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COAGULATION DISORDERS AFTER TRAUMATIC BRAIN INJURY

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Isolated traumatic brain injury (TBI) is often associated with abnormalities in coagulation parameters. Prehospital fluids exceeding 2,000 ml may be associated with coagulation disorders in patients with TBI. The aim of this study was to investigate the incidence of coagulation disorders, to establish its relation to the outcome, and to establish a correlation between prehospital fluid infusion and development of coagulation disorders.

The study included 82 patients with isolated brain injury. Coagulation parameters were determined using the values of prothrombin time (PT), activated partial thromboplastin time (APPT) and platelet count. We also analyzed a correlation between prehospital administered fluid and the occurrence of coagulopathy.

Pearson's correlation analysis showed that in terms of survival, there was no significant difference between the groups (group A OR 37 CI 0.11-1,27; group B OR 0,48 CI 0,16-1,49; group C OR 0,69, CI 0,24-1,98), but it also indicated that prehospital fluid administered in a larger amount was in a negative correlation with the treatment outcome (-0,240).

The results of our studies have confirmed the correlation of coagulation abnormalities with the lethal outcome in patients with TBI. Administration of more than 1,500 ml of fluid is associated with more frequent occurrence of coagulation disorders and with poor outcome.

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Key words: *traumatic brain injury, coagulation disorders*

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Introduction

Isolated traumatic brain injury (TBI) is often associated with abnormalities in coagulation parameters, although the incidence of such disturbances remains poorly defined. The presence of coagulation disorder has been linked to the progression of both hemorrhagic and ischemic lesions (1, 2) and is associated with increases in morbidity and mortality (3-7). The mechanisms underlying coagulopathy after TBI are still poorly understood. The most commonly accepted hypothesis of the pathogenesis of coagulopathy after TBI implies alterations in local and systemic coagulation and fibrinolytic pathways secondary to the release of tissue factor (TF) (8-10), disseminated intravascular coagulation (11-13), platelet dysfunction, (14-16), and activation of protein C pathways secondary to hypoperfusion (17, 18).

Early recognition of coagulopathy is of value in predicting the occurrence of delayed brain injury and may contribute to prevention of bleeding disorders (19).

Most studies report about early coagulopathy after the arrival at the emergency department, which was associated with increased morbidity and mortality (20). Although the prevalence of coagulopathy increases after the patient admission at the emergency department, there is a lack of evidence about delayed coagulopathy (21-23).

Recent findings also suggest that prehospital fluids exceeding 2,000ml may independently be associated with coagulopathy in patients with isolated blunt TBI (24).

The aim of this study was to investigate the incidence of early and delayed coagulopathy, and to establish a relation to the outcome; in addition, the aim was to establish a correlation between prehospital fluid infusion and development of coagulopathy.

Material and methods

The study included 82 patients of both genders and various ages with isolated brain injury. In all the patients, on admission to the ICU, the level of consciousness using the Glasgow comma score was assessed. Coagulation parameters were deter-

mined using the value of prothrombin time (PT), activated partial thromboplastin time (APPT), and platelet count.

Using the Marshal and Marshal modified scale, radiological classification of brain injury was performed. The study did not include patients with injuries of other organs or organ systems.

Coagulopathy is defined as an extension of aPTT > 40 seconds and < or PT, or platelet count less than 120x109.

Patients were divided into two groups: a group which manifested coagulopathy and a group in which coagulopathy was not detected. The group with coagulopathy was divided into three groups: group A, consisting of patients who had coagulopathy upon their arrival, group B which included patients who developed coagulopathy after 24 hours, and group C which comprised patients in whom coagulopathy occurred 48 hours after the injury.

A correlation with the outcome was made, both of the groups with and without coagulation, as well as the analysis of outcome in subgroups with coagulopathy. In addition, we also analyzed a correlation between prehospital administered fluid and the occurrence of coagulopathy.

Statistical analysis was performed using the standard programs for data processing - MS EXCEL and software packages R. Using descriptive statistical analysis, the following statistical parameters were shown: number, percentage, arithmetic mean, standard deviation and median interval of variation (min-max).

Using analytical statistical methodology, we measured the statistical significance of mutual differences in the frequency of occurrence of certain characteristics in patients who had been divided into groups. The tests were performed (by Chi-square test). Comparisons of mean values between the groups were performed by t-test for independent samples or by nonparametric Mann-Whitney test (in case CV > 30%). For the purpose of measuring the relation of certain characteristics we performed the correlation analysis.

Results

The study included 82 patients with isolated severe traumatic brain injury. The presence of coagulopathy on hospital admission was registered in 17 % (20.7) patients with average age of 50.35 years (± 16.71) and its average value was GCS 5 (GCS 3-7); 11 patients were males (64.7%) and 6 patients were females (35.3%). Sixty-five patients on admission had no signs of coagulopathy. Their demographic characteristics are shown in Table 1.

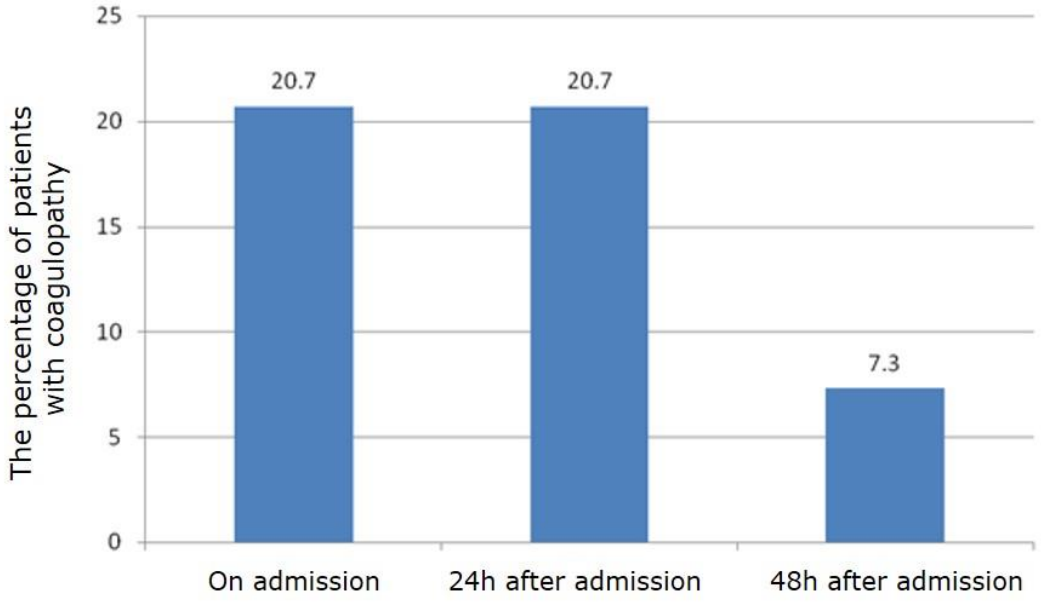
Table 1. Condition on admission

	Coagulopathy	**No coagulopathy**	**p**
N (%)	17 (20.7%)	65 (79.3%)	n.s.
Age	50.35 ± 16.71	48.28 ± 16.08	n.s.
Male	11/17 (64.7%)	42/65 (64.6%)	n.s.
Female	6/17 (35.3%)	23/65 (35.4%)	
GCS (mediana)	5	5	n.s
GCS (range)	3-7	3-8	
Pre - hospital fluid	1,102.94 ± 905.96	780.77 ± 804.66	n.s.

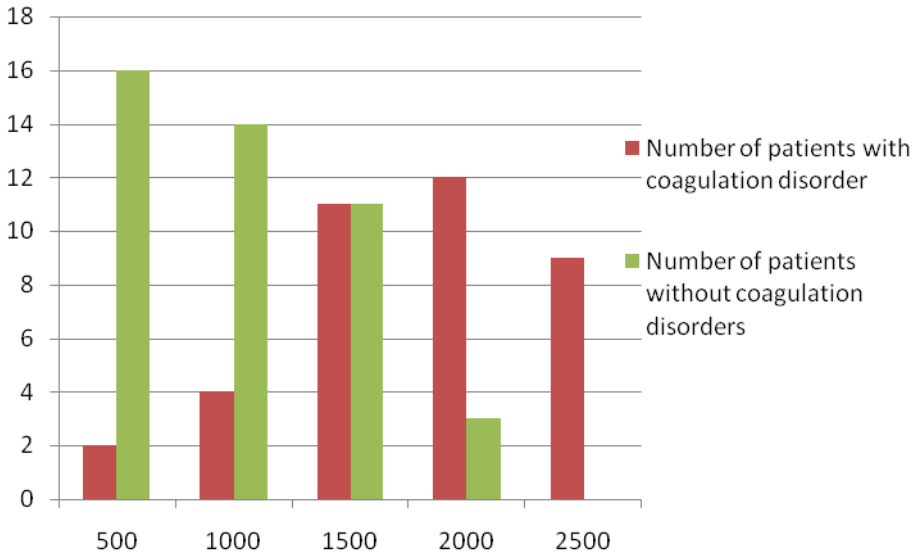
n.s. value of parameters among patients with coalugopathy and without coagulopathy do not differ significantly

There was no statistically significant difference found in the age structure and structure by gender. Also, the values of the Glasgow comma score were not statistically significant in the examined groups, which is also shown in Table 1.

Graph 1 shows the distribution of coagulation disorder development. The correlation between the development of bleeding disorders and the administered prehospital liquid is shown in Graph 2.



Graph 1. Distribution of patients with coagulopathy on admission, 24 hours after, and 48 hours after the admission



Graph 2. Number of patients with coagulation disorders compared to prehospital fluid administration

In the examined groups, there was no difference in gender and age distribution as well as in the values of GCS on admission.

Pearson's correlation analysis showed that in terms of survival, there was no significant difference between the groups (group A OR 37 CI 0.11-1,27; group B OR 0,48 CI 0,16-1,49; group C OR 0,69, CI 0,24-1,98), but it also indicated that prehospital fluid administered in a larger amount was in the negative correlation with the treatment outcome (-0,240).

Discussion

The mechanism of development of coagulopathy after TBI is still controversial. It is believed that excessive release of tissue factor (TF) leads to the activation of the mechanisms of blood coagulation. TF, previously known as thromboplastin, is a protein that is localized in subendothelial tissue, leucocytes and platelets (25). A release of TF from the injured brain tissue thus leads to excessive acti-

vation of the mechanism of blood coagulation. A loss of balance between the mechanisms of coagulation, on the one hand, and those of anticoagulation, on the other hand, leads to the development of systemic coagulopathy. Also, it is considered that the level of coagulation is in direct proportion to the serum level of TF (26).

The occurrence of coagulation disorders after an isolated injury of CNS leads to a higher incidence of lethal outcome in patients who exhibit the disorder, which has been confirmed in a number of studies (27). The current theories indicate that liberated TF acts locally on the arterioles and venules forming microthrombi dimension 10-600 micrometers (28), which leads to ischemia of the brain parenchyma near the trauma. In the studies of Stain et al., a significant correlation between severity of ischemic changes and density of intravascular microthrombosis was found (27, 29, 30). The reduction of blood flow in the vicinity of contusion change is also attributed to microthrombosis. The local microthrombosis may occur as a delayed one and is considered to be the main cause of secondary ischemic brain injuries (31). Liberated TF can have its effect also on distant parts of the brain, as well as on distant organs considering that it passes through a damaged blood brain barrier (BBB). In that way, the ischemic changes may develop in almost all organs, thus creating conditions for the development of multiple organ failure syndrome (MOF) (31).

Besides the undeniable correlation with the lethal outcome, there is a clear link between coagulation and increase in intracranial hemorrhagic masses, which leads to a greater damage of the CNS tissue and more severe neurological sequelae (32, 33), but could also lead to the lethal outcome. Allard et al. observed a four-fold increase in mortality (32% compared to 8%) in patients with hemorrhagic progression of the injury, on the successive CT images. Their study found that the greatest risk is associated with those injured patients in whom the aPTT was increased on admission. Other coagulation parameters had lower correlation with progression of hemorrhage (34).

The presence of coagulopathy ranges from 10-86%. It is considered that the reason for the discrepancy lies in the definition of coagulopathy which was accepted by the authors, but also in a common consideration of early and delayed coagulation. One of our study objectives was the analysis of the time of the appearance of coagulation disorders and their correlation with the outcome. In our study, the early coagulopathy, which we defined as a disorder of one of the followed coagulation parameters on admission to the ICU, occurred in 20,7%. After 24 hours, the number of patients with coagulopathy was doubled (20,7% of the patients developed some form of coagulopathy), while on the third day, the occurrence of the disorder was found in additional 7.31% of the patients. Such dynamics of appearance of the disorder was also found in other studies i.e. data from a large German Registry of Patients (34) which are related to 3,000 patients indicating that during the first 24 hours the number of patients with coagulopathy doubled, and that coagulopathy was possible to detect also three days after the trauma (35).

In our study, there was no occurrence of coagulopathy after the third day, although in the available literature one may find data on a possibility of its occurrence even six days after the trauma (36, 37). Such data impose an obligation of subsequent laboratory analysis in relation to dynamics of disorder development.

Our study was designed to perform the analysis among the group with early coagulopathy, the group with coagulopathy detected after 24 hours, and the group where coagulopathy occurred after 48 hours, but also to make a correlation with the outcome. In terms of survival, there was no statistically significant difference between the groups of patients with early coagulopathy and both groups with delayed coagulopathy (the group with early coagulopathy on admission OR 37 CI 0.11-1,27; the group with delayed coagulopathy after 24 hours OR 0,48 CI 0,16 - 1,49; the group with delayed coagulopathy after 48 hours OR 0,69, CI 0,24-1,98). Our results show that the presence of coagulopathy is sufficient, while dynamics of its occurrence does not affect the appearance of undesirable outcome.

Our data showed that prehospital administration of 1,500 ml of fluid was associated with increased occurrence of coagulation disorders, while Pearson's correlation analysis showed that the amount of prehospital administered fluid was in a negative correlation with the survival (-0,240).

Treatment of patients with confirmed TBI involves treatment that includes prevention of secondary brain injuries. That means maneuvers which increase the arterial pressure, since even short episodes of hypotension are associated with significantly poorer prognosis (38). However, the excessive administration of fluids also has adverse effects probably due to dilution of coagulation factors, which leads to the development of a state similar to DIC and also correlates with poor outcome. Patients who were administered prehospital fluid had worse prognosis compared to the group of patients who did not receive prehospital fluid. Data presented by Meagele et al. (24) among other demonstrate that prehospital administration of more than 2,000 ml of fluid is associated with more frequent occurrence of coagulation disorder. Our study determined that administration of more than 1,500 ml of fluid is associated with more frequent occurrence of coagulation disorders and with poor outcome. The lack of our study is in the absence of data on the values of TA during transportation so that it is not possible to discuss the reasons for prehospital administration of fluids. Current recommendations suggest that in patients with TBI prehospital maintenance of systolic blood pressure above 110 mmHg is necessary. Yet, for definitive determination of desired value of TA, more extensive study is required.

Conclusion

The results of our studies have confirmed the correlation of coagulation abnormalities with the lethal outcome in patients with TBI, regardless of the chronology of its creation. Early or postponed coaglation disorder is directly connected with increased

mortality. This conclusion imposes an obligation of a regular, routine control coagulation status.
Prehospital condition of the patient often requires the use of physiological solution. However, if the situation allows, it is desirable to control the intake of fliuds and limit their amount to 1500-2000ml.

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POREMEĆAJ KOAGULACIONE FUNKCIJE NAKON NEPENETRANTNIH POVREDA MOZGA

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Izolovana nepenetrantna povreda mozga (TBI) često se povezuje sa abnormalnostima koagulacionih parametara. Prehospitalno data tečnost, koja prelazi 2.000 ml, može biti povezana sa poremećajima koagulacije kod bolesnika sa TBI. Cilj ove studije bio je da se ispita učestalost pojave poremećaja koagulacije i da se uspostavi odnos sa ishodom, kao i da se uspostavi korelacija između prehospitalno administrirane tečnosti i pojave poremećaja koagulacionih parametara.

Studija je obuhvatila 82 bolesnika sa izolovanom povredom mozga. Parametri koagulacije su definisani korišćenjem vrednost PT, apt i trombocita. Takođe, analizirali smo povezanost prehospitalno ordinirane tečnosti i pojave koagulacionih poremećaja.

Pirsonova analiza korelacije je pokazala da u pogledu preživljavanja nije bilo značajne razlike između grupa (grupa A ili 37 CI 0,11-1,27; grupe B ili 0,48 CI 0,16-1,49; grupi C ili 0,69, CI 0,24-1,98), ali je, takođe, ukazala da je prehospitalno ordinirana tečnost u količini većoj od 1500 ml u negativnoj korelaciji sa rezultatima lečenja (-0,240).

Rezultati naših istraživanja potvrdili su povezanost poremećaja koagulacije sa smrtnim ishodom kod bolesnika sa TBI. Administracija više od 1500 ml tečnosti je povezana sa češćim pojavama poremećaja koagulacije i sa lošim ishodom.

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Ključne reči: *nepenetrantna povreda mozga, poremećaj koagulacije*

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PSYCHOLOGICAL CHARACTERISTICS OF PATIENTS WITH PSORIASIS: OUR EXPERIENCE

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In consultative psychiatry, we have also noticed negative emotional reactions and mood disorders in patients with psoriasis. In this paper, we wanted to determine the presence of psychological symptoms and psychiatric disorders among patients with psoriasis and an association between psychological traits and severity of psoriasis.

We examined 30 patients with psoriasis, using a consecutive method of patient selection. The severity of psoriasis was determined by the PASI score. Psychological assessment was done by the application of an unstructured clinical interview, M.I.N.I, for psychiatric disorders as well as KON-6 inventory for psychological traits: extroversion, somatization, and neuroticism. Pearson`s linear correlation was used to determine the relation between t-values of psychological dimensions and the values of PASI score.

One quarter of the sample had mild depression, anxiety and panic disorder. Patients with mild psoriasis had lower neuroticism, and those with extroversion had lower tendency to somatization.

Our patients described feeling tension, discomfort, and shyness due to their low self–esteem. A low degree of psychiatric comorbidity is probably due to the sample size.

Mild psoriasis is associated with low neuroticism, and further follow-up of the patients is needed to examine the psychological and medical outcome in relation to the severity of psoriasis.

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Key words: *psoriasis, psychological characteristics*

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Introduction

In the field of liaison psychiatry, psychodermatology deals with a psychological aspect of dermatologic disorders. Skin and nervous system have mutual embryonic origin, enabling psychophysiological mechanisms to disturb the neuro-immuno-endocrine functions and take part in dermatologic disease (1).

Psychodermatology refers to the three groups of disorders:

1.Dermatological conditions that are worsened or initiated by stress: urticaria, alopecia areata, psoriasis, acnae, dermatitis saeborrhioica.

2.Psychiatric conditions with dermatologic consequences due to self-harming behavior: dermatitis artefacta, excoriation neurotica, trihotilomania.

3.Secondary developed psychological reactions to skin diseases affecting the appearance: negative emotions, psychiatric syndromes associated with vitiligo psoriasis, ichtiosis, tumors, and others changes of the exposed skin (face, neck, hands).

Psoriasis is a chronic inflammatory skin condition, and usually it is associated with psychological issues. During treatment, dermatologist is in a position to notice such changes in a patient's behavior as well as their emotional reactions. A connection between slow recovery, reoccurrences, and long duration of the disease with chronic distress and unfavorable life events is well known (2). Chronic distress, anxiety, depression, suppressed inner tension through limbic activation and hypothalamic-pituitary adrenal stimulation increase catecholamine and sympathetic autonomic function as well as cortisol dysfunction (3). Patients with psoriasis have increased stress response to social stressors and higher cortisol levels compared to healthy controls (4). This vulnerability to stress might increase the vulnerability to

the onset or worsening of psoriasis. The other important link is decreased brain-derived neuroptophic factor (BDNF) in psoriatic patients due to sensitivity to stress. This neurotrophin is important for neuroplasticivity, neuronal growth, cognitive processing and copping mechanisms. Psychological stress could influence a decrease of BDNF in patient plasma (5). Negative emotions and distress reactions may precede the occurrence of the psoriasis or follow the illness as psychological consequences of disturbed look of their skin. Visible skin manifestations are the basis of lower self-esteem and psychopathological reactions, when psychological help is important.

In consultative psychiatry, we have also noticed negative emotional reactions and mood disorders in patients with psoriasis. In this paper, we wanted to determine the presence of subjective psychological symptoms and psychiatric disorders among patients with psoriasis. The second aim was to find an association between psychological traits and severity of psoriasis.

Material and methods

We have examined 30 patients with psoriasis of both genders, using a consecutive method of patient selection. They all had a chronic form of the disease diagnosed by a dermatologist in the previous 1-3 years. Participants gave their written consent for participating in the study. After their regular check-up in an ambulatory setting, the dermatologist determined the severity of the disease by the psoriasis area severity index (PASI) score. Light forms of disease were ranged 0-18, moderate 19-36, severe 37-54, very severe 55-72. Evaluation was based on the appearance of the lesion surface, the presence of erythema, and indurations and desquamation of the affected area. Then, the dermatologist referred patients to psychiatrist for further evaluation.

Psychiatric assessment included:

1. The use of unstructured clinical interview to get information about subjective psychological symptoms, their duration and intensity, and also to establish a therapeutic relation with patients. The participants fulfilled demographic questionnaire after the interview.

2. Mini International Neuropsychiatric Inter view (6), (M.I.N.I.) is a questionnaire designed for diagnosing psychiatric disorders. Two psychiatrists used the scale to confirm the presence or absence of psychiatric disorder.

3. Neurotic personality inventory KON-6 test is a self-rated questionnaire (7) which includes the following subscales: ALFA (it measures the level of neuroticism and a tendency to anxiety reactions); EPSILON (the level of extroversion and energetic level of functioning) HI (tendency to somatization).

Our study is only part of wider research, considering psychiatric evaluation of other chronic inflammatory diseases. In this paper, we present only data of patients with psoriasis. An association with other diseases will follow.

We analyzed data by SPSS for Windows, version 16. Pearson`s linear correlation was used to determine a correlation between t-values of psychological dimensions and values of PASI score for the whole sample and in the subgroup with low PASI score.

Results

Our patients were in their thirties, most of them were men, unemployed, and almost two thirds of the sample had no emotional partner at the time of the assessment. According to M.I.N.I. and by comparing the diagnosis with criteria in the ICD-10 manual, over 70 % of participants had no psychiatric condition. Chronic mood disturbance (mild depression) was the most frequent diagnosis, while panic disorder was present only in one patient. A mild form of skin condition was present in one third of the subjects, whereas the rest of them had moderate and severe forms of psoriasis (Table 1).

Table 1. Demographic characteristic, psychiatric diagnosis, and PASI score of the participants

Variables	N(%) 30
Age	32.33
Men	21 (70.00)
Living with partner	12 (40.00)
Employed	8 (26.66)
Without psychiatric disorder	22 (73.33)
Anxiety disorder	3 (10.00)
Dysthymia	4 (13.33)
Panic disorder	1 (3.33)
PASI <18	10 (30.00)
PASI (18-72)	20 (66.66)

We assessed the psychological characteristics of the subjects and compared their t-values too see a relation between psychological dimensions and PASI score in the whole group. The value of $p < 0.05$ was considered significant. Considering the whole sample, there was a correlation only between the psychological variables: extroversion (EPSILON) and tendency to somatization (HI), meaning that extroverts have lower tendencies to somatization (Table 2).

For further analysis, we divided the participants into two groups. The first group included patients with a mild form of psoriasis (PASI < 18). The second group included the moderately severe and very severe forms of the disease (PASI 19-72). We compared the severity of psoriasis with psychological dimensions. Only in subjects with the light form of psoriasis we found the same negative correlation between extroversion and tendency to somatization.

There was a negative correlation, with statistical significance, between low PASI score and ALFA score – neuroticism (Table 3). Subjects with mild psoriasis were less prone to neurotic (anxiety) reactions.

There was no correlation between the PASI score and psychological traits for the whole sample and for the more severe form of psoriasis.

Table 2. A correlation between psychological dimensions and PASI score for the sample

		EPS	HI	ALFA	PASI
EPSILON	Pearson's correlation	1.000	-0.545*	0.096	-0.112
	Sig. (2-tailed)	0	0.013	0.687	0.693
	N	30	30	30	30
HI	Pearson's correlation	-0.545*	1.000	0.394	0.231
	Sig. (2-tailed)	0.013	0	0.085	0.326
	N	30	30	30	30
ALFA	Pearson's correlation	0.096	0.394	1.000	0.195
	Sig. (2-tailed)	0.687	0.085	0	0.410
	N	30	30	30	30
PASI	Pearson's correlation	-0.112	0.231	0.195	1.000
	Sig. (2-tailed)	0.639	0.326	0.410	0
	N	30	30	30	30

EPSILON-Extroversion
HI-somatization
ALFA-neurozicism
PASI- psoriasis area severity index

Table 3. A correlation between PASI score and psychological characteristics in the group with a light form of psoriasis

		EPS	HI	ALFA	PASI
	Pearson's correlation	1.000	-0.674*	0.028	-0.294
EPSILON	Sig. (2-tailed)	0	0.033	0.939	0.410
	N	10	10	10	10
	Pearson's correlation	-0.674*	1.000	0.572	-0.194
HI	Sig. (2-tailed)	0.033	0	0.084	0.591
	N	10	10	10	10
	Pearson's correlation	0.028	0.572	1.000	-0.500*
ALFA	Sig. (2-tailed)	0.939	0.084	0	0.135
	N	10	10	10	10
	Pearson's correlation	-0.294	-0.194	-0.500*	1.000
PASI<18	Sig. (2-tailed)	0.410	0.591	0.135	0
	N	10	10	10	10

EPSILON-Extroversion
HI-somatization
ALFA-neurozicism
PASI- psoriasis area severity index

Discussion

In our consultative psychiatry practice, we have noticed some psychological issues coexisting with psoriasis, particularly in forms affecting uncovered parts of the body. A chronic skin disease has a negative impact on the appearance and quality of life and may precipitate psychological reactions or

psychiatric comorbidity. The researches in this field accentuate that vulnerability to stress contributes to maintenance of the skin disease and mental health problems as well (8). Our group of patients had chronic skin condition in the most active period of their lives. Still, most of them were neither married nor had a partner. This could be a result of their prudency and insecurity in social relations due to the appearance of their skin. Psychiatric interview included the questions about emotional reactions to everyday situations, unfavorable events in their lives, and the onset or exacerbation of psoriasis. Most of the subjects could not associate the beginning of the disease with a particular stress event, but with precipitating undesirable circumstances and chronic distress. This finding is different from the research of Hunter et al. from 2013 (2). They found a temporal relationship between stress events and exacerbation of psoriasis. Similar findings reported in Scandinavian research (9) showed subjective association between acute distress and exacerbation of psoriasis. Their subjects also emphasized anxiety traits, lack of assertiveness and depression. Our patients described feeling tension, discomfort, and shyness due to their low self-esteem. According to the psychiatric interview, they also have less coping abilities in social situations. In younger patients, we have noticed a tendency to social isolation and stigmatization, even with mild and moderate forms of the disease. Anxiety is the main symptom of distress reactions and neurotic disorders. Considering anxiety disorders, only one patient had panic attacks with secondary agoraphobic complication and avoidance behavior. He avoided leaving home due to a fear of fainting, but on a deep psychological level due to shame and fear of rejection because of his physical appearance. Chronic anxiety was present in 10% of the sample. The patients were prone to emotional hypereactivity to everyday problems. Their sensitivity in social relations and general inhibition are the basis of a tendency to distress reactions. Skin discomfort, scratching and inching were accompanied with worry, emotional tension and general anxiety, as was found in a prospective study of Verhoeven et al. from 2009 (10). In our sample, 13% of patients had a chronic mood disorder - mild depression, with subjective evaluation of the coexistening depression and exacerbation of psoriasis. This result differs from those of large studies because of the small sample. In a Danish study (11), five million people were evaluated for depression. Individuals with severe form of psoriasis and with other medical comorbidity had a greater risk for the onset depression in a five-year prospective follow-up. In another cohort study done in the United Kingdom, patients with severe psoriasis were at greater risk of depression, anxiety and suicidality (12). The investigators concluded that early detection and treatment of psychiatric disorders is important for better control of the disease. Our patients were young adults, without other medical condition at the time of the assessment, but future evaluation of the same group may show a different medical and psychiatric outcome.

We expected more patients with a psychiatric diagnosis, but the majority of patients with psoriasis had some psychological symptoms, without fulfilled criteria for psychiatric disorders. A small number of respondents may be the reason for only few psychiatric syndromes associated with psoriasis. In order to determine the psychological characteristics of our patients, we used a KON-6 questionnaire and measured some personality traits: neuroticism, extroversion, and somatization. There was no correlation between t-values of the tests and PASI score. In our sample, we did not detect a relationship between the severity of psoriasis and some personality dimensions. Analysis of the subgroup PASI < 18 showed that less neuroticism was associated with a light form of the skin condition. We can assume that in a larger sample, moderate and severe psoriasis would be in relation with higher neuroticism. Considering other two dimensions, our patients with a tendency to extroversion had less somatization. The former psychological characteristic is a typical mechanism present in psychosomatic disorders, meaning that psychological stress is experienced on the somatic level of functioning (13), contributing to somatic/ dermatological illness and quality of life (14). In our further evaluation, we will see if subjects with extroversion have better prognosis in relation to subjects with a tendency to somatization.

A limitation of our study is a small number of respondents and lack of a prospective follow-up of patients. Our experience in consultative psychiatry indicates the presence of negative emotions in patients with psoriasis, often of subclinical level, without a diagnosis of a psychiatric condition. These results point to the necessity to analyze a larger number of patients with different forms of psoriasis in order to evaluate the impact of psychological characteristics on the prognosis and quality of life. Stress reduction intervention (15) and counseling can be helpful for improving their psychological issues and a possible course of a skin condition.

Conclusion

Our patients with psoriasis had a low degree of psychiatric comorbidity, mild depression and anxiety disorders. Psychological symptoms in the majority of patients are: somatic tension, worry, and insecurity in social relations. Patients with a light form of psoriasis had less neuroticism.

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PSIHOLOŠKE KARAKTERISTIKE BOLESNIKA SA PSORIJAZOM: NAŠA ISKUSTVA

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U konsultativnoj psihijatrijskoj praksi primetili smo negativne emocionalne reakcije i poremećaje raspoloženja kod bolesnika sa psorijazom. U našem radu smo želeli da utvrdimo prisustvo psiholoških simptoma i psihijatrijskih poremećaja među bolesnicima sa psorijazom i odnos između psiholoških crta i težine psorijaze.

Pregledali smo 30 ambulantnih bolesnika sa psorijazom po metodi uzastopnih odabira. Težinu psorijaze smo odredili putem PASI skora. Za psihološko ispitivanje smo upotrebili: nestrukturisani klinički intervju, M.I.N.I. upitnik za postavljanje psihijatrijske dijagnoze, KON-6 inventar za određivanje crta ličnosti. Pirsonovom linearnom korelacijom smo odredili odnos između t-vrednosti psiholoških dimenzija sa vrednostima PASI skora.

Četvrtina uzorka je imala blagu depresiju, anksiozni i panični poremećaj. Bolesnici sa blagom formom psorijaze su imali manji neuroticizam, oni sa ekstroverzijom su imali manje izraženu somatizaciju.

Naši bolesnici su zbog niskog samopoštovanja osećali napetost, nelagodnost, stidljivost. Mali psihijatrijski komorbiditet je verovatno zbog malog uzorka ispitanika.

Blaga psorijaza je povezana sa manjim neuroticizmom i potrebno je dalje praćenje ispitanika da bi se ispitao psihološki i medicinski ishod u odnosu na težinu psorijaze.

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Ključne reči: *psorijaza, psihološke karakteristike*

Original article

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EVALUATION OF THE IMPORTANCE OF PERCUTANEOUS LIVER BIOPSY IN NEWLY DIAGNOSED DIFFUSE AND FOCAL LIVER LESIONS

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Percutaneous liver biopsy (PLB) is an important diagnostic procedure in routine clinical practice because it allows for a fast pathohistological diagnosis. The aim of this study was to assess the importance of PAB in the diagnosis of newly recognized diffuse and focal liver lesions. This retrospective study included 277 patients who underwent PLB between January 2006 and December 2015. After the initial single dose of midazolam sedation, interventions were conducted using local infiltrative anesthesia (2-8 mL lidocaine 2% with adrenaline) under the guidance of ultrasound or computerized tomography, using the transabdominal or transthoracic approach, depending on the lesion site. Fine 14-20 gauge needles were used. In 52 patients referred with the diagnosis of indeterminate diffuse liver lesions who underwent PLB and histopathological analysis, the following results were obtained: 35 patients had steatosis hepatis (67.3%), 12 patients were with cirrhosis (23.7%), and 5 patients had hepatocellular carcinoma (9%). Of 164 with the diagnosis of primary liver tumors (164), the presence of malignant tumors was confirmed in 140 patients (85.3%), while the remaining 24 patients (14.7%) had benign lesions. From the total of 42 patients with the referral diagnosis of metastatic liver disease, colorectal carcinoma metastases were confirmed in 31 patients (73,8%), while ovarian cancer metastases were diagnosed in 6 patients (14,3%). As a minimally invasive interventional radiology procedure, PLB is an indispensable tool that allows for a fast diagnosis and decision-making in patients with diffuse and focal liver lesions.

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Key words: *percutaneous liver biopsy, liver lesions, diagnosis*

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Introduction

Significant development of interventional radiology in the past few decades has resulted, among other things, in the introduction of numerous diagnostic and therapeutic procedures for the patients with digestive system diseases. Percutaneous liver biopsy (PLB) is an important diagnostic procedure in routine clinical practice. It allows for a fast non-surgical pathohistological diagnosis and is used in patients with suspected, newly discovered diffuse and focal liver lesions. The main features of this procedure are its minimal invasiveness, low incidence of complications and most commonly uneventful post-procedural recovery, which are the reasons why these interventions are usually performed on an outpatient basis, contributing thus to cost-effectiveness as well. The most common complication of these interventions is bleeding. The mortality rate is very low. Due to an increase of routine application of PLB, its importance in the diagnostic algorithm of liver disease should be evaluated.

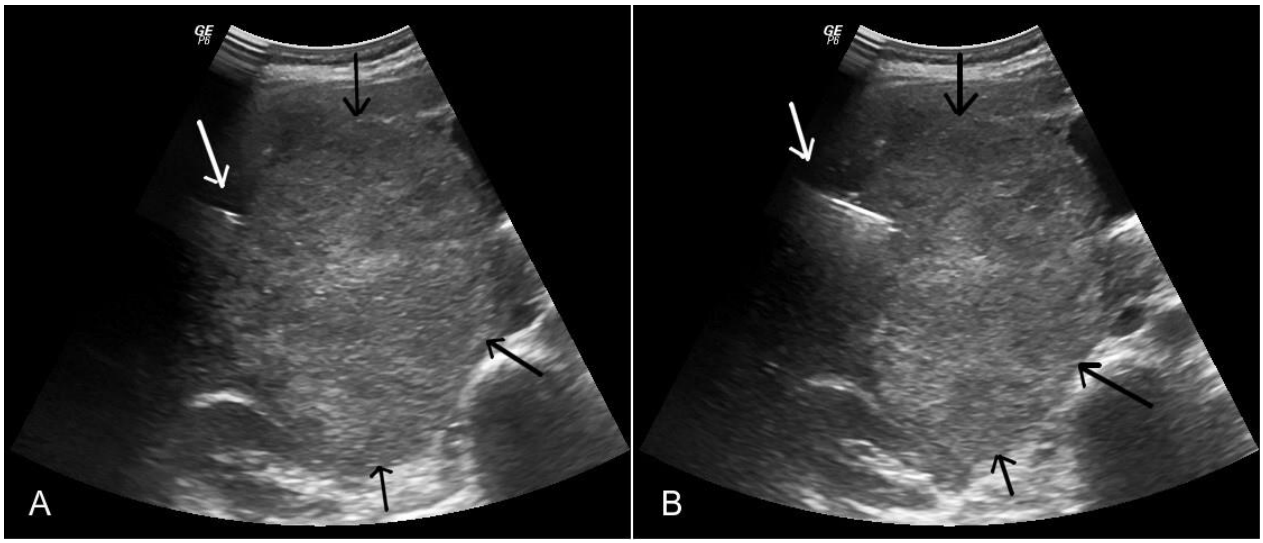
The aim of the study

The aim of our study was to assess the role of PAB in the diagnosis of newly recognized diffuse and focal liver lesions.

Material and methods

This retrospective study included 277 patients who underwent PLB between January 2006 and December 2015. In all the patients, the initial imaging diagnosis of liver tumor(s) was made at the Department of General Surgery, Clinical Center Niš, while the interventional radiological procedures were carried out at the Institute of Radiology, Clinical Center Niš.

There were 162 men (58.4%) and 115 women (42.6%) aged 59 years on the average (range, 19-78 years). After the initial radiological diagnosis, standard preprocedural preparation was carried out, including blood type determination and laboratory tests: complete blood count, blood clotting tests and general serum biochemical analysis - glucose, electrolytes, nitrogenous products, liver function tests, amylase, CRP. The criteria for exclusion were patient non-cooperation, significantly altered coagulation status (platelet count < 60,000/mm3, INR > 1.5, hemophilia, recent use of anticoagulants, fibrinolytic and antiplatelet agents), suspected liver hydatid cysts and hemangioma, obesity, infection of the anterior abdominal wall or right pleural space, unavailability of blood components and derivatives, previous interventions in less than 7 days and small lesions with highly suspected or evident involvement of large perihepatic blood vessels (inferior vena cava, hepatic veins on their origin from inferior vena cava and portal vein). After the initial sedation with a single dose of midazolam, interventions were conducted using local infiltrative anesthesia (2-8 mL 2% lidocaine with adrenaline) under the guidance of ultrasound or computerized tomography, using the transabdominal or transthoracic approach, depend-ing on the position of the lesion. Fine needles of dif-ferent diameters (14-20 gauge) were used (Figure 1).



*Bard biopsy system with MAGNUM type of gun and needle for core-biopsy (16 gauge)

Figure 1. Two ultrasound images of a large liver tumor percutaneous biopsy
(white arrow depicts needle, black arrows depict tumor):
A – biopsy needle introduced into the liver parenchyma;
B – needle tip in the tumor during the sample taking

Results

Initial referral radiological imaging diagnoses indicating PLB in analyzed patients are shown in Table 1.

Of 52 patients referred with the diagnosis of indeterminate diffuse liver lesions after PLB and histopathological analysis, the following results were obtained: 35 patients had liver steatosis (67.3%), 12 patients had cirrhosis (23.7%), and 5 patients had hepatocellular carcinoma (HCC) (9%). The data are shown in Table 2.

After PLB and histopathological analysis in patients with the diagnosis of primary liver tumors (164), the presence of malignant tumors was confirmed in 140 patients (85.3%): hepatocellular carcinoma (HCC) in 101 and cholangiocellular carcinoma (CHC) in 39 patients, while the remaining 24 patients (14.7%) had benign lesions (focal nodular hyperplasia - FNH, adenoma, haemangioma). The results are summarized in Table 3.

From the total of 42 patients with the referral diagnosis of metastatic liver disease, 31 were with suspected metastases of colorectal carcinoma (CRC), and 7 were with suspected ovarian cancer metastasis. In the first subgroup of patients, the diagnosis of CRC metastases was confirmed in 31 patients (73,8%), while in the other subgroup the diagnosis of ovarian cancer metastases was confirmed in 6 patients (14,3%). The remaining five had benign lesions (Table 4).

Table 1. Distribution of referral diagnoses.

The referral diagnosis	Primary liver tumor	Metastatic liver tumor	Liver cirrhosis	Wilson's disease	Liver cystadeno-carcinoma	Diffuse liver lesion	Caroli disease
Number/ percentage	164 (59,2%)	42 (15,2%)	10 (3,6%)	2 (0,7%)	6 (2,2%)	52 (18,8%)	1 (0.4%)

Table 2. Distribution of patients with the diagnosis of indeterminate diffuse liver lesions after performed PLB

Unknown referral diagnosis	Steatosis hepatis	Cirrhosis hepatis	HCC
Number/ percentage	35 (67,3%)	12 (23,7%)	5 (9%)

Table 3. Histopathology results

Primary liver tumors	Benign liver tumors	Malignant liver tumors	
Number/ percentage	24 (14,7%)	140 (85,3%)	
		HCC	CHC
		101 (72,1%)	39 (27,9%)

Table 4. Patients with metastatic liver diseases.

Metastatic liver tumors	Metastases of CRC	Metastases of ovarian cancer	Benign liver lesions
Number/ percentage	31 (73,8%)	6 (14,3%)	5 (11,9%)

There were no lethal outcomes. The incidence of post-interventional syndrome was 11.2% (31 patients) and this included transient symptoms such as pain in the right hypochondrium lasting up to 12 hours and requiring analgesia with metamizol-sodium (4,5 g per patient on the average) and/or nausea which required antiemetic therapy. For 19 of these patients (61,3%), it was necessary to complete the intervention with percutaneous drainage of the Morison's pouch or subhepatic space due to uncertain hemostasis at the biopsy site, but in all of these patients the drain was removed after 24-hour observation, when the possibility of bleeding and other leakage was ruled out.

There was no need for the administration of vitamin K, fresh frozen plasma, platelets or whole blood transfusions in any patient. Two patients required additional second-act percutaneous drainage for post-procedural low productivity (less than 100 ml/24h) biliary fistula from the site of the biopsy which was followed by spontaneous regression after 4 days in both of the patients and subsequent drain removal. The drains in all the patients were removed after normal follow-up abdominal ultrasound. In our patients there was no significant post-interventional fever which would require treatment. Immediately before the intervention all the patients received single dose prophylactic antibiotics (2 g ceftriaxone).

For all the patients the procedure was performed on an outpatient basis (admission in the morning, release in the evening, after a full-day observation), except in those with post-procedural syndrome (31 patients), who were hospitalized, and released from the clinic after 24 hours because of discomfort and/or drainage, or after 4 days in case of 2 patients with biliary fistulas. Other less common but reported complications were not encountered (such as sepsis, reaction to the anesthetic agent, breaking of the biopsy needle, iatrogenic perforation of neighboring hollow organs with peritonitis, intrahepatic arteriovenous fistula, pneumothorax, hematoma in the abdominal wall at the site of cannulation). None of the patients required operative reintervention (laparotomy).

Discussion

It is believed that the first aspiration liver biopsy was done by Erlich in 1883, while the first diagnostic PLB was published in 1923 (1). Since then, the technical aspect of the procedure has been, naturally, significantly modified. Therefore, during the last three decades, this intervention has taken a central place in the diagnostic algorithm of liver disease. Due to a very low mortality rate (from 0.01 to 0.17%) and relatively low morbidity, this procedure is now widely used routinely, even in smaller health facilities by interventional radiologists, gastroenterologists and surgeons (2). The progress in the field of medical technology, especially radiological imaging techniques, contributes significantly to the expansion of PLB application, and has caused a significant expansion of the indication areas for this procedure. However, despite the growing experience, there are still significant differences in the basic principles of PLB application, especially in terms of the strategic position of this diagnostic intervention, as evidenced by the lack of standardized protocols in almost all institutions.

Bearing in mind that, although at very low incidence rates, PLB may be accompanied by a variety of complications, even when it is implemented by an experienced physician, according to recommendations, it should be performed only when a pathohistological finding is necessary for definitive diagnosis and treatment initiation or continuation. In patients with acute hepatitis of unknown etiology, PLB may be indicated for differential diagnosis between viral and other causes of inflammation (e.g., medication damage), while in those with chronic viral hepatitis, the indications for PLB may be different: monitoring of the inflammation activitiy (e.g., assessment of the so-called Hepatitis Activity Index, a necro-inflammation/necrosis „scoring"–system) (3), monitoring of morphologic response of liver tissue to therapy, or a pathological diagnosis of suspected tumor lesions in the field of viral inflammation (e.g., HCC). Similarly, the primary diagnostic and follow-up diagnostic value of PLB (differential diagnosis and monitoring of the evolution - "staging" of the disease) is undoubtful in patients with other benign liver diseases, such as Wilson's degeneration, Caroli's disease, hepatic steatosis, cirrhosis (alcoholic or primary biliary), primary sclerosing cholangitis, specific infections (e.g., tuberculosis), etc.

The indication for PLB in the patients with focal liver tumefactions largely depends on the clinical circumstances in which radiological diagnosis was made. By far the largest number of patients with both solitary and multifocal tumor lesions of the liver are recruited during the oncological follow-up following surgery for primary CRC. However, unfortunately, metastatic liver disease is an expected and very common form of progression of colorectal malignancy, thus in most of these patients PLB is not necessary, due to high clinical suspicion and assuredness about the metastatic origin of these lesions. The situation is completely the opposite for de novo discovered tumors of the liver with radiological (US, MSCT or NMR) features of metastases in patients without data on any primary malignant disease. In these patients PLB is a diagnostic method of choice for establishing histopathological diagnosis and therefore detection of primary malignancies. It is similar in patients with primary malignant tumors of the liver with or without jaundice (HCC or CHC), regardless of the possibility of operative treatment. In these patients PLB may have a different diagnostic importance: for histopathological confirmation of the diagnosis to perform curative resection, for the application of neoadjuvant (preoperative) oncological therapy or for the implementation of definitive oncological therapy (for inoperable cases). Mixed cystic-solid malignant lesions (e.g. cystadenocarcinoma) require greater vigilance and skilfulness in performing PLB, considering that a solid component suitable for a biopsy may be a very small part of the lesion, and therefore may not be accessible for percutaneous approach. Caution when indicating PLB in suspected malignant liver lesions is required, because of bleeding risk as well as documented risk of dissemination of tumor cells along the biopsy route (4). Benign liver lesions (e.g. FNH, hemangioma, etc.) are very rare indications for PLB for two reasons: they are mainly characterized by a clear radiological presentation and can be detected with high accuracy using MSCT or NMR, or are commonly associated with complications (e.g., bleeding from hemangioma or due to present amyloidoses).

Both the type and incidence of post-procedural complications in our patients are consistent with the literature data. The pain is by far the most common component of „post-interventional" syndrome, including our own study (5). Major bleeding is very rare (0,35-0,5%) (6), in contrast to the so-called subclinical bleeding that may occur in up to 23% of patients and does not require treatment (blood supplementation or re-intervention), which was also the case in our patients (7). The reduction of unwanted post-procedural developments is achieved by precise radiological diagnosis, clear indications, good selection and adequate pre-procedural preparation of the patients, as well as by closely observing the technical principles involved in the performance of the procedure (the correct choice of access and equipment used, appropriate needle and analgesia with sedation).

Conclusion

Rapid development of medical knowledge and related technologies have made PLB an indispensable tool in the diagnosis of many liver diseases. Although it is primarily an interventional radiology procedure, PLB is nowadays used by physicians of numerous other specialties (gastroenterologists, surgeons, oncologists) in their daily routine. However, more research with systematized results is needed to contribute to the strategic standardization of PLB in the diagnostic algorithm.

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PROCENA ZNAČAJA PERKUTANE BIOPSIJE JETRE U DIJAGNOSTICI NOVOOTKRIVENIH DIFUZNIH I FOKALNIH LEZIJA JETRE

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Perkutana aspiraciona biopsija (PAB) jetre predstavlja važan dijagnostički postupak u svakodnevnoj kliničkoj praksi, jer omogućava neoperativno postavljanje patohistološke dijagnoze. Cilj rada bio je da se utvrdi značaj PAB jetre u dijagnostici novootkrivenih difuznih i fokalnih lezija jetre. Sprovedena je retrospektivna studija koja je obuhvatila 277 bolesnika kojima je urađena PAB jetre u periodu između januara 2006. godine i decembra 2013. godine. Nakon inicijalne sedacije pojedinačnom dozom midazolama, intervencije su obavljane korišćenjem lokalne infiltrativne anestezije (2-8 mL 2% lidokaina sa adrenalinom) pod vođstvom ultrazvuka ili kompjuterizovane tomografije, transabdominalnim ili transtorakalnim pristupom, u zavisnosti od lokalizacije lezije. Korišćene su punkcione igle različitog promera (14-20 gauge). Od 52 bolesnika sa dijagnozom neidentifikovane difuzne lezije jetre, nakon izvršene PAB jetre i patohistoloških analiza, dobijeni su sledeći rezultati: 35 bolesnika imalo je steatosis hepatis (67,3%), kod 12 je dokazana ciroze jetre (23,7%), a kod 5 bolesnika postojanje hepatocelularnog karcinoma (9%). Od 164 bolesnika sa uputnom dijagnozom primarnih tumora jetre, potvrđeno je prisustvo malignih tumora kod 140 bolesnika (85,3%), dok su preostala 24 (14,7%) dokazano imala benigne lezije. Od ukupno 42 bolesnika sa uputnom dijagnozom metastatske bolesti jetre, metastaze KRK su potvrđene kod 31 bolesnika (73,8%), dok su metastaze karcinoma ovarijuma potvrđene kod 6 bolesnika (14,3%). Iako primarno interventna radiološka procedura, PAB jetre je nezamenjivo oruđe u dijagnostici novootkrivenih difuznih i fokalnih lezija jetre.

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Ključne reči: *perkutana biopsija jetre, lezije jetre, dijagnostika*

Review article

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RADIOLOGICAL DIAGNOSIS OF MALIGNANT TUMORS OF THE ORAL CAVITY

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The oral cavity and oropharynx represent the topmost parts of the digestive tract, which is unique due to both its complex anatomy and tissue structures localized in a small area. In the head and neck region, oral carcinomas are characterized by high prevalence and mortality, multifactorial etiology and delayed diagnosis. Their prognosis, as in other tumors, depends on the disease stage. More than 90% of the mouth and oropharynx malignant tumors are histopathologically diagnosed as squamous cell carcinomas. Their clinical diagnosis is based on the inspection and palpation, and cranial nerve neurological examinations. The use of computerized tomography (CT) and magnetic resonance imaging (MRI) is a key step in the staging of oral cavity tumors and adequate therapy planning. The knowledge of radiological anatomy and pathology of this region is of great importance in making adequate diagnostic conclusions.

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Introduction

The oral cavity and oropharynx represent the topmost parts of the digestive tract, which is unique due to both its complex anatomy and tissue structures localized in a small area (1). Its main role is food intake, chewing and food preparation for swallowing. Although the sinonasal complex is the main respiratory pathway, the mouth can be considered as a secondary respiratory pathway with a crucial role in phonation. Lip movement allows for different functions such as: speech, coughing and facial expression. The sense of taste is located in the mouth (2).

According to the International classification of diseases and related health problems, tenth revision, issued by the World Health Organization (WHO), oral cavity carcinomas are classified as follows: lip (excluding the skin of the lip), tongue, gum, floor of the mouth, palate and carcinomas of other unspecified parts of the mouth (cheek mucosa, vestibule of the mouth, retromolar area and small salivary glands), excluding carcinoma of the large salivary gland (3). As for the head and neck area, oral cavity carcinomas, characterized by high prevalence and mortality, multifactorial etiology and delayed diagnosis, represent a public health issue, especially in the developing countries (4). Additionally, the treatment of these carcinomas represents a great therapeutic challenge due to poor prognosis of late stage disease, as well as due to the influence of therapeutic treatments on the mouth and pharynx functions.

The oral cavity and pharynx carcinomas are the sixth most frequent malignant tumors worldwide and seventh in Serbia. Their incidence varies with the geographical region. The highest incidence has been found in South Asia, Pacific islands, Latin America and in parts of the Central and Eastern Europe (5).

More than 90% of mouth and oropharynx malignant tumors are histopathologically diagnosed as squamous cell carcinomas (SCCs). Less frequently occurring are the verrucous carcinomas, small salivary gland carcinomas, lymphomas, sarcomas and other malignant neoplasms. According to incidence by regions, squamous cell carcinoma of the oral cavity is the most frequent one, followed by the anterior two thirds of the tongue and floor of the mouth (6).

Clinical evaluation of the oral cavity carcinoma is based on clinical examinations, inspection and palpation, with the addition of the cranial nerve neurological examination. Utilization of computerized

tomography (CT) and magnetic resonance imaging (MRI) represents the main diagnostic procedure in the staging of oral cavity tumors and adequate therapeutic approach planning.

Oral cavity squamous cell carcinoma etiology

Oral cavity SCC etiology is multifactorial and according to the available global evidence, the known risk factors for these tumors could be divided into two main groups: modifiable (risk behavior related) and non-modifiable ones (sociodemographic) (7).

The main etiological factors for the carcinomas of this region are tobacco and alcohol consumption. They can act separately, but their combined influence is boosting the carcinoma incidence, especially if larger amounts are consumed (a large number of cigarettes/alcoholic drinks per day/week) in a prolonged period of time. Moreover, quitting smoking after the initial diagnosis of oral cavity carcinoma and therapy initiation increases survival rates (7).

Recent studies in the USA have revealed a connection between the incidence of orofacial carcinomas and sexually transmitted human papilloma virus (HPV) infection, especially HPV16 (8). The combined analysis of the International head and neck cancer epidemiology consortium (INHANCE) found a correlation between the carcinomas of these regions and risky sexual behavior and HPV infection (9).

The most important non-modifiable etiological factors for the development of oral region carcinomas are low socioeconomic status, age and gender. Oral carcinoma is more frequent in males than in females and around two thirds of all oral cavity carcinomas belong to this type. In parallel, there is an increased risk of this carcinoma in older people, occuring mostly at the age of 50 and over (7,8).

Radiological diagnosis of squamous cell carcinoma of the oral cavity

Early diagnosis of oral cavity carcinoma is a public and health priority, since early discovery contributes to the decrease of mortality and better prognosis during and after treatment.

Although oral cavity SCCs, due to their localization, could be detected by inspection, most of them are discovered in advanced disease stages. Persistent ulcerations and bleeding in the mouth, presence of a mass, swallowing and speech difficulties and ear pain are the most frequent symptoms of oral and oropharyngeal carcinomas. However, some tumors, especially those of the tongue region, can be asymptomatic for a long period of time. Superficial areas with mucosa lesions, such as leukoplakia or erythroplakia, detected at clinical examinations are often the sites with dysplasia or even invasive carcinoma, when the final diagnosis is based on biopsy findings.

Adequate choice of therapy and assessment of disease prognosis involve determination of the grade of the disease. This includes the use of imaging techniques such as CT and MRI. These radiological methods are the ones most frequently utilized, due to their reliability in the estimation of tumor spread, vascular infiltration and metastases to the surrounding tissues. Positron emission tomography (PET) together with the imaging techniques provide combined functional (metabolic) and anatomical information, resulting in a precise diagnosis and a greater chance for improvement of the patient condition (10).

The main advantages of CT compared to MRI are shorter examination period, fast image processing, availability, lesser impact of movement artefacts, and better visualization of destructive bone lesions. Exposure to high x-ray radiation doses, as well as artefacts originating from dental implants, represent the disadvantages of this radiological method.

Depending on CT itself, scan protocols can vary. Native (native-phase) and postcontrast scans are usually performed from the skull base to the top edge of the manubrium (chest bone), with a great capability of image reconstruction. Axial sections at the oral cavity and oropharynx levels should be parallel, while coronal sections should be perpendicular to the hard palate plane. Due to the fact that oral cavity carcinomas are accompanied by tumors of the pharynx, larynx and tracheobronchial tree, the area to be scanned should be expanded to the chest cavity as well (6, 11).

On the other hand, MRI examination allows a better visibility of the soft tissue structures, i.e. tumors, their spread, bone marrow and neurovascular infiltration, especially for the detection of small changes. However, long examination periods, impact of movement artefact, limited access and high cost are the main shortcomings of this method. A high level of cooperation with the patient is needed in order to perform MRI, thus it is not applicable in claustrophobic patients, those with high dyspnea and with metal implants. Standard MRI examination involves the same scan area as in CT, in axial, sagittal and coronal planes. T1W, T2W and STIR, with additional postcontrast T1W with fat suppression and diffusion imaging (DWI) sequences are used. Postcontrast T1W sequence provides the best visualization of tongue tumors, as well as their perineural spreading. Tumor spread to the lateral tongue muscles is best visualized with axial and coronal T2W sequences, in combination with postcontrast sequence. On the other hand, tumor spreading to the bone marrow is best seen using native T1W, STIR and postcontrast T1W sequences. The presence of cancer cells in lymph nodes is estimated using T2W, coronal STIR and postcontrast T1W sequential diffusion (DWI) sequences (11).

When CT is applied, these tumors appear as badly limited areas and with a homogenous density increase after contrast injection. Larger tumors are mostly heterodense due to necrosis.

On T1W MR images tumors appear hypointense or isointense with muscles, whereas on T2W MR images tumors are mostly hyperintense. Solid tumors present as a signal increase after gadolinium contrast application, while the areas of necrosis inside the tumor tissue remain hypointense. Precontrast T1W images are useful for the differentiation between tumor and surrounding fatty tissues, detec-

tion of spread to the bone and neurovascular structures.

Pathways of spread of oral cavity squamous cell carcinoma

Oral SCCs most frequently spread in one of the following three pathways: (a) by direct invasion through the mucosal surface, muscles or bones; (b) by lymph; or (c) by perineural dissemination (12).

a) Direct invasion – initial mucosal spread is best seen during a physical examination. Superficial lesions are radiologically invisible, whereas submucosal and deeper structure dissemination into the fatty and muscle tissues is visible.

The main shortcoming of both of these radiological examinations is low specificity, i.e. one cannot make the difference between tumor tissue and inflammatory changes. Cortical bone invasion is represented as a discontinuation or an erosion with hyperdense edges on CT image or hypointense on all MRI sequences. Subtle cortical bone erosion can be best detected in the bone window during CT examination, while MRI is the most suitable for visual assessment of the extent of medullar invasion. Low signal intensity on native T1W, high intensity on T2W with increased signal intensity after contrast application can correspond to tumor invasion of the bone marrow, but the same image can be seen in inflammation, peritumoral edema and osteomyelitis.

b) Lymphogenic dissemination – large percentage of the oral cavity and oropharynx carcinomas is initially manifested with a change on the neck that corresponds to enlarged lymph nodes, frequently I-III level.

Most of the tumor - affected nodes are enlarged. Nodes with size larger than 1 cm in their transversal shorter diameter, except of those of II level (12 mm), are considered to be pathological. Nodal morphology assessment is also an important criterion. The ratio between longitudinal and transversal nodal diameters is normally higher than two, while pathologically affected nodes are mostly round. Unclear node margins with an increase in the local fatty tissue density on CT examination, and high signal intensity with fat suppression in T2W sequence and postcontrast T1W MRI favor extracapsular spreading. The grouping of three or more border sized lymph nodes in the primary tumor region strongly points to lymph node metastases. Central necrosis represents the most reliable radiological criterion in neck lymph node metastasis diagnostics independent of the tumor size. It is represented as a central hypodense/hypointense area with the edge postcontrast intensity increase.

c) Perineural dissemination – this type of tumor spreading is a characteristic of invasive mouth SCCs and is defined as a malignant cell dissemination via nerve fibers. During CT examination, increased neural opening and surrounding fatty tissue obliteration can be seen. Increase and postcontrast intensification of the nerve and surrounding fat signals is seen on MRI. Muscular denervation atrophy is manifested as a loss of muscle volume and its replacement with fatty tissue.

TNM classification of oral cavity squamous cell carcinoma

Head and neck tumor TNM staging system is an anatomical estimation of the primary tumor spreading, dissemination to the locoregional lymph nodes and the presence of distant metastases. Stage T is defined by the tumor size, depth of invasion and the affected vital structures. In VIIth issue of TNM classification, T4 stage is divided in to T4a and T4b and it depends on the affected vital structure and suitability for surgical treatment. The presence of lymph node metastases (N), their size, number, as well as the presence of extracapsular spreading are the most important prognostic factors for SCC of this region. Distant metastases (M) include the lymph nodes that are affected by tumor tissue cells, except the level VII. Their incidence increases with tumor grade. The most frequent localization of M in carcinomas of this region are the lungs, less frequently the liver and brain (13).

Having in mind that SCC has a different incidence for different regions of the oral cavity and has unique regional spreading patterns, the following text will be dedicated to this topic (6, 12).

Squamous cell carcinoma of the lip

Mucosal carcinoma, i.e. carcinoma of the inner lip surface is the most common malignant neoplasm of this region, representing 40% of all oral cavity SCCs. The lower lip is predominantly affected, between the middle line and labial commissure, while the upper one is rarely affected. There are three morphological types: exophytic, ulcerative and verrucous. These tumors slowly progress, with rare regional lymph node metastases occuring much later compared to other oral cavity carcinomas. More than a half of patients during the initial radiological examination has a tumor mass larger than 1.5 cm. Native CT and MRI examinations of patients with these tumors show a tumor mass with undefined margins with or without an area of ulceration, while the postcontrast image reveals variable density/signal intensity increase. In the beginning, bone erosion could most frequently be seen at the alveolar ridge region in the bone window during CT examination. Larger tumors could affect the mandible or the mental nerve directly, infiltrate the skin and subcutaneous fatty tissue of the buccal region, floor of the mouth and masticatory space.

Squamous cell carcinoma of the oral part of the tongue

This type of carcinoma commonly originates from the lateral edge of the middle 1/3 of the tongue or from the ventral side and its prognosis is generally better compared to the same tumor of the tongue base which has a lower histological degree of differentiation (Figure 1). Infiltrative, ulcerative or exophytic growth forms can be seen, while most often it is clinically manifested when its dimensions are higher than 2 cm, with the presence of enlarged lymph nodes of the I and II level. Infiltrative carcinomas initially affect lateral tongue muscles (hyo-

glossus, palatoglossus and styloglossus) and spread towards the medial part of the tongue, affecting the genioglossal muscle and lingual septum, ending at the contralateral side. Large tumors arising from the lateral tongue edge can spread towards the tonsillar sulcus, tonsillar fossa, base of the tongue, floor of the mouth, mandible and submandibular spaces, while those from the ventral side spread directly to the floor of the mouth or the base of the tongue.In addition to the standard axial view, the coronal and sagittal MR views are of greater help here, since they give more information concerning the lesion size as well as the state of the neurovascular structures. Perineural spreading along the lingual nerve is responsible for the pain sensations in the ipsilateral ear in patients in the advanced stages.

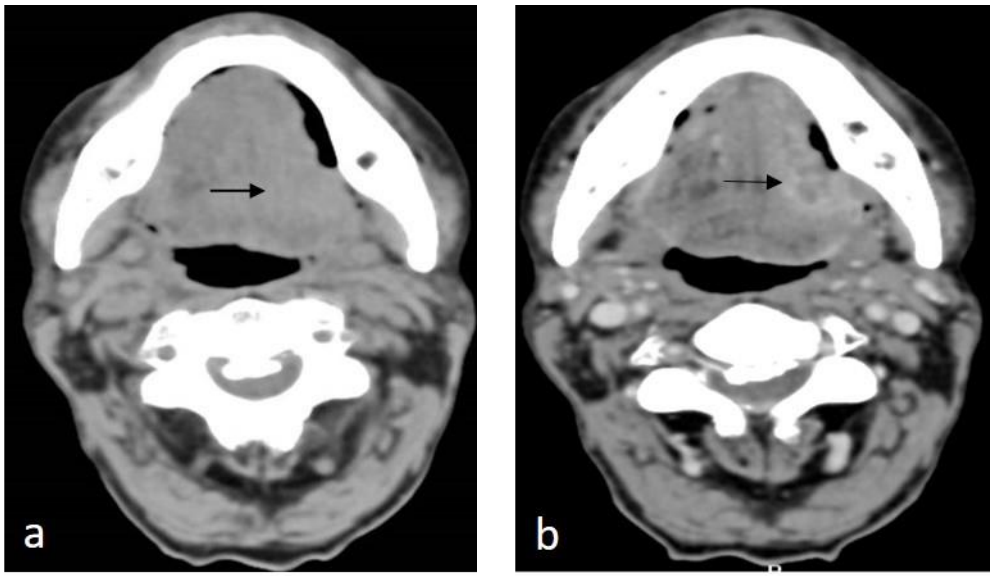

Figure 1. Squamous cell carcinoma of the tongue, native (a) and postcontrast (b)*

*Native axial CT image, with the presence of a homogenous tumor mass of the left tongue half (arrow) asymmetrically compared to the right side (a). Axial CT image where the increase of postcontrast density of the left tongue half (arrow) can be seen corresponding to the tumorous mass (b).

Squamous cell carcinoma of the floor of the mouth

This type of tumor originates from the mucosa that overlays the space between the gums of the lower jaw and the bottom side of the tongue. Most of the tumors are found on the anterior part of the mouth floor. In the beginning, the lesion is very small, while with the progress characteristic papillary or exophytic formation, spreading in different directions, can be found. Often one of the first disease manifestations is the consequence of direct spreading of the tumor, causing the obstruction of the Warthon's duct and submandibular gland sialadenitis. Tumor spreads in both superior and posterior directions affecting the anterior surface and the base of the tongue, as well as to other neurovascular structures. In the anterior and lateral directions, SCC spreads to the gingival mucosa, mandible and medial pterygoid muscle. In the advanced stages, the process can expand outside the oral cavity, most frequently to the parapharyngeal and masticator spaces. Regional I and II level lymph node metastases, predominantly unilateral, occur later compared to the metastases of SCC of the tongue.

Squamous cell carcinoma of the retromolar triangle

Although this is a small region of the mouth, covered with mucosa and located behind the last molar in the mandibular ramus, retromolar triangle represents a crossroads between the oral cavity, oropharynx, soft palate, buccal space, floor of the mouth, masticator and parapharyngeal spaces. Carcinoma of this location can spread to some of the surrounding structures (Figures 2 and 3), including the tonsils, base of the tongue, to the temporal muscle via pterygomandibular raphe, medial to the pterygomandibular space, in the inferior and anterior direction to the floor of the mouth and pterygopalatine fossa, respectively.

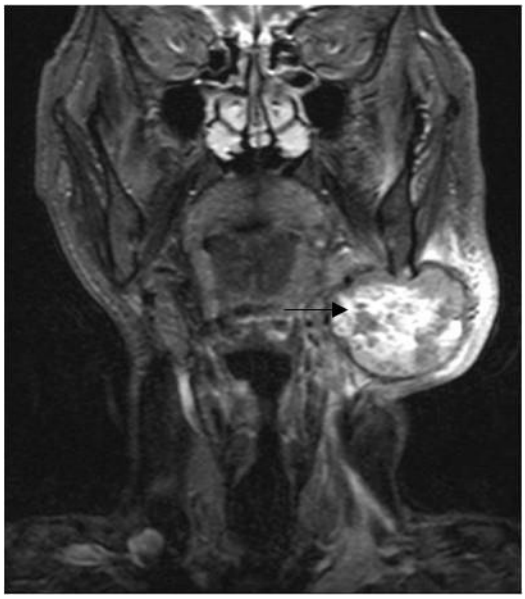

Figure 2. Squamous cell carcinoma of the mandibular gingiva on the left side**

**Coronal T2W MR image with fat suppression where a neoplastic change of the mandibular gingiva on the left side can be seen (pathohistological confirmation) spreading to the floor of the mouth and the equilateral neck region (arrow).

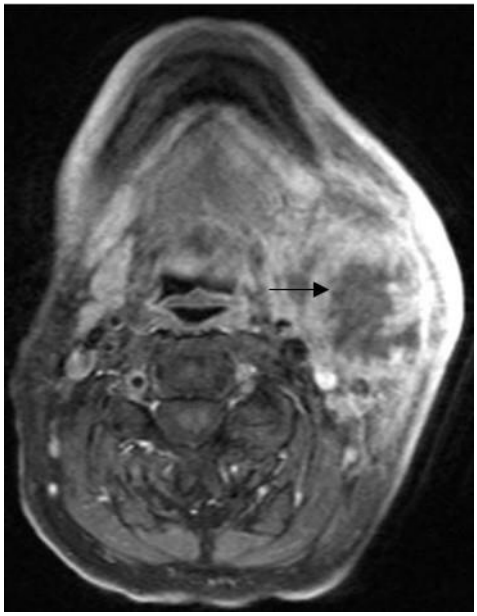

Figure 3. Squamous cell carcinoma of the mandibular gingiva on the left side[2]***

***Axial T1W MR image showing a peripheral postcontrast tumorous mass signal intensity increase with areas of central necrosis, surrounding tissue infiltration and spreading to the retromolar triangle (arrow).

Squamous cell carcinoma of the buccal region and gums

Even though only 10% of all oral cancers are localized in these regions they represent a potential danger for the surrounding bone structures and their invasion. Buccal tumors are most frequently arising from the lateral margin of the mouth and spread through submucosa, alongside buccinator muscle towards pterygomandibular raphe and bone cortex, affecting masticatory space, the skin, tonsils and soft palate. Regional metastases occur early affecting the submandibular, facial, parotid and periauricular lymph nodes. SCC of the gums arises from the edge of premolars and molars, where the most common radiological sign is the bone destruction with metastases present in level I and II lymph nodes.

Squamous cell carcinoma of the hard palate

Tumors of this region can occur in the middle or lateral parts of hard palate. It is of great importance to estimate the degree of bone invasion and to exclude perineural spreading, which could also be seen in adenoid cystic carcinoma and lymphoma. The tumor can spread along the greater and lesser palatine nerve into the pterygopalatine fossa, from where along the maxillary nerve through the foramen rotundum, progresses through infraorbital foramen or along the Vidian's nerve in the Vidian channel. In the advanced stage of the disease the invasion of the upper jaw, nasal cavity, buccal mucosa, tongue or retromolar triangle can occur. Lymphogenic dissemination affects facial, retropharyngeal lymph nodes and level II lymph nodes.

Atypical forms of squamous cell carcinoma

Atypical forms of SCC are the variants with special histological and immunohistochemical characteristics, such as verrucous carcinoma or basaloid squamous cell carcinoma (6,12,14).

Verrucous carcinoma or Ackerman's tumor typically occur on the buccal mucosa or lower jaw gums in older males that consume tobacco. Clinical appearance of these tumors is in a form of papilla, where in the early stage it has an excellent prognosis after the surgical treatment. Locally invasive forms can affect the mandible, while the metastases in the regional lymph nodes almost never occur.

Basaloid squamous cell carcinoma occurs in males in their sixth or seventh decades with the history of pronounced tobacco and alcohol consumption. It represents a exophytic ulcerative formation, most frequently in the region of the floor of the mouth and has a bad prognosis. The diagnosis of these tumors is a late one and the tumors aggressive behavior is reflected in perineural invasion, regional and distant metastases in the lungs, liver, bones, brain and skin. Compared to other types of the same region carcinomas, basaloid squamous cell carcinoma shows a high percentage of recidives, distant metastases and shorter survival rates.

Other malignant tumors of the oral cavity

The tumors with the highest incidence in this group are small salivary gland tumors and lymphomas.

Small salivary gland carcinomas are the second most common tumors of the oral cavity. It is considered that there is around 500-1000 small salivary glands in the mouth and oropharynx, especially in the region of soft and hard palate where, on their junction, these tumors occur most frequently. Opposite to SCC, these carcinomas occur in younger persons, in their fifth and sixth decade, with an equal distribution between the genders. They are characterized with a slow growth rate and most commonly with local invasion and better prognosis. Histologically two forms can be distinguished: adenoid cystic carcinoma, which is the most common mucoepidermoid carcinoma. There are three types of adenoid cystic carcinoma: cribriform (grade I), tubular (grade II) and solid (grade III). Depending on the grade of malignancy, characteristic submucosal and perineural spreading, along the maxillary or mandibular nerve, can be encountered. CT examination reveals a homogenous postcontrast density increase, with possible signs of hard palate bone erosion and an expansion of the grater palatine and round opening. Using MRI, low signal intensity in T2W points to the high grade tumors with high cellularity, while high signal intensity using the same sequence corresponds to low grade types and have a better prognosis. On the postcontrast T1W image the increase in tumor and maxillary nerve intensity can be observed. Regional lymph node metastases are rare, which is the opposite to mucoepidermoid carcinomas where metastases are frequent.

Although Hodgkin and non-Hodgkin lymphomas are common in the head and neck region, primary lymphomas of the oral cavity and oropharynx are rare and they most commonly affect the structures of Waldeyer's ring. In the oral cavity the nodal form of non-Hodgkin lymphoma in the submandibular space can occur. It is represented with multiple, enlarged and mostly non-necrotic lymph nodes, on both sides at the level I. They are revealed by the homogenous postcontrast signal intensity increase, except in the case of the high grade form with central necrosis (12, 14).

Conclusion

Although the presence of oral cavity carcinomas, due to their localization, can be detected in early stages by inspection, most of the diagnosed cases are in advanced stages of the disease. Due to the mentioned issues the involvement of contemporary radiological examination, such as computerized tomography and magnetic resonance imaging, during the diagnosis is of vast importance. Their use is necessary for disease staging, which influences further therapeutical treatment.

Reliability and precision of these imaging methods depends on the adequate radiological criteria application, especially TNM staging system, optimal scanning techniques, with the necessary knowledge of the radiological anatomy and pathology of this region.

The application of positron emission tomography, together with the imaging techniques, would allow more precise diagnosis and a greater chance for improvement in the patient's state.

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RADIOLOŠKA DIJAGNOSTIKA MALIGNIH TUMORA USNE DUPLJE

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Usna duplja, zajedno sa usnim delom ždrela, predstavlja najviši deo digestivnog trakta, jedinstven po složenoj anatomiji i tkivnoj strukturi unutar malog prostora. Posmatrajući oblast glave i vrata, oralne karcinome odlikuju visoka učestalost i mortalitet, multifaktorijalna etiologija i najčešće kasno postavljena dijagnoza. Njihova prognoza, kao i kod većine tumora, zavisi od stadijuma bolesti. Preko 90% svih malignih tumora usta i orofarinksa histopatološki pripada skvamocelularnom karcinomu. Njihova klinička procena se zasniva na inspekciji i palpaciji, uz neurološku procenu stanja kranijalnih nerava. Upotreba kompjuterizovane tomografije (CT) i magnetne rezonancije (MR) ključna je u definisanju raširenosti karcinoma usne duplje i planiranju adekvatnog terapijskog postupka. Poznavanje radiološke anatomije i patologije ove regije od presudne je važnosti u donošenju adekvatnog zaključka.

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Ključne reči: *maligni tumori usne duplje, skvamocelularni karcinom, CT, MR*

Case report **UDC: 616-006.8**
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EXTRAMAMMARY PAGET'S DISEASE OF THE INGUINUM: A CASE REPORT

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Extramammary Paget's disease (EMPD) is a rare intra-epithelial malignancy that is occasionally associated with an invasive adenocarcinoma component as well as other secondary cancers. The diagnosis is confirmed by the presence of Paget's cells on histopathological examination of a tissue specimen. The standard treatment method of inguinal EMPD, as well as EMPD in other areas, is surgical resection. We present a patient with extra-mammary Paget's disease, who developed the erythematous eruption in the right inguinal region.

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Key words: *Extramammary Paget's disease, inguinum, diagnosis, treatment*

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Introduction

Extramammary Paget's disease (EMPD) is a rare intra-epithelial malignancy that is occasionally associated with an invasive adenocarcinoma component as well as other secondary cancers (1). EMPD can generate from all areas characterized by a high density of apocrine glands, as the axilla, inguinum, anus and perianal region, vulva in women, penis and scrotum in men. The lesion is located deep within the dermal to epidermal layer, and underlying carcinoma of the sweat glands has been suggested as a possible etiology (2).

EMPD schoud be differentiated from benign papulosquamous diseases, squamous cell carcinoma, and melanoma. Very often, it is mistaken for eczema or contact dermatitis. The diagnosis is confirmed by the presence of Paget's cells on histopathological examination of a tissue specimen. The standard treatment method of inguinal EMPD, as well as EMPD in other areas, is surgical resection (1).

We present a patient with extramammary Paget's disease, who developed an erythematous eruption in the right inguinal region.

Case report

A 75-year-old male presented with a 8-month history of recurrent, itchy, eczematous erosion, and indurated patchy lesion in right inguinal area (Figure 1a). The patient had been initially treated for an erythematous, pruritic lesion in the inguinum with topical corticosteroids. He had been treated with the steroids for 7 months. On physical examination, there was a centrally eroded, scaly erythematous lesion, 9 x 11 cm in diameter, on his right inguinal area, spreading to the scrotum and base of the penis.

Figure 1a. Skin lesions in the right inguinal area

No lesions were seen in the other apocrine-bearing regions, including breasts, perianal area, or external auditory canals. Superficial lymph nodes were not palpable in the inguinal regions. The results

of complete blood counts and blood chemistry examinations, including carcinoembryonic antigen (CEA), were normal. There was no evidence of internal malignancy on clinical, X-ray, abdominal and pelvic MSCT examinations.

The patient underwent wide excision of the skin lesion with a 2-cm margin to the macroscopic normal tissue, and primary closure. Surgical margins were histopathologically negative and the wound healed without complications. (Figure 1b).

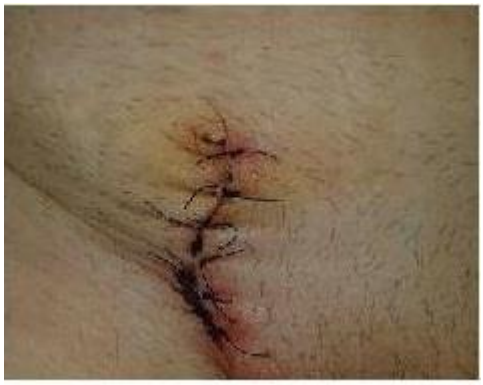

Figure 1b. Final result of the previous case

Histopathological examination of a biopsy specimen of the skin lesion revealed an infiltration of the epidermal-dermal junction by Paget cells, that are atypical glandular-type cells, larger than the adjacent keratinocytes and with fine granular amphophilic to basophilic cytoplasm. The cytoplasm is paler than that of adjacent keratinocytes. The nucleus is oval with one or more prominent nucleoli. (Figure 2).

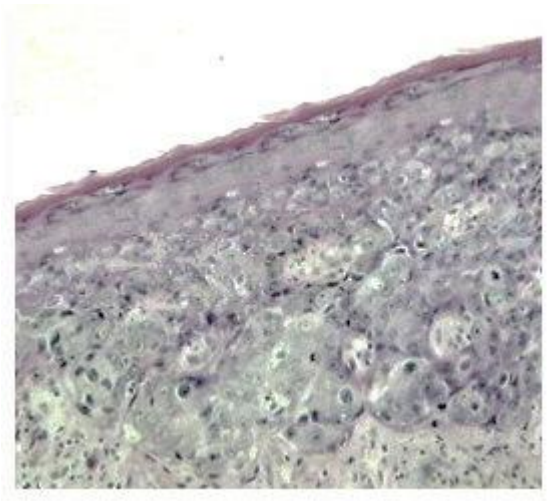

Figure 2. Histology of the excised specimen

Although the surgical margins were negative for malignancy, lymph nodes from the inguinal, external iliac, internal iliac and obturator regions were negative for malignancy by ultrasaund (US) and MSCT examination, and postoperative radiotherapy and chemotherapy were not recommended.

During the one-year follow up, local recurrence and metastasis on US and MSCT examinations were not present.

Discussion

Extramammary Paget's Disease is a rare neoplastic lesion. The lesion generally appears as eczema and the most frequently reported symptom is the itch. Because of these poor clinical features, there is usually a delay in its diagnosis, based on the typical biopsy histological pattern. (1).

The most common site of EMPD is the vulva, and more than 200 cases have been reported. The second most common site is the perianal region, with more than 86 cases documented (3). Other locations include the perineum, scrotum, axilla, and eye lids. We present a patient with rare inguinal EMPD. EMPD generally occurs between the ages of 50 and 80, most frequently in Caucasians. In Japan, men are affected with extra-mammary Paget's disease twice as often as women (4), whereas women are predominantly affected in western countries (2).

The symptoms of the disease are not specific. Most patients report itching, burning, and soreness. A small number of patients may be asymptomatic. The presence of inguinal pain, bleeding, and tumor formation are reported to be more common in patients affected by invasive disease. The signs and symptoms are skin lesions, often mistaken for eczema, that may be itchy or painful (1). Our patient also had non specific simptoms and had been initially treated for an erythematous, pruritic lesion in the inguinum with topical corticosteroids for 7 months.

Extramammary Paget's disease is usually seen in isolation and is associated with an underlying invasive malignancy in about 12% of the cases. It is associated with an underlying adnexal malignancy in about 24% of the cases (5). It has a good prognosis in absence of malignancy, but may result in a poor quality of life because of frequent recurrences with the necessity of ablative therapies and anxiety about possible cancerization. Rarely, EMPD can be invasive or associated with adenocarcinoma or other kinds of cancer (6). We did not find any underlying malignacy after abdominal MSCT examination.

In conclusion, inguinal EMPD is a rare disease, as has been described in the currently available literature. It is usually associated with adnexal and visceral malignancies and generally has a poor prognosis due to its progressive course if not treated early. Early biopsy is very important for correct diagnosis in patients who fail to respond to conventional topical therapy. The standard treatment of inguinal EMPD is surgical resection.

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EKSTRAMAMARNA PAGETOVA BOLEST INGVINUMA

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Ekstramamarna Pagetova bolest (EMPB) predstavlja retki intraepitelni maligni tumor koji je povezan sa invazivnim adenokarcinomima, kao i sa drugim sekundarnim karcinomima. Dijagnoza se potvrđuje prisustvom Pagetovih ćelija na histopatološkom ispitivanju uzoraka tkiva. Standardna metoda lečenja za preponsku EMPB, kao i za EMPB u drugim područjima, je hirurška resekcija. Predstavljamo bolesnika s ekstramamarnom Pagetovom bolešću, koji je razvio eritematozne erupcije u desnoj imgvinalnoj regiji.

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Ključne reči: *ekstramamarna Pagetova bolest, ingvinum, dijagnoza, tretman*

Review article

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MEDICAL LAW AND HEALTH LAW – IS IT THE SAME?

Nikola Todorovski

This is a review of literature on the current position of medical and health law in the legal system of the Republic of Serbia and the world in general. The article defines the similarities and differences of medical and health law with respect to forensic medicine, as well as the similarities and differences between medical and health law, from the point of view of practical, clinical application and possible ethical dilemmas. The reviewed literature suggests that the knowledge of these facts can be crucial for the improvement and full implementation of medical and health law in the legal and health system with the aim of ensuring the quality of health services and exercising the rights of all participants in their provision. On the other hand, full knowledge and application of these branches of law in the field of healthcare activity would additionally contribute to the humanization of legal science.

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Key words: *medicine, law*

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Definition of medical and health law

Development of medicine requires development of law branch, known as medical law, which will ensure the quality and rights of all participants in medical service system. Beside the term of medical law there is wider term – health law (1). While the medical law covers an area of regulations relating to the medical operations, the industry operators, the procedures involved in the medical operations, characteristics of medical experts performing medical operations, as well as the relations that occurred while performing medical activities, the health law regulates a wide area of activities, not only medical activities, but also the procedures that are being implemented, the need and necessity of the procedure, professionals who carry out the procedure (1,2). The medical law also covers regulative of drugs and other medical devices. Health law covers a wide area of regulations concerning health, procedural matters and organization of the public health system. From the everyday practice the medical law finds its utility in relation to patients' health, such as: life, body integrity, health, self-determination, as well as personal dignity (2,3).

The review of literature suggests that there are opened questions about similarities and differences of medical and health law especially from the practical implementations, both in law practice and medical industry. The recognized similarities might be as followed: medical law is the branch of law which concerns the prerogatives and responsibilities of medical professionals and the patients, health law is the federal, state, and local law, rules, regulations and other jurisprudence among providers, payers and vendors to the health care industry and its patients; and delivery of health care services; all with an emphasis on operations, regulatory and transactional legal issues (4). On the other side there are some clear differences. Thus, medical law concerns system of medical industry, qualifications of those that provide medical service and their relations to the repaint of medical care. Health care is a wider discipline that concerns all legal acts to human health. This discipline covers the area of procedures, patients, doctors, informed consent and fact that matters to human health (5).

Position of medical law has been elevated to the highest positions, by the European law system implemented by each state, EU members and Council of Europe members. International sources of medical law are the European Convention of Human Rights, Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine: Convention on Human Rights and Biomedicine (6). On the contrary, the current position of medical law in Serbia is not so good. It is at the very beginning in Serbian law system, adjusting to the EU Directives. The

Serbian sources of Medical and Health law are the Constitution (Gazette of RS", no. 98/2006), Law on Health care ("Gazette of RS", no. 107/2005, 72/2009 - Law, 88/2010, 99/2010, 57/2011, 119/2012, 45/2013 - Law 93/2014, 96/2015 and 106/2015); Law on Health Insurance ("Gazette of RS", no. 107/2005, 109/2005 - corr., 57/2011, 110/2012 - decision, 119/2012, 99/2014, 123/2014, 126/2014 - decision, 106/2015 and 10/2016), Law on Organ Transplantation ("Gazette of RS", no. 72/2009) etc (7), as well as the series of health law highlights through the history, as it is specified in Table 1 and Table 2 (8).

Table 1. Basic characteristics of medical and health law

BASIC CHARACTERISTICS	
Medical law	**Heath law**
Medical law covers an area of regulations relating to the medical operations, the industry operators, the procedures involved in the medical operations, characteristics of medical experts performing medical operations, as well as the relations that occurred while performing medical activities. the health law regulates a wide area of activities, not only medical activities, but also the procedures that are being implemented, the need and necessity of the procedure, professionals who carry out the procedure, regulative of drugs and other medical devices **M**edical law finds its utility in relation to patients' health, such as: life, body integrity, health, self-determination, as well as personal dignity	**H**ealth law regulates a wide area of activities, not only medical activities, but also the procedures that are being implemented, the need and necessity of the procedure, professionals who carry out the procedure, covers a wide area of regulations concerning health, procedural matters and organization of the public health system. **H**ealth care is a wider discipline that concerns all legal acts to human health. **C**overs the area of procedures, patients, doctors, informed consent and fact that matters to human health.

Table 2. The key Health Law Highlights through the history

Year	Event
1767.	Slater v. Baker and Stapleton, CB Eng Rptr (UK) (medical experimentation)
1803.	Percival's Medical Ethics published (original title, Medical Jurisprudence)
1809.	Commonwealth v. Thompson, 6 Mass. 134 (wrongful death, quackery)
1818.	First medical licensure statute enacted in Massachusetts
1823.	Theodoric Beck's Elements of Medical Jurisprudence published
1840.	Medical malpractice litigation appears in the United States
1860.	John J. Elwell's A Medico-Legal Treatise published
1905.	Jacobson v. Massachusetts, 197 U.S. 11 (no right to refuse smallpox vaccination)
1946–1947.	Doctors' Trial at Nuremberg (Nuremberg Code set forth in the judgment)
1955.	American College of Legal Medicine founded
1966.	Medicare and Medicaid enacted
1972.	American Society of Law and Medicine founded
1973.	Roe v. Wade, 410 U.S. 113 (right to terminate pregnancy)
1990.	Cruzan v. Director, Missouri Department of Health, 497 U.S. 261 (right to refuse life-sustaining treatment)
1997.	Washington v. Glucksberg, 521 U.S. 702, and Vacco v. Quill, 521 U.S. 793 (no right to physician-assisted suicide)
2010.	Patient Protection and Affordable Care Act enacted
2012.	National Federation of Independent Business v. Sebelius (upheld all of the Patient Protection and Affordable Care Act as constitutional except the penalty for states that do not expand their Medicaid programs)

Table 3. Timeline of Major Milestones in Global Health Law Development

Year	Regulatives	
1892.	Adoption of the International Sanitary Convention (predecessor to the International Health Regulations)	Historical predecessors to contemporary WHO instruments
1893.	Adoption of the International List of Causes of Death (predecessor to the International Classification of Diseases)	
1948.	Adoption of Nomenclature with Respect to Diseases and Causes of Death	WHO treaties: Conventions or Regulations
1951.	Adoption of the International Sanitary Regulations (predecessor to the International Health Regulations)	
1955.	Launch of the global program to eradicate malaria	WHO Global Campaigns cosponsored with partners
1959.	Launch of the global program to eradicate smallpox	
1978.	Adoption of the Declaration of Alma-Ata ("Health for All") by the International Conference on Primary Health Care	
1981.	Adoption of the International Code of Marketing of Breast-Milk Substitutes	WHO Nonbinding Normative Instruments
1988.	Launch of the global program to eradicate polio	WHO Global Campaigns cosponsored with partners
1999.	Launch of Vision 2020, a global initiative to eliminate avoidable blindness by the year 2020	WHO Nonbinding Normative Instruments
2000.	Adoption of the Millennium Declaration and Millennium Development Goals	U.N. Nonbinding Resolutions and Declarations
2001.	Publication of the Global Strategy for Containment of Antimicrobial Resistance Adoption of the Declaration of Commitment on HIV/AIDS	WHO Nonbinding Normative Instruments U.N. Nonbinding Resolutions and Declarations
2003.	Adoption of the Framework Convention on Tobacco Control Launch of the 3 by 5 Initiative (HIV treatment for 3 million patients by 2005)	WHO treaties: Conventions or Regulations WHO Global Campaigns cosponsored with partners
2004.	Adoption of the Global Strategy on Diet, Physical Activity, and Health	WHO Nonbinding Normative Instruments
2005.	Adoption of the Revised International Health Regulations	WHO treaties: Conventions or Regulations
2006.	Adoption of the Political Declaration on HIV/AIDS (5-yr follow-up) Launch of the Stop TB Strategy	WHO Nonbinding Normative Instruments U.N. Nonbinding Resolutions and Declarations
2009.	Adoption of the Global Action Plan for the Prevention and Control of Noncommunicable Diseases	WHO Nonbinding Normative Instruments
2010.	Adoption of the Global Code of Practice on the International Recruitment of Health Personnel Adoption of the Global Strategy to Reduce the Harmful Use of Alcohol	
2011.	Launch of the Pandemic Influenza Preparedness Framework Adoption of the Political Declaration on the Prevention and Control of Noncommunicable Diseases • Adoption of the Political Declaration on HIV/AIDS (10-yr follow-up)	WHO Nonbinding Normative Instruments U.N. Nonbinding Resolutions and Declarations
2012.	Adoption of a resolution promoting universal health coverage worldwide	U.N. Nonbinding Resolutions and Declarations
2013.	Launch of the Mental Health Action Plan	WHO Nonbinding Normative Instruments
2014.	Adoption of the Sustainable Development Goals	U.N. Nonbinding Resolutions and Declarations

Law and clinical implications

Medical law regulative contributes precise definition of legal rules that define the medical industry, service providers, and the rights of doctors and patients in the health system. Health law implies the broader term regulations concerning human health. The regulation governs procedures and relationships concerning human health. All relationships, procedures and their influence are connected to certain rights or legal actions and are related to human health. Developing medical and health law will precisely determine the rights and obligations of the providers of medical care and users of health services (5). Regulations will determinate the precise procedures that take place in health institutions, the conditions for performing the procedure, the staff that will provide this type of service, thereby the ability of jeopardizing the patients' rights would be reduced to a minimum (9).

From this point of view the future of medical and health law will be considered as branch of law to develop in the direction of the precise definition of relations between medical service providers and patients. Precise definition of the relationship will improve the implementation and protection of patients' rights and the rights of doctors and health personnel. Medical procedure, the iuridization of medical procedures, informed consent, consequences and expectations, are areas of health law, which must be precisely regulated for the purpose of exercising and protecting the rights of patients (10).

As the pivotal parameters of medical and health law development (Table 3) (8) the following has been recognized: specificity of medical treat ments, which includes interventions, such as therapeutic procedures (as it has been in the past) and also diagnostic procedures; the medicine is developed as the collective medicine depending on technical science development; human factor and medical profession rules (11).

Implementation of medical and health law, both in medical practice and law practice, we can recognize at, for example, organ transplantation. First of all, a doctor must discuss with their patients about this issue before the need of tissues and organs arise as a part of advance care planning (5, 9-11). Organ donation involves several issues. Of particular concern is to avoid any conflict between medical care of potential donor and needs of potential recipient (11). The care of potential donors must be separated from the care of potential recipients. Potential donor's physician should not be responsible for the care of potential recipient and should not be involved in retrieving the organs and tissues. Another set of issues involves financial incentives to encourage organ donation. The financial incentive must not be support to organ donation, although increasing organ donation is a noble goal this shouldn't be the decisive factor for organ donation, which can bring humans as commodities (11, 12).

Legal, ethical and moral decisions

As the essential in the medical and health law three types of decisions that can be made by doctors are recognized: legal, ethical and moral (13). Legal decisions are decisions where the doctor has no choice at all. Ethical decisions are those that the law leaves to the medical profession to regulate, and thus reflect the corporate morality of the profession. A moral decision is the one which is entirely uninhibited by anything other than the conscience of the individual doctor.

The type of decision that is recognized as a Legal Decision is the one where the law and regulation govern acceptable conduct. Usually it does this when the law recognizes the issues to relate to patients right, and in that way enables the matter to be used as justification for regulating medical conduct. Reflection of this law influence to medicine is the most clarified in the context of informed consent. As a consequence of that influence, and also the influence of court decisions, especially in European law systems, changed the importance of emphasis and prioritization of patients' rights to autonomy, than being based on duties of the doctor as it was considered in the past (12,13). This influence contributed to enhanced recognition of both the ethical aspects of informed consent and patients' autonomy (11-14). The importance of protecting the autonomy is particularly shown in the case of Chester vs. Afshar. This case led to The House of Lords (Great Britain) to declare that if the law does not protect the autonomy, it must be changed. This could not be limited only to informed consent. As an example of importance of patients' autonomy can be found in case of Ms B vs. A in the NHS Trust. Ms B was maintained on ventilator, so she felt her quality of life was so poor that she wanted to die. She asked her doctors to do that thing. Ms B's doctors refused to cease the ventilator, arguing that this kind of decision might be identified as killing her, and it was 'unethical' from their point of view (12-14). She went to court to force the doctors to stop the ventilation. The court applied simple and ground legal rules. Doctor must respect the wishes of patient, in situation when there is a patient with sound mind, and properly informed about procedure, respect the autonomy of patient. Ms B refused to be on ventilator so doctors must cease it. The ventilation ceased and Ms B died. What is of interest to us here is the fact that the medical profession tried to claim the issue of the desirability of Ms. B's survival as its own, by defining it as 'ethical' in nature (15). The court recognized the patients' right of autonomy, therefore forced doctors to cease the ventilator. In this case we can see that the law took the matter with the ethical content and defined it as legal, as it is approached in the paper of Foster and Miola (13, 15).

The type of Professional Medical Ethical decisions are those that the law decides are best resolved by medical profession itself, as it is defined in the

same paper (13-16). But there must be made a distinction: when there are 'ethical' issues involved, it can not be considered that it is the best way to decide by the medical profession. More 'ethical' issues requires the law to take control of making the decision, due to issues other than appropriate performance of medical skills, as it is mentioned in the paper of Foster and Miola (13).

The type of moral decision is the one where it is left to an individual to make a decision, and this type of decision is correctly referred to as being moral in nature. Moral decision must not be harmful for the patient. The rights protected then are related not to the patient but to the doctor (13,16).

In conclusion, medical law is a branch of law covering the wide authorities of medical industry, providers and medical service users, rights of patients and doctors. Health law is a discipline expanding its authorities, which may be wider than that of medical law, concerning the regulations related to human health. Within its scope, a health law regulates the procedures related to human health, binding together basic human rights and legal actions. Based upon the findings presented here, it could be said that there are differences between medical and health law. Although medical and health law are often being perceived separately, these should not be strictly separated, because both of the disciplines contribute to the humanization of law.

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MEDICINSKO I ZDRAVSTVENO PRAVO – DA LI JE ISTO?

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Rad se bavi pregledom literature na temu trenutne pozicije medicinskog i zdravstvenog prava u pravnom sistemu Republike Srbije i u svetu uopšte. U radu se definišu sličnosti i razlike medicinskog i zdravstvenog prava u odnosu na sudsku medicinu, ali i sličnosti i razlike između medicinskog i zdravstvenog prava i to sa stanovišta praktične, kliničke primene i mogućih etičkih nedoumica. Pregledana literature sugeriše da poznavanje ovih činjenica može biti ključno za unapređenje i potpunu implementaciju medicinskog i zdravstvenog prava u pravni i zdravstveni sistem sa ciljem osiguravanja kvaliteta zdravstvene usluge i ostvarivanje prava svih učesnika u njihovom pružanju. S druge strane, potpuno poznavanje i primena ovih grana prava u oblasti zdravstvene delatnosti dodatno bi doprinela humanizaciji pravne nauke.

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Review article

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THE UNDERLYING CONCEPT OF MOTIVATION IN MEDICAL ENGLISH TEACHING

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There is irrefutable evidence that medical English cannot be taught at the same level as general English language. The aim of studying English at this career specific level is acquiring contextually-based practical use of language within the given domain, rather than focusing on grammar and structure. The teacher's job is to design an appropriate curriculum that can be adjusted to satisfy the needs of the educational institution, while enabling students to perform medical jobs in a qualified, safe and competent manner. If asked to identify the strongest influence on language learning, motivational factors would appear at the top of few teachers' lists. Nevertheless, motivation is rooted in human behaviour so profoundly, that we often fail to realize its underlying presence. This paper attempts to shed light, i.e. demystify the somewhat metaphysical concept of motivation, as well as to demonstrate how gravely and unjustly neglected the notion of motivation has been.

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Key words: *motivation, teaching, medical English, methodology*

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Introduction

It is a well-known fact that medical English cannot be taught at the same level or by using the same methods as general English language. The language of medicine and health care is of idiosyncratic magnitude, laden with academic expressions, colloquialisms, acronyms and abbreviations alike. As for gaining medical English proficiency, like in all scopes of English, the higher the applicable value of the material learned, the higher the levels of student motivation.

When it comes to motivational factors during a course of language teaching, motivating students is both an immense challenge, and one of the most serious sources of difficulties. Motivation is, in fact, the primary impetus and the driving force behind this demanding process. People often take motivation for granted, reducing its actual importance and omnipresence, and yet, without proper motivation, even those with outstanding abilities will fail short of achieving long-term goals.

It is the teacher's job to design an appropriate curriculum which will serve as a framework that can be developed in order to satisfy the needs of the educational institution in question. It is of paramount importance that the knowledge thus acquired enables students to perform their jobs safely and competently within the scope of their expertise.

The importance of student motivation

The word motivation derives from the Latin root "motivus"- a moving cause. As the name suggests, motivation is what moves us to perform. It is the purpose or psychological cause for our actions, the desire or willingness to do something. From the most trivial activities such as getting out of bed in the morning, to far more complex endeavours such as acquiring a new language for specific purposes - motivation is virtually ubiquitous.

Motivation is an absolute prerequisite to successful language learning. Maintaining a high level of motivation over the entire process is the best suited way to ensure the process is the most effective. When it comes down to medical English acquisition, motivation is a psychological drive that spurs us towards achieving a goal - whether it is mastery of a language, communicative competence, or even basic communication skills (1). Teachers are facing a tremendously difficult task of setting the favourable micro-climate by promoting positive attitudes and the students' self-esteem, while providing their own

emotional involvement. Both teachers and students seem to have reached the consensus that the main focal point for motivation (and demotivation alike) is the teacher. It can certainly look like a Sisyphean task at times, but good teachers have a plethora of methods, resources, and tricks up their sleeves to maximize the ultimate results.

What better way to motivate your students than to guarantee them a closer contact with all relevant factors from their field of expertise. One way is to use materials both useful and relevant for their future careers, while ensuring they are going to learn as efficiently as possible (2). Misconceptions about motivation as a learner-centered phenomenon were present for a long time, while the teacher's function was seen predominantly as a provider for materials and conditions for learning. The tables have turned, however, and teachers nowadays are expected to encourage learners to take ownership in learning, help them identify and decide for themselves the relevant learning goals, while providing continuous support (2). It is vital to perceive the class not as a unified group, but as a set of individuals different from one another, and address them correspondingly.

There are two basic kinds of motivation, the integrative and the instrumental. The integrative motivation reflects on the desire to integrate into the wholesome culture of the target language, while the instrumental concentrates on achieving concrete aims such as: promotions in career, the ability to understand foreign literature, getting a job abroad, or any other pragmatic goal (3). Intrinsic motivation has a number of relevant factors: the level of aspiration (can be rigid or adjustable, depends heavily on the level of ambition, getting a D on an exam can be either a catastrophe, or a cause for celebrations of Biblical proportions for two different students), pleasantness (we humans are designed to be more prone to remembering/acquiring something pleasant than something very unpleasant, while least likely to remember something we are indifferent to; it explains how we first remember the words we are most interested in, than those really long medical expressions that seem impossible to grasp at first, while having many problems with those small, often monosyllabic words that always mean something), sincere intention to learn something (46 forced repetitions have a lesser effect than 6 repetitions with a real intention to learn), interest (which grows proportionally as we start to connect the new material with something interesting, getting to know its significance and its practical use in real life, i.e. in medical practice for EMP students).

The concept of motivation is so incredibly complex, it takes a respectable number of interrelated disciplines (psychology, sociology, linguistics, etc.) to arrive anywhere near reasonable understanding of its different facets. However, being omnipresent as it is, we often fail to recognize the importance of motivation in the process of foreign language teaching/learning. The more the students see their progress, the more they want to do even better. On the other hand, perceptions of failure demotivate.

Students' language skills cannot be improved without their genuine engagement, while motivation is seen as the crucial element for success in the classroom and beyond. There is only so much a teacher can do, the rest is up to students. Students need to develop learner autonomy in order to succeed in the course, later classes and in future jobs. The teacher's attitude can enhance or inhibit autonomy and motivation. Finally, educators must not forget the culture of students nor ethical considerations of their profession to encourage and eventually allow students to take control of their own learning. Other factors include: how important it is for students to feel autonomous, classroom methodology, selection of appropriate medical materials, and helping students overcome their natural anxiety, particularly during classroom activities such as oral presentations and test taking.

There is a whole range of possibilities for teachers to increase motivation in the classroom. Generally speaking, they fall into the before mentioned categories of intrinsic and extrinsic motivation. As far as the former is concerned, the topics ought to be in the sphere of interest of a particular learning group, and not to force any topic the students are not interested, under no circumstances whatsoever. Students' curiosity must be stimulated at all times by providing challenging, and yet achievable goals in order for them to feel how much they are progressing. When considering extrinsic motivation, teachers can help students establish high (and yet achievable) expectations, and also help them develop positive (and yet realistic) attitudes towards language learning.

Overcoming obstacles

One of teachers' main concerns is to help students perceive why they are learning the language and what possibilities it can open for them in their professional lives. Upon realizing the connection between what they are learning in the classroom, and what they can apply it to practically in medicine, most students continue to work even harder. Acquiring medical terminology and its concise clear manner does not happen overnight; it is a painstaking process that takes lots of practice, causes a lot of frustrations, and demands a great deal of patience; the teacher's role is to convince them that the process is going to be worthwhile in the end, while giving them positive feedback throughout the entire two-way process.

It is not always possible for a person who is not a professional linguist to express himself/herself precisely in the target language, therefore, the students ought to be encouraged to think of synonyms and alternative ways to express their thoughts in verbal communication, with the teacher as a mere facilitator. The important thing is that the message is communicated and understood in a safe and correct way. The goal of medical English must be acquisition and applications of language rather than memorization or direct focus on vocabulary, grammar and syntax. Lest anyone wonders why that is the case, it suffices to say that when you make a mistake in syntax, case, spelling, 3rd person singular, (or any other grammatical category for that matter), you might provoke a sneer, giggle or smile. But when

you make a mistake in medical English terminology, it might have life-threatening implications for your patient. Therefore, it is a very important challenge for all the parties involved, because one day it might mean the difference between life and death.

It is next to impossible for a layman English learner to comprehend all the acronyms and abbreviations used in career specific medical jargon. It is basically a language of its own. Teaching and learning are dynamic intertwined processes and feed off each other. Even with the developed curriculum, it serves merely as a framework within which the students are supposed to thrive. Whereas the goals of the curriculum are somewhat fixed, extra attention needs to be paid to students' interests and needs, which provide valuable feedback. Therefore, it is essential that the curriculum is adjusted to any particular group of students, while bearing in mind that it is prone to modification, as their needs and goals might change over the period of time. The teacher also needs to be highly flexible. Medical English needs vary from student to student, but each person will have specific demands to meet their individual desired results (4).

The teacher needs to be a master of strategies, there are no unique approaches that work with everyone, one needs to adjust constantly. A couple of techniques for creating positive classroom environment do stand out, though. These include: using structural formats (e.g. making smaller groups), carefully choosing content (as not to estrange students from the subject, or kill their interest), initiating semi-controlled activities such as group discussions (not entirely controlled as not to stifle the students' creativity and sense of self-reliance). Those who are more interested in professional development seem to be more committed than those with short-noticed aims such as purchasing careers abroad as this diverse teaching field offers lucrative opportunities.

Medical English poses a great challenge given the fact that, unlike in teaching general English, students have more knowledge in the field of expertise. Therefore, symbiosis is required, and coordination and cooperation are crucial. The teacher's role is to guide and facilitate, not to be the centre of attention. Moreover, one approach is never enough. Hence, a combination of several usually makes the highest impact. Those must be carefully implemented and interwoven to achieve the most satisfactory results for both parties involved. Personal sensitivity from the teacher is highly desirable, as is the overall resilience and versatility that covers a wide spectrum of methodological alternatives.

Novel approaches to methodology

A foreign language teacher plays a specific role in learners' education: by teaching foreign language, we are also teaching them foreign cultures, opening gates to the whole world for them. Activities related to the target culture facilitate language acquisition drastically, open up new horizons and stimulate their curiosity, thus contributing to their motivation for learning the language in question significantly. Given such impetus, at that point, students will usually take initiative and explore further on their own (5). Medical English language teaching demands a novel and modern approach. It appears that earlier methods notoriously applied to students of medicine predominantly focused on acquiring the language while embedding health terminology as a subsidiary contribution. However, nowadays it is believed that it should be vice versa.

Every course starts with a curriculum, which is designed to help doctors and medical students develop listening, speaking, reading and writing skills in English. It aims to give the students the opportunity to learn specialized vocabulary and expressions, to activate passive vocabulary, to learn the English pronunciation of medical terms and to increase fluency and confidence in using English in professional contexts. The four skills of reading, writing, listening and speaking can be taught through the use of various materials ranging from research papers and case presentations to medical interviews and works of literature (6). The goal of learning English at this level is not to learn grammar and structure primarily, but to acquire and use the language of practice and social relations within the career (7).

Medical English teacher will find a way to teach students, but also to encourage independent learning. This can be achieved provided the teacher does not lack enthusiasm and commitment towards the target language, the field of medicine and its noble causes, but even more importantly to the learners. Students need to feel appreciated and supported by teachers who love their jobs, and who want to establish a good rapport with them. A stimulating classroom atmosphere and integrating students into class performances will inevitably lead to higher levels of stamina, which is absolutely essential in the language learning process.

Medical students, nurses, doctors and international health care workers worldwide who do not speak English as their native language are filling up medical English classes. Schools, individual health care workers, and government bodies are looking for teachers who specialize in teaching medical terminology, concepts, and attitudes to instruct these people. Whether to enable communication of specialists in scientific exchanges thus enabling scientific progress, facilitating worldwide access to learning, or continuing studies in English in one of the 50 English speaking countries among approximately 375 million people who speak the language, dominant position of medical literature in English is unquestionable.

We have to bear in mind that students are intelligent adult people, self-conscious and dedicated to a noble goal, and it is the teacher's task to pave their way to the top by offering their best. EMP teachers act as managers, facilitators, organizers, monitors and helpers, which sometimes requires nerves of steel, large amounts of trust and empathy. In order to do that, they must be willing to interact not only with their students, but also with experts from the medical field.

The material used in EMP should cover all the relevant topics to the required field of medicine. In order to trigger the interest and hold the attention of the students it must come in various forms: medical articles, audio materials, videos, a combination of

academic preparation that includes behavioral and cognitive approaches. Lessons would ideally be empirically and contextually based to provide immediate opportunities to use the acquired knowledge. In interactive classes that promote discussion, cooperation and team work, students will benefit by achieving the sense of improvement and accomplishment. The use of authentic materials is just as important as making the lectures interesting. Again, this is the teacher's task. Through your own behavior, course design and teaching practices, you can create classroom conditions that encourage engagement and motivation to learn on a variety of levels (8).

The traditional teacher-student roles are a thing of the past. The new approach requires substantial sensitivity when it comes to teaching methods and a wide array of methodological alternatives. EMP teachers have far greater responsibility than general English teachers. One must integrate medical knowledge and the mastery of English language in order to pass on all the skills and findings regarding proficiency in medical terminology, procedures and concepts necessary for practicing medicine in a native English speaking country. It is a prestigious vocation to be a guide to future doctors, to people in whose hands so many lives will be placed.

Conclusion

Modern methodology recognizes the importance of motivational factors, and puts them very high on the list of priorities. One of the teachers' main concerns is to help students perceive why they are learning the language and what possibilities it can open for them. Upon realizing the connection between what they are learning in the classroom, and what they can apply it to practically in their professional lives, most students continue to work even harder. Activities related to the target culture facilitate language acquisition drastically, open up new horizons and stimulate their curiosity, thus contributing to their motivation for learning the language in question significantly. Given such impetus, at that point, students will usually take initiative and explore further on their own. Medical English teachers will find a way to teach students, but also to encourage independent learning. A stimulating classroom atmosphere and integrating students into class performances will inevitably lead to higher levels of motivation, which is essential in a language learning process. The teacher’s role in increasing students’ motivation is crucial, and through a variety of teaching methods students can be not only motivated positively, but also remain motivated for long periods of time.

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FUNDAMENTALNI KONCEPT MOTIVACIJE U NASTAVI MEDICINSKOG ENGLESKOG JEZIKA

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Postoje nepobitni dokazi da se medicinski engleski ne može predavati na klasičan način. Cilj učenja engleskog na ovom specifičnom medicinskom nivou je sticanje kontekstualne praktične upotrebe jezika u okviru datog domena, uz manji fokus na gramatiku i strukture. Posao nastavnika je i osmišljavanje odgovarajućeg programa prilagođenog da zadovolji potrebe obrazovne ustanove, kao i da omogući studentima da obavljaju medicinske poslove na kvalitetan, siguran i kompetentan način. Ako bi trebalo da se izabere najbitniji uticaj na učenje stranog jezika, motivacioni faktori bi se našli na vrhu liste malog broja nastavnika. Pa ipak, motivacija je toliko ukorenjena u ljudskom ponašanju da često prenebregnemo njeno fundamentalno prisustvo. Ovaj rad pokušava da rasvetli, tj. demistifikuje metafizički koncept motivacije, kao i da ukaže koliko je nepravedno faktor motivacije do sad bio zanemarivan.

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Ključne reči: *motivacija, podučavanje, medicinski engleski, metodika*

Review article

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PATHOPHYSIOLOGICAL ASPECTS OF OLIGOELEMENT SUPPLEMENTATION IN ATHLETES

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The precondition for achieving top athletic results is full health and psychophysical readiness. Essential oligoelements are necessary for normal biochemical and physiological processes, utilization of energy and building of tissues, as well as for optimal functioning of the muscles and their harmonious relationship with other systems. Trace elements play an important role in energy metabolism during strenuous physical activity, and in the conditions of increased oxygen demand, free radical production, activity of scavenger enzymes, and anti-oxidant protection.

Athletes may have iron deficiency due to decreased dietary intake, blood loss or increased needs due to physical activity, however, supplementation is not justified in terms of improving sports performance, and may even be harmful. Copper has particular importance in biological processes of energy metabolism, iron homeostasis and antioxidant protection. Additional amounts of copper of 0.5-3.0 mg per day are recommended to athletes, although high doses do not have ergogenic properties. Moreover, athletes have a greater need for zinc. Additional amounts of zinc supplements are recommended, 15-50 mg per day. The deficiency of manganese occurs most frequently in malnourished people, while the need for selenium in well-trained athletes increase depending on the energy consumption.

Sport activity, especially when it comes to long-term extreme efforts, increases the need for micronutrient substances. Athletes need to ensure a balanced diet and oligoelement supplementation to meet their needs, increased in relation to the recommended daily intake.

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Introduction

Oligoelements (trace elements) are substances whose concentration does not exceed 250 μg/g of weight and whose decrease below a certain value leads to a reduction of physiologically important functions, because they are part of organic systems which perform vital functions. According to their importance in the diet, as recommended by the experts of the World Health Organization, trace elements can be divided into essential, elements that are likely essential, and elements that are potentially toxic, but can be essential in very small concentrations (1, 2).

Essential trace elements are necessary for normal biochemical, physiological processes in the body, growth, maintenance of health and longevity, and their lack is causing disease, while correcting the deficiency eliminate the disease. This group includes: iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), chromium (Cr), molybdenum (Mo), iodine (I) and selenium (Se). The group of elements that are likely essential comprise: manganese (Mn), silicon (Si), nickel (Ni), bromium (Br), and vanadium (V). Among the elements that are potentially toxic but may be essential in very small concentrations are: fluorine (F), lead (Pb), cadmium (Cd) and mercury (Hg). A pathophysiological division of oligoelements is made according to their biological role in the organism as follows: hematopoietically active (Fe, Cu, Co), active enzyme components (Zn, Mn, Mo, Cr, Se, V), and halogen elements (J, F, Br) (1-4).

A prerequisite for achieving top results in disciplines that require physical strength and stamina is full health and psychophysical fitness of athletes. Full health is necessary for optimal muscle function and harmonious relationship of musculoskeletal, neuro-endocrine, immune and other systems.

Clear criteria and recommendations exist on the necessary macronutrient intake and their relation

to diet for proper functioning of the body and maximum mental and physical readiness. Although trace elements are measured in micrograms or milligrams, and rarely grams, they are essential for energy utilization, building tissue materials and proper functioning of all the above mentioned systems. This imposes the need for intensive studying of the role and importance of micronutritive materials (vitamins and trace elements).

Experts from the World Health Organization (WHO) and Food and Drug Administration of the United States of America (United States Food and Drug Administration - US FDA) are trying to formulate recognizable standards that determine the daily needs for trace elements, which are defined as the recommended daily allowance (Recommended Daily Intake - RDI). Their efforts are shared by the research teams around the world in order to adopt national and international standards. Despite intense research, there is still a lot of unknown facts when it comes to the impact of vitamins and trace elements to some specific functions related to stamina, physical strength and capability (1, 4).

The body supply with minerals that are present in large quantities, such as Ca, Mg, P, K and C is achieved easily in terms of well-balanced diet, as these substances are present in food in sufficient quantities. Regarding trace elements, the situation is quite different. Some trace elements are present in a small number of foods which can be the cause of their insufficient intake and deficiencies.

Lack of most trace elements in the body is not easily visible and recognizable in part because we still do not know enough about certain elements and possible symptoms of their deficiency, and partly because there are very complex interactions between the individual trace element, that can change and mask the clinical manifestations of deficiency.

Over the long term strenuous physical activity energy transport in skeletal muscle increases by 20 to 100 times compared to the inactive state. There is an increased demand for the substances that provide energy which is accompanied by increased needs for certain macronutrients, vitamins and trace elements. Many trace elements play a key role in energy metabolism during strenuous physical activity (3). Enormous physical activity also increases oxygen consumption, production of free radicals, the activity of the enzyme "cleaners" and the need for antioxidant vitamins (C, E, and beta-carotene) and trace elements (5).

In addition, many trace elements are intensively lost in sweat or urine during physical exertion. It should be mentioned that between certain trace elements there is a strong synergism, meaning that some trace elements are necessary for the proper operation of others. Some trace elements may enter into mutual interaction which should also be taken into account (3, 4, 6, 7).

It is believed that most athletes do not need mineral supplementation as a balanced diet is rich enough and meet the increased needs. However, some groups of athletes are at risk to develop trace element or macronutrient mineral deficiencies. These are the persons involved in sports where low body mass is important for success (gymnasts, ice skaters, jockeys) or sports that require exactly the right body weight due to the category (boxers, weightlifters, wrestlers). Despite intense training, these athletes are very often on reduction diets to achieve the required weight loss to compete. A food intake below 2000 kcal per day can lead to inadequate intake of essential substances such as vitamins and trace elements (1, 4).

The deficiency in trace elements can also occur in the period of rapid growth in adolescents who participate in sports as well as in people who suffer from eating disorders. In athletes, most common eating disorders are borderline forms of bulimia and anorexia. Poor nutrition is the most common cause of deficiencys in athletes. Lack of knowledge about the principles of proper nutrition is a common culprit, as well as the lack of time to prepare quality meals and susceptibility to advertising that favor different supplements and foods of poor quality (1, 8, 9).

The impact of micronutrient deficiency on physical strength and abilities was the subject of numerous studies. Commonly, there is decreased physical fitness and strength associated with the deficiency of a number of oligoelements and vitamins due to prolonged lack of dietary intake, which results in a decrease in their tissue concentrations (1, 8, 10, 11).

The great part of this knowledge was gained in the forties of the twentieth century, until the introduction of the practice of processed foods enrichment with iron and vitamins. In modern conditions, the research of micronutritive substance deficiencies is carried out in experimental conditions in animals or in developing countries where foods enrichment has not been conducted (8, 10, 11).

Today, the generally accepted opinion is that the deficiency of iron, thiamine, riboflavin, vitamin B6, C and E vitamins (individually or combinations) certainly reduces physical strength and fitness. Investigation of the effects of other micronutritive substances on physical strength and fitness give conflicting results (1, 5, 7).

Persons involved in sports, either professional or amateur, are aware of benefits brought about by proper nutrition and optimal health. Lots of information offered by the popular press and media very often are not fully supported by scientific studies and valid evidence. It is wise to consult a physician when making a decision on food supplement use.

Iron (Fe) and the needs of athletes for iron

Iron plays an important role in the body because it participates in oxidation-reduction reactions and allows the transport of oxygen and constitutes a component of metalloenzymes.

In addition to the most known function in transferring oxygen via hemoglobin, the iron allows the running of a number of important functions as a cofactor of many enzymes. Through myoglobin it allows movement, performs modification of collagen and elastin (lysyl and prolyl hydroxylase), supports immune function (myeloperoxidase), detoxify xenobiotics (cytochrome P-450). It is involved in cellular transport of energy: in the production of energy (cytochromes and aconitase), in aerobic metabolism (activities of α-glycerilphosphate dehydrogenase),

allows electron transfer (iron-sulfur oxidoreductase), has a role in gluconeogenesis (carboxykinase), exhibits antioxidant activity (catalase) and participates in the synthesis of DNA and RNA (ribonucleotide reductase) (1, 4, 8, 9).

The recommended daily intake (RDI) of iron for adults is 18 mg per recommendation from the 1995 year (1, 2). Iron is abundantly represented in meat, offal, egg yolk, chicken, fish, whole grains, legumes, blueberries, blackberries and leafy vegetables. It is easier to exploit iron from foods of animal origin. In this case, the 25% of the heme Fe is absorbed, compared to only 3-15% of vegetables Fe, as well as supplementation. The iron absorption promotes vitamin C. It is therefore recommended intake of juices and vegetables with meat (6).

The absorption of iron is reduced in the presence of: tea, coffee, calcium carbonate, clay and the reduced acidity of gastric fluid. Iron interacts with zinc and vitamin E. Control mechanisms of absorption and transport of iron are highly specialized and do not allow the spontaneous release of Fe ions in the body (6).

Disorders of iron metabolism may be of a type that lack or deficiencys and excessive intake and intoxication.

Iron deficiency is the most common nutritional disorder. The main risk group for Fe deficiency are females of reproductive age, then children and adolescents (12, 13). The deficiency may occur in the following conditions: pregnancy, malnutrition (anorexia, diet), the elderly, with blood loss (menorrhagia, irregular bleeding, ulcers, hemorrhoids, the use of salicylates, the use of non-steroidal anti-inflammatory drugs, tumors of the digestive tract of various localization, frequent blood donation, thalassemia), gastrectomy, malabsorption syndrome, congenital hemorrhagic syndromes, paroxysmal nocturnal hemoglobinuria and hemolysis in athletes who practice running (6, 8, 10, 11).

Signs and symptoms of iron deficiency are primarily related to the development of sideropenic (microcytic) anemia. These people complain of frequent fatigue and exhaustion, have poor memory, altered peripheral sensitivity (paresthesia), reduced work capacity and tolerance to cold. Their skin is pale, with a pearly white sclera, the hair is frayed and nails are brittle. There may be gastrointestinal discomfort, glossitis, angular stomatitis, dysphagia and edema. Loss of immunity is accompanied by increased propensity to infection and eczema. There is tachycardia and shortness of breath, while in more severe conditions systolic murmur above the ictus cordis can be heard together with electrocardiographic disorders (signs of ischemia). There may be a secondary hormonal disorders, while growth retardation is observed in children (2, 14).

Excessive iron intake usually occurs by the accidental intake of excessive amounts of iron supplements. The first event after oral intake is stomach pain, because iron as a corrosive substance damages gastrointestinal tract epithelium. This is followed by nausea and vomiting, bloody diarrhea and in severe cases of hepatocellular necrosis with hepatic failure (15). Metabolic acidosis can be developed, also depression of cardiac function with a drop in blood pressure and CNS depression to coma (16, 17).

Long-term accumulation of iron in the body (hemochromatosis) as an aquired disorder is usually caused by hematological disorders (thalassemia and anemia). Iron is in these people predominantly deposited and damages the liver, heart and pancreas (18). Excessive iron deposition was observed in athletes involved in professional cycling, because of long-term excessive doses of iron supplementation (19).

Athletes may have iron deficiency due to decreased dietary intake, blood loss or increased needs due to physical activity. Endurance athletes are at greater risk for iron deficiency due to the disproportion between its absorption from foods and exercise-induced loss (12).

Physiological changes during exercise can create a false picture of the reduction of hemoglobin, ferritin and iron. These changes include hemodilution and stimulation of erythropoiesis. The phenomenon of transient decrease in hemoglobin concentration exists at the beginning of training, especially in endurance sports (in runners or swimmers) and is explained by the rapid expansion of plasma volume in relation to the mass of red blood cells, affected by osmosis and hormonal response. In some cases, reticulocytosis and macrocytosis are the consequences of hemolysis. The mechanisms that lead to hemolysis differ depending on type of sport, and the best known cause is the mechanical trauma of peripheral capillaries and consequent fragmentation of erythrocytes, as in runners (13, 20).

It was found that athletes lose iron through bleeding from the gastrointestinal tract, especially the long distance runners. Top athletes have an increased loss of blood through the intestine, but this phenomenon is usually compensated by increased absorption of dietary iron (13). In the study of Stewart et al. (21) 83% of runners had occult blood in the stool after the competition. Of course, athletes who have iron deficiency anemia experienced a significant decline in sport performance, working capacity and VO2max. Their iron supplementation is clearly justified, which is proved by improving athletic performance after treatment (6, 12, 22).

Measurement of ferritin levels is used for a general assessment of the amount of iron in the body, however, the expression of ferritin in the serum is also determined by other factors, for example inflammation. Serum ferritin concentration declines during and after exercise, which was interpreted as a lack of iron after exercise. The level of ferritin in elite athletes is often low, however the iron deficiency is not common (6, 12). Currently the lower limit of ferritin concentration, that would determine the need for iron compensation, has not been standardized yet. According to a study Rodenberg (22) if ferritin is below 35 ng/ml the treatment of iron should be considered (6).

Instead of ferritin, other parameters can be used for assessing of iron reserves in the body. These include serum transferrin receptors (sTfR), for which was found to more accurately shows the need of bone marrow for iron (13, 23).

Athletes are recommended to take iron in an amount of 10 - 25 mg per day depending on their status. A dose above 100 mg per day increases the risk of infection and multiple toxic effects can be expressed (stomach pain, constipation, black stained feces and melena). Mega-doses of iron are certainly anti-ergogenic (10). Supplementation with Fe is not justified in terms of improving sports performance, and may even be harmful (13, 23).

Copper (Cu) and the needs of athletes for copper

The basic role of copper in the body is determined by the activity of two key enzymes that contain this element, and are involved in aerobic metabolism: cytochrome-C-oxidase and superoxide dismutase. Copper has many functions in the body: antioxidant (superoxide dismutase and ceruloplasmin), performs metallothionine (tyrosinase) induction, it is involved in intracellular energy production (cytochrome c oxidase), the formation of disulfide bonds (thiol oxidase), synthesis of collagen (lysyl oxidase), catecholamines, neurotransmitters (monoamine oxidase) and hemoglobin, as well in the process of blood clotting (coagulation factor IV) (1-3, 8, 24).

The current (1995 year) recommended daily intake (RDI) of copper for adults is 2.0 mg (from 0.6 to 0.7 mg/1000 kcal). A dose of 10 mg is toxic (1).

Copper is mostly present in seafoods (mussels, clams, squid), legumes, stone fruits, seeds, cereals, offal (kidney, liver, brain) and potatoes (1-4).

Copper absorption is reduced by excesive intake of calcium, phosphate, iron, zinc, cellulose fibers, fructose and raw meat. The favorable effect on copper absorption have breast milk, histidine and other amino acids (1-3, 24).

A copper deficiency may occur in children, women, the elderly, pregnant women, in long-term total parenteral nutrition, malnutrition, Menke's disease, Wilson's disease and Ehlers-Danlos syndrome, in premature infants (accumulates near the end of pregnancy) (1-4). The signs and symptoms of copper deficiency are hypochromic microcytic anemia (not improving on the administration of iron), growth failure and weight loss, depigmentation (role in melanogenesis), retarded growth, decreased reproductive ability, low immunity, reduced elasticity of blood vessels (blocks the synthesis of elastin causing rigidity of arteries); possible rupture of the aorta, neuropathy, electrocardiographic disorders. The signs of copper deficiency in cattle are decreased appetite, osteoporosis and bone demineralization, hypotonia and hypothermia (1-3, 9).

The causes of copper excess in the body are infections (part of the acute phase proteins), nutritional anemia (pernicious), aplastic anemia (within leukosis), endocrine disorders, liver cirrhosis (inability to store and reduced secretion of the bile) and physiologic (pregnancy), use of contraceptives and estrogen therapy with testosterone and progesterone (2, 24).

Copper has special significance in physical activity in biological processes of energy metabolism, iron homeostasis and antioxidant protection (24).

The sheer physical activity affects copper homeostasis and may interfere with antioxidant activity. Various studies have found that copper concentrations in the blood are high (25) or normal (26). Strenuous exercise leads to an increase in reactive oxygen species and inflammatory reaction with initiation of acute phase response which lead to increased release of ceruloplasmin. On the other hand, intense sweating leads to an immediate reduction of the copper concentration, but not a deficiency. Another proposed possibility is that copper is used for the synthesis of antioxidant enzymes, which is stimulated by long-term physical exercise (24, 27).

Athletes are recommended additional amounts of copper from 0.5-3.0 mg per day. Although it is considered that a daily dose of 10 mg may be toxic, even a single dose of 100 mg has not been found to be toxic in athletes. Large doses of copper do not have ergogenic properties. Because high concentrations of iron and zinc have a negative impact on copper homeostasis, use of supplements that do not take into account this interaction, may undermine the essential elements of its functions related to physical activity, which often occurs in practice (8, 10, 24, 28).

Zinc (Zn) and the needs of athletes for zinc

Zinc is essential for the operation of over 200 enzymes (29). Zinc metalloenzymes have a catalytic, structural and regulatory role. Zink is a component of many enzyme systems: lactate dehydrogenase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, thereby participating in the processes of synthesis and degradation of carbohydrates, proteins, nucleic acids and fats (1, 4).

In addition to these processes, zinc plays a role in cell replication and differentiation (regulates transcription), function of cell membranes, regulation of pH (coal anhydrase), cellular motility and internal transport. It is required for glucose utilization and insulin secretion, cellular immune response (T lymphocytes); acts as an antioxidant (superoxide dismutase) and regulates hormone metabolism (production, deposition and secretion of growth hormone, thyroid, gonadotropins, sex hormones, prolactin and corticosteroids) (1, 4, 30, 31).

Zinc RDI for an adult amounts to 15 mg. The doses above 25 mg can cause anemia and deficiency of copper (1). Zinc is present in the following foods: milk, eggs, red meat, organic meats, wheat germ, seeds, soybeans, brewer's yeast, stone fruits, beans, spinach, in legumes, potatoes, wine and seafood. Zinc absorption is improved by: red meat, EDTA, citrate, methionine, cysteine, histidine, lysine, and glycine. Foods that decrease zinc absorption are: excessive calcium, iron, copper, oxalates, spinach, phytates, foods rich in cellulose and whole grain products (28, 32).

Primary zinc deficiency has been described in rare hereditary disease Acrodermatitis enteropatica followed by growth retardation and hypogonadism in

the presence of gastrointestinal, dermatological and neurological symptoms (1, 9, 33).

The secondary zinc deficiency can develop in various types of malabsorption (ulcer, ulcerative colitis, Crohn's disease, malnutrition in children, pregnant women, the elderly) and increased urinary excretion (2, 4, 9).

The following signs and symptoms of zinc deficiency are identified: growth retardation, delayed bone maturation, delayed sexual maturation, impaired immunity, anorexia, gastrointestinal symptoms and diarrhea, dermatitis, eczema, skin ulcers, acnae, seborrhea, alopecia, hypogeusia (impaired sense of taste), night blindness, impaired reproductive ability, disorders of the musculoskeletal system, slow wound healing, and changes in behavior (1, 34).

It is believed that athletes have a greater need for zinc from people who are not exposed to strenuous physical activity. Increased demand is due to increased production of erythrocytes, caused by increased hemolysis, loss of zinc through the sweat, increased fatty acid metabolism during physical activity, numerous interactions of zinc in the metabolism of iron, as well as the extra testosterone that the athletes needed for muscle development (1- 3, 7-9).

It has been shown that physical activity affects the decrease in the amount of zinc in the body. In the usual diet there are small amounts of zinc. Zinc deficiency usually presents in athletes who are engaged in marathon, running for longer and shorter runs, wrestling, gymnastics and dance (8, 10, 11, 28).

Intense endurance training will increase the plasma concentration of zinc immediately after exercise. It is assumed that the reason is the transition of zinc from the damaged myofibrils of contracting skeletal muscle in the extracellular space. After a short-term increase in the concentration, zinc is eliminated from the blood via urinary excretion, or the re-distribution to the liver under the influence of cytokines (7, 35, 36).

Athletes are recommended to take zinc supplementation of 15-50 mg per day. Zinc exerts toxic effects, which are not disturbing to a dose of 500 mg daily for adults. Large doses of zinc interact with the metabolism of copper and do not have ergogenih effects. The doses above 25 mg can cause anemia and deficiency of copper (8, 10, 11, 28).

Manganese (Mn) and the needs of athletes for manganese

Manganese performs the activation of the following enzymes: glycosyltransferases, manganese -superoxide dismutase, pyruvate carboxylase, phos phoenolpyruvate carboxykinase arginase and glutamine synthetase (1-4). Manganese influences the growth and regeneration of bone, cartilage, and connective tissue through the synthesis of glycosa-minoglycans and proteoglycans. Through the synthesis of glycoproteins it plays a role in the immune system and mucus production, while through the superoxide dismutase it participates in antioxidant protection. It is necessary in the metabolism of carbohydrates (gluconeogenesis) and the production of urea (1-3, 8, 9).

The RDI for manganese is 5.0 mg for adults. It is found in whole grains, black tea, coffee, chocolate, products made of whole grain cereals, seeds, stone fruits, soybeans, liver and fruits. The absorption of manganese is hindered by an excess of calcium, phosphate, iron, zinc, fibers (cellulose, pectin, phytate), oxalate, antacids (alkalinity) and achlorhydria. Manganese absorption enhance vitamin C and hem of meat (1, 9, 37).

Manganese deficiency is most common in underweight people (hospitalized, people on a diet, the elderly), in malabsorption syndrome, Down's syndrome, lupus erythematosus, epilepsy and chronic use of antacids. Signs of manganese deficiency are disorders of skeleton and cartilage (osteoathritis, osteoporosis, fractures) and delayed wound healing (1-4).

Athletes usually take supplementation of 2.0 to 5.0 mg of manganese daily. Ergogenic potential of manganese has not been tested yet, but it probably does not exist. Manganese is considered the least toxic of all of the trace elements, when ingested orally (9, 28, 37).

Selenium (Se) and the needs of athletes for selenium

Selenium is important for the transport of electrons in tissue breathing. As a component of glutathione peroxidase, it participates in the processes of peroxidation. It is important as an antioxidant and replaces the antioxidants vitamin E and C. Selenium is involved in the inactivation of heavy metals and biological transformation of xenobiotics (28). RDI for selenium for adults is 70 mg (1). Foods that contain selenium in lerger amounts are liver, brewer's yeast, broccoli, tomato, onion, tuna, herring, bran, wheat germ and grains (9, 38).

Foods low in selenium causes growth retardation, impaired fetility, degenerative changes in the liver and muscles (38, 39).

Increased intake of selenium causes acute or chronic poisoning. Most often, professional poisonings of workers in the industry of paints and varnishes occur, or with selenium oxide vapor. Poisoning is characterised by respiratory tract irritation and pulmonary edema. Contact dermatitis may occur on the skin. Characteristic symptoms and signs of selenium poisoning are metallic taste and smell of garlic in exhaled air, which is derived from dimethyl selenide, which is produced in the liver (28, 38, 40).

It seems that the need for selenium in well-trained athletes increase depending on the energy consumption, but not in a linear way. In their study, Margaritis et al. (41) have reported that selenium intake in a quarter of men and two thirds of female athletes was inadequate. An additional increase of selenium in the diet is recommended for athletes, in the amounts of 50 to 100 ng (8, 10, 11, 28).

Iodine (J) and the needs of athletes for iodine

Iodine is a component of thyroid hormones and is necessary for the functioning of thyroid hormones (thyroxine and triiodothyronine). RDI for

adults is 150 μg. Foods that contain a lot of iodine are sea salt, iodized salt, seafood, onion, vegetables that is grown on soil rich in iodine, and walnuts (1-3).

Iodine deficiency occurs in endemic areas (Jošanica). Symptoms and signs of iodine deficiency are goiter, cretinism (mental retardation, dwarfism), spastic dysplegia, hypothyroidism, myxedema, apathy, fatigue, cardiovascular disease, hypothermia and constipation (1-3, 8, 9).

Athletes needs for iodine are higher than other people's, because lots of iodine is excreted in sweat. The results showed that the loss of iodine in physical activity amounted to 146 ng per day. Athletes are recommended supplementation with 50 to 200 ng of iodine per day. Ergogenic iodine potential is equal to zero (4, 9, 28).

Chromium (Cr) and the needs of athletes for chromium

Chromium improves the utilization of glucose and acts as a factor of glucose tolerance, because of which it potentiates insulin action. RDI is 120 mg for adults. The main source of chromium are whole grains and meats (1-3).

The lack of chromium occurs in: malnutrition, prolonged total parenteral nutrition, pregnancy, trauma, intensive exercise and excessive consumption of simple sugars. Symptoms of chromium deficiency are glucose tolerance disorders, hyperglycemia and hypoglycemia, hyperinsulinemia, hyperlipidemia, fatigue, diabetes mellitus type 2 and cardiovascular diseases (1, 2, 4, 7).

It has been shown that serum concentrations of chromium increase immediately after exercise in runners (after 10km) and remain elevated for up to 2 times after exercise, and are accompanied by an increased secretion of chromium in the urine for 1 day (42).

Although there are several theories and studies about incread muscle mass and strength while reducing body fat as a result of chromium supplementation, there are no reliable results to date (7). Athletes are recommended to consume from 200 to 800 mg of chromium per day. Trivalent chromium, which is located in the food is non-toxic even at levels 100 times greater than the recommended amounts. Hexavalent chromium or chromate is highly toxic and is known to have carcinogenic potential (3, 28, 43).

Conclusion

Trace elements are important substances involved in the metabolism of energy and cellular structural components, and thus directly or indirectly affect the physical strength and fitness of athletes. Optimal presence of micronutrients allows optimal functioning of the body. Playing sports, especially in situations where athletes are exposed to prolonged extreme efforts, increase the need for both macronutrient and micronutrient substances. Supplementation with trace elements should be carried out in athletes when there is a deficiency or boundary deficiency. A balanced diet and trace elements supplementation should be provided to athletes, satisfying their increased demand in relation to the recommended daily intake (RDI). Supplementation with megadoses of trace elements is not recommended because of possible toxic effects. During supplementation, attention has to be taken regarding possible interactions between trace elements.

A balanced diet and professional supplementation with trace elements, when necessary, provide better physical effect and fitness, together with the achievement of top athletic results.

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PATOFIZIOLOŠKI ASPEKTI SUPLEMENTACIJE OLIGOELEMENTIMA KOD SPORTISTA

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Preduslov za postizanje vrhunskih sportskih rezultata je puno zdravlje i psihofizička utreniranost. Esencijalni oligoelementi su neophodni za normalno odvijanje biohemijskih i fizioloških procesa, iskorišćavanje energetskih i gradivnih materija i optimalno funkcionisanje samih mišića i njihovu skladnu povezanost sa drugim sistemima. Oligoelementi imaju važnu ulogu u energetskom metabolizmu tokom naporne fizičke aktivnosti, kao i stanjima povećane potrebe za kiseonikom, produkcije slobodnih radikala, aktivnosti skavendžer enzma i antioksidantne zaštite.

Sportisti mogu imati deficit gvožđa zbog smanjenog dijetarnog unusa, gubitka krvi ili povećanih potreba usled fizičke aktivnost, međutim, suplementacija nije opravdana radi samog poboljšanja sportske uspešnosti, čak može biti i štetna. Poseban značaj bakar ima u biološkim procesima energetskog metabolizma, homeostaze gvožđa i antioksidantne zaštite. Sportistima se preporučuju dodatne količine bakra od 0,5 do 3,0 mg dnevno, iako velike doze nemaju ergogena svojstva. Takođe, sportisti imaju veće potrebe za cinkom i preporučuje im se suplementacija dodatnom količinom od 15 do 50 mg dnevno. Deficit mangana se najčešće javlja kod pothranjenih osoba, dok se potrebe za selenom kod dobro utreniranih sportista povećavaju u zavisnosti od energetske potrošnje.

Bavljenje sportom, posebno kada se radi o dugotrajnim ekstremnim naporima, povećava potrebe za mikronutritivnim materijama. Sportistima treba obezbediti izbalansiranu ishranu i suplemantaciju oligoelementima koja će zadovoljiti njihove povećane potrebe u odnosu na preporučeni dnevni unos.

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Ključne reči: *mikronutrijenti, fizička aktivnost, gvožđe, bakar, cink, preporučeni dnevni unos*

Original article

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THE ROLE OF SERUM LEVEL OF TUMOR MARKER CA 125 IN DISTINGUISHING BENIGN FROM MALIGNANT OVARIAN TUMORS IN POSTMENOPUSUAL WOMEN AND CORRELATION WITH SONOGRAPHIC FINDING

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Malignant ovarian tumors occur at all ages, including early childhood, but also advanced old age, with the total incidence dramatically increasing with age. Tumor markers for early detection of ovarian carcinoma are used in ovarian cancer examination.

The aim of the study was to examine the degree of correlation between sonographic findings and the levels of serum tumor marker Ca 125, and to study a correlation of preoperative sonographic findings and serum marker level CA 125 with intraoperative finding and patohistopathological results.

The study was based on the prospective-retrospective study model involving 60 postmenopausal women diagnosed with the presence of ovarian tumor.

The following medical tests and examinations were performed for all patients: anamnestic analysis of the medical record, that is the history of the disease with the data on age, parity, duration of menopause, the use of oral contraceptives and symptomatology, small pelvis sonography, lab parameters - Ca 125 with referent ranges up to 35 ml/U. Laparotomy was used as an operative procedure in all patients. All material obtained operatively underwent histopathological treatment.

The group of patients with malignant tumors of high statistical significance showed considerably higher average CA125 values.

Among subjects with benign tumors, the dominant tumor structure was cystic, as opposed to the mixed-type tumors in malignant tumors. To this effect, the parameter of tumor structure is a serious factor in distinguishing between benign and malignant ovarian tumors.

Tumor location is, with high statistical significance, more often bilateral in subjects with histopathologically proven malignant tumors, while it is predominantly unilateral in benign tumors.

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Key words: *CA125, ovarian tumors, postmenopausal, sonographic finding*

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Introduction

Malignant ovarian tumors occur at all ages, including early childhood, but also advanced old age, with the total incidence dramatically increasing with age. The risk of ovarian carcinoma formation increases after the age of 40, with the incidence peak between 50 – 55 years of age. Ovarian carcinoma is in the fifth place immediately behind breast, lung, rectosigmoid colon and lymphoma cancer, with the highest mortality rate among the gynecologic cancers (1, 2).

Survival rate for ovarian cancer depends on the stage when the disease is detected. In this respect, survival is 93% in stage I, 70% in stage II, 37% in stage III and 20% in stage IV. Three-quarters of newly discovered ovarian carcinomas are in stage III and IV, where the five-year survival rate is below 50%. This leads to the conclusion how important early detection and treatment of ovarian cancer are.

The development of ovarian cancers is influenced by many factors. First of all, hereditary, environmental factors, prior pregnancies, breast feeding, oral contraceptives, infertility, substitution hormone therapy, oncogenic viruses, etc.

It is thought that malignant tumors can develop in many ways. The most likely path of development is by means of a benign epithelial neoplasm developed from serous inclusion cyst, developed again by the invagination of the serosa. This is followed by the malignant transformation into epithelium of the benign cyst or the epithelium in serous inclusion cyst can turn malignant without a previous benign phase or, in turn, ovarian serous cells may develop into epithelial malignancy de novo without the formation of the serous inclusion cyst. For a long time, it has been widely accepted that, in time, benign epithelial ovarian cysts may turn malignant (3).

Precisely, due to this complex and insufficiently clarified pathogenesis and unknown etiology, the incidence of malignant ovarian tumors cannot be prevented. Only early diagnosis in asymptomatic women and modern therapeutic approach have proven effective in this serious illness.

Tumor markers for early detection of ovarian carcinoma are used in ovarian tumor examination.

In the past 20 years, various methods for ovarian cancer detection (Papanicolaou test, peritoneal cytology examination, etc.) have been applied, but have not proven to be good enough. The latest methods of immunoscintigraphy, nuclear magnetic resonance and computed tomography may detect small cancers, but their invasiveness and price prevent them from becoming the main screening tests. The methods examined today as screening methods are: bimanual gynecological examination, ultrasound, tumor markers.

Bimanual examination has its advantages: it is relatively easy, it can fit into the already existing cervical screening program, and it does not require special equipment, thus the costs are low. However, neither the sensitivity nor the specificity of this test has been known so far.

The majority of studies looking into the value of ultrasound in the diagnosis of ovarian tumors included women with symptoms who were about to undergo the laparotomy due to suspected ovarian masses. They confirmed the concurrence between ultrasonography and operative findings regarding the size, position and characteristics of the ovarian tumor. Many researchers have tried to use the ultrasound to characterize benign and malignant tumors. However, a criterion with 100% specificity for malignant ovarian tumors has not been described so far. With all this in mind, Sassone and associates published a study in 1991 presenting a scoring system for an objective description of pelvic disease based on the transvaginal ultranosographic characterization of the alteration (4). The proposed scoring system, used both for ovaries and extrauterine pelvic masses of unknown origin, is based on the following four criteria: the structure of interior tumor wall (1-4 points), thickness of the tumor wall (1-3 points), the presence and thickness of septa (1-3 points), 4 echogenicity (1-5 points). In order to obtain a scoring threshold that best separates malignant ovaries from the rest, the sensitivity and specificity for each score from 5-13 is calculated, based on which the curve is formed, the shape of which showed that the total score of 9 points best distinguishes between benign and malignant lesions.

Primarily those are serum levels of alpha-fetoprotein markers (AFP), CA 125, lactate dehydrogenase (LDH), CEA, and inhibin B. If there is suspicion that the tumor is hormone active and produces certain hormones, hormone analyses are performed and they serve as tumor markers. The most common are β HCG, estradiol and testosterone. In addition, tumor markers are used to monitor the effect of the therapy, as well as for the early detection of the recurrence of certain ovarian tumors (5).

CA 125 is antigenic determinant of high molecular weight glycoprotein recognized by the monoclonal antibody OC125. CA 125 is highly sensitive, but not a specific marker for tumors of ovarian epithelial cells. It may have elevated values in many intraperitoneal processes such as endometriosis, pregnancy, small pelvis inflammatory disease, Crohn's disease or other malignant abdominal tumors.

Ca 125 antigen may be detected in serum using radioimmunoassay, and serum levels are higher than 35 ml/U in over 80% of women with ovarian cancer (5). Bast and associates (6) also showed that only 1 % of healthy women had serum Ca 125 levels higher than 35 ml/U (6). Elevated levels may, however, be related to a benign gynecological pathology. However, the incidence of these benign conditions in postmenopausal women, the group that is most at risk of getting ovarian cancer, is low. A more detailed analysis however shows dependence on the stage of the disease. The disease spread outside the ovary is associated with the elevated levels of CA 125 in the serum in over 90% of cases. In the case where the tumor is restricted to ovarian tissue, CA 125 levels in the serum are increased only in 50% of cases (6).

Given that a high degree of specificity is required for the prospective screening program for ovarian cancer and given the link between CA 125 and non-malignant pathology, the positive predictive value of elevated serum CA 125 for this disease is considered too small to use only CA 125 as a screening test. The specificity could be enough if CA 125 were combined with ultrasonography. Such screening program is used in large centers worldwide, including the Royal London Hospital since 1985 (6).

Aims

The aim of this study was to examine the degree of correlation between the ultrasound finding with respect to the level of serum tumor marker Ca 125 and the correlation of preoperative ultrasonography and the level of serum CA 125 marker with intraoperative finding and pathohistopathological results.

Material and methods

The study was based on the prospective-retrospective study models involving 60 postmenopausal women diagnosed with the presence of ovarian tumor.

The research was carried out in the following

institutions: Clinic for Gynecology and Obstetrics at the Clinical Center Niš, Women's Health Service of the Health Center Niš, Clinic for Gynecology and Obstetrics of the Clinical Center Kragujevac, and Clinic for Gynecology and Obstetrics „Narodni front" from Belgrade.

All patients underwent the following tests and examinations:

- Anamnestic health card analysis, history of the disease with the data on age, parity, duration of menopause, the use of oral contraceptives and symptomatology;
- Ultrasonography of the small pelvis;
- Lab parameters - Ca 125 with reference range up to 35 ml/U.

Laparotomy was used as an operative procedure in all patients. All material obtained operatively has undergone histopathological treatment.

The standard descriptive statistical methods were used in statistical data processing (mean value, standard deviation, representation in percentages, degrees of freedom). The assessment of the distribution type was carried out using Kolmogorov-Smirnov test. The assessment of the distribution significance was carried out using the parametric t-test and non-parametric x^2 statistical test, using a standard significance level.

Results

The reference ranges of all laboratories where the levels of serum Ca 125 tumor markers were tested are up to 35 U/L. Average Ca 125 values in the group of benign ovarian tumors amounted to 39.67 U/L, while in the group of women with malignant ones they amounted to 556.6 U/L, which is a difference in values with outstandingly high statistical significance.

In over one half of the subjects with benign tumors, Ca 125 levels were within the reference ranges up to 35 U/L. High marker values (over 100 U/L) were determined in 4 women from this category, whereby the presence of endometrial tumors was found in histopathological preparations after the laparotomy. Simultaneously, similar marker values were also found in the category of malignant tumors in 9 out of 15 subjects, as shown in Table 1 and 2.

Table 1. Distribution of values of CA 125 tumor marker

CA 125 m/U	Benign tumors	Malignant tumors
< 17.4	14	0
17.5 – 35	13	0
36 – 52.5	7	0
52.6 – 99	7	2
100 – 999	4	9
1000 >	0	4
Σ	45	15

Table 2. A correlation between the levels of tumor markers Ca 125 measured in benign and malignant tumors, with standard deviations in both categories

	$\overline{X}$	SD	Σ
Benign	39.67	± 34.8	45
Malignant	556.6	± 499.3	15

*T – test = - 7.08; p < 0.01 – significantly high statistical significance, df = 58

Tumor location is predominantly bilateral in the case of malignant tumors, while unilateral in benign ones, resulting in high statistical significance as shown in Table 3. The size of benign tumors is 7 cm on average, while the malignant tumors in the examined group of patients were over 9 cm, which makes high statistical significance, as shown in Tables 4 and 5.

Table 3. A correlation between the location of benign and malignant tumors, with standard deviations in both categories

	$\overline{X}$	SD	Σ
Benign	4.4	1	45
Malignant	2	13	15

$x^2 \rightarrow \infty$; p > 0.001 – high significance, df = 1

Table 4. The size of the tumor in the examined group of women

Tumor size in cm	Benign tumors	Malignant tumors
< 3 cm	0	0
4 – 5	8	0
6 – 7	23	2
8 – 9	7	5
> 10	7	8
Σ	45	15

Table 5. A correlation between the size of benign and malignant tumors, with standard deviations in both categories

	$\overline{X}$	SD	Σ
Benign	6.96	± 3.11	45
Malignant	9.04	± 4.12	15

T test = -2.8: p = 0.006 – high statistical significance, df = 58

The wall was significantly thicker in benign changes in 39 subjects and it amounted to 3 and more millimeters. In contrast, in malignant tumors, the thickness of the tumor wall in 13 out of 15 subjects was 2 and below 2 mm, which has high statistical significance, as shown in Table 6.

Table 6. A correlation between the wall thickness of benign and malignant tumors, with standard deviations in both categories

	≤ 2 mm	≥ 3 mm	Σ
Benign	6	39	45
Malignant	13	2	15

$x^2 = \rightarrow \infty$; p < 0.001 – high statistical significance, df = 1

There are significant differences in the appearance of the wall of the change. While in benign ovarian tumors the interior of the wall is smooth, in malignant tumors the interior of the wall is uneven and with numerous excrescences, as shown in Table 7.

Table 7. A correlation between the wall structure of benign and malignant tumors, with standard deviations in both categories

	Smooth	Uneven	Σ
Benign	40	5	45
Malignant	7	8	15

x^2 = 11.8, p < 0.001 – high statistical significance, df = 1

Discussion

A large number of previously conducted studies (7-9) showed that Ca 125 tumor marker was not specific enough in the differentiation of benign from malignant ovarian tumors. Van Calster and associates point out in their paper that serum Ca 125 value are more often false positive in premenopausal women compared to postmenopausal women, but that in both groups the ultrasonographic classification of changes in the ovary is by far a more reliable criterion for distinguishing between benign and malignant tumors (7). The average Ca 125 values in the examined population were considerably higher in the group of patients with malignant tumors X = 556.6 U/L versus X = 39.6 U/L in benign tumors. Hence, there is a high statistical significance. The results of this study are complementary to the results of the study led by Dr. Edward E. Partridge, University of Alabama and Birmingham (10). This study showed that Ca 125 values of more than 35 U/L could be considered suspicious (indicative of tumor), and that values greater than 65 U/L were a reliable predictor for tumor malignancy in asymptomatic menopausal women in combination with transvaginal ultrasound examination.

Differentiation between benign and malignant ovarian tumors is most important both for the patient and the physician. In most institutions, the operative procedure (laparoscopy or laparotomy) depends on the assessment of the malignancy of the change, but malignancy can be safely excluded by histopathological confirmation only (11, 12). Therefore, many prognostic models for the differentiation of malignant from nonmalignant ovarian tumors, including Doppler criteria, have been published so far and show significant validity (12-15).

In ultrasound diagnostics and in estimation of malignant potential for ovarian tumors, many scoring systems have been created that did not find broad application in clinical practice (10, 16). However, all data suggest that in terms of location in malignant tumor processes, the tumor is most often bilateral, in contrast to dominant one-sidedness in benign conditions. It is that assumption that was proven in the presented study. In addition, the size of benign tumors is up to 70 mm, while the malignant tumors in the examined group of patients are over 90 mm.

Benign ovarian tumors are primarily cystic in structure, in contrast to the mixed-type structure in malignant tumors. The thickness of the ovarian tumor wall is considerably higher in benign tumors, which speaks in favor of their cystic and clearly limited structure. There are also significant differences in the appearance of the wall of the tumor alteration. While the interior of the wall is smooth in benign tumors, it is uneven and with numerous excrescences in malignant tumors. Papillary projection is a significant ultrasound sign of tumor malignancy. The degree of malignancy is proportional to the number of these papillary formations (16). Granberget al. showed that the risk of malignancy is 3-6 times higher in unilateral cysts with papillary formations compared to unilateral cysts without these formations, which makes the conservative tracking of these cysts unacceptable (16).

As an addition to the ultrasonographic morphological image of the tumor, other factors such as family history, the presence of free fluid in the pouch of Douglas and the presence of subjective symptoms should be taken into account when deciding on the optimal treatment. When it comes to benign alterations in the conducted study, the presence of free fluid in the pouch of Douglas was sporadically determined, while in malignant ovarian conditions this is one of almost regular clinical and ultrasound findings.

Conclusion

Average Ca 125 values were far higher in the group of patients with malignant tumors with high statistical significance. Among subjects with benign tumors, the dominant tumor structure was cystic, as opposed to mixed-type structure tumors in the malignant ones. In that sense, the parameter of tumor structure was a significant factor in distinguishing between malignant ovarian tumors.

Tumor location is, with high statistical significance, more often bilateral in subjects with histopathologically proven malignant tumors, while it is predominantly unilateral in benign tumors.

The size of benign tumors was around 70 mm on average, while the malignant tumors in the examined group of patients were over 90 mm. Based on this, tumor size is a reliable factor in distinguishing between benign and malignant ovarian tumors. The thickness of the wall of benign tumors is higher in relation to that of malignant ones, and this is a parameter of high statistical significance.

The presence of free fluid in the pouch of Douglas is rare in benign ovarian tumors, and as a rule, where determined, it is associated with the ruptures of the tumor wall (cyst) and most often it is below 50 ml, while in malignant ovarian tumors the

presence of free fluid is quite frequent, whereby the quantity is multiple times higher, and often filling the entire volume of Douglas.

The obtained results clearly demonstrated that detailed ultrasonography of the small pelvis and adnexa and the levels of serum tumor marker Ca 125 are reliable parameters for the differentiation of benign from malignant ovarian tumors in postmenopausal women.

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ULOGA SERUMSKOG NIVOA TUMORSKOG MARKERA CA 125 U RAZLIKOVANJU BENIGNIH OD MALIGNIH TUMORA JAJNIKA KOD ŽENA U POSTMENOPAUZI I KORELACIJA SA ULTRAZVUČNIM NALAZOM

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Maligni tumori jajnika javljaju se u svim životnim dobima, uključujući i rano detinjstvo, ali i duboku starost, pri čemu ukupna incidencija dramatično raste sa godinama života. U ispitivanju tumora jajnika koriste se tumorski markeri za njihovo rano otkrivanje.

Cilj rada bio je ispitivanje stepena korelacije ultrazvučnog nalaza u odnosu na nivoe serumskog tumorskog markera Ca 125 i ispitivanje korelacije preoperativnih ultrasonografskih nalaza i nivoa serumskog markera CA 125 sa intraoperativnim nalazom i patohistopatološkim rezultatima.

Istraživanje je definisano po modelima prospektivno-retrospektivne studije, koja obuhvata 60 žena u postmenopauzi kod kojih je postavljena dijagnoza postojanja tumora jajnika.

Svim bolesnicama su urađene sledeće pretrage i pregledi: anamnestička analiza kartona, odnosno, istorije bolesti sa podacima o godinama starosti, paritetu, trajanju menopauze, upotrebi oralnih kontraceptiva i simptomatologiji, ultrasonografski pregled male karlice, laboratorijski parametri: Ca 125 sa referentnim vrednostima do 35 ml/U. Kod svih bolesnica primenjena je laparotomija kao operativna procedura. Operativno dobijen material je histopatološki obrađen.

Prosečne vrednosti CA125 bile su daleko veće u grupi bolesnica sa malignim tumorima, sa visokom statističkom značajnošću.

Među ispitanicama sa benignim tumorima dominantna građa tumora bila je cistična, nasuprot tumorima mešovite građe kod malignih tumora. U tom smislu, parametar građe tumora je ozbiljan činilac u razlikovanju benignih od malignih tumora jajnika.

Lokalizacija tumora je sa visokom statističkom značajnošću češće bilateralna kod ispitanica sa histopatološki dokazanim malignim tumorima, dok je kod benignih tumora pretežno unilateralna.

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Ključne reči: *CA 125, tumori jajnika, postmenopauza, ultrazvučni nalaz*

Original article

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DISTHYROID ORBITOPATHY

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A correlation of autoimmune Graves' hyperthyroiditis and mild forms of autoimmune Graves' ophthalmopathy (GO) occurs in approximately 50% of patients, while severe form of autoimmune Graves'ophthalmopathy occurs in 3-7% of all patients. Around 80% of Graves' ophthalmopathy cases occur in association with hyperthyroidism, although not all coincide with the onset of hyperthyroid symptoms. Because an increase in orbital content compresses the eye, elevated intraocular pressure, protrusion of the eye and/or isolated optic nerve neuropathy can be developed. The aim of our work was to demonstrate that autoimmune Graves' disease of the thyroid gland in patients without other autoimmune diseases is in correlation with an increased risk of protrusion of an eye and increased eye pressure.

The research included a group of 42 patients (84 eyes) who were divided into two equal groups. The first group consisted of patients with Graves' disease of the thyroid gland, without other autoimmune diseases, while the second, control group consisted of patients who did not have autoimmune disease. All patients were subjected to a complete ophthalmological and internal examination in the morning hours. The χ^2test and Fisher exact test were used in statistical analysis. Patients with Graves' disease of the thyroid gland had statistically higher values of intraocular tension and values of protrusion of patients of control group ($p < 0.001$). Elevated intraocular pressure was measured in 14 eyes of patients with autoimmune hyperthyroidism (16.67%) and 5 eyes of patients of the control group (5.95%). Mild protrusion was found in 12 eyes in the patients with autoimmune hyperthyroiditis (14.29%) and 4 eyes in the control group patients (4.76%). Patients with the disease of thyroid gland have a higher risk of the intraocular pressure increase and protrusion of the eye. Because of that, it is necessary to do a complete ophthalmological and internal examination of patients with Graves' hyperthyroiditis in order to prevent glaucoma.

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Key words: *Graves' ophthalmopathy, protrusion, increased eye pressure*

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Introduction

The eye disease which has generally been associated with autoimmune thyroiditis has been known by many names: Graves' ophthalmopathy (GO) or von Basedow's disease, thyroid eye disease (TED), or thyroid associated orbithopathy (TAO)(1). These terms indicate that the etiopathogenesis of the condition is not fully elucidated and that the disease has numerous and varied clinical presentations and some features are more sight-threatening than others (2). Around 80% of cases of GO occur in association with hyperthyroidism, although not all coincide with the onset of hyperthyroid symptoms. The rest of 20% of cases of GO occur in association with autoimmune hypothyroidism or autoimmune eutiroidism (3). The most frequent symptoms in the beginning of GO disease are characterized by pain, swelling, redness, watering, retraction of the upper eyelids, conjunctivitis, bulging eyes, double vision and occasionally decreased vision (4). GO has many clinical appearances with more or less important clinical signs (5). Diagnosis and differential diagnosis of Graves' disease (GD) include eye changes, autoimmunity of thyroid gland and exclusion of other autoimmune coexisting diseases such as vitiligo, celiac disease, autoimmune liver disease, myasthenia gravis, sclerosis multiplex, rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome and systemic sclerosis (6).

GO may occur before or after the onset of an overt thyroid disease, and it can start suddenly or more slowly (7). Even though some patients undergo spontaneous remission of symptoms within a year, many need treatment (8). The first step of treatment aims to regulate thyroid hormone levels and implement smoking cessation (9). Lubricating eye drops are used to avoid damage to the eye (10). GO is a rare but treatable disease that causes a significant decrease in quality of life (11). The main antigen, which is the same for both thyroid gland and orbit, is called thyrotropin receptor (TR), and it is important for the mechanism involved in the pathogenesis of GO (12). TR-stimulating GD immunoglobulins induce hyaluronan synthesis and enhance adipogenesis by differentiating orbital fibroblasts (13). In active disease, the accumulation of inflammatory cells and interstitial edema of extraocular muscles occurs, in first line m.rectus internus and m.rectus inferior. The muscles may be in increase for two or three times (14). In pathogenesis of glaucoma the most important role was the increase of orbital volume, and compression of bulbus oculi, orbital veins and nervus opticus into the orbital apex. The drainage of anterior chamber fluid is with difficulties (15).

This division allows us a different approach to the treatment of ophthalmopathy (16). The active disease lasts for 18-24 months, especially in patients, younger than 40 years. Signs of the active disease are pain, swelling, redness, watering, retraction of the upper eyelids, conjunctivitis, bulging eyes, and double vision. Signs of inactive disease are proptosis and higher palpebral aperture. In older patients, synthesis of glycosaminoglycans is dominant (17).

Methods

This study was conducted from December, 2013 to June, 2015. The study included 21 patients with autoimmune hyperthyroiditis from Department of Internal Disease, treated with hormonal therapy and 21 patients without autoimmune diseases from Department of Physical Medicine and Rehabilitation Military Medical Center. All patients were subjected to a detailed internist and ophthalmological examination, including detailed history of previous diseases or injuries, with the aim to exclude any other autoimmune diseases.

The first group consisted of 16 female and 5 male patients, aged from 33 to 65 years. Some of them had other chronic diseases, 15 patients had arterial hypertension, 6 insulin-independent diabetes mellitus and 9 of them were smokers.

The second group of patients served as a control group, consisting of 21 patients, 11 women and 10 men, from 30-65 years. All patients were without autoimmune disease, whereas among other chronic diseases there were 5 smokers, 17 with arterial hypertension and 4 insulin-independent diabetes mellitus patients.

Ophtalmology examinations were performed in the morning and included examination of visual acuity, color vision, measurement of proptosis with HERTEL-exophthalmometer, examination of eyes motility, upper lid retraction, examination on a slit lamp HAAG-STREIT, intraocular pressure measurement by the contact applanation tonometer, and fundus examination. Measuring with HERTEL-exophthalmometer, with a snug mechanism and preferably a square angle which sits against the orbital rim, was done in a sitting position (18). Pathological values of proptosis were 20mm and higher intraocular pressure (IOP) was 22mmHg and over (19). Values of proptosis from 21 to 23 mm were small, from 24 to 27 were moderate, and values over 28mm were large (20). In a statistical analysis, the χ^2test and Fisher exact test were used. During a review with the slit–lamp, the presence or absence of conjunctival plica semilunaris was noted and redness of the caruncula excluded any microbial inflammation. We detected some changes on the optic nerve by fundus examination, using ophthalmoscopy through dilating pupil by the application of the mydriatic agent tropicamide.

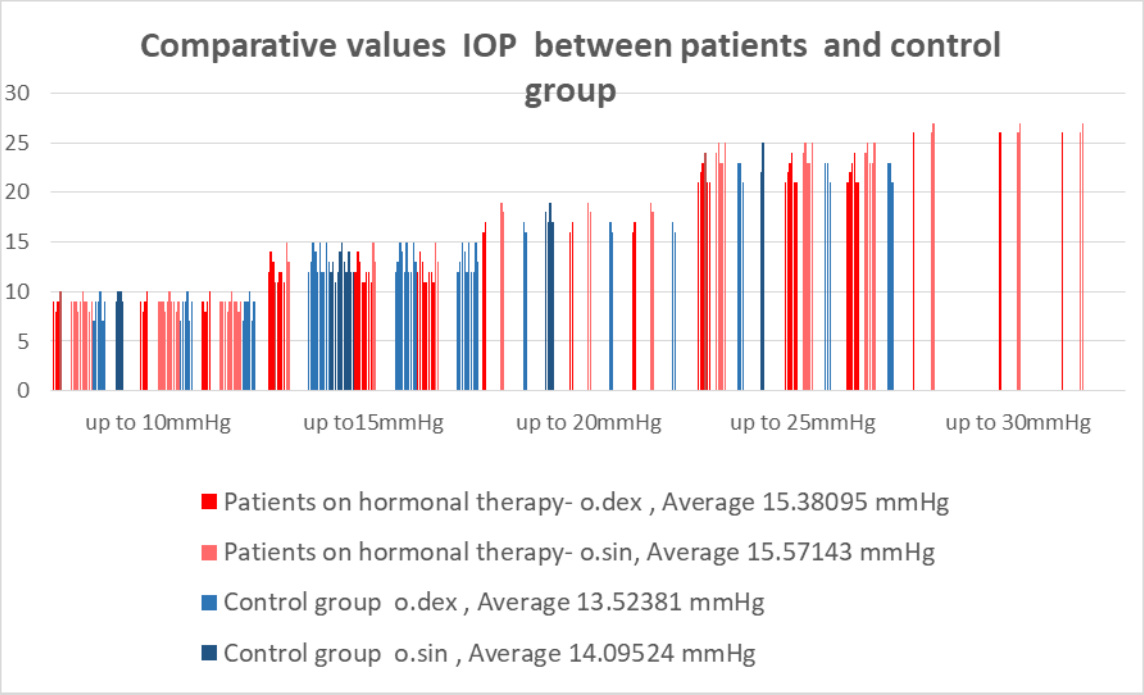
Results

In a group of 42 patients in total (84 eyes), IOP was increased in 14 eyes on hormonal therapy (16.67%), and in 5 eyes in the control group (5.95%). The average IOP in patients on hormonal therapy was 20.73 mmHg for the right eye and 20.94 mmHg for the left one. In control group, the average IOP was 15.94 mmHg for the right eye and 18.13 mmHg for the left eye (Graph 1).

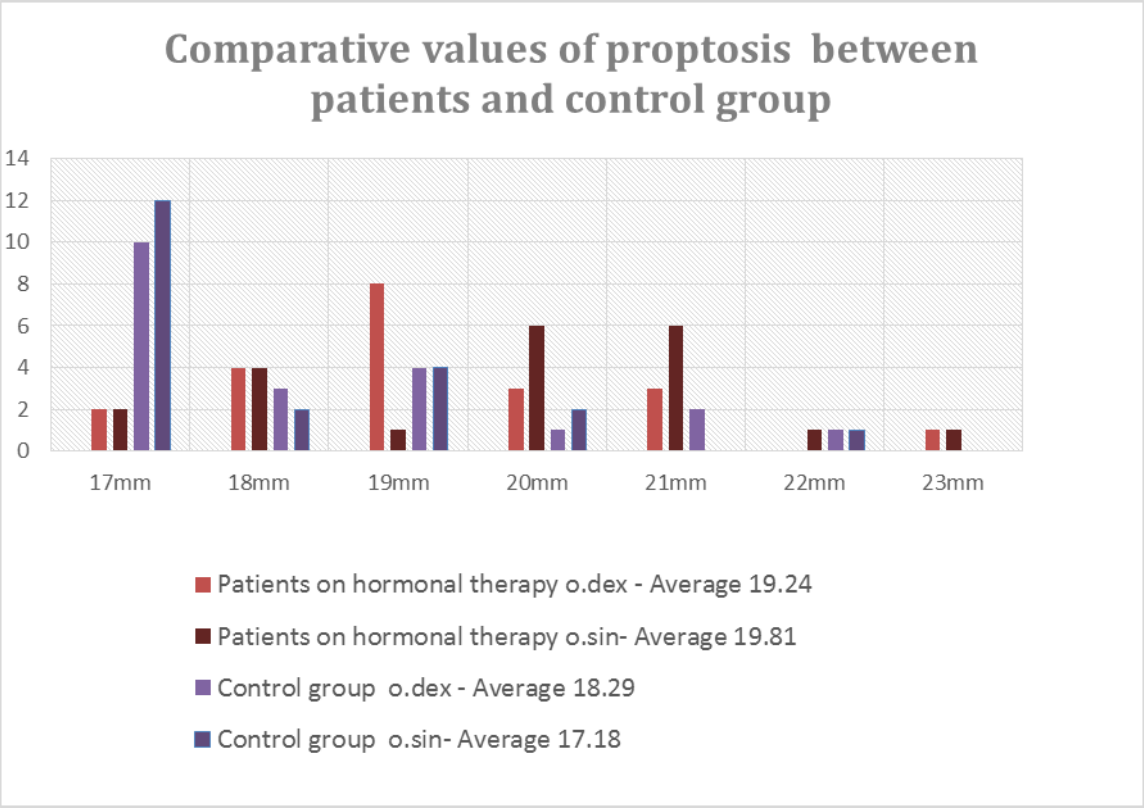
Graph 2 shows the values of proptosis in patients on hormonal therapy and the control group. The average value of proptosis in the patients on hormonal therapy was 19.24 mm for the right eye, and 19.80 mm for the left eye. The average value of proptosis in the patients in control group was 18.29 mm for the right eye, and 18.00 mm for the left eye. Out of the total group of 42 patients (84 eyes) we examined, 12 eyes from the group on hormonal therapy were with mild proptosis (14.29%), while in the control group 4 eyes were with slight proptosis (4.76%).

Discussion

Hashimoto's thyroiditis - goiter is an autoimmune disease associated with ophthalmopathy, too. In Hashimoto thyroiditis - goiter, changes of the eye are less pronounced, they are slowly progressing and less conspicuous. Goiter is discreet, and it does not indicate a disease (21). Genetics, pregnancy, stress, deficiency of jodine (J) or (Se) in a nutrition, excess of radioactive J, usually entered by therapy per os, various toxins in environment, radioactivity in the living surrounding, cosmic radiation and yersinia enterocolitica are the factors which can be associated with it (22).



Graph 1.



Graph 2.

Tobacco smoke has a big influence on the level of activity of an autoimmune inflammation, triggering the immune system (23). Smokers are at an increased risk of activating autoimune processes (24). Physicians have to be aware of the emotional aspects of patients with Graves' ophthalmopathy, since various kinds of neuroses are frequent (25). In developed countries, a routine thyroid testing of

pregnant women, regular screening of neonates, children and young people is mandatory, because hormone deficiency can lead to brain and organic defects of intrauterine baby, slow growth and slow psychological development (26).

Nowadays, in the modern age, the thyroid gland dysfunctions are diagnosed much earlier, hence endocrinological medical treatment can start immediately at the first sign of proptosis (27). With hormonal therapy conducted on time, from the very beginning of the disease, we are able to suppress autoimmune inflammation (28). If the therapy starts early, a regression of all signs can be accomplished. After Graves' ophthalmopathy has been diagnosed, we should choose an indication for a local medical treatment. Only a few patients (5-10%) develop a severe disease form that requires aggressive treatment. Medical therapy is not effective enough at this stage of the disease (29). Glucocorticoids represent a major therapeutical modality for severe, active ophthalmopathy (30). For differential diagnosis there are other conditions, such as inflammable ophthalmopathy, orbital cellulitis, myositis, chronic progressive external ophthalmoplegia, orbital tumors, neurosarcoidosis, myastenia gravis, carotid-cavernous fistula and conjunctivitis allergica.

Conclusion

Patients with GO are exposed to an increased risk of developing proptosis and increased IOP that can lead to glaucoma. Complete ophthalmic examination is necessary for all patients with GO because of the possibility for the development of isolated inflammation localized in the orbital apex, without other signs of proptosis, disturbed ocular motility, or increased IOP.

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DISTIROIDNA ORBITOPATIJA

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Povezanost autoimunog Grejvsovog hipertiroiditisa i blage forme Grejvsove autoimune oftalmopatije (GO) javlja se kod priblizno 50% bolesnika, dok se teška forma javlja kod 3-7% bolesnika. Oko 80% slučajeva Grejvsove oftalmopatije povezano je sa autoimunim hipertiroidizmom, iako nije u vezi sa pojavom prvih simptoma bolesti. Zbog uvećanog sadržaja orbite koji vrši kompresiju na oko može se javiti povišen očni pritisak, protruzija oka i/ili kompresivna neuropatija optičkog nerva. Cilj našeg rada bio je da ukažemo da kod bolesnika sa autoimunim Grejvsovim oboljenjem štitne žlezde, bez drugih autoimunih oboljenja, postoji povećan rizik za nastanak protruzije oka i povišen očni pritisak.

Istraživanje je rađeno na grupi od 42 bolesnika (84 oka) podeljenoj u dve jednake grupe. Prvu grupu činili su bolesnici sa Grejvsovim oboljenjem štitne žlezde bez drugih autoimunih oboljenja, dok su drugu, kontrolnu grupu, činili bolesnici koji nemaju autoimuno oboljenje. Svim bolesnicima je urađen kompletan internistički i oftalmološki pregled u prepodnevnim satima. U statističkoj obradi je korišćen χ^2 -test i Fišerov egzaktni test. Bolesnici sa Grejvsovim oboljenjem štitne žlezde imaju značajno više vrednosti očnog pritiska i izraženiju protruziju očiju od bolesnika kontrolne grupe ($p < 0,001$). Povišen očni pritisak je izmeren kod 14 očiju kod bolesnika sa Grejvsovim oboljenjem štitne žlezde (16,67%) i kod 5 očiju kod bolesnika iz kontrolne grupe (5,95%). Blaga protruzija je nađena kod 12 očiju bolesnika sa Grejvsovim oboljenjem štitne žlezde (14,29%) i kod 4 oka u kontrolnoj grupi (4,76%). Bolesnici sa oboljenjem štitne žlezde imaju veći rizik od nastanka povišenog očnog pritiska i protruzije oka. Zbog toga je kod bolesnika sa Grejvsovim hipertiroidizmom potrebno uraditi kompletna internistička i oftalmološka ispitivanja u cilju prevencije glaukoma.

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Ključne reči: *Grejvsova oftalmopatija, protruzija, povišen intraokularni pritisak*

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THE INFLUENCE OF CANCER PAIN ON THE QUALITY OF LIFE IN PATIENTS WITH ADVANCED CERVICAL CANCER: ONE-YEAR SINGLE CENTER EXPERIENCE

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The aim of the study was to investigate the incidence of pain in patients at various stages of inoperable cervical cancer, establish clinical phenotype of pain, as well as the degree of impact of pain on quality of life and its indicators.

The study included 102 patients with a pathohistological finding of inoperable cervical cancer. A numerical scale (NRS) was used to determine the severity of the pain. The following parameters of quality of life were observed: appetite, sleep, mood, social interaction and general activity. Patients assessed the degree of pain on a scale from zero to ten for each of these parameters. By adding these values, the score (0-50) defining the quality of life was obtained. The impact of pain on the quality of life was determined before specific oncological treatment and three months after therapy.

Before therapy, scores of pain effects on appetite, sleep, mood, social interaction, general activity, as well as quality of life were significantly higher in patients with severe and the worst possible pain than in patients with mild (ANOVA and Tukey test: $p < 0.001$) and moderate pain ($p < 0.01$). The score of impact of pain on the quality of life after therapy was significantly higher in patients with the worst possible pain (48.57 ± 1.81) than in patients with mild (4.50 ± 10.79; $p < 0.001$), moderate (15.56 ± 17.34; $p < 0.001$) and severe pain (17.61 ± 21.88; $p < 0.01$).

Cancer pain reduces the motive for treatment, affects basic parameters such as appetite, sleep, mood, social interaction and general activity. All this significantly reduces the quality of life and performance status, both before and after the application of adequate therapeutic procedures.

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Key words: *pain, cervical cancer, life quality*

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Introduction

Pain is defined as an unpleasant sensory and e-motional experience associated with actual or potential damage of tissue or the sensation described in terms of such damage (1). Pain is an individual and subjective feeling modulated by physiological, psychological and other factors, such as previous experience, culture, fear and anxiety. In a wider sense, "the pain is all that the patient says hurts him" (2).

Long and strong pain may completely change all reactions of a person, inhibit motivations and lead to the loss of will to live. It is the most common cause of suffering of patients with malignant disease (3,4). It occurs in all stages of malignancy, but its frequency and intensity increases with the progression of malignant disease (3).

Cancer pain significantly affects the quality of life of patients by changing all the components (physical, social, psychological and spiritual) (5, 6). This pain reduces the motive for treatment, affects basic parameters such as appetite, sleep, mood and social interaction (7). All these factors of impaired quality of life by feedback increase the severity of pain, which is why the severity of pain usually does not correlate with the severity of painful sensation. Long-standing uncontrolled pain has a very negative effect because it is perceived by patients as a signal of progression of the disease, causes doubt in healing, and leads to hopelessness and depression (4,8). This creates a "vicious circle" that pain affects the possi-

bility of healing, and limited treatment options reduce the therapeutic response leading to disease progression, which inevitably results in an increase of the pain intensity (9,10).

Women with advanced cervical cancer are known to experience pain and quality of life deficits as a result of disease progression, and quality of life has been reported as poor and generally lower than published norms in patients with advanced cervical cancer (11,12).

Therefore, the aim of the research was to study the incidence of pain in different stages of advanced cervical cancer, determine the clinical phenotype of pain, as well as the degree of impact of pain on the quality of life and its indicators.

Patients and methods

This is a prospective study conducted between January 2010 and July 2011 at the Oncology Clinic, Clinical Center Niš, University of Niš, Serbia. The study followed the tenets of the Declaration of Helsinki. An informed consent was obtained from each patient after they were explained the nature of the study.

The research included 102 female patients with pathohistologically verified advanced cervical cancer in FIGO stages II-b, III-a, III-b, IV-a and IV-b. Patients were consecutively enrolled as they presented at the Oncology Clinic. All the patients underwent a complete clinical and paraclinical examination as follows: before being referred to the oncology team, the patients underwent a standard clinical procedure (gynecological examination, Papanicolaou test, colposcopy, cervical biopsy and endocervical curettage). After the pathohistological verification of malignity (13), the stage of the disease was determined according to the FIGO classification (14). In order to estimate the degree of the local and metastatic spread of the tumor, the following diagnostic procedures were used: two-plane chest x-ray, abdominal and pelvic ultrasound, computed tomography (CT) (if necessary), and magnetic resonance (MR) (in exceptional cases). Laboratory analyses included the determination of the sedimentation rate, hematological parameters, and parameters related to the liver and kidney function.

Patients with intellectual incapacity to answer to the proposed questionnaire for the pain evaluation were not included in this study.

According to the consulting body decision, the patients were referred to a specific oncological treatment which included local or systemic therapy for the treatment of the underlying disease, and palliative radiation therapy was carried out in an attempt to control locoregional disease and anti-dolorous effect. All patients were also treated with analgesic therapy according to the current WHO guidelines (15, 16).

Pain Evaluations

Medical history was obtained for each patient and it included data related to the presence or absence of pain, its character, localization, irradiation, duration, primary therapy effect, etc. To determine the intensity of pain, numerical rating scale (NRS) was used (0-10), where 0 denotes the absence of pain and 10 the worst possible pain. The patients with the intensity of pain from 1 to 4 belonged to the group of patients with mild pain. The patients with the intensity of pain rated as 5 and 6 were assigned to the group of moderate pain, whereas the patients with the intensity of pain from 7 to 9 belonged to the group of severe pain.

According to the intensity of pain, the patients were randomly divided into five groups: group I – 34 patients without pain; group II – 16 patients with mild pain; group III – 27 patients with moderate pain; group IV – 18 patients with severe pain; group V – 7 patients with worst possible pain.

The determination of the intensity of pain was done at the time of establishing the diagnosis i.e. before the introduction of any cancer treatment, during treatment (in the middle of the therapy cycle), and after cancer treatment (at the first control examination three months later).

Quality of life assessment

Along with determining the pain intensity, the influence on the parameters that are important for the quality of life was evaluated. The parameters of quality of life included: appetite, sleep, mood, social interaction and general activity. Patients assessed the degree of pain on a scale from zero to ten for each of these parameters, and by adding these values, the score (0-50) defining the quality of life was obtained. The score was low in patients with good quality of life, and reached maximum in patients with the poorest quality of life. The impact of pain on the quality of life was determined before specific oncological treatment and three months after the therapy. All these assessments were based on the own assessment of the patients themselves.

Statistics

The comparison of mean values of numerical data between the groups of examinees was done using an analysis of variance (ANOVA) and Tukey's post hoc test with Kramer's modifications for unequal sample sizes. The comparison of frequencies of certain categories of data between the groups was performed by Mantel-Haenszel chi-square test or Fisher's exact test in cases when some of the expected frequencies of data were lower than 5. To test the effects of certain type of therapy on the changes in the intensity of pain, repeated measures ANOVA was applied together with Greenhouse-Geisser test. The essessment of coincidence between the values of pain intensity before and after treatment was done by calculating the Kappa coefficient. Assessment of the impact of pain intensity on the indicators of life quality was carried out by linear regression analysis. The coefficients of linear regression - B, as well as the borders of their 95% confidence interval (95% IP) were calculated. Testing of the significance of regression coefficients was done by Student's t-test. The threshold for statistical significance was set at $p <$

0.05 or 5%. Data were analyzed using PASW Statistics version 18.0.

Results

One hundred and two patients with pathohistologically verified advanced cervical cancer participated in the research undertaken in the period from January 2010 to July 2011. Their basic clinical characteristics are given in Table 1.

Clinical characteristics of pain are presented in Table 2.

The presence of pain before, during and after cancer treatment in all the patients is presented in Graph 1. Sixty-eight (66.7%) patients had pain prior to treatment, 61 (59.8%) patients in the course of treatment, and 37 (36.3%) patients reported pain after treatment. There is a statistically significant difference in the presence of pain before and after treatment ($p < 0.001$) and during and after treatment ($p < 0.01$).

The influence of pain on mental health is presented in Table 3. In patients with pain affecting mental health causing fear and depression, there were no significant differences between groups.

The impact of pain on the quality of life prior to therapy is presented in Table 4. The scores of impact of pain on appetite, sleep, mood, social interaction, general activity, and quality of life before therapy were significantly higher in patients with severe and the worst possible pain than in patients with mild (ANOVA and Tukey test: $p < 0.001$) and moderate pain ($p < 0.01$). The scores of impact of pain on sleep, general activity, as well as quality of life before therapy were significantly higher in patients with moderate pain than in patients with mild pain ($p < 0.001$ and $p < 0.01$).

Regression analysis also showed that the intensity of the pain was statistically significant ($p < 0.001$) before the therapy, affecting all indicators of quality of life. Any score increase in the intensity of pain for 1 was associated with a significant increase in the impact of pain on the overall quality of life by 4,815 (95% IP: 4,349 to 5,281) (Graph 2).

The influence of pain on the quality of life after therapy is given in Table 5. The score of pain impact on appetite after therapy was significantly higher in patients with the worst possible pain (9.29 ± 1.89) than in patients with mild (2.67 ± 4.62; $p < 0.05$) and moderate severe pain (3.67 ± 3.20; $p < 0.01$). The score of impact of pain on mood after therapy was significantly higher in patients with the worst possible pain (10.00 ± 0.00) than in patients with moderate pain (6.73 ± 2.79; $p < 0.05$).

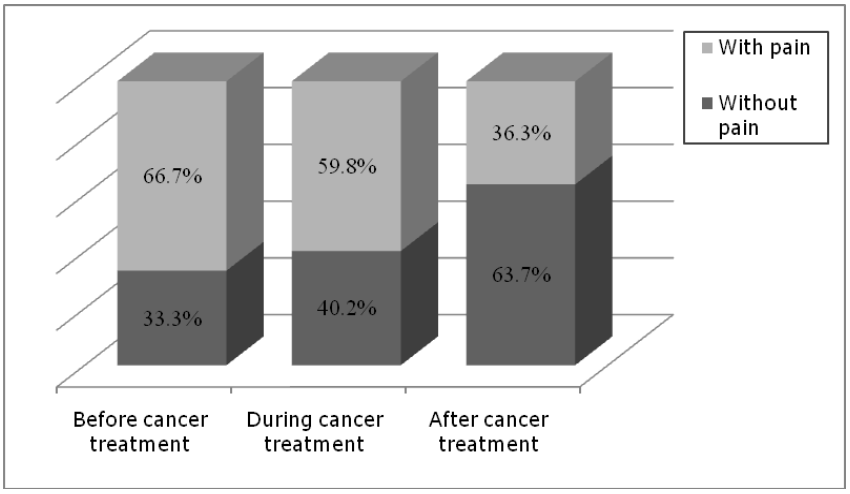
Table 1. Clinical characteristics of patients presented by groups

Characteristic	Group					Total
	No pain (n = 34)	Mild pain (n = 16)	Moderate pain (n = 27)	Severe pain (n = 18)	Worst possible pain (n = 7)	(n = 102)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Age	61.12 ± 8.77	53.75 ± 8.33	60.33 ± 11.41	47.83 ± 11.66	64.00 ± 11.43	57.61 ± 11.31
FIGO stage of the disease						
II b	17 (50.0)	7 (43.8)	3 (11.1)	2 (11.1)	0 (0.0)	29 (28.4)
III a	2 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)
III b	15 (44.1)	9 (56.3)	21 (77.8)	16 (88.9)	5 (71.4)	66 (64.7)
IV a	0 (0.0)	0 (0.0)	2 (7.4)	0 (0.0)	0 (0.0)	2 (2.0)
IV b	0 (0.0)	0 (0.0)	1 (3.7)	0 (0.0)	2 (28.6)	3 (2.9)
Histological type of the tumor						
Planocellular carcinoma	31 (91.2)	15 (93.8)	24 (88.9)	16 (88.9)	6 (85.7)	92 (90.2)
Adeno carcinoma	3 (8.8)	1 (6.3)	3 (11.1)	2 (11.1)	1 (14.3)	10 (9.8)
Histological grade						
G 1	4 (11.8)	3 (18.8)	5 (18.5)	4 (22.2)	0 (0.0)	16 (15.7)
G 2	30 (88.2)	12 (75.0)	22 (81.5)	14 (77.8)	7 (100.0)	85 (83.3)
G 3	0 (0.0)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)

Table 2. Clinical characteristics of pain

Characteristic	Group				Total
	Mild pain (n=16)	Moderate pain (n=27)	Severe pain (n=18)	Worst possible pain (n=7)	(n=102)
	N (%)	N (%)	N (%)	N (%)	N (%)
Duration of pain before treatment					
Less than 2 weeks	4 (25.0)	3 (11.1)	0 (0.0)	0 (0.0)	7 (10.3)
2 to 4 weeks	5 (31.3)	6 (22.2)	6 (33.3)	0 (0.0)	17 (25.0)
1 to 3 months	4 (25.0)	9 (33.3)	3 (16.7)	5 (71.4)	21 (30.9)
More than 3 months	3 (18.8)	9 (33.3)	9 (50.0)	2 (28.6)	23 (33.8)
Rhythm of pain					
Constant	5 (31.3)	14 (51.9)	15 (83.3)	3 (42.9)	37 (54.4)
Occasional	11 (68.8)	13 (48.1)	3 (16.7)	4 (57.1)	31 (45.6)
Time of occurrence of pain					
In the morning	0 (0.0)	1 (3.7)	0 (0.0)	0 (0.0)	1 (1.5)
In the afternoon	1 (6.3)	4 (14.8)	0 (0.0)	0 (0.0)	5 (7.4)
In the evening	3 (18.8)	3 (11.1)	0 (0.0)	2 (28.6)	8 (11.8)
At night	4 (25.0)	4 (14.8)	9 (50.0)	0 (0.0)	17 (25.0)
During the whole day	8 (50.0)	15 (55.6)	9 (50.0)	5 (71.4)	37 (54.4)
Localization of pain					
Pelvis	16 (100.0)	22 (81.5)	16 (88.9)	6 (85.7)	60 (88.2)
Hips	3 (18.8)	10 (37.0)	9 (50.0)	7 (100.0)	29 (42.6)
Lumbar spine	7 (43.8)	18 (66.7)	12 (66.7)	7 (100.0)	44 (64.7)
Thigh	1 (6.3)	2 (7.4)	4 (22.2)	5 (71.4)	12 (17.6)
Quality of pain					
Throbbing	3 (18.8)	0 (0.0)	3 (16.7)	0 (0.0)	6 (8.8)
Stabbing	3 (18.8)	7 (25.9)	6 (33.3)	3 (42.9)	19 (27.9)
Picking	1 (6.3)	5 (18.5)	3 (16.7)	2 (28.6)	11 (16.2)
Burning	2 (12.5)	7 (25.9)	1 (5.6)	2 (28.6)	12 (17.6)
Tiring	0 (0.0)	2 (7.4)	0 (0.0)	0 (0.0)	2 (2.9)
Dull	7 (43.8)	6 (22.2)	5 (27.8)	0 (0.0)	18 (26.5)
Factors alleviating the pain					
Warmth	0 (0.0)	2 (7.4)	6 (33.3)	0 (0.0)	8 (11.8)
Massage	2 (12.5)	2 (7.4)	1 (5.6)	1 (14.3)	6 (8.8)
Pressure	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)
Sitting	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.5)
Lying	9 (56.3)	21 (77.8)	8 (44.4)	6 (85.7)	44 (64.7)
Walking	3 (18.8)	2 (7.4)	3 (16.7)	0 (0.0)	8 (11.8)
Factors aggravating the pain					
Pressure	4 (25.0)	4 (14.8)	3 (16.7)	0 (0.0)	11 (16.2)
Sitting	1 (6.3)	9 (33.3)	0 (0.0)	0 (0.0)	10 (14.7)
Standing	0 (0.0)	4 (14.8)	3 (16.7)	2 (28.6)	9 (13.2)
Lying	3 (18.8)	0 (0.0)	3 (16.7)	0 (0.0)	6 (8.8)
Walking	6 (37.5)	9 (33.3)	9 (50.0)	3 (42.9)	27 (39.7)
Getting out of bed	2 (12.5)	1 (3.7)	0 (0.0)	2 (28.6)	5 (7.4)

The score of impact of pain on social interaction after therapy was significantly higher in patients with severe (7.50 ± 3.51) and the worst possible pain (9.29 ± 0.95) than in patients with mild (0.00 ± 0.00; $p < 0.01$) and moderate pain (3.67 ± 3.70; $p < 0.01$). The score of impact of pain on the quality of life after therapy was significantly higher in patients with the worst possible pain (48.57 ± 1.81) than in patients with mild (4.50 ± 10.79; $p < 0.001$), moderate (15.56 ± 17.34; $p < 0.001$) and severe pain (17.61 ± 21.88; $p < 0.01$).The results of the regression analysis show that, the intensity of the pain after the therapy was statistically significant ($p < 0.001$) affecting all indicators of the quality of life. Any increase of the score of the pain intensity for 1 after therapy was associated with a significant increase of the impact of pain on the overall quality of life by 4,907 (95% IP: 4,604 to 5,209) (Graph 3).



Graph 1. The presence of pain before, during and after cancer treatment

Table 3. The influence of pain on mental health

Influence	Group				Total (n=68)
	Mild pain (n=16)	Moderate pain (n=27)	Severe pain (n=18)	Worst possible pain (n=7)	
Pain causes depression	6 (37.5%)	14 (51.9%)	12 (66.7%)	4 (57.1%)	36 (52.9%)
Pain causes fear	7 (43.8%)	14 (51.9%)	11 (61.1%)	6 (85.7%)	38 (55.9%)

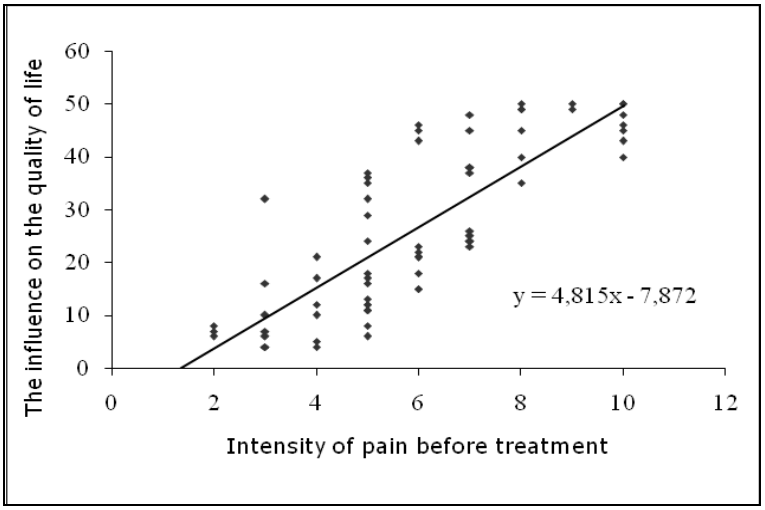
Table 4. The influence of pain on the quality of life before cancer treatment

Influence	Group					Total (n=102)
	No pain (n=34)	Mild pain (n=16)	Moderate pain (n=27)	Severe pain (n=18)	Worst possible Pain (n=7)	
The influence of pain on appetite	-	0.63 ± 2.03	2.15 ± 3.62	5.83 ± 4.06	8.14 ± 2.27	3.38 ± 4.13
The influence of pain on sleep	-	2.75 ± 2.02	6.04 ± 2.90	8.50 ± 2.31	10.00 ± 0.00	6.32 ± 3.36
The influence of pain on mood	-	4.06 ± 2.43	5.78 ± 2.68	8.39 ± 2.62	9.86 ± 0.38	6.49 ± 3.10
The influence of pain on social interaction	-	1.00 ± 2.42	2.33 ± 3.49	7.00 ± 3.99	8.29 ± 1.70	3.87 ± 4.23
The influence of pain on general activity	-	2.13 ± 2.63	5.81 ± 2.68	8.89 ± 1.41	9.71 ± 0.76	6.16 ± 3.48
The influence of pain on the quality of life	-	10.56 ± 7.64	22.11 ± 11.73	38.61 ± 10.25	46.00 ± 3.70	17.48 ± 17.74

Discussion

The results of our research have shown that pain is a common symptom in patients with advanced cervical cancer. Before being referred to cancer treatment, 66.7% of patients reported pain. As cancer treatment is the best way to treat cancer pain, since it decreases the tumor mass and thus alleviates and eliminates the pain, the number of patients having this pain decreased (from 59.8% during therapy to 36.3% after therapy).

The influence of pain on the mental health of patients with advanced carcinoma is very prominent (17, 18). Studies have shown that pain is closely associated with anxiety, fear and depression in patients with advanced malignant disease (19, 20).



Graph 2. The influence of pain on the quality of life before cancer treatment

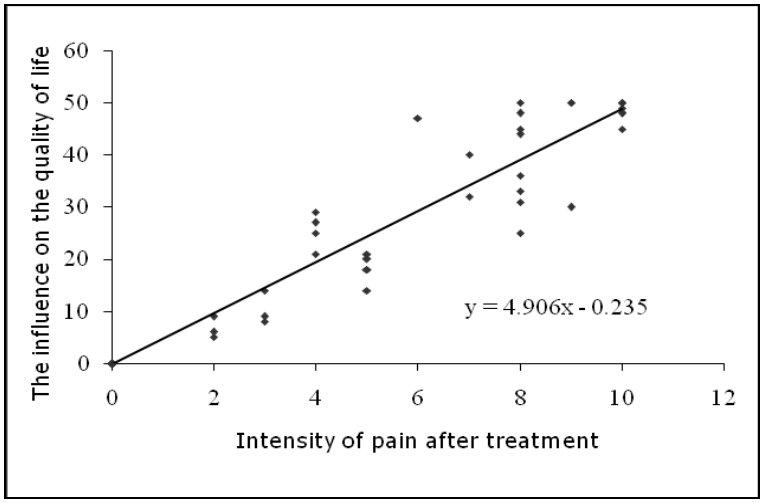
Table 5. The influence of pain on the quality of life after cancer treatment

Influence	Group					Total (n=102)
	No pain (n=34)	Mild pain (n=16)	Moderate pain (n=27)	Severe pain (n=18)	Worst possible pain (n=7)	
The influence of pain on appetite	1.50 ± 3.00	2.67 ± 4.62	3.67 ± 3.20	7.25 ± 3.62	9.29 ± 1.89	5.19 ± 4.03
The influence of pain on sleep	5.25 ± 2.22	6.33 ± 5.51	7.27 ± 2.87	8.63 ± 2.26	10.00 ± 0.00	7.78 ± 2.90
The influence of pain on mood	2.75 ± 1.50	7.67 ± 2.52	6.73 ± 2.79	8.88 ± 1.36	10.00 ± 0.00	7.46 ± 2.88
The influence of pain on social interaction	0.00 ± 0.00	0.00 ± 0.00	3.67 ± 3.70	7.50 ± 3.51	9.29 ± 0.95	4.86 ± 4.29
The influence of pain on general activity	2.00 ± 2.45	7.33 ± 3.06	6.67 ± 3.04	7.38 ± 3.70	10.00 ± 0.00	7.00 ± 3.45
The influence of pain on the quality of life	11.50 ±5 .69	4.50 ± 10.79	15.56 ± 17.34	17.61 ± 21.88	48.57 ± 1.81	16.60 ± 19.67

When cancer pain persists and worsens, it can serve as a sign of the progression of disease and can create a sense of hopelessness because patients fear that their lives are not worth continuing or patients lose the meaning of living if they must live in pain (21). In our study, depression appears to be most frequent in the group of patients with severe pain (66.7%), while it is least frequent in the group with mild pain (37.5%). Fear as important parameter for the mental health of patients shows high positive correlation with pain intensity and is present in 43.8% of patients with mild pain, in 51.9% of patients with moderate pain, in 61.1% of patients with severe pain, and even in 85.7% of patients with the worst possi ble pain.

Pain significantly affects the quality of life of patients with malignant disease (3). Many research results showed that pain was significantly correlated with appetite, mood, quality of sleeping, fatigue, pain intensity, daily activity, side effect, general appearance, and support from family (23) and the relationship between pain and quality of life was found to be reciprocal (24). If pain was not relieved, the patient's quality of life would certainly decrease. The results obtained in this study show poorer quality of life in proportion to the increase in pain intensity.

Before specific oncologic therapy, the scores of impact of pain on appetite, sleep, social interaction, general activity, and quality of life were significantly higher in patients with severe and the worst possible pain than in pati-ents with mild (ANOVA and Tukey test: $p < 0.001$) and moderate pain ($p < 0.01$).



Graph 3. The influence of pain on the quality of life after cancer treatment

After complete specific oncology therapy the distribution of the pain impact on the quality of life between some groups changes, the quality of life in all groups was noticed, except in the group with the worst possible pain where there was tendency of deterioration of the quality of life with the almost maximum score (48.57 ± 1.81). This finding also influenced the overall quality of life of all patients with pain, so there was no significant difference relatied to quality of life before and after therapy.

The score of impact of pain on appetite after therapy was significantly higher in patients with the worst possible pain (9.29 ± 1.89) compared to patients with mild (2.67 ± 4.62; $p < 0.05$) and moderate pain (3.67 ± 3.20; $p < 0.01$). The impact of pain on mood after therapy was significantly higher in patients with the worst possible pain (10.00 ± 0.00) than in patients with moderate pain (6.73 ± 2.79; $p < 0.05$). Unexpectedly, the pain showed to have great influence on the mood in a group of patients with mild pain, which may be explained by the disappointing expectation that all ailments will atop after the treatment. Also, painful sensations caused by the applied therapy (25, 26) are especially superposed in this group. They can explain the onset of pain after treatment in patients who felt no pain at the time of establishing the diagnosis.

The score of impact of pain on social interaction after therapy was significantly higher in patients with severe (7.50 ± 3.51) and the worst possible pain (9.29 ± 0.95) compared to patients with mild (0.00 ± 0.00; $p < 0.01$) and moderate pain (3.67 ± 3.70; $p < 0.01$).

Regression analysis also showed that the intensity of pain had statistically significantly ($p < 0.001$) high impact prior to therapy on all indicators of quality of life, as well as on the overall quality of life. Even after therapy, the intensity of the pain was statistically significant ($p < 0.001$), affecting all the indicators of quality of life and the overall quality of life.

Conclusion

The results of our study have demonstrated that pain is a common symptom in patients with advanced cervical cancer. Cancer pain reduces the motivation for treatment, affects basic parameters such as appetite, sleep, mood, social interaction and general activity. All this significantly reduces the quality of life and performance status, both before and after the application of adequate therapeutic procedures. The results of our research show poorer quality of life in proportion to the increase in pain intensity. A larger series of patients and a longer follow-up period are required to test the results of this research using also more comprehensive questionnaires for assessing the quality of life.

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Conflict of interest

Authors declare that they have no conflict of interest.

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UTICAJ KANCERSKOG BOLA NA KVALITET ŽIVOTA BOLESNICA SA INOPERABILNIM KARCINOMOM GRLIĆA MATERICE: NAŠE JEDNOGODIŠNJE ISKUSTVO

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Cij rada bio je ispitati učestalost pojave bola kod bolesnica u različitim stadijumima inoperabilnog karcinoma grlića materice, utvrditi klinički fenotip bola, kao i stepen uticaja bola na kvalitet života i na njegove pokazatelje.

Ispitivanjem su obuhvaćene 102 bolesnice sa patohistološki verifikovanim inoperabilnim karcinomom grlića materice. Za određivanje jačine bola korišćena je numerička skala (NRS). Kao parametri kvaliteta života sagledavani su apetit, san, raspoloženje, odnos sa drugima i opšta aktivnost. Bolesnice su davale ocenu od nula do deset za svaki od ovih parametara, a sabiranjem tih vrednosti dobijen je skor (0-50) koji definiše kvalitet života. Uticaj bola na kvalitet života određivan je pre započinjanja specifičnog onkološkog lečenja i tri meseca nakon kompletiranja terapije.

Pre terapije skorovi uticaja bola na apetit, san, raspoloženje, odnos sa drugim ljudima, opštu aktivnost, kao i kvalitet života bili su značajno veći kod bolesnica sa jakim i najjačim mogućim bolom nego kod bolesnica sa umerenim (ANOVA i Tuki test: $p < 0,001$) i srednje jakim bolom ($p < 0,01$). Skor uticaja bola na kvalitet života posle terapije bio je značajno veći kod bolesnica sa najjačim mogućim bolom (48,57 ± 1,81) nego kod bolesnica sa umerenim (4,50 ± 10,79; $p < 0,001$), srednje jakim (15,56 ± 17,34; $p < 0,001$) i jakim bolom (17,61 ± 21,88; $p < 0,01$).

Kancerski bol smanjuje motiv za lečenje, utiče na osnovne performanse kao što su apetit, san, raspoloženje, odnos sa drugim ljudima i opštu aktivnost. Sve ovo značajno smanjuje kvalitet života i performans status, kako pre tako i nakon primene adekvatnih terapijskih procedura.

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Ključne reči: *bol, cervikalni karcinom, kvalitet života*

Review article

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PARAMETRIC VERSUS NONPARAMETRIC TESTS IN BIOMEDICAL RESEARCH

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Despite the wide use of statistics in biomedical research, simple ideas are sometimes misunderstood or misinterpreted by medical research workers, who have only limited knowledge of statistics. This article deals with basic biostatistical concepts and their application to enable postgraduate medical students and researchers to analyze and interpret their study data and to critically interpret published literature. The adequate choice of statistical tests has a strong influence on data interpretation. Understanding this choice is important for critical evaluation of biomedical research. The question often arises on whether to use parametric or nonparametric test. If we are planning a study and trying to determine how many patients/cases to include, a nonparametric test will require a slightly larger sample size to have the same power as the corresponding parametric test. In summary, nonparametric procedures are useful in many cases and necessary in individual, but they are not the perfect solution. Fortunately, the most frequently used parametric analyses have their non-parametric counterparts. This can be useful when the assumptions of a parametric test are violated and we can thus choose a nonparametric alternative instead.

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Key words: *Biomedicine, parametric tests, nonparametric tests*

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Introduction

Few people among medical students and physicians understand the differences between parametric and nonparametric statistics and most do not realize how important it is to make the right choice (1, 2). Statistics is basically a way of thinking about data that are changeable. Despite the wide use of statistics in biomedical research, simple ideas are sometimes misunderstood or misinterpreted by medical research workers, who have only limited knowledge of statistics. This article deals with basic biostatistical concepts and their application to enable postgraduate medical students and researchers to analyze and interpret their study data and to critically interpret published literature. We will try to explain the differences between parametric and nonparametric statistics and why it is crucial to know which type of test is appropriate to use and in what situations.

The adequate choice of statistical tests strongly influences data interpretation. Understanding this choice is important for the critical evaluation of biomedical research. The question often arises on whether to use one or another test.

Biostatistics: The concept

Statistics is just a methodology and without scientific application it has no purpose. Statistics may thus be defined as a discipline concerned with the analysis of numerical data derived from a group of statistical elements. These statistical elements may be human beings, animals, or other organisms. Biostatistics is a branch of statistics applied to biological or medical sciences. Biostatistics covers the applications and contributions not only from health, medicines and nutrition, but also from the fields such as epidemiology, biology and genetics (3). Biostatistics involves various stages, like setting the hypothesis, collection of data and application of statistical analysis. In order to draw valid conclusions, researchers should know about the data obtained during the research, its distribution, and its analysis.

The first step, before considering any statistical analyses, is data research. Statistical methods for analysis mainly depend on the type of data. Generally, data present the picture of variability and central tendency. Therefore, it is very important to under-

stand the types of data. There exist three types of data: nominal, ordinal, and interval data. Nominal or categorical data simply assigned "names" or categories are based on the presence or absence of certain attributes/characteristics without any ranking between the categories (4). For example, humans are categorized by gender as males or females; by marital status as married, not married, widowed and divorced. Ordinal data, also called ordered, are the type of data which are expressed as scores or ranks. There is a natural order among categories, and they can be ranked or arranged in an order (4). For example, burns may be classified into four ranks and another example is the APGAR score. Interval data (continuous data) are the third type, which are characterized by an equal and definite interval between two measurements (some of the examples are weight, hemoglobin, body mass index).

The next step is to choose an adequate test for the analyses based on the type of collected data and some key features of that data. Hence, looking at the data, we are looking at data distributions to estimate the center, shape and spread and describe how the validity of many statistical procedures relies on an assumption of approximate normality (5). There are several statistical tests that can be used to assess whether the data are derived from a normal distribution. The most popular are the Kolmogorov-Smirnov test and the Shapiro-Wilk test (6). These normality tests take into account both the skewness and kurtosis of the data, and, therefore, the application of normality tests is recommended. These tests compare the observed data to quantiles of the normal (or other specified) distribution. The null hypothesis for each test is H0: Data follow a normal distribution, versus H1: Data do not follow a normal distribution. If the test is statistically significant (e.g., $p < 0.05$), then data do not follow a normal distribution, and a nonparametric test should be used.

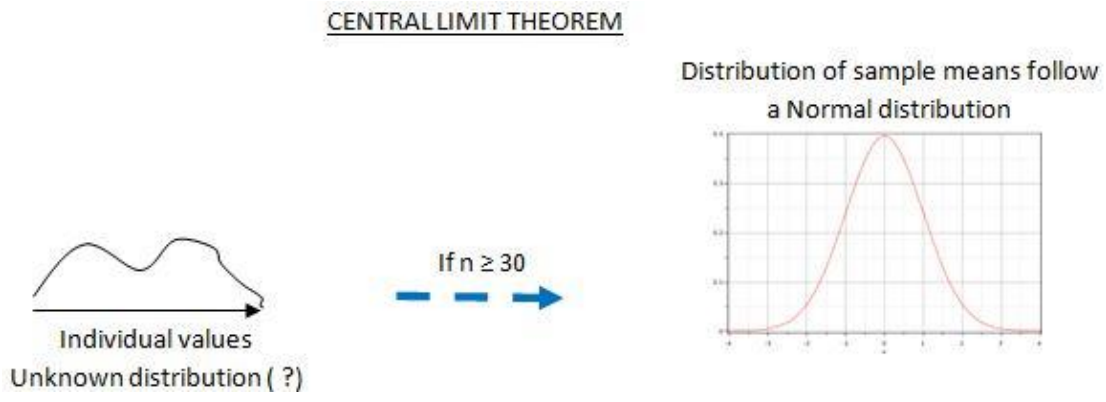
Therefore, we will try to explain the difference between parametric and nonparametric procedures. The principal difference for parametric versus nonparametric is:

- If measurement scale is nominal or ordinal, then we use nonparametric statistics;
- If we are using interval or ratio scales, we use parametric statistics.

Parametric tests

We can freely say that most people who use statistics are more familiar with parametric than nonparametric techniques. Parametric tests are based on the assumption that the data follow a normal or "bell-shaped" distribution. Parametric methods are often those for which we know that the population is approximately normal, or we can approximate using a normal distribution after we invoke the Central Limit Theorem. There are two parameters for a normal distribution: the mean and the standard deviation. Parametric tests are usually appropriate when examining either interval data or ratio data.

Altman states that „parametric methods require the observations within each group to have an approximately Normal distribution ... if the data do not satisfy these conditions ... a nonparametric method should be used" (7). According to the Central Limit Theorem (Graph 1), when the sample size is larger than 30, normality is not a main condition for a standard t (Student) or z hypothesis test: even though the individual values within a sample might follow an unknown, non-normal distribution, the sample means (as long as the sample sizes are at least 30) will follow a normal distribution.



Graph 1. Central Limit Theorem

Methods

If normality tests do not provide evidence for normal distribution, the data can be transformed to more normally distributed data. In some cases, the transformation of data will make it better to match the assumptions. To transform the data, we perform a mathematical operation on each observation, and then use these transformed numbers in our statistical test. The most popular transformations are the log and square-root transformations (8). In situations when we cannot make the data more normally

distributed, we will select an equivalent nonparametric test. Commonly used parametric tests are described below.

Student t-Test:

The Student t-test is likely the most commonly applied parametric test. It was developed by a statistician William Sealy Gosset, who developed the „t-statistic" and published it under the „Student" pseudonym (9). A single sample t-test is used to determine whether the mean of a sample is different from a known average. A 2-sample t-test is used to establish that the means of two populations are equal. "Repeated measures" t-test is used to determine the differences between two responses measured on the same statistical units. One should know the mean, standard deviation, and number of samples to calculate the test statistic. In a data set with a large number of samples, the critical value for the Student t-test is 1.96 for an alpha of 0.05, and 2,58 for an alpha of 0.01, obtained from a t-test table.

The z-Test:

The z-test is very similar to the Student t-test. However, with the z-test, the variance of the standard population, rather than the standard deviation of the study groups, is used to obtain the z-test statistic. Using the z-chart, like the t-table, we can see what percentage of the standard population is outside the mean of the sample population. If, like the t-test, greater than 95% of the standard population is on one side of the mean, the p-value is less than 0.05 and statistical significance is achieved. The disadvantage of this test is that it should not be used if the sample size is less than 30.

The one-way analysis of variance (ANOVA):

The one-way analysis of variance (ANOVA) is used to determine whether there are any statistically significant differences between the means of three or more independent (unrelated) groups. The test statistic for ANOVA is called the F-ratio. As with the t- and z-statistics, the F-statistic is compared with a table to determine whether it is greater than the critical value. In interpreting the F-statistic, the degrees of freedom for both the numerator and the denominator are required. The degrees of freedom in the numerator are the number of groups minus 1, and the degrees of freedom in the denominator are the number of data points minus the number of group.

Further, to determine which specific groups differed from each other, we need to use a post hoc test (Bonferroni, Tukey, Duncan…), which represents a t-test modification. Two way ANOVA, also called two factors ANOVA, determines how a response is affected by two factors.

Pearson Correlation Coefficient:

The correlation coefficient (r) is a value that tells us how well two continuous variables from the same subject correlate to each other. An r value may have values from -1 to +1: +1 means the data are completely positively correlated, an r of 0 means that the variables are completely random, and an r of -1 is completely negatively correlated. It is important to note that in biomedical research r could not be +1 or -1, because between the variables there is not any functional but statistical association. Further, the crucial thing to remember is that this is only an association and does not imply a cause-and-effect relationship.

Nonparametric tests

In biomedical sciences data often does not follow normal distribution (10) and the sample sizes are often small. Nonparametric tests are a satisfactory alternative to parametric tests for the data where there are skewness, extreme asymmetries and multimodality, especially in small samples. These tests are also called "distribution free tests" and represent statistical techniques for which we do not have to make any assumption of parameters for the population we are studying. According to Robson (11), non-parametric tests are usually appropriate when examining ordinal or nominal data when the assumptions of parametric test have not been achieved. A non-parametric statistical test is also a test whose model does not specify conditions about the parameters of the population from which the sample was taken. It does not require measurements as strong as that required for parametric tests. Non-parametric tests are generally appropriate when the data being examined is ordinal or nominal and is based on a small population sample or does not have a clear Gaussian function. In general, the measure of central tendency in nonparametric testing is median. Commonly used non-parametric tests are described below.

Pearson's chi-squared test

The Chi-square test is a non-parametric test of proportions. This test is not based on any assumption or distribution of any variable. This test, though different, follows a specific distribution known as Chi-square distribution, which is very useful in research. We use this test to determine whether there is a significant difference between the expected and observed frequencies in one or more categories. This test is used to investigate whether distributions of categorical variables differ from one another (10). The Chi-Square test of Independence is used to determine if there is a significant relationship between two nominal (categorical) variables. The frequency of one nominal variable is compared with different values of the second nominal variable. The data can be displayed in an RxC contingency table, where R is the row and C is the column. It has no alternative in parametric testing.

Mann-Whitney U test

This test is a nonparametric alternative for independent student t-test. It is used for continuous data, to compare the means of two independent or unrelated samples for significant differences. To com-

pute the U-test, data is ranked ordered and combined into a single dataset. This combination is used to determine if the rank ordering is random or clustered. If the data points of the sample are clustered, then there is evidence of a significant difference between the sample means. Conversely, randomly distributed rank ordered data would be the evidence that there is no significant difference between the means of the samples.

Wilcoxon signed-rank test

Wilcoxon signed-rank test is a nonparametric test that can be used to determine whether two dependent samples were selected from populations having the same distribution. It compares two related samples, matched samples, or repeated measurements on a single sample to assess whether their population mean ranks are different. It is used as an alternative to the paired Student's t-test, t-test for matched pairs, or t-test for dependent samples, when the population cannot be assumed to be normally distributed (12, 13).

Kruskal-Wallis test

Kruskal-Wallis test is a nonparametric test used for comparing two or more independent samples of equal or different sample sizes. It extends the Mann–Whitney U test when there are more than two groups. This test is the nonparametric equivalent of the ANOVA which can be used for both continuous and ordinal-level dependent variables. However, like most non-parametric tests, the Kruskal-Wallis Test is not as powerful as the ANOVA.

The Friedman test

The Friedman test is a non-parametric test for testing the difference between several related samples. This test is an alternative to Repeated measures analysis of variances which is used when the same parameter has been measured under different conditions on the same subjects.

Spearman rank correlation

Spearman rank correlation is a nonparametric alternative to the Pearson correlation coefficient. It assesses how well the relationship between two variables can be described using a monotonic function (10). This test measures the strength and direction of association between two ranked variables. Spearman rank correlation has less power than the Pearson correlation coefficient, and in situations when we can choose between the two, Pearson correlation coefficient is a better option.

Differences between parametric and nonparametric tests

The „power" of a nonparametric test is lower than that of its parametric counterpart. This means that to detect any given effect at a specified significance level, a larger sample size is required for nonparametric compared to parametric tests (13). They are generally less statistically powerful than the analogous parametric tests when the data are truly approximately normal. "Less powerful" means that there is a smaller probability that the procedure will tell us that two variables are associated with each other when they in fact are really associated. Some people also debate if non-parametric tests are most appropriate when the sample sizes are small. However, when the data set is large, (e.g. $n > 30$), the Central Limit Theorem can be used, so it often makes little sense to employ nonparametric tests.

Another disadvantage associated with nonparametric tests is that their results are often more difficult to interpret than the results of parametric tests. Many nonparametric tests use data ranking values instead of using the actual data, hence the difference in mean ranks between two groups very often does not really contribute to our intuitive understanding of the data.

Non-parametric tests are appropriate for very small samples. However, if sample sizes as small as $N = 5$ are used, nonparametric tests have no alternatives. Non-parametric tests can treat samples made up of observations from several different populations, can treat data which are in ranks as well as data whose seemingly numerical scores have the strength in ranks. They are available to treat data which are classificatory, and are easier to learn and apply than parametric tests.

If we are planning a study and trying to determine how many patients/cases to include, a nonparametric test will require a slightly larger sample size to have the same power as the corresponding parametric test. In summary, nonparametric procedures are useful in many cases and necessary in individual, but they are not the perfect solution.

Fortunately, the most frequently used parametric analyses have their non-parametric counterparts. This can be useful when the assumptions of a parametric test are violated and therefore we can choose the nonparametric alternative. The examples are shown in Table 1.

Conclusion

The tests outlined here are commonly used in clinical studies. Understanding these tests will provide some framework for analyzing test results when critically reading journal articles. Inappropriate use of statistical tests will lead to incorrect conclusions. In general, we should try to avoid non-parametric tests whenever possible (because they are less powerful). In conclusion, the next time when you are having doubts about which test to employ, you should consult a statistician.

Table 1. Parametric tests and nonparametric counterparts

Statistical Tests		
Parametric test	**Corresponding nonparametric test**	**Purpose of test**
t test for independent samples	Mann-Whitney U test; Wilcoxon rank-sum test	Compares two independent samples
Paired *t* test	Wilcoxon matched pairs signed-rank test	Examines a set of differences
One way- *ANOVA*	Kruskal-Wallis analysis of variance by ranks	Compares three or more groups
Two way- *ANOVA*	Friedman Two way analysis of variance	Compares groups classified by two different factors
Pearson correlation coefficient	Spearman rank correlation coefficient	Assesses the linear association between two variables

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PARAMETARSKI NASUPROT NEPARAMETARSKIM TESTOVIMA U BIOMEDICINSKIM ISTRAŽIVANJIMA

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Uprkos širokoj upotrebi statistike u biomedicinskim istraživanjima, jednostavne ideje su ponekad pogrešno shvaćene ili pogrešno tumačene od strane medicinskih naučnih radnika, koji uglavnom imaju ograničeno znanje iz statistike. Ovaj članak se bavi osnovnim konceptima biostatistike i njene primene kako bi se studentima postdiplomskih studija medicine i istraživačima omogućilo da analiziraju i kritički tumače svoje podatke i dostupnu literaturu. Adekvatan izbor statističkih testova ima bitan uticaj na interpretaciju podataka. Razumevanje ovog izbora je bitno za kritičku procenu rezultata biomedicinskih istraživanja. Pitanje koje se često postavlja je da li koristiti parametarski ili neparametarski test. Ukoliko planiramo da sprovedemo određenu studiju i pokušavamo da utvrdimo koliko bolesnika/slučajeva je potrebno uključiti u nju, neparametarski test će zahtevati veću veličinu uzoraka da bi postigao istu snagu kao i odgovarajući parametarski test. Ukratko, neparametarskih testovi su korisni i neophodni u mnogim slučajevima, ali oni često nisu savršeno rešenje. Srećom, najčešće korišćene parametarske analize imaju svoje neparametarske ekvivalente. Ovo saznanje je korisno u slučaju kada raspred nije po tipu normalnosti, te stoga biramo neparametarsku alternativu.

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Ključne reči: *biostatistika, parametarski testovi, neparametarski testovi*

Case report **UDC: 616.42-006.44**
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NASOPHARYNGEAL PLASMABLASTIC LYMPHOMA: A CASE REPORT

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Plasmablastic lymphoma (PBL) is a rare aggressive subtype of non-Hodgkin's lymphoma (NHL). It occurs predominantly in older patients and HIV infected individuals and shows a predilection for the oral cavity. This case report describes a presentation of PBL in the nasopharynx of an older male patient. Due to its unusual immunophenotype and rare occurence it is often misdiagnosed by pathologists. The biggest challenge in the differential diagnosis of PBL is the distinction from plasmablastic (anaplastic) plasma cell myeloma, as the morphological and immunophenotypic features of these two entities overlap.

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Key words: *plasmablastic lymphoma, nasopharynx, immunohistochemistry*

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Introduction

Plasmablastic lymphoma (PBL) is a rare aggressive subtype of non-Hodgkin's lymphoma (NHL). Initially described in 1997, it occurs predominantly in older patients and HIV infected individuals and shows a predilection for the oral cavity.

Infection with Epstein-Barr virus (EBV) and human herpesvirus 8 (HHV8) has been proven in PBL tissue samples. Plasmablastic lymphoma has also been found outside the oral cavity, in the nasopharynx, lung, skin, various soft tissues, maxilarry sinus, intestines, heart (1). Although the majority of cases occur in immunodeficient patients, in a recent meta-analysis approximately 35% of cases occurred in immunocompetent individuals (2,3). The majority of patients reported were middle-aged men who were around 50 years old. However, the patients with HIV infection tend to have an earlier onset of the disease – they were around 38 years old. In rare cases, PBL is the initial presentation of HIV infection. The distribution of the disease in patients who have previously received organ transplants differs: lymph nodes and skin are the most common sites, with less frequent involvement of the oral cavity/jaw and gastrointestinal tract. Most cases of PBL present with advanced-stage disease (Ann Arbor stage III or IV) (4).

We present a case of plasmablastic lymphoma localised in the nasopharynx.

Patient

The 63-year-old male was referred from a general hospital outside Niš. He reported one sided nasal congestion and epistaxis for a period of few months. The patient's HIV status was unknown. During standard examination, endoscopy showed a pedunculated flesh-colored nasopharyngeal mass, 2 cm in its greatest diameter. The mass was surgically removed without any complications.

The surgically removed specimen was formallin fixed and paraffin embedded. Using standard procedures, H&E slides were made. After the initial microscopic examination without immunohistochemistry, the preliminary diagnosis was carcinoma sinonasalis dediferentiatum (anaplasticum). In order to confirm the diagnosis, the material was sent to the Center for Pathology and Pathological Anatomy, Clinical Center Niš, Serbia.

Immunohistochemistry was performed using the Dako Autostainer with Envision(+) Detection Kit at the Center of Pathology and Pathological Anatomy in Niš. Histologically, there were groups of densely packed oval and round shaped cells with scant reddish cytoplasm and eccentrically placed nucleus (Figure 1), with large areas of geographic necrosis. Some cells resembled plasmablasts. Determination of proliferative activity by immunohistochemistry was performed quantitetively by counting immunoreactive tumor cells in the most intensely stained areas in two high-power fields (x 400) by using ImageJ program cell counter, on 400 cells.

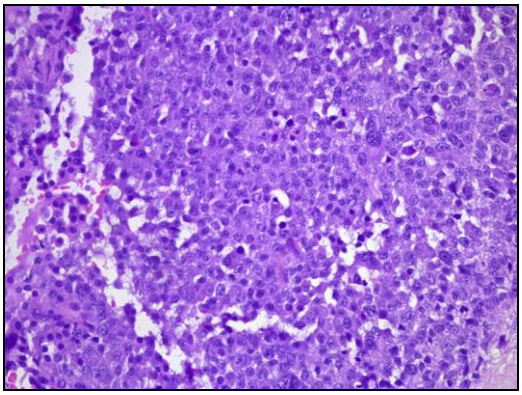

Figure 1. Nasopharyngeal tumor composed of oval and round shaped cells with eccentric nuclei and abundant eosinophilic cytoplasm, in a diffuse sheet-like and cohesive growth pattern. Apoptotic bodies and mitotic figures can also be seen. HE, Obj.x40

The Ki-67 index was defined as the percentage of immunoreactive tumor cells out of the total number of tumor cells. The Ki-67 index value was around 40% (Figure 2.A). Tumor cells were positive for CD138, CD38 (Figure 2.B), MUM-1 (Figure 2.C), lambda light chains positive (Figure 2.D) and negative for kappa light chains, CD56, EMA, LCA, cyclin D1, CD20, CD3, ALK, CD21, CD23 and CK5/6. Based on the morphological features and immunohistochemical profile, the diagnosis of plasmablastic lymphoma was made.

The patient was symptom-free at the time of this study, awaiting for his first treatment trial.

Discussion

Generally accepted treatment guidelines for plasmablastic lymphoma have not yet been established and treatment regimes largely vary and are usually a matter of physician discretion. The treatment consists mainly of chemotherapy, with the occasional use of radiotherapy (5).

Plasmablastic lymphoma is characterized by monomorphic cellular proliferation of round to oval-shaped cells with either centrally or eccentrically placed nuclei and abundant eosinophilic cytoplasm in a diffuse sheet-like and cohesive growth pattern, with large areas of geographic necrosis, and has two morphologic subtypes, monomorphic and plasmacytic.

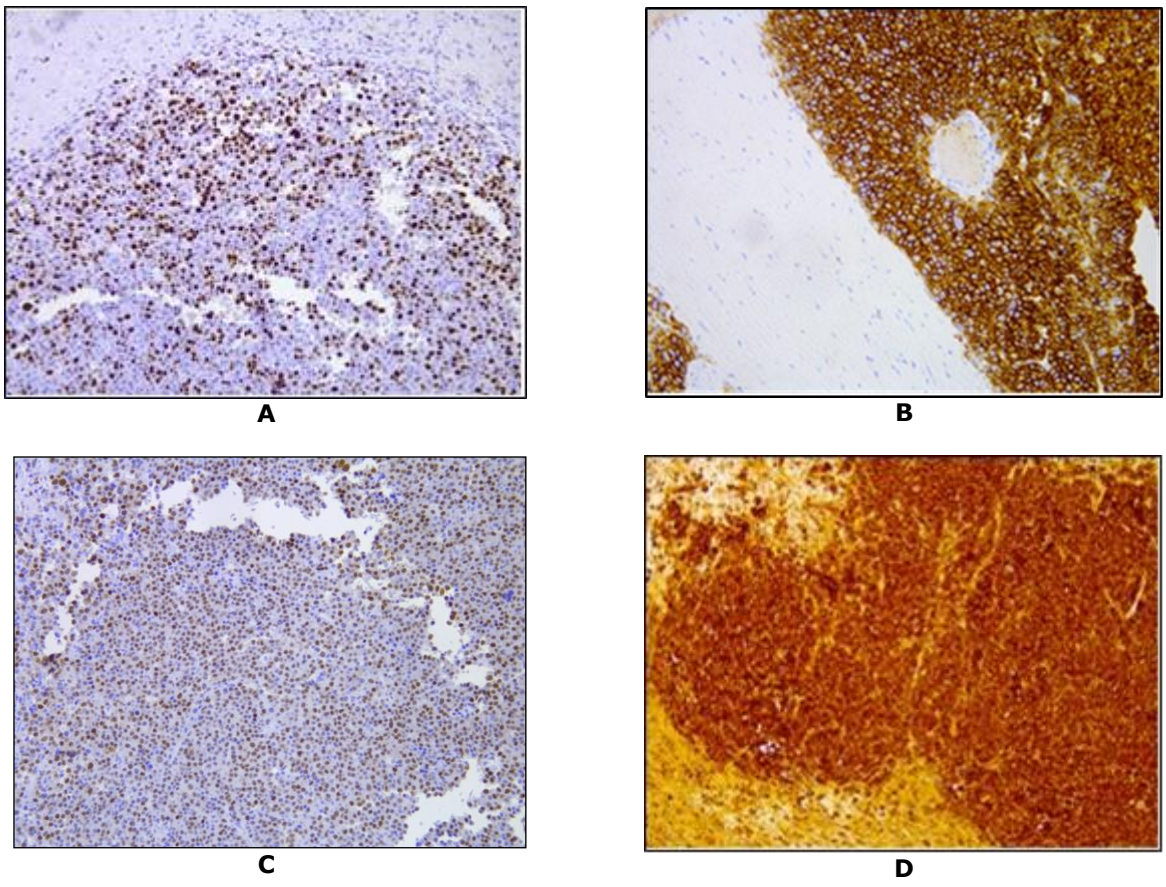

Figure 2. Immunohistochemical profile of nasopharyngeal tumor cells:
A) Ki-67 positive nuclei of tumor cells. Obj.x20.
B) CD38 positive tumor cells. Obj.x20.
C) MUM-1 positive nuclear stain. Obj.x20.
D) Neoplastic cells show intense positive lambda light chains staining. Obj. x20

Apoptotic bodies and mitotic figures are frequent, and tangible-body macrophages are easily detectable leading to a starry-sky appearance. PBL is a high-grade B-cell lymphoma that arises from post-germinal center B-cells and usually expresses the characteristic immunophenotype of plasmacytoid terminally differentiated B-cells. As plasmablasts acquire plasma-cell markers (i.e. VS38c, CD38, MUM1 /IRF4, CD138, EMA), they lose the leukocyte common antigen (CD45) and their B-cell markers CD20, CD79a, PAX5, and a high proliferation rate is reflected by Ki67 expression > 80%. Cytoplasmic immunoglobulins are expressed in near 70% of cases. Interestingly, these lymphomas might express epithelial and endothelial markers such EMA and CD31, respectively, posing some problems in differential diagnosis with poorly differentiated solid tumors. Recently, an immunohistochemistry staining for PRDM1 /BLIMP1 and XBP1 has been proposed to identify PBL (6).

The biggest challenge in the differential diagnosis of PBL is the distinction from plasmablastic (anaplastic) plasma cell myeloma, since the morphological and immunophenotypic features of these 2 entities overlap (7). A positive HIV status, EBV positive neoplastic cells, as well as high values of Ki67 proliferation index favor the diagnosis of PBL. On the other hand, clinical parameters and laboratory findings such as: renal dysfunction, a significant paraprotein, osteolytic lesions, hypercalcemia, and diffuse bone marrow involvement support the diagnosis of plasmablastic plasma cell myeloma (8). However, some cases occuring in HIV positive patients have overlapping features with plasma cell myelomas, such as lytic bone lesions and monoclonal serum immunoglobulins. In some cases a firm distinction cannot be made, and a descriptive diagnosis such as plasmablastic neoplasm, interdeterminate between plasmablastic lymphoma and anaplastic plasmacytoma may be acceptable. CD56 expression tends to occur more frequently in plasma cell neoplasms, but it also occurs in some PBLs and therefore cannot be used as a definitive criterion. In our case, tumor cells were CD56 negative. Cyclin D1 is negative in PBL but positive in a subgroup of patients with plasma cell myeloma. In our case, tumor cells were cyclin D1 negative.

Another important entity in the differential diagnosis of PBL is diffuse large B-cell lymphoma (DLBCL) with plasmacytoid differentiation, in which the absence of immunosuppression and lack of EBV infection are much more common than in PBL. Anaplastic lymphoma kinase (ALK) is an enzyme the expression of which defines a form of DLBCL that can often exhibit plasmablastic features. The presence of ALK expression by immunohistochemistry and identification of ALK gene rearrangement typically establish the diagnosis of ALK-positive DLBCL. Large B-cell lymphomas with plasmablastic features may occur as a rare transformation of small B-cell lymphoid neoplasms, mainly chronic lymphocitic leukemia and follicular lymphoma (9). Extracavitary/solid variant of primary effusion lymphoma (PEL) can closely resemble PBL, but these tumors are by definition positive for the HHV8 virus. Diffuse large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease is similarly positive for HHV8 by definition (10). In our case, tumor cells were ALK negative.

Lastly, we should also consider blastic plasmacytoid dendritic cell neoplasm (BPDCN) in the differential diagnosis of this tumor. Blastic plasmacytoid dendritic cell neoplasm is a clinically aggressive tumor, which frequently presents as cutaneous lesions and subsequently progresses to bone marrow (BM) involvement and leukemic dissemination (11). However, Dunlap et al. presented a case of BPDCN in the paranasal sinus. Immunophenotypic profile including a CD4+/CD56+/CD123+ population of cells in the absence of expression of B-cell (CD19, CD20, and CD79a), T-cell (CD3, cCD3, and CD5), or myelomonocytic (myeloperoxidase, lysozyme, CD14, and CD64) -specific antigens is needed for a diagnosis of BPDCN (12). In our case, tumor cells were CD21 and CD23 negative.

Conclusion

In our paper, a rare case of nasopharyngeal plasmablastic lymphoma was presented. Plasmablastic lymphoma is usually found in older patients and immunocompromised persons, with a predilection for oral cavity. Due to its unusual immunophenotype and rare occurence it is often misdiagnosed by pathologists. The biggest challenge in the differential diagnosis of PBL is its distinction from plasmablastic (anaplastic) plasma cell myeloma, as the morphologic and immunophenotypic features of these two entities overlap.

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NAZOFARINGEALNI PLAZMABLASTNI LIMFOM: PRIKAZ BOLESNIKA

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Plazmablastni limfom (PBL) je redak agresivni podtip nehočkinovog limfoma (NHL). Javlja se najčešće u usnoj duplji starijih bolesnika i HIV pozitivnih osoba. Prikazan je slučaj plazmablastnog limfoma u nazofarinksu starijeg muškarca. Zbog specifičnog imunofenotipa i retke incidencije ovog tumora česte su greške u postavljanju dijagnoze od strane patologa. Najveći izazov u diferencijalnoj dijagnozi plazmablastnog limfoma jeste njegovo razlikovanje od plazmoblastnog (anaplastičnog) plazmoćelijskog mijeloma, zbog morfoloških i imunofenotipskih osobina koje se preklapaju.

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Ključne reči: *plazmablastni limfom, nazofarinks, imunohistohemija*

Review article

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VITAMIN B COMPLEX AS A POTENTIAL THERAPEUTICAL MODALITY IN COMBATING PERIPHERAL NERVE INJURY

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Injuries of the peripheral nerves represent a large-scale problem in the modern world. They lead to significant consequences considering working ability and quality of life due to restricted recovery, especially of motor function. Different therapeutical approaches have been used in order to improve motor recovery. A significant number of studies showed some beneficial effects of different B complex vitamins on motor regeneration. Different experimental animal models were used in these studies, as well as within in vitro studies. In this paper, we will present the effects of B complex vitamin therapy on peripheral nerve regeneration after injury.

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Key words: *peripheral nerve injury, motor recovery, therapy, B vitamins*

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Introduction

Injuries of the peripheral nerves represent a significant problem in the modern world (1). The incidence of peripheral nerve injuries in developed countries is high, around 20 out of 100,000 people per year (2), which is around 300,000 injuries in Europe per year (3). After injury, damage to the motor, sensory or autonomic function of the nerve occurs, or in the worst case, the loss of all of these functions. Injuries may be caused by different causes, including bone fracture, ischemia, penetration of a foreign body, after infection, injection of narcotic drugs or after the use of drugs. The number of peripheral nerve injuries is constantly increasing due to the increase in traffic accident rates, industrial traumatism, and injuries at work. Trauma of the peripheral nerves results in a significant neurological deficit and almost always leaves a certain percentage of disability (4, 5). Such a trauma most commonly occurs in young male subjects in their most productive age (6) and therefore creates major problems, since the recovery is slow and usually incomplete. These injuries often make it impossible for a person to return to their original job and are therefore significant as a major socio-economic problem. It is estimated that 2-3% of all injuries are peripheral nerve injurie (6, 7). Injuries of the nerves of the upper limbs are more common, and they usually affect n. ulnaris and n. medianus, while injuries of the lower limbs affect n. ischiadicus, n. peroneus, n. tibialis, and n. femoralis.

Peripheral nerve injury and animal models

Generally, peripheral nerves may be injured in closed or open injuries. Open violations are more frequent, even though closed injuries account for a significant percentage.

Based on the severity of an injury, there are several degrees of peripheral nerve injury. Seddon (8) concludes that three degrees can be distinguished to neuropraxia, axonotmesis, and neurotmesis. Neuropraxia is a physiological block of conductivity, where the nerve is anatomically and histologically normal. It occurs most often due to blunt trauma, stretching, compression, and ischemia. Recovery is complete and spontaneous. Axonotmesis involves breaking the continuity of the axon without damaging the trunk of the nerve. Recovery is spontaneous but time-consuming, surgery is not indicated. Neurotmesis is a lesion of axons and connective tissue, complete or incomplete. A surgical intervention is in-dicated with the aim of reconstructing the nerve. On the other hand, Sunderland (9) provides a

more precise division of the lesions at five levels, where II, III, and IV levels are comparable to axonotmesis.

In order to discover the mechanisms of successful and unsuccessful reinnervation, animal models are used (10). Additionally, dysfunction studies require appropriate and functionally applicable measurement methods. Finally, the fact should be considered that successful functional regeneration can depend on various factors in different experimental models.

The model of facial nerve injuries as a pure motor nerve model, the facial nerve model, over the past decade has enabled the collection of a large number of data on the cellular and molecular responses of motoneurons and their surroundings. (11). The disadvantage of this model is a low recovery rate after reconstruction (12).

Sciatic nerve is a mixed nerve, containing motor and sensory axons. Motor recovery after injury reaches a maximum of 40% of normal function. This fact, in addition to the fact that the assessment of motor function recovery is difficult, makes the model of sciatic nerve injury limited in assessing the recovery of motor function of the peripheral nerve (13). The disadvantage of this model is a high degree of complications such as automutilation, the appearance of skin ulceration and joint contractures, and therefore is not a good model for studying the recovery of motor function of the peripheral nerve (13).

Femoral nerve is a mixed nerve that contains motor fibers which innervate m. quadriceps femoris as well as sensory axons for skin innervation. After transecting the motor branch, there are equal chances to establish correct and incorrect reinnervation, so the model of femoral nerve injury is good for the analysis of recovery of the motor component of the nerve (2). There is also a pure motor nerve model, as shown in the study by Nedeljković and colleagues (14), in which the section of the femoral nerve motor branch (innervating m. quadriceps femoris) is performed at a distance from bifurcation, leaving a sensitive branch intact, in order to exclude the possibility of wrong sprouting of the axon from the motor to the sensitive part.

Peripheral nerve regeneration

The response of the peripheral nervous system to injury is the induction of a self-repair process, and this is an essential difference between the peripheral and central nervous system (15, 16). Repair can occur through remyelination, collateral sprouting distally from preserved axons and regeneration from the site of injury (17).

Peripheral nerve regeneration is a complex process of cell-molecular interactions and structural changes in the proximal and distal stumps of the injured nerve, subsequently providing a meaningful functional recovery for patients. The proximal part of the injured nerve undergoes Wallerian degeneration up to the first node of Ranvier and then each injured axon elaborates multiple daughter axons. At the same time, the distal part of the injured nerve undergoes the same process of Wallerian degeneration, which is essential as a preparation phase for the axon regeneration process, in order to eliminate the molecules that could interfere with regeneration. Wallerian degeneration involves the invasion of macrophages that ingest myelin and initiate the Schwann cell mitosis. After the cytoskeleton and cell membrane are destroyed, Schwann cells degrade myelin. Further, after cleansing, the regeneration takes place from the proximal to the distal end of the nerve (18). Schwann cells help regenerating axons to cross the injury site from the proximal to distal part of the nerve. The exceptional ability of Schwann cells is the ability to change their phenotype and to dedifferentiate when they lose contact with axons. Therefore, after peripheral nerve injury, there is a reduced expression of molecular markers that are characteristic feature of mature Schwann cells. Between the first and fifth day after the injury, Schwann cells start to proliferate and the maximum of their activation is reached about the fourth day, and then decreases during the following weeks. This proliferation plays a key role during Wallerian degeneration (18). The secondary phase of proliferation takes place during the regenerative process. As they proliferate within the endoneurium membrane, Schwann cells form the Bungner bands, providing thus a favorable environment for axon regeneration. Denervated Schwann cells increase the expression of fibronectin, laminin, tenascin, and some proteoglycans, which form a favorable environment for axon elongation. They also increase the expression of several neurotrophic factors, such as nerve growth factor, brain-derived neurotrophic factor, neurotrophin 4, glial cell-derived neu rotrophic factor, and insulin-like growth factor 1. Schwann cells reduce the production of myelin proteins, as well as some other trophic factors (19). When reinnervation occurs, suppression of the expression of neurotrophic factors and their receptors occurs, and Schwann cells are in a steady state (20). A low level of functional recovery after the use of acellular nerve grafts for nerve reconstruction after transection indicate that active Schwann cells are crucial to axon regeneration. The capacity of Schwann cells to maintain pro-regenerative phenotype during long periods of time explains the limited capacity of chronic denervated nerves to maintain axonal regeneration, indicating the importance of early nerve reparation and strategies that could accelerate the recovery (21).

Treatment of peripheral nerve injuries

Peripheral nerves have an innate capacity for the induction of repair process. However, this capacity is limited and this process is seldom successful, and surgical intervention following injury is almost always required. Consequently, surgery is the first treatment for most peripheral nerve injuries and the current gold standard treatments are either direct microsurgical nerve repair or autologous nerve grafts. However, the regeneration of motor and sensory function often remains incomplete because full functional recovery is dependent upon many factors including the patient age, trauma location, injury severity, and presence of other diseases and conditions with an adverse impact on nerve regeneration.

Therefore, the development of alternative repair strategies that complement current established surgical procedures is needed (22). Additionally, although there are different animal models and studies of peripheral nerve regeneration process, the best treatment of peripheral nerve injury is still debated (23). Bearing in the mind all the above facts, the studies of the effects of neuroprotective agents that could potentially increase axonal regeneration following peripheral nerve damage, especially if axonal integrity cannot be preserved, are needed. Vitamins of the B complex are possible candidates because they are infinitely renewable and amenable to molecular manipulation. There are various additional therapeutic approaches in peripheral nerve regeneration, but this paper will describe the use of vitamins of the B complex.

B vitamins as potential treatment modality for repairing peripheral nerve injuries

Vitamins are dietary components which are necessary for life and play an important role in health. B vitamins act as coenzymes in a substantial proportion of enzymatic processes and play key interacting roles in the majority of cellular functions (24). According to that, B vitamins are important for normal functioning of the nervous system as well (25). Due to its positive effects on the nervous system, both central and peripheral, they are often used in the treatment of various pathological conditions of the nervous system (26, 27). In this chapter, the functions of B vitamins and some of their effects upon the nervous system will be presented.

Vitamin B1 (Thiamine) is essential for normal growth and development. It expresses a positive effect upon the digestive, cardiovascular, and, especially nervous system. Vitamin B1 deficiency in humans causes the occurrence of cardiovascular diseases (Beriberi) and neurological disease (Wernick-Korskoff syndrome, Parkinson and Alzheimer disease) (28).

Vitamin B2 (Riboflavin) is a water-soluble vitamin present in two coenzyme forms of riboflavin, flavin mononucleotide and flavin adenine dinucleotide, playing important roles in enzymatic reactions. Riboflavin exerts neuroprotective effects in some neurological disorders (Parkinson disease, migraine, and multiple sclerosis) through its role in some pathways such as antioxidation, myelin formation, mitochondrial function, and iron metabolism. Hoan and colleagues (26) have shown that vitamin B2 improved behavioral outcome and reduced lesion volume, edema formation, and GFAP expression following traumatic brain injury.

Vitamin B3 (Nicotinamide), in the form of coenzymes, participate in many important redox reactions of the cell metabolism, such as cell respiration, the oxidation energy important molecules, biosynthesis of fatty acid and steroids, as well as in the oxidation of glucose-6-phosphate into ribose-5-phosphate in the pentose path. Further, as a coenzyme, B3 is important in DNA replication and repair, as well as in cell differentiation. Nicotinamide shows some neuroprotective effects in animal ischemia models (29, 30).

Vitamin B5 (Pantothenic acid) represents a functional part of coenzyme A. Coenzyme A is important for the synthesis of fatty acids, cholesterol, and acetylcholine. Lack of vitamin B5 leads to peripheral nerve damage, referred to as „burning feet syndrome" (31).

Vitamin B6 includes a group of related compounds: Pyridoxine, Pyridoxal, and Pyridoxamine. They are metabolized in the body to pyridoxal phosphate, which acts as a coenzyme in many important reactions in the blood, nervous system, and skin. In this way, vitamin B6 in amino acid metabolism is a rate-limiting cofactor in the synthesis of neurotransmitters, including dopamine, serotonin, γ-aminobutyric acid, noradrenaline and melatonin hormone (25). It is assumed that increased levels of pyridoxal can have neuroprotective effects (32).

Vitamin B7 (Biotin) plays a key role in glucose metabolism and haemostasis, including the regulation of hepatic glucose uptake, gluconeogenesis (and lipogenesis), insulin receptor transcription, and pancreatic β-cell function (33). Therefore, vitamin B7 has influence to the brain that is particularly sensitive to the delivery and metabolism of glucose (25).

Vitamin B9 (Folate) and Vitamin B12 (Cobolamin) are inextricably linked due to their complementary roles in the "folate" and "methionine" cycles (25). Vitamin B12 is required for the normal functioning of the nervous system and its deficiency causes damage to white matter of the brain and spinal cord, resulting in peripheral neuropathy (34). It has been shown in vivo that vitamin B12 is the most effective of all B vitamins in the regeneration of peripheral nerve after trauma and reconstruction. Scalabrino and Peracchi (35) showed that methylcobalamin (MeB12), a methylated cobolamin analogue, promotes conversion of homocysteine to methionine and expresses a stronger affinity for nervous tissues than other analogues, including cyanocobalamin. That is why MeB12 is prescribed to ameliorate various neuropathies (36, 37). The positive effect of vitamin B12 includes several actions: (i) use of MeB12 promote neurite outgrowth, regeneration and conduction of nerves after trauma by the activation of ERK1/2 and Akt protein kinase (38); (ii) MeB12 promotes Schwann cell proliferation and migration (39), which is essential in providing a permissive environment for axonal growth (36); (iii) MeB12 treatment enhances the final outcome of end-to-side neurorrhaphy, but not the excessive enumeration of invading collaterals (40); (iiii) MeB12 boosts the maturation of ingrowing axons to establish an effective connection, so that larger axons tend to prevail as the rats' survival lengthens (40); (iiiii) it also enhances axon myelination. Liao and colleagues (40) showed that the mean value of axon diameters in their MeB12-treated group is more than doubled compared to PBS-treated animals.

Recently, the therapy with assorted combinations of B vitamins has been investigated as an efficient method for the treatment of peripheral neuropathies, neuroregeneration, particularly in the regeneration of injured nerves. Vitamins of the B group are widely used in the treatment of peripheral neuropathies. Spinal cord ischaemia may cause long-lasting neuropathic pain in addition to other severe

problems. The mechanisms underlying neuropathic pain remain elusive and effective treatments of neuropathic pain are currently unavailable. B vitamins, such as B1, B6 and B12, are capable of antinociception in experi-mental animals with acute and chronic pain evoked by electrical, chemical and thermal stimulation, pri-mary neuronal injury and diabetes (41, 42, 43). In 2006, Caram-Salas et al. (42) showed that the com-bination of vitamin B1 and vitamin B12 (analog cya-nocobalamin) and dexamethasone reduced spinal nerve ligation induced allodynia in rats (approxima-tely 90%), indicating a synergistic interaction be-tween either vitamin B1 or vitamin B12 and dexamethasone and suggesting a possibility of clinical use of these drugs in the treatment of neuropathic pain in humans. The combination of B1, B6, and B12 syner-gistically inhibited thermal hyperalgesia, and their repetitive administration produced long-term inhibition of thermal hyperalgesia and suggested possible clinical utility of B vitamins in the treatment of neuropathic painful conditions following injury, inflammation, degeneration or other disorders in the nervous systems in human beings (41). Jolivalt and colleagues (2009) (43) showed the positive effects of B vitamins cocktails (B1, B6 and B12) on functional and behavioral disorders of diabetic rats that suggested their potential for use in the treatment of painful diabetic neuropathy. In addition, studies demonstrated that certain B vitamins, especially B6 and B12, can protect neurons from certain injuries (44, 45). The dose of these vitamins is important as well. Okada and colleagues (38) showed that high dose vitamin B12 had the potential to treat peri-pheral nerve injury. Recent studies have suggested that the use of a vitamin B combination (B1, B2, B3, B5, B6, and B12) in high doses after the transection of motor branch of rat femoral nerve contributes to the prevention of damage progression on one hand, and on the other, it promotes and accelerates the regeneration of the damaged nerve, so that their application in the treatment of peripheral nerve injury is justified. The vitamin B complex therapy ap-plied in high doses immediately postoperatively leads to the reduction of muscle atrophy, improved recovery of EMG parameters, reduction of nuclear density of the injured nerve and appropriate muscle, which all lead to the improved recovery of peripheral nerve motor function (14). Some authors investigated the combination of vitamin B12 with Dexamethasone and showed that this combination promote (i) regeneration of myelinated nerve fibers; (ii) proliferation of Schwann cells, (iii) recovery of sciatic functional index and sensory nerve conduction velocity (46). In line with all these data is the paper showing that the tissue levels of vitamin B complex and vitamin B12 in the injured sciatic nerve were significantly greater at 1 and 12 hours after experimental nerve injury, while they were significantly lower at 7 days than in the control group (47).

Conclusion

The effectiveness of B vitamins, alone or in different combinations, in the treatment of central and peripheral nervous system injury has been increasing, highlighting its importance in the development of new researches. This review showed the efficacy of B vitamins in the neuroregeneration process, elucidating a possible therapeutic potential in the treatment of peripheral nerve injury. However, even with the evidence that B vitamins can act on different targets and accelerate nerve regeneration, additional validated evidence is required to determine more intrinsic mechanisms of B vitamins effects in different peripheral nerve injury models.

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VITAMINI B KOMPLEKSA KAO POTENCIJALNI TERAPIJSKI MODALITET U LEČENJU POVREDA PERIFERNOG NERVA

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Povrede perifernih nerava predstavljaju problem velikih razmera u savremenom svetu. Posledice po radnu i životnu aktivnost su velike usled ograničenih mogućnosti za regeneraciju, pogotovo motorne funkcije. Različiti terapeutski pristupi su pokušali da poboljšaju rezultate motorne regeneracije. Značajan broj studija je pokazao povoljan efekat različitih vitamina B kompleksa na regeneraciju perifernog nerva. U navedenim istraživanjima su korišćeni različiti eksperimentalni animalni modeli, kao i *in vitro* studije. U ovom radu će biti prikazan uticaj terapije vitaminima B kompleksa na regeneraciju perifernog nerva nakon povrede.

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Ključne reči: *povreda perifernog nerva, motorni oporavak, terapija, B vitamini*

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ANGIOGRAPHIC CORRECTED TIMI FRAME COUNT CAN PREDICT LEFT VENTRICULAR REMODELING AFTER ACUTE ANTERIOR MYOCARDIAL INFARCTION IN PATIENTS WITH TIMI 3 FLOW IMMEDIATELY AFTER PRIMARY PCI ON PROXIMAL LEFT ANTERIOR DESCENDING CORONARY ARTERY

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The aim of this study was to evaluate coronary flow in the LAD coronary artery immediately after primary PCI in patients with acute anterior myocardial infarction, using the quantitative Corrected TIMI Frame Count (CTFC) method, and to compare coronary flow velocity with ST segment elevation resolution of electrocardiogram, echocardiographic left ventricular function parameters, and clinical outcomes during hospitalisation and after 12 months. Ninety eight patients with successful mechanical myocardial reperfusion, who achieved TIMI 3 flow and who were not planned for further revascularisaton, out of 156 consecutive patients with first anterior myocardial infarction, were included in this study. There were 44 patients in the group with faster TIMI 3 flow (CTFC ≤ 27), of whom 14 had PCI on the proximal segment LAD artery, 16 on medial and 14 on distal segment, and 54 patients in the group with slower TIMI 3 flow (CTFC 28-40) of whom 18 patients had intervention on proximal segment LAD artery, 22 on medial and 14 on distal segment.

The patients with primary PCI on proximal LAD segment with faster TIMI 3 flow achieved significantly more often complete ST segment elevation resolution at 90 minutes after PCI (50%), compared to those with slower TIMI 3 flow (17%, $p < 0.025$). The patients with PCI on proximal LAD artery segment who had faster TIMI 3 flow, showed after 12 months a significantly lower echocardiographic end-systolic volume index (ESVI) 31.3 ± 6.7 ml/m^2, compared to those with intervention on the proximal LAD coronary with slower TIMI 3 flow 37.2 ± 6.5 ml/m^2 ($p < 0.025$). Faster TIMI 3 flow in the infarction artery was accompanied with a more complete ST segment resolution in acute phase and lesser left ventricular remodeling after 12 months, only if the culprit lesion was localized in the proximal LAD artery segment.

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Key words: *percutaneous coronary intervention, myocardial infarction, remodelling*

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Introduction

The aim of reperfusion therapy in acute myocardial infarction is to open the occluded coronary artery as early as possible and to reduce myocardial necrosis. By reducing myocardial infarction size, left ventricular dysfunction can be prevented and clinical outcome of a patient can be improved. Efficient mechanical reperfusion by primary PCI involves establishing rapid blood flow in the infarction epicardial coronary artery, but also adequate microvascular flow and myocardial perfusion. In clinical practice, the flow in infarction artery is evaluated qualitatively on coronary arteriography by the TIMI flow classification (1, 2, 3). Quanification of coronary blood flow using the Corrected TIMI Frame Count (CTFC) method can make the assessment more precise, objective and reproducibile. The aim of this study was to estimate the coronary flow in the LAD coronary at the end of primary PCI in patients with acute anterior myocardial infarction, using the quantitative Corrected TIMI Frame Count CTFC method. This analysis includes patients with successful mechanical myocardial reperfusion and TIMI 3 flow in the infarcted

artery after primary PCI. Coronary flow velocity in the LAD artery was compared to electrocardiographic findings of ST segment elevation resolution at 90 minutes after PCI, and with the maximum value of myocardial necrosis biochemical marker CK-MB. Coronary flow velocity in the LAD coronary was also compared to the left ventricular ejection fraction (EF) and end-systolic volume index (ESVI), determined by echocardiography during hospitalization and after 12 months. Coronary flow velocity in the LAD artery at the end of primary PCI was also compared to clinical outcomes: mortality, myocardial reinfarction and repeated LAD revascularization, during hospitalization and after 12 months.

Methods

Coronary flow in the LAD coronary was quantified by the Corrected TIMI Frame Count CTFC method using mostly the RAO caudal view, which represented correctly the beginning of artery and also distal LAD bifurcation ("moustache") on the apex (1, 2). Angio frame, when contrast entering LAD vessel touched both walls of the artery for the first time, was taken as the first frame. Angio frame was taken as the last frame when contrast started entering the branch of distal bifurcation of LAD artery, and the number of frames was counted during contrast going between two reference points of LAD. The correction was made according to registration speed and the value of CTFC was expressed related to the speed of 30 frames/sec. CTFC correction was also done by the division by 1.7, due to the lentgh of the LAD artery. An analysis was done at the end of percutaneous intervention, after intracoronary aplication of 200 μg of nitroglycerine (4). CTFC measurement was done three times and the average value was calculated. Based on the values of CTFC (all patients in this study had TIMI 3) the patients were divided into two groups. The first group included patients with fast TIMI 3 flow and CTFC ≤ 27, and the other group had slower TIMI 3 flow and CTFC from 28 to 40. Coronary flow with CTFC > 40 was, by definition, categorized as TIMI 2 flow, and these patients were not included in this study.

ST segment elevation resolution was quantified in the precordial lead with highest elevation, prior to percutaneous intervention and ST elevation resolution was estimated at the end of PCI and after 90 minutes. ST segment elevation resolution ≥ 70% was considered to be complete, from 30% to 69% as partial resolution, and a < 30% as the absence of resolution. The size of myocardial necrosis was estimated using the maximum value of biochemical marker of necrosis CKMB.

Echocardiographic assessment of the left ventricular contractile function was performed using the area lentgh method and left ventricuar ejection fraction (EF) and volume indexes at the end of diastole (EDVI) and end of systole (ESVI) were measured during hospitalization, and after 12 months. Clinical outcome of patients was evaluated during hospitalization and after 12 months, analyzing the occurrences of death, reinfarction, repeated revascularization, by PCI intervention or aortocoronary bypass, and hospitalization due to heart failure in 12 months.

The significance of the difference of cathegorical (qualitative) parameters between the patient groups was determined by $\chi 2$ test. The significance of numerical parameter difference between the patient groups was determined using the Student t-test. The difference was considered to be significant if $p < 0.05$.

Results

One hundred fifty six patients with first acute anterior myocardial infarction and primary PCI performed on the left anterior descending artery within six hours of chest pain onset, were analyzed. Manual aspiration catheter was used in 27 patients (17%), direct stenting was performed in 63 patients (40%) and stenting after predilation in 93 patients (60%). At the end of percuataneous coronary procedure and implantation of 1.3 ± 0.4 stents (72% BMS, 28% DES), TIMI 3 flow in the LAD artery was present in 133 patients (85%). Fourteeen patients (9%) had TIMI 2 flow and 9 patients (6%) had TIMI 0-1 flow. Pre-PCI baseline coronary flow in the infarction artery was TIMI flow 0-1 in 82 patients (53%), TIMI 2 flow in 29 patients (19 %) and TIMI 3 flow in 45 patients (28%). A staged PCI intervention on another coronary artery was done in 25 patients (19%) with TIMI 3 flow, while 10 patients (8 %) were sent for additional surgical revascularization of the myocardium. The patients with previous myocardial infaction and also with previous PCI were not included in this study. This study included 98 patients with acute anterior myocardial infarction and successful mechanical myocardial reperfusion who achieved TIMI 3 flow in the LAD artery at the end of primary PCI. Primary PCI was performed on the proximal LAD artery segment in 32 patients, on medial LAD segment in 38, and on distal LAD segment in 28 patients. In order to participate in this study the patients were not to be treated additionally by further revascularization of myocardium (PCI or aortocoronary bypass surgery). The patients were observed while hospitalized and followed up after discharge in the following 12 months.

In the group with fast TIMI 3 flow in the LAD artery after primary PCI and Corrected TIMI Frame Count CTFC ≤ 27 there were 44 patients, aged 58.5 ± 10.5 years, 33 male and 11 female, and 14 patients had percutaneous intervention on the proximal segment LAD artery, 16 on the medial and 14 on the distal segment. Pre-PCI baseline coronary flow in these patients was TIMI flow 0-1 in 18 patients (41%), TIMI 2 flow in 11 patients (25 %) and TIMI 3 flow in 15 patients (34%). In the group with slower TIMI 3 flow CTFC 28-40 there were 54 patients, aged 58.1 ± 10.3 years, 44 male and 10 female, and 18 patients had intervention on the proximal segment LAD artery, 22 on the medial and 14 on the distal segment. Pre-PCI baseline coronary flow in these patients was TIMI flow 0-1 in 24 patients (44%), TIMI 2 flow in 13 patients (24 %) and TIMI 3

flow in 17 patients (32%). The groups did not differ significantly in relation to age and gender. In the group with CTFC ≤ 27, 18 patients (41%) out of 44 acheived complete resolution of ST segment elevation of 70% and more, 11 patients (25%) resolution of ST segment 30-69%, while 15 patients (34%) had ST resolution below 30% (Table 1).

Table 1. ST segment elevation resolution and size of myocardial necrosis

ST segment resolution ECG	TIMI 3 CTFC ≤ 27	TIMI 3 CTFC 28 - 40	Significance
PCI LAD			
Number of patients n	44	54	
≥ 70%	18 (41%)	18 (33%)	ns
30-69%	11 (25%)	16 (30%)	ns
< 30%	15 (34%)	20 (37%)	ns
PCI prox LAD			
N	14	18	
≥ 70%	7 (50%)	3 (17%)	p < 0.025
30-69%	4 (29%)	8 (44%)	ns
< 30%	3 (21%)	7 (39%)	ns
PCI med LAD			
N	16	22	
≥ 70%	5 (31%)	7 (32%)	ns
30-69%	4 (25%)	6 (27%)	ns
< 30%	7 (44%)	9 (41%)	ns
PCI dist LAD			
N	14	14	
≥ 70%	6 (43%)	8 (58%)	ns
30-69%	3 (21%)	2 (14%)	ns
< 30%	5 (36%)	4 (28%)	ns
Size of myocardial necrosis			
PCI LAD CK MB max U/L	108.7 ± 48.2	116.2 ± 43.5	ns
PCI prox LAD CK MB max U/L	107.5 ± 46.2	117.8 ± 40.2	ns
PCI med LAD CK MB max U/L	109.1 ± 54.2	119.3 ± 44.2	ns
PCI dist LAD CK MB max U/L	109.4 ± 41.2	109.3 ± 42.2	ns

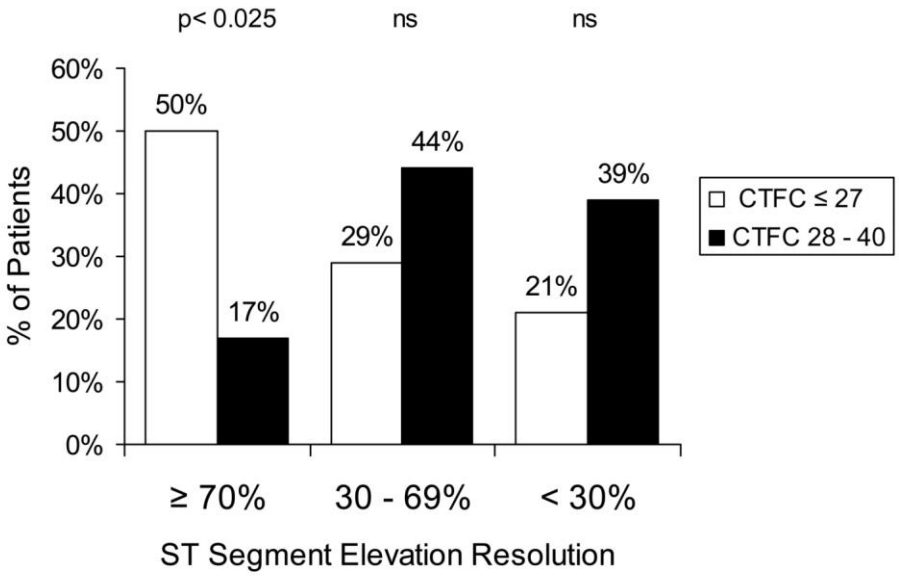
In the group of patients with CTFC 28-40, 18 patients (33%) out of 54 had complete resolution of 70% and more, 16 patients (30%) had partial resolution of ST segment elevation 30-69%, while 20 patients (37%) acheived ST resolution of less than 30%. There were no statistically significant differences in ST segment elevation resolution degree, between the groups with fast and slower TIMI 3 flow.

The analysis of ST segment elevation resolution was done considering also the LAD segment site of the culprit lesion and PCI. In the group with fast TIMI 3 flow and CTFC ≤ 27, 14 patients had PCI on the proximal LAD artery segment, and in 7 out of these (50%) a complete ST segment elevation resolution of 70% or more was achieved (Graph 1) in 4 patients (29%) ST elevation resolution of 30-69%, and in 3 patients (21%) ST segment resolution was below 30%.

In the group of patients with slower TIMI 3 flow and CTFC 28-40, PCI intervention on proximal LAD segment was done in 18 patients and complete ST segment resolution of 70% and more was achieved in 3 patients (17%), partial resolution of 30-69% in 8 patients (44%) and in 7 patients (39%) ST segment resolution was below 30%.

The patients with primary percutaneous intervention on proximal LAD segment who had fast TIMI 3 coronary flow at the end of PCI achieved significantly more often complete ST segment resoultion (> 70%) at 90 minutes after PCI, compared to those with slower TIMI 3 flow (p < 0.025). In the group with CTFC ≤ 27, primary PCI was done in 16 patients on the medial segment LAD artery and 5 of these patients (31%) had a complete ST segment elevation resolution (> 70%), 4 patients (25%) showed ST resolution of 30-69%, and 7 patients (44%) had ST segment resolution below 30% (Table 1).

In the group of 22 patients with CTFC 28-40 and PCI on the medial segment LAD artery, 7 patients (32%) had complete ST segment elevation resolution (> 70%), 6 patients (27%) had a partial resolution of 30-69%, and 9 patients (41%) showed ST segment resolution of less than 30%.



Graph 1. ST segment elevation resolution 90 minutes after primary PCI on the proximal LAD artery in relation to coronary flow velocity at the end of PCI. ST segment elevation resolution 90 minutes after primary PCI on proximal LAD in relation to coronary flow velocity at the end of PCI, in patients with TIMI 3 flow. Patients with fast TIMI 3 flow (CTFC ≤ 27) had more frequent complete reperfusion (p<0.025), compared to the patients with slow TIMI 3 flow (CTFC 28-40).

In the group with CTFC ≤ 27, primary PCI was done in 14 patients on the distal segment LAD artery and 6 of these patients (43%) had a complete ST segment elevation resolution (> 70%), 3 patients (21%) showed ST resolution of 30-69%, and 5 patients (36%) had ST segment resolution of less than 30%.In the group of 14 patients with CTFC 28-40 and PCI on the distal segment LAD artery, 8 patients (58%) had complete ST segment elevation resolution (> 70%), 2 patients (14%) had a partial resolution of 30-69%, and 4 patients (28%) showed ST segment resolution of less than 30%. In the patients with primary PCI on the medial LAD artery segments and also on distal LAD segments, there were no statistically significant differences in ST segment elevation resolution degree between the groups with fast TIMI 3 flow and slow TIMI 3 flow (Table 1).In our study of patients with TIMI 3 flow at the end of primary PCI on the LAD artery, a fast TIMI 3 flow (CTFC ≤ 27), compared to slow TIMI 3 flow (CTFC 28-40), was accompanied by a greater degree of ST segment elevation resolution only in the subgroup of patients with PCI on the proximal LAD artery segment. The biochemical marker of myocardial necrosis CKMB did not show any significant difference in its maximum value (Table 1) between the patients with fast TIMI 3 flow 108.7 ± 48.2 U/L and patients with slow TIMI 3 flow 116.2 ± 43.5 U/L. The analysis of the sub-groups of patients with intervention on the proximal, medial and distal LAD artery segments did not show any significant difference of the maximum value of CKMB between the patients with fast TIMI 3 flow and slow TIMI 3 flow at the end of primary PCI.

Echocardiographic examination was done during hospitalization, 2.5 ± 1.2 days after primary PCI, using the 2D area length method. In the group of patients with fast TIMI 3 flow at the end of primary PCI, the ejection fraction was 51.1% ± 11.4% (Table 2) and was not significantly different in relation to those with slow TIMI 3 flow 50.4% ± 10.8%. There was no significant difference in the left ventricular endsystolic volume index ESVI between the groups of patients with fast TIMI 3 flow 33.9 ± 7.8 ml/m^2 and slow TIMI 3 flow 35.7 ± 8.1 ml/m^2. There was also no significant difference in ejection fraction EF and left ventricular ESVI between the patients with fast TIMI 3 flow and slow TIMI 3 flow, in relation to the localization of the culprit lesion and PCI on the proximal, medial or distal LAD artery segment (Table 2). There was not any significant difference in mortality during hospitalization betwen the patients with fast TIMI 3 flow 2.3% and slow TIMI 3 flow 1.9% (ns) (Table 2), and also in myo-cardial reinfarction 6.8% and 5.6 % (ns), respectively.

Table 2. Left ventricular function and clinical outcome during hospitalization

2D Echocardiography Area lentgh method	TIMI 3 CTFC ≤ 27	TIMI 3 CTFC 28 - 40	Significance
PCI LAD			
Number of patients n	44	54	
EF %	51.1 ± 11.4	50.4 ± 10.8	ns
ESVI ml/m^2	33.9 ± 7.8	35.7 ± 8.1	ns
PCI prox. LAD			
N	14	18	
EF %	50.1 ± 10.8	49.5 ± 11.2	ns
ESVI ml/m^2	34.2 ± 7.5	36.3 ± 7.2	ns
PCI med. LAD			
N	16	22	
EF %	51.8 ± 11.9	51.0 ± 10.6	ns
ESVI ml/m^2	33.7 ± 7.8	35.3 ± 8.1	ns
PCI dist LAD			
N	14	14	
EF %	51.3 ± 8.9	50.6 ± 8.7	ns
ESVI ml/m^2	33.8 ± 6.2	35.5 ± 6.2	ns
Clinical outcome			
Mortality	1 (2.3%)	1 (1.9%)	ns
Reinfarction	3 (6.8%)	3 (5.6%)	ns

EF ejection fraction, ESVI of left ventricular end-systolic volume index

At the end of twelve month follow up period, there was not any significant difference in mortality between the groups with fast TIMI 3 flow 6.8% and slow TIMI 3 flow 7.4% (ns) (Table 3) at the end of primary PCI on the LAD artery. There was not any significant difference in combined major adverse coronary events: mortality, reinfarction, repeated target lesion revascularization and hospitalization due

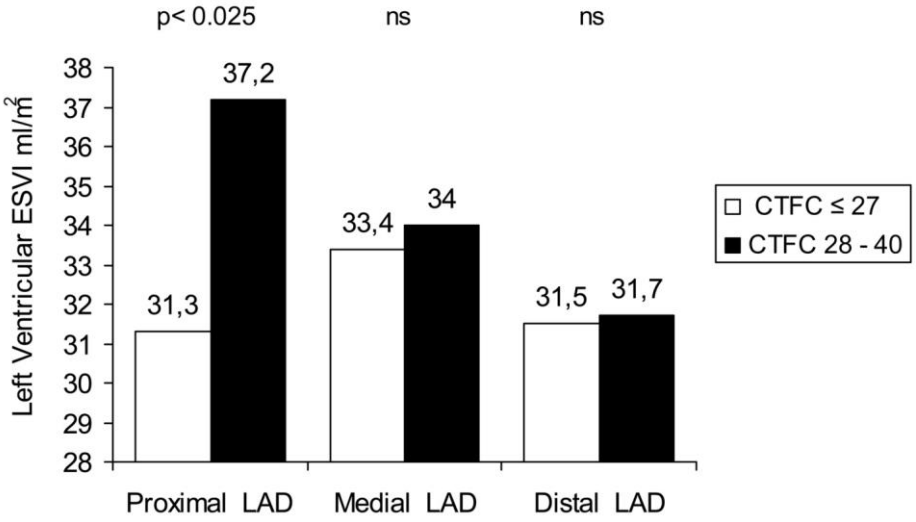
Table 3. Left ventricular function and clinical outcome after 12 months

2D Echocardiography Area lentgh method	TIMI 3 CTFC ≤ 27	TIMI 3 CTFC 28 – 40	Significance
PCI LAD			
Number of patients n	41	50	
EF %	55.3 ± 8.7	53.7 ± 8.8	ns
ESVI ml/m^2	32.3 ± 6.9	34.1 ± 6.7	ns
PCI prox. LAD			
N	13	17	
EF %	54.1 ± 9.8	53.2 ± 10.2	ns
ESVI ml/m^2	31.3 ± 6,7	37.2 ± 6.5	p < 0.025
PCI med. LAD			
N	15	20	
EF %	55.6 ± 10.2	54.1 ± 10.6	ns
ESVI ml/m^2	33.4 ± 7.1	34.0 ± 7.0	ns
PCI dist. LAD			
N	14	14	
EF %	58.1 ± 8.9	53.8 ± 8.6	ns
ESVI ml/m^2	31.5 ± 6.5	31.7 ± 6.1	ns
Clinical outcome			
Mortality	3 (6.8%)	4 (7.4%)	ns
Combined outcome mortality, reinfarction, repeated target lesion revascularisation	7 (15.9 %)	9 (16.7%)	ns

EF ejection fraction, ESVI end-systolic volume index of left ventricle

to heart failure between the groups with CTFC ≤ 7, 15.9%, and CTFC 28-40, 16.7% (ns).

After twelve months, the left ventricular ejection fraction in the group with fast TIMI 3 flow at the end of primary PCI was 55.3% ± 8.7% and was not significantly different from the group with slow TIMI 3 flow 53.7% ± 8.8%, (ns). Left ventricular end-systolic volume index after 12 months was not significantly different between the patient groups with fast TIMI 3 flow 32.3 ± 6.9 ml/m^2 and slow TIMI 3 flow 34.1 ± 6.7 ml/m^2, (ns). Left ventricular end-systolic volume index and ejection fraction between the patient groups were analyzed in relation to the culprit lesion and PCI segment localization in the LAD artery. The patients with primary PCI on the proximal LAD artery and fast TIMI 3 flow at the end of pro-cedure, had significantly lower ESVI of 31.3 ± 6.7 ml/m^2 (Graph 2) compared to those with intervention on the proximal LAD artery and slow TIMI 3 flow of 37.2 ± 6.5 ml/m^2 (p < 0.025).



Graph 2. Left ventricular end-systolic volume index ESVI after 12 months in relation to coronary flow velocity at the end of primary PCI and culprit lesion LAD artery segment localization. Patients with slow TIMI 3 flow (CTFC 28-40) showed left ventricular remodeling and significantly greater left ventricular end-systolic volume index ESVI (p<0.025), compared to those with fast TIMI 3 flow (CTFC ≤ 27), only in the group with primary PCI on the proximal LAD artery segment.

Ejection fraction after 12 months was not statistically significantly different between the patients with primary PCI on the LAD artery proximal segment with fast TIMI 3 flow of 54.1% ± 9.8% and slow TIMI 3 flow of 53.2% ± 10.2%, (ns) (Table 3).

After 12 months follow up of patients with primary PCI on the medial LAD artery segment, the group with fast TIMI 3 flow did not show a significant difference in ejection fracton 55.6% ± 10.2% and ESVI 33.4 ± 7.1 ml/m^2 (Table 3), compared to the group with slow TIMI 3 flow EF 54.1% ± 10.6%, (ns) and ESVI 34.0 ± 7.0 ml/m^2 (ns). Left ventricular end-systolic volume index and ejection fraction after 12 months did not show a significant difference in patients with the culprit lesion and PCI on the LAD artery distal segment in relation to coronary TIMI 3 flow velocity at the end of primary PCI (Table 3).

Discussion

Corrected TIMI Frame Count CTFC method for assesing of coronary flow velocity at the end of primary PCI can be used to evaluate the efficacy of mechanical reperfusion and to predict clinical outcomes and patient prognosis (2, 3, 5-8). Different physiological and technical factors can influence coronary flow velocity and therefore result interpretation should be done carefully (4). Nitroglycerine increases coronary flow velocity in the infarction artery and CTFC evaluation is done at the end of primary PCI, after intracoronary application of a standard dose of 200 μg of nitroglycerine. ST segment resolu-tion of electrocardiogram after primary PCI is related to the efficacy of culprit lesion PCI treatment and infarction artery flow velocity, as well as microvascular perfusion in the territory at risk and myocardial viability. It has been shown that earlier and more complete ST segment resolution of electrocardiogram after primary PCI is followed by smaller size myocardial necrosis, better contractile left ventricular function and more favourable clinical outcome (9). In some patients a rapid epicardial flow after primary percutaneous intervention is not followed by a coresponding ST segment resolution of electrocardiogram, and there are patients with compromised microvascular flow and inadequate myocardial perfusion despite patient epicardial coronary arteries (10, 11). Microvascular circulation can be damaged by a prolonged ichemia but also by reperfusion after in-

farction artery opening. Swelling of endotelial cells with capillary lumen narrowing has been reported. Myocardial interstitial oedema can cause external capillary compression and compromised microcirculation. Micro embolisation of thrombotic material and detritus of ruptured aterome can cause microvascular obstruction. Distal macroembolisation and microembolisation can occur as a consequence of percutaenous coronary intervention but also during thrombolysis, either spontaneous or pharmacological. Leukocyte and trombocyte accumulation in the capillaries in the infarction area impair microcirculatory flow and activate inflammation process. Vasospasm induced by vasoactive substances released from microvascular platelet plugs can further compromise myocardial flow.

Our study shows that faster flow TIMI 3 flow in the infarction epicardial artery was accompanied with a more complete ST segment resolution on electrocardiogram 90 minutes after primary PCI, only in the case of intervention on the proximal LAD artery segment. As for interventions on the medial and distal LAD artery segments, a faster flow in the infarction artery was not accompanied by a significantly better ST segment resolution. In patients with TIMI 3 flow at the end of primary PCI of the LAD artery, a slightly faster coronary flow was accompanied with a significantly better ST segment resolution only in patients with more proximal culprit lesion and more extensive jeopardized myocardial territory. There was no significant difference in the maximum value of released myocardial necrosis marker CKMB, between the patient groups with faster and slower TIMI 3 coronary flow. Mechanical myocardial reperfusion is followed by a faster washout of myocardial necrosis marker out of the infarction area and earlier rise of serum CKMB.

A larger myocardial necrosis with more extensive myocardial infarction size can be followed by the process of left ventricle dilation and remodelling, worsening the global contractile function with unfavorable clinical outcomes. Left ventricular remodeling occurs more often after an extensive anterior myocardial infarction. After primary PCI, a faster CTFC flow in the infarction artery is followed by less frequent left ventricular remodeling (3, 5). Echocardiograpy is a suitable tool for left ventricle function follow up after myocardial infarction. In our study of patients with TIMI 3 flow at the end of primary PCI of the LAD artery, a slower coronary flow was accompanied by echocardiographic findings of more frequent left ventricle remodeling at 12 months only in the patients with culprit lesions in the proximal LAD artery segment, with more extensive anterior myocardial teritory jeopardized.

Limitations

This study did not involve examination of the microvascular flow and myocardial perfusion in the infarction region. It has been shown that fast epicardial flow after primary PCI is not always accompanied by efective myocardial perfusion due to possible impairement of microvascular flow (9-11). Longer time elapsed from the onset of chest pain to reperfusion induces greater myocardial necrosis and more severe impairement of microvascular flow. Reduced myocardial perfusion increases myocardial necrosis and unfavourably influences left ventricular contractile function and clinical outcomes. Microvascular flow and myocardial perfusion can be estimated by angiography using the TIMI Myocardial Perfusion Grade (TMPG) method. Perfusion in the microvascular bed can also be assesed using contrast echocardiography, nuclear radioisotopic techniques, as well as nuclear magnetic resonance method. Coronary flow velocity in the infarction artery before primary PCI can also influence post-procedural results, as well as clinical outcomes (12-14).

Conclusion

In this study of patients with TIMI 3 flow, at the end of primary PCI of the LAD artery in acute anterior myocardial infarction, faster flow in the infarction artery was accompanied by a more complete ST segment resolution on electrocardiogram, compared to the patients with slower TIMI 3 flow, only if the culprit lesion site and performed intervention were on the proximal LAD artery segment. In these patients with TIMI 3 flow, a discrete difference in the achieved coronary flow velocity influenced significantly ST segment resolution degree only in patients with more proximal culprit lesion and with more extensive myocardial territory jeopardized. There was no significant difference in the maximum value of released myocardial necrosis marker CKMB, between the patients with faster and slower target artery TIMI 3 flow.

The patients with slower TIMI 3 flow at the end of primary PCI on the LAD artery showed after 12 months more frequent dilation and remodeling of the left ventricle and increased ESVI, compared to those with faster TIMI 3 flow only if the culprit lesion and intervention were on the proximal LAD artery segment. Our study of 98 patients with acute anterior myocardial infarction and TIMI 3 flow at the end of primary PCI did not show any significant differences in clinical outcomes during hospitalization and after 12 months, between the patients with faster and slower TIMI 3 flow. CTFC assessment can be used in further risk stratification of patients with TIMI 3 flow at the end of primary PCI on the proximal LAD artery segment in acute myocardial infarction.

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BRZINA KORONARNOG PROTOKA CTFC MOŽE PREDVIDETI REMODELOVANJE LEVE KOMORE POSLE INFARKTA MIOKARDA KOD BOLESNIKA SA TIMI 3 PROTOKOM NAKON PRIMARNE PERKUTANE KORONARNE INTERVENCIJE NA PROKSIMALNOM SEGMENTU PREDNJE DESCEDENTNE ARTERIJE

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Cilj ispitivanja bio je da se proceni brzina koronarnog protoka u prednjoj descedentnoj arteriji LAD neposredno nakon primarne perkutane koronarne intervencije kod bolesnika sa akutnim infarktom prednjeg zida, korišćenjem korigovanog broja angiografskih slika CTFC, i da se brzina protoka u infarktnoj arteriji uporedi sa rezolucijom elevacije ST segmenta elektrokardiograma, ehokardiografskim parametrima funkcije leve komore i sa kliničkim ishodom u toku hospitalizacije i nakon 12 meseci. U ispitivanje je uključeno devedeset osam bolesnika sa uspešnom mehaničkom reperfuzijom miokarda, koji su postigli TIMI 3 protok u infarktnoj arteriji i koji nisu bili planirani za dalju revaskularizaciju miokarda, od ukupno 156 konsekutivnih bolesnika sa prvim infarktom prednjeg zida. U grupi sa bržim TIMI 3 protokom (CTFC ≤ 27) bilo je 44 bolesnika, od kojih je 14 imalo intervenciju na proksimalnom segmentu LAD, 16 na medijalnom i 14 na distalnom segmentu LAD. U grupi sa sporijim TIMI 3 protokom (CTFC 28-40) bilo je 54 bolesnika, od kojih je 18 imalo intervenciju na proksimalnom segmentu LAD, 22 na medijalnom i 14 na distalnom segmentu LAD. Bolesnici sa primarnom PCI na proksimalnom segmentu LAD i bržim TIMI 3 protokom (CTFC ≤ 27) značajno su češće (50%) postigli kompletnu rezoluciju ST segmenta elektrokardiograma, 90 minuta nakon PCI, u poređenju sa bolesnicima sa PCI na proksimalnom segmentu LAD i sporijim TIMI 3 (CTFC 28-40) protokom (17%, $p < 0,025$). Bolesnici sa PCI na proksimalnom segmentu LAD i bržim TIMI 3 protokom (CTFC ≤ 27) su nakon 12 meseci imali značajno manji ehokardiografski endosistolni volumen indeks (ESVI) 31,3 ± 6,7 ml/m^2, u poređenju sa bolesnicima sa PCI na proksimalnom segmentu LAD i sporijim TIMI 3 protokom 37,2 ± 6,5 ml/m^2 ($p < 0,025$). Brži TIMI 3 protok u infarktnoj arteriji, nakon primarne PCI, bio je udružen sa češćim postizanjem kompletne rezolucije ST segmenta elektrokardiograma u akutnoj fazi, i sa manje ispoljenim remodelovanjem leve komore nakon 12 meseci, samo ukoliko je infarktna koronarna lezija bila lokalizovana na proksimalnom segmentu LAD.

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Ključne reči: *perkutana koronarna intervencija, infarkt miokarda, remodelovanje*

Case report

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DRAINAGE OF PLEURAL SPACE BY APICAL APPROACH AS A STEP BEFORE DEFINITIVE SURGICAL RESOLUTION OF SPONTANEOUS PNEUMOTHORAX RECURRENCE: A CASE REPORT

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Pneumothorax represents the presence of air and/or gases in the pleural space. Spontaneous pneumothorax is divided into: primary (PSP), occuring in the healthy lung, and secondary (SSP), that occurs together with some understanding lung disease. PSP is thought to occur due to a rupture of the similar to emphysema change (ELC) in the lungs and/or diffuse pleural porosity. Pleural space drainage is one of the most useful and most commonly used procedures in the treatment of PSP. The recurrence of PSP is an indication for surgical treatment. In the following case study, it has been shown that the combined approach to treating PSP recurrence with thoracic drainage through the 1st intercostal space from above, and the minimally invasive surgical approach, is purposeful and desirable.

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Key words: *primary spontaneous pneumothorax, recurrent pneumothorax, thoracic drainage, pleuractomy, pleural abrasion*

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Introduction

Pneumothorax represents the presence of air and/or gases in the pleural space. The term pneumothorax was first introduced by Itard in 1803 and Lannec in 1819, and the diagnosis of spontaneous pneumothorax was made by Kjaergaard in 1932 (1, 2). [Spontaneous pneumothorax is divided into: primary (occuring in the healthy lungs) and secondary (in the space of some of the existing lung diseases) (3)]. The exact cause of the occurrence of primary spontaneous pneumothorax (PSP) is still unknown. PSP is thought to occur due to a rupture of similar to emphysema change (ELC) in the lungs and/or diffuse pleural porosity (4, 5). The most common symptoms and signs of the formation of primary spontaneous pneumothorax are: sudden chest pain, dyspnoea, dry hacking cough, accelerated deep breathing, paroxysmal tachycardia, and weakness and fatigue (6). PSP treatment can comprise: conservative, pleural space puncture, pleural space drainage by pigtail catheter or thoracic drainage, pleural space drainage combined with Heimlich valve, drainage of pleural space combined with pleurodesis, VATS combined with chemical pleurodesis, VATS with pleuractomy and/or parietal pleura abrasion, open thoracotomy combined with chemical pleurodesis, open thoracotomy with pleuractomy and/or abrasion of the parietal pleura (7).

Pleural space drainage is one of the most useful and commonly used procedures in the treatment of PSP, and in chest surgery in general (8). The first description of the thoracic drainage was given by Hippocrates (9). In the 14th century, the drainage of pleural space was performed by Guy de Chauliac, in the 18th century by Boerhave, and Hewett in 1876 was the first to apply a completely closed system of thoracic drainage (10, 11). A widespread use of thoracodrainage was introduced during World War II (12). Standard techniques of thoracic drainage include the placement of the thoracic drain laterally at the level of the "safety triangle" (the space limited by the front edge of the m. latissimus dorsi, the lateral edge of the m. pectoralis major, the horizontal line above the level of the nipple, with a top in the base of the axilla), in the 2nd intercostal space (ICS) of the medioclavicular line and apical approach, through the 1st ICS from above (13, 14). Depending on the type and method of PSP treatment, recurrences may

range from 0% -50% of cases (6,15-17). The recurrence of PSP is an indication for surgical treatment (3).

Case study

Patient I.P., 23 years old, was admitted to our facility with a clinical and Rtg case of a second PSP episode on the left (Figure 1).

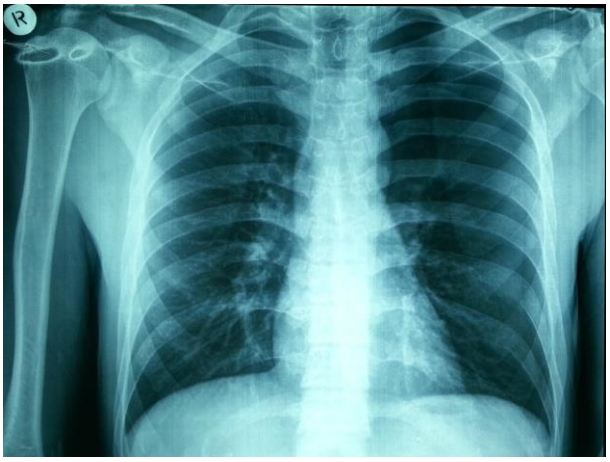

Figure 1. Chest Rtg P-A at admission - recurrent primary spontaneous pneumothorax on the left

He stated that the day before admission, at rest, he felt a sudden, sharp pain on the left side of the chest, which was followed by dry cough and a feeling of lack of air. Previously, three months before, drainage of the left pleural space was made in the middle axillary line, in the 5th ICS on the left side due to the first episode of the PSP. Immediately after admission, the left-hand pleural space was redrained through the 1st ICS from above (Figure 2). After infiltration of 10 mL of 2% Lidocaine, a skin and subcutaneous tissue cut was made at the level of the joint of the inner and middle third of the line that joins the processus spinosus vertebrae prominens and acromion (four transverse fingers, about 4 cm from the vertebrae prominens), on two transverse fingers (about 2 cm) from the front edge m. Trapezium (Figures 3 and 4). Then, the trocar was introduced from above, strictly straight, through the 1st ICS, and thoracic drain N020 Ch was inserted in the left pleural space. The drain was fixed, connected to underwater drainage and continuous vacuum aspiration of - 20 cm H_2O.

Initially, air was given. Analgesic and antibiotic therapy was administered. Immediately after drainage, the general condition of the patient was stabilized, and subjectively, he did not complain of difficult breathing. Since it was a relapse of PSP, an indication for surgical treatment was established. The next day the patient underwent surgery. An access to the left pleural space was enhanced by video-assisted mini-thoracotomy through the 5th ICS. On the tip of the lungs, there were emphysema-like changes and scars of previously ruptured bullae. Atypical resection of the top left lung by tissue stapler was performed, and then partial pleuralctomy to the level of thoracotomy incision and abrasion of the remaining parietal pleura.

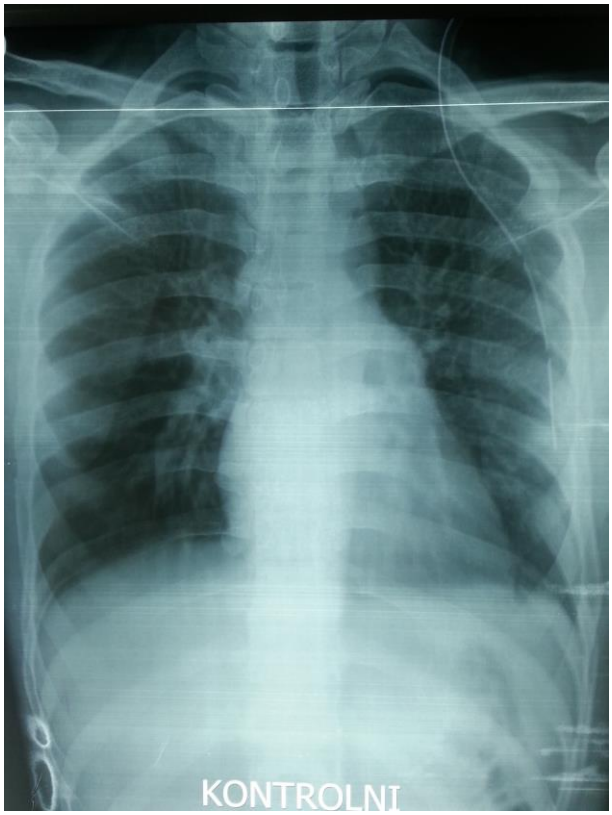

Figure 2. Chest Rtg P-A after drainage through the 1st intercostal space above

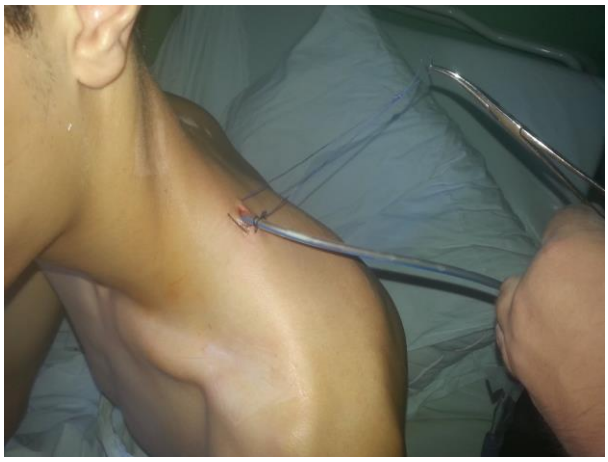

Figure 3. Thoracic drain placed apically on the left side of the chest

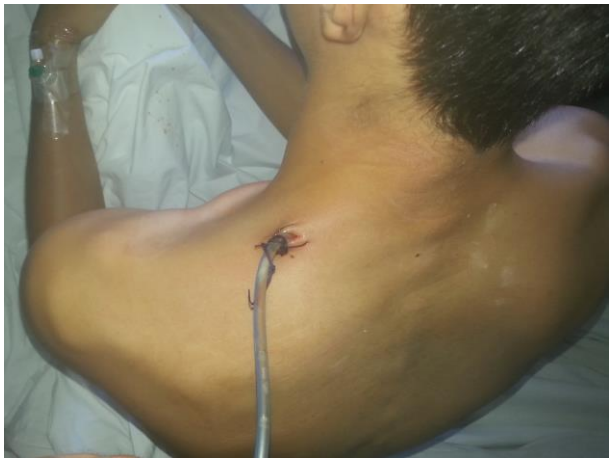

Figure 4. Thoracic drain placed apically on the left side of the chest

At the end of the operation, thoracic N028 Ch drain was inserted in the left pleural space, and the wound was closed in layers. Direct postoperative procedure flowed neatly. In a series of control Rtg, P-A resection of the lungs was performed, there was no air loss (Figure 5).

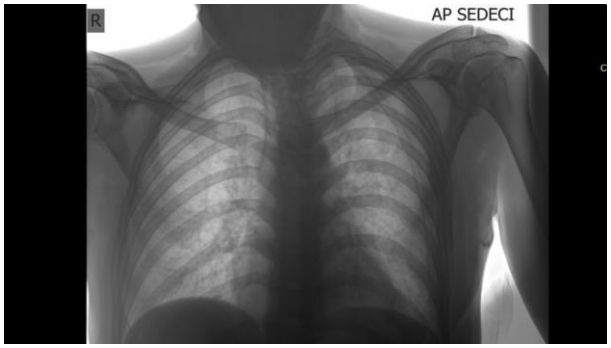

Figure 5. Chest Rtg P-A immediately after surgery

Blood loss was negligible (< 100 mL). The wound after minitoracotomy was healing per primam. The thoracic drain was removed and on the seventh postoperative day he was released for home treatment. During the check-up, the patient stated that he subjectively felt well. Rtg P-A corresponded to the operation performed, the lung resection was complete. Recurrence of spontaneous pneumothorax has not occurred even after two years of surgery. The patient returned completely to his everyday activities.

Discussion

The most common place of chest drainage is the 5th ICS of the middle axillary line within the "triangle of safety" (14). However, if surgical treatment is necessary after the drainage of the pleural area, the cutaneous thoracotomy incision should be at the level of the laterally placed thoracic drain. As the place through which the thoracic drain is placed is considered to be "dirty", it makes the planning and operational approach much more difficult. Also, among the potential complications of the lateral drainage of the chest, most frequent is placement of thoracic drain in a oblique fissure, and consequently difficult and incomplete lung resection (18). Chest drainage through the 1st ICS from above at the level of scapular line, by the apical approach demands an experienced surgeon, but is therefore: relatively simple to perform, less painful, does not bother the patient during lying, early patient mobilization is facilitated, the top of the lung re-expansion is easier, the cosmetic effect is better and, most importantly, on the side of the chest remains a "clean" space if surgical treatment is required (Figure 6) (14).

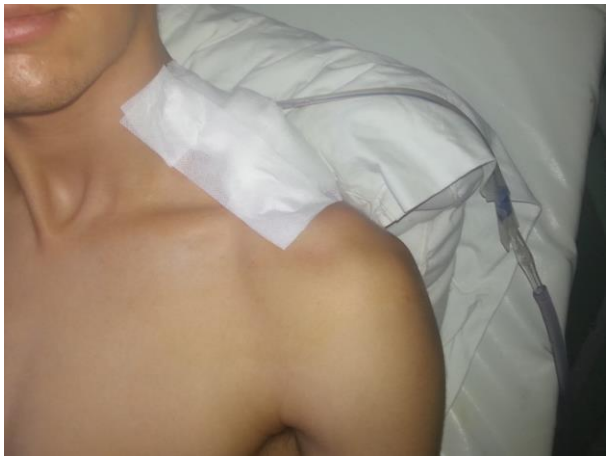

Figure 6. Thoracic drain placed apically on the left side of the chest

Contraindications to drainage through the 1st ICS are relative: the presence of pleural adhesions, patient is refusal, patient is collapse during the preparation and execution of the procedure, coagulopathy or therapy with oral anticoagulants and infection at the site of the placement of drain (19). Potential complications during drainage by apical approach can be (similar to lateral drainage) infectious caused by technical causes (of which the most undesirable are: a. subclaviae injury, Horner syndrome, n. phrenicus and n. ulnaris injuries (18). It has been proven that catheters and drains of smaller diameter are more comfortable for the patient, more precise, with a lower rate of infectious complications and with significantly better clinical effect as compared to large-diameter drains (20, 21).

In the described case, the indication for surgical treatment was clear, but it was preoperatively necessary to relieve the patient from the discomforts caused by the relapse of the PSP. First of all, it was decided to place the thoracic drain of small diameter by apical approach. In this way, all the abovementioned advantages of apical access to the pleural space were utilized, and the patient was free from the discomfort. After the stabilization of the patient, the planned operation was performed. A video-assisted mini-toracotomy has proven to be very useful as it

is less traumatic and provides good visibility and access to the pleural space. The atypical resection of the top by ELC tissue stapler, with partial pleurectomy and abrasion of the remaining parietal pleura was performed in accordance with the recommendations and principles of modern clinical practice (22). From the presented case, it can be seen that the combined approach to treatment of PSP recurrence with thoracic drainage through the 1st ICS from above, and a minimal-invasive surgical approach, is worthwhile. The patient recovered very quickly and completely and returned to his previous life activities. The recurrence of PSP was not registered even after two years from the operative treatment.

Conclusion

Primary spontaneous pneumothorax occurs due to a rupture of the similar to emphysema changes in the lungs and/or diffuse pleural porosity. The drainage of the pleural space is one of the most useful and commonly used procedures in the treatment of pneumothorax. Chest drainage by apical approach is simple to perform, is less painful, does not bother the patient during lying, early mobilization of the patient is facilitated, the top of the lung re-expansion is easier, the cosmetic effect is better and, most importantly, on the side of the chest remains a "clean" space, if surgical treatment is required. Recurrence of primary spontaneous pneumothorax is an indication for surgical treatment. In the above presented case study, it has been shown that the combined approach to the treatment of PSP recurrence with thoracic drainage through the 1st intercostal space from above, and a minimally invasive surgical approach, is purposeful and desirable.

Disclosure:
The authors declare no conflict of interest.

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DRENAŽA PLEURALNOG PROSTORA APIKALNIM PRISTUPOM KAO KORAK PRE DEFINITIVNOG HIRURŠKOG REŠAVANJA RECIDIVA SPONTANOG PNEUMOTORAKSA: PRIKAZ SLUČAJA

Milorad Pavlović[1], Bojan Ilić[1], Desa Nastasijević-Borovac[2], Senada Pavlović[3], Dušica Ilić[4], Miloš Stanković[1], Miloš Milojković[1]

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Pneumotoraks predstavlja prisustvo vazduha i/ili gasova u pleuralnom prostoru. Spontani pneumotoraks se deli na: primarni (PSP), na terenu zdravih pluća i sekundarni (SSP), na terenu neke od postojećih bolesti pluća. Smatra se da PSP nastaje zbog rupture u plućima, nalik promena sličnih emfizemu (ELC) i/ili difuzne pleuralne poroznosti. Drenaža pleuralnog prostora je jedna od najkorisnijih i najčešće primenjivanih procedura u lečenju PSP. Pojava recidiva PSP predstavlja indikaciju za hirurško lečenje. U prikazu slučaja koji sledi pokazano je da je kombinovani pristup lečenju recidiva PSP torakalnom drenažom kroz prvi međurebarni prostor odozgo i minimalno-invanzivnim hirurškim pristupom svrsishodan i poželjan.

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Ključne reči: *primarni spontani pneumotoraks, recidiv pneumotoraksa, torakalna drenaža, pleurektomija, abrazija pleure*

Review article

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PLASMINOGEN ACTIVATOR INHIBITOR 1 (PAI-1) AS A POTENTIAL DIAGNOSTIC AND THERAPEUTIC TARGET

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The plasminogen activator inhibitor type 1 (PAI-1) is the main inhibitor of tissue plasminogen activator and urokinase type plasminogen activator in the blood. Besides the key regulatory role in fibrinolysis, plasmin and its activators and inhibitors are responsible for the processes of extracellular matrix turnover and remodeling, cellular adhesion and migration, thus they participate in many pathophysiological processes such as thrombosis, fibrosis, atherosclerosis, cancer spread, and other. The measurement of PAI-1 expression and its levels is suggested for a risk factor assessment in certain diseases. Also, PAI-1 is being considered a potential therapeutic target that could modify disease development and progression. The aim of this work is to outline significant findings regarding PAI-1 application in diagnostics, risk factor assessment, and pathogenetic treatment of different diseases.

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Key words: *plasminogen system, extracellular matrix, fibrogenesis, cancer metastasis*

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Introduction

The plasminogen activator inhibitor type 1 (PAI-1) is a serine protease inhibitor, specifically – tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA). Plasminogen activators convert plasminogen into plasmin, a key regulator of fibrinolysis, which is responsible for fibrin degradation and, in conjunction with matrix metalloproteinases, for tissue stroma turnover and remodeling (1-3). Besides important role in fibrinolysis, PAI-1 is involved in the processes of extracellular matrix (ECM) remodeling that include cellular adhesion and migration, fibrogenesis, angiogenesis, etc. (4-6).

Many cells can produce and secrete PAI-1, such as endothelial cells (ECs), macrophages, adipocytes, smooth muscle cells (SMC), hepatocytes, and fibroblasts. It is partly stored in platelets and when in bloodstream it may be active or complexed with t-PA or vitronectin (VN), that stabilizes it and prolongs its half-life (2, 6).

Increased or decreased PAI-1 levels and expression were determined in different diseases and are suggested to have modifying role in these diseases pathogenesis (atherosclerosis, fibrosis, cancer spreading, obstetric complications, etc.). It is well known that elevated PAI-1 levels are associated with venous and arterial thrombus formation, and subsequently thromboembolism. Nevertheless, PAI-1 was shown to participate in complex processes of vascular and stromal remodeling. It influences cellular responsiveness, particularly migration (6-8). Taken together, measuring of PAI-1 levels is supported as a risk factor assessment for certain disease states and potential therapeutic target, that could modify disease development and progression.

Considering previously mentioned PAI-1 functions, the aim of the study was to outline findings regarding PAI-1 application in diagnostics, risk factor assessment, and pathogenetic treatment of different diseases.

PAI-1 and vascular wall injury and thrombosis

Plasminogen activator inhibitor 1 has an important role in the modulation of injury reparation process through the control of plasmin mediated ECM remodeling, cell migration and apoptosis. Primary response to vascular endothelial injury requires ECs and SMCs migration and proliferation. Various local factors may influence PAI-1 function, particularly adhesion/deadhesion responses of SMCs in migra-

tion process (7-11). Additionally, vascular injury is associated with inflammation, thus increased endothelial permeability and *in situ* activation of coagulation system components, as well as macrophage specific u-PA overexpression (6).

Plasminogen activator inhibitor 1 levels were shown to correlate with neointima formation upon fibrin deposition and thrombus formation at the injury site (murine animal model). At the same time, PAI-1 promoted VN dependent SMC-fibrin interacttions necessary for cellular motility in the healing process. On the contrary, PAI-1 deficient mice had significantly attenuated neointima formation compared to controls (12). Interestingly, PAI-VN complex hindered thrombin mediated SMC proliferation, implying a complex dynamic interaction of plasminogen and coagulation system components (13).

Specific PAI-1 inhibitors are an emerging drug class which could affect ECs and SMCs migration. The specific PAI-1 inhibitor (PAI-039, tiplaxtinin) hindered SMC migration, intimal hyperplasia and inflammation in murine model of adverse vascular remodeling. PAI-039 showed no effect on PAI-1-deficient SMCs nor ECs and re-endothelization after endothelium denuding vascular injury. Proposed explanation is a significantly lower expression of low-density lipoprotein receptor-related protein 1 (LRP-1), a motogenic PAI-1 receptor, on ECs than SMCs. The results suggest that PAI-1 could be an important therapeutic target that modulates neointimal hyperplasia and vascular stenosis (8). However, therapies using PAI-1 antagonists should be used with caution, because of the PAI-1 mediated direct effect on vascular integrity and permeability. By controlling VE-cadherin cellular trafficking, PAI-1 maintains ECs junctions. *In vivo* PAI inhibition showed vascular leakage in the zebrafish hindbrain embryos as well as decreased transendothelial resistance and disrupted ECs junctions in human umbilical vein endothelium (14).

Increased PAI-1 serum levels were found to be associated with atherosclerosis, coronary artery disease, myocardial infarction (MI), and cerebrovascular events (CVE) (4,15-17). The importance of fibrinolytic potential in cerebrovascular disease was investigated in the Framingham Heart Study offspring cohort, among individuals without prior CVE. PAI-1 and t-PA levels had a strong unadjusted linear correlation with incident CVE and were found predictive of CVE after accounting for established risk factors (15). The prospective EPICOR study determined significantly increased risk of acute coronary syndrome and ischemic stroke in individuals with highest PAI-1 levels compared to the lowest, after adjustment for sex, age, insulin and other metabolic variables (18).

Various cytokines, growth factors and hormones can induce PAI-1 gene transcription, such as interleukin-1, tumor necrosis factor-α (TNF-α), transforming growth factor β (TGFβ), thrombin, angiotensin II, etc. (1, 19, 20). Important inherited predisposition for increased PAI-1 expression is 4G/5G polymorphism in promoter of the PAI-1 gene (SERPINE1). The prevalence of 4G allele was found higher in coronary artery disease, preeclampsia, and pulmonary thromboembolism patients. This is reported as a risk factor for MI in Caucasian and Asian populations. The polymorphism was associated with early-onset cardiovascular risk and male sex (21, 22). The 4G/4G PAI-1 genotype correlated with a higher risk of thrombosis, particularly in vessels of internal organs such as the portal veins (22). However, several studies found no association of 4G/4G polymorphism with thrombotic disorders (23, 24), pointing to a necessary additional prothrombotic risk factors for disease to be manifested.

A fine control is necessary to prevent excess fibrin deposition in placental vessels and intervillous spaces, for successful endometrial vascular remodeling and angiogenesis, during implantation. Pregnancy is hypercoagulable state, thus the thrombophilic predisposition may become evident. Pathologic tendency towards hemorrhage or thrombosis is a risk factor for miscarriages. Females with obstetric complications (severe preeclampsia, placental abruption, fetal growth restriction, and stillbirth) had increased incidence of 4G/4G polymorphism than females with normal pregnancies (25). On the other hand, Said et al. (26) found no association between 4G/5G polymorphism and increased risk of serious adverse pregnancy outcome in asymptomatic nulliparous women. PAI-1 effects on adverse pregnancy outcome are at least additive to other inherited and/or acquired thrombophilic factors (mutations in coagulation factor V (FV), prothrombin (FII), methylenetetrahydrofolate reductase, fibrin-stabilizing factor (FXIII), autoantibodies, etc.) (27).

Although major guidelines do not recognize 4G/5G PAI-1 polymorphism as a significant factor for anticoagulants use, many medical centers consider it for assessment of combined heterozygosity risk. In cases of a single venous thromboembolism and known combined heterozygosity (FV, FII) the guidelines of relevant medical associations recommend prophylactic or therapeutic doses of low molecular weight heparin (LMWH) or unfractionated heparin, both antepartum and postpartum (28). The recommendations are subjected to individual variations between patients. Certainly, the best strategy for prevention of venous thromboembolism and related pregnancy complications is by following the guidelines.

PAI-1 and tissue fibrosis

Elevated PAI-1 levels have been associated with tissue fibrosis, while PAI-1 deficiency showed protection from stress-induced tissue fibrosis of different organs. Increased PAI-1 expression is observed in the early phases of tissue injury and responds to the intensive cellular migration. In induced proliferative glomerulonephritis (animal model), PAI-1 was expressed by mesangial cells at the margins of glomerular lesions between 8-24h post injury (6, 29).

Fibrotic renal disease is characterized by increase in TGF-β, angiotensin II, and PAI-1 transcri-

ption and protein levels. These three factors are in complex relationship where angiotensin II upregulates PAI-1 gene via angiotensin receptor antagonist (AT) 1 receptor, promotes TGF-β and collagens I and III expression (30). TGF-β is a multifunctional protein with strong pro-fibrotic action. It stimulates PAI-1 and collagen expression, while PAI-1 was described to increase TGF-β expression, via ERK/MAPK signaling, or may suppress TGF-β levels, through the lack of plasmin dependent TGF-β conversion into active form (6,31-33).

Therapies that apply angiotensin II converting enzyme (ACE) or AT inhibitor are applied in fibrogenic conditions. AT1 and aldosterone receptor antagonism were shown to reduce PAI-1 levels (34). The reduction lasts longer with ACE inhibitors when used in short term compared to AT1 receptor antagonist (35). Interestingly, the use of high tissue penetrating ACE inhibitors after MI had greater reduction of PAI-1 levels than low tissue penetrating medications. However, the potential beneficial effects of this change need to be elucidated (36). Also, the use of ACE inhibitor reduces morning PAI-1 levels. Namely, PAI-1 plasma levels exhibit a circadian variation, with its highest concentration in the morning and lowest in the afternoon (1, 37) which accordingly influences diurnal variation of fibrinolytic activity (38). Homozygosity for the D allele in the ACE gene was shown to increase PAI-1 gene expression, and in synergy with 4G polymorphism further increases PAI-1 plasma levels. These genotypes were associated with more frequent recurrent miscarriages and thus the application of LMWH is suggested to prevent uteroplacental insufficiency (27).

Future fibrosis treatment might involve antibodies that target TGF-β (39). Interruption of integrin αvβ6 protects against tubulointerstitial fibrosis after unilateral ureteral obstruction in mice. This integrin binds and activates latent TGF-β1. Mice lacking αvβ6 had less kidney injury than wild type, seen through the lower collagen content, and PAI-1 and TGF-β1 mRNK levels. Tubulointerstitial fibrosis was restored after 2 weeks' treatment with aldosterone or angiotensin II in αvβ6 (-/-) mice suggesting TGF-β1 independent pathway of fibrosis induction. The fibrosis correlated with increased PAI-1 expression. Robust macrophage infiltration in αvβ6 (-/-) mice with hindered fibrosis, including the infiltration reduction in angiotensin-restored fibrosis, point to the partial role of these cells in fibrogenesis and dependence on additional factors, such as PAI-1 expression (40).

Free radicals are involved in renal fibrogenesis by activation of profibrogenic mediators, among other TGF-β. Oxidative injury increases PAI-1 expression while increased intracellular antioxidants showed its inhibition. These results point that oxidative stress lowering therapies would have beneficial effect in slowing down the process of fibrosis (6, 41).

Likewise, significant PAI-1 influence is determined in the pathogenesis of lung fibrosis. Several mechanisms are described to enhance fibrosis after alveolar epithelium injury involving plasminogen system. Those are PAI-1 to VN binding and decreased cell motility, prolonged PAI-1 and VN accumulation, inhibition of fibrin and matrix degradation. Therefore, pharmacological inhibitors of PAI-1 or siRNA therapy should be considered in its treatment (6).

Several reports described a direct correlation of PAI-1 levels and steatosis, obesity, and other metabolic disturbances (42, 43). Activation of PAI-1 by TGF-β leads to the progression of steatohepatitis. Significant reversal of progressive fibrosing steatoheaptitis was achieved in mice model by using fenofibrate (a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist). The reversal was mediated by the adenosine monophosphate-activated protein kinase (AMPK) induced small heterodimer partner (SHP) gene expression, in a PPARalpha-independent manner, and was followed by marked decrease in PAI-1 transcription. The pathway is specific for SHP and AMPK signaling. The results suggest a potential therapeutic option for AMPK activators in ameliorating hepatic syndromes (44).

The use of an antifibrotic drug (IFN-α 2a) led to reduced collagen and PAI-1 production in a rat model of liver fibrosis. Also, the administration of Pirfendione (used for treating idiopathic pulmonary fibrosis) was shown to significantly reduce liver fibrosis and demonstrated higher active cell regeneration. The gene expression of collagens, TGF, Smad-7, and PAI-1 were considerably decreased in this model (45).

PAI-1 and cancer

Malignant cells may exploit plasminogen system, aiming to modify microenvironment for its progression. This is partly explained through the enhanced plasmin *de novo* generation by malignant cells, which further induces pericellular proteolysis and ECM degradation, outside-in signaling, activation of TGF-β, etc. (33, 46). High PAI-1 concentrations in cancer microenvironment markedly accelerate migration of invasive cell lines (47, 48).

The American Society of Clinical Oncology has recommended u-PA and PAI-1 as cancer biomarkers for assessment of adjuvant chemotherapy application in node-negative breast cancer patients (49). High local PAI-1 levels were determined in the primary tumor tissue of solid cancers and correlated with disease recurrence. Individuals with the highest PAI-1 values had significantly increased risk of colorectal and breast cancer compared to those with the lowest values, and importantly, the associations remainned after adjustment for sex, age, insulin or other metabolic variables (18).

Because of their role in cancer migration, invasion and metastasis, plasminogen system components are considered an important target for anticancer treatment. Many different approaches for anticancer treatment have been analyzed so far, or are under investigation, such as development of u-PA inhibitors, soluble u-PA receptor, monoclonal antibodies, antisense oligodeoxynucleotides, and others. The approaches are mainly directed toward u-PA cancer activity. The first u-PA inhibitors are tested in oncology in combination with chemotherapy (46, 50, 51). Furthermore, combined evaluation of u-PA and

PAI-1 in breast cancer was shown to improve the clinical risk assessment. Patients with high u-PA/PAI-1 ratio had significantly increased early relapse risk, and a benefit from adjuvant therapy is recommended in these high-risk patients (48).

Interesting usage of a novel tumor targeting drug carrier was investigated by Li et al. (52) with the concept that takes advantage of specific u-PA receptor overexpression in malignant cells. They generated recombinant protein, human serum albumin fused with the amino-terminal urokinase fragment, that enables binding to u-PA receptor. Human albumin has been used as a drug carrier, and the tumor-killing potential of this fused protein complexed with cytotoxic agent was demonstrated in a mouse model. Besides the accumulation of the cytotoxic agent, the complex can be also useful for tumor specific imaging probe.

Even though PAI-1 is a natural inhibitor of u-PA, and thus should have cancer-inhibiting effect, it can enhance tumor growth by acting via several mechanisms such as inhibition of apoptosis, cell proliferation and promotion of angiogenesis (53, 54).

One of the investigated therapeutic approaches is application of aptamers, oligonucleotides or peptides that bind to a specific target molecule. RNA aptamers that target PAI-1 were demonstrated to inhibit extracellular and intracellular PAI-1. Intracellular PAI-1 levels are increased in cancer cells and are thought to contribute cancer progression. Aptamer transfected human breast cancer cells line showed decreased PAI-1 and u-PA protein levels, as well as cancer cell migration and invasion. However, these cells' line medium expressed slight pro-angiogenic effect, while when in human umbilical vein ECs, the aptamer decreased endothelial tube formation, and subsequently angiogenesis (54).

Micro RNA-143 was reported to significantly suppress lung metastasis of osteosarcoma in a mouse model. PAI-1 and matrix metalloproteinase-13 (MMP-13) genes are direct targets of miR-143. *In vitro* osteosarcoma cells that were transfected with this silencing RNA showed downregulation of PAI and suppressed cell invasion, but not proliferation. Also, miR-143 injection into the primary osteosarcoma lesion inhibited lung metastasis. Higher expressing miR-143 cells had poorer PAI-1 expression and belonged to the metastasis negative group. PAI-1 knockdown resulted in downregulation of MMP-13 expression, a MMP that participate in tumor osteolysis. Taken together, the results indicate PAI-1 and MMP-13 genes as potential therapeutic targets for the prevention of lung metastasis (55).

In conclusion, development of new diagnostic and therapeutic strategies is necessary particularly for those patients that do not or poorly respond to the current treatments. Targeted therapy is an evolving field that seems to be increasingly used in the future. Plasminogen system represents a significant target for therapeutic intervention of vascular pathology, fibrosis, and tumor growth and metastasis. Regarding the interaction complexity of plasminogen system components and additional factors of specific disease conditions, therapeutics design requires detailed and thorough understanding of pathogenetic mechanisms.

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PLAZMINOGEN AKTIVATOR INHIBITOR 1 (PAI-1) KAO MOGUĆI CILJ DIJAGNOSTIČKIH I TERAPIJSKIH POSTUPAKA

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Plazminogen aktivator inhibitor tip 1 (PAI-1) je glavni inhibitor tkivnog aktivatora plazminogena i urokinaznog tipa aktivatora plazminogena u krvi. Pored ključne regulatorne uloge u fibrinolizi, plazmin i njegovi aktivatori i inhibitori su odgovorni za procese prometa i remodelovanja ekstraćelijskog matriksa, ćelijsku atheziju i migraciju, tako da učestvuju u puno patofizioloških procesa, kao što su tromboza, fibroza, ateroskleroza, širenje kancera i drugih. Merenje ekspresije i nivoa PAI-1 se sugeriše u proceni faktora rizika pojedinih bolesti. Takođe, PAI-1 se smatra mogućim terapijskim ciljem koji bi modifikovao razvoj i progresiju bolesti. Cilj ovog rada bio je da istakne značajna otkrića vezana za upotrebu PAI-1 u dijagnostici, proceni faktora rizika i patogenetskom tretmanu različitih bolesti.

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Ključne reči: *sistem plasminogena, ekstraćelijski matriks, fibrogeneza, metastaza kancera*

Review article

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THE ROLE OF ULTRASOUNDS IN PLANNING AND DEVELOPING AIRWAY MANAGEMENT STRATEGIES

Ivana Zdravković

Ultrasounds represent one of great innovations in the field of medicine in the last century; thanks to technological development, instrumentation design and portability, they became widespread used in many fields of medicine, including anesthesia. Apart for consolidated role for central venous lines placement and for regional anesthesia, where they succeeded to reduce complication rate while increasing performance, they are now more and more frequently used also for airway management, for different purposes. They are powerful tools for second level airway assessment, with reference to evaluation of anatomy and difficult airway management prediction; they are also used for tube position confirmation (especially in the field of emergency), for cricothyroideal membrane identification, for evaluation of glottic diameter prior to extubation, for airway devices position evaluation (such as LMA), for tube size choice (especially in pediatric patients), for preprocedural evaluation during tracheostomy and for endobronchial diagnosis of lung pathology.

Principal applications of ultrasounds in airway management, including literature analysis and identification of evidence based indications are discussed.

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Key words: *ultrasounds, airway management, intubation, tracheostomy, cricothyrotomy*

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Introduction

Due to technological development, diagnostic and interventional ultrasounds (US) have a crucial role in many areas of medicine, and thanks to devices miniaturization and portability, they are more and more used also by non- Radiologists, such as Anesthesiologists.

In 1999 Hatfield and Bodenham predictively affirmed that ultrasounds could represent "a developing area which is likely to expand rapidly when clinicians appreciate the true potential of such technique" (1), but for sure it was out of their imagination that less than 15 years later, US technology would have been so represented in Anesthesiologists' hands. Central and peripheral lines, arterial lines, peripheral and central blocks, FAST protocols for emergency abdominal exploration, lungs echo exploration and fast echocardiography represents only some of the common and widespread applications of US in Emergency Department, Anesthesia and Intensive Care.

In the last years, thanks to the above mentioned technical development, the use of US for airway management has gained a certain success and diffusion, representing an attractive and pluripotential field of application for this easy, safe, low cost and low skill (at least for basic use) technology.

The first ultrasound instrument was introduced in the early 1950s, but it was only in the 1960s that similar units became available for limited, primarily experimental use. In the early 1980s, there had been significant improvement in the technology to the extent that real-time ultrasound was developed, and real-time scanning was one of the most significant factors conditioning applications and large use of US (2). The American College of Emergency Physicians (ACEP) offered its first course specifically dedicated to emergency applications of ultrasound in 1990, and in 2001, ACEP published the Emergency Ultrasound Guidelines, which pertain to the scope of practice and clinical indications for emergency ultrasonography (3). Nevertheless, despite such earliest reports dealing with US applications in clinical medicine include the description of soft-tissue imaging of the pretracheal structures and anterior tracheal wall (4), the first detailed reports of using US to assist in various applications in airway management date from only a few years ago, that is why this peculiar field of US application is still to be well studied and

established, and it probably represents the future for development of such a technique (5).

Technical aspects of US for airway management

Further readings are available to better understand principles of US (6), and also to consider recently published papers which might higlight potential biological US side effects (7). Specific probes are required for airway study, such as Linear 7 - 12 MHz and Convex 2 - 6 MHz probes (8); for this reason, typically, vascular-type probes with high frequencies (> 7.5 MHz) and high resolution are used (9). The main concern for use of US in the airway is represented by US reflectance attissue-air interface (and air is the most represented element inside normal airways), as because of an-echoicity of air. So, due to what is called the very high acoustic impedence of air, US cannot directly depict the inside of any air-filled organ: as a result, despite a very good view of larynx, trachea, epiglottis, cricoid cartilage, which are clearly echoic, a limited view can be obtained for whatever is in the background. As a result, while we can easily have a good view, due to their superficial position, of the frontal and lateral walls of nearly all upper airway segments, the cuff of an endotracheal tube is hardly detected, if not inflated with fluid (US reflective) (1). Airway "sonoanatomy" regards different structures: US can image the floor of the oral cavity and its lateral wall with vertical and diagonal scansions; the lateral walls of the nasal cavity are only rarely visible (only if if the maxillary sinuses are filled with liquid), while the larynx could be seen as a musculocartilaginous structure situated below the hyoid bone which remains very clearly visible by US as hyperechoic structure (cartilage) reciprocally connected by isoechoic membranaceous ligaments with visible air below. US could also be used to explore vocal cords, which could only be examined by preoperative endoscopy (10), and they could also be powerful instrument to assess and provide follow up of postoperative vocal cords dysfunctions (11). Tracheal rings, down from the cricoid cartilage, are easily visible by US in vertical or transversal section together with the differently echoic pretracheal tissue.

Applications of US for airway assessment

US approach might represent a low cost/low skill and noninvasive test for routine second level study of the airway: as an operative strategy we could suggest using US in all cases where some suspects arise after an inspective clinical study of the patient. A recent study compares clinical and ultrasonographic airway examination (12), concluding that upper airway US is capable of providing detailed anatomic information and has numerous potential clinical applications. An interesting paper by Sustic (9) underlines a number of attractive advantages for US compared with competing imaging techniques or endoscopy, starting from advantage that US are widely available, portable, repeatable, relatively inexpensive, pain-free, and safe.

Ezri and Coworkers (13) higkighted the role of obesity as independent predictor of difficult intubation with an elegant US-based study, providing a neck thickness/circumference cut-off value and confirming that US better than increased body mass index per se could predict difficult laryngoscopy. On this pathway, US have been used for diagnosis of airway masses, anatomical deformities (1, 8, 14) and last but not least for Obstructive Sleep Apnoea Syndrome (OSAS) diagnosis and consequential airway management implications (8, 15). The use of US still remains open questions an isolated tool for predicting difficult laryngoscopy, but on the other hand it could easily be second line instrument to perform more accurate predictions (16).

US could also be used to preoperatively assess diameter of endotracheal tube to be used, which could be of particular interest in children due to their different airway anatomy; different papers have been published with no homogeneous results, indicating not high predictive value but starting from point that same formulas used for tube diameter prediction result poorly effective (17).

Applications of US for airway management: ET tube position

According to worldwide airway management guidelines, (18),insertion of an endotracheal tube must be followed by mandatory position confirmation test to exclude inadvertent or unrecognized esophageal or endobronchial intubation; due to poor effectiveness of simple chest auscultation (though bilateral and extended), exhaled CO2 detection or direct fiberoptic view through the endotracheal tube universally remain the gold standard techniques for correct intubation confirmation. From this point of view, US, despite the afrementioned technical limitations for direct detection of tracheal tube cuff, might represent a simple and low cost alternative, especially in some settings such as out of hospital emergency, out of operatory room anesthesia or Intensive Care Unit, all places where where both CO2 and fiberoptic scopes could not always be primarily or immediately available (19). When using ultrasounds, due to limitations of the technique itself, inflation with fluid together with bubbles or leaving a malleable stylet in the tube might provide the direct view of tube cuff inside the trachea (immediately below tracheal rings image) (20). This technique is not universally used or accepted, so the most important application for US to detect tracheal tube position is indirect assessment of lungs expansion. In this way, US provide a reliable and specific method to assess correct endotracheal intubation (21) by observation of correct and simultaneous expansion of both pleura, lungs (lung sliding sign) and diaphragm, thus resulting in indirect quantitative and qualitative indicators of lung ventilation and high specific confirmation (22) of correct intubation (23,24). This approach might also help in excluding bronchial intubation (pleuropulmo-

nary and diaphragmatic movements remain unilateral) (25) or using US to assess correct selective intubation during one lung ventilation and adequate tube choice to perform selective or superselective procedures (26). Differently, if tube is positioned in esophagus or not correctly in trachea, lung expansion is not observed, and particularly the simultaneous pleural movement is missing: this results in the so called lung pulse sign, with lungs moving syncronically to hearbeat due to missed tidal-volume related cyclic expansion.

Alternative and indirect test for corrected intubation assessment is upper cervical esophagus visibility: normally US could not detect esophagus, because its virtual lumen is collapsed in absence of content: differenlty, when esophageal intubation occurs and the tracheal tube cuff is in esophagus, it becomes visible alongside the tracheal rings. To summarise, correct intubation detection could be confirmed with US either directly (but with some technical interventions with styletted tube or fluid-fille tube cuffs) or indirectly, which is the most common way to perform this test: correct intubation is confirmed with presence of some signs (lung sliding) or through their absence (no lung pulse, no esophagus visible) (27).

Finally, US might also result safe and effective in case of non operative room intubation or during patients transport or external interventions (25). New and recent studies are currently performed to assess in an evidence based manner the effectiveness and the potential role for US for correct endotracheal assessment (28), whereas actual informations seem very promising.

Applications of US for airway management: emergency cricothyrotomy

The opportunity to "see through walls" with US was highly appreciated by anesthesiologists with regiopnal anesthesia techniques and with central venous lines placement (29); following this "line", it was almost natural thinking of using US guided approach to identify cricothyroid membrane and to locate optimal puncture site for emergency cricothyrotomy.

Starting from the point that most important rea-son for failure of emergency tracheal access is difficultty to properly locate the cricothyroideal membrane, especially in obese patients and parturients (30,31), different papers have studied this opportunity on realistic models (32). Results seem promising, but probably US are not yet so promptly and commonly available to allow a full setup in useful time lags to allow a safe and effective emergency airway access, whereas the best option remains a correct and prudential preprocedural identification of potential cannot intubate – cannot oxygenate situations (33). From this point of view, preliminary US-supported airway evaluation including preliminary identification of landmarks, and specifically cricothyroid membrane, could be a great tool to practice and to increase procedural safety. As a consequence, in the setup of preoperative airway evaluation with US, cricothyroideal membrane identification should be a mandatory procedural step.

Applications of US for airway management: percutaneous tracheostomy

Differently from emergency tracheal access, preprocedural US represent a well known (34), effective, interesting and well recognised technique for elective (percutaneous) tracheotomy. US have been described to allow correct site identification for tracheostomy (35), preliminary recognition of neck masses (36) or blood vessels at risk for critical emhorrage if punctured and correct approach in relationship to a physiological (isthmus) or pathological thyroid gland (37). US might also be used to check bilateral and regular lung expansion during ventilation after the tracheostomic cannula has been inserted in position (38). On the other hand, it is important to remind that in case of percutaneous techniques, the use of US is not at all alternative for periprocedural use of fiberoptic bronchoscope, the use of which should not be abandoned, either before, during or after tracheotomy is performed.

Applications of US for airway management: pre/post-extubation evaluation

Interesting airway uses of US have been described in postoperative setting or in ICU, for evaluation of (long time) time intubated patients scheduled for weaning or extubation. US have been used to assess readiness for extubation and to try to prevent post-extubation complications. In the first case, US have been used starting from the principle that respiratory movements and excursions of the diaphragm, liver, and spleen directly correlate with respiratory muscular strength, so extubation outcome could someway be predirect in correlation with such muscles performance and endurance. Considering that US can easily explore respiratory muscles activity (39), they can be used to assess weaning performance during spontaneous/supported breathing trials and to follow-up post- extubation performance, as elegantly demonstrated in a recent paper (40).

US might also be used during extubation to assess laryngeal structures conditions, with particular reference to airway caliber and evenctual swelling/edema at vocal cords level, which might result in post extubation stridor (PES) and respiratory distress after extubation, requiring reintubation (which could also not be easy). A study by Lakhal and Coworkers (41) showed a good correlation between magnetic resonance and US airway caliber studies, and different other papers suggest US approach to increase safety for easy and non- invasive assessment of airways at extubation (42).

US for fast estimation, delivery of local anaesthetics to the airway, LMA cuff placement and other airway related procedures

Other interesting applications of US in the field of airway management might regard correct placement and cuff pressure monitoring with LMA (1, 43) or other extraglottic devices once in position, and to allow safe jugular vein cannulation with these devices in position (44). They can also be used to perform US-guided airway anaesthesia techniques for the approach to superior laryngeal nerve close to thyroid cartilage (45) and very interestingly to assess gastric content, which could be extremely used when facing full stomach patient or patients suspected for risk of aspiration, with important potential implications on anaesthesia technique to be performed (8, 46, 47).

Recently important support has come from ultrasound for endobronchial or peribronchial lung pathology, thanks to opportunity to combine bronchoscopy and echography to perform EBUS (endobronchial ultrasound) and to address trans- bronchial fine needle aspiration biopsy (TB-FNA) (48).

Future directions

Starting from the evidence of easeness of US approach, costs, instruments diffusion and lack of (known) side effects, we can easily hypothesize extended use of US in many fields of medicine, including larger applications in anesthesia. As in other technology applications a hidden risk might be represented by loss of "blind" techniques skills because of ability to perform only "US revealed" procedures; this means maintainance of traditional skills together with oriented and targeted learning of new techniques, including US approach. At the moment, probably, one of the greatest challenges faced by critical care physicians in widely adopting ultrasound is the requirement for wide- spread ultrasound education in order to reach proficiency and ensure safety; this can be (easily) obtained implementing an ultrasound curriculum in the course of training for residents, and, more challenging, education of those already in practice (5,49), who could not be prone to accept new techniques or, even worse, to be taught on something which, easy in the appearence, requires anyway a learning curve and a suffuicient practice.

In the end, we might say that US sound really good!

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ULOGA ULTRAZVUKA U PLANIRANJU I RAZVOJU STRATEGIJA ZA REŠAVANJE PROBLEMATIČNOG DISAJNOG PUTA

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Ultrazuk predstavlja jednu od najznačajnijih inovacija na polju medicine prošlog veka; zahvaljujući razvitku tehnologije, dizajnu uređaja i njihovoj portabilnosti, široko su upotrebljavani u mnogim granama medicine, uključujući i anesteziju. Osim utemeljene upotrebe kod plasiranja centralnih venskih katetera i kod izvođenja regionalne anestezije, gde je upotreba ultrazvuka značajno smanjila broj komplikacija i podigla nivo uspešnosti, ultazvuk se sve više primenjuje kod obezbeđivanja otežanog disajnog puta i to u različite svrhe. Ultrazvuk je moćno oruđe za sekundarni nivo procene disajnog puta, naročito preporučen za evaluaciju anatomskih nepravilnosti i predikciju otežanog disajnog puta. On se takođe upotrebljava za verifikaciju pozicije tubusa (naročito u urgentnim slučajevima), za identifikaciju krikotireoidne membrane, merenje dijametra glotisa pre ekstubacije, proveru pozicije uređaja za obezbeđivanje disajnog puta (kakve su laringealne maske), za izbor veličine tubusa (specijalno kod pedijatrijskih bolesnika), izvođenje procedura, kao što je traheostomija, kao i za endobronhijalnu dijagnozu bolesti pluća. U ovom radu su obrađeni osnovni principi primene ultrazvuka u menadžmentu problematičnog disajnog puta, kao i pregled literature, uključujući osnovne i proširene indikacije.

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Ključne reči: *ultrazvuk, obezbeđivanje disajnog puta, intubacija, traheostomija, krikotireoidektomija*

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa — International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obele-žavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legen-di moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

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Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

Izbegavati citiranje prethodnih saopštenja (personal communication) ukoliko ona ne obezbeđuju esencijalne rezultate koji još nigde nisu objavljeni. U ovom slučaju, neophodno je u zagradi navesti ime osobe i datum usmenog saopštenja rezultata. Za objavljivanje ovih podataka neophodno je pismeno odobrenje autora.

Kriterijumi za pisanje referenci korišćenih u radu

U ovom pregledu biće obrađena pravila za pi-sanje literaturnih referenci samo za najčešće korišćene tipove publikacija.

Članci u časopisima

1. Standardni članak u časopisu

Navesti prvih šest autora, ukoliko ih je više iza šestog dodati **et al.** ukoliko je referenca na engleskom jeziku ili **i sar.** ukoliko je referenca na srpskom jeziku.

Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996; 124(11):980-3.

Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood–leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.

2. Organizacija kao autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;164:282-4.

3. Članak bez poznatih autora

Cancer in South Africa (editorial). S Afr Med J 1994;84:15.

4. Volumen sa suplementom

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.

5. Broj sa suplementom

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

6. Volumen sa više delova

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995;32(Pt 3):303-6.

7. Broj sa više delova

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994;107(986 Pt 1):377-8.

8. Časopisi sa brojem bez volumena

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995;(320):110-4.

9. Časopisi bez volumena i broja

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993:325-33.

10. Reference u obliku apstrakta ili prethodnih saopštenja

Enzensberger W, Fischer PA. Metronome in Parkinson's disease (letter) Lancet 1996;347:1337.

Clement J, De Bock R. Hematological complica–tions of hantavirus nephropathy (HVN) (abstract). Kidney Int 1992; 42:1285.

Udžbenici i monografije

11. Monografija

Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

12. Autori kao urednici

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

13. Organizacija kao autor i izdavač

Institute of Medicine (US). Looking at the future of the Medicaid program. Washington: The Institute; 1992.

14. Poglavlje u knjizi

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

15. Conference proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

16. Conference paper

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors.

MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

17. Istraživački ili tehnički izveštaji

Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

Sponzorisani izveštaji (Issued by performing agency)

Field MJ, Tranquada RE, Feasley JC, editors. Health services research: work force and educational issues. Washington: National Academy Press; 1995. Contract No.: AHCPR282942008. Sponsored by the Agency for Health Care Policy and Research.

18. Magistarske i doktorske disertacije

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Druge vrste publikovanog materijala

Neobjavljeni materijal

19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.

Elektronski zapisi

20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm

21. Monografija u elektronskom formatu

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

22. Kompjuterski podaci

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

PROPOZICIJE ZA PISANJE RADOVA U ACTA MEDICA MEDIANAE

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Priloge u vidu teksta, tabela i ilustracija (grafikoni, crteži i dr.) ne unositi u tekst članka, već na kraju teksta, na posebnim stranicama obeleženim u gor-njem levom uglu sa "Tabela, Grafikon, Ilustracija" i arapskim brojem redosledom pojavljivanja u tekstu (npr. Tabela 1, Grafikon 1 i dr.) i svakoj se daje kratak naslov. Kratka objašnjenja i skraćenice daju se u fusnoti. Za fusnotu koristiti sledeće simbole: *, **, ***, #, ##, ###, …itd. Tabele, grafikone i ilustracije treba praviti korišćenjem nekog od programa iz Microsoft Office paketa. Izbegavati upotrebu boja kod izrade grafika.

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