicide rates are high in many countries. With regard to age, suicide rates are highest in elderly people of both sexes in almost all regions of the world [2, 6, 7].

In Serbia, from the early 1950s to the mid-2010s, the total number of suicides exceeded 75.000. Recent data from the Statistical Office of the Republic of Serbia showed that the average age-standardized mortality rate was 17.4 suicides per 100.000 inhabitants in the whole country. The highest rate was in Vojvodina region with 22.7 suicides per 100.000 inhabitants, and the lowest was in South and East Serbia with 14.0 suicides per 100.000 inhabitants. Gender and age differences in suicide deaths in Serbia follow the world trend. In the last two decades, out of the total number of suicides, 70.7% were committed by males, and only 29.3% by females. In addition, 48.1% of person who committed suicide were aged 60 years or over [8].

Suicide risk factors can be divided into proximal and distal factors. Distal factors increase the predisposition, whereas proximal act as precipitants [9]. Psychiatric disorders represent one of the most important proximal risk factor. Previous studies reported that psychiatric disorders are present in about 80 - 90% of persons who committed suicide. Moreover, recent findings confirmed that persons with more than one psychiatric disorder are at higher risk for suicide [10–12]. Additionally, it has been reported that functional psychiatric disorders (major depressive disorder, bipolar disorders, schizophrenia, etc.) are associated with higher suicide risk than organic psychiatric disorders (epilepsy and brain injury) [13].

Recent studies have reported the suicide mortality rate of Serbian population in different time periods related to age and sex [14 - 16]. On the other hand, there are no previously reported findings about the socio-demographic characteristics of persons with psychiatric disorders who committed suicide. Therefore, this study aims to investigate the socio-demographic characteristics of persons with psychiatric disorders who committed suicide and to compare them with the same characteristics of persons without registered psychiatric or physical disorders in the territory of the City of Niš in the period from 2001 to 2010. Furthermore, this study investigates the annual trend growth of suicide deaths and the differences in the incidence of specific psychiatric disorders among the persons who committed suicide.

Material and methods

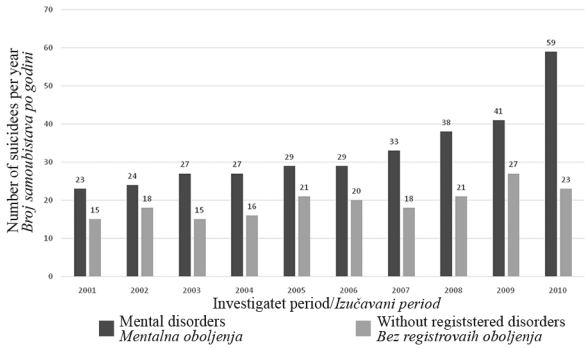
This retrospective study included 524 persons (264 males and 260 females) who committed suicide in the territory of the City of Niš, Republic of Serbia, in the period from 2001 to 2010. Data on persons who died of suicide and self-inflicted injury were obtained from the Police Directorate for the City of Niš, Ministry of Internal Affairs, and from the Statistical Office of the Republic of Serbia, Department of Demography.

The study sample was divided into two groups. The first group included 330 persons (169 males and 161 females) who committed suicide with previously diagnosed psychiatric disorders according to the International Statistical Classification of Diseases and Related Health Problems (ICD-X) [17]. The second

Table 1. Persons who committed suicide included in the study
Tabela 1. *Osobe koje su počinile samoubistvo uključene u istraživanje*

Study group/*Ispitivana grupa*	Type of disorder/*Vrsta poremećaja*	N
Persons with psychiatric disorders *Osobe sa mentalnim poremećajima*	Depressive episodes/*Depresivne epizode* (ICD, *IKB*-V-F 32)	104
	Adult peresonality disorders/*Poremećaji ličnosti* (ICD, *IKB*-V-F 60-63)	103
	Disorders due to alcohol abuse *Poremećaji usled upotrebe alkohola* (ICD, *IKB*-V-F10)	69
	Opioid-related disorders *Poremećaji usled upotrebe opoidnih sredstava* (ICD, *IKB*-V-F 11)	33
	Schizophrenia/*Shizofrenija (ICD, IKB-V-F-20-21)*	21
Persons without disorders *Osobe bez poremećaja*		194
Total/*Ukupno*		524

ICD - International classification of diseases/*IKB – Internacionalna klasifikacija bolesti;* N - number of persons/*broj osoba*



Graph 1. Numer of suicides in the investigated period
Grafikon 1. *Broj samoubistava u izučavanom periodu*

group included 194 persons (95 males i 99 females) without diagnosed psychiatric or physical disorders who committed suicide **(Table 1)**. Using family interviews, medical and police records, we extracted details about the age, sex, marital status, level of education, source of income, criminal record, history of previous suicide attempts, and methods of suicide of each person who committed suicide.

Statistical analysis was performed using IBM SPSS Statistics for Windows Software (Version 20.0< IBM Corp, Armonk, NY, USA). The results were presented as frequencies and percentages. The Pearson's chi-squared test was conducted to assess statistical significance between categorical data. All p values under .05 were considered significant.

Results

Of the total number of committed suicides (524), 63% (330, 169 males and 161 females) were committed by persons with psychiatric disorders, while the rest of 37% (194, 95 males and 99 females) were committed by persons without registered psychiatric of physical disorders.

Depression (104, 31.5%) and personality disorders (103, 31.2%) were the most frequently diagnosed psychiatric disorders among the persons who committed suicide. On the other hand, only 21 persons were diagnosed with schizophrenia (6.36%) **(Table 1)**.

The number of suicide deaths increased annually in both groups within the investigated period. In 2001, the number of suicides was 0.65 times more frequent among the persons with psychiatric disorders compared to persons without registered disorders. Moreover, in 2010, the observed ratio was 2.57 **(Graph 1)**.

The average age of persons with psychiatric disorders was 49.86 ± 18.11 years, and in the group of persons without registered disorders it was 55.82 ± 25.23 years **(Table 2).** The observed difference was statistically significant (p<0.001).

Persons with psychiatric disorders who committed suicide had higher level of education (296, 89.7%) than persons without registered disorders (136, 70.1%). In addition, a higher number of persons without any education or only with elementary school were observed among the persons without registered disorders (58, 29.9%) compared to persons with psychiatric disorders (34, 10.3%) **(Table 2).** These differences were statistically significant (p<0.001).

The analysis of the marital status of persons who committed suicide showed that persons with psychiatric disorders were 4.18 times more often divorced compared to persons without registered disorders. Additionally, persons with psychiatric disorders were 1.98 times more frequently married or in non-marital relationships. Roughly, the same number of persons who committed suicide in both groups were widowed **(Table 2)**. The observed differences were statistically significant (p<0.001).

A significantly higher number of persons with psychiatric disorders had an income or pension (223, 67.6%), while persons without registered disorders were dependent or received a social welfare (107, 55.1%) **(Table 2)**.

A previous suicide attempt was 8.13 times more frequent among the persons with psychiatric disorders, compared to persons without disorders (p<0.001) **(Table 2)**. Although persons with psychiatric disorders were more frequently criminally offended compared to persons without disorders, a significant difference was not found (p=0.053) **(Table 2)**.

In both investigated groups hanging and intentional self-poisoning, using drugs or liquid substances, were the most common methods of suicide. However, differences between methods of suicide were not statistically significant among the observed groups (p=0.210) **(Table 3)**.

Discussion

It is well known that numerous factors contribute to suicide. Previous psychological autopsy case-control studies reported a strong relationship between suicide and psychiatric disorders [10–12]. The results of Harris & Barraclough [13] suggest that the risk of suicide is 5 to 15-folds higher in persons with psychiatric disorders. Moreover, previous studies showed that specific psychiatric disorders were associated with a higher risk of suicide [18, 19]. Mood disorders (RR = 13.4), substance-related disorders (RR = 5.2), personality disorders (RR = 4.5) and psychotic disorders (RR = 6.6) are the most common psychiatric disorders among the persons who commit suicide [18]. These results are in accordance with the study of Ferrari et al. [19] who estimated that the relative risk of suicide in an individual with a major depressive disorder was 19.9 (OR = 9.5-41.7), with schizophrenia 12.6 (OR = 11.0-14.5), and with alcohol abuse 9.8 (OR = 9.0-10.7).

Our findings are in agreement with the previously reported results. Of 330 persons with diagnosed psychiatric disorders who committed suicide in the territory of the City of Niš, 104 (31.5%) were with depression, and 103 (31.2%) were with personality disorders. Schizophrenia was the least frequent psychiatric disorder registered in our sample (21, 6.36%). Depression is recognized as the leading diagnosis associated with suicide, occurring in almost two-thirds of cases [20].

Table 2. Socio-demographic characteristics of persons who committed suicide
Tabela 2. *Sociodemografske karakteristike osoba koje su počinile samoubistvo*

Socio-demographic characteristics *Sociodemografske karakteristike*	Persons with psychiatric disorders/*Osobe sa mentalnim poremećajima N (%)*	Persons without disorders *Osobe bez poremećaja N (%)*	p value *p vrednost*
Sex/*Pol*			
Male/*Muški*	169 (51.2%)	95 (49%)	p=0.620[a]
Female/*Ženski*	161 (48.8%)	99 (51%)	
Age (year)/*Starost (godine)* (Mn±SD)	49.86±18.11	55.82±25.23	p=0.002[a]
Level of education/*Stepen obrazovanja*			
Illiterate/*Nepismen*	10 (3.0%)	13 (6.7%)	p<0.001[a]
Elementary school/*Osnovna škola*	24 (7.3%)	45 (23.2%)	
High school/*Srednja škola*	168 (50.9%)	103 (53.1%)	
Faculty/*Fakultet*	128 (38.8%)	33 (17.0%)	
Marital status/*Bračni status*			
Single/*Samac*	87 (26.4%)	57 (29.4%)	p<0.001[a]
Married/*Bračna zajednica*	75 (22.7%)	41 (21.1%)	
Extramarital relationship *Vanbračna zajednica*	26 (7.9%)	16 (8.2%)	
Divorced/*Razveden*	92 (27.9%)	22 (11.3%)	
Widowed/*Udovac*	50 (15.2%)	58 (29.9%)	
Source of income/*Izvor prihoda*			
Salary/*Plata*	162 (49.1%)	35 (18.0%)	p<0.001[a]
Pension/*Penzija*	61 (18.5%)	52 (26.8%)	
Social welfare/*Socijalna pomoć*	21 (6.4%)	20 (10.3%)	
Dependent/*Izdržavano lice*	86 (26.1%)	87 (44.8%)	
Previous suicide attempts/*Prethodni pokušaji samoubistva*			
No/*Ne*	135 (40.9%)	170 (86.7%)	p<0.001[a]
Yes, once/*Da, jednom*	140 (42.4%)	19 (9.8%)	
Yes, more than once/*Da, više puta*	55 (16.7%)	5 (2.6%)	
Criminal record/*Krivično kažnjavan*			
No/*Ne*	248 (75.2%)	157 (80.9%)	p=0.053[a]
Yes/*Da*	82 (24.8%)	37 (19.1%)	

[a] Pearson Chi – square test/[a] *Pirson Hi – kvadrat test,* Mn – mean/*srednja vrednost,* SD – standard deviation/*standardna devijacija,* N – number of persons/*broj ispitanika*

A particularly disturbing fact is that depression is increasing among the young people [21]. A recent systematic review of Hawton et al. [22] identified the following risk factors for suicide in persons with depression: male gender, family history of suicide or psychiatric disorder, history of attempted suicide, hopelessness, and comorbid disorders such as anxiety, personality disorder, drug and alcohol abuse. This is in accordance with the number of persons with personality disorders, drug and alcohol abuse who committed suicide in our study. Compared with the group without registered disorders, these pathologies may increase the risk of suicide in persons with mental disorders [22]. Additionally, Coryell & Young [23] reported that clinical predictors of suicide in persons with a major depressive disorder include a history of attempted suicide, high level of hopelessness, and high suicidal tendencies.

Our findings indicate a slightly higher suicide rate in males than in females, with an average age of 55.83 years. These results are in accordance with the previously reported findings [3, 6, 7]. There are several explanations for this specific ratio. Basically, males are more impulsive and aggressive compared to females. Additionally, males choose more effective methods of suicide than women [24]. From the cultural aspect, less social acceptance of suicide attempts in males causes more suicide deaths and fewer attempts that are converse compared to females [25]. Increased alcohol and psychoactive substance abuse [26], suppression of emotions [27], and non-acceptance of medical care [28] contribute to higher suicide rate in males.

Our findings also showed that suicide is more common among elderly people in both investigated groups. These results are in line with previously reported results which suggest that suicide deaths are more common in advanced age [3, 6, 7]. Moreover, recent studies have shown that high percentage of people older than 65 years suffered from depres-

Table 3. Methods of suicide
Tabela 3. *Načini izvršenja samoubistva*

Methods of suicide *Način izvršenja samoubistva*	Persons with psychiatric disorders/*Osobe sa mentalnim poremećajima N (%)*	Persons without disorders N/*Osobe bez poremećaja N (%)*
X60-65, X68-69 Self-poisoning by drugs or by exposure to liquid substances/*Trovanje zboupotrebom droga i izlaganjem tečnim supstancijama*	83 (25.2%)	51 (26.3%)
X70 Hanging/*Vešanje*	116 (35.2%)	67 (34.5%)
X71 Drowning and submersion/*Davljenje i potapanje*	31 (9.4%)	30 (15.5%)
X72-75 Firearm and explosive material/*Oružje i ekploziv*	60 (18.2%)	28 (14.4%)
X80 Jumping from height/*Skok sa visine*	40 (12.1%)	18 (9.3%)

N – numbers of persons/*broj osoba*

sion [20]. A diagnosed depression additionally increases the risk of suicide among the elderly. Another obvious problem is that in the majority of cases, depression is unrecognized due to the symptoms of other diseases in the elderly and side effects of various drugs [29]. Apart from this, the risk of suicide in old people increases with alcohol abuse [30], and a higher rate of comorbidity [31].

Socio-demographic data showed that persons with psychiatric disorders who committed suicide had a higher level of education, personal source of income, were more frequently divorced, and have already attempted to commit suicide compared to persons without registered disorders. Previous studies indicate that divorced persons had higher suicide rates and increased risk of suicide compared to married persons [32, 33]. Marriage has a protective role, providing social and emotional stability and easier integration into the community. Divorce or loss of a spouse may increase the risk of suicide [33]. Previous studies reported that suicide is more common among the persons with lower level of education and poor socioeconomic status [6, 7]. Our findings showed that the majority of persons who committed suicide had a personal source of income, whereas data of Statistical Office of the Republic of Serbia showed that the lowest incomes were found in the South and East Serbia [34]. Higher incidence of well-educated persons who committed suicide can be explained by the fact that females in Serbia have higher level of education than males [34].

For every suicide, there are many more people who attempt suicide every year. A prior suicide attempt is the single most important risk factor for suicide in the general population. Persons who have attempted suicide are at significantly higher risk of suicide in the future [2, 4]. Our study showed that persons with psychiatric disorders who committed suicide more frequently attempted to commit suicide compared to persons without registered disorders (59.1% versus 12.4). These findings are in accordance with the previously published results [21, 22], that showed a higher suicide rate among the persons with psychiatric disorders.

We did not observe a significant difference in suicide methods between the study groups. In both groups, the most common suicide method was hanging, followed by intentional self-poisoning. The most lethal methods of suicide are firearms and hanging. Highly lethal methods are more commonly used in committing suicide, while less lethal methods are more frequent in suicide attempts [35]. In general, men tend to choose more violent and highly lethal methods, whereas women often choose methods which are less violent and less lethal [36]. The previous study in Serbian population showed that the leading methods of suicide were by hanging and firearms, which is in line with our findings [16]. Furthermore, our results are in agreement with previously published results worldwide [6, 7].

There is strong evidence that suicide is preventable [37, 38]. According to World Health Organization reports, world-wide evidence-based interventions for suicide prevention are classified into universal, selective and indicated interventions [39]. We assume that additional screening tests [40–42] among the most vulnerable risk groups (i.e. males, older persons and persons with registered psychiatric disorders) should decrease the rate of committed suicides.

Conclusion

In conclusion, persons with psychiatric disorders committed suicide more frequently compared to persons without registered disorders in the territory of the City of Niš in the period from 2001 to 2010. Depression was the most commonly registered psychiatric disorder among the persons who committed suicide. Socio-demographic characteristics of persons with psychiatric disorders indicate that suicide deaths occurred more frequently among males, divorced, with personal sources of income and higher level of education. Furthermore, persons with psychiatric disorders attempted to commit suicide more frequently than persons without registered disorders.

Although no way exists to predict those who will commit suicide, more epidemiological studies should help to recognize population groups prone to self-injuring. It is necessary to establish a national suicide prevention strategy with specific goals to decrease suicidal behavior in all risk groups, especially among the persons with mental disorders.

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EVALUATION OF KNOWLEDGE ON DOPING IN SPORTS AMONG SERBIAN GENERAL PRACTITIONERS

PROCENA NIVOA INFORMISANOSTI SRPSKIH LEKARA OPŠTE MEDICINE O DOPINGU U SPORTU

Dušan ANTIĆ

Summary

Introduction. In the case of illness or injury, athletes, like other patients, seek medical care from general practitioners. Athletes, however, need to be aware of anti-doping regulations. The aim of this study was to evaluate the knowledge and attitudes to doping in sports among general practitioners in Vojvodina. **Material and Methods.** This cross-sectional study included 276 participants of both sexes and different ages. The data collection was performed using a questionnaire, as a non-standard research technique. The statistical analysis correlated the gathered data in regard sex and age of examinees. **Results.** Only a small number of respondents is well informed about both the *List* of *Prohibited Doping Substances and Methods* and *the Law on Prevention of Doping in Sports* (10.5% and 8%, respectively). Also, only 2.5% of examinees thought they were qualified to treat athletes. Correct answers that furosemide, pseudoephedrine and tamoxifen are prohibited in sports were given by a small number of respondents (36.6%, 56.9% and 29%, respectively). On the other hand, the fact that inhaled salmeterol, inhaled corticosteroids, enalapril and diclofenac are allowed in sports, was known by 42%, 40.6%, 60.9% and 52.9% of respondents, respectively. Doctors had different attitudes towards doping in sports. **Conclusion.** The results of this study showed that general practitioners have insufficient knowledge on different aspects of doping in sports. Since they treat all patients, including athletes, their knowledge should be on a higher level in order to avoid accidental doping. The obtained results point to the need for further education of general practitioners on doping in sports.

Key words: Doping in Sports; Athletes; General Practitioners; Surveys and Questionnaires; Health Knowledge, Attitudes, Practice

Sažetak

Uvod. Sportisti, kao i svi drugi pacijenti, u slučaju bolesti ili povrede, javljaju se lekaru radi lečenja i ordiniranja terapije. S druge strane, sportisti podležu antidoping pravilima. Cilj ovog istraživanja je da se proceni nivo informisanja, znanje i stavovi o dopingu lekara opšte medicine sa teritorije Vojvodine. **Materijal i metode.** Istraživanje je realizovano kao transverzalna studija empirijskog karaktera i uključivalo je 276 ispitanika različitog pola i godina starosti. Za prikupljanje podataka korišćen je anketni list kao nestandardna istraživačka tehnika. Statističkom analizom rezultata poređeni su rezultati u odnosu na pol i godine ispitanika. **Rezultati.** Mali broj ispitanika je inforamisan o *Listi zabranjenih supstanci i metoda u sportu*, kao i o *Zakonu o sprečavanju dopinga u sportu* (10,5% i 8%). Samo 2,5% njih smatra da su dobro pripremljeni da leče pacijente koji su sportisti. Tačan odgovor da su furosemid, pseudoefedrin i tamoksifen zabranjeni u sportu dalo je 36,6%, 56,9% i 29% ispitanih lekara. Sa druge strane, da su salmeterol unet inhalacijom, inhalatorni kortikosteroidi, enalapril i diklofenak dozvoljeni u sportu tačno je odgovorilo 42%, 40,6%, 60,9% i 52,9% ispitanika. Učesnici su imali polivalentne stavove po pitanju dopinga u sportu. **Zaključak.** Rezultati ovog istraživanja pokazali su da su lekari opšte medicine nedovoljno informisani o različitim aspektima dopinga u sportu. Budući da su uključeni u lečenje pacijenata koji mogu biti sportisti, njihovo znanje o dopingu bi trebalo da je na višem nivou. Rezultati ovog istraživanja bi mogli da budu dobra smernica za planiranje aktivnosti koje bi mogle da poboljšaju edukaciju lekara opšte medicine o dopingu u sportu.

Ključne reči: dopingovanje u sportu; sportisti; lekari opšte prakse; ankete i upitnici; znanje o zdravlju, stavovi, praksa

Introduction

Doping in sports is as old as sports, but it grew remarkably during the 20th century, especially during the 1990s with development of amphetamines (1950s) and anabolic steroids (1960s) [1], as well as peptide hormones such as human growth hormone (HGH) (1980s) and erythropoietin (1990s) [2]. Recently, gene doping has been identified as a major problem in sports, and the extent of its long-term health consequences is difficult to predict, but likely to be substantial [3]. The international fight against doping took a giant step forward in 1999, with the foundation of the World Anti-Doping Agency (WADA), and after that with publishing of the World Anti-Doping Code (first edition) in 2003. The *List of Prohibited Doping Substances and Methods* is published every year, outlining substances and methods prohibited in competition and out of competition.

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Abbreviations
HGH – human growth hormone
WADA – World Anti-Doping Agency
GP – general practitoners
TUE – Therapeutic Use Exemption

General practitioners (GPs) are a vital part of the sport chain. In their everyday practice they prescribe medicines, and they have to be very careful when the patient is an athlete. Athletes are liable to anti-doping rules and procedures, prohibited in sports. GPs may negligently prescribe prohibited drugs to the athletes, which results in positive doping tests and penalties for the athletes [4, 5]. According to the WADA Code [6], an athlete is always held responsible for violating anti-doping rules, regardless of whether he was familiar with them or he did it out of ignorance.

Moreover, GPs can be involved in sports by [7]:
– Looking after athletes (i.e. in a sporting club)
– Informing athletes about prohibited substances (i.e. for self-medication)
– Being asked to be stakeholders in prevention actions
–Etc.

All around the world, GPs are more or less involved in sports. Doctors do not appear to have much knowledge on the subject of doping, as underlined by a study conducted in the Netherlands, including 1000 GPs, according to which 85% of respondents admitted that they were not familiar with banned substances or their side effects [8]. Another study included 400 GPs in West Sussex, Great Britain; 12% of the respondents answered that physicians can prescribe anabolic steroids for non-medical reasons, and only 35% of them knew that the Prohibited List appears in the British National Formulary [9]. Many physicians are faced with doping in their everyday work. More than 30% of French GPs [10, 11], 28% of Irish GPs [12], 18% of Senegalese doctors [13], 12% of Austrian GPs [14], as well as Dutch GPs [8], were asked to prescribe banned substances to athletes over the last 12 months, and 18% of British GPs [9] were asked to prescribe particularly anabolic steroids during the same period. Physicians were aware of their role in doping prevention [8, 11, 12, 14, 15], even though most of them considered themselves poorly trained in this domain [11].

According to information from 2008, 12% of Serbian doctors who worked in sports associations were GPs [16].

Based on everything stated above, it can be concluded how significant it is, not only for athletes, coaches, and sport physicians as well, but also for GPs, to have basic knowledge and current information resources on doping.

The aim of this research was to establish the level of knowledge and attitudes towards doping among general practitioners in Vojvodina. Moreover, the aim was also to correlate respondents' answers with respect to sex and age.

Material nad Methods

This survey was a cross-sectional study with empirical characteristics. According to power analysis (sample size of n= 975, confidence level 95%, confidence interval 5%), the study included 276 participants of both sexes and different age.

The participants in this survey were primary care physicians - general practitioners (GPs). The author decided to conduct the survey among GPs from Vojvodina, province of Serbia, with its established healthcare system functioning within the healthcare system of Serbia. GPs who took part in the survey were randomly selected from the list of GPs from Vojvodina, provided by the Ministry of Health of the Republic of Serbia (276 out of 975).

Table 1. Knowledge on doping among GPs
Tabela 1. *Informisanost lekara opšte medicine o dopingu*

Questions *Pitanja*	Answers/*Odgovori*						χ^2-test	ANOVA
	Yes *Da*		No *Ne*		I don't know *Ne znam*		p	p
	n	%	n	%	n	%		
Are you informed about the *Prohibited List of Substances and Methods* for 2014?/*Da li ste upoznati sa Listom zabranjenih supstancija i metoda u sportu za 2014. godinu?*	29	10.5	237	85.9	10	3.6	0.045	0,281
Are you informed about the *Law on Prevention of Doping in Sports*?/*Da li ste upoznati sa Zakonom o sprečavanju dopinga u sportu?*	22	8.0	231	83.7	23	8.3	0.925	0,729
Do you think that your knowledge on doping, gained during your previous education or otherwise, is sufficient to treat athletes?/*Da li mislite da imate dovoljno znanja iz oblasti dopinga, stečenog školovanjem, usavršavanjem ili na neki drugi način, koje bi moglo biti korisno prilikom lečenja pacijenata koji su sportisti?*	7	2.5	216	78.3	53	19.2	0.001	0,031

Table 2. Knowledge of respondents about specific substances
Tabela 2. *Znanje ispitanika o ponuđenim supstancijama*

Is this substance allowed in sports?/*Da li je dozvoljena upotreba u sportu sledećih supstancija:*	Answers/*Odgovori*						χ^2-test	ANOVA
	Yes/*Da*		No/*Ne*		I don't know/*Ne znam*		p	p
	n	%	n	%	n	%		
Furosemide/*Furosemid*	79	28.6	101	36.6	96	34.8	0.415	0,110
Salmeterol (inhaled)/*Salmeterol unet inhalacijom*	116	42.0	77	27.9	83	30.1	0.930	0,167
Pseudoephedrine/*Pseudoefedrin*	52	18.8	157	56.9	67	24.3	0.503	0,995
Corticosteroids (inhaled)/*Inhalacioni kortikosteroidi*	112	40.6	85	30.8	79	28.6	0.578	0,038
Diclofenac/*Diklofenak*	168	60.9	26	9.4	82	29.7	0.927	0,201
Enalapril/*Enalapril*	146	52.9	29	10.5	101	36.6	0.465	0,833
Tamoxifen/*Tamoksifen*	32	11.6	80	29.0	164	59.4	0.881	0,145

The data collection was done through a questionnaire, a non-standard research technique. The questionnaire included 15 items. The closed-ended questions covered general information about doping, pharmaceutical agents GPs may be in contact with on everyday basis (drugs in the questionnaire were from the group of most prescribed medicines in Serbia), and doctors' attitudes to doping. The author supervised the completion of the questionnaires, and assurance of anonymity and confidentiality were given to all participants. The study was conducted in the period December 2014 - May 2015.

The gathered data were processed and analyzed using a computer program SPSS for Windows - version 16.0 (SPSS Inc. USA). Chi-square test was used for comparisons between groups with respect to sex, and analysis of variance (ANOVA) with respect to age (LSD Post Hoc multiple comparisons). The $p<0.05$ was considered as statistically significant.

Results

The study included 276 participants. The mean age was 38.22 ± 7.79 years (range 26 – 60 years). The female to male ratio was 71.4% and 28.6%, respectively.

Our participants' general knowledge about doping is presented in **Table 1.** A great number of participants was not informed about the *Prohibited List* (85.9%) and *Law on Prevention of Doping in Sports* (83.7%). There was a statistically significant difference between the given answers of male and female physicians to the question about the List (positive answers – 15.2% and 8.6%, respectively) (p=0.045). When asked if they thought that previous knowledge about doping (from school, faculty, specialization, or some other way) was helpful in work with athletes, only 2.5% of them answered positively, whereas 19.2% answered *I don't know* (difference between the mean age of the participants answering this question showed a statistical significance in all groups – positive answer - 42.86 years, neutral answer - 36.09, and negative answer - 38.60, p=0.031); 3.8% of male and 2% of female physicians (p=0.001) gave positive answers.

When it comes to questions related to the knowledge about specific substances which are regularly used in the treatment of different diseases, but also as doping **(Table 2)**, almost one third of all answers were *I don't know.* Only a small number of examinees gave correct answers that furosemide, pseudoephedrine and tamoxifen were prohibited in sports (36.6%, 56.9% and 29%, respectively). On the other hand, examinees knew that inhaled salmeterol, inhaled corticosteroids, enalapril and diclofenac were allowed in sports: 42%, 40.6%, 60.9% and 52.9%, respectively. Elder participants gave more positive answers about inhaled corticosteroids than neutral answers (39.26 vs. 36.39, p=0.012).

The attitudes of GPs to doping are shown in **Table 3**. A large number of GPs who participated in the survey (84.1%) thought that doping was the greatest evil in sports worldwide. Half of respondents agreed that most professional athletes used a prohibited substance at least once in their career (63.3% of male and 44.7% of female physicians, respectively, p=0.039). In regard to the impact of doping on athletes' health, not less than 92.4% of respondents thought that doping has negative effects on health, whereas 71.4% of them found that athletes often do not know about the consequences doping may have. The majority (94.8%) agreed that GPs need more education in the field of doping (95.9% of female and 86.1% of male physicians, respectively, p=0,006).

Discussion

The results of this study show how well GPs in Vojvodina are informed on doping in sports. There have been no published papers concerning GPs' knowledge on this subject in Serbia.

The results obtained through this survey carried out among physicians from Vojvodina may reflect the knowledge and attitudes of physicians in the whole Serbia. However, the question of possible regional variations within Serbia (which has not been studied so far) could be the subject of some future research.

General practitioners answered questions about doping and doping substances, but also about their attitudes to doping. The results show that GPs have

Table 3. Attitudes of respondents to doping
Tabela 3. *Stavovi ispitanika o dopingu*

Attitudes/*Stavovi*	Answers (%) *Odgovori (%)*			χ^2-test	ANOVA
	I agree *Slažem se*	I am not sure *Nisam siguran/na*	I do not agree *Ne slažem se*	p	p
Doping is the greatest evil in modern sport. *Doping je najveće zlo u savremenom sportu.*	84.1	14.1	1.8	0.121	0,168
Most of the high level athletes have used doping at least once in their career./*Većina vrhunskih sportista je koristila doping bar jednom tokom svoje karijere.*	50.0	35.1	14.9	0.039	0,278
Doping has negative impact on the athletes' health. *Doping utiče negativno na zdravlje sportiste.*	92.4	7.6	0	0.134	0,983
Athletes are often unaware of the risks which doping may have on their health./*Sportisti često ne znaju kakve posledice po zdravlje može imati doping sredstvo.*	71.4	16.7	11.9	0.254	0,521
GPs need more education in the field of doping. *Lekarima opšte medicine treba više edukacije iz oblasti dopinga.*	93.1	6.2	0.7	0.006	0,959

insufficient knowledge about different aspects of doping.

Results presented in **Table 1** indicate that a small number of respondents is well informed about basic documents on doping, such as the Prohibited List and the *Law on Prevention of Doping in Sports.* These results come as no surprise, because Serbian GPs do not have lots of opportunities to read about them. In some countries, GPs know where they can find the List and most of them have it (i.e. BNF, Vidal Dictionary, etc.) [9, 11, 12, 17]. Serbian GPs can only get information about doping through the media or some other source and that is probably why they are not confident if they should be treating sick athletes. Due to the fact that men watch sports more than women [18], it is presumed that they have an opportunity to get more information about doping and prohibited substances than women.

According to the answers related to GPs' knowledge whether certain substances are allowed in sports, it can be concluded that Serbian GPs have insufficient knowledge about that issue. It is connected with the previously mentioned answers showing that many of them have not been informed about the *Prohibited List.* The questionnaire included questions on medicines which are regularly prescribed to patients. For example, furosemide, pseudoephedrine and tamoxifen are on the *Prohibited List* [19] and athletes taking them would be sanctioned for breaking the anti-doping rules if they didn't have a granted TUE (Therapeutic Use Exemption). Furosemide is a diuretic and it is often prescribed by GPs. Diuretics are commonly abused substances in sports [20], and the following example is related to diuretics. In 2010, a Serbian handball player was sanctioned for doping, due to the fact that hydrochlorothiazide (diuretic) was found in his urine [21]. His defense was based on the fact that he used hydrochlorothiazide for regular treatment of high blood pressure (documented by the player) and because of that his doctor should have informed him whether it was forbidden for athletes. He said that he would have made an application for TUE if he knew that the substance was on the *Prohibited List.* However, the player received a 10 month ineligibility sanction imposed by the Handball Federation of Serbia for breaking the anti-doping rule, although it was done out of ignorance. That was, apparently, aberration of the article 14, paragraph 2, of *Serbian Law on Prevention of Doping in Sports*, which says: "A physician who prescribes prohibited doping substances for the purpose of treatment is obliged to inform the athlete about it, if he/she is advised to continue sports activities" [22]. Pseudoephedrine is a nasal decongestant, also on the *Prohibited List*. It is often used to relieve nasal congestion caused by colds, allergies, and hay fever. There are many medications containing pseudoephedrine on the market, and they are easily obtained by athletes. On the other hand, pseudoephedrine is also a psycho-stimulant, and that is the reason why it is on the *Prohibited List*. Some authors believe that athletes use pseudoephedrine only as a decongestant [23], whereas other authors think that athletes mainly used it as a performance-enhancing drug, not as a decongestant [24, 25]. Nevertheless, as mentioned before, athletes are sanctioned if a doping substance is found in their sample, although it might have been done out of ignorance.

Other substances included in the questionnaire were diclofenac, enalapril, inhaled corticosteroids and inhaled salmeterol, which are not on the *Prohibited List*. In contrast to the respondents' knowledge about diclofenac and enalapril (more than 50% correct answers), less than half of them gave correct answers that both inhaled corticosteroids and inhaled salmeterol are allowed in sports. Both medicines are used in the treatment of asthma. Bearing in mind the prevalence of asthma in general population [26, 27], but also among athletes [28–30], it is presumed that

QUESTIONNAIRE/*UPITNIK*

Sex/*Pol:*

Age/*Godine starosti:*

1. Please answer the following questions/*Molim Vas da zaokružite odgovor na sledeća pitanja:*

Are you informed about the Prohibited List of Substances and Methods for 2014?
Da li ste upoznati sa Listom zabranjenih supstanci i metoda u sportu za 2014. godinu?

Yes/*Da* No/*Ne* I don't know/*Ne znam*

Are you informed about the Law on Prevention of Doping in Sports?
Da li ste upoznati sa Zakonom o sprečavanju dopinga u sportu?

Yes/*Da* No/*Ne* I don't know/*Ne znam*

Do you think that your knowledge on doping, gained during your previous education or otherwise, is sufficient to treat athletes?/*Da li mislite da imate dovoljno znanja iz oblasti dopinga, stečenog školovanjem, usavršavanjem ili na neki drugi način, koje bi moglo biti korisno prilikom lečenja pacijenata koji su sportisti?*

Yes/*Da* No/*Ne* I don't know/*Ne znam*

2. Is this substance allowed in sports?/*Da li je dozvoljena upotreba u sportu sledećih supstanci?:*

Furosemide/*Furosemid*	Yes/*Da*	No/*Ne*	I don't know/*Ne znam*
Salmeterol (inhaled) *Salmeterol unet inhalacijom*	Yes *Da*	No *Ne*	I don't know *Ne znam*
Pseudoephedrine *Pseudoefedrin*	Yes *Da*	No *Ne*	I don't know *Ne znam*
Corticosteroids (inhaled) *Inhalacioni kortikosteroidi*	Yes *Da*	No *Ne*	I don't know *Ne znam*
Diclofenac/*Diklofenak*	Yes/*Da*	No/*Ne*	I don't know/*Ne znam*
Enalapril/*Enalapril*	Yes/*Da*	No/*Ne*	I don't know/*Ne znam*
Tamoxifen/*Tamoksifen*	Yes/*Da*	No/*Ne*	I don't know/*Ne znam*

3. What is your attitude to doping in sports?/*Molim Vas da iskažete mišljenje o sledećim stavovima (tvrdnjama):*

Doping is the greatest evil in modern sport./*Doping je najveće zlo u savremenom sportu.*

I agree / *Slažem se* — I am not sure / *Nisam siguran/na* — I do not agree / *Ne slažem se*

Most of the high level athletes have used doping at least once in their career.
Većina vrhunskih sportista je koristila doping barem jedanput tokom svoje karijere.

I agree / *Slažem se* — I am not sure / *Nisam siguran/na* — I do not agree / *Ne slažem se*

Doping has negative impact on the athlete's health.
Doping utiče negativno na zdravlje sportiste.

I agree / *Slažem se* — I am not sure / *Nisam siguran/na* — I do not agree / *Ne slažem se*

Athletes are often unaware of the risks which doping may have on their health.
Sportisti često ne znaju kakve posledice po zdravlje može imati doping sredstvo.

I agree / *Slažem se* — I am not sure / *Nisam siguran/na* — I do not agree / *Ne slažem se*

GPs need more education in the field of doping.
Lekarima opšte medicine treba više edukacije iz oblasti dopinga.

I agree / *Slažem se* — I am not sure / *Nisam siguran/na* — I do not agree / *Ne slažem se*

some patients in the doctor's office may be athletes with asthma. However, severe asthma attacks are treated with oral corticosteroids, which are banned in sports. There is some confusion about whether corticosteroids are allowed in sports, but only corticosteroids administered by oral, intravenous, intramuscular and rectal routes are banned in sports [19].

The majority of examined GPs thought that doping is the greatest evil in modern sport, and half of them believed that most high level athletes used doping at least once in their career. These results show the negative feelings of people about doping, regarding the fact that during and after many sporting events media report on doping of some competitors. Besides, speed, strength and endurance performances in different sports are continuously getting better, and physical appearance of certain athletes may sometimes also raise suspicion [31]. That is why some GPs have doubts whether many sports successes and wins were made with the help of doping. According to the results of the study and regarding the fact that men follow sports more than women [18] and get lots of information about this subject (including pieces of information about doping positive cases), it is presumed that men have more doubts about sports results, whereas women seem to idealize athletes and have more belief in the spirit of sport and fair-play [32].

When it comes to the connection between doping and health, many partitipants are aware that doping is a health risk, but also think that athletes are often not aware of it. It is good that general practitioners are aware that health has no price and that it can be put at risk by doping. It is known that some GPs were faced with requests of athletes to prescribe banned substances, especially anabolic steroids [9–14]. Therefore, GPs have an important role in doping prevention [14, 15, 17]. On the other hand, athletes themselves sometimes have a different point of view. More than 40 years ago, Dr. Gabe Mirkin asked more than 100 competitive runners if they would take a "magic pill" that guaranteed them an Olympic gold medal but would also kill them within a year, and found that more than one-half of athletes responded that they would take the pill [33]. Similarly, in our previous study including Serbian high level athletes, we found that 5.6% of them admitted they would take a doping drug in order to advance their sports career even though it could have negative consequences on their health (7.5% didn't know) [34].

Our results were compared with the results of other authors. Only 34.5% of French GPs were aware of the French Law concerning protection of health of athletes and fight against doping [11], whereas even less [8%] of our respondents were aware of the *Serbian Law on Prevention of Doping in Sports*. In regard to the *List of Prohibited Substances and Methods in Sport*, one out of ten (10.5%) partitipants in this study were informed about it, and much more than that, 46% of team doctors in Malawi (almost half of team doctors were GPs) were informed about the same [35]. One third of British GPs [9] knew that the *List of Prohibited Substances and Methods* was available in the British National Formulary (BNF), and 73% of French GPs [11] and 33% of Irish GPs [12] possesed such a list.

A great number (65%) of GPs from West Sussex answered correctly that inhaled salmeterol and inhaled corticosteroids are allowed in sports [9] with respect to our examinees (42% and 40.6%, respectively).

Lots of French GPs [11] stated that most records have been broken due to doping (83.5%) and that most of the great champions resort to doping (73%), whereas half of the Serbian GPs believe that most professional athletes used a doping agent at least once in their career.

In the present study, the majority of respondents agreed that doping has negative impact on athlete's health (92.4%) and, also, that athletes often do not know about the consequences which doping may have on their health (71.4%). Similarly, 87% of French GPs [10], and 80% of Senegalese doctors [13] believe that doping is a public health problem. A large number of French GPs [10] (83%) and Dutch GPs [8] (85%), consider themselves poorly trained in the field of doping, as well as 65% of Austrian GPs [14] and 69% of team doctors from Malawi [35]. A small number (9%) of Irish GPs [12] felt adequately trained to treat athletes, which is more than respondents in our study (2.5%). Furthermore, the Irish GPs [12] (86%) feel they need further training in relation to doping, and 93.1% of our examinees felt the same.

Conclusion

The results of this study showed that general practitioners in Vojvodina have insufficient knowledge about doping. Since all patients, including athletes, seek for their help, their knowledge should be on a higher level in order to avoid accidental doping. The respondents' answers showed small differences with respect to sex and age.

These results point to the need for further education of general practitioners on doping in sports. This should include organization of seminars and symposia for general practitoners on this and related topics, as well as lectures on doping in sports for medical students.

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THE ROLE OF OXIDATIVE STRESS MARKERS IN PREGNANCY INDUCED HYPERTENSION

ZNAČAJ MARKERA OKSIDATIVNOG STRESA KOD HIPERTENZIJE U TRUDNOĆI

Dragica DRAGANOVIĆ[1], Branka ČANČAREVIĆ ĐAJIĆ[1] and Dragica JOJIĆ[2]

Summary

Introduction. This article investigated the role of oxidative stress in the etiology of pregnancy induced hypertension. The aim of this study was to determine the degree of oxidative stress, and the level of thiobarbituric acid reactive substance in the blood of pregnant women with and without pregnancy induced hypertension and to correlate these parameters with clinical parameters during pregnancy and delivery. **Material and Methods.** This prospective study was performed at the *University Clinical Centre of the Republic of Srpska*. It included 200 pregnant women - 100 with pregnancy induced hypertension, and 100 healthy normotensive pregnant women between 28 to 40 weeks of gestation. **Results**. Pregnant women with pregnancy induced hypertension had significantly higher median levels of oxidative stress marker: thiobarbituric acid reactive substance of 36.7 μmol compared to the control group of 13.2 μmol. Pregnant women with pregnancy induced hypertension presenting with complications had significantly higher thiobarbituric acid reactive substance mean levels of 41.6 μmol compared with pregnant women without complications. The highest thiobarbituric acid reactive substance level of 43.9 μmol was found in pregnant women with Hemolysis, Elevated, Liver Ensimes, Low Plateles syndrome. **Conclusion.** The study showed that thiobarbituric acid reactive substance, as an oxidative stress marker, may be used in clinical practice in the assessment of the severity of complications and as an indicator for timely delivery in women with pregnancy induced hypertension. Further studies and a larger study sample of pregnant women with severe hypertension are necessary to confirm this conclusion.

Key words: Oxidative Stress; Hypertension, Pregnancy-Induced; Biomarkers; Pregnancy Complications; Pregnancy Outcome; Thiobarbituric Acid Reactive Substances

Sažetak

Uvod. Ispitivana je uloga oksidativnog stresa u etiologiji hipertenzije u trudnoći. Cilj ovog istraživanja bio je da se utvrdi stepen oksidativnog stresa, nivo tiobarbiturne kiseline - nivo reaktivnih supstancija tiobarbiturne kiseline u krvi trudnica sa hipertenzijom u trudnoći i korelacija tih parametara sa kliničkim parametrima toka trudnoće i porođaja. **Materijal i metode.** Istraživanje je sprovedeno kao prospektivna studija u Univerzitetskoj bolnici Kliničkog centra Banja Luka. Obuhvatilo je 200 trudnica, od kojih je bilo 100 trudnica ispitivane grupe, trudnice sa hipertenzijom, i 100 zdravih trudnica od 28. do 40. gestacijske nedelje. **Rezultati.** Trudnice sa hipertenzijom imale su znatno više srednje vrednosti markera oksidativnog stresa, vrednosti reaktivnih supstancija tiobarbiturne kiseline od 36,7 μmol u odnosu na trudnice kontrolne grupe od 13,2 μmol. Trudnice sa hipertenzijom izazvanom trudnoćom, koje su imale komplikacije, imale su znatno više srednje vrednosti reaktivnih supstancija tiobarbiturne kiseline od 41,6 μmol u odnosu na trudnice bez komplikacija. Najvišu vrednost od 43,9 μmol imale su trudnice sa sindromom hemolize povećanim jetrenim enzimima, niskim trombocitima. **Zaključak.** Ovo istraživanje pokazuje da bi se nivo reaktivnih supstancija tiobarbiturne kiseline, kao marker oksidativnog stresa, mogao primenjivati u kliničkom radu za procenu težine kliničke slike i vremena prekida trudnoća sa hipertenzijom, ali bi to svakako zahtevalo dodatno istraživanje sa većim brojem trudnica pre svega sa teškom hipertenzijom.

Ključne reči:oksidativni stres; trudnoćom indukovana hipertenzija; biomarkeri; komplikacije u trudnoći; ishod trudnoće; TBARS

Introduction

Pregnancy induced hypertension (PIH) is one of the most common and serious conditions in obstetrics. It puts in danger both the mother and the fetus [1–11]. Up to now, the etiology and pathogenesis of PIH are still unclear. Even today, the most significant cause of maternal death, apart from hemorrhages and infections, is pregnancy induced hypertension. According to many authors, PIH accounts for about 12% of total maternal mortality [11–13], whereas according to Shapiro it is about 11% [14]. The highest mortality is associated with the most severe complications such as Hemolysis, Elevated, Liver Ensimes, Low Pla-

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Abbreviations

PIH – pregnancy induced hypertension
TBARS – thiobarbituric acid reactive substance
CGO – *Clinic of Gynecology and Obstetrics*
CCBL – *Clinical Centre of Banja Luka*
DIC – dissmeninated intrascular coagulation
MDA – malondialdehyde

teles (HELLP) syndrome, placental abruption, eclampsia, edema and pulmonary embolism, kidneys failure, disseminated intravascular coagulation (DIC), and brain hemorrhages [15–17]. High maternal mortality and morbidity is followed by high fetal mortality and morbidity. Perinatal mortality is mostly associated with iatrogenic preterm birth, intrauterine growth retardation, as well as acute or chronic fetal stress [18, 19].

Nowadays, the role of oxidative stress in the etiology of PIH is being researched, and the results show that it may have a significant role in the development of preeclampsia, since it damages the endothelium of placenta, its vascularization and immune response [20–23]. In normal pregnancy, oxidative stress increases a bit, but there is no increase in free radicals. Recently, a great attention is being paid to lipid peroxidation, which actually is oxidative damage of lipids and increased formation of lipid peroxides, and its final product is malondialdehyde. Nowadays, malondialdehyde (MDA) is used in many expert researches as an oxidative stress marker, i.e. in the assessment of lipid peroxidation [24–27]. In accordance with the current literature findings, it is presumed that PIH is a condition of extremely increased oxidative stress [34]. This study was conducted to analyze the existence and degree of oxidative stress, i.e. lipid peroxidation, correlation of these parameters with clinical parameters of maternal and fetal outcomes in patients with pregnancy induced hypertension. Current medical literature does not contain a comprehensive analysis of all these parameters together.

The aim of this research was to determine levels of thiobarbituric acid reactive substance (TBARS) in blood of pregnant women with pregnancy induced hypertension and to analyze the correlation of TBARS parameters with clinical parameters during pregnancy and delivery in pregnancies with pregnancy induced hypertension.

Material and Methods

The study was performed at the Clinic of Gynecology and Obstetrics (CGO), of the University Hospital, Clinical Centre of Banja Luka (CCBL), according to current standards and regulations of the Ethics Committee. It included 200 pregnant women, 28 - 40 weeks of gestation, who were admitted to the Department of Perinatology of the CGO in Banja Luka. All pregnant women gave birth at the Maternity Ward of the CGO in accordance with medical indications. These were single pregnancies, accurately dated, with no other pathology. Test results were performed at the Institute of Laboratory Diagnosis and the Pediatric Clinic, of the University Hospital, CCBL in Banja Luka.

The pregnant women were divided into two groups: the study group of 100 pregnant women with the diagnosis of pregnancy induced hypertension based on clinical, laboratory and ultrasound evidence; the control group of 100 pregnant women without clinical, laboratory and ultrasound evidence of pregnancy induced hypertension.

During hospital stay, all the pregnant women were treated in the same way: they were advised to reduce salt intake by half, eat protein-rich food, have strict rest and oxygenation, blood pressure and respiratory rate were measured every 4 hours, unless unregulated blood pressure required more frequent measurement, filled in a blood pressure chart for each patient. PIH is classified as mild hypertension if it equals or is higher than 140/90 mmHg, and severe hypertension if it equals or is higher than 160/110 mmHg. Selection of pregnant women of the control group was performed after collection of data for the study group, and the groups were matched in terms of maternal age and gestational age as much as possible.

The 4 most common complications associated wit PIH were analyzed in all patients: HELLP syndrome, eclampsia, placental abruption and hemorrhages. After delivery, the most common maternal complications were: hemorrhages, infections, kidney damages, central nervous system damage, etc.

Fetal outcome was assessed based on the Apgar score at the first and fifth minute (0 to 7 is considered low - dead fetus, severe and mild asphyxia, and 8 to 10 is excellent).

Thiobarbituric acid reactive substance (TBARS) is an oxidative stress marker, which quickly and strongly reacts to malondialdehyde. Due to the simplicity of this oxidative stress marker, i.e. being a lipid peroxidation marker, it was used in this research. Blood tests were performed in all pregnant women, with PIH and the control group, for biomarkers of oxidative stress, TBARS levels, using the spectrophotometric method based on the concentration of MDA, product of lipid peroxidation, at the Institute of Laboratory Diagnosis and Pediatric Clinic, of the University Hospital, Clinical Centre of Banja Luka. TBARS is a very sensitive method for quantitative determination of lipid peroxidation. The principle of this screening method is that two molecules of thiobarbituric acid reactive substance react with one molecule of malondialdehyde, creating a compound, which is determined by spectrophotometry. Five milliliters of venous blood were collected from the cubital vein of each patient and put into a sterile tubes. TBARS levels were determined as an equivalent of MDA standard, in accordance with recommendations of the producer (Oxi Select TBARS Analisa Kit (MDA quantification), and results were acquired by spectrophotometry at 532 nm. In accordance with the recommendations, and minimal and maximal values, the results were divided into intervals for detailed analysis and comparison: low level interval up to 20 μmol, medium level interval from 20 to 40 μmol, and high level interval of TBARS over 40 μmol.

Results were analyzed and presented through descriptive statistics and adequate statistical tests using the SPSS analytic-statistical software kit. In normal division, t–test of independent samples was used, and in cases where the basic group significantly deviated

Table 1. Complications in the group with PIH and in the control group
Tabela 1. *Prisustvo komplikacija kod trudnica sa TIH* i kontrolne grupe*

		Complications/*Komplikacije*			
		Placental abruption *Abrupcija placente*	Eclampsia *Eklamsija*	HELLP	Hemorrhage *Hemoragija*
Group with PIH/*Trudnice sa TIH*	No/*Ne*	92	98	96	96
	Yes/*Da*	8	2	4	4
Control group/*Kontrolna grupa*	No/*Ne*	100	100	100	100

**TIH = Trudnoćom indukovana hipertenzija*

from the normal division, we applied the Mann-Whitney U-test. We used Hi-square (χ^2) and Fisher test, and contingency 2 x 2 table, and *Yates*' *correction was made* for continuity of smaller samples [28].

Results

All the gathered results of pregnant women with PIH and the control group of healthy pregnant women, were analyzed in detail in accordance with anthropometric parameters of pregnancy and delivery.

The mean gestational age at delivery in pregnant women with PIH was 261.9 days, whereas in the control group it was 273.9 days. The Mann-Whitney U test showed a high statistical significance p=0.00; a significantly lower gestational age was found in pregnant women with PIH in comparison to the control group. In regard to age distribution, hypertensive pregnant women were statistically significantly older compared to the control group, p=0,005; the mean age of hypertensive pregnant women was 31.2 years, whereas in the control group it was 28.9 years. In regard to parity, there was no statistically significant difference between the two groups: there was an equal number of primiparas and multiparas. Regarding the number of abortions, there was no statistically significant difference between the two groups: there was an equal number of pregnant women without abortions and pregnant women with one or more abortions in both groups. The fetal outcomes and Apgar scores at the first minute showed a high statistical significant difference, p=0.002; pregnant women with PIH delivered children of significantly lower Apgar score (0 - 7) in comparison to healthy pregnant women. The analysis of arterial tension, showed that the mean systolic pressure of pregnant women with PIH was 160 mmHg, and, in the control group it was 120 mmHg, whereas the mean diastolic pressure of pregnant women with PIH was 110 mmHg, and 70 mmHg in the control group. The percentage of pregnant women with severe PIH was 59%, whereas 49% had mild PIH.

Complications during pregnancy and delivery in pregnant women with PIH and the control group

Table 1 shows the most common complications during pregnancy and delivery. In pregnant women with PIH, there was a total of 18 complications present in 14 women. Four women had 2 complications each. In pregnant women with PIH, there were 8 with placental abruption, 4 with hemorrhages, 2 with eclampsia and 4 with HELLP syndrome. In the control group, on the other hand, there were no complications whatsoever.

We analyzed the presence of complications during pregnancy and delivery related to the height of blood pressure. Out of 100 women with PIH, 59 women had severe PIH, and 41 had mild PIH.

Table 2 shows complications in patients with mild and severe PIH. There were four pregnant women having two complications each. The Fisher test showed a high statistically significant difference (p = 0.007) related to the presence of complications in pregnant women with PIH, those with mild and severe PIH. Pregnant women with severe PIH, presented with a significantly larger number of complications.

The analysis of the maternal outcome showed that there were no lethal outcomes in either group, being the worst complication after delivery. There were no complications after delivery in the control group, whereas there were 5 women with PIH with complications.

TBARS – an oxidative stress marker in pregnant women with PIH and in the control group

We compared the levels of oxidative stress marker - TBARS, byproduct of lipid peroxidation in pregnant women with PIH, and in the control group. The Mann-

Table 2. Complications in the group with PIH
Tabela 2. *Prisustvo komplikacija kod pacijentkinja sa TIH**

Complications *Komplikacije*	Group with PIH/*Trudnice sa TIH*		Total *Ukupno*
	Mild/*Blaga*	Severe/*Teška*	
No/*Ne*	40	46	86
Yes/*Da*	1	13	14
Total/*Ukupno*	41	59	100

**TIH - trudnoćom indukovana hipertenzija*

Table 3. TBARS levels in the group with PIH and and in the control group
Tabela 3. *Vrednosti TBARS* kod trudnica sa TIH** i kontrolne grupe*

Study group/*Ispitivane grupe*	N	Min.	Max.	Range	Median	Mean	SD
Group with PIH/*Trudnice sa TIH*	100	21.70	61.70	40.00	36.7500	36.6860	8.29359
Control group/*Kontrolna grupa*	100	9.10	25.80	16.70	13.2000	13.4220	2.74493
Total/*Ukupno*	200	9.10	61.70	52.60	21.7500	25.0540	13.18904

**TBARS – reaktivne supstancije tiobarbiturne kiseline; **TIH - trudnoćom indukovana hipertenzija*

Table 4. TBARS levels in the group with PIH in regard to the height of blood pressure
Tabela 4. *Vrednosti TBARS* kod trudnica sa TIH** prema visini krvnog pritiska*

Group with PIH/*Trudnice sa TIH*	N	TBARS ($\bar{x}$)	p
Mild PIH/*Blaga TIH*	41	30.3000	0.000
Severe PIH/*Teška TIH*	59	41.1237	
Total/*Ukupno*	100	36.6860	

**TBARS – reaktivne supstancije tiobarbiturne kiseline; **TIH - trudnoćom indukovana hipertenzija*

Table 5. TBARS levels in the group with PIH in comparison to pregnancy and delivery complications
Tabela 5. *Vrednosti TBARS* kod trudnica sa TIH** u odnosu na komplikacije trudnoće i porođaja*

Complications/*Komplikacije*	N	TBARS ($\bar{x}$)	p
No/*Ne*	86	35.8826	0.008
Yes/*Da*	14	41.6214	
Total/*Ukupno*	100	36.6860	

**TBARS – reaktivne supstancije tiobarbiturne kiseline; TIH - trudnoćom indukovana hipertenzija*

Whitney U test showed a high statistically significant difference in levels of TBARS in pregnant women with PIH (Md = 36.75, n = 100) in comparison to the control group (Md = 13.20, n = 100), U = 6.000, z = -12.203, p = 0.000 with r = 0.863. Pregnant women with PIH had significantly higher levels of TBARS oxidative stress marker. The mean value was 36.7 μmol in PIH group, in comparison to 13.2 μmol in the control group. The highest TBARS level was found in women with PIH and it was 61.7 μmol, whereas in the group of healthy pregnant women, it was 25.8 μmol. The lowest level of TBARS in women with PIH was 21.7 μmol, and 9.10 μmol in the control group, which is highly statistically significant (**Table 3**).

We also analyzed the levels of TBARS related to the height of blood pressure in women with mild and severe PIH. The Mann-Whitney U test showed a high statistically significant difference in TBARS levels in women with mild PIH (Md = 29.20, n = 41) and women with severe PIH (Md = 41.80, n = 59), U = 272.000, z = -6.571, p = 0.000 with r = 0.657. The pregnant women with severe PIH had significantly higher levels of TBARS in comparison to pregnant women with mild PIH: TBARS in mild PIH - 30.30 μmol, and in severe PIH – 41.12 μmol (**Table 4**).

TBARS - an oxidative stress marker in pregnant women with PIH and in the control group related with complications during pregnancy and delivery

The Mann-Whitney U test revealed a high statistically significant difference in TBARS levels in women with PIH presenting with complications (Md = 35.95, n = 86) and women with PIH without complications (Md = 43.35, n = 14), U = 334.000, z = -2.663, p = 0.008 with r = 0.266. The pregnant women with PIH accompanied with complications, had significantly higher levels of TBARS; the mean TBARS level was 41.6 μmol, i.e. in high level interval of TBARS, in comparison to pregnant women without complications (**Table 5**).

The analysis of the most common complications in pregnant women with PIH and their comparison with the mean levels of TBARS showed the highest level of 43.9 μmol in pregnant women with HELLP syndrome. All pregnant women with complications had mean level of TBARS in the high level interval, i.e. over 40 μmol (**Table 6**). We got positive correlation of these pregnancy and delivery parameters with levels of TBARS.

Discussion

Former studies dealing with oxidative stress indicate that pregnancy is a state of physiological, slightly increased oxidative stress in comparison to healthy non-pregnant women [20, 21, 29, 30]. Oxidative stress may have a significant role in the development of preeclampsia since it starts damaging the endothelium of placenta, vascularization, and immune response [24–27].

Complications associated with PIH are very difficult and severe; they can put at risk the maternal and the fetal life. The gathered results show that there were no complications during pregnancy and delivery in the

Table 6. TBARS mean levels in the group with PIH in comparison to complications
Tabela 6. *Srednje vrijednosti TBARS* kod trudnica sa TIH** u odnosu na komplikacije*

Complication/*Komplikacije*	N	TBARS ($\bar{x}$)
Placental abruption/*Abrupcija placente*	8	40.8125
Eclampsia/*Eklamsija*	2	40.9500
HELLP	4	43.9250
Hemorrhage/*Hemoragija*	4	421500
Total/*Ukupno*	18	36.6860

**TBARS – reaktivne supstancije tiobarbiturne kiseline; **TIH - trudnoćom indukovana hipertenzija*

control group. On the other hand, in the group of pregnant women with PIH, including a total of 100 pregnant women, there were 18 complications in 14 women, 4 women had 2 complications each. Most complications referred to placental abruption - 8, hemorrhages - 4, HELLP-syndrome – 2, and eclampsia - 2. Our results are in agreement with literature data [31–33]. The latest findings on the pathogenesis and etiology of PIH [34], indicate that there are different views on the beginning of the disease, severity of symptoms and complications. The analysis of the occurrence of complications in regard to the height of blood pressure showed a high statistically significant difference in the group of pregnant women with mild or severe PIH. The pregnant women with severe PIH had significantly higher number of complications – a total of 17 in 14 pregnant women, while 4 women with severe PIH had 2 complications each. Only 1 pregnant woman with mild PIH presented with 1 complication. These results are also in agreement with literature data [34–37].

The analysis of maternal outcome in women with PIH and in the control group shows that there was no statistically significant difference between the examined groups. In the group of women with PIH, 5 had complications, whereas in the control group there were no complications whatsoever. It is important to say that there were no cases of maternal lethal outcome, which is the worst complication after delivery. Although the difference was not statistically significant, the Fisher test, p=0.059, value was at the limit of statistical significance between women with PIH and the control group. The obtained results indicate that the patients were provided with good prenatal diagnosis, pregnancy and delivery management, as well as timely delivery.

In the control group, the mean systolic pressure was 120 mmHg, and diastolic 70 mmHg. In the group of women with PIH, the mean systolic pressure was 160 mmHg, and diastolic 110 mmHg. The highest systolic pressure was measured in women with PIH - 220 mmHg, and diastolic 160 mmHg.

In regard to the levels of TBARS, as an oxidative stress marker, there was a high statistical difference of $p < 0.01$. Pregnant women with PIH had a significantly higher mean value of TBARS in comparison to the control group. The highest level of TBARS in the group with PIH was 61.7 μmol, whereas in the control group it was 25.8 μmol. The lowest level of TBARS in women with PIH was 21.7 μmol, and in the control group it was 9.10 μmol. The mean level of TBARS in the control group was 13.2 μmol, whereas in women with PIH, it was 36.7 μmol. The obtained results of this research clearly indicate to extremely increased oxidative stress in PIH, i.e. we proved increased level of lipid peroxidation and hence the possibility of its prevention in pregnancy.

We also analyzed the mean levels of TBARS, which proved to be extremely high in hypertensive pregnant women, especially in women with the most severe PIH. Previous analyses showed that the most severe complications and the highest number of complications were found in women with severe preeclampsia (PIH). TBARS may be used as a preclinical parameter in hypertensive pregnant women for the purpose of timely diagnosis and in the best maternal and fetal interests.

The analysis of TBARS and complications in the examined groups shows a high statistically significant difference (p=0.008) of TBARS levels in pregnant women with PIH with complications, and in pregnant women with PIH without complications. The mean level of TBARS in pregnant women with PIH with complications was 41.62 μmol. The pregnant women with PIH and accompanied complications had significantly higher levels of TBARS in comparison to pregnant women with PIH without complications. Our results show that the highest level of TBARS of 43.92 μmol was found in pregnant women with HELLP syndrome, which is one of the most severe complications of PIH, whereas women with hemorrhages come next with 42.15 μmol.

Conclusion

In conclusion, our research shows that pregnant women with pregnancy induced hypertension have extremely high levels of oxidative stress and lipid peroxidation. Pregnant women with pregnancy induced hypertension and the most severe complications during pregnancy and delivery had extremely high levels of thiobarbituric acid reactive substance, and the highest levels were found in pregnant women with Hemolysis, Elevated, Liver Ensimes, Low Plateles syndrome.

This study shows that thiobarbituric acid reactive substance, as an oxidative stress marker, may be used in clinical practice in the assessment of the severity of complications and as an indicator for timely delivery in women with pregnancy induced hypertension. Surely, further studies with larger study samples of pregnant women with severe hypertension are needed to confirm this conclusion.

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CASE REPORTS
PRIKAZI SLUČAJEVA

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Case report
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BILATERAL SIMULTANEOUS ANTERIOR CRUCIATE LIGAMENT RUPTURE: A CASE REPORT AND LITERATURE REVIEW

ISTOVREMENO OBOSTRANO KIDANJE PREDNJEG UKRŠTENOG LIGAMENTA: PRIKAZ SLUČAJA I PREGLED LITERATURE

Miroslav MILANKOV[1,2], Miodrag VRANJEŠ[2], Zlatko BUDINSKI[1,2] and Mile BJELOBRK[2]

Summary
Bilateral simultaneous anterior cruciate ligament ruptures are very rare and only a few cases have been previously reported in the orthopedic literature. We present a case of simultaneous bilateral ACL tears in an actor who sustained injuries after landing from a guitar jump during a theatre performance. The patient underwent a two-stage bilateral ACL reconstruction using bone-patellar-tendon-bone *autograft.*
Key words: Anterior Cruciate Ligament; Anterior Cruciate Ligament Reconstruction; Rupture; Rupture, Spontaneous; Knee Injuries; Bone-Patellar Tendon-Bone Grafts

Sažetak
Istovremeno obostrano kidanje prednjeg ukrštenog ligamenta kolena je retko i samo nekoliko slučajeva ove povrede je do sada prikazano u ortopedskoj literaturi. Prikazujemo slučaj istovremene, obostrane povrede prednjeg uklrštenog ligamenta kolena koji je nastao pri doskoku za vreme „gitarskog skoka" tokom pozorišne predstave, koji je lečen rekonstrukcijom predneg ukrštenog ligamenta kost−ligament čašice−kost kalemom u dva vremena.
Ključne reči: prednji ukršteni ligament; rekonstrukcija prednjeg ukrštenog ligamenta; ruptura; spontana ruptura; povrede kolena; kost-ligament čašice-kost kalem

Introduction

Anterior cruciate ligament (ACL) injuries are rather common in adolescents and young adults. They are mostly associated with recreational, competitive and professional sports activities. The incidence of ACL injuries in general population ranges from 0.01 – 0.08% inhabitants/year, but it is much higher in athletes playing multidirectional sports [1]. The overall incidence of bilateral ACL ruptures at two separate times is 2.4 – 6% [2–4]. Simultaneous bilateral ACL ruptures are rare, and only a few cases have been previously reported in the orthopedic literature (**Table 1**) [5–10].

Looking into these case reports, many questions have arisen about the mechanism of injury and the optimal treatment. The aim of this study is to present a case of simultaneous bilateral ACL tears in an actor who was injured during a theatre performance after landing from a guitar jump. He was treated surgically with staged bilateral ACL reconstruction using bone-patellar-tendon-bone (BTB) autograft.

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Case Report

A 30-year-old male professional actor, 174 cm tall and weighing 74 kg, body mass index (BMI) 24.4, with no significant past medical or family history of collagen disorders, was injured during a theatre performance on a punk-rock concert while playing the bass guitar. Prior to the injury, he had a very exhausting period with very little sleep (3 – 5 hours per night). During the performance, he was wearing a very heavy costume, and ill-fitting, untied shoes. The floor was wet and made of rubber, with many cables lying around. After he jumped high in the air, with his left leg extended to the front and his right leg fully bended behind, he hit his right buttock **(Figure 1)**, landed back on the floor, and heard a 'pop' sound in both knees. He felt severe pain in both knees, and could not get up. Ultimately, he felt like his legs were not his own, and when he attempted to walk, his every step was very unstable – his legs felt like rubber. At that point he decided to go to the

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Abbreviations

ACL – anterior cruciate ligament
BMI – body mass index
MCL – medial collateral ligament
EC – emergency center
NWI – notch width index
PTS – posterior tibial slope

Figure 1. Bilateral ACL injury occurred during a theatre performance, after landing from a 'guitar jump'; the left knee is extended, the left hip is flexed, and both right hip and knee are flexed
Slika 1. *Istovremeno obostrano kidanje prednjeg ukrštenog ligamenta je nastalo za vreme predstave u pozorištu prilikom doskoka „gitarskog skoka", pri čemu je levo koleno bilo ispruženo, levi kuk savijen, dok su desno koleno i kuk bili savijeni*

Emergency Center (EC). In the EC, he had mild bilateral knee effusions, but there was no collateral instability, neither in full extension nor at 30° flexion, but the medial collateral ligament (MCL) femoral attachment was tender to palpation. Positive Lachman test with soft endpoint was present in both knees. The posterior drawer test was negative. His neurovascular status was intact. Bilateral knee X-rays (anteroposterior and lateral) were normal. Magnetic resonance imaging of both knees revealed the following: left knee - complete ACL tear, partial tear of medial collateral complex (MCL and medial part of the retinaculum at the femoral attachment grade I - II), discrete lesion of the medial meniscus, without a real meniscal tear and discrete impaction lesion of the posterior, lateral tibial plateau **(Figures 2a, b and c)**; right knee - partial tear of ACL located in the proximal third, partial tear of MCL grade I - II, complex tear of the posterior horn of the medial meniscus, partial lesion of the posterolateral corner and 'kissing' impaction lesions of the lateral femoral condyle and posterior medial tibial plateau **(Figures 3a, b and c)**. The posterior tibial slope in both knees was 8 degrees, the notch width index (NWI) in both knees was 0.22 (NWI is defined as the ratio of the width of the intercondylar notch (18 mm) to the width of the distal femur at the popliteal groove (79 mm)).

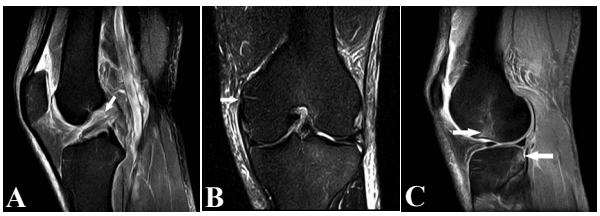

Figure 2. Magnetic resonance images of the left knee
Slika 2. *Magnetno-rezonantni prikaz levog zgloba kolena*
a) Sagittal T2w fat-saturated image of the left knee demonstrates a complete ACL tear (arrow)
a) Sagitalni T2wFS tomogram levog zgloba kolena prikazuje kompletnu rupturu (strelica) prednjeg ukrštenog ligamenta
b) Coronal T2w fat-saturated image of the left knee demonstrates a partial thickness MCL tear at the level of the femoral attachment (arrow)
b) Koronalni T2wFS tomogram levog zgloba kolena prikazuje parcijalnu rupturu unutrašnjeg bočnog ligamenta u nivou femoralnog pripoja ligamenta (strelica) unutrašnjeg bočnog ligamenta
c) Sagittal T2w fat-saturated image of the left knee showing a minor bone impaction at the posterior lip of the lateral tibial plateau (arrow)
c) Sagitalni T2wFS tomogram levog zgloba kolena prikazuje minimalnu koštanu impakciju u nivou posteriornog ruba lateralnog platoa tibije (strelica)

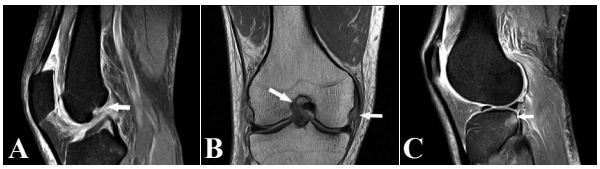

Figure 3. Magnetic resonance images of the right knee
Slika 3. *Magnetno-rezonantni prikaz desnog zgloba kolena*
a) Sagittal 2w fat-saturated image of the right knee showing a complete ACL tear (arrow)
a) Sagitalni T2wFS tomogram desnog zgloba kolena prikazuje kompletnu rupturu prednjeg ukrštenog ligamenta (strelica)
b) Coronal Proton density weighted image of the right knee showing partial medial collateral complex lesion (arrow)
b) Koronalni PDw tomogram desnog zgloba kolena prikazuje parcijalnu kompleksnu leziju medijalnog kolateralnog kompleksa (strelica)
c) Sagittal T2w fat-saturated image of the right knee demonstrates recent „kissing" bone impactions at the anterior weight-bearing portion of the lateral femoral condyle, and at the posterior lip of the tibial plateau (arrows)
c) Sagitalni T2wFS tomogram desnog zgloba kolena prikazuje nedavnu „kissing" koštanu impakciju u anteriornom aspektu noseće artikularne površine lateralnog kondila femura i na posteriornom rubu tibijalnog platoa (strelice)

Table 1. A review of cases with simultaneous bilateral ACL rupture reported in the international literature
Tabela 1. *Pregled objavljenih slučajeva istovremenog obostranog kidanja prednjeg ukrštenog ligamenta*

	Gender	Age	Mechanism	INW (mm)	DFW (mm)	(NWI)	Magnetic resonance images	ACL reconstruction	Additional operation
Maywood and Hechtman *Am J Knee Surg 1995*	F	15	A cheerleader jumped from ground level, height of approximately 30 inches, and landed with her feet 6 to 10 inches apart.	L 12 R 16	72 77	0.15 0.22	Right knee: ACL rupture, bone contusion lat tibial plateau, posterior horn med men, sprain of the deep fibres MCL Left knee: ACL rupture sprain MCL, capsular separation of posterior horn MM	Right knee: 2 weeks after injury, BTB autograft Left knee: 3 months after injury, BTB autograft	
Sanchis-Alfonso and Tintó-Pedrerol; *Knee Surg Sports Traumatol Arthrosc 2000*	F	26	Skiing injury. She had lost her balance, fell backward with sudden bilateral internal tibial rotation with hyperflexed knees, the hips being below the knees, and without release of the ski boot bindings.	R 17 L 16	- -	0.23 0.25	-	staged bilateral ACL reconstruction, quadrupled STG autografts	
Stilger *Athletic Therapy Today 2000*	F	21	A gymnast fell and landed with bilaterally flexed knees and a simultaneous valgus stress to both knees and subsequent varus stress to the left knee.	-	-	-	Right knee: a torn ACL, a grade 2+ MCL tear, a tear of posterior horn of the lateral meniscus, and an incomplete inferior margin tear of the medial meniscus. Left knee: a torn ACL, a grade 2 LCL tear, and anterior horn tear of the medial meniscus.	7 weeks after injury simultaneous bilateral ACL reconstruction, BTB autograft.	Right knee: debridement of a cyclops lesion and a partial meniscectomy of the medial meniscus
Tifford and Jackson *Arthroscopy 2001*	F	17	A cheerleader, her both lower extremities were widely abducted and the knees fully extended. During descent, her limbs were adducted back to neutral as she landed on her feet. The patient landed awkwardly, her feet slid on the gravel and she described her knees "buckling inwards" producing a valgus force at both knees.	L 14 R 14	83 83	0.16 0.16	Both knees ACL rupture medial meniscal tears involving the posterior horns, lateral femoral condyle and lateral tibial plateau bone contusion, and large joint effusions.	9 weeks after injury simultaneous bilateral ACL reconstruction quadrupled STG autograft.	
Lukas and Baue *Aktuelle Traumatologie 2004*	M	50	A plumber fell into a channel with a fully extended knee.	-	-	-	Right knee: ACL rupture and lesion medial mensicus Left knee: only ACL rupture	2 days after injury ACL reconstruction not done Right knee: 11 weeks after injury, BTB autograft Left knee: 5 month after injury BTB autograft	
	M	24	During landing, a skier sat on the rear of his skis.	-	-	-	Right knee: ACL rupture and lesion medial mensicus Left knee: only ACL rupture		
Saadat et al. *Orthopedic Reviews 2014*	F	23	Bilateral knee injuries with a fall while skiing.	-	-	-	Both knees mid-substance ACL ruptures, partial thickness radial tear of the posterior horn of the lateral menisci, MCL sprain, subchondral fractures of the lateral femoral condyles, and interstitial tears of the proximal gastrocnemius muscle and left knee non-displaced fibular head fracture of the left fibular styloid process	Left knee: one week after initial injury tibial anterior allograft Right knee: 8 weeks post-injury, tibial anterior allograft	Right knee arthroscopy with debridement of a small Cyclops lesion.
our case	M	30	A guitar jump. After he had jumped high in the air, with his left leg extended to the front and his right leg fully bended behind, he hit his right buttocks, landed back on the floor.	18	79	0.22	Left knee: complete tear of ACL, partial tear of medial collateral complex (MCL and medial part of the retinaculum at the femoral insertion grade I-II), discrete lesion of the medial meniscus, without a real meniscal tear and discrete impaction lesion of the posterior, lateral tibial plateau. Right knee: partial tear of ACL located in the proximal third, partial tear of MCL grade I-II, complex tear of the posterior horn of the medial meniscus, partial lesion of the posterolateral corner and "kissing" impaction lesions of lateral femoral condyle and posterior medial tibial plateau.	Right knee: 6 weeks post-injury, BTB autograft Left knee: 4 month after injury right knee BTB autograft	

INW – Intercondylar notch width/*širina interkondilarnog useka butne kosti*, DFW – Distal femoral width/*širina donjeg okrajka butne kosti,* NWI – notch width index/*odnos između širine interkondilarnog useka i širine donjeg okrajka butne kosti*

The surgery was delayed for 6 weeks during which the patient had physical therapy. The first surgery involved right ACL reconstruction with bone-patellar tendon-bone BTB autograft fixed using titanium interference screws, and medial meniscectomy of the right knee. Four months later, ACL reconstruction of the left knee was done. One year after the second surgery, the patient started playing recreational soccer again. Five years later, the patient has stable knees with full range of motion, good quadriceps femoris muscle strength, bilaterally, and negative Lachman and pivot shift tests. The patient is still an actor and plays recreational soccer without any limitations.

Discussion

In the period from January 2003 to December 2014, 3.400 ACL reconstruction surgeries were performed at the Department of Orthopaedic Surgery and Traumatology, Clinical Center Vojvodina. Among these 3.400 patients, 73 patients (2.14%) had bilateral knee surgery. Only one patient (0.029%) had bilateral ACL injury requiring ACL reconstruction surgery, which is in concordance with the existing literature data describing this type of injury-only in 7 cases [5–10]. Saadat et al. [10] reported that the majority of experienced sports medicine specialists have never seen a patient with bilateral simultaneous ACL ruptures, and of those most have only seen a single case.

The risk factors associated with ACL injuries are multiple and can be divided into intrinsic and extrinsic, or those that can or cannot be altered. The extrinsic risk factors, which can be altered, include environmental conditions such as the playing surface, footwear, weather conditions and type of sport [11]. Our patient's injury occurred after landing a jump onto a wet surface while wearing untied shoes. The intrinsic risk factors can be divided into anatomic, hormonal, neuromuscular and familial [12]. Numerous anatomic variables include intercondylar notch width, posterior tibial slope, increased BMI, landing kinematics, female sex, and anatomic alignment [11]. Anatomic differences between males and females include Q-angles, pelvis width, effects of hormones (primarily estrogen), the incidence of injuries and ligament strength, neuromuscular and biomechanical factors, previous knee injuries and age. All of them may be potential risk factors for ACL injury [12]. Simultaneous ruptures of bilateral ACLs have been described in 4 females, mean age 20 (range - 15 – 26) and two males aged 50 and 25 years. Our patient was a 25-year-old male, which fits into the reported age group.

Anatomic variations of both proximal tibia and intercondylar notch are considered risk factors for a primary ACL injury [13]. Posterior tibial slope (PTS) is an angle between the articular surface of the tibia in the knee joint and tibial axis, and it normally ranges between 7 and 10 degrees [14]. A greater PTS with axial loading generates a greater anterior translation of tibia [15]. As the ACL is the main stabilizer of this movement, this results in its over-tightening that leads to increased internal tibial rotation which generates a greater force on ACL [16]. DeJour and Bonnin [15] looked into lateral knee X-rays and found that for every 10 degree increase in PTS, there was a 6 mm increase in the anterior tibial translation during a single-legged stance. Webb et al. [17] showed that patients who underwent ACL reconstruction surgery and had increased PTS (of 9.9° on average) sustained more re-ruptures of the ACL graft and ACL ruptures of the contralateral knee in comparison to the patients with less steep PTS (8.5°). PTS in our patient was 8 degrees and no comparison has been made to other reported cases since this information was not provided.

Shelbourne et al. [18] examined the relationship between intercondylar notch stenosis and noncontact ACL injuries and concluded that the notch of 15 mm width or less, puts a person at higher risk for contralateral ACL tearing than the notch of 16 mm width or wider. Souryal and Freeman [19] reported that an NWI of less than 0.18 in women is significant for intercondylar notch stenosis, and that athletes with a stenotic intercondylar notch are 26 times more likely to sustain a noncontact ACL tear than those with a normal sized notch. On the other hand, Teitz et al. [20] suggested that the NWI alone is not critical in patients with an unilateral ACL tear. Intercondylar notch width less than 16 mm was reported in three females with simultaneous bilateral ACL ruptures [5, 8, 9]. Our patient did not have a stenotic intercondylar notch.

The ACL injury mechanisms have been investigated through a variety of research models, including retrospective interviews, medical report reviews, and video studies. However, due to multiple study limitations, it is not possible to directly determine the mechanism of ACL injury [21]. Most reported injury mechanisms involve noncontact mechanisms during weight-bearing conditions, such as landing from a jump, sudden deceleration while running, with or without a change in direction and the knee movements in multiple planes [22]. In many cases patients reported that the injury happened when the injured foot was in contact with the ground, the knee went into valgus with either internal or external rotation, while the knee was in hyperextension or shallow flexion [23]. In three patients with simultaneous injury of bilateral ACL, the injury mechanism involved landing with both knees extended. Two girls were cheerleaders [5, 9], and one man was a plumber who fell into a channel [6]. Stilger [7] reported a female gymnast who fell and landed bilaterally on flexed knees. Also, two females and one male sustained a simultaneous bilateral ACL injury while downhill skiing, while both hips and knees were maximally flexed [6, 8, 10]. Bilateral ACL injury in our case occurred during a theatre-performance, while landing from a jump with the left knee being extended and left hip being flexed and both right hip and knee being flexed ('guitar jump'). Based on the tenderness along the femoral insertion of MCL and magentic resonance image (MRI) findings which showed partial MCL tear, it can be assumed that the knee went into valgus during landing with rotation. Just looking into the location of the bone bruise, one cannot comment on the mechanism of the injury.

The final challenge associated with simultaneous bilateral ACL rupture is the treatment approach. Tifford

and Jackson [9] and Stilger et al. [7] showed that simultaneous bilateral ACL reconstructions are more time- and cost-effective compared to staged bilateral ACL reconstructions. In our opinion, simultaneous ACL reconstruction of both knees is overly aggressive; standing on both feet post-operatively is difficult and mobility is reduced with a great risk for arthrofibrosis and tromboembolia, so we opted for 2-stage bilateral ACL reconstruction [5, 6, 8, 10]. The optimal timing for reconstructive ACL surgery varies among authors. Tifford and Jackson [9] performed simultaneous bilateral ACL reconstruction 7 weeks after the initial injury, while Stilger et al. [7] did it after 9 weeks, when the swelling subsided and the range of motion and muscle strength were almost back to normal. In our case, the first ACL reconstruction was done 6 weeks after the injury, which is longer compared to other authors who performed staged reconstruction within the first two weeks from the injury [5, 6, 10]. The second ACL reconstruction was done 4 months after the injury, which is similar to other reported time frames (from 2 to 5 months) [5, 6, 10].

In addition to the timing and surgery planning, there is a wide choice of grafts used for ACL reconstruction. Of those who preferred performing a simultaneous reconstruction, Tifford and Jackson [8] used a hamstring autograft and Stilger et al. [7] used a bone-patellar tendon-bone autograft. Of those who opted for a staged reconstruction, Maywood and Hechtman [5], and Lukas and Bauer [6], used bone-patellar tendon-bone autograft, Sanchis-Alfonso and Tintó-Pedrerola [8] used hamstring autograft, and Saadat et al. [10] used an allograft.

Conclusion

Simultaneous bilateral anterior cruciate ligament rupture is a very rare injury, which occurs through noncontact mechanisms, such as landing from a jump while the knees are hyperextended or hyperflexed and in valgus position at the same time. Although simultaneous bilateral anterior cruciate ligament reconstruction has its advantages, being time- and cost-effective, in our opinion staged anterior cruciate ligament reconstruction is less aggressive and better tolerated by the patients.

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Case report
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POSTERIOR WALL MYOCARDIAL INFARCTION: A CASE REPORT

INFARKT ZADNJEG ZIDA MIOKARDA: PRIKAZ SLUČAJA

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Summary

Introduction. Acute myocardial infarction is a clinical manifestation of coronary disease which occurs when a blood vessel is narrowed or occluded in such a way that it leads to irreversible myocardial ischemia. ST segment depression in leads V1–V3 on the electrocardiogram points to the anterior wall ischemia, although it is actually ST elevation with posterior wall myocardial infarction. In the absence of clear ST segment elevation, it may be overlooked, leading to different therapeutic algorithms which could significantly affect the outcome. **Case report.** A 77 year-old female patient was admitted to the Coronary Care Unit due to prolonged chest pain followed by nausea and horizontal ST segment depression on the electrocardiogram in V1–V3 up to 3 mm. ST segment elevation myocardial infarction of the posterior wall was diagnosed, associated with the development of initial cardiogenic shock and ischemic mitral regurgitation. An emergency coronarography was performed as well as primary percutaneous coronary intervention with stent placement in the circumflex artery, the infarct-related artery. Due to a multi-vessel disease, surgical myocardial revascularization was indicated. **Conclusion.** Posterior wall transmural myocardial infarction is the most common misdiagnosis in the 12 lead electrocardiogram reading. Routine use of additional posterior (lateral) leads in all patients with chest pain has no diagnostic or therapeutic benefits, but it is indicated when posterior or lateral wall infarction is suspected. The use of posterior leads increases the number of diagnosed ST segment elevation myocardial infarctions contributing to better risk assessment, prognosis and survival due to reperfusion therapy.

Key words: Myocardial Infarction; Electrocardiography; Diagnosis; Coronary Angiography; Risk Assessment; Myocardial Revascularization; Myocardial Reperfusion; Diagnostic Errors; Prognosis

Sažetak

Uvod. Akutni infarkt miokarda je klinički oblik koronarne bolesti koja nastaje pri suženju koronarnog krvnog suda ili njegovoj okluziji koji dovode do nastanka ireverzibilne ishemije miokarda. Elektrokardiografski registrovana depresija ST segmenta u odvodima V1–V3 ukazuje na ishemiju prednjeg, iako se zapravo radi o ST eleviranom infarktu miokarda zadnjeg zida. Izostanak jasne elevacije ST segmenta doprinosi da se on previdi, što vodi drugačijem terapijskom algoritmu koji u značajnoj meri može da utiče na ishod. **Prikaz slučaja.** Pacijentkinja starosti 77 godina primljena je u jedinicu intenzivne kardiološke nege zbog tegoba u vidu prolongiranog bola u grudima, praćenog mučninom, i elektrokardiografski registrovane horizontalne denivelacije ST segmenta u V1–V3 do 3 mm. Postavljena je dijagnoza ST eleviranog infarkta miokarda zadnjeg zida koji se komplikovao razvojem incipijentnog kardiogenog šoka i ishemijske mitralne regurgitacije. Urađena je urgentna koronarografija i primarna perkutana koronarna intervencija sa implantacijom stenta u *ramus circumfexus* koja je bila infarktna arterija, a zbog višesudovne koronarne bolesti indikovana je hirurška revaskularizacija miokarda. **Zaključak.** Transmuralni infarkt miokarda zadnjeg zida je najčešće previđena dijagnoza prilikom tumačenja 12-kanalnog elektrokardiograma. Rutinska upotreba dodatnih posteriornih (lateralnih) odvoda kod svih bolesnika sa bolom u grudima nema dijagnostički i terapijski benefit, ali je indikovana kod sumnje na infarkt posteriornog ili lateralnog zida. Upotreba posteriornih odvoda povećava broj dijagnostikovanih ST eleviranih infarkta miokarda, što doprinosi boljoj proceni rizika, prognozi i preživljavaju zahvaljujuću reperfuzionoj terapiji.

Ključne reči: infarkt miokarda; elektrokardiogram; dijagnoza; koronarna angiografija; procena rizika; revaskularizacija miokarda; reperfuzija miokarda; greške u dijagnostikovanju; prognoza

Introduction

Acute myocardial infarction is a clinical manifestation of coronary disease which occurs when a blood vessel is narrowed or occluded in such a way that it leads to irreversible myocardial ischemia [1, 2]. Acute ST elevation myocardial infarction (STEMI) is diagnosed based on clinical presentation, new-onset persistent elevation registered on electrocardiogram (ECG) in at least two leads i.e. transitory ST elevation longer than 20 minutes, and positive cardiospecific enzymes [3]. In certain cases chest pain along with the signs of ischemia on ECG and cardiospecific enzymes can have origins other than coronary, like in myopericarditis [4]. Equivalents to ST elevation myocardial infarction represent a great diagnostic problem. They are: ST segment elevation in aVR lead indicating left main coronary artery stenosis, the left bundle branch block in myocardial infarction, and posterior myocardial infarction [5, 6]. In

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Abbreviations

ECG	– electrocardiogram
CCU	– Coronary Care Unit
STEMI	– ST elevation myocardial infarction
pPCI	– primary percutaneous coronary intervention
RCx	– circumflex artery
LAD	– left anterior descending artery
PDA	– posterior descending artery
OM	– obtuse marginal
RCA	– right coronary artery
MI	– myocardial infarction

these cases it is rather difficult to diagnose transmural myocardial infarction because their electrocardiographic manifestations do not show clear signs of lesions. The existence of ST depression in $V_1 – V_3$ leads on ECG strictly points to the anterior wall myocardial ischemia although it is actually the ST elevation myocardial infarction [3, 7]. In this way misdiagnosis can be made leading to inadequate reperfusion therapeutic algorithms which may significantly influence the outcome and cause complications. One of the most common complications, especially of the posterior wall myocardial infarction, is significant ischemic functional mitral regurgitation [9].

This paper presents a case of a patient admitted due to acute myocardial infarction of strictly posterior region which was complicated by the development of cardiogenic shock and acute ischemic mitral insufficiency. The condition was recognized and adequately treated.

Case report

A 77 year-old female patient was admitted to the Coronary Care Unit (CCU) at the Institute of Cardiovascular Diseases of Vojvodina due to prolonged feeling of chest pain followed by nausea and horizontal ST segment at $V_1 – V_3$ up to 3 mm registered by ECG **(Figure 1)**. Discomforts began two hours before admission as first manifestations of coronary disease. From risk factors for ischemic disease she confirmed having high blood pressure and insulin independent diabetes mellitus.

On admission, the patient's general condition was critical. She was in cardiogenic shock, conscious, adynamic, with difficulties in communication, hypotensive, with normal heart rhythm. Her skin was pale, cold and sweaty.

ST segment elevation posterior myocardial infarction was diagnosed in accordance with the clinical presentation and registered ECG changes **(Figure 2)**.

An emergency coronarography was immediately performed, registering occlusion of the circumflex artery (RCx) medial segment as the infarct-related artery. Significant lesions at the left anterior descending artery (LAD) segment were also registered as well as at the right coronary artery (RCA) - posterior descending artery (PDA) **(Figure 3)**.

Primary percutaneous coronary intervention was performed along with stent placement at the RCx medial segment. It was followed by balloon dilatation of

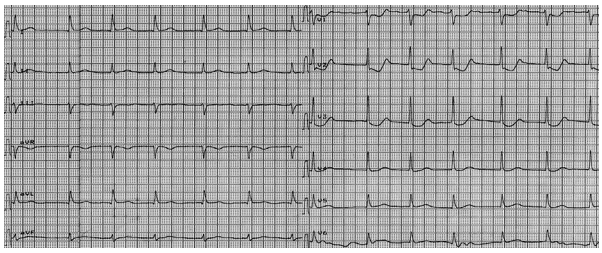

Figure 1. ECG taken after admission to the CCU – registered horizontal ST segment depression up to - 3 mm in V1−V3
Slika 1. *Elektrokardiogram snimljen nakon prijema u koronarnu jedinicu − registrovana je horizontalna denivelacija ST segmenta do 3 mm u V1−V3*

obtuse marginal (OM) branch establishing a satisfactory anterograde flow **(Figure 4)**.

After the intervention, the patient was clinically stabile. Echocardiography, which was performed a day after the intervention, registered lower left ventricular ejection fraction (EF 48%) and mediobasal inferior and mediobasal inferolateral hypokinesis, as well as severe ischemic mitral insufficiency.

The patient's case was presented at the Board of Cardiologists and Cardiac Surgeons and surgical myocardial revascularization and repeated echocardiography examination of mitral insufficiency, four weeks after myocardial infarction, were indicated, but prior to the surgery.

Since the control echocardiography registered gradual recovery of the mitral valve function with decrease of regurgitation volume, a myocardial revascularization was performed. The patient underwent triple aortocoronary bypass surgery (left internal mammary artery - LAD, RCx - OM1 and RCA) without mitral valve correction six weeks after myocardial infarction.

After surgical treatment the patient had no discomforts. Two years after myocardial infarction, control echocardiography showed mild to moderate mitral regurgitation.

Discussion

Posterior myocardial infarction is caused by necrosis of the posterior, infra-atrial part of the left ventricle located beneath the atrioventricular sulcus. Pathophysi-

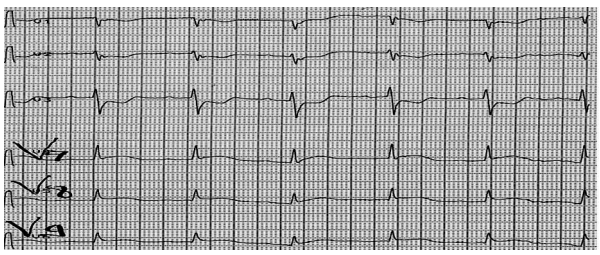

Figure 2. ECG showing posterior (lateral) leads and V7 - V9 elevation
Slika 2. *Elektrokardiogram prikazuje zadnje (lateralne) odvode sa prisutnom elevacijom u V7−V9*

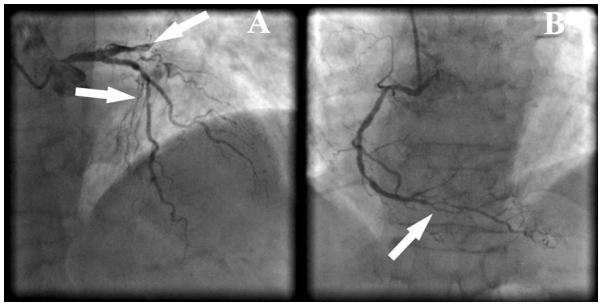

Figure 3. In the left image, the upper arrow shows an occlusion of medial RCx segment, while the lower arrow shows significant lesions of medial LAD segment. In the right image the arrow shows a significant lesion on the RCA – PDA
Slika 3. *Na Slici s leve strane, gornja strelica pokazuje okluziju medijalnog segmenta r. circumflexus, dok donja strelica pokazuje značajnu leziju u medijalnom segmentu r. interventricularis anterior. Na slici sa desne strane strelica prikazuje značajnu leziju r. interventricularis posterior i a. coronariae dextrae*

ologically, there is a significant stenosis or occlusion of the RCx, while in some patients the infarct related artery is the RCA - its PDA [16–18].

Clinical presentation, risk factors and differential diagnosis of posterior myocardial infarction do not differ from other localizations of myocardial infarction. When the diagnosis is made, ECG interpretation has to correlate with the clinical presentation and laboratory findings i.e. characteristic increase of cardiospecific enzymes. It should be emphasized that ECG changes can be uncharacteristic (complete left bundle branch block, changes in avR lead), making the diagnosis even more difficult [5, 8].

Depending on the anatomy of coronary arteries and location of blood vessels, posterior myocardial infarction is commonly accompanied with inferior and/or lateral wall infarction, thus causing a large infarction area with a high risk of complications such as left ventricular dysfunction, right ventricular infarction, ischemic mitral regurgitation, arrhythmias and fatal outcome [8, 9].

After the 17-segment model of the left ventricle was adopted based on echocardiography and magnetic resonance imaging data, it was recommended that segment 4 should be named inferobasal instead of posterior [21]. Both terms are still being used in the literature to indicate the localization of myocardial infarction.

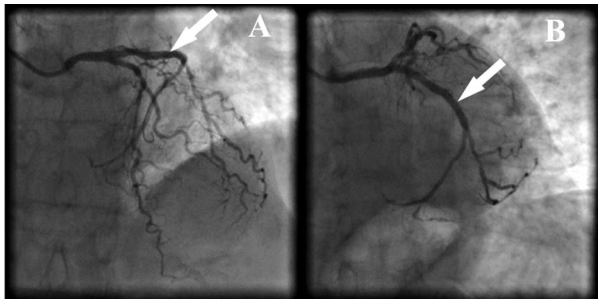

Figure 4. Arrows are pointing to the area of the implemented stent with optimal results
Slika 4. *Strelice pokazuju segment gde je implantiran stent, sa postignutim optimalnim rezultatom*

It is assumed that posterior myocardial infarction accompanied by infarction of other localizations accounts for around 15–21%, while isolated posterior yocardial infarction is rarely found [10–12].

Acute ischemia that is typically a consequence of coronary artery occlusion is associated with ST segment elevation on ECG leads, whose positive poles are located over the ischemic region and with reciprocal ST depression in leads whose positive poles are oriented in the opposite direction [13]. The posterior left ventricular wall is not shown directly on the 12-lead ECG, but the posterior wall electrical activity in $V_1 – V_3$ leads is reflected as a "mirror image", including ST depression, tall R-wave and tall T-wave, which could be signs of STEMI - ST elevation, Q-wave and negative T-wave [14]. Placing additional leads V_7 - V_9 on the posterior chest wall assures the posterior wall myocardial infarction to be diagnosed, since the registered ST segment elevation in leads $V_7 – V_9$ clearly indicates the posterior STEMI. Leads are placed on the fifth interocostal area: V_7 on the left posterior axillary line, V_8 on the tip of left scapula and V_9 on the left paraspinal region.

According to the recommendations of the European Society of Cardiology from 2012, the ST elevation in additional leads $V_7 – V_9 \geq 0.05$ mV is important (≥ 0.1 mV in men younger than 40 years of age) in diagnosing posterior myocardial infarction. When there is a suspicion, usage of these leads should be considered (class IIa, C level of evidence), while echocardiography can help in making a diagnosis, but cannot postpone angiography (class IIb, C level of evidence) [3].

Placing electrodes on the back of the thorax in routine practice increases the number of registered posterior myocardial infarctions so the incidence of all myocardial infarctions is considered to be 3.3% [15].

Additional ECG leads are not always necessary. According to Boden et al., if there is a horizontal ST depression, it is acute posterior MI in 100% of patients, as compared with patients with non-Q myocardial infarction where ST depression is registered in the same leads, but down-sloping. However, 5% of diagnosed non ST-segment myocardial infarction are posterior MI, where reperfusion therapy would be justified if the posterior leads were used [10].

In a study of Satture et al., including 1083 patients with suspected acute myocardial infarction, 87 (8%) had ST elevation myocardial infarction, diagnosed using a 12 lead ECG, 15 lead ECG, 2D echo and cardiospecific enzymes. The posterior wall was a more common localization of infarction than it was considered to be (38% of all STEMI). It was shown that posterior MI is associated with a high six-month mortality, similar to the mortality of anterior wall infarction (around 21%). A moderate mitral insufficiency was also registered in 35% of patients with posterior MI on admission, while six-months later it was registered in 3.1% of patients [22].

Insufficient knowledge of all listed electrocardiographic criteria, as well as the absence of additional

leads contributes to the failure to recognize posterior ST elevation myocardial infarction and thus to the delay of reperfusion therapy. It also leads to an increased risk of potentially fatal consequences such as ischemic mitral insufficiency, left ventricular free wall rupture, and even fatal outcome [9, 19–20].

In our case there were clear electrocardiographic criteria that indicted STEMI of the posterior region, which was complicated by the development of cardiogenic shock and severe ischemic mitral regurgitation. Since early diagnosis was correct, the patient received an adequate therapeutic algorithm, in other words mechanical reperfusion and stent placement in the infarct-related artery. Timely revascularization of blood vessel contributed significantly to the patient's clinical stabilization as well as to later gradual mitral valve recovery.

Conclusion

Posterior wall transmural myocardial infarction is the most common misdiagnosis in 12 lead electrocardiogram interpretation. Routine use of additional posterior (lateral) leads in all patients with chest pain shows no diagnostic or therapeutic benefits. It is recommended only in selected cases, especially in myocardial infarction of the inferior and/or lateral wall. The use of these leads increases the number of diagnosed myocardial infarctions, leading to better risk assessment, prognosis and survival, due to reperfusion therapy.

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Case report
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HEPATOCELLULAR CARCINOMA PRESENTING WITH PYREXIA AND LEUKOCYTOSIS: A CASE REPORT

HEPATOCELULARNI KARCINOM UDRUŽEN SA PIREKSIJOM I LEUKOCITOZOM: PRIKAZ SLUČAJA

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Summary

Introducion. This type of hepatocellular carcinoma is characterized by fever and persistent leukocytosis. **Case report.** This is a report of a patient with a long term fever accompanied by persistent leukocytosis. Abdominal ultrasonography revealed a focal lesion in the left hepatic lobe, whereas, computed tomography/magnetic resonance imaging findings were consistent with a liver abscess. The patient received therapy for liver abscess, without improvement. He underwent left lobe segmentectomy 3, with histological features of hepatocellular carcinoma (pseudoglandular type). **Conclusion.** In patients with focal hepatic lesions accompanied with raised temeparature and persistent leukocytosis, without adequate therapeutic response, this clinicopathological type of hepatocellular carcinoma should be considered.

Key words: Carcinoma, Hepatocellular; Leukocytosis; Fever; Liver Abscess; Treatment Outcome; Diagnosis, Differential; Tomography, X-Ray Computed; Magnetic Resonance Imaging; Signs and Symptoms

Sažetak

Uvod. Poseban kliničkopatološki tip hepatocelularnog karcinoma je oblik koji se klinički manifestuje povišenom telesnom temperaturom uz perzistentnu leukocitozu. **Prikaz slučaja**. Prikazan je slučaj pacijenta sa dugotrajnom povišenom telesnom temperaturom praćenom perzistentnom leukocitozom kod koga je ultrasonografski uočena fokalna promena u levom režnju jetre, a koja je radiološki ukazivala na apsces jetre. Kako na primenjenu adekvatnu terapiju za apsces jetre nije došlo do regresije promene, učinjen je operativni zahvat - segmentektomija III levog režnja i histološki nalaz je ukazao da se radi o hepatocelularnom karcinomu (histološki pseudoglandularni tip). **Zaključak**. Kod pacijenta sa fokalnom promenom u jetri i povišenom telesnom temperaturom uz perzistentnu leukocitozu koja ne reaguje na adekvatnu terapiju, uvek treba misliti i na ovaj poseban kliničkopatološki oblik hepatocelularnog karcinoma.

Ključne reči: hepatocelularni karcinom; leukocitoza; pireksija; apsces jetre; ishod lečenja; diferencijalna dijagnoza; CT; MRI; znaci i simptomi

Introduction

Hepatocellular carcinoma (HCC) is the most common primary liver carcinoma, according to most authors accounting for about 80% of all liver cancers; it is the fifth most common cause of death in the male, and the seventh most common cause of death in the female population, while the 5 year survival rate is about 15%. It is associated with a nubmer of risk factors, while its prevalance is different in various regions of the world. HCC mostly develops in the condition of chronic inflammation that has led to liver cirrhosis, so the etiologic factors of chronic liver disease overlap with the factors which account for the development of HCC, like hepatitis C and B virus, alcohol consumption, autoimune hepatitis, afla toxin, and recently non-alcoholic liver steatosis [1–3].

Hepatocellular carcinoma is often diagnosed after the appearance of clinical signs and symptoms, mostly thanks to screening programmes for cirrhotic patients and patients with chronic viral hepatitis, sometimes after repeated ultrasound check ups and evaluation of alpha-fetoprotein (AFP) levels, although it has proven to be an incompletely sensitive and specific marker for a succesful follow up and diagnosis. Accurate diagnosis of HCC is made by imaging techniques, specific computed tomography/magnetic resonance imaging (CT/MR) findings (so called nonivasive criteria), or by histological analysis of biopsy tissue samples [4–7].

The clinical presentation of HCC is commonly associated with the underlying liver disease, and sometimes it is very hard to differentiate the symptoms of HCC from those of chronic liver disease.

It is well known that HCC is associated with the following symptoms: hepatomegaly, vascular bruit, abdominal pain, portal vein thrombosis, gastrointestinal bleeding, jaundice, caval invasion, palpable

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Abbreviations

HCC – hepatocellular carcinoma
CRP – C-reactive protein
CT – computed tomography
MRI – magnetic resonance imaging
HbsAg – hepatitis C surface antigen
FP – feto protein

mass, ascites, ankle edema, etc. Also, laboratory liver tests are in accordance with paraneoplastic syndrome (hypoglicemia, hypocalcemia, erythrocytosis, thrombocytosis etc.), which may be accompanied by skin and neurologic manifestations. HCC may be associated with asthenia, anorexia, weight loss, nausea and fever, all of which may be primary non-specific symptoms [8].

Fever of unknown origin may be the first symptom. It is usually intermittent and accompanied by leukocytosis [9, 10]. Imaging studies play a crucial role in order to rule out liver abscess, or HCC. Hepatocellular carcinoma has different signs and symptoms, depending on the size of the tumor, invasion of vascular structures, presence of cirrhosis and presensce of metastases.

Case report

A 69-year-old male patient presented with a two-month history of a burning in the upper part of the abdomen, sweating and raised body temperature (37.5 to 38°C) which usually occurred in the late afternoon. On examination, the patient seemed in relatively good condition, without fever (36.3°C), in a state of cardiopulmonary compensation, without abdominal pain or palpable mass. Laboratory tests revealed leukocytosis of 18.4 (diff white blood cells: neutrophils 74.9%, lymphocytes 14.8%, monocytes 6.9%) and C-reactive protein (CRP) level of 48.7 (reference value below 5). Abdominal ultrasonography was performed (Aplio SSA-770A; Toshiba Medical, Tokyo, Japan), showing an enlarged, fatty liver, with a focal oval lesion in the left hepatic lobe, 38 mm in diameter, not clearly visible, due to the patient's obesity and aerocolia; the lesion was

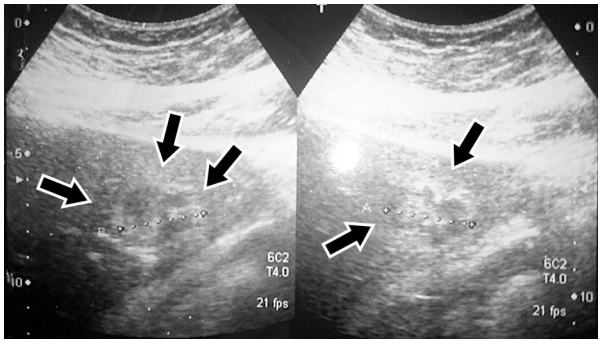

Figure 1. Ultrasonography: A focal oval hypo/hyperechoic lesion in the left hepatic lobe
Slika 1. *Ultrasonografija. Fokalna ovalna hipo/hiperehogena promena u levom režnju jetre*

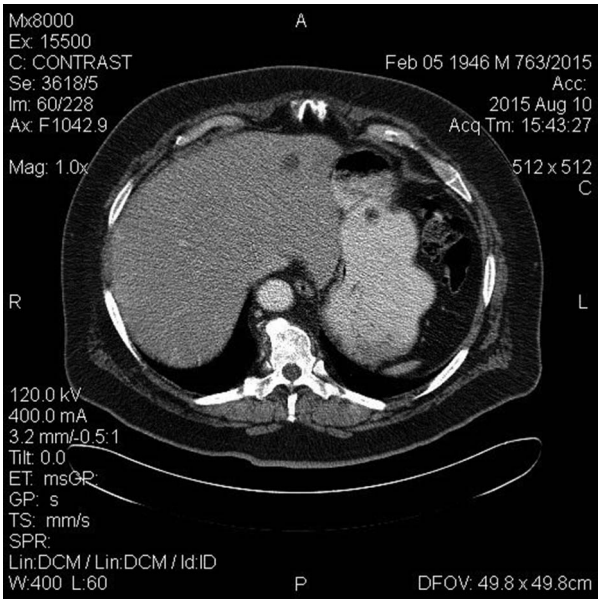

Figure 2. Computed tomography: Axial arterial phase tomogram. An oval heterogeneous hypodense lesion in the left liver lobe with contrast enhancement
Slika 2. *Kompjuterizovana tomografija. Aksijalni tomogram u arterijskoj fazi. Ovalna heterogena hipodenzna promena u levom režnju jetre sa postkontarstnim pojačanjem*

strongly suggestive of 2 structures, one of which was hyperechoic, the other hypoechoic (**Figure 1**).

The previous medical history showed a chronic heart condition. As part of his routine check-ups, he had an abdominal CT scan a year prior to febrility, without pathological findings.

Although he performed a few examinations due to fever of unknown origin, the laboratory results only showed a persistent leukocytosis of 14,59 – 21,70 (with a neutrophilia of 85.2%) despite antibiotic therapy. At that time all the other examined parameters were within reference values.

Since the focal lesion in the left hepatic lobe was of non-specific etiology, a Multidetector CT (MDCT) was performed (MX8000; Phillip Healthcare, Best, Netherlands) revealing an oval heterogeneous hypodense lesion in the left hepatic lobe, 35 x 25 mm in size. On post-contrast studies, the lesion mostly showed enhancement in the arterial phase while one smaller part of the lesion, 14 mm in diameter, remained hypodense, without washout signs in portal venous and venous phases (**Figure 2**). The differential reading suggested the lesion could be a liver abscess, but MRI was indicated.

The patient received the following therapy: Longaceph 2g parenterally + Metronidazole 3 x 400 mg per os. In the meantime, MRI was performed (1.5 T Avanto; Siemens Healthcare, Erlangen, Germany), showing a well demarcated cystic formation, 14 mm in diamter in the left lobe (S3), surrounded by a zone of inhomogeneous liver tissue with a maximum diameter of 35 mm. The cystic lesion was only slightly

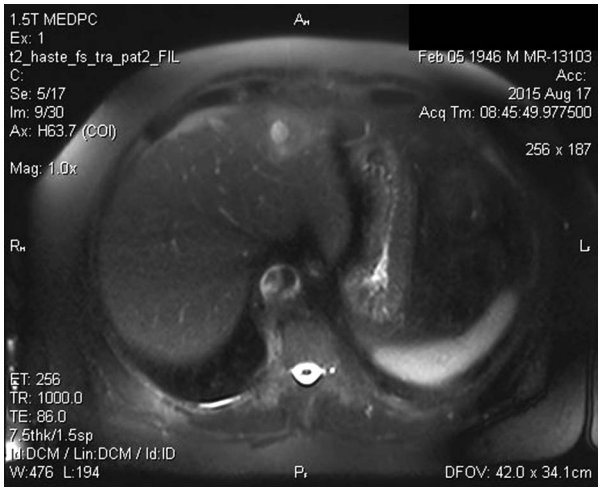

Figure 3. MRI scan: Axial T2 weighted haste image. A well demarcated cystic lesion surrounded by inhomogeneous liver tissue in the left liver lobe

Slika 3. *Magentno rezonantni pregled. Aksijalni* ***T2W haste*** *tomogram. Jasno ograničena cistična formacija okružena zonom inhomogenog jetrenog parenhima u levom režnju jetre*

visible on T1 weighted tomograms with a high DWI signal, while the zone of the surrounding inhomogeneous tissue was predominantly T2W hyper/-T1W slightly hypointense. After the administration of a paramagnetic contrast, there was a linear increase in signal intensity of the cystic wall, as well as an increase in signal intensity of the peripheral zone to isointensity with liver parenchyma. On delayed postcontrast sequences there was no evidence of a washout effect. In consclusion, the lesion was primarily characterized as a liver abscess, without a certain possibility to exclude other etiology (**Figure 3**).

After the results of the MRI were read, an abdominali surgeon was consulted, and the patient was referred to the Clinic of Infectious Diseases, where the patient was hospitalized and treated for a liver abscess. During the hospitalization, the patient recieved Azaran 2 x 2g parenterally and Metronidazole 3 x 500 mg per os for 8 days, followed by 8 days of Tazobactam 4.5 g x 4. A control CT scan was performed 3 weeks later (Somatom sensation 64; Siemens Healthcare, Erlangen, Germany) and showed a persisting inhomogeneous lesion in the left liver lobe (S3), 33 x 28 mm in diameter, with a hypodense zone of liquid density, along with peripheral enhancement in arterial, portal venous and venous phases (**Figure 4**). The lesion was characterized as an abscess. Since there was no significant change in morphology or size of the lesion, the patient was discharged and referred to a gastroenterologist.

A control utrasound was performed 10 days later (Aplio SSA-770A; Toshiba Medical, Tokyo, Japan), still showing a focal lesion in the left liver lobe, about 4 cm in diameter, with irregular and spiky margins, oval in shape, with a thin hyperechogenic

Figure 4. Control CT scan: Venous phase axial tomogram. Persisting inhomogeneous hypodense lesion with a central cystic zone along with a rim enhancement in the left liver lobe

Slika 4. *Kontrolna kompjuterizovana tomografija. Aksijalni tomogram u venskoj fazi. Perzistirajuća nehomogena hipodenzna promena sa centranom cističnom zonom i rubnim postkontrastnim pojačanjem u levom režnju jetre*

capsule, with an inhomogeneous structure and a few smaller lesions of different echogenicity (characterized as a nodule within a nodule) (**Figure 5**). Then, with a persistent leukocytosis, the laboratory results showed: sedimentation rate (SE) 58/108, CRP 59,16, without signs of anemia, alpha feto protein (FP) level within reference values, hepatitis B surface antigen (HbsAg) and anti hepatitis C virus (HCV) negative.

The patient was once more examined by an abdominal surgeon, who suggested surgical treatment. The operation was performed abou 4 months after

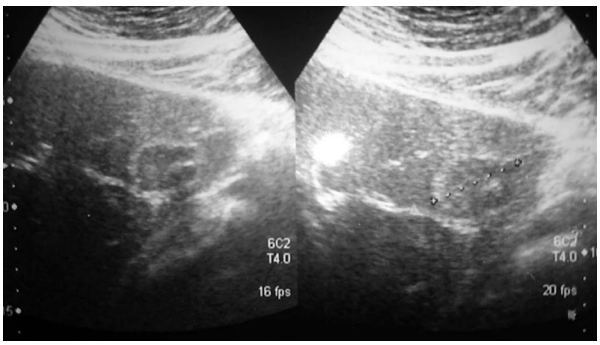

Figure 5. Control ultrasonography: Persistent focal oval heteroechoic lesion in the left hepatic lobe

Slika 5. *Kontrolni ultrasonogram. Perzistentna fokalna ovalna heteroehogena lezija u levom režnju jetre*

the fever started, and 2 months after the lesion was seen on the first ultrasound examination (laparotomia med. superior. segmentectomia lobi hepatis sin. III). The patient's postoperative recovery was uneventful and gradually he started feeling better. The histopathological report showed that it was a case of hepatocellular carcinoma of pseudoglandular type, HG2, pT1NxMx (imunohistochemical profile: Hepar 1 positive), with evidence of smaller fields of necrosis in the tumor tissue and no pathohistologic characteristics of an abscess. The surroinding liver parenchyma was with signs of micro and macroglandular cirrhosis. The patient was referred to the Oncologic Committee of the Institute of Ooncology where a control regimen was planned. A control CT scan of the abdomen was performed 5 months after surgery, and it showed no areas of detectabile pathologic density, and the patient was afebrile.

Discussion

There are sporadic cases of HCC described in literature, with clinical manifestations including mainly intermittent fever and leukocytosis [8–10]. Even before the era of CT and MRI, there were rare cases of inflammatory pseudotumors or abscesses associated with increased body temperature and leukocytosis, confirmed to be rare types of HCC mimicing liver abscess after pathohistological analysis of tissue samples obtained from operated or deceased patients. Among the five published cases, two were patients with cirrhosis, all paitients were male (aged 43–79), all with negative (HBsAg), Alpha FP within reference values, except in one case just before death [9]. All patients had intermittent high body temperature (from 36.5 to 39°C), associated with leukocytosis of 15.000 to 100.000 or more, without any response to antibiotics, which was also the case in our patient. Massive necrosis was present in all 5 cases, while our patient presented only with small fields of necrosis in the tissue of pseudoglandular HCC, without pathomorphologic signs of abscess. In most cases of histologically confirmed HCC, it was a poorly differentiated tumor. There are theories that these are tumors with sarcoid degeneration [10]. Even though the main parts of the tumor were proven to contain sarcomatoid malignant cells, with fields of trabecular cells and areas of necrosis, it was a case of a poorly differntiated HCC, more so than a combination of sarcoma and HCC [9].

All these tumors were hypovascular on angiography and a possible explanation for this is that the tumor cells were not producing the angiogenic factors, so the lack of neovascularization was the cause of tumor cell necrosis [9].

It is known that tumor-induced granulocytosis can be explained by the production of granulocyte growth factors by tumor cells, while high body temperature is explained by the production of pyrogenic factors of malignant cells or macrophages in response to tumor necrosis.

Due to the aforementioned facts, patients with this type of tumor present with different clinical signs and symptoms compared to those with other forms of HCC. Raised body temperature occurs in 12% of patients suffering from HCC across Europe, 17% in Japan, only 2% in China, and as much as 35 % of black patients in Africa. This leads to the assumption that etiologic factors also have a role in the pathogenesis of this special type of liver carcinoma [8].

With all this in mind, this type of HCC is special not only histologically, but also clinically, and it is considered a separate clinical and pathological entity. Nowadays, the diagnosis of HCC can be made by radiologic criteria only (CT/MRI), by strictly following imaging protocols, even without a biopsy performed, if familiar and specific findings are established [11–14].

Conclusion

In conclusion, we can safely say that even though we have sophisticated medical imaging techniques and established criteria for the diagnosis of hepatocellular carcinoma, when it comes to nonspecific findings in extensive liver lesions, with a clinical presentation mimicking liver abscess, including long-term intermittent fever and persistent leukocytosis, one should always have in mind this special clinical and pathological entity.

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BIOMARKERS OF ENDOTHELIAL DYSFUNCTION IN CARDIOVASCULAR DISEASES

BIOMARKERI ENDOTELNE DISFUNKCIJE U BOLESTIMA KARDIOVASKULARNOG SISTEMA

Sonja SMILJIĆ[1], Milica MIJOVIĆ[2] and Slađana SAVIĆ[3]

Summary

Introduction. Endothelial dysfunction is the result of numerous infectious or noninfectious acute and chronic diseases, mechanical damage, hemodynamic imbalance and effects of certain drugs. Endothelial dysfunction can be assessed by determining biomarkers (adhesion molecules, inflammatory cytokines and growth factors, and noninvasive visualization biomarkers). **Intercellular adhesion molecule -1 and vascular cellular adhesion molecule.** Adhesion molecules mediate the interaction of cells with the extracellular matrix, as well as with other cells. It is shown that adhesion molecules and molecules of the extracellular matrix are markers of endothelial dysfunction and they are involved in the pathogenesis of atherosclerosis. **P-selectin and E-selectin.** Determination of these two mediators is important not only in the evaluation of endothelial damage in acute inflammation, but in other chronic non-infectious conditions such as atherosclerosis. **C-reactive protein.** C-reactive protein reduces the transcription of endothelial nitric oxide synthase at the level of the endothelial cells and 'destabilize' their messenger ribonucleic acid, thus leading to a reduction of the synthesis of nitric oxide, in the basal and stimulated conditions, which is significant for the development of endothelial dysfunction as a part of the formation of subclinical atherosclerosis. **Vascular endothelial growth factor, fibrinogen and thrombomodulin.** Vascular endothelial growth factor is a cytokine that stimulates angiogenesis for the purpose of revascularization of ischemic tissues, and mediates in a variety of functions of endothelial cells, including proliferation, migration, invasion, survival and permeability. Noninvasive visualization biomarkers. Determination of intima-media complex thickness and calcium score index by computed tomography are considered clinically reliable methods in prevention and early diagnosis of coronary artery disease and acute myocardial infarction, changing the basic concepts of prevention. **Conclusion.** At this point it is not easy to say what clinical significance are listed biomarkers of endothelial dysfunction in determining the risk for cardiovascular disease.

Key words: Biomarkers; Endothelium, Vascular; Risk Factors; Cardiovascular Diseases; C-Reactive Protein; Selectins; Intercellular Adhesion Molecule-1; Vascular Cell Adhesion Molecule-1; Vascular Endothelial Growth Factor A; Thrombomodulin

Sažetak

Uvod. Endotelna disfunkcija je posledica brojnih infektivnih ili neinfektivnih akutnih ili hroničnih bolesti, mehaničkog oštećenja, hemodinamskog disbalansa i delovanja nekih lekova. Endotelna disfunkcija se može procenti određivanjem biomarkera endotelne disfunkcije (adhezijskih molekula, selektina, faktora rasta i zapaljenja, neinvazivnim vizuelizacionim biomarkerima). **Intercelularni adhezioni molekul-1 i vaskularni adhezioni molekul.** Adhezioni molekuli posreduju u interakciji ćelija sa ekstracelularnim matriksom, kao i sa drugim ćelijama. I za adhezijske molekule i za molekule ekstracelularnog matriksa pokazano je da su markeri endotelne disfunkcije te da su uključeni u patogenezu ateroskleroze. **P-selectin and E-selectin.** Određivanje ova dva biomarkera značajno je u proceni oštećenja endotela u akutnom zapaljenju, ali i u drugim hroničnim neinfektivnim stanjima, poput ateroskleroze. **C/rektivni protein.** C-reaktivni protein smanjuje transkripciju eNOS-a na nivou endotelnih ćelija i "destabiliše" njihovu mRNK, dovodeći na taj način do smanjenja sinteze azot-monoksida, u bazalnim i stimulisanim uslovima. **Vaskularni endotelni faktor rasta, fibrinogen i trombomodulin.** Vaskularni endotelni faktor rasta je citokin koji stimuliše angiogenezu u svrhu revaskularizacije ishemičnih tkiva, a posreduje u brojnim funkcijama endotelnih ćelija, uključujući proliferaciju, migraciju, invaziju, preživljavanje i permeabilnost. Neinvazivni vizuelizacioni biomarkeri. Klinički pouzdani u prevenciji i ranoj dijagnozi bolesti koronarnih arterija i akutnog infarkta miokarda jeste oderđivanje intimomedijalnog kompleksa i određivanje Ca Scor indeksa pregledom kompjuterizovanom tomografijom, koji su izmenili bazične koncepte mogućnosti prevencije. **Zaključak.** U ovom trenutku rano je reći kakvu kliničku vrednost imaju pojedini biomarkeri endotelne disfunkcije u određivanju rizika za oboljevanje od kardiovaskularnih bolesti.

Ključne reči: biomarkeri; vaskularni endotel; faktori rizika; kardiovaskularne bolesti; CRP; selektin; intercelularni adhezijski molekul-1; vaskularno celularni adhezijski molekul-1; vaskularni endotelni faktor rasta; trombomodulin

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Abbreviations

ED – endothelial dysfunction
ICAM-1 – intercellular adhesion molecule-1
VCAM-1 – vascular cellular adhesion molecule-1
VEGF – vascular endothelial growth factor
CRP – C-reactive protein
LFA – lymphocyte function associated antigen 1
ND – nitric oxide
IL – interleukin
IMP – intima media complex

Introduction

Endothelial dysfunction (ED) is an early, usually asymptomatic phase of endothelial cell damage which results in disruption of its numerous functions. During this process, various toxic substances induce the loss of protective mechanisms of the endothelium, including: anti-platelet and anti-inflammatory functions. It affects proliferation, migration and survival of endothelial cells, and endothelial permeability change [1]. ED effects are first manifested at the microvascular level of different tissues in any organ system, in extremely large number of infectious and non-infectious, acute or chronic diseases. The vascular endothelium is a single-cell layer forming the inner lining of the blood vessel which has a protective function but it can be compromised by activation of cytokines and other inflammatory mediators. ED is also caused by other potentially damaging factors: mechanical damage, hemodynamic imbalance and certain medications [2]. It is well known that aging and diabetes mellitus are two independent risk factors for the development of ED, while the concept of low-intensity chronic inflammation includes other conditions that can lead to ED, as well as infectious or non-infectious diseases, hypertension, dyslipidemia, smoking, etc. [3]. Each of these conditions may be initiated by the expression of adhesion molecules, enhanced release of cytokines, chemokines, growth factors and other inflammatory mediators that promote migration and activation of inflammatory cells.

Important biomarkers and mediators of ED are endothelial adhesion molecules, growth factors, and inflammatory factors. They participate not only in local, but in systemic response to tissue damage, as well. The ED biomarkers include: intercellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), P- and E-selectin, vascular endothelial growth factor (VEGF) and C-reactive protein (CRP) [4]. Recent studies indicate a significant contribution of ED in the pathogenesis of atherosclerosis and cardiovascular disease (CVD). Atherosclerotic plaque formation includes a greater number of cells: platelets, endothelial cells, activated monocytes, macrophages and smooth muscle cells. Macrophages and T-lymphocytes play a key role in the growth and changes of atherosclerotic plaque, mediated by the secretion of growth factors that stimulate the proliferation of smooth muscle cells and secretion of the extracellular matrix, proinflammatory cytokines, interferon-γ or interleukin-1, with a simultaneous activation of enzymes that break down the extracellular matrix, such as metalloproteinases, causing weakening of the fibrous part of the plaque [5].

Numerous studies have found a positive correlation between ED and coronary artery disease. It is believed that ED, alone, without coronary disease and without evidence of coronary artery spasm, is caused by the acute coronary syndrome, unstable angina and myocardial infarction, without ST segment elevation. One of the potentially most important factors in the development of ED is insufficient production of nitric oxide (NO), vasodilator of endothelial origin [6]. The term "microvascular coronary dysfunction" has become a distinct clinical entity, which refers to ischemia with clinical signs of angina, defined by the triad of chest pain, evidence of ischemia by stress testing, and non-obstructive coronary artery disease.

Currently there is no gold standard for the assessment of ED. Ultrasound measured flow-mediated dilatation (FMD) of the brachial artery has proven to be a useful non-invasive method. Venous occlusion plethysmography, determination of the intima-media complex thickness, pulse wave velocity and peritoneal equilibrium test (PET) are some of the methods, but rarely used in practice. The future of the assessment of ED is in determining the level of biomarkers, adhesion molecules (VCAM, ICAM-1, selectins), and VEGF, fibrinogen, thrombomodulin and CRP in the blood.

Intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1

Vascular cellular adhesion molecule-1 and ICAM-1, (also known as CD54) are adhesion molecules of similar structure and function. VCAM-1 gene contains seven immunoglobulin domains and it is expressed on all blood vessels, regardless of their size. However, upon stimulation of endothelial cells by cytokines its concentration is significantly increased. In addition to the endothelial cells, VCAM-1 is expressed in neurons, smooth muscle cells, fibroblasts and macrophages [4].

Synthesis and secretion of ICAM-1 can be induced by interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), and it functions as a ligand for lyphocyte function associated antigen 1 (LFA-1) (integrin) receptor found on leukocytes. Upon activation, leukocytes bind to endothelial cells via the ICAM-1/LFA-1, and this allows them to migrate into the tissues. Elevated levels of ICAM-1 were observed in patients with type II diabetes mellitus, cardiovascular diseases, graft dysfunction, oxidative stress, abdominal obesity, hypertension, liver disease and some malignant diseases. Intercellular adhesion molecule-1 promotes angiogenesis and studies have shown that it can be an indicator of activation or damage of endothelial cells [7].

Adhesion molecules mediate the interaction of cells with the extracellular matrix, as well as with other cells. It is shown that both adhesion molecules and extracellular matrix molecules are markers of ED and are involved in the pathogenesis of atherosclerosis. Also, immunohistochemical analysis of

atherosclerotic plaque demonstrated the presence of a number of adhesion molecules [5]. At the site of inflammation, proinflammatory cytokines lead to increased expression of ICAM-1 on vascular endothelium and activation of leukocyte integrins, which results in the adhesion of leukocytes to endothelial cells and migration to the site of inflammation. VCAM-1 expression is found in small blood vessels after stimulation of endothelial cells with cytokines, and patients with coronary heart disease showed higher levels of VCAM-1 than those with normal epicardial coronary arteries [8]. In the previous studies of ED and coronary artery disease biomarkers, the best tested was ICAM-1. People with higher basal levels of ICAM-1 had a two-fold risk of developing cardiovascular disease and in the absence of other factors for cardiovascular disease, which marked this biomarker of ED as a potential indicator of future ischemic incident; further researches could confirm its clinical value in determining cardiovascular risk and mortality. An increase in the value of ICAM-1 has been established within the first 12 - 24 hours after acute myocardial infarction and during ischemia and reperfusion [8]. Studies have also shown a connection between higher values of ICAM-1 and the occurrence of reperfusion arrhythmias. The serum concentration of this biomarker is reduced in individuals using medications, such as angiotensin converting enzyme inhibitors (ACE inhibitors) and calcium channel blockers. VCAM-1 may be independently elevated in asymptomatic smokers as well, while the elevated value of this adhesion and E-selectin were observed in both active and former asymptomatic smokers [10].

It has been determined that patients with elevated plasma VCAM-1 concentration and stable coronary heart disease are at higher risk of future cardiovascular events. In patients hospitalized for acute coronary syndrome, higher values of this marker are correlated with intrahospital complications, including a fatal myocardial infarction [5, 8, 9]. Both of these markers of plasma endothelial dysfunction are closely associated with total mortality from cardiovascular disease.

There are several factors that lead to elevation in plasma concentrations of adhesion molecules: hypertension, immunosuppressive therapy, autoimmune disease and graft rejection [11]. In experimental conditions, both types of adhesion molecules are expressed on the endothelium of the aorta in the regions predisposed to atherosclerosis. They can be easily measured in plasma by enzyme-linked immunosorbent assay (ELISA test) and thus represent potential biomarkers indicating endothelial activation and vascular inflammation.

P-selectin and E-selectin

P-selectin and E-selectin play a major role in the migration and chemotaxis of lymphocytes in the tissues and neutrophils in the formation of thrombus in atherosclerosis. Determination of these two mediators is important not only in the evaluation of endothelial damage in acute infectious inflammation, but in other chronic non-infectious conditions such as atherosclerosis, as well. They were found in Wiebel-Palade bodies of the endothelial cells and alpha-granules of platelets. These molecules are important in the activation of T- and B-lymphocytes and adhesion of platelets, monocytes and neutrophils, which play a central role in the accumulation of neutrophils within the thrombus [10].

E-selectin expression is found only on endothelial cells after activation of proinflammatory cytokines (interleukin-1, tumor necrosis factor) or endotoxin. Although elevated values of P-selectin are indicators of future coronary events in women, and elevated basal levels of ED mediators are observed in men, studies have not shown a sufficient degree of predictive value for their clinical use. It is believed that E-selectin is an important biomarker for monitoring ischemia and necrosis after acute myocardial infarction. In case of acute ischemic event, high levels of ICAM-1, VCAM-1, P- and E- selectin may be found, all of which remain elevated over the next six months, followed by a return to the baseline. In case of myocardial revascularization, the value of ICAM-1 and P-selectin remain elevated for about 24 months after the surgical procedure [12].

C-reactive protein

C-reactive protein is a biomarker of inflammation and ED. A CRP test helps in determining ED, which separates it from other serum indicators of ED. CRP is one of the leading acute phase proteins of inflammation and a marker of systemic inflammatory response. It is elevated in certain chronic diseases, including atherosclerosis, and it is believed that this protein, synthesized in the liver, causes and prolongs the inflammatory component of atherosclerosis by activation of a number of molecules present in/on endothelial cells which participate in the process of atherogenesis [13].

The physiological role of this protein is to identify potentially toxic autogenous substances released from damaged tissues, to bind them and then detoxify them or remove from the blood. A considerable number of studies have indicated that high-sensitivity CRP (hsCRP) is one of the most significant independent predictors for the occurrence of adverse vascular events [8, 14]. CRP reduces eNOS transcription at the level of the endothelial cells, and destabilizes eNOS mesenger ribonucleic acid (mRNA), thus leading to a reduction of NO synthesis, in the basal and stimulated conditions, which is significant for the development of ED as part of the subclinical atherosclerosis [6]. This molecule also stimulates the release of endothelin (ET-1) and IL-6, elevates the expression of adhesion molecules, stimulates monocyte chemoattractant protein-1 and facilitates the entry of LDL into macrophages, thus participating in their transformation into foam cells, which is an important stage in the atherogenesis [14]. Researches show that CRP facilitates apoptosis of endothelial cells, which is described as part of the mechanism of ED. In addition to direct effects on the promotion of endothelial acti-

vation, CRP also indirectly inhibits bone-marrow derived endothelial progenitor cells and thus prevents repair of the endothelium. Besides the formation of plaque, CRP has a role in its further maintenance, leading to elevated expression of receptors for angiotensin and on vascular smooth muscle cells (*in vivo* and *in vitro*), stimulating their proliferation, neointimal formation and reactive oxygen species.

Statins exhibit positive effects on elevated levels of CRP in patients with statin-treated hyperlipidemia, which makes them useful substances in the primary prevention of coronary heart disease in patients with slightly elevated or normal lipidogram values [15]. Many studies have determined a correlation between elevated CRP levels and progression of atherosclerosis. In particular, a positive correlation was observed between the above mentioned, the severity of coronary artery disease and the risk for development of acute coronary syndrome. However, researchers disagree over the connection between cardiovascular diseases and CRP. CRP is an indicator of ED, but it is not clear whether it is a predictor of cardiovascular events, given the heterogeneity of causes and mechanisms of ED occurrence.

Vascular endothelial growth factor, fibrinogen and thrombomodulin

Vascular endothelial growth factor (VEGF) is a cytokine which promotes angiogenesis and ischemic tissue revascularization, and mediates in a variety of functions of endothelial cells, including proliferation, migration, invasion, survival and permeability. During the intracoronary thrombus formation, platelets release significant amounts of VEGF, and there is a significant positive correlation between the serum values of VEGF and platelet count and the size of thrombus in patients with STEMI myocardial infarction. Higher values of this factor were recorded in acute coronary events in relation to chronic ischemic conditions, and it is believed that the reason for this is under-developed collateral circulation in patients who develop an acute incident. It was determined that heparin simultaneously binds VEGF and endothelial cells, thus suppressing ED and lowering serum concentration of VEGF [16, 17].

A high level of fibrinogen is associated with an increased risk for developing cardiovascular disease in both men and women, but some differences were detected in relation to gender. The level of plasma fibrinogen is elevated in menopause, during the use of oral contraceptives, and pregnancy, although the hormone replacement therapy affects its reduction [18]. The latest European guidelines on cardiovascular disease prevention in clinical practice recommend determination of fibrinogen as a part of risk assessment in patients with low or moderate risk for developing cardiovascular disease [18].

Thrombomodulin (TM) is traditionally thought to be a biomarker of ED. It is a transmembrane glycoprotein which is expressed on vascular endothelial cells and represents a thrombin receptor. A high level of thrombomodulin is associated with elevated level of plasminogen activator inhibitor (plasminogen activator inhibitor-1, PAI-1) [20, 21]. Thrombomodulin and von Willebrand factor are transferred to plasma and their high values are found in patients with endothelial dysfunction suffering from type II diabetes mellitus.

Noninvasive visualization

Noninvasive imaging biomarkers are a valuable tool in the diagnosis of cardiovascular diseases. The determination of intima-media complex thickness (IMC) and Ca Score Index by computed tomography examination are considered clinically reliable methods in prevention and early diagnosis of coronary artery disease and acute myocardial infarction, affecting the change in the basic concepts of prevention.

An integral part of the Doppler ultrasonography of blood vessels is determining intima-media complex thickness. IMC is measured in common, internal carotid arteries, or on the walls of the carotid bulb. In clinical practice the significance of this parameter is well defined, and since normal IMC values vary significantly according to gender, age and ethnicity, they are defined for different geographic areas [22].

Calcium accumulation in the walls of blood vessels is an active cellular process and an integral part of atherosclerosis. In physiological conditions, the wall of the coronary artery does not contain calcium. The presence of calcium in the coronary arteries is a sign of coronary artery disease. There is a direct linear relationship between the calcium concentration and the degree of atherosclerosis, which is determined by Ca Score Index. Detection of calcium in the walls of coronary arteries is a certain sign of some degree of atherosclerosis [23].

Conclusion

Endothelial dysfunction, manifesting as a subclinical atherosclerosis, occurs long before structural changes to the vessel wall are apparent, and represents an independent risk factor for future occurrence of cardiovascular events. The tissue damage is a result of the accumulation of inflammatory cells. Also, an activation mechanism leads to the expression of different adhesion molecules and chemokines as signalling molecules involved in the recruitment of inflammatory cells in the target tissues. If such changes occur in the myocardium, it leads to the development of fibrosis, which manifests by diastolic dysfunction. Endothelial cells play a leading role in the process of inflammation, because they represent a direct barrier between the target tissue and circulating inflammatory cells. If the cell is stimulated by the pro-inflammatory signals: cytokines, endotoxin, modified lipids or reactive oxygen species, it causes activation of an inhibitor necrosis factor of kappa B kinases and the expression of pro-inflammatory mediators: intercellular adhesion molecule-1, vascular cellular adhesion molecule-1, monocyte chemoattractant protein-1, interleukin-6 and others. At the same time, it

inhibits nitric oxide production. These endothelial changes lead to chronic inflammation in the walls of blood vessels and play a key role in the development of atherosclerosis. Future researches should be directed towards the clarification of the possible role of a signalling molecules mediators of endothelial dysfunction, as potential predictors of future cardiovascular events, both in persons diagnosed with cardiovascular disease, and in those who still have asymptomatic atherosclerosis. The mechanisms of action of certain endothelial dysfunction markers are still not fully elucidated and require further research to understand their role and the mode of operation in all respects. At this point, it is too soon to evaluate the clinical significance of mediators and biomarkers of endothelial dysfunction in determining the risk for cardiovascular disease.

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THE ROLE OF PHYSICAL THERAPY IN THE TREATMENT OF POST-TRAUMATIC CONTRACTURE OF THE ELBOW IN CHILDREN

ZNAČAJ FIZIKALNE TERAPIJE U LEČENJU POSTTRAUMATSKIH KONTRAKTURA ZGLOBA LAKTA KOD DECE

Maja BOGDAN[1] and Aleksandra MIKOV[2]

Summary

Introduction. Elbow fractures account for 7 – 9% of all fractures in children, while supracondylar humeral fractures are the most common fractures in the elbow joint region. Most of these fractures are seen in children between 5 and 8 years of age. **Management of elbow injuries.** Orthopedic treatment of elbow injuries includes conservative and/or surgical treatment, and outcomes are much better in children than in adults. The main contributing factors to the development of elbow contractures include the severity of the trauma, intra-articular damage, and length of immobilization. The literature is still limited in terms of the time required for regaining good elbow range of motion after contracture. **Complication of elbow injuries.** Neurological deficits may be treated conservatively, by electrical stimulation, vitamin therapy and intensive exercises. **Physical therapy of elbow contracture.** Some authors showed that, in uncomplicated cases of supracondylar elbow fractures, physical therapy is not necessary in order to regain adequate range of motion. On the other hand, according to guide recommendations, after post-traumatic elbow immobilisation and reduced mobility, patients should undergo occupational and physical therapy as soon as possible after the period of immobilization. It has been proven that combined active and passive therapeutic programs significantly improve the range of motion of the elbow joint. **Conclusion**. The therapeutic approach to post-traumatic contracture of the elbow should be individual, patiently and properly dosed, with follow-up of pathogenesis and kinesiology disorders. However, physicians should be aware of the fact that particular attention should primarily be paid to the prevention of contractures.

Key words: Elbow Joint; Elbow Joint Injuries; Contracture; Physical Therapy Modalities; Child; Treatment Outcome; Range of Motion; Occupational Therapy; Immobilization; Neurologic Manifestations

Sažetak

Uvod. Prelomi lakta čine 7−9% svih preloma kod dece, dok su suprakondilarni prelomi ramene kosti najčešći prelomi u predelu lakta. Većina ovih preloma se dešava kod dece uzrasta 5−8 godina. **Lečenje povreda lakta.** Ortopedsko lečenje povreda lakta uključuje konzervativni i/ili hirurški tretman, a ishod je mnogo bolji kod dece nego kod odraslih. Faktori koji određuju nastanak kontrakture lakta su ozbiljnost povrede, intraartikularna oštećenja i dužina imobilizacije. Literatura je još uvek ograničena po pitanju vremena potrebnog za uspostavljanje adekvatnog obima pokreta u zglobu lakta nakon nastale kontrakture. **Komplikacije povreda lakta.** Neurološki deficiti se mogu tretirati konzervativno elektrostimulacijom, vitaminskom terapijom i intenzivnim aktivnim vežbama. **Fizikalna terapija kontrakture lakta**. Neki autori su pokazali da, u nekomplikovanim slučajevima suprakondilarnih preloma lakta, fizikalna terapija nije potrebna da bi se postigao adekvatan obim pokreta. S druge strane, prema preporukama vodiča, pacijente sa posttraumatskom imobilizacijom lakta i smanjenom pokretljivošću u zglobu trebalo bi lečiti radnom i fizikalnom terapijom što je pre moguće posle perioda imobilizacije. Naučno je dokazano da kombinovani aktivni i pasivni terapijski programi značajno poboljšavaju obim pokreta u zglobu lakta. **Zaključak**. Terapijski pristup kontrakturi lakta trebalo bi da bude individualan, pažljivo i prikladno doziran, uz praćenje patogenetskih i kineziooloških poremećaja. Međutim, doktori treba da budu svesni da više pažnje treba posvetiti prevenciji kontrakture.

Ključne reči: lakatni zglob; povrede lakta; kontrakture; fizikalna terapija; dete; ishod lečenja; opseg pokreta; okupaciona terapija; imobilizacija; neurološki deficiti

Introduction

Post-traumatic joint contractures are the most common complication of the locomotor apparatus injuries. A 50% reduction of elbow range of motion can decrease the upper extremity function by nearly 80% [1]. Elbow fractures account for 7–9% of all fractures in children, while supracondylar humeral fractures are the most common fractures in the elbow joint region. Most of these fractures are seen in children between 5 and 8 years of age [1, 2].

Management of elbow injuries

Orthopedic treatment of elbow injuries includes conservative and/or surgical treatment, and out-

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comes are much better in children than in adults [1]. The selection and implementation of treatment depends on the clinical presentation and x-ray findings [2]. Surgical treatment of non-dislocated transcondylar and supracondylar elbow fractures involves cast immobilization with the forearm in pronation for no longer than 3 weeks [3, 4]. According to the recent literature, the safest and most effective method in the treatment of a dislocated elbow fracture is a closed (orthopedic) reduction with percutaneous pins within 12 hours after admission [3, 5, 6]. After closed reduction and pin fixation, the pins are normally removed 3 – 4 weeks later [7]. During the acute phase of fracture healing, in the first two weeks, it is important to apply cold packs to reduce inflammation. During the fibroelastic (2 – 6 weeks after injury) and remodelling phase, the most important are passive, active and active assisted exercises combined with heat procedures [8].

Complications of elbow injuries

Complications associated with supracondylar elbow fractures are either early (vascular, neurological injuries, compartment syndrome) or late (malunion, contracture) [7]. The most common neurological lesions include contusions, strains or compressions of the median and radial nerves, with reduced muscular function and sensory disturbances in the innervation zone [9]. Primary lesions are the direct result of the traumatic injury, and secondary lesions occur during the surgical treatment, during the manipulation of fractured bones, as well as due to edema of the surrounding tissue [10]. The majority of nerve injuries associated with supracondylar fractures or their surgical management are transient neurapraxias and can be managed expectantly. If there is concern over iatrogenic injury, then a thorough assessment with a consultant's input is required for consideration of the nerve exploration [7].

A contracture is a rare complication caused by a combination of intrinsic and extrinsic factors. The extrinsic factors include: contracture of the joint capsule and ligaments, edema, muscle weakness, extra-articular osteophytes, and ectopic ossification. The intrinsic factors are intra-articular adhesions, osteophytes, cartilage defects, hemarthrosis and hematomas, causing muscle fibrosis [11, 12]. The factors contributing to the development of elbow contractures include the severity of the trauma, intra-articular damage, and length of immobilization [11].

Physical therapy of elbow contracture

The goal of elbow contracture treatment is to provide patients with pain-free, functional range of motion of the joint. However, the literature is still limited in terms of the time required to regain good range of motion in the elbow joint after contracture [13]. Some authors reported that, in uncomplicated cases of supracondylar elbow fractures, physical therapy is not necessary in order to regain adequate range of motion [4, 14]. In addition, a study from 2015, showed that full range of motion after supracondylar elbow fractures may be achieved in 12 months, with or without physical therapy [15]. On the other hand, according to a guide from 2007, patients with post-traumatic elbow immobilization and reduced mobility should undergo occupational and physical therapy as soon as possible after the period of immobilization [8]. It has been proven that combined active and passive therapeutic programs significantly improve the range of motion of the elbow joint [16–18]. Early closed reduction, along with active and active assisted exercises, showed positive results in many studies [3, 12, 19, 20]. Contractures due to extrinsic causes and lasting less than a year, have a better prognosis and response to physical therapy [11].

The biophysical effects of heat increase the extensibility and blood flow of the tissue, reduce pain and muscle spasms [15]. Wilk et al. recommended the use of heat procedures, high voltage galvanic and transcutaneous neuromuscular stimulation, as best methods for preparing tissues for passive or active stretching [21]. Some authors have concluded that deep thermal procedures, especially diathermy, are more effective in reducing pain and increasing the range of motion than superficial procedures [22–24]. Draper (2014) reported that pulsed short-wave diathermy warms up a larger surface than ultrasound, which is ideal for treating contractures of major joints [25].

Heat should be applied before and during stretching. It is very important to use adequately dosed and manually stable force that does not cause muscle spasm, soft tissue damage or paresthesia [26]. It is recommended to apply manual force for twenty seconds and repeat it 4 or 5 times [27]. Passive movements should be followed by active movements. The patient should begin with low weight, submaximal effort and high repetitions [11].

Peripheral nerves injuries must be treated as well. In the further course of monitoring the recovery of nerve injury, clinical and electromyographic analyses are of great importance [28]. Neurological deficits may be treated conservatively, by electrical stimulation, vitamin therapy, and intensive exercises. There are several different opinions about the treatment of iatrogenic nerve injuries, but most iatrogenic and fracture-related nerve injuries are transient neurapraxias, which mostly disappear spontaneously in 2–3 months [28–31]. It is believed that the beginning of the nerve injury treatment has no effect on the time of recovery [32]. In cases with no clinical or electromyographic signs of nerve recovery after 3–6 months, surgical exploration and neurolysis are indicated [28, 33, 34]. Several studies showed that isolated neurological lesions are benign conditions which must be monitored and treated conservatively, by electrical stimulation and active exercises, while complete sensor-motor pa-

ralysis followed by vascular disorders requires a surgical intervention [31, 35, 36]. Treatment selection, conservative or surgical, should be individually tailored, depending on the localization, degree of dislocation, and neurologic deficit.

Corrective splints can also be used in the treatment of elbow contractures. It is recommended to wait until sufficient healing and fracture stability occurs, prior to initiating splinting to regain range of motion (ROM) in order to avoid pain, inflammation, ligamentous insufficiency, and heterotopic ossification. Splinting is most effective if initiated in the first 3 months, moderately effective after 3 to 6 months, and has variable effectiveness when initiated 6 to 12 months post injury [8]. Dynamic corrective splints operate under the principle of creep and are usually used from 8 to 12 hours per session [8, 37]. Adverse effect of splints is compression of the ulnar nerve and therefore they should be monitored and adjusted [38]. Static progressive splints operate under the principle of stress relaxation force and are used to regain range of motion [8]. Persistent and patient use of dynamic and static progressive splints is recommended for up to a year, or until the progression of the range of motion stops regardless of the etiology [39, 40]. Dynamic early rehabilitation using continuous passive motion (CPM) exercise device and dynamic splints, has great advantages compared to other therapeutic modalities and may prevent surgical treatment, and thus reduce the cost of treatment [41, 42].

Conclusion

In children and adults different factors affect reduction of mobility, pain, and treatment outcome. Any delay in the rehabilitation program may cause disability and have a negative impact on further mental and physical development of a child. The therapeutic approach to post-traumatic elbow contracture should be individual, patiently and properly dosed, with follow-up of pathogenetic and kinesiology disorders. However, physicians should be aware of the fact that particular attention should be paid to the prevention of contractures.

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IN MEMORIAM
IN MEMORIAM

Prof. dr LAZAR LEPŠANOVIĆ (1932-2016)

Prof. dr Lazar Lepšanović, redovni profesor interne medicine na Medicinskom fakultetu u Novom Sadu i redovni član Akademije medicinskih nauka Srpskog lekarskog društva preminuo je l6. oktobra 2016. godine u Novom Sadu.

Rođen je u učiteljskoj porodici u Dobanovcima, školovao se u Zemunu i Beogradu, a profesionalnu karijeru je započeo u Domu zdravlja u Inđiji. Na Kliniku za interne bolesti došao je l962. godine gde je niz godina pred penzionisanje obavljao funkciju upravnika Klinike za endokrinologiju, dijabetes i bolesti metabolizma. Doktorirao je l977. godine s tezom *Funkciono stanje kore nadbubrežnih žlezda odraslih osoba obolelih od šećerne bolesti*. Usavršavao se u više stranih zemalja, najviše u Francuskoj na Univerzitetu *Klod Bernar* u Lionu. U toku svoje profesionalne delatnosti na Klinici za endokrinologiju, dijabetes i bolesti metabolizma neprestalno je radio na usavršavanju dijagnostičkih i terapijskih procedura koristeći se znanjem stečenim u toku brojnih studijskih putovanja i boravcima na poznatim ustanovama u inostranstvu. U saradnji sa prof. dr Teodorom Kovačem osnovao je Antidijabetesnu službu pri Klinici za endokrinologiju, dijabetes i bolesti metabolizma koja je pružala kompletnu zaštitu obolelim od šećerne bolesti.

Uporedo sa radom na Klinici bavio se i nastavnim radom na Medicinskom fakultetu u Novom Sadu. Započeo je kao asistent na predmetu Interna medicina, a potom je njegova univerzitetska pedagoška karijera išla sve do zvanja redovnog profesora. Predavao je po pozivu na svim medicinskim fakultetima tadašnje Jugoslavije, ali i u inostranstvu.

Pored zdravstvene i prosvetne delatnosti, prof. Lepšanović nalazio je vremena i za naučni rad koji obuhvata sve oblasti kliničke endokrinologije i dijabetologije, ali se posebno bavio bolestima koje su specifične za našu sredinu, kao što su šećerna bolest, gojaznost i posebno poremećaji metabolizma lipida.

Prof. Lepšanović je osnivač jedne potpuno nove naučne oblasti, lipidologije gde mu je idealni saradnik bila supruga Ljiljana. Pisao je brojne publikacije iz te oblasti, kao i prve knjige sa ovom problematikom. Prof. dr Lazar Lepšanović je objavio preko 450 naučnih radova, napisao je oko 20 knjiga, a učestvovao je i u pisanju brojnih udžbenika iz oblasti interne medicine.

Bio je član Akademije medicinskih nauka Srpskog lekarskog društva, a u jednom mandatu obavljao je i funkciju potpredsednika Akademije. Bio je i predsednik Društva lekara Vojvodine, član Srpskog lekarskog društva, Udruženja endokrinologa Jugoslavije, Udruženja za borbu protiv ateroskleroze, Udruženja za proučavanje gojaznosti, Naučnog društva za istoriju zdravstvene kulture Vojvodine i Jugoslovenskog odbora za lipide. Pored toga, bio je i član brojnih udruženja u inostranstvu, kao što je Evropsko društvo za aterosklerozu, Evropsko udruženje za proučavanje dijabetesa, Internacionalno udruženje za proučavanje gojaznosti, Švajcarsko endokrinološko društvo, Nemačko udruženje za proučavanje gojaznosti. Aktivan je bio i u Matici srpskoj kao član Odbora za prirodne nauke.

Neumorno je radio u redakcijama časopisa Medicinski pregled, Srpski arhiv, *Endocrinologia Jugoslavica*, *Pharmaca*, Bilten odbora za lipide i dr.

Angažovao se i na popularisanju medicine pišući članke i knjige o šećernoj bolesti i aterosklerozi. Najpoznatije su *Vodič kroz šećernu bolesti*, *Povišen holesterol – kako ga sniziti*, *Povišeni holesterol i ateroskleroza*. Inicirao je i bio urednik popularnog časopisa za obolele od šećerne bolesti *Me Dij*.

Ovakva profesionalna i naučna delatnost nije ostala nezapažena u sredini u kojoj je živeo i radio. Dobitnik je Oktobarske nagrade Novog Sada, Zlatne plakete za životno delo Udruženja univerzitetskih profesora i naučnika Srbije, Nagrade za životno delo Srpskog lerkarskog društva, Zlatne medalje Udruženja internista Jugoslavije, nagrade Zbora liječnika Hrvatske *Vuk Vrhovac* i Nagrade za naučnoistraživački rad Društva lekara Vojvodine. Poslednja nagrada koju je dobio je *Veliki pečat* Srpskog lekarskog društva.

Slobodna sam da na kraju, kao neko ko je ceo svoj radni vek proveo uz prof. Lepšanovića, kažem par reči o njemu kao čoveku, lekaru i učitelju. Krasile su ga brojne vrline. Ne samo njegovo medicinsko znanje, već i smiren, human i strpljiv pristup bolesniku doprineli su da postane omiljeni lekar kome su se obraćali s punim poverenjem. Za njega nije postojalo radno vreme − bio je spreman da u svakom trenutku dođe na Kliniku i obiđe teško obolele. Već samo svojom pojavom, prijatnim nastupom i predanošću poslu bio je primer brojnim mladim lekarima. Studentima, svojim saradnicima lekarima i medicinskim sestrama nesebično i u svakoj prilici je nenametljivo prenosio svoje znanje i iskustvo. Imao je vremena za svakog, da sasluša probleme, da dâ savet i sugestije, a njegove kritike, uvek opravdane, nikada nisu zvučale kao kritike. Mnogi danas ugledni lekari bili su njegovi učenici. Njegovo široko obrazovanje i dobra informisanost činili su ga prijatnim i zanimljivim sagovornikom po pitanjima, kako iz struke, tako i iz istorije, likovne i muzičke umetnosti, politike i aktuelnih kretanja i dešavanja u našem društvu. Bio je poznat kao veliki poštovalac i redovni posetilac pozorišnih predstava, kao i ljubitelj klasnične muzike.

Supruzi Ljiljani je sada sigurno najteže jer su jedno drugome bili oslonac i skladno su se nadopunjavali. Neka joj uteha budu albumi sa fotografijama načinjenim na brojnim putovanjima na koja su zajedno išli, knjige koje su zajedno pisali i brojni prjatelji i poštovaoci lika i dela prof. dr Lazara Lepšanovića.

Profesor Lazar Lepšanović nije više mađu nama. Ostaju uspomene na jednog istinskog humanistu kakav se retko sreće i koga će se sećati njegovi bolesnici, učenici, saradnici i brojni prijatelji. Hvala mu za sve što je učinio i slava mu.

Prof. dr Tatjana Ivković Lazar

UPUTSTVO ZA AUTORE

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Radove koji su pristigli u časopis *Medicinski pregled* pregleda jedan ili više članova Uređivačkog odbora Časopisa. Oni radovi koji su napisani prema pravilima Časopisa šalju se na anonimnu recenziju kod najmanje dva recenzenta, stručnjaka iz odgovarajuće oblasti biomedicine. Načinjene recenzije radova pregleda glavni urednik ili članovi Uređivačkog odbora i one nisu garancija da će rad biti prihvaćen za štampu. Materijal koji je pristigao u časopis ostaje poverljiv dok se rad nalazi na recenziji, a identitet autora i recenzenata su zaštićeni, osim u slučaju ako oni odluče drugačije.

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Rukopis

Opšta uputstva

Tekst rada treba da bude napisan u programu *Microsoft Word* za *Windows*, na A4 formatu stranice (sve četiri margine 2,5 cm), proreda 1,5 (isto važi i za tabele), fontom *Times New Roman*, veličinom slova 12 *pt*. Neophodno je koristiti međunarodni sistem mernih jedinica (*SI*), uz izuzetak temperature (° *C*) i krvnog pritiska (*mmHg*).

Rukopis treba da sadrži sledeće elemente:

1. Naslovna strana

Naslovna strana treba da sadrži: kratak i sažet naslov rada, bez skraćenica, skraćeni naslov rada (do 40 karaktera), imena i prezimena autora (ne više od 6) i afilijacije svih autora. Na dnu strane treba da piše ime, prezime i titula autora zaduženog za korespondenciju, njena/njegova adresa, elektronska adresa, broj telefona i faksa.

2. Sažetak

Sažetak ne može da sadrži više od 250 reči niti skraćenice. Treba da bude strukturisan, kratak i sažet, sa jasnim pregledom problema istraživanja, ciljevima, metodama, značajnim rezultatima i zaključcima.

Sažetak originalnih i stručnih članaka treba da sadrži uvod (sa ciljevima istraživanja), materijale i metode, rezultate i zaključak.

Sažetak prikaza slučaja treba da sadrži uvod, prikaz slučaja i zaključak.

Sažetak preglednih članaka treba da sadrži Uvod, podnaslove koji odgovaraju istima u tekstu i Zaključak.

Navesti do 10 ključnih reči ispod sažetka. One su pomoć prilikom indeksiranja, ali autorove ključne reči mogu biti izmenjene u skladu sa odgovarajućim deskriptorima, odnosno terminima iz *Medical Subject Headings*, *MeSH*.

Sažetak treba da bude napisan na srpskom i engleskom jeziku. Sažetak na srpskom jeziku trebalo bi da predstavlja prevod sažetka na engleskom, što podrazumeva da sadrži jednake delove.

3. Tekst članka

Originalni rad treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima istraživanja), Materijal i metode, Rezultati, Diskusija, Zaključak, spisak skraćenica (ukoliko su

korišćene u tekstu). Nije neophodno da se u posebnom poglavlju rada napiše zahvalnica onima koji su pomogli da se istraživanje uradi, kao i da se rad napiše.

Prikaz slučaja treba da sadrži sledeća poglavlja: Uvod (sa jasno definisanim ciljevima), Prikaz slučaja, Diskusija i Zaključak.

Uvod

U poglavlju Uvod potrebno je jasno definisati predmet istraživanja (prirodu i značaj istraživanja), navesti značajne navode literature i jasno definisati ciljeve istraživanja i hipoteze.

Materijal i metode

Materijal i metode rada treba da sadrže podatke o vrsti studije (prospektivna/retrospektivna, uslove za uključivanje i ograničenja studije, trajanje istraživanja, demografske podatke, period praćenja). Detaljno treba opisati statističke metode da bi čitaoci rada mogli da provere iznesene rezultate.

Rezultati

Rezultati predstavljaju detaljan prikaz podataka koji su dobijeni istraživanjem. Sve tabele, grafikoni, sheme i slike moraju biti citirani u tekstu rada i označeni brojevima po redosledu njihovog navođenja.

Diskusija

Diskusija treba da bude koncizna, jasna i da predstavlja tumačenje i poređenje rezultata studije sa relevantnim studijama koje su objavljene u domaćoj i međunarodnoj literaturi. U poglavlju Diskusija potrebno je naglasiti da li su postavljene hipoteze potvrđene ili nisu, kao i istaknuti značaj i nedostatke istraživanja.

Zaključak

Zaključci moraju proisteći isključivo iz rezultata istraživanja rada; treba izbegavati uopštene i nepotrebne zaključke. Zaključci koji su navedeni u tekstu rada moraju biti u saglasnosti sa zaključcima iz Sažetka.

4. Literatura

Potrebno je da se literatura numeriše arapskim brojevima redosledom kojim je u tekstu navedena u parentezama; izbegavati nepotrebno velik broj navoda literature. Časopise bi trebalo navoditi u skraćenom obliku koji se koristi u *Index Medicus* (*http://www.nlm.nih.gov/tsd/serials/lji.html*). Pri citiranju literature koristiti Vankuverski sistem. Potrebno je da se navedu svi autori rada, osim ukoliko je broj autora veći od šest. U tom slučaju napisati imena prvih šest autora praćeno sa *et al*.

Primeri pravilnog navođenja literature nalaze se u nastavku.

Radovi u časopisima

∗ Standardni rad

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ Organizacija kao autor

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ Bez autora

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ Volumen sa suplementom

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ Sveska sa suplementom

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ Sažetak u časopisu

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Knjige i druge monografije

∗ Jedan ili više autora

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ Urednik (urednici) kao autor (autori)

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ Poglavlje u knjizi

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ Zbornik radova sa kongresa

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

∗ Disertacija

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Elektronski materijal

∗ Članak iz časopisa u elektronskom formatu

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ Monografija u elektronskom formatu

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ Kompjuterska datoteka

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Prilozi (tabele, grafikoni, sheme i slike)

BROJ PRILOGA NE SME BITI VEĆI OD ŠEST!

Tabele, grafikoni, sheme i slike se postavljaju kao posebni dokumenti.

– Tabele i grafikone bi trebalo pripremiti u formatu koji je kompatibilan programu u kojem je napisan tekst rada. Slike bi trebalo poslati u jednom od sledećih oblika: *JPG*, *GIF*, *TIFF*, *EPS*.

– Svaki prilog mora biti obeležen arapskim brojem prema redosledu po kojem se navodi u tekstu rada.

– Naslovi, tekst u tabelama, grafikonima, shemama i legende slika bi trebalo da budu napisani na srpskom i engleskom jeziku.

– Nestandardne priloge označiti u fusnoti uz korišćenje sledećih simbola: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– U legendi slika trebalo bi napisati korišćeno uveličanje okulara i objektiva mikroskopa. Svaka fotografija treba da ima vidljivu skalu.

– Ako su tabele, grafikoni, sheme ili slike već objavljene, navesti originalni izvor i priložiti pisano odobrenje autora za njihovo korišćenje.

– Svi prilozi će biti štampani kao crno-bele slike. Ukoliko autori žele da se prilozi štampaju u boji, obavezno treba da plate dodatne troškove.

6. Dodatne obaveze

AUTORI I SVI KOAUTORI RADA OBAVEZNO TREBA DA PLATE GODIŠNJU PRETPLATU ZA ČASOPIS *MEDICINSKI PREGLED*. U PROTIVNOM, RAD NEĆE BITI ŠTAMPAN U ČASOPISU.

INFORMATION FOR AUTHORS

Medical Review publishes papers (previously neither published in nor submitted to any other journals) from various fields of biomedicine intended for broad circles of doctors.

Since January 1th, 2013 the Medical Review has been using the service e-Ur: Electronic Journal Editing. All users of the Registration system, i.e. authors, reviewers, and editors have to be registered users with only one e-mail address. Registration should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/user/register.

Manuscript submission should be made on the web address:

http://aseestant.ceon.rs/index.php/medpreg/

A SUPPLEMENTARY FILE, WITH THE STATEMENT THAT THE PAPER HAS NOT BEEN SUBMITTED OR ACCEPTED FOR PUBLICATION ELSEWHERE AND A CONSENT SIGNED BY ALL AUTHORS, HAVE TO BE ENCLOSED WITH THE MANUSCRIPT.

Authors may not send the same manuscript to more than one journal concurrently. If this occurs, the Editor may return the paper without reviewing it, reject the paper, contact the Editor of the other journal(s) in question and/or contact the author's employers.

Papers should be written in English language, with an abstract and title page in English, as well as in Serbian language.

All papers submitted to ***Medical Review*** are seen by one or more members of the Editorial Board. Suitable articles are sent to at least two experts to be reviewed, thier reports are returned to the assigned member of the Editorial Board and the Editor. Revision of an article gives no guarantee of acceptance and in some cases revised articles are rejected if the improvements are not sufficient or new issues have arisen. Material submitted to *the Journal* remains confidential while being reviewed and peer-reviewers' identities are protected unless they elect to lose anonymity.

Medical Review publishes the following types of articles: editorials, original studies, preliminary reports, review articles, professional articles, case reports, articles from history of medicine and other types of publications.

1. Editorials – up to 5 pages – convey opinions or discussions on a subject relevant for the Journal. Editorials are commonly written by one author by invitation.

2. Original studies – up to 12 pages – present the authors' own investigations and their interpretations. They should contain data which could be the basis to check the obtained results and reproduce the investigative procedure.

3. Review articles – up to 10 pages – provide a condensed, comprehensive and critical review of a problem on the basis of the published material being analyzed and discussed, reflecting the current situation in one area of research. Papers of this type will be accepted for publication provided that the authors confirm their expertise in the relevant area by citing at least 5 self-citations.

4. Preliminary reports – up to 4 pages – contain scientific results of significant importance requiring urgent publishing; however, it need not provide detailed description for repeating the obtained results. It presents new scientific data without a detailed explanation of methods and results. It contains all parts of an original study in an abridged form.

5. Professional articles – up to 10 pages – examine or reproduce previous investigation and represent a valuable source of knowledge and adaption of original investigations for the needs of current science and practice.

6. Case reports – up to 6 pages – deal with rare casuistry from practice important for doctors in direct charge of patients and are similar to professional articles. They emphasize unusual characteristics and course of a disease, unexpected reactions to a therapy, application of new diagnostic procedures and describe a rare or new disease.

7. History of medicine – up to 10 pages – deals with history with the aim of providing continuity of medical and health care culture. They have the character of professional articles.

8. Other types of publications – The journal also publishes feuilletons, book reviews, extracts from foreign literature, reports from congresses and professional meetings, communications on activities of certain medical institutions, branches and sections, announcements of the Editorial Board, letters to the Editorial Board, novelties in medicine, questions and answers, professional and vocational news and In memoriam.

Preparation of the manuscript

The complete manuscript, including the text, all supplementary material and covering letter, is to be sent to the web address above.

The covering letter:

– It must contain the proof given by the author that the paper represents an original work that it has neither been previously published in other journals nor is under consideration to be published in other journals.

– It must confirm that all the authors meet criteria set for the authorship of the paper, that they agree completely with the text and that there is no conflict of interest.

– It must state the type of the paper submitted (an original study, a review article, a preliminary report, a professional article, a case report, history of medicine).

The manuscript:

General instructions.

Use Microsoft Word for Windows to type the text. The text must be typed in font *Times New Roman*, page format A4, space 1.5 (for tables as well), margins set to 2.5 cm and font size 12pt. All measurements should be reported in the metric system of the International System of Units – SI. Temperature should be expressed in Celsius degrees (°C) and pressure in mmHg.

The manuscript should contain the following elements:

1. The title page.

The title page should contain a concise and clear title of the paper, without abbreviations, then a short title (up to 40 characters), full names and surnames of the authors (not more than 6) indexed by numbers corresponding to those given in the heading along with the full name and place of the institutions they work for. Contact information including the academic degree(s), full address, e-mail and number of phone or fax of the corresponding author (the author responsible for correspondence) are to be given at the bottom of this page.

2. Summary.

The summary should contain up to 250 words, without abbreviations, with the precise review of problems, objectives, methods, important results and conclusions. It should be structured into the paragraphs as follows:

– Original and professional papers should have the introduction (with the objective of the paper), materials and methods, results and conclusion

– Case reports should have the introduction, case report and conclusion

– Review papers should have the introduction, subtitles corresponding to those in the paper and conclusion.

The authors should provide up to 10 keywords below the summary. These keywords will assist indexers in cross-indexing the article and will be published with the summary, but the authors' keywords could be changed in accordance with the list of Medical Subject Headings, MeSH of the American National Medical Library.

The summary should be written in both languages, English as well as Serbian. The summary in Serbian language should be the translation of the summary in English; therefore, it has to contain the same paragraphs.

3. The text of the paper.

The text of original studies must contain the following: introduction (with the clearly defined objective of the study), materials and methods, results, discussion, conclusion, list of abbreviations (if used in the text) and not necessarily, the acknowledgment mentioning those who have helped in the investigation and preparation of the paper.

The text of a case report should contain the following: introduction (with clearly defined objective of the study), case report, discussion and conclusion.

Introduction contains clearly defined problem dealt with in the study (its nature and importance), with the relevant references and clearly defined objective of the investigation and hypothesis.

Materials and methods should contain data on design of the study (prospective/retrospective, eligibility and exclusion criteria, duration, demographic data, follow-up period). Statistical methods applied should be clear and described in details.

Results give a detailed review of data obtained during the study. All tables, graphs, schemes and figures must be cited in the text and numbered consecutively in the order of their first citation in the text.

Discussion should be concise and clear, interpreting the basic findings of the study in comparison with the results of relevant studies published in international and national literature. It should be stated whether the hypothesis has been confirmed or denied. Merits and demerits of the study should be mentioned.

Conclusion must deny or confirm the attitude towards the 0based solely on the author's own results, corroborating them. Avoid generalized and unnecessary conclusions. Conclusions in the text must be in accordance with those given in the summary.

4. References are to be given in the text under Arabic numerals in parentheses consecutively in the order of their first citation. Avoid a large number of citations in the text. The title of journals should be abbreviated according to the style used in Index Medicus (http://www.nlm.nih.gov/tsd/serials/lji.html). Apply Vancouver Group's Criteria, which define the order of data and punctuation marks separating them. Examples of correct forms of references are given below. List all authors, but if the number exceeds six, give the names of six authors followed by 'et al'.

Articles in journals

∗ *A standard article*

Ginsberg JS, Bates SM. Management of venous thromboembolism during pregnancy. J Thromb Haemost 2003;1:1435-42.

∗ *An organization as the author*

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension 2002;40(5):679-86.

∗ *No author given*

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

∗ *A volume with supplement*

Magni F, Rossoni G, Berti F. BN-52021 protects guinea pig from heart anaphylaxix. Pharmacol Res Commun 1988;20 Suppl 5:75-8.

∗ *An issue with supplement*

Gardos G, Cole JO, Haskell D, Marby D, Pame SS, Moore P. The natural history of tardive dyskinesia. J Clin Psychopharmacol 1988;8(4 Suppl):31S-37S.

∗ *A summary in a journal*

Fuhrman SA, Joiner KA. Binding of the third component of complement C3 by Toxoplasma gondi [abstract]. Clin Res 1987;35:475A.

Books and other monographs

∗ *One or more authors*

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

∗ *Editor(s) as author(s)*

Danset J, Colombani J, eds. Histocompatibility testing 1972. Copenhagen: Munksgaard, 1973:12-8.

∗ *A chapter in a book*

Weinstein L, Shwartz MN. Pathologic properties of invading microorganisms. In: Soderman WA Jr, Soderman WA, eds. Pathologic physiology: mechanisms of disease. Philadelphia: Saunders; 1974. p. 457-72.

∗ *A conference paper*

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

∗ *A dissertation and theses*

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Electronic material

∗ *A journal article in electronic format*

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 1 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htmArticle

∗ *Monographs in electronic format*

CDI, clinical dermatology illustrated [monograph on CD-ROM]. Reevs JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego:CMEA;1995.

∗ *A computer file*

Hemodynamics III: the ups and downs of hemodynamics [computer program]. Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

5. Attachments (tables, graphs, schemes and photographs).

THE MAXIMUM NUMBER OF ATTACHMENTS ALLOWED IS SIX!

– Tables, graphs, schemes and photographs are to be submitted as separate documents, on separate pages.

– Tables and graphs are to be prepared in the format compatible with Microsoft Word for Windows programme. Photographs are to be prepared in JPG, GIF, TIFF, EPS or similar format.

– Each attachment must be numbered by Arabic numerals consecutively in the order of their appearance in the text

– The title, text in tables, graphs, schemes and legends must be given in both Serbian and English languages.

– Explain all non-standard abbreviations in footnotes using the following symbols *, †, ‡, §, | |, ¶, **, † †, ‡ ‡ .

– State the type of color used and microscope magnification in the legends of photomicrographs. Photomicrographs should have internal scale markers.

– If a table, graph, scheme or figure has been previously published, acknowledge the original source and submit written permission from the copyright holder to reproduce it.

– All attachments will be printed in black and white. If the authors wish to have the attachments in color, they will have to pay additional cost.

6. Additional requirements

SHOULD THE AUTHOR AND ALL CO-AUTHORS FAIL TO PAY THE SUBSCRIPTION FOR MEDICAL REVIEW, THEIR PAPER WILL NOT BE PUBLISHED.