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SENSITIVITY OF DIAGNOSTIC METHODS AND TNM CLASSIFICATION IN STAGING OF GASTRIC CARCINOMA

Aleksandar V. Zlatić[1], Nebojša Ignjatović[1,2], Miodrag N. Djordjević[1], Aleksandar Karanikolić[1,2], Ivan M. Pešić[1], Biljana Radovanović-Dinić[2]

Preoperative staging in patients with malign gastric disease is of crucial importance for its multimodal treatment. The research included a group of 65 patients with gastric carcinoma. Targeted preoperative diagnostic procedures were performed as a basis for determining the preoperative and postoperative (TNM) degree. The diagnostic techniques used for the clinical and statistical examinations included ultrasonography (US), multi-slice computerized tomography (MSCT) and endoscopic ultrasound (EUS). The sensitivity of MSCT used for preoperative T status, compared to the postoperative findings, amounted to 39%, while the EUS sensitivity used for an assessment of the depth of tumour infiltration for T status amounted to 83%. The ultrasound examination could not detect enlarged lymph nodes in 58 out of 65 subjects with gastric carcinoma who had intra-operatively detected enlarged nodes, while a realistic positive finding of spreading malign process was detected in lymph nodes N1 in three patients and N2 in four patients. Ultrasound examination proved to be the least sensitive, with the detection rate of the affected lymph nodes in gastric carcinoma patients of only 11%. The MSCT proved as a reliable diagnostic technique in 43% of the preoperative assessments of malign process spreading into lymph nodes. Preoperatively, M0 status was found in 67.21% of the patients, while it was intraoperatively confirmed in 62.29%. The M0 status was preoperatively underestimated in 8.1% (M0 into M1) of patients.

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Key words: *staging, gastric carcinoma, diagnostic methods*

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Introduction

The frequency of gastric carcinoma is in gradual decline in many countries, including Serbia. However, gastric carcinoma is still the most frequent cause of death in all digestive tract carcinoma cases, which points to the practical relevance of the research. Numerous difficulties have been linked with timely diagnosis of the disease, while the success of surgical treatment depends primarily on its early detection. A long clinical period is one of the basic gastric carcinoma characteristics (up to several months) (1). For this reason, a number of patients (according to some statistics, as many as 50%) seek surgical help too late, at an incurable stage of the disease. The prognosis of the course of the disease thus depends on early diagnosis but also on the type of the applied surgical intervention. A preoperative diagnosis is important for determining all elements indicating the extent of how radical a surgical intervention should be in order to guarantee a longer survival rate (2).

For that purpose, all patients should be examined by contrast radiography, by applying high-sensitivity barium and gastric gas distention fibre gastroscopy, ultrasonography (US), multi-slice computerized tomography (MSCT) and endoscopic ultrasonography (EUS)(3,4).

In addition to these most significant diagnostic procedures, there is a range of auxiliary diagnostic techniques which can contribute to early gastric carcinoma diagnosis. These include, primarily, NMR in a strictly indicated framework, assessment of gastric juice acidity level, determining the carcino-embryonal antigen concentration (tumour markers), cytological examination of gastric juice, routine laboratory analyses, etc (7,8).

Patients and methods

The research included a group of 65 patients with gastric carcinoma (35 male and 30 female, aged 37-83 years) (Table 1), treated at the General

Surgery Clinic of the City of Niš, in the period January 1, 2011 – November 1, 2012. The patients were subjected to targeted preoperative diagnostic procedures, used as a basis for defining a preoperative, intraoperative and final staging (pTNM). Targeted diagnostic procedures, clinical examinations and statistical data processing were performed in all patients.

Table 1. Age structure of the patients with gastric carcinoma by gender

Gender	Number	Average age	Age (Range)
Men	35	65 ± 9	37-83
Women	30	59 ± 13[a]	37-78
Total	65	62 ± 11	37-83

[a]p < 0,05

Ultrasonography (US) of abdomen and gastroduodenal region was used for the detection of metastases and involvement of regional lymph nodes. SIMENS ACUSON X 300 was used in this research.

TOSHIBA AQULION MS Computerized tomography (MSCT) was used for determining the extent to which the lymph nodes were affected and in order to verify metastases.

Endoscopic ultrasound (EUS) was applied for determining intramuscular expansion of carcinoma and identification of metastases in regional lymph nodes. The examinations were performed using a 12MHz probe.

The applied TNM classification was the one presented by the International Union against Cancer (UICC) in the seventh edition of American Joint Committee on Cancer (AJCC/UICC TNM classification, 7th ed. 2010, XV, 649 p), which is the most frequently used one, by most tertiary health institutions worldwide.

The statistical processing was performed by descriptive, parametrical and non-parametrical statistics. One-Way ANOVA program was used for comparison of parametrical variables, while the frequency was tested by Spearman's $\chi 2$ test. The correlations was tested by the Spearman's correlation coefficient $\rho > 0.01$ as a non-parametrical version of the Pearson's correlation coefficient. Error risk discrepancies of not more than 5% were considered as statistically significant for discarding the zero hypothesis. Method sensitivity level was determined as the ratio of truly positive findings with the sum of truly positive and falsely negative findings.

Results

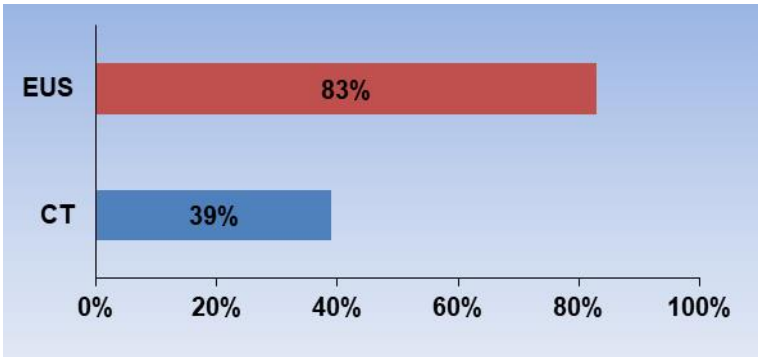
A comparative overview of preoperative and post-operative findings by TNM classification is provided in this section.

A comparison between the preoperative and postoperative (final findings) tumour staging of gastric carcinoma showed a significant, mid-level coincidence with the Spearman's rank correlation coefficient $\rho = 0.638$ ($p < 0.001$) (Table 2).

Table 2. Postoperative tumour staging compared to the preoperative finding

Preoperative tumour staging	Postoperative tumour staging				
	T1	**T2**	**T3**	**T4**	**Total**
T1	1	1	-	-	2
T2	-	1	4	1	6
T3	-	7	10	6	23
T4	-	-	-	34	34
Total	1	9	14	41	65

The postoperative staging coincided with the preoperative one in 83% of the patients examined by endoscopic ultrasonography (EUS). Preoperative T findings by multi-slice computerized tomography (MSCT) performed in 40 subjects, coincided with the pT status in 39% of the subjects (Graph 1).



Graph 1. EUS and CT sensitivity in preoperative diagnosis of tumour expansion into the gastric wall, compared to the postoperative PT finding

Table 3 shows a comparison of the preoperative staging with the postoperative findings in enlarged lymph nodes. Although the findings significantly correspond with each other, the value of Spearman's rank correlation coefficient of $\rho = 0.360$ ($p < 0.05$) indicates a low correlation of pre - and postoperative finding of tumor expansion to lymph nodes.

Table 3. Preoperative staging of lymph node involvement in gastric carcinoma patients, compared to the postoperative finding

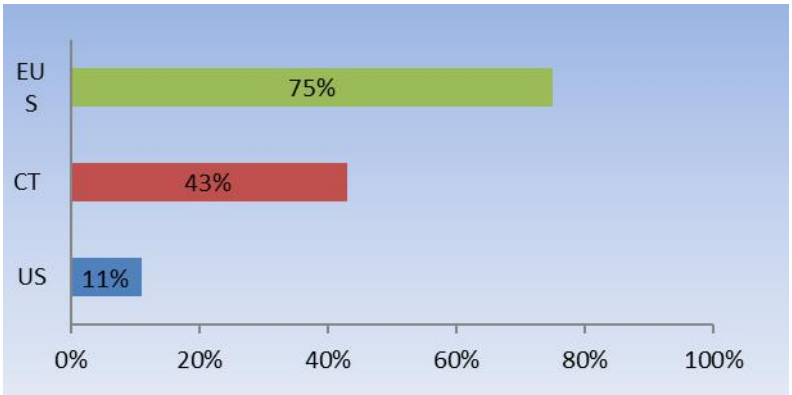
Preoperative staging of lymph nodes	Postoperative staging of lymph nodes			
	N0	N1	N2	Total
N0	2	14	7	23
N1	2	7	9	18
N2	-	-	24	24
Total	4	21	40	65

Ultrasonography could not detect enlarged lymph nodes in 58 out of 65 subjects, in which the enlarged lymph nodes were confirmed intraoperatively, whereas malign process expansion to lymph nodes was evident in 8 subjects (N1 in 4 and N2 in 4, respectively). Ultrasonography showed the lowest sensitivity in determining the stage of lymph node involvement in gastric carcinoma patients (11%). The MSCT proved as a reliable diagnostic technique in 43% of the preoperative assessments of malign process spreading into the lymph nodes. Out of the 65 gastric carcinoma patients examined by EUS, the finding was truly positive in 49, while in 16 patients the N0 finding was falsely negative. Thus, EUS proved to be sensitive in the detection of malign process expansion into the lymph nodes in 75% of the cases.

The CT sensitivity rate in preoperative lymph node staging was 43%. This method confirmed a truly positive finding of the presence of malignant process in lymph nodes in 27 out of 65 subjects, while it was truly negative in 5 patients (7.7%) and falsely negative in 33 patients (50.8%), compared to the postoperative finding.

Graph 2 shows the sensitivity rate of abdominal ultrasonography, EUS and CT in detecting the affected lymph nodes in gastric carcinoma patients.

Table 4 shows the preoperative findings of distant metastases in gastric carcinoma subjects, compared to the postoperative findings. The absence of distant metastases was postoperatively confirmed in 42 patients (67.21%), whereas distant metastases were found in 23 of the subjects (32.79%). However, in two out of the 65 subjects, distant metastases were determined only postoperatively, so it was intraoperatively confirmed in 62.29%. The applied diagnostic procedures (abdomen echosonography, CT, lung radiography) for preoperative distant metastasis staging in gastric carcinoma patients proved to be sensitive in 91.9% of the cases, compared to the final finding and diagnosis.



Graph 2. Sensitivity of abdominal ultrasound, endoscopic ultrasound and CT in preoperative detection of the degree of malign process expansion to lymph nodes in gastric carcinoma patients, compared to the postoperative finding

Table 4. Preoperative finding of distant metastases in gastric carcinoma patients, compared to the postoperative finding

Preoperative finding of distant metastases	Postoperative finding of distant metastases		
	M0	M1	Total
Not defined	1	-	1
M0	41	2	43
M1	-	21	21
Total	42	23	65

Discussion

Preoperative staging in patients with malignant gastric disease is of crucial importance for its multi-modal treatment. Today, it tends to go beyond a simple staging of the depth of tumour infiltration into the gastric wall (T), staging of the degree of regional lymph node involvement (N), and presence or absence of distant metastases (M). A contemporary approach to this problem includes an assessment of the prognostic factors, such as Ras protein, p53 tumour suppressor gene, growth factor receptors, tumour proliferation-related antigens, as well as protolithic factors. Recent techniques include both radiographic processing - routine ultrasonography (US), endoscopy and endoscopic ultrasound (EUS), multi-slice computerized tomography (MSCT) and surgical laparoscopy and laparoscopic ultrasonography (1).

T staging and method sensitivity

A comparison of preoperative staging with the postoperative finding of gastric carcinoma expansion has shown a significant but medium degree of matching with the Spearman's correlation coefficient $\rho = 0.638$ ($p < 0.001$) (Table 2).

In line with improved experience, multi-slice computerized tomography (MSCT) is increasingly used for detecting gastric carcinoma. Coburn et al. (2010) have reported that the use of CT scanning for gastric carcinoma detection has increased from 28% of the subjects in 1982 to 84% in 2007. Approximately 2/3 of the scanning in both groups have indicated carcinoma. In cases of gastric carcinoma, MSCT most frequently shows thickening of the gastric wall. Gastric wall thickness varies from 0.5 to 4 cm and correlates with tumor penetration (2). MSCT use for accurate gastric carcinoma T staging is still controversial. MSCT is obviously a less precise method than exploration and can be misleading in staging. Chen et al. (2007) have reported a comparative study of 75 patients in which T status staging was performed by MSCT, and consequently by surgery. The findings of this study indicate that as many as 31% of the subjects were graded lower than the actual status, while the MSCT overestimated it in 16% (3). In our research, MSCT sensitivity use for preoperative T staging was 39%, compared to the postoperative finding.

Our research has shown 83% of the EUS sensitivity regarding T staging of tumor infiltration depth (Graph 1). Shil et al. (2015) have concluded that EUS T staging was 78%. The accuracy rate for T1 amounted to 80% (in 20% of the cases it was overestimated), which is a high staging sensitivity, according to these authors. T staging proved to be reliable in 63% of the cases for T2 stage, while it amounted to 95% and 83% for T3 and T4 stage, respectively (4). Fairweather et al. (2015) have

found 81% of EUS in T1 staging, while it was 71%, 87% and 79% for T2, T3 and T4 staging, respectively (5). The obtained percentage of EUS sensitivity in our research is in accordance with such findings of other authors, regardless of the relatively low number of subjects in the series (Graph 1). T staging EUS sensitivity range from 71% to 95% (4) can be, perhaps, explained by a difficulty in discerning between T2 (subserous infiltration) from T3 stage. These two stages are clear indicators of a distinction between localized and progressed tumors. The EUS findings can sometimes be both falsely positive and falsely negative. Sometimes, it is difficult to make a distinction among tumorous tissue, inflammation-induced changes of the surrounding connecting tissue or the surrounding fibrous alterations (1). A comparison of MSCT, EUS and intraoperative finding with the final pTNM staging of the depth of gastric tumor invasion, performed by Pech et al. (2006), indicates that T staging was done correctly in 42% of the cases by MSCT, while the results were correct in 71% and 55% by EUS and intraoperative surgical finding, respectively (6). Similarly, Mehmedović et al. (2014) reported that EUS finding coincided with the surgical finding in as many as 92% compared to significantly lower matching rate of MSCT finding (only 42%) (7). With regard to T3, i.e., T4 stating, Hallinan and Venkatesh (2013), have emphasized the fact that 88% of the T3 and T4-staged tumors have metastases in lymph nodes, indicating that the concomitant T stage can be an important criterion for detecting the nature of endoscopically examined lymph nodes (8).

N staging and method sensitivity

A comparison of preoperative staging results by abdominal ultrasonography, computerized tomography and endoscopic ultrasonography with the final pN status has led us to the following findings:

The ultrasound examination could not detect enlarged lymph nodes in 58 out of 65 subjects with gastric carcinoma who had intraoperatively detected enlarged nodes, while a realistic positive finding of spreading malign process was detected in lymph nodes N1 in 3 patients and N2 in 4 patients (Table 3.). Ultrasound examination proved to be the least sensitive, with the detection rate of the affected lymph nodes in gastric carcinoma patients of only 11%.

In our research, the MSCT proved as a reliable diagnostic technique in 43% of the preoperative assessments of malign process spreading into lymph nodes (Graph 2). Van Vliet et al. (2007) compared the MSCT sensitivity for preoperative staging with the sensitivity of preoperative abdominal ultrasonography in detecting tumor invasion to the surrounding lymph nodes. In a series of 95 subjects the sensitivity of affected lymph node detection for preoperative MSCT was 26.6%, whereas for abdominal ultrasound it was 20% (9). A comparison between these results and our findings suggests that neither MSCT nor US can be considered as reliable methods for detecting potential tumor invasion to the surrounding, primarily, perigastric lymph nodes. We also suggest that MSCT is a slightly more superior method of verification of advanced tumor in N2 stage and detection of distant metastases. Our research has indicated particularly poor performance of abdominal ultrasound as a method of detection of nodal metastases.

Endoscopic ultrasound (EUS) sensitivity to detecting an invasion of the malign process into the surrounding lymph nodes is 75% in total. Shil et al. (2015) suggest that when it comes to N staging, EUS is significantly sensitive in 78-87% (4). However, this method can only detect the lymph nodes in immediate proximity to the gastric wall. N2 stage, i.e. lymph nodes farther than 3cm from the primary tumor can hardly be diagnosed by EUS. Griniatsos et al. (2011) found a correlation of T stage with the number and focalization of lymph nodes (10). Similarly to Javaid et al. (2004), this group of authors concludes that in T3 stage 88% of the lymph nodes are positive (14). Hallinan and Venkatesh (2013) preoperative endoscopic ultrasonography (EUS) on 254 consecutive patients with gastric carcinoma and compared the findings with the final pathohistological results. EUS yielded correct N sta-ging in 66%, while the accuracy in N0 stage was as high as 93%. However, in N1 and N2 staging (64% and 52%, respectively), EUS proved to be less reliable (8).

M staging and method sensitivity

The M status was determined by RŐ (rentgen) US and MSCT. Preoperatively, M0 status was found in 67.21% of the patients, while it was intraoperatively confirmed in 62.29%. The M0 status was preoperatively underestimated in 8.1% (M0 into M1). The M1 status was confirmed intraoperatively in the patients with secondary invasion of the disease (Table 4).

Considering the natural progression capacity of gastric cancer, there is a 27-37% probability that peritoneal implants can be detected during laparotomy (M1), although they have not been shown by the previously performed CT. Since the subjects with distant metastases are usually prone to intraoperative hemorrhage and obstruction by tumor mass is present in high percentage, which is most frequently lethal, we believe that this might be a strong argument to suggest that preoperative video laparoscopy is done in patients with advanced gastric carcinoma (11).

Due to embryological gastric rotation, gastric carcinoma gives metastases not only to the lymph nodes of the large and small omentum, but also into the truncus coeliacus nodes as well as retroperitoneal space along large blood vessels. A tumor can spread per continuitatem into the liver, pancreas, intestines, colon, and sometimes spleen. Rarely, in about 2% of the cases, gastric cancer can give metastases into bones. In women, metastases are frequent into ovaries (Krukenberg's tumor). According to Lauren, different histological tumor types give different metastases (13). The intestinal type, predominantly results in liver and lymph node metastases, while the diffuse type goes predominantly to peritoneum. Considering the pathways of invasion, abdominal MSCT seems to be necessary. An issue with MSCT is that it can detect peritoneal metastases

only in the presence of ascites (1). Our research has shown CT sensitivity of 57%, which is a considerable percentage. Metastases smaller than 10 mm in diameter pose a considerable problem. Since liver metastases detection depends on their size, they can be detected by ordinary MSCT in 50-60% of the cases. If MSCT is combined with arterial portography, the percentage of reliable metastases detection in liver increases and goes up to 81%, and if NMR is used, the sensitivity is up to 72% (12). However, intraoperative and laparoscopic ultrasound is still considered to be the most reliable method for the detection of liver metastases (1).

Conclusion

Preoperative T staging is confirmed by the intraperative one in about 57.5% and by the postoperative staging in about 52.5% of the cases. EUS sensitivity in the staging the depth of tumor infiltration is 83%, while the CT sensitivity is 39%, which recommends EUS as a more sensitive T staging method.

Preoperative assessment of carcinoma invasion to lymph nodes is confirmed by intraoperative and postoperative staging (final pathological verification) in 34% and 32%, respectively. Transcutaneous ultrasonography is sensitive in assessing gastric cancer invasion into lymph nodes in 11%. T has verified the findings in lymph nodes in 43%, while EUS has verified the findings in the affected lymph nodes in 75% when it comes to perigastric lymph nodes not distant more than 3cm. EUS has proved to be a very reliable method for N0 and N1 status verification, while CT has shown superior results in N2 staging verification.

Detection of distant metastatic deposits (M staging) by US and MSCT is accurate in high percentage (97%), which decreases the possibility for intraoperative "surprises". These diagnostic procedures have a significant contribution to better planning of surgical treatment degree of radicality.

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SENZITIVNOST DIJAGNOSTIČKIH METODA I TNM KLASIFIKACIJA U STAGING KARCINOMA ŽELUCA

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Preoperativni staging kod bolesnika sa malignim oboljenjem želuca je od presudnog značaja za njegov multimodalni tretman. Istraživanje je obuhvatilo grupu od 65 bolesnika sa karcinomom želuca. Ciljane preoperativne dijagnostičke procedure su obavljene kao osnova za određivanje preoperativnog i postoperativnog (TNM) stadijuma. Dijagnostičke tehnike koje su se koristile za klinička i statistička ispitivanja uključivale su ultra zvuk (UZ), multi-slajs kompjuterizovanu tomografiju (MSCT) i endoskopski ultrazvuk (EUZ). Senzitivnost MSCT-a u određivanju preoperativnog T statusa u poređenju sa postoperativnim nalazima iznosio je 39%, dok je osetljivost EUZ-a koji smo koristili za procenu dubine tumorske infiltracije za T stadijum iznosila 83%. Pregled ultrazvukom (UZ) ne može da detektuje uvećane limfne noduse kod 58 od 65 bolesnika sa karcinomom želuca i intraoperativno utvrđenim lifnim nodusima, dok je kod tri bolesnika dobijen realno pozitivan nalaz širenja malignog pocesa u limfne noduse N1 i kod četiri bolesnika u N2. Ultrazvuk je pokazao najnižu senzitivnost u detekciji zahvaćenih limfnih nodusa kod obolelih od karcinoma želuca koja je iznosila samo 11%. MSCT je kao pouzdana dijagnostička tehnika koja pokazuje tačnost u 43% kod preoperativnog određivanja stepena proširenosti malignog procesa na okolne limfne noduse. Preoperativno je M0 status utvrđen kod 67,21% bolesnika, a intraoperativno kod 62,29%. M0 status je preoperativno potcenjen kod 8,1% bolesnika (M0 u M1).

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Ključne reči: *staging, karcinom želuca, dijagnostičke metode*

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ANALYSIS OF DRUG PRESCRIBING IN PATIENTS ON HEMODIALYSIS TREATMENT

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Potentially inappropriate prescribing (PIP) of drugs is defined as the use of drugs whose potential damage can overcome benefits. Elderly patients (65 years and older) with renal insufficiency are at particular risk, because they take a lot of drugs, and for the usage of the same, the patients need to have great knowledge and skills.

To identify the risk factors contributing to potentially inappropriate prescribing of drugs in elderly patients with renal insufficiency.

The study was designed as an observational case-control study. The research was carried out at the Clinic of Nephrology, Clinical Center Nis, Serbia. The data were collected from the medical files of elderly patients undergoing chronic hemodialysis treatment, as well as by interviewing. The cases were patients in whom the potentially inappropriate prescribing of drugs was determined by Beers criteria, and the controls were patients who used properly prescribed medications. The risk factors for PIP were established by univariant and subsequent ly by multivariate logistic regression.

The study included 68 patients older than 65 years who were on chronic hemo-dialysis treatment, 41 (60.3%) of them were men and 27 (39.7%) women. The average age of the studied population was 72.71 ± 5.92 years, among which the youngest patient was 65 and the oldest 85 years old. According to Beers criteria, PIP was found in 14 (21%) patients. A significantly higher number of drugs was given to the patients in whom the potentially inadequate prescription of medication was established ($Z = 2.650$; $p = 0.008$).

The patients to whom the drugs were potentially inappropriately prescribed had a significantly higher number of comorbidities compared to other patients ($\chi 2 = 2.636$; $p = 0.008$).

This study showed that patients who have multiple comorbidities and take multiple drugs are at a substantially greater risk of having at least one drug potentially inadequately prescribed. PIP often results in the occurrence of toxic or side effects, and ultimately damage to the body.

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Key words: *potentially inappropriate prescribing of drugs, Beers criteria, polypharmacy, elderly patients*

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Introduction

Choosing the right drug in order to produce the desired therapeutic effect is an every day challenge for health workers (1). Although in practice there is a tendency for rational prescribing, errors and omissions are relatively common (2).

Potentiallly inappropriate prescribing (PIP) of drugs is defined as the use of medicines which potential damage can overcome the profit (3), or when the pharmacotherapy does not meet the medical standards. PIP results in more frequent adverse drug effects (ADE), hospitalization and a higher degree of health care usage, and higher cost of treatment (4).

Elderly patients (65 years and over) are at greater risk of potentially inappropriate prescribing of drugs because the physiology of their organism cha-

nges as they grow older (3), which results in frequent illness, and therefore the more frequent use of drugs and very often, the use of several medications at the same time, whose dose must be adjusted to the weakened functions of excretory organs (5). Elderly patients with renal insufficiency are at additional risk, because they receive more drugs than others and their doses must be precisely adjusted, and some drugs, even more, should be completely avoided (6,7).

PIP is a global problem, due to higher prevalence. Numerous studies have considered precisely the PIP among elderly people and found that the prevalence goes up to 79% depending on the criteria used and depending on the country where the study was conducted (5).

There is the question: how can we figure out which drug is inappropriate? There are two types of methods for identification of PIP- implicit and explicit. Implicit methods rely on clinical experience, they are more reliable because they can be used for each patient individually, while explicit methods are based on several criteria through which a potentially inappropriate medication prescribing in elderly patients is confirmed, therefore they are faster and easier, requiring less clinical assessment, but on the other hand less reliable. Due to easier usage, explicit methods widely applied (6), and commonly used are:

1. Beers criteria - a list of drugs which have much higher potential risks than benefits for people over 65 years (8)
2. STOPP criteria ("Screening Tool of Older Persons Potentially Inappropriate Prescriptions") - medicines to be avoided in elderly patients
3. START criteria ("Screening Tool to Alert doctors to the right Treatment") - medicines to be used in elderly patients on the basis of the evidence (9).

In essence, the PIP includes two aspects: 1. PIM (potentially inappropriate medication) when the drug is prescribed without adequate indications or when it is about the use of an indicated drug that in some situations can be more harmful than beneficial to the patient, and 2. PPO (potentially prescribing omissions) not prescribing the drug even though there is a clear indication (10).

Aim

The aim of this study was to reveal the factors that lead to PIP in elderly patients with renal insufficiency.

Methods

The study was designed as an observational case-control study.

The data were collected from medical files and by interviewing patients at the Clinic of Nephrology, Clinical Center Niš, in the period from June 2015 to June 2016. Inclusion criteria were patients aged 65 or older and end stage renal insufficiency treated with hemodialysis (HD). Exclusion criteria were cognitive impairment, psychiatric disorders, and the participation of patients in other clinical studies.

Data collected through the questionnaire included the following variables: socio-demographic characteristics of the patients (age, gender, height and weight, place of residence, whether they live alone or with someone, education level), previous treatment in the last year (the number of hospitalizations, duration of hospitalization, potentially adverse effects), laboratory, habits (smoking, coffee, alcohol), if they read the drug instructions, if they ask the doctor about adverse drug reactions and interactions, whether the doctors give them the information about adverse drug reactions and interactions; comorbidities, all the drugs that a patient receives (total number and individual drugs), how long the patient is on hemodialysis, how many times a week he goes to dialysis and how long one dialysis treatment lasts. Potentially inappropriate prescribing of at least one drug (PIP) was determined according to the Beers criteria.

The approval for this study was obtained from the Ethics Committee of the Medical Faculty, the University of Niš.

Entering, tabular and graphical presentation of the data was performed by using MS Office Excel program. The results of the statistical analysis were presented in tables. Statistical calculations were performed by using the SPSS version 22.

The data were primarily described by absolute numbers, relative numbers (%), mean and standard deviation (SD). The normality of distribution was analyzed by Kolmogorov-Smirnov test. The Mann Whitny U test was used for comparison of continuous variables, and hi-square test for comparison of categorical variables. Univariate and subsequent multivariate logistic regression were used to determine the risk factors for potentially inappropriate prescribing of drugs by Beers criteria. The results were considered statistically significant if the probability of the null hypothesis was less than 0.05.

Results

The study included 68 patients older than 65 years which were on chronic hemodialysis (HD) treatment, of which 41 (60.3%) were men and 27 (39.7%) women. The average age of the studied population was 72.71 ± 5.92 years, including the youngest patient who was 65 and the oldest 85 years old. The age structure of men and women was not statistically significantly different (72.63 ± 6.29 vs. 72.81 ± 5.41, $p = 0.903$).

According to Beers criteria PIP was found in 14 (21%) patients.

Table 1. Distribution of variables in relation to Beers criteria

Variables		*Potentially inappropriate prescribing of drugs according to Beers criteria* No	Yes	Z/χ^2	p
Total number of drugs		3,67 ± 2,16	6,14 ± 3,27	2,650	0,008
Length of therapy		1,61 ± 0,94	1,15 ± 0,36	1,594	0,111
Number of comorbidities		1,20 ± 0,73	1,78 ± 0,69	2,636	0,008
Other	**no**	43(79,6)	7(50,0)		
comorbidities	**yes**	11(20,4)	7(50,0)	3,608	0,058
HD internship	**≤ 5 years**	26(48,1)	12(85,7)		
	od 5 do 10	19(35,2)	2(14,3)		
	> 10	9(16,7)	0(0,0)	6,713	0,035
Reading of drug instructions	**no**	20(77,3)	4(16,7)		
	yes	34(83,3)	10(22,7)	0,077	0,782
Whether they ask the doctor	**no**	36(66,7)	5(35,7)		
about adverse drug reactions (ADR)	**yes**	18(33,3)	9(64,3)	3,250	0,071

Z- Mann Whitney U test

Table 1. shows the results of comparison of the investigated variables between groups of patients with at least one drug potentially inadequately prescribed and the group of patients with no PIPs. In patients receiving significantly greater number of drugs, potentially inadequate prescription of drugs, according to Beers criteria, was confirmed (Z =2.650; p = 0.008). Patients who were confirmed potentially inadequate prescription of drugs had a significantly higher number of comorbidities compared to patients who were prescribed medications appropriately ($\chi2$ = 2.636; p = 0.008). Other comorbidities were more frequent in patients with potentially inadequately prescribed drugs ($\chi2$=5.015; p=0.025).

HD internship significantly differed among the examined groups of patients ($\chi2$ = 6.713; p = 0.035). It was further observed that a significantly greater number of patients in whom PIP was registered were on dialysis for less than 5 years ($\chi2$ = 6.364; p = 0.011).

Reading of drug instructions was not significantly different among the examined groups ($\chi2$ = 0.077; p = 0.782), nor whether the patient asked the doctor about the side effects of drugs and their interactions ($\chi2$ = 3.250; p = 0.071). Further, it did not turn out statistically significant whether the patient was also suffering from some other disease ($\chi2$ = 3.608; p = 0.058), neither did the length of therapy ($\chi2$ = 1.594; p = 0.111).

Table 2. Univariate logistic regression in relation to the potentially inappropriate prescription of drugs according to the Beers criteria

	B	SE	OR	95%CI	p
Total number of drugs	0,372	0,132	1,450	1,119 - 1,880	0,005
Length of therapy	-0,802	0,472	0,448	0,178 - 1,131	0,089
Number of comorbidities	1,034	0,425	2,812	1,222 - 6,472	0,015
Other comorbidities	1,363	0,632	3,909	1,132 - 13,500	0,031
HD internship	-1,671	0,745	0,188	0,044 - 0,811	0,025
Reading of the drug instructions	-0,386	0,655	0,680	0,188 - 2,456	0,556
Asking the doctor about adverse drug reactions (ADR)	-1,281	0,628	0,278	0,081 - 0,951	0,041

Univariate logistic regression analysis confirmed, as a significant independent factors associated with PIP by Beers criteria, the total number of drugs, number of comorbidities, other comorbidities, HD internship and asking the doctor about ADR. With each new drug the risk for PIP is increased by 45% (OR = 1.450, p = 0.005). Each new comorbidity increases the risk of potentially inappropriate drug prescription almost three times (OR = 2.812, p = 0.015), while the other comorbidities increased the risk almost 4 times (OR = 3.909, p = 0.031). Patients with less HD internship have a higher risk of the occurrence of PIP (OR = 0.188, p = 0.025), as well as the patients who do not ask doctors about ADR (OR = 0.278, p = 0.041).

Table 3. Multivariate logistic regression of examined variables in relation to potentially inappropriate prescribing of drugs by Beers criteria

	B	SE	OR	95%CI	p
Total number of drugs	0,317	0,165	1,373	0,995 - 1,896	0,048
Number of comorbidities	0,077	0,596	1,080	0,336 - 3,475	0,897
Other comorbidities	0,514	0,827	1,673	0,331 - 8,463	0,534
HD intrnship	-1,389	0,852	0,249	0,047 - 1,325	0,103
Asking the doctor about ADR	-1,017	0,722	0,362	0,088 - 1,488	0,159

Multivariate logistic regression was conducted for assessing the connection between studied factors and the likelihood of the risk for potentially inappropriate prescribing of drugs determined by Beers criteria (Table 3). The multivariate included all the variables that were distinguished as individual statistically independent factors: the total number of drugs, number of comorbidities, other comorbidities, HD internship and questioning the doctor about ADR. The model with all predictors was statistically significant $\chi 2$ ((5, N= 68) = 18.680, p = 0.002), which indicates that the model makes distinction between the subjects who are at risk and those who are not. The model entirely ex-plains between 24.0% (R squared Cox and Snel) and 37.6% (R squared Naagelkerkea) of risk for having PIP and correctly classifies 86.8% of cases. Only total number of drugs that a patient received has significant influence on risk from PIPs (OR = 1.373, p = 0.048).

Discussion

To our best knowledge, this is the first study to analyze the factors that contribute to potentially inappropriate prescribing of drugs in elderly patients on hemodialysis. Out of the 68 patients who took part in our study, in 14 (21%) of them PIP was found, which is considerably less than in some other studies (11–13), although it is difficult to compare them because they included different population in the study, and used different criteria and guidelines for determination of the PIP.

Out of the 14 patients with potentially inappropriate prescribing of drugs, 10 patients (71%) have one PIP, two have 2 (14.3%) PIPs and two patients (14.3%) have 3 PIPs. From a total of 19 potentially inappropriately prescribed drugs, 52.3% were benzodiazepines, 31.6% methyldopa and one ticlopidine and zolpidem.

Our study showed that patients with a greater number of comorbidities, those who are on shorter program of hemodialysis and patients who do not ask their physicians about adverse drug reactions and interactions are more likely to have PIP.

The optimal outcome of medical treatment is certainly a priority for every doctor. Treatment of elderly patients (65 years and above) is particularly challenging because they are a special group of patients. Aging is associated with an increase in the number of chronic diseases, and therefore with a greater number of patients taking drugs (4, 14), which, according to our study represents one of the most important factors that contribute to potentially inappropriate prescribing. The results of our study indicate that with each newly prescribed drug the risk of PIP increases by 45% (OR = 1.450, p = 0.005). The results of many other studies are also in accordance with the results of our study (4, 10, 14–19).

In the study (16) the polypharmacy is present in 70% of patients. The main reason for this, as Wang and colleagues cite, is the increasing of morbidity with age. They also stated, as an important fact, that doctors follow the guidelines of clinical research which does not involve elderly patients.

Another study showed that a high prevalence of polypharmacy in the elderly people, leads to a higher rate of adverse drug reactions, a greater possibility that the patient has at least one PIP, and therefore a greater likelihood of treatment failure (20).

The results of our studies further show that

with the increase in the number of comorbidities increases the likelihood that the patient consumes potentially inappropriately prescribed medication. With every new disease, the risk of occurrence of PIP by Beers criteria, is almost three times higher than in patients with a small number of diseases(OR = 2.812, p = 0.015). This is also confirmed by the study (21), as well as numerous other studies that actually claim that, with the increasing number of comorbidities, the number of drugs that the patient takes also increases, and therefore, a chance that the patient gets potentially inappropriately prescribed drug is incomparably higher (4, 14–16).

According to our study, patients who had shortly been on dialysis (< 5 years) were more likely to have potentially inappropriately prescribed drug (OR = 0.188, p = 0.025). Since we did not find similar studies, this information did not have what to be compared with.

Also according to our survey, the patients who asked physicians about adverse drug reactions and interactions among the drugs had a smaller risk of being prescribed the potentially inappropriate drug (OR = 0.278, p = 0.041). Gordon and his associates, in their study (22), showed that patients are a part of the decision making process regarding their treatment, but they also stated that it is known that counseling by physicians is only one phase of the patients' participating in the conducting of their treatment according to their needs.

Finally, our study also had some limitations. Data on drugs that patients were taking were taken from their medical files, as well as directly from the patients during the survey. Considering that these are elderly patients, suffering from serious illnesses, and many of them with associated numerous comorbidities, it is possible that data on the number of drugs they took was not accurate because many of them could not remember the names of all the medicines they were taking, as well as those which they took in the last year.

Also, it should be borne in mind that the criteria that indicate potentially inappropriate prescribing of drugs, such as the Beers criteria that we used in our study, are general. They do not take into account each person, considering his nationality, weight, and body mass index (23). People, after all, differ among themselves, and each patient should be seen as a separate individual. What is, under valid criteria, potentially inadequately prescribed remedy, perhaps suits to some of the patients. If the guidelines require that the dosage of a certain drug should be reduced, perhaps this dosage is still insufficient to some patients.

Conclusion

We believe that the results of our study will raise the awareness about the high prevalence of PIP in elderly patients on chronic hemodialysis treatment and its harmful consequences. PIP needs to be paid special attention in patients with a greater number of comorbidities, those who are on shorter program of hemodialysis and patients who do not ask their physicians about adverse drug reactions and interactions. Early detection of PIP plays a key role in improving the quality of treatment, and reducing the costs of treatment.

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ANALIZA PROPISIVANJA LEKOVA KOD BOLESNIKA KOJI SE NALAZE NA HRONIČNOM TRETMANU HEMODIJALIZE

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Potencijalno neadekvatno propisivanje (Potentially inappropriate prescribing, PIP) lekova se definiše kao upotreba lekova čija potencijalna šteta može nadvladati dobit. Stariji bolesnici (65 godina i više) sa bubrežnom insuficijencijom su pod posebnim rizikom, jer uzimaju mnogo lekova za čije je doziranje potrebno mnogo znanja i veštine.

Cilj rada bio je da se utvrde faktori rizika koji doprinose potencijalno neadekvatnom propisivanju lekova kod starijih bolesnika sa bubrežnom insuficijencijom.

Studija je dizajnirana kao opservaciona studija slučaj/kontrola. Istraživanje je sprovedeno na Klinici za nefrologiju Kliničkog centra Niš. Prikupljanje podataka je vršeno iz istorija bolesti starijih bolesnika koji se nalaze na hroničnom tretmanu hemodijalize, kao i anketiranjem. Slučajevi su bili bolesnici kod kojih je utvrđeno potencijalno neadekvatno propisivanje lekova prema Beers kriterijumima, a kontrole bolesnika kod kojih su lekovi adekvatno propisani. Univarijantnom i naknadno multivarijantnom logističkom regresijom utvrđeni su faktori rizika za pojavu PIP-a kod ovih bolesnika.

U istraživanju je učestvovalo 68 bolesnika starijih od 65 godina koji su na hroničnom programu hemodijalize, od kojih je 41 (60,3%) muškarac i 27 (29,7%) žena. Prosečna starost ispitivane populacije iznosila je 72,71 ± 5,92 godina; najmlađi bolesnik imao je 65, a najstariji 85 godina. Prema Beers kriterijumu, PIP je utvrđen kod 14 (21%) bolesnika. Značajno veći broj lekova primali su bolesnici kod kojih je utvrđeno potencijalno neadekvatno propisivanje lekova ($Z = 2,650$; $p = 0,008$). Bolesnici kojima su lekovi pote-ncijalno neadekvatno propisivani imali su značajno veći broj komorbiditeta u odnosu na ostale bolesnike ($\chi 2 = 2,636$; $p = 0,008$).

Ova studija je pokazala da su bolesnici koji imaju više komorbiditeta i primaju više lekova pod značajno većim rizikom da im bar jedan lek bude potencijalno neadekvatno propisan. Potencijalno neadekvatno propisivanje često ima za posledicu pojavu toksičnih ili neželjenih dejstava, i u krajnjem, oštećenje organizma.

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Ključne reči: *potencijalno neadekvatno propisivanje lekova, Beers kriterijum, polifarmacija, stariji bolesnici*

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VISUAL FUNCTION AND QUALITY OF LIFE IN PSEUDOPHAKIC PATIENTS

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Loss of visual function due to cataract can be a major obstacle to doing everyday activities and can decrease quality of life.

The aim of the paper was to determine the impact of cataract surgery on the visual function and quality of life in pseudophakic patients. Two hundred twenty-two patients were examined, 242 cataract surgeries were performed, and surgery was done on both eyes in 40 patients. Postoperative questionnaires scores VF-14 (87.85 ± 17.10 med95.80), CATQ (13.72 ± 6.30 med12.00) and EQ-5D (5.78 ± 1.47 med5.00) were significantly better after surgery.

Pseudophakic patients have statistically better preoperative and postoperative VF-14 scores (76.23 ± 18.09 med79.75; 90.89 ± 15.66 med97.90) regarding the patients with second eye cataract. CATQ scores in pseudophakic patients are poorer preoperatively (21.19 ± 7.25 med21.00) and postoperatively (12.63 ± 6.17 med10.00) regarding the patients in the cataract group.

Pseudophakic patients have poorer EQ-5D preoperative and postoperative scores (7.54 ± 1.64 med7.00; 5.66 ± 1.35 med5.00) regarding the patients with second eye cataract.

Pseudophakic patients have significantly better visual function and quality of life before and after surgery, with regards to patients with second eye cataract.

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Key words: *senile cataract, visual function, quality of life*

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Introduction

Senile cataract is the leading and most easily eliminated cause of visual impairment in the world. According to the World Health Organization (WHO), up to 45 million people are blind (about 0.7% of the world's population) and 180 million have low vision. Cataract is the leading cause of blindness (47.8%) and approximately 20 million people are blind due to cataract. Low vision becomes a significant problem in developed countries because the population is getting older, and also because of the chronic diseases, and in developing countries it is mostly caused by infectious agents (1-3).

The existence of cataract is an obstacle in obtaining information from the environment and in doing everyday activities; also, it is obvious that the quality of vision is essential for maintaining the quality of life. Monocular visual acuity was the only indication for surgery and a parameter for assessing postoperative outcomes.

Patients with the same monocular visual acuity often have neither the same visual needs nor the same visual function. Therefore, using special visual function assessment questionnaires in patients with cataract is recommended. There is a strong and highly significant correlation between subjective ophthalmological symptoms and the level of quality of life (HR-QoL) (4, 5).

HR-QoL instruments include the processing of subjective data that measure patient's ability to perform daily activities, to take part in social activities, the level of emotional status and some other dimensions of everyday life (6). Ful of understanding the functionality of patients with cataract involves, beside the measurement of visual acuity, the use of special instruments for assessing and measuring quality of life, on which depends the need for sur-

gery. A patient with cataract has lower visual acuity, contrast sensitivity, and altered color vision. All this could have an impact on daily life and subjective perception of independence.

In the last twenty years, a lot of useful questionnaires with items that patients respond by gradation of subjective perception of difficulties in performing specific everyday activities were developed (7). The most widely used questionnaires for assessing quality of life and surgical outcomes are Visual Functioning Index (VFI i VF-14), CatQuest, Visual Activities Questionnaire (VAQ),Cataract Score Scale (CSS). Measuring visual function and quality of life become an integral part of patient care and it is an imperative to develop a valid, accurate and brief questionnaire.

Aims

The purpose of this study was to determine an impact of senile cataract surgery on the visual function and quality of life in pseudophakic patients.

Material and methods

This is a prospective study in which 202 patients were exeminated, 242 cataract surgeries were performed, and surgery was done on both eyes in 40 patients at the eye department at HC Kosovska Mitrovica and the Clinic for Eye Diseases in Nis.

The patients were divided into two groups; common to both groups is that they consist of patients who had cataract in one eye. However, these groups differed regarding the status of cataract in the other eye, as that information could affect the outcome – the first group (cataract) had cataract in the other eye, and the second group consisted of patients who had cataract surgery on the other eye and they were pseudophakic (pseudophakia).

Monitoring parameter is a visual function via the results of applied standardized instruments (VF-14 and Catquest -9SF). Eventually, each patient was asked about the subjective feeling of quality of life (questionnaire EQ-5D).

Patients were screened again, one month after surgery. The results are shown in tables, as a statistically significant result was interpreted as $p < 0.05$.

Results

Visual function and quality of life, before and after cataract surgery, are displayed using the results of measurement instruments (VF-14, CATQ and EQ-5D).

Visual function and quality of life, analyzed with these questionnaires in all patients, regardless of the group, are significantly better after cataract surgery. After cataract surgery, the level of difficulties related to visual function is significantly lower, and the quality of life is significantly better (Table 1).

Table 1. Preoperative and postoperative results of the VF14, CATQ and EQ5D questionnaires in all patients

	Arithmetic mean	SD	Median	Minimum	Maximum	Results
VF14 1	71.89	20.50	72.90	16.70	100.00	Z = -12.509 p < 0.001
VF14 2	87.85	17.10	95.80	25.00	100.00	
catq 1	.26	8.01		7.00	37.0023	23.00
catq 2	13.72	6.30	12.00	6.00	36.00	
EQ5D 1	7.78	1.67	8.00	5.00	12.00	Z = -12.109 p < 0.001
EQ5D 2	5.78	1.47	5.00	5.00	12.00	

Average postoperative results are statistically significantly better compared to preoperative results. The average postoperative outcome of the VF-14 (87.85 ± 17.10 med95.80) is significantly better the preoperative one (71.89 ± 20.50 med72.90). Also, the postoperative CATQ outcome (13.72 ± 6.30 med12.00) is statistically significantly better than preoperative (23.26 ± 8.01 med23.00). Postoperative EQ-5D outcome (5.78 ± 1.47 med5.00) is significantly better than the preoperative one (7.78 ± 1.67 med8.00).

Descriptive statistics and the results of the statistical analysis of VF-14 questionnaire before and after surgery, divided by groups, are shown in Table 2.

Table 2. Preoperative and postoperative results of the VF-14 questionnaire with regard to the group

		Group					Results
		Arithmetic mean	SD	Median	Minimum	Maximum	
VF14 1	cataract	68.73	21.61	66.70	16.70	100.00	Z = -2.577 p = 0.010
	pseudophakia	76.23	18.09	79.75	25.00	100.00	
VF14 2	cataract	85.64	17.81	92.70	25.00	100.00	Z = -3.263 p = 0.001
	pseudophakia	90.89	15.66	97.90	41.60	100.00	

Patients with pseudophakia in the other eye have significantly better results of VF-14 questionnaire, preoperatively and postoperatively, compared to patients with cataract in the other eye.

The average preoperative result of VF-14 questionnaire in pseudophakia group (76.23 ± 18.09, med 79.75) and postoperative (90.89 ± 15.66 med97.90) are significantly better than the average preoperative (68.73 ± 21.61 med66.70) and postoperative (85.64 ± 17.81 med92.70) results in the cataract group.

The same analysis was done for CATQ outcomes and the results are displayed in Table 3.

Table 3. Preoperative and postoperative results of the CATQ questionnaire with regards to the group

		Group					Results
		Arithmetic mean	SD	Median	Minimum	Maximum	
catq 1	cataract	24.76	8.22	26.00	7.00	37.00	Z = -3.503 p < 0.001
	pseudophakia	21.19	7.25	21.00	9.00	36.00	
catq 2	cataract	14.52	6.29	13.00	6.00	36.00	Z = -3.226 p = 0.001
	pseudophakia	12.63	6.17	10.00	6.00	36.00	

Patients with pseudophakia in the other eye have poorer CATQ scores, preoperatively and postoperatively, compared to the cataract group. This means they have less difficulties related to visual function.

The average preoperative CATQ scores (21.19 ± 7.25 med21.00) and postoperative ones (12.63 ± 6.17 med10.00,) in patients with pseudophakia are lower compared to preoperative (24.76 ± 8.22 med26.00) and postoperative CATQ scores (14.52 ± 6.29 med13.00) in patients with cataract.

EQ-5D scores in groups, before and after surgery, are descriptively presented in Table 4.

Table 4. Preoperative and postoperative results of the EQ-5D questionnaire with regards to the groups

		Group					Results
		Arithmetic mean	SD	Median	Minimum	Maximum	
EQ5D 1	cataract	7.95	1.68	8.00	5.00	12.00	Z = -3.503 p< 0.001
	pseudophakia	7.54	1.64	7.00	5.00	12.00	
EQ5D 2	cataract	5.87	1.54	5.00	5.00	12.00	Z = -3.226 p = 0.001
	pseudophakia	5.66	1.35	5.00	5.00	11.00	

Patients with pseudophakia in the other eye have poorer EQ-5D scores, preoperatively and postoperatively, compared to the group with cataract. This means they have less difficulties related to quality of life, compared to the cataract group.

The average preoperative EQ-5D scores in pseudophakia group (7.54 ± 1.64 med7.00) and postoperative (5.66 ± 1.35 med5.00) are lower compared to the preoperative EQ-5D scores (7.95 ± 1.68 med8.00) and postoperative ones (5.87 ± 1.54 med5.00) in the cataract group.

To determine the influence of the group on the quality of life, it was necessary to create new variables that represent the difference between the results of visual function and quality of life. Delta is the difference for each of the parameters. Further analysis is testing the significance of differences between groups after the change of parameters. Descriptive statistics is presented in Table 5.

Table 5. Difference between analyzed parameters

	Group	N	Mean	SD	Median	Minimum	Maximum	Results
ΔVF14	cataract	140	16.91	16.69	14.60	-22.90	70.80	Z = -0.399 p= 0.690
	pseudophakia	102	14.66	11.54	12.50	-10.40	54.10	
	Total	242	15.96	14.75	12.50	-22.90	70.80	
ΔCATQ	cataract	140	-10.24	6.94	-10.00	-27.00	7.00	Z = -1.947 p = 0.052
	pseudophakia	102	-8.56	5.73	-8.00	-23.00	1.00	
	Total	242	-9.53	6.50	-9.00	-27.00	7.00	
ΔEQ5D	cataract	140	-2.08	1.76	-2.00	-6.00	4.00	Z = -1.075 p = 0.283
	pseudophakia	102	-1.88	1.41	-2.00	-6.00	.00	
	Total	242	-2.00	1.62	-2.00	-6.00	4.00	

Based on the results it was found there is a greater change in before-after at all three parameters in the cataract group, but the difference is statistically significant only in the CATQ questionnaire.

Discussion

After cataract surgery, all patients, regardless of the group, had significantly improved visual function and quality of life. There was statistically significantly lower level of difficulties related to visual function and statistically significantly better quality of life. After surgery, all patients regardless of the group, have significantly less difficulties in doing everyday activities, better quality of life and improved visual function.

According to the literature, 90.9% of patients have improvement in visual function and quality of life after cataract surgery on one eye, and this percentage was higher after cataract surgery on the other eye (8).

Patients with pseudophakia in the other eye had significantly better VF-14 scores, preoperatively and postoperatively, compared to patients with cataract in the other eye. This suggests that patients with pseudophakia had significantly less difficulties in doing everyday activities, before and after surgery, compared to patients in the cataract group.

Patients with pseudophakia in the other eye had significantly less difficulties in doing daily activities (reading newspapers and books, performing fine manual work, cooking, watching TV) than patients with cataract in the other eye. The high level of visual function in pseudophakic eye was achieved in the other eye as well as after cataract surgery. Cataract in the other eye was the reason why patients in the cataract group had more difficulties related to the visual function, before and after surgery, compared to the patients with pseudophakia.

In the results published by other authors, patients had a significant improvement of visual function after cataract surgery, and the average preoperative VF-14 score was 82.6 and postoperative 94.8. Improvement of quality of life was significantly linked to the satisfaction with surgical outcome (9).

Patients with pseudophakia in the other eye have poorer CATQ score, preoperatively and postoperatively, compared to the cataract group. This suggests that they have less difficulties related to visual function and less dificulties in doing daily activities (reading newspapers, prices, movement on unfamiliar terrain) before and after surgery, compared to patients in the cataract group. The high level of visual function was achieved after cataract surgery in the other eye, and high level of visual function was already achieved in the operated, pseudophakic eye.

Cataract in the other eye was the reason why the patients in the cataract group had more difficulties related to the visual function, before and after surgery, compared to the patients with pseudophakia.

In the published study, 846 patients underwent cataract surgery on both eyes and the average preoperative CATQ score was -0.22 ± 1.96, and postoperative -3.69 ± 2.28. After cataract surgery on both eyes, 91.5% of patients had significantly less difficulties related to visual function, and 7.2% achieved poor Catquest score (10).

Also, the results of other studies suggest that patients who underwent cataract surgery on both

eyes, with or without comorbidities, have less difficulties related to visual function. Improvement of visual function after cataract surgery on one eye was similar as improvement of visual function in the other, tretaed eye, with or without comorbidities.

Postoperative CATQ score of the first treated eye, with or without comorbidities, are 1.92 and 3.57, respectively, and of the other one 1.44 and 2.94, respectively (11). Of the total number of patients with comorbidities in the tretaed eye, 25.6% of patients had significantly less difficulties related to visual function after surgery, 12.2% slightly lower, 37.8% lower, 15.6 % were without improvement.

Patients with pseudophakia in the other eye have poorer EQ-5D score, before and after surgery, compared to the cataract group. Regarding the mobility, self care, daily activities, pain and discomfort, anxiety and depression there were less difficulties compared to patients with cataract in the other eye, preoperatively and postoperatively. That means they have less difficulties related to the quality of life, preoperatively and postoperatively, compared to the cataract group. Pseudophakic patients had better quality of life, before and after surgery, compared to the patients with cataract in the other eye.

Pseudophakic patients preoperatively had less difficulties related to visual function and quality of life compared to patients with cataract in the other eye. After cataract surgery, pseudophakic patients had less benefits compared to patients in the cataract group. It is evident that the level of benefits is higher in cataract group in all three questionnaires but only significantly higher in the CATQ questionnaire.

Conclusion

Visual function and quality of life, analyzed with these questionnaires and in all patients, regardless of the group, are significantly better after cataract surgery. After cataract surgery, all patients, regardless of the group, have significantly less difficulties in performing daily activities and significantly better quality of life.

Patients with pseudophakia in the other eye have significantly better VF-14 score, preoperatively and postoperatively, compared to patients with cataract in the other eye.

Patients with pseudophakia in the other eye have poorer CATQ score, preoperatively and postoperatively, compared to the cataract group. This suggests they have less difficulties related to visual function, before and after surgery, compared to the cataract group.

Patients with pseudophakia in the other eye have poorer EQ-5D score, preoperatively and postoperatively, compared to the cataract group. This suggests they have less difficulties related to quality of life, before and after surgery, compared to the patients with cataract in the other eye.

There was a greater change in the value of the three observed parameters before-after in the cataract group but the difference was only statistically significant in the CATQ questionnaire.

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VIDNA FUNKCIONALNOST I KVALITET ŽIVOTA BOLESNIKA SA PSEUDOFAKIJOM

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Smanjenje ili gubitak vidne funkcije zbog katarakte je prepreka u obavljanju svakodnevnih aktivnosti i dovodi do pada kvaliteta života bolesnika. Cilj rada bio je utvrditi uticaj hirurgije katarakte na vidnu funkcionalnost i kvalitet života bolesnika sa pseudofakijom. Anketirano je 202 bolesnika kod kojih su urađene 242 hirurške intervencije, odnosno 40 bolesnika je operisalo oba oka. Postoperativni rezultati svih analiziranih upitnika kod svih bolesnika i to: VF-14 (87,85 ± 17,10 medijana 95,80), CATQ (13,72 ± 6,30 medijana 12,00) i EQ-5D (5,78 ± 1,47 medijana 5,00) statistički su značajno bolji od preoperativnih. Bolesnici sa pseudofakijom imaju statistički značajno bolje i preoperativne (76,2 3± 18,09 medijana 79,75) i postoperativne (90,89 ± 15,66 medijana 97,90) rezultate, VF-14 upitnika u odnosu na bolesnike koji su na drugom oku imali kataraktu. Vrednosti CATQ upitnika bolesnika sa pseudofakijom su niže i pre (21,19 ± 7,25 medijana 21,00) i posle (12,63 ± 6,17 medijana 10,00) operacije katarakte u odnosu na bolesnike u grupi sa kataraktom. Bolesnici sa pseudofakijom imaju niže i preoperativne (7,54 ± 1,64 medijana 7,00) i postoperativne (5,66 ± 1,35 medijana 5,00) vrednosti EQ-5D upitnika u odnosu na bolesnike koji su na drugom oku imali kataraktu. Bolesnici sa pseudofakijom imaju značajno bolju vidnu funkcionalnost i kvalitet života i pre i posle operacije u odnosu na bolesnike koji su na drugom oku imali kataraktu.

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Ključne reči: *senilna katarakta, vidna funkcionalnost, kvalitet života*

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THE INFLUENCE OF A TIME PERIOD ON BONDING STRENGTH OF PLACED BRACKETS

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One of the factors that can influence the bond strength of the placed brackets can be the length of the orthodontic therapy.

The aim of this in vitro experimental study was to examine the bond strength between bonded orthodontic metal brackets fixed with different adhesives and the surface of the teeth enamel at different time intervals.

Three different types of adhesives were used in this study: Heliosit (Ivoclar Vivadent, Lichenstein), Fuji Ortho LC (Japan), System 1+ (Dentaurum, Germany) and their impact on the bond strength of the bonded orthodontic brackets at different time intervals.

Average bond strengths in all three examined groups showed an increase 15 days after bonding the orthodontic brackets, and then a slight decrease in a bond strength of the brackets 30 days after their placement.

The results of the examined average bond strengths lead to a conclusion that the mutual characteristic of all three examined materials is that the bond strength is the weakest 24 hours after the bracket placement; after 15 days it reaches its maximum in strength, and 30 days later it decreases in all three groups.

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Key words: *adhesive materials, orthodontic brackets, bond strength*

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Introduction

Buonoccore introduced the procedure of enamel conditioning into restorative dentistry in 1955, which was the first attempt of establishing the micromechanical bond between adhesive materials and enamel structure. The process of enamel etching causes the formation of qualitative and quantitative microporous surface, which provides a micromechanical bond of enamel with an adhesive agent (1).

Adhesiveness, and thus the quality of the materials of enamel and their mutual interaction is determined by many factors such as physical and chemical properties of the substrate to be joined, adherents (enamel) and adhesive material, oral environment, physical stress, temperature changes, electrochemical reaction of saliva, eating, chewing habits (2).

Regardless of the value and diversity of adhesives used in orthodontic treatment with fixed appliances, in order to obtain an optimal bond, there is no material that fully meets chemical, physical and biological properties of dental structures. The main task of adhesives is to form a strong, permanent bond between the hard dental tissues and restorative materials. This bond can be achieved by mechanical retention, chemical adhesion or a combination of both (3).

The basic principle of adhesion means that the liquid adhesive must come into intimate contact with the substrate to facilitate the attraction of molecules and thus enable either chemical adhesion formation or micromechanical bond. In order to achieve adequate adhesive bond between the material and hard tissues of the tooth, binders must adhere to and remain in place in the presence of enamel fluid and vital tissue to withstand chemical, thermal and mechanical stresses in the oral cavity (1, 4).

In addition to the necessary compatibility, adhesive applied to the conditioned surface must have a lower surface tension and have low viscosity and thus allow the penetration of an adhesive into the prepared surface micropores (4 – 6).

Due to the specific structure of enamel, a stable bondage requires pretreatment or conditioning of the hardest human tissue. Surface pretreatment is usually performed by agents which alter the surface enamel structure and ensure favorable conditions for the physical and chemical forces during the application of the adhesive material (7).

Hydroxyapatite dissolution and selective dissolving of enamel prism endings occur during the pretreatment of enamel with acid. That way we obtain a porous surface with micropores resembles the channel system which subsequently flows into the lower viscous resin (bond) (8).

Etching of enamel surface of the tooth solutions with different acids represents the physical-chemical process that increases the active surface creating microcracks into which the bond and the adhesive material should penetrate into. Concentration and type of acid play an important role in ensuring good adhesion between enamel and adhesive materials. Attitudes about the duration of the procedure are still inconsistent, but it is considered that the optimal time is 30 seconds and it can be extended in fluoritic teeth and the elderly (4-7).

Acid applied to the enamel structure provokes dissolving of hydroxyapatite and a process of demineralization. Efficacy of etching depends on: orientation of surface prisms to the surface plane (demineralisation is faster if acid acts parallely on longitudinal prism axis and not if acid acts perpendicularly to a direction of a prism); acid and its concentration; etching time (length of acid effect) (4, 8).

To understand the purpose of adhesive materials, it is necessary to know that the adhesive must be resistant to the environment it is in, it must adhere firmly, it must be liquid enough, be tolerant to the smallest amount of dirt, and without discontinuity. When it comes to orthodontic materials, the first ones that appeared in orthodoncy were adhesives based on epoxy resin. Knowing that this kind of adhesive could not provide resistant bonds in oral environment (cavity), acrylic adhesives appeared despite their weaker chemical affinity. Modern generation of acrylic adhesives, which are based on cross-linking monomers, has advantages owing to its firmness and resistance to the conditions in the mouth cavity (9-12).

Each adhesive must achieve a balance between two opposing phenomena, that is to be sufficiently liquid to penetrate into the fissures and microcracks of the surface enamel of the teeth and secondly, to be viscous enough to allow good positioning of brackets (13, 14).

Aims

The aim of this experimental study *in vitro* was to test the strength of the bonded orthodontic metal brackets fixed with various sealants and surface enamel of teeth at different time intervals.

Methods

Tests were carried out on 90 extracted human premolars divided into three groups. Criteria for the selection of the teeth were: intact enamel surface which was not previously exposed to chemical agents (hydrogen peroxide), no cracks due to the pressure of pliers in extraction and without caries. Preparation of biomaterials was made by collecting, short teeth storing in 4% formalin solution, and rinsing them with sterile saline. The teeth were cleaned and polished with rubber bands for 10 seconds.

Three different adhesives were used for brackets fixation: light-curing adhesive (Heliosit, Ivoclar Vivadent, Lichtenstain), auto-curing adhesive (Dentaurum, System 1+, Germany), and glass-ionomer cement (Fuji Ortho LC, Japan).

The samples were divided into three groups. Each experimental group consisted of three subgroups of ten teeth, (n = 10), in order to determine the differences within the group (in the length of the placed brackets of 1, 15 and 30 days), as well as among the groups in terms of testing the material within the same time frame in the following manner:

- The first group (n = 30) consisted of samples where light-curing adhesive Heliosit (Ivoclar-Vivadent, Lichtenstein) was used. This group of experimental samples previously underwent enamel conditioning with 37% phosphoric acid for 30 seconds, then bonding of brackets and adhesive polymerization in a time interval of 40 seconds per tooth using Heliolux GT (Ivoclar Vivadent, Lichtenstein) polymerization lamp.
- The second group (n = 30) consisted of samples in which the used adhesive material was Dentaurum (System 1+, Germany).
- The third group (n = 30) consisted of samples in which glass-ionomer cement Fuji Ortho LC was used and it is important to mention that it can be used without prior conditioning of enamel and without curing light.

All specimens were stored in a water bath at 37°C for 1, 15 and 30 days.

All the teeth were sealed with metal orthodontic brackets for premolars Discovery (Dentaurum, 022 Roth, Germany), with an average area of the bracket base of 14.7 mm^2.

The process of bonding and application of orthodontic brackets to enamel surface included the process of enamel etching with 37% of phosphoric acid (in the first two groups) for 30 seconds, and then the tooth was thoroughly rinsed with water under pressure. After drying, the next steps of the procedure were bonding the etched surface of the tooth enamel and applying the adhesive to the surface of the bracket base and its positioning on the chemically created retentive place on the tooth surface.There was no conditioning of the enamel surface in the third group of teeth but the manufacturer recommended leaving a thin film of water on the surface on which the bonding was performed. The excess of the adhesive which was pushed out while it was being applied and pressed on the tooth was removed with a sharp scaler.

Storing of the tooth material was done within the standardised time in a pressurised chamber and humid environment in order to prevent dehydratation (100% humiditi at 37°C).

The process of debonding and removal of preapplicated orthodontic brackets included the adequate preparation of the tooth. The strength of the force required to separate the bracket from the tooth surface was measured by fixing the samples using the upper and lower pairs of terminals in electronic dynamometer - Zwick 1445 (Control laboratory ADO "Tiger", Pirot; Figure 1), while the tensile force was generated at a constant speed of 1mm/min.

Figure 1. Electronic dynamometer - Zwick 1445, Control laboratory ADO "Tigar" – Pirot

The direction of the force was gingival-occlusal (Figure 2). The device automatically recorded the force with an accuracy of 0.1 N. Individual force value was divided by the total area of the bracket (expressed in mm^2), which represented the size of the contact surface. In this way, all values were expre-ssed in N/mm^2, ie. megaPascals (MPa).

Figure 2. Dental sample placed in an electronic dynamometer

All studies were carried out at the Department of Orthodontics, Faculty of Medicine in Niš, Univeristy of Niš and Control Development Laboratory ADO "Tigar" in Pirot.

Results

The comparative analysis of the results of all three experimental groups showed differences in the strength of placed bonded brackets among the groups. The analysis was performed in order to study the differences within the group as a function of time (the length of the placement of brackets in the duration of 1, 15 and 30 days), as well as among the groups in terms of testing the materials within the same time frame.

The results and basic statistical peremetres of bond strengths of debonded brackets of all groups are shown in Table 1.

Table 1. Bond strenght of investigated adhesive materials after 1,15 and 30 days

Material	N	Time (days)	Bond strenght (MPa)	KV (%)
Heliosit	10	1	3.92 ± 0.37	9.45
		15	5.62 ± 0.55	9.77
		30	4.06 ± 0.27	6.69
Dentaurum	10	1	4.22 ± 0.28	6.68
		15	9.05 ±0 .53	5.83
		30	7.70 ± 0.70	9.14
Ortho Fuji LC	10	1	4.86 ± 0.62	12.69
		15	8.76 ± 1.03	11.73
		30	8.71 ± 0.83	9.49

The table shows mean values ± SD.

The average value of the bond strength of bonded brackets System 1+ adhesive material after 24 hours was 4.22 MPa, after 15 days it reached the value of 9.05 MPa, and on 30th day it was 7.7 MPa. The attached notes show that the weakest bond strength in this group was recorded after 24 hours from the placement of brackets, the value of the tested bond strength was the strongest after 15 days, and then there was a decline in the value of the test after 30 days.

The average value of the bond strength of the brackets bonded with Fuji Ortho LC adhesive material after 24 hours was 4.86 MPa, after 15 days the value was 8.76 MPa and after 30 days the value of the tested bond strength was 8.71 MPa. The weakest bond strength of bonded brackets was observed 24 hours after the bracket placement, the maximum average value was reached after 15 days, and after 30 days this value decreased slightly.

The results of the comparative analysis of bond strengths of all three adhesive materials after 24 hours from the bracket placement are shown in Table 2.

Table 2. Bond strenght of investigated adhesive materials after 24 hours

Material	N	Bond strenght (MPa)	Median	Min-Max	Analysis of variance
Heliosit	10	3.92 ± 0.37	4.0	3.2 - 4.4	F = 11.578 p < 0.001
Dentaurum	10	4.22 ± 0.28	4.2	3.8 - 4.7	
Ortho Fuji LC	10	4.86 ± 0.62	5.0	3.9 - 5.8	

The table shows mean values ± SD.

Variance analyses tested the average value of bond strength (in MPa) in all groups as a function of the way the brackets were placed onto the tooth surface and statistically significant difference was obtained ($F = 11.578$, $p < 0.001$). Post hoc analysis showed that the groups in which brackets were placed with Heliosit adhesive material (3.92 MPa) and System 1+ (4.22 MPa) did not differ significantly during the first 24 hours from their bonding, and that the strength of bond in the group using Fuji Ortho LC adhesive was significantly higher than in the previous two (4.86 MPa).

The results of the comparative analysis of bond strengths of all three adhesive materials after 15 days of brackets placing are shown in Table 3.

Table 3. Bond strenght of investigated adhesive materials after 15 days

Material	N	Bond strenght (MPa)	Median	Min-Max	Analysis of variance
Heliosit	10	5.62 ± 0.55	5.6	4.7 - 6.6	F = 66.342 p < 0.001
Dentaurum	10	9.05 ± 0.53	9.0	8.2 - 10.2	
Ortho Fuji LC	10	8.76 ± 1.03	8.5	7.5 - 10.9	

The table shows mean values ± SD.

Variance analyses tested the average value of bond strength (in MPa) in all groups as a function of the way the bracket was placed onto the tooth surface and a statistically significant difference ($F = 66{,}342$; $p < 0.001$) was obtained. Post hoc analysis showed that the groups where the brackets were bonded with System 1+ (9.05 MPa) and Fuji Ortho LC (8.76 MPa) adhesive material do not differ significantly, while the group of the bonded brackets using Heliosit adhesive (5, 62 MPa) was significantly different from the previous two in terms of reducing the value of the bond strength after 15 days of their placement.

Table 4. Bond strenght of investigated adhesive materials after 30 days

Material	N	Bond strenght (MPa)	Median	Min-Max	Analysis of variance
Heliosit	10	4.06 ± 0.27	4.0	3.7 - 4.5	F = 143.276 p < 0.001
Dentaurum	10	7.70 ± 0.70	7.7	6.6 - 8.8	
Ortho Fuji LC	10	8.71 ± 0.83	8.5	7.7 - 10.6	

The table shows mean values ± SD.

The results of comparative analysis of bond strengths of all three adhesive materials after 30 days of brackets placing are shown in Table 4.

Variance analyses tested the average value of bond strength (in MPa) in all groups as a function of ways of placing the orthodontic brackets and a statistically highly significant difference ($F = 143,276$, $p < 0.001$) was obtained. Post hoc analysis showed that all groups differ significantly in bond strength.

Discussion

Since the orthodontic therapy with fixed appliances can be a long and complex process, there is a great interest connected to numerous factors that can influence the bond strength of the placed brackets. One of the important factors is the duration of the orthodontic therapy and its influence on the behaviour of the basic elements of the fixed appliances under different conditions.

Although many authors (4, 6, 7, 9-12) have criticized *in vitro* studies, the majority of them agree that laboratory tests must meet the following requirements: to focus on clinically relevant properties (usually on the bond strength), to be reproductive and able to compare materials, to fully expose the tested materials to oral cavity, to be tolerated by clinical subjects for a long time, be appropriate to be used for a wider range of clinical subjects in the field of specific dentition and occlusion, and to be relatively inexpensive.

Despite these facts, most dental materials research continues *in vitro*, precisely because it is difficult to test materials, and then put them back in the oral environment. There are numerous *in vitro* methods and great efforts have been made to carry out such studies *in vivo* in order to get more realistic results (10-13).

It should be noted that there is currently no universally accepted minimal clinical strength of bonded orthodontic brackets. Results of any *in vitro* studies should be presented carefully, especially in predicting clinical performance. Previous studies (14-18) dealing with shear bond strength between the tooth surface and adhesive materials have shown that the bond strength of bonded brackets should be in the range of 3-7 MPa.

Results shown by Ajlouni et al. (15) showed significantly higher values in bond strength after 24 hours compared to the results in our study, while the values of the bond strength after 30 days showed a significant decline compared to the results in this study.

Ruse et al. (16) showed similar results. He tested the bond strength of the cyanoacrylate adhesive after 1, 7 and 30 days. He recorded an increase in bond strength within the first 24 hours (25%), then the bond strength dropped dramatically to day 30. Summing up the results of our study, it is evident that there is a correlation of values in the strength of bonds among the three treatment groups up to 15 days, followed by a decline in the value of the bond strengths.

Comparing the results of our study with the results presented by Chamda and Stein (17) who tested the bond strength of the adhesive material (Concise) based on the chemical nature, as is common in our study System 1+ (Dentaurum), which showed lower values of the bond strength of brackets bonded after 24 hours, an increase in the bond strength occurred in the later period. Results of the bond strength obtained in this study using a polymerization adhesive material (Heliosit) showed a significantly lower values in the first 24 hours in relation to the aforementioned.

Tests of Wendl and Droschl (18) have shown that the bond strength of brackets bonded directly to the tooth surface decreases in the first 24 hours, while the results that Cacciafesta et al. (19) and Movahhed et al. (20) obtained using a short-term (15 min) exposure of teeth in a humid environment showed greater values in the bond stregth.

The values of the results of our study show lower values in the group where this adhesive was tested. It is interesting to mention that the author compared the strength of the bonded brackets with Ortho Fuji LC and adhesive autopolymerized System 1+ (Dentaurum) *in vivo*. Presently, there is a total of 220 brackets in two groups of 110 teeth, and patients were observed over a period of 12 months. Significantly higher bracket bonding failure was in a group of glass ionomer cement (Fuji Ortho LC) 34.5%, compared to the second group, where the percentage of unsuccessful bracket bonding was 9% (+ System1). In contrast to Cacciafesta et al. (19), Fricker (21) tested the percentage of unsuccessful placing of brackets onto teeth surface in 10 patients (a total of 60 brackets), using the same adhesive materials (Fuji Ortho LC and System 1 +) and anterior teeth of the upper and lower jaw. Results have shown that under *in vivo* conditions, no significant difference was present in an unsuccessful bonding brackets with Ortho Fuji LC (5%), and System 1 + (8.3%). The study was coducted over a period of 12 months.

Placing brackets onto enamel surface of the tooth should be strong enough not to cause their unwanted and premature separation of the teeth, and demineralization should be minimal during their removal after the completion of orthodontic treatment. The most common procedure for bonding is the use of a suitable acid in a given time interval. Variations in concentration, etching time, acid used in etching of enamel are very important factors in achieving the adequate bond strength and minimal damage to the enamel (22-27).

Conclusion

Based on the results of this study, it can be concluded that the highest bond strength was recorded in the group that used the System 1+ adhesive material after 15 days of placing the orthodontic brackets. The minimum strength values were recorded in the group that used Heliosit Ivoclar Vivadent

24 hours after placing the orthodontic brackets. Also, the bond strength of the bonded brackets was the weakest in the first 24 hours after their placement in all three groups, which should be taken into account when deciding on the appropriate time load brackets.

The maximum mean value of the bond strength in three treatment groups was observed after 15 days of brackets bonding, a reduction in bond strength occured 30 days after their placement, whereby it should be noted that the strength of such bonds is in the range of adequate strength recommended by other authors.

Note

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UTICAJ VREMENSKOG PERIODA NA JAČINU VEZE POSTAVLJENIH METALNIH ORTODONTSKIH BRAVICA ZA ZUBE

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Kao jedan od faktora uticaja na jačinu veze postavljenih ortodontskih bravica za zube može biti dužina trajanja ortodontske terapije.

Cilj ove eksperimentalne studije *in vitro*, bio je da ispita jačinu veze između bondiranih ortodontskih metalnih bravica fiksiranih različitim adhezivnim sredstvima i površine gleđi zuba u različitim vremenskim intervalima.

U ovoj studiji korišćena su tri različita tipa adhezivnih materijala Heliosit (Ivoclar-Vivadent, Lichtenstain), Fuji Ortho LC (Japan), System 1+ (Dentaurum, Germany) i njihov uticaj na jačinu veze bondiranih ortodontskih bravica za zube u različitim vremenskim intervalima.

Prosečne jačine veza u sve tri ispitivane grupe pokazuju porast nakon 15 dana od bondiranja ortodontskih bravica za zube, a zatim i neznatno smanjenje u jačini veze nakon 30 dana od njihovog postavljanja.

Na osnovu rezultata ispitivanih prosečnih vrednosti jačina veza može se zapaziti zajednička karakteristika sva tri ispitivana adhezivna materijala, a to je da je nakon 24 sata od postavljanja bravica, jačina veza najslabija, nakon 15. dana, vrednosti jačine veza dostižu svoj maksimum, dok nakon 30. dana, opada u sve tri ispitivane grupe.

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Ključne reči: *adhezivni materijali, ortodontske bravice, jačina veze*

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THE CORRELATION BETWEEN BODY MASS INDEX AND THYROID STIMULATING HORMONE IN EUTHYROID PERSONS

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The aim of the study was to determine the correlation between body mass index (BMI), thyroid stimulating hormone (TSH), and thyroid hormones in euthyroid persons. The study included 396 euthyroid participants. The subjects with already established disease of the thyroid function were excluded. In all subjects we measured weight, height and determined BMI. According to BMI values, the subjests were divided into three groups: group A – BMI up to 24.9 (normal weight); group B - BMI from 25 to 29.9 (overweight); group C - BMI over 30 (obesity). In all subjests we determined serum TSH and free thyroxine (FT4) levels and antibodies to thyroid peroxidase (TPOAb). According to TSH levels, the subjects were divided into two groups: TSH up to 2.5, and TSH higher than 2.5. There is an increase in TSH levels with increasing of BMI. In group C, TSH values were significantly higher than in the groups A and B, and were also higher than the mean values of TSH in all subjects. TSH level in group B were slightly higher compared to the group A but there is no statistically significant difference. TPOAb values increase with increasing of BMI. The mean value of BMI was significantly lower in the group of patients with TSH values lower than 2.5, compared to the mean value of BMI in the group of patients with TSH values higher than 2.5. The relationship of BMI and TSH points to significant positive correlations of two parameters, except in group A. We found a significant degree of positive correlation between BMI and TSH, which remains within the normal range. There are also significant changes in the of TPOAb values. There is no significant changes in the FT4 levels.

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Key words: *euthyroid persons, BMI, TSH*

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Introduction

Nowadays, weight gain is a global epidemiological problem and is associated with numerous cardiovascular and metabolic diseases. Increasing body mass index (BMI) has probably resulted in the shortening of life expectancy. The etiology of obesity is multifactorial, and among other things, some endocrine diseases may be the cause of weight gain. Hypothalamic-pituitary-thyroid axis controls and influences the energy and oxygen consumption, metabolic rate, thermogenesis and weight. Hyperthyroidism is usually accompanied by the loss of body weight, while in hypothyroidism body weight increases. Subclinical hypothyroidism in recent years is gaining in importance due to its frequency and potential impact on the incidence of cardiovascular and metabolic diseases. Even small differences in thyroid function under normal hormone levels can have a significant impact on the regulation of body weight. All this points to the great importance of the rela-tionship between thyroid function and weight.

The relationship of body weight to TSH is increasingly gaining in importance, but the obtained data vary, from the fact that there is a positive correlation between BMI and TSH, to the one that there is no correlation at all. Increased body weight and obesity will rather lead to changed thyroid function than vice versa. The aim of the study was to determine the correlation between BMI, TSH and thyroid hormones in euthyroid persons.

Methods

The study included 396 euthyroid persons, 236 women and 160 men, aged 27 to 64 years. We excluded the subjects with already established disease of the thyroid function. In all subjects, weight, height and BMI were determined. Body weight and height were measured on the same digital weight machine. BMI is defined as body weight in kg divided by the square of body height in meters. According to BMI

values, the subject were divided into three groups: group A – BMI up to 24.9 (normal weight); group B - BMI from 25 to 29.9 (overweight); group C - BMI over 30 (obese). All the subjects had serum TSH and free thyroxine (FT4) levels determined as well as antibodies to thyroid peroxidase (TPOAb). According TSH levels, all subjects were divided into two groups: TSH levels up to 2.5 and TSH levels higher than 2.5.

All hormones levels were assessed at the Institute of Biochemistry, Clinical Center Niš on Beckman UNICELL DXI 600, using a chemiluminiscent method. The reference ranges for TSH are 0.40-4.60mU/l, for FT4 7-16pmol/l, and TPOAb < 14. The results were expressed as mean ± standard errors mean (S:E:M:). Significance of differences between the groups was determined using the Least Significant Difference (LSD) test. Statistically significant differences were assumed at $p \leq 0.05$. Pearson's coefficient was used to determine a correlation between TSH, FT4 and BMI in all groups.

Results

Mean values of the levels of TSH, FT4 and TPOAb per group are shown in Table 1.

An increase in TSH levels with increasing BMI was noticed. In group C, TSH values were significantly higher than those in groups A and B as well as mean TSH values in all the subjects. TSH values in group B were slightly higher compared to the group A, but there was no statistically significant difference. TPOAb values increase with increasing of BMI. The values in group C were significantly higher than the values in the groups A and B and mean values obtained for all subjects. The mean value of BMI was significantly lower in the group of patients with TSH levels lower than 2.5 compared to the mean value of BMI in the group of patients with TSH higher than 2.5. (Table 2).

The relationship between BMI and TSH shows positive correlations between the two parameters in all groups. Correlation was not significant only in group A (Table 3).

Table 1. TSH, FT4 and TPOab values in all groups

	Group A	Group B	Group C	All
TSH (mU/l)	1.89 ± 0.67	2.06 ± 1.25	2.47 ± 1.78	2.12 ± 2.06 *
FT4 (pmol/l)	11.58 ± 2.64	11.04 ± 2.06	10.32 ± 2.89	11.14 ± 2.72
TPOab	4.6 ± 2.5	5.7 ± 3.0	25.8 ± 6.5	10.3 ± 5.3 *

*p < 0.05

Table 2. BMI and FT4 values according to TSH

	TSH < 2.5mU/l	TSH > 2.5mU/l
BMI	26.3 ± 3.1	29.7 ± 4.6 *
FT4 (pmol/l)	11.44 ± 2.58	10.22 ± 2.71

*p < 0.01

Table 3. Correlation between BMI and TSH in all groups

Group	All	A	B	C	TSH < 2.5	TSH > 2.5
ρ	0.151	0.016	0.101	0.273	0.113	0.237
p	0.021	0.31	0.043	0.007	0.042	0.0009

Discussion

The link between thyroid hormone levels and body weight is well known, with weight gain in hypothyroidism and decrease in body weight in hyperthyroidism. Changes of TSH levels were observed with increasing of BMI in euthyroid persons. In our study, we determined the connection between TSH values and the values of BMI in euthyroid persons. It was observed that with increasing of BMI, TSH levels also increase, but remain within normal limits. In all the groups, there is an increase of medium TSH, which reached statistical significance. An increase of TSH levels is significant in groups with higher BMI values, indicating significant degree of correlation between TSH and BMI in overweigt group and in the group with obesity. Similar changes were observed in the TPOab values, while there was no significant difference in FT4 levels. Some authors have found decreasing values of FT4 and a negative correlation between BMI and FT4 (1). There is no change in the triiodothyronine, T3, levels and its value is not correlated with the values of BMI, although increasing of BMI shows an increase in T3/T4 relationship (2). Most authors presented similar results with an increase in TSH levels with increasing of BMI, with no significant change in the FT4 value. There are few works that show no difference in TSH with increasing of BMI (3).

Our results show no difference between men and women, while some authors obtained significant increase in TSH level in a group of obese women.

(4). It is not clear whether weight changes affect thyroid function and in what way. It is possible that the increase in adipose tissue and insulin resistance affect the thyroid function (5). According to some authors, visceral adipose tissue plays a far more significant role in changing the TSH levels than the subutaneous adipose tissue (6). This is indicated by a positive correlation between TSH values and waist and hip circumference and waist / hip ratio (7). On the other hand, there is a possibility that even small changes in the thyroid function within the normal range may play a role in the change of body weight. It is possible that there is an ability of the adipose tissue to change the expression of TSH receptors (8), so that there is a certain degree of hormone resistance, similar to the insulin resistance.

Some authors describe the connection between leptin and TSH so that leptin may be one of the mediators that cause an increase of TSH in obesity (9). However, most authors have not found a significant correlation between leptin and TSH in obese patients, although both hormones show a positive correlation with the increase in body weight (10). TSH production is also influenced by transmitters that influence body weight and satiety, and they primarily include neuropeptide Y and α-melanocyte-stimulating hormone. The presence of TSH receptors on adipocytes suggests that a positive correlation of TSH and BMI can have an important physiological role and also point to a possible existence of the hypothalamic-pituitary-adipocyte axis, with numerous metabolic consequences. As thyroid hormones and TSH have a significant role in the processes of lipolysis and thermogenesis, the changes in their values can be part of adaptation process in obesity. On the other hand, an increase in TSH level can stimulate the release of adipocytokines from adipose tissue and contribute to the proinflammatory state and an increase in insulin resistance in obesity (11). Synthesis of TSH and thyroid hormones can be affected by the magnification of caloric intake and fat mass.

Sari et al. have shown in women that reduction in body weight of 10% leads to decreased TSH levels (12). There is also an impact of smoking on the connection between TSH and BMI. Some authors find a significant positive correlation between BMI and TSH only for non-smokers, while in smokers it loses significance (11). The impact of smoking on thyroid function is well-known, also affecting changes in body weight. The lack of results may lies in the fact that lean body mass has not been determined but BMI only. Lean body mass is probably the major determinant for l-thyroxine level, and if increased, it may require an increased TSH stimulation of the thyroid gland to maintain the normal thyroid hormone levels (13).

Conclusion

In our study we found a significant degree of positive correlation between BMI and TSH, which remains within the normal range. There are also significant changes in the value of TPOAb levels. No significant changes in FT4 levels were found.

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KORELACIJA INDEKSA MASE TELA I STIMULIRAJUĆEG HORMONA ŠTITNE ŽLEZDE KOD EUTIREOIDNIH OSOBA

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Cilj rada bio je utvrditi korelaciju između indeksa telesne mase (body mass index, BMI), stimulirajućeg hormona štitne žlezde (TSH) i tireoidnih hormona kod eutireoidnih osoba. U ispitivanje je uključeno 396 eutiroidnih osoba. Nisu uključene osobe sa poznatim oboljenjem tiroidne funkcije. Kod svih ispitanika izmerena je telesna težina i visina i određen BMI. Prema vrednostima BMI podeljeni su u tri grupe; do 24,9 normalno uhranjeni, grupa A; od 25-29,9 sa prekomernom težinom, grupa B i preko 30 gojaznih, grupa C. Kod svih ispitanika su određivane vrednosti serumskog TSH, slobodnog tiroksina (FT4) i antitela na tiroidnu peroksidazu (TPOab). Prema vrednostima TSH ispitanici su podeljeni u dve grupe, TSH do 2,5 i preko 2,5. Zapaža se porast vrednosti TSH sa porastom BMI. Vrednosti TSH su statistički značajno veće u grupi C u odnosu na grupu A i B, kao i u odnosu na srednje vrednosti TSH svih ispitanika. Vrednosti TSH u grupi B su nešto veće o odnosu na grupu A, ali nema statistički značajne razlike. Vrednosti TPOab rastu sa porastom BMI. Srednja vrednost BMI je značajno niža u grupi ispitanika sa vrednostima TSH manjim od 2,5 u odnosu na srednju vrednost BMI u grupi ispitanika sa vrednostima TSH većim od 2,5. Odnos BMI i TSH pokazuje pozitivnost korelacije dva parametra, osim u grupi A. Utvrdili smo značajan stepen pozitivne korelacije BMI i TSH, koji ostaje u okviru granica normalnih vrednosti. Postoje i značajne promene vrednosti TPOab. Nema značajnih promena vrednosti FT4.

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Ključne reči: *eutireoidne osobe, BMI, TSH*

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STUDY OF ESSENTIAL METALS IN SELECTED MEDICINAL PLANT FROM SERBIA

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In this paper, we have studied uptake and accumulation of essential major and trace metals present in the six selected medicinal plants from Serbia, and their transfer from soil to plant. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was used to analyze the metals: Na, K, Mg, Ca, Mn, Fe, Cu, and Zn in plant and soil samples. Selected plants accumulate a sufficient amount of studied metals, except Cu. They are not accumulator of Na, Ca and Fe, neither are they tolerant to Na and Mn, except Dittrichia graveolens, tolerant species to all examined metals. The tested plant species can be important in human diet as a source of the essential elements of importance for the optimal functioning of the human body.

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Key words: *essential metals, medicinal plant, ICP-OES*

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Introduction

Medicinal plant preparations have a significant role in the pharmaceutical markets and health care sector of the 21st century (1). Herbs do not only provide organic constituents of medicinal value, but also metals required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health (2). On the other hand, essential metals can produce toxic effects when the metal intake is in high concentration (3). The World Health Organization (WHO) recommends that medicinal plants, as the raw materials for the finished medicinal products should be checked for the presence of heavy metals. The ability of plants to selectively accumulate metals varies in different species and is subjected to certain geochemical characteristics depending on the type of soil (4). For these reasons, the study of the metal contents accumulation in medicinal plants from Serbia is very important.

Aim

The objective of the present study was to deter-mine the content of essential major and trace metals (Na, K, Mg, Ca, Mn, Fe, Cu, and Zn) present in the six selected medicinal plants from Serbia and their transfer from soil to plants.

Materials and Methods

Chemicals

All chemicals and reagents were of analytical reagent grade and were purchased from the Sigma–Aldrich Chemical Company. Standard solutions used in the ICP-OES techniques were purchased from Alfa Aesar, Germany. The certified reference material BCR-701 was purchased from the Community Bureau of Reference (5).

Sample collection

Throughout this work, abbreviations were used in tables to indicate the growing area of the different plant populations. Six different medicinal plant species were used in the study: Thymus glabrescens Willd. (Tg.), Thymus pulegioides L. (Tp.), Satureja kitaibelii Wierzb. ex Heuff. (Sk.), Libanotis montana Crantz subsp. leiocarpa (Heuff.) Soó. (Apiaceae) (Lm.), Peucedanum longifolium Waldst. & Kit. (Pl.) and Dittrichia graveolens (L.) Greuter (Dg.). A voucher specimens were deposited at the Her-barium of the Department of Botany, Faculty of Biology, University of Belgrade-Herbarium Code BEOU. The sampling areas were located in the east Serbia: Kravlje village

(1.)-Tg., Suva planina mountain (2.)-Tp., Sićevo gorge Nature Park (3.)-Sk., Vidlič mountain (4.)-Lm., Rtanj mountain (5.)-Pl. and Stara planina mountain (6.)-Dg. Samples from seven different specimens from each population and soil from the rhizosphere were collected and transported to the laboratory where they were submitted to analysis.

Soil and plant metal analysis

Soil samples were dried at 60 °C and sieved (≤ 2 mm). The total metal content was analyzed in the same soil fraction after grounding samples and submitting them to an acid digestion (HF, HNO3 and HCl). The soil bioavailable metal content was analyzed using 0.05 mol/dm3 EDTA (pH 7) solution, at a ratio of 1:25 (soil/solution). The samples were mixed for 5 h and centrifuged at 10,000 × g for 10 min.

The metal content in plant tissue was analyzed after washing the plants with distilled water and drying them at 60 °C for 48 h. The leaves were excised from the stems and submitted to extraction (HNO_3, H_2O_2 and HCl). Metals were quantified in ICP-OES (Thermo Scientific iCAP 6500 Duo ICP spectrometer). The certified reference material BCR-701 from the Community Bureau of Reference was treated by ICP-OES analysis (5).

The soil–plant transfer coefficient (TC) and bioconcentration factor (BF) were used to analyze the relationship between soil and plant metal content. TC was calculated as the concentration of metal in plant over that in soil (TC = [metal plant]/[metal soil]) and it represented the capacity of a species to accumulate an element. BF was based on the ratio of metal in plant to that in a bioavailable form in soil (BF = [metal plant]/[EDTA-extractable metal in soil]) and it represented the tolerance for a potential toxic element in the absence of toxicity symptoms. A plant could be considered to be an accumulator for a particular metal when TC > 1 and tolerant to certain metal when BF > 1 (6). The experimental results were expressed as mean ± standard deviation of three replicates.

Results

Metal contents of soils in total and EDTA-extractable forms were summarized in Table 1 and Table 2. Concentrations of studied metals were within the specified soil values (7). In the group of major metals, the largest number (four) of the highest concentration values (μg/g) was recorded in the locality of Sićevo gorge Nature Park: Ca 154250.35 in the total form and Na, 25.28; Mg, 606.23 and Ca, 78283.89 in the EDTA-extractable form. The six highest concentration values of studied trace metals (μg/g) were found in the locality of Vidlič mountain; in the total form: Fe, 15411.14; Cu, 17.05 and Zn, 54.53, and in the EDTA-extractable form: Mn, 305.35; Cu, 0.06 and Zn, 2.19. It was interesting that the highest level of Ca in both total and EDTA-extractable forms was in Sićevo gorge Nature Park. On the other hand, the highest concentrations of Cu and Zn in both total and EDTA-extractable forms were in the mountain Vidlič.

Table 1. Soils' total metal content (μg/g)

Metals	Localities					
	1.	2.	3.	4.	5.	6.
Na	2162.47±41.95	2410.86±46.77	718.69±13.94	815.47±15.82	1266.73±24.57	289.06±5.61
K	6880.97±56.42	7315.09±59.98	4030.65±33.05	6740.61±55.27	4997.30±40.98	10871.18±89.14
Mg	3229.80±53.29	2459.54±40.58	1332.23±21.98	1466.49±24.20	1159.37±19.12	1463.56±24.15
Ca	99821.98±1078.08	10251.92±110.73	154250.35±1665.90	22115.18±238.84	25586.59±276.33	14308.62±154.53
Mn	918.11±6.61	1280.25±9.21	736.86±5.31	1044.43±7.52	820.68±5.91	11.03±0.08
Fe	13516.51±227.08	14745.34±247.72	8156.82±137.03	15411.14±258.91	9669.43±162.44	4786.97±80.42
Cu	16.11±0.14	11.46±0.10	5.79±0.05	17.05±0.15	10.45±0.09	2.25±0.02
Zn	40.70±0.64	25.97±0.41	23.67±0.37	54.53±0.86	42.93±0.68	8.45±0.13

Table 2. Soils' metal content in EDTA-extractable forms (μg/g)

Metals	Localities					
	1.	2.	3.	4.	5.	6.
Na	18.71±0.36	10.64±0.21	25.28±0.49	12.19±0.24	12.69±0.25	5.59±0.11
K	313.81±2.57	73.73±0.60	282.40±2.32	232.78±1.91	188.04±1.54	108.88±0.89
Mg	446.67±7.37	223.25±3.68	606.23±10.01	509.63±8.41	257.91±4.26	103.89±1.71
Ca	76973.40±831.31	3762.50±40.64	78283.89±845.47	10463.60±113.01	13620.71±147.10	829.93±8.96
Mn	207.52±1.49	252.24±1.81	227.95±1.64	305.35±2.20	263.84±1.90	4.60±0.03
Fe	2.08±0.03	3.04±0.05	2.83±0.04	2.60±0.04	4.83±0.08	26.63±0.44
Cu	0.01±0.00	0.01±0.00	0.01±0.00	0.06±00	0.01±0.00	0.01±0.00
Zn	0.01±0.00	0.59±0.01	0.01±0.00	2.19±0.03	0.31±0.01	0.34±0.01

The result of the levels of examined metals in selected plants was presented in Table 3. Selected plants accumulated a sufficient amount of studied metals, except Cu. Also, it could be said that T. glabrescens and P. longifolium accumulated lower Na content in comparison with normal range described in plants (8). Plant species D. graveolens accumulated four highest contents, from eight investigated metals (μg/g): Na, 27.00; K, 12333.56; Mn, 50.85 and Fe, 132.76.

Table 3. Metal content in plant leaves (μg/g)

Metals	Localities					
	Tg.	Tp.	Sk.	Lm.	Pl.	Dg.
Na	3.20±0.06	6.55±0.13	6.17±0.12	6.33±0.13	3.52±0.07	27.00±0.52
K	9848.73±80.76	7805.78±64.00	5789.57±47.47	8011.79±65.69	5891.94±48.31	12333.56±101.14
Mg	1624.03±26.80	3609.30±59.55	2446.64±40.37	2199.26±36.29	1314.96±21.70	1370.80±22.62
Ca	8755.53±94.56	9638.73±104.10	12292.58±132.76	10545.32±113.89	7010.00±75.71	11672.56±126.06
Mn	21.18±0.15	18.24±0.13	19.85±0.14	36.35±0.26	42.71±0.31	50.85±0.37
Fe	88.43±1.49	123.90±2.08	50.40±0.85	42.82±0.72	24.40±0.41	132.76±2.23
Cu	4.16±0.04	3.67±0.03	2.76±0.02	6.03±0.05	1.80±0.02	2.49±0.02
Zn	21.17±0.33	25.49±0.40	15.56±0.25	22.35±0.35	12.16±0.19	12.26±0.19

To estimate metal accumulation and tolerance of wild plant populations, the transfer coefficient (TC) and the bioconcentration factor (BF) were calculated as indicated in the previous section. As shown in Table 4, TC values revealed that the selected plant species was not an accumulator of Na, Ca and Fe. Curiously, TC values were dependent on studied populations. For instance, only D. graveolens was an accumulator for Mn, Cu and Zn. BF values (Table 5) suggested that studied plants were not tolerant to Na and Mn, except D. graveolens, tolerant species to all examined metals. Despite a lower tolerance of plants to Na and Mn, it should be noted that the adverse impact of these metals was not observed during their collection.

Table 4. Soil–plant transfer coefficient (TC) for selected plants growing in different soils

Metals	Localities					
	Tg.	Tp.	Sk.	Lm.	Pl.	Dg.
Na	0.0015	0.0027	0.0086	0.0078	0.0028	0.0934
K	1.4313	1.0671	1.4364	1.1886	1.1790	1.1345
Mg	0.5028	1.4675	1.8365	1.4997	1.1342	0.9366
Ca	0.0877	0.9402	0.0797	0.4768	0.2740	0.8158
Mn	0.0231	0.0142	0.0269	0.0348	0.0520	4.6102
Fe	0.0065	0.0084	0.0062	0.0028	0.0025	0.0277
Cu	0.2582	0.3203	0.4764	0.3538	0.1722	1.1081
Zn	0.5201	0.9815	0.6575	0.4098	0.2831	1.4511

Table 5. Soil–plant bioconcentration factor (BF) for selected plants growing in different soils

Metals	Localities					
	Tg.	Tp.	Sk.	Lm.	Pl.	Dg.
Na	0.17	0.62	0.24	0.52	0.28	4.83
K	31.38	105.87	20.50	34.42	31.33	113.28
Mg	3.64	16.17	4.04	4.32	5.10	13.19
Ca	0.11	2.56	0.16	1.01	0.51	14.06
Mn	0.10	0.07	0.09	0.12	0.16	11.05
Fe	42.52	40.76	17.81	16.47	5.05	4.99
Cu	415.89	367.10	276.00	100.51	180.03	249.01
Zn	2116.83	43.20	1556.35	10.20	39.21	36.06

Discussion

Selected medicinal plants from Serbia exhibit significant antibacterial activity (9-11). Environmental and health effects of metal exposure from soil depend on the mobility and availability of the elements. Total metal levels in soil reflect the soil's geological origin and weathering. Certainly, chemical forms of the metals are very important, which may affect their environmental mobility and availability to

organisms (12). The metal content of the soils included in this study had a different origin. Soils from Vidlič mountain and Rtanj mountain had the highest chemical similarity in terms of total metal content. Metals after EDTA-extraction are considered the most mobile and potentially bioavailable forms present in soils, and may best capture the anthropogenic contribution of greatest possible concern for human exposure (13). Soils from Kravlje village and Sićevo gorge Nature Park had the highest chemical similarity in terms of EDTA-extractable metal content.

Leaves, organs with photosynthetic activity accumulate higher quantities of metals. As we said, investigated plants accumulated a sufficient amount of studied metals, except copper. The comparison of our results with the results of other authors showed a considerable agreement regarding the heavy metals content in the plants from the region of Southeast Serbia (14). Compared with published data, it is also important to note that in our study a significantly lower content of Na was established in the plant leaves (15).

High concentrations of Na, Ca and Mn in EDTA-extractable forms resulted in the reduction of tolerance of these metals (BF < 1), which may be manifested in their toxicity (12). D. graveolens may be considered as K, Mn, Cu and Zn accumulator and it is tolerant to these four metals, as deduced from TC and BF values obtained in this study. Moreover, D. graveolens is tolerant to all the metals examined. In addition to chemical and ecological aspects, the study of plant-soil interaction can help in the design of modern herbal medicinal products.

Conclusion

The present study gives a new perspective on the chemical composition of selected medicinal plants from Serbia and their corresponding soil. Within the group of major metals, the four highest concentration values were recorded in the locality of Sićevo gorge Nature Park, while the six highest concentration values of studied trace metals were found in the locality of Vidlič mountain. Selected plants accumulate a sufficient amount of studied metals, except copper. They are not accumulator of Na, Ca and Fe, neither are they tolerant to Na and Mn, except D. graveolens, a tolerant species to all examined metals. Analysis of the metal content in the plant material confirms that the tested plant species can be important in human diet as a source of the essential elements of importance for the optimal functioning of the human body at a biochemical level.

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PROUČAVANJE ESENCIJALNIH METALA U ODABRANIM MEDICINSKIM BILJKAMA IZ SRBIJE

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U radu je proučavano usvajanje i akumulacija esencijalnih makro i mikro elemenata u šest odabranih medicinskih biljaka iz Srbije i njihov transfer iz zemljišta u biljku. Za analizu metala: Na, K, Mg, Ca, Mn, Fe, Cu i Zn u uzorcima zemljišta i biljaka korišćena je optičko-emisiona spektroskopija sa induktivno-spregnutom plazmom (ICP-OES). Odabrane biljke akumuliraju dovoljnu količinu proučavanih metala, osim Cu. One nisu akumulatori Na, Ca i Fe, takođe, nisu tolerantne na Na i Mn, osim vrste Dittrichia graveolens, koja je tolerantna na sve ispitivane metale. Proučavane biljne vrste mogu biti značajne u ljudskoj ishrani kao izvor esencijalnih elemenata potrebnih za optimalno funkcionisanje ljudskog organizma.

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Ključne reči: *esencijalni metali, medicinska biljka, ICP-OES*

Review article

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MECONIUM ILEUS

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Meconium ileus is congenital mechanical obstruction of the small intestine appearing as a result of the amended meconium. It is responsible for a third of small bowel obstruction in infants and is manifested in about 30-40% of children with cystic fibrosis. Patients with mutations in the CFTR gene and cystic fibrosis have abnormal chloride conductance through external cell membranes resulting in precipitation of thick secret in the respiratory tract, pancreas, liver, intestines and sweat glands. Maternal history and ultrasound during pregnancy allow the prediction which children will have the risk of meconium ileus. Meconium ileus occurs in 2 formats: a simple (67%) and complex (33%). The most common complications are: volvulus, atresia, meconium peritonitis, pseudocystic formation or perforation of the colon. The conservative treatment is based on the application of hyper or iso-osmolar contrast. A contrast enema is performed during fluoroscopy, gradually increasing intraluminal pressure in order to avoid possible perforation. In case of failure of conservative treatment, further treatment must be surgical. There are several surgical options of treatment (most commonly: Mikulicz, Bishop-Koop and Santulli) applied in order to provide the lowest possible bowel resection and enterostomy formation for possible postoperative irrigation. Long-term survival of patients with meconium ileus and cystic fibrosis is 83-90%.

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Key words: *meconium ileus, neonates, treatmen*

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Introduction

Meconium ileus is congenital mechanical obstruction of the small intestine appearingas a result of the amended meconium (1). It was first described in 1905 (2), and in 1938, Anderson described and explained the connection between cystic fibrosis and meconium ileus (3). It is responsible for a third of small bowel obstruction in infants, manifested in about 30-40% of children with cystic fibrosis (4). Meconium ileus is the earliest clinical sign of cystic fibrosis, and because of this patients are diagnosed and treated in the neonatal age (5). Cystic fibrosis is an autosomal recessive disorder that appears in the white race in 1 of 29 live births, while it is rare in the black race (1/17 000) (3). Genetic substrate comprises a mutation in the CFTR (cystic fibrosis transmembrane conductance regulator) gene encoding the activity of chloride ion guide. Patients with CFTR gene mutations and cystic fibrosis have abnormal chloride conductance through external cell membranes, resulting in precipitation of thick secretions in the respiratory tract, pancreas, liver, intestines and sweat glands (6). Meconium ileus may be associated with intestinal atresia and congenital defects of the anterior abdominal wall (7).

Incidence

Meconium ileus is the cause in 9-33% of all neonatal intestinal obstruction, with an incidence of 1:2500 newborns. It is the third most frequent cause of neonatal intestinal obstruction, after ileo and duodeno-jejunal atresia and malrotation (2). It occurs in about 30-40% of children with cystic fibrosis (4).

Pathogenesis

Meconium ileus is always associated with cystic fibrosis, an autosomal recessive disease which is typical dysfunctional transfer of chloride ions across epithelial cells. This is due to quantitative or qualitative CFTR protein related disorders affecting multiple organ systems: intestines, pancreas, sweat glands, liver and salivary glands. The pancreas is the most

commonly affected organ because of the retention of juices and progressive atrophy of the acinar cells occurring in fetal life, in contrast to pulmonary mucosal plaque in the lower respiratory tract with a respiratory insufficiency during puberty. In 1989, the gene CFTR is detected. It is normally located on the apical membrane of epithelial cells of the stomach to the colon. The mutation of the CFTR gene on chromosome 7 is responsible for the development of cystic fibrosis. The most common mutation is ΔF508 and can be identified by DNA test used in neonates as well as family members who are considered to be potential carriers of the gene (2).

Clinical signs

One of the signs is polyhydroamnion during pregnancy, found in high intestinal obstruction and in 20% of mothers. The existence of fetal bowel hyperechogenicity on ultrasound, associated with bowel dilatation or ascites may indicate intestinal obstruction. Meconium begins to fulfill the small intestine during the 20th week of gestation, so the identification of meconium ileus before that period is rare (7). History of mother's ultrasound during pregnancy allows the prediction that the children will have the risk of meconium ileus (3). Meconium ileus occurs in two forms: a simple (67%) and complex (33%). The most common complications are: volvulus, atresia, meconium peritonitis, pseudocystic formation or perforation (1). If abdominal distension, intermittent vomiting with mixture of bile, and without meconium stools occur in the first 8 hours of life, it is a simple form of meconium ileus. By clinical examination it is possible to palpate dilated intestinal loops in the right lower abdominal quadrant; furthermore digital rectal examination found an empty rectum. Unlike simple forms, complex forms of meconium ileus occur in the first 24 hours of life of the newborn. The abdomen is distended with pronounced veins of the abdominal wall. Bowel necrosis and perforation resulting in peritonitis may arise as complications. Sometimes after meconium perforation, a pseudocyst can occur as a palpable mass in the right lower abdomen, and the skin over the mass is edematous and transparent (1,2).

Diagnosis

Neonates with meconium ileus are often born with distension of the abdominal wall, which is the only difference between children with bowel obstruction in which distension occurs after air swallowing. Peristaltic waves are visible, and can often be palpable as solid gyrus intestine. In many children, digital rectal examination found an empty rectum. Diagnosis of meconium ileus based on clinical examination includes radiological examination of the abdomen. Intestinal distension a dominant sign. Characteristic radiographic signs are distended intestinal convolutions, without or with little air in the intestines above meconium content. The most common sign is in the right hemiabdomen, present like air between meconium content that resembles the appearance of a soap bubbles. Radiographic signs of complicated meconium ileus may be different. Diffuse calcification in the abdomen present on the plane radiograph of the newborn indicates intrauterine intestinal perforation and meconial peritonitis (3). Meconium ileus is associated with cystic fibrosis, and the most important test in the diagnosis of cystic fibrosis is a sweat test which measures the concentration of sodium chloride. The concentration of these chloride ions must be greater than 60 mEq / L in 100 mg of sweat. In newborns, it is often difficult to collect sufficient amount of sweat in order to do the necessary analysis, so sometimes it is necessary to repeat the test several times. Children with cystic fibrosis as well as heterozygous carriers of the gene can be revealed with this method. Increased serum immunoreactive trypsin (IRT) may indicate cystic fibrosis, but must be confirmed by other tests (2).

Differential diagnosis

Differential diagnosis of all causes of distal intestinal obstruction should be considered. They include: Hirschsprung's disease, small bowel atresia, meconium plug syndrome and small left colon neonate (8). Meconium ileus, distal intestinal obstruction and constipation can often be different varieties that accompany cystic fibrosis (9).

Treatment

Meconium ileus can be treated conservatively and surgically. The first step in treatment of meconium ileus requires installation of nasogastric tube, antibiotic prophylaxis and correction of dehydration, electrolyte and hypothermia. The conservative treatment includes applying of hyper or iso-osmolar contrast. Recent studies show the effectiveness of the gastrografin of 20-50%. The goal of contrasting enemas is to soften and moisturises meconium for easier bowel emptying. Contrast enema is applied under the control of fluoroscopy, gradually increasing intraluminal pressure in order to avoid possible perforation. Fifty percent of children treated with contrast enema do not require further treatment, while some may need to repeat enema. Radiological images are repeated after 3, 6, 12, 24 and 48 hours to monitor the occurrence of possible complications (2.10). Conservative treatment is applied if there is no intestinal atresia, volvulus, gangrene bowel perforation and meconium peritonitis. If conservative treatment is not successful, surgical treatment is optimal option for cases of complicated meconium ileus. The goal of surgery is the evacuation of meconium from the intestines and the preservation of greater length of the intestines (10). There are several surgical techniques (Mikulicz, Bishop-Koop and Santulli enterostomy) for postoperative intestinal irrigation that can be performed even in extremely small birth-waight premature infants (11). In the next step, closure of the stoma and intestinal anastomosis should be planned (1). In some cases, it is possible to perform segmental intestinal resection with primary anastomosis creation (12).

Prognosis

Meconium ileus is the first sign that points to the existence of cystic fibrosis. In complicated forms of meconium ileus, obstruction of the small intestine may occur, while in uncomplicated forms of conservative treatment complications are rarely seen. In recent years, survival of newborns with meconial meconium ileus has improved and reached about 90% thanks to intensive care, improved surgical techniques and medical treatment. Long-term survival of patients with meconium ileus and cystic fibrosis is 83-90% (2).

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MEKONIJALNI ILEUS

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Mekonijalni ileus je kongenitalna mehanička opstrukcija tankog creva koja je nastala kao posledica izmenjenog mekonijuma. Odgovoran je za trećinu opstrukcija tankog creva kod novorođenčadi, a manifestuje se u oko 30-40% dece sa cističnom fibrozom. Bolesnici sa CFTR mutacijama gena i cističnom fibrozom imaju nenormalnu provodljivost hlorida preko spoljnih ćelijskih membrana, što rezultira precipitacijom gustog sekreta u respiratornom traktu, pankreasu, jetri, crevima i znojnim žlezdama. Anamneza majke i ultrazvučne pretrage tokom trudnoće omogućavaju predikciju koja će deca imati rizik od nastanka mekonijalnog ileusa. Mekonijalni ileus se javlja u dva oblika: jednostavni (67%) i komplikovani (33%). Od najčešćih komplikacija navode se: volvulus, atrezija, mekonijalni peritonitis, pseudocistična formacija ili perforacija kolona. U konzervativnom lečenju primenjuje se hiper ili iso-osmolarni kontrast. Kontrastna klizma se primenjuje pod kontrolom fluoroskopije, postepenim povećanjem intraluminalnog pritiska kako bi se izbegla moguća perforacija. U slučaju neuspeha konzervativnog lečenja, dalje lečenje je hirurško. Postoji nekoliko hirurških opcija lečenja (najčešće korištene su: po Mikuliczu, Bishop-Koopu i Santulliju), a koje se svode na najmanju moguću resekciju creva i formiranje enterostomije kroz koju će biti moguća postoperativna irigacija. Dugoročno preživljavanje bolesnika s mekonijalnim ileusom i cističnom fibrozom iznosi 83-90%, ali je moguća pojava fibrozne kolonopatije i endokrine disfunkcije.

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Ključne reči: *ileus mekonijalis, neonates, lečenje*

Original article

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TOTAL HIP REPLACEMENT REHABILITATION: RESULTS AND DILEMMAS

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The number of candidates for total hip replacement (THR) is steadily increasing. Judging by the clinical results and implant longevity, THR is one of the most common and most successful orthopedic interventions of all times. Material, design, surgical techniques and subsequent rehabilitation continue to evolve. Choice of the prosthesis and fixation technique depends on the patients' bone structure and joint stability and their individual characteristics, such as age, weight and level of activity. Rehabilitation after THR is as important as the surgery. Rehabilitation protocols vary with the type of endoprosthesis. There is some controversial evidence about the differences in the surgical approach, the role of the preoperative education and exercises, as well as the implementation of the most efficient rehabilitation protocol. Despite many uncertainties and dilemmas, most studies have shown that majority of patients are satisfied with their arthroplasty results.

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Key words: *total hip replacement, total hip arthroplasty, rehabilitation, exercise*

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Introduction

The hip joint is one of the most important joints in the human body. It is one of the largest, but also one of our most flexible joints that allows a greater range of motion than all other joints in the body except for the shoulder. The damage of the hip joint and its function is a complex medical problem. As a major weight-bearing joint, together with the knee joint, it is most susceptible to osteoarthritis. Chronic pain, stiffness and limited mobility are just some of the possible symptoms. In addition to severe OA, femoral neck fractures, RA, post-traumatic arthritis and avascular necrosis are the most common indications for total hip replacement (THR) surgery.

Thanks to the accomplishments of modern medicine, it is possible today to resolve a number of pathological conditions by replacing the damaged hip joint with endoprothesis. THR surgery is a safe and effective procedure that can relieve pain, increase mobility, and help patients enjoy normal, everyday activities and regain their former quality of life. The basic goal of modern medicine is that endoprosthesis should completely replace the hip joint and restore its function. Despite technical complexity, THR is one of the most common and most successful orthopedic interventions, according to the clinical results and based on the implant duration.

Physicians first developed modern THR surgery in the early 1960s. Since 1962, improvements in joint replacement surgical techniques and technology have substantially increased the effectiveness of THR, making it one of the most successful operations. Nowadays, according to the Agency for Healthcare Research and Quality and the National Joint Registry, more than 300.000 THRs are performed in the United States annually (with 80.000 THRs in England) and the procedure has become more common in younger patients.

Objectives

The aim of the paper is to review systematically and determine the clinical significance of diseased hip joint replacement, taking into account different types and characteristics of the hip endoprosthesis, to present the main treatment methods, pos-

sible complications, as well as evidence-based physical medicine and rehabilitation procedures and overall patient satisfaction.

Materials and methods

A search of the PubMed database was conducted using the keywords „total hip replacement", „total hip arthroplasty", „rehabilitation" and „exercise". The study included the papers published in English in the last 30 years dealing with the etiology, treatment, surgical approaches, most common complications, exercise and rehabilitation of patients after THR.

Results and discussion

The search identified 2,447 publications in the last 30 years, 490 of which were considered potentially relevant for the research based on the title and abstract.

It is generally known that osteoarthritis, rheumatoid and traumatic arthritis, femoral neck fractures, pathologic fractures, congenital hip diseases, avascular necrosis (commonly as the result of failure of earlier reconstructive surgery) or joint instability, could be the reason for THR.

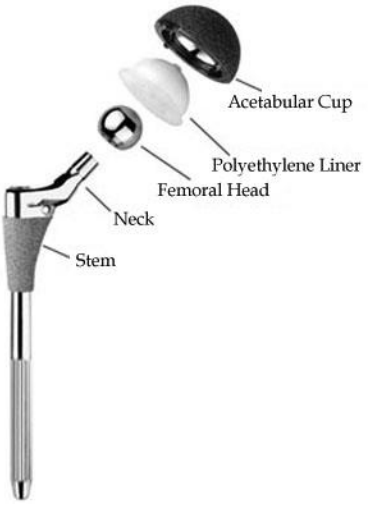
Being a major weight-bearing joint, the hip is very prone to osteoarthritis. The incidence rates of OA increase with age. Hip OA occurs more often in the female population, especially after the age of fifty, due to obesity, hormonal imbalance, congenital and acquired deformities and trauma.

Pain, stiffness, crepitation, reduced range of motion, shorter leg, walking difficulties, limping, difficulties in performing everyday activities are just some of the possible symptoms of OA. Loss of the joint space, subchondral sclerosis and cysts, subluxation, irregularity of the joint surface are all typical radiographic signs of OA.

Conservative treatment options for OA patients involve a multimodal approach, involving patient education, medication, modification of activity and weight loss, using of cane or walkers, and physical therapy procedures, which can prevent or postpone THR. NSAIDs, COX-2 inhibitors, glucosamine and chondroitin supplements are most commonly used medications for the treatment of OA. Intra-articular injections of corticosteroids and viscosupplementation are sometimes used as well.

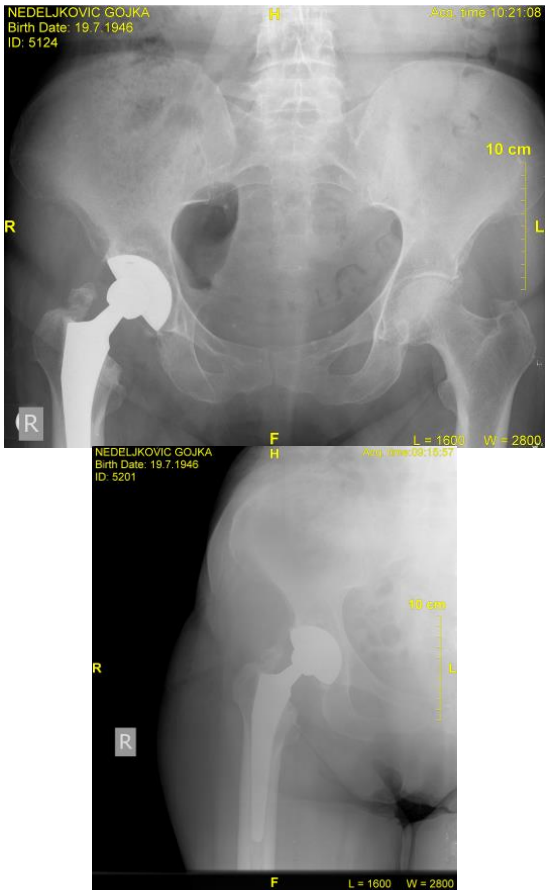
Today, lots of different types of hip endo prosthesis are available. Possible surgical methods of treatment include: hemiarthroplasty, resurfacing arthroplasty and total hip replacement. Hip replacement surgery can be performed as a resurfacing arthroplasty (a bone-preserving procedure), half (hemi) arthroplasty, where only the femoral head is replaced, or total, where both acetabulum and femoral head are replaced (Picture 1). Hemiarthroplasty is usually per-formed on the hip after a subcapital fracture of the neck of the femur.

Prosthetic implants have to be durable, inert and firmly fixed to the skeleton. The prostheses are



Picture 1: THR implants

of various designs and may be fixed to the remaining bone by cement, press fit or bone in-growth. The type of fixation – cemented, ceme-ntless or hybrid (Picture 2) – determines the post-operative weight bearing ability.



Picture 2: Total hip replacement, hybrid fixation. X-rays of a patient from picture 3.

As for the choice of implants, cemented endoprostheses are recommended for elderly patients (over 65 years old) because of their better early fixation, while cementless technique is now a preferred method for younger, more active patients, who are expected to walk with protected weight-bearing for the first 6 weeks after the surgery.

The results differ regarding the duration of endoprostheses. With regard to implant longevity, the advances in both femoral cementing techni-ques and the design of cemented stems have re-sulted in near perfect survivorship at 10 years (98%) and good survivorship at 25 years (93%). Comparable survival rates have been reported using cementless techniques for the femoral component. In the acetabular component, 10-year survival rates were similar as for cemented (95%) and cementless (95–100%) technique, but at 15 years cementless technology superseded cemented techniques (70–95% for cemented, versus 85–94% for cementless) (1).

Different techniques are being used for surgical approach: lateral, anterolateral, posterolateral, minimally invasive surgery and greater trohanter osteotomy. In standard THR, the incision is 8 to 10 inches long, compared to 2 to 5 inches in a minimally invasive approach. Aside from smaller incisions and blood loss, minimally invasive surgery is technically more demanding, due to a limited operative field. In comparison to traditional THR, some of the studies have shown advantages in favor of the minimally invasive approach 6 and 12 weeks postoperatively. However, up to now there have been no prospective randomized clinical studies that could definitely show the superiority of minimally invasive procedure. More evidence and better evaluation of minimally invasive hip replacement will be necessary before this technique could be recommended for more widespread clinical use. For that reason, conventional approaches to THR are still considered to be the gold standard (2). Also, a higher dislocation rate was reported in the mini-incision group.

Computer-assisted hip navigation for THR offers the potential for more accurate placement of hip components and control of leg length and offset. Although it is a brand new method without long-term data, preliminary results are encouraging regarding improved accuracy of the acetabular cup placement compared with conventional manual techniques, so it might become the technique of choice in the near future (3).

Possible complications of THR include: infection, leg length discrepancy, nerve palsy, deep vain thrombosis, improper implant fixation, joint instability, prosthetic hip dislocation, loosening of the prosthesis, osteolysis, periprosthetic fractures, etc. So far, there has been some evidence about the number of complications and lower functional gains in obese patients after THR. Functional improvements usually occurred, but the obese patients generally did not reach the same level of physical function in comparison to the patients with a lower BMI score. Also, uncontrolled obesity after THR was related to aggravated comorbidities and excessive long term healthcare costs (4).

There has been a lack of empirical data to support the type of sport activities that are safe and feasible for patients after THR, and current recommendations are based more on clinical experience and personal preferences, than on a prospective and retrospective analysis (5). Most authors approve of low impact sporting activities such as walking, swimming, stationary biking, bowling, dancing, rowing and golf. On the other hand, contact sports, running, jogging, jumping, high impact aerobics, football, baseball, snow-boarding, weight lifting, parachooting are not allowed for THR patients. Most surgeons recommend that patients should return to the most advisable activities 3 to 6 months after their surgery (6).

The role of preoperative education remains inconclusive to a degree. Studies have generally shown shorter hospital stay, less analgesic use, less anxiety and fear in patients who attended preoperative education. Further, patients' participation in the preoperative educational programs might significantly reduce overall costs of primary THR procedures (7). In some studies, preoperative education reduced anxiety, but did not improve postoperative outcome (8).

Physical therapy procedures and rehabilitation of THR patients are as important as the surgical intervention. The main goal of rehabilitation after THR is pain reduction, restoration of the function, muscle strength and mobility, regaining a satisfactory range of motion in the hip joint necessary for everyday activities (Picture 3), thus achieving functional, economic and enduring gait and altogether better quality of life. The optimal treatment strategy following THR remains unknown.

Picture 3: Excellent functional recovery of a patient with the right THR.

There are many rehabilitation protocols, individually adjusted to patients based on the type of endoprosthesis. It is not known which protocol is the most effective one, whether inpatient, outpatient or home-based rehabilitation treatment produces better long-term results and provides greater patient satisfaction (1).

The role of the preoperative kinesitherapy is disputable; most studies have shown improvements in the preoperative functional status (9), but not postoperative results, considering the recovery time,

length of hospital stay and possible complications (10).

Some authors confirmed the effectiveness of treadmill training with partial body-weight support in addition to usual exercise program for THR patients (11). Certain studies have revealed that treadmill training program helps THR patients to achieve a more symmetrical gait (12). When comparing treadmill training with partial body-weight support to conventional physical therapy in ambulatory patients with THR, treadmill training proved to be more effective than conventional physical therapy at restoring symmetrical independent walking after hip replacement (13).

Ergometer cycling after THR is also an effective means of achieving significant and clinically important improvement in patient health-related quality of life and personal satisfaction (14). Some authors think that ergometer cycling should also be incorporated into the standard rehabilitation protocol.

Functional exercises (strengthening, active range of motion, balancing, stair climbing and gait training) are essential after primary THR. Exercise therapy following THR is considered to be important during initial postoperative care, but till date only a few evidence-based recommendations have been presented. There has been a substantial disagreement among rehabilitation professionals regarding exercise therapy prescriptions. Surgeons and therapists differ in their recommendations about weight-bearing and resistance training. Physiotherapists and exercise therapists prefer a more conservative approach with a delayed start of weight-bearing and resistance training, which is in contrast to the current literature evidence (15). In contrast to some evidence that preoperative kinesitherapy might be of benefit, authors agree about the absolute necessity of regular physical exercise after THR. In addition to other advantages, exercise should be able to increase bone density and prosthesis fixation and decrease the risk of falls (16).

On top of numerous uncertainties concerning THR patient rehabilitation, most studies have shown that over 86% of the operated are satisfied with arthroplasty results (17, 18), in particular the elderly female patients (especially concerning pain relief) and those with poorer preoperative results (1).

Bearing in mind a number of dilemmas and controversy about different aspects of THR, and a lot of insufficient data to reach a firm conclusion about the most efficient rehabilitation protocol, more information is needed for better understanding of the issues these patients are faced with, so further research (with the long-term results) should be conducted, especially taking into account the rising number of younger, active patients who undergo THR. More evidence-based recommendations about beneficial exercise therapy dosages and components are needed so that reasonable guidelines and standards for postoperative treatment could be established (15).

Conclusion

Total hip replacement has completely revolutionized the nature of treatment of arthritic hip, producing better pain relief, functional recovery, and substantial quality of life improvement. It is considered to be one of the most common and most successful orthopedic interventions of all times.

Although today implant duration is in most cases 15 years and more, the material, design, surgical techniques and postoperative rehabilitation continue to develop. Selection of the prosthesis and fixation techniques depends on patient individual characteristics, while the type of endoprosthesis determines the rehabilitation protocol. Physical therapy and rehabilitation of THR patients play a significant role in regaining the mobility and strength. It has not been established yet which rehabilitation protocol is the most efficient, whether inpatient, outpatient or home-based rehabilitation treatment provide greater patient satisfaction and best long-term results. Besides the various uncertainties concerning THR patient rehabilitation, most studies have shown that over 86% of patients are satisfied with arthroplasty results, especially the uncertaintics elderly populations.

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REZULTATI I DILEME U REHABILITACIJI BOLESNIKA SA ENDOPROTEZOM KUKA

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Sudeći prema kliničkim rezultatima i vremenu trajanja implantata, zamena obolelog zgloba kuka endoprotezom jedna je od najčešćih i najuspešnijih ortopedskih intervencija. Broj kandidata za ugradnju endoproteze kuka u stalnom je porastu. Materijali, dizajn, hirurške tehnike i rehabilitacija koja sledi nastavljaju da se razvijaju. Rehabilitacija je veoma važna i ne zaostaje za samim operativnim zahvatom. Izbor endoproteze i načina fiksacije zavisi od individualnih karakteristika pacijenata; stanja lokomotornog sistema, godina, telesne težine, fizičke aktivnosti. U zavisnosti od vrste endoproteze, postoji više različitih rehabilitacionih protokola. Kontroverzni su dokazi o razlikama u hirurškom pristupu, ulozi preoperativne edukacije i vežbi, kao i o primeni najefikasnijeg rehabilitacionog protokola. Uprkos brojnim nedoumicama, različite studije pokazuju da je najveći broj pacijenata zadovoljan rezultatima aloartroplastike kuka.

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Ključne reči: *totalna endoproteza kuka, aloartroplastika kuka, rehabilitacija, vežbe*

Review article

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NEW DRUGS FOR THE TREATMENT OF DYSLIPIDEMIA

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Dyslipidemia is the leading risk factor for the development of atherosclerosis and associated consequences, such as coronary heart disease, ischemic cerebrovascular and peripheral vascular disease. These diseases are the major cause of mortality in the world and in Europe as well, where they are responsible for around 45% of all deaths. Treatment of dyslipidemia includes the use of statins, ezetimibe, fibrates, niacin, bile acids sequestrants and omega-3 fatty acids. Although statins play the major role in dyslipidemia treatment by reducing the risk of cardiovascular (CV) events by 30%, there is a need for additional new drugs that reduce the residual risk even more. PCSK9 inhibitors, apolipoprotein B (apoB) synthesis inhibitors, MTP inhibitors and CETP inhibitors are already approved for the specific indications, or are in the advanced stages of clinical investigation. Two PCSK9 inhibitors, alirocumab and evolocumab are approved for use in combination with statins for the treatment of heterozygous familial hypercholesterolemia (FH), but also in patients with clinical atherosclerotic CV diseases who require additional low-density lipoprotein cholesterol (LDL-C) level reduction. In addition, evolocumab is approved for use in patients with homozygous FH. Mipomersen, apoB synthesis inhibitor, lomitapide, and oral MTP inhibitor are currently approved in the treatment of patients with homozygous FH as an adjunct to the maximum tolerated doses of statins and other lipid-lowering drugs. Although the new lipid-lowering agents produce significant LDL-C level reduction, more clinical studies are necessary to confirm their efficacy and safety in dyslipidemia treatment.

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Key words: *dyslipidemia, PCSK9 inhibitors, mipomersen, lomitapide, CETP inhibitors*

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Introduction

Dyslipidemia, characterized by increased levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides, and low levels of high-density lipoprotein cholesterol (HDL-C) are the major risk factors for the development of atherosclerosis and associated diseases as well (coronary artery, ischemic cerebrovascular and peripheral vascular disease). They are the major cause of morbidity and mortality among middle-aged adults as well as in the elderly (1).

Statins are the first line drugs used in the dyslipidemia treatment. They generally reduce the LDL-C level by 25-50% and have a range of pleiotrophic effects as well. Intestinal cholesterol absorption inhibitors (ezetimibe) and bile acid sequestrants (not registered in Serbia)reduce LDL-C level in combination with statins by 15-20% and 10-20%, respectively. The nicotinic acid acts by HDL-C augmentation (not registered in Serbia), while fibrates and omega 3 fatty acids lower triglycerides blood level as well.

According to the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS), the latest guidelines for dyslipidemia management shows that statins are the drugs of choice for the treatment of hyperlipoproteinemia. If the goal is not reached by the use of statins, the combination of statin and ezetimibe, or a bile acid sequestrant, as a second line, should be considered as an adjunct. In patients with statin intolerance, ezetimibe or bile acid sequestrants should be used. In a group of high-risk patients, with persistent high LDL-C despite the maximal tolerated statin dose, in combination with ezetimibe or in patients with statin intolerance, the use of a new group of lipid-lowering agents, like PCSK9 inhibitors, should be considered (2).

It is important to emphasize that in spite of statin effectiveness in reducing the risk of CV events by 30%, residual risk still remains, indicating that there is a need for additional drugs to reduce the residual risk further more.

New drugs for dyslipidemia treatment include proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, apolipoprotein B (apoB) synthesis inhibitors, microsomal triglyceride transfer protein (MTP) inhibitors and cholesteryl ester transfer protein (CETP) inhibitors, that are either already approved for the use in specific indications or are in the advanced stages of clinical investigation. The aim of our work was to make an overview of these new groups of drugs in the treatment of dyslipidemia with an overview of their mechanism of action, adverse effects and results of clinical trials, as well as their current place in the therapy.

Methods

With a view of collecting data about new lipid-lowering agents, we used several sources of information. Most data were collected by searching a large database MEDLINE:

(http://www.ncbi.nlm.nih.gov/pubmed),

using the key words "dyslipidemia", "hyperlipidemia" and "familial hypercholesterolaemia" in combination with "PCSK9 inhibitors" "alirocumab", "evolocumab", "mipomersen", "MTP inhibitors", "CETP inhibitors", "new drugs" and "clinical trials". In databases, from 2001-2016, we searched for: review articles relating to the new lipid-lowering agents and clinical studies which investigated the effectiveness and side effects of the new lipid-lowering agents (original articles), as well as the latest guidelines for the treatment of dyslipidemia. Each paper was reviewed and further analyzed. The references of the found articles were examined in order to identify additional studies. Priority was given to manuscripts published in the past 5 years, randomized placebo-controlled trials, and meta-analyses. Overall, 7 review papers and 19 clinical studies were used.

PCSK9 inhibitors

Two PCSK9 inhibitors, alirocumab and evolocumab, are approved for the use in combination with statins for the treatment of heterozygous familial hypercholesterolemia (FH), as well as in patients with clinical atherosclerotic CV diseases who require additional LDL-C level reduction (3, 4). Evolocumab is also approved for the use in the patients with homozygous FH (4). It should be noted that these two drugs were registered in the Republic of Serbia, evolocumab in December 2016, and alirocumab in April 2017. The clinical development program for bococizumab was discontinued in November 2016 because of an unanticipated attenuation of LDL-C lowering over time, as well as a higher level of immunogenicity and higher rate of injection-site reactions than are shown with the other agents in this class (5). Few different PCSK9 inhibitors (e.g. monoclonal antibodies LGT-209, RG-7652) are currently in the phase I or II clinical studies (1).

In October 2016, ESC/EAS Task Force consensus statement for the use of PCSK9 inhibitor treatment in patients at very high risk of recurrent cardiovascular events with poorly controlled LDL-C levels was published. It is recommended that the treatment with a PCSK9 inhibitor may be considered in very high risk patients with clinical atherosclerotic CV diseases, including those with progressive form of CV disease or diabetes mellitus (with target organ damage or a major CV factor); or in patients with severe FH without atherosclerotic CV diseases with substantially elevated LDL-C levels despite maximal statin/ezetimibe therapy. In addition, patients with verified statin intolerance may be also considered for treatment with these agents (6).

PCSK9 is a serine protease that plays a central role in cholesterol metabolism in the liver by enhancing the degradation of LDL receptors (7, 8). Serum level of LDL-C is controlled predominately by the hepatic LDL receptors. When LDL-C binds to LDL receptor, the complex undergoes endocytosis, thus removing LDL-C from the circulation. After releasing LDL-C, LDL receptor is then either degraded or recycled back to the cell surface (8). Circulating PCSK9 binds to the LDL receptors directing them to the lysosomes for degradation and preventing the receptor recycling process after internalization. By inhibiting this protein, PCSK9 inhibitors reduce LDL receptors degradation and increase expression of LDL receptors resulting in LDL-C level reduction (Figure 1) (1, 8).

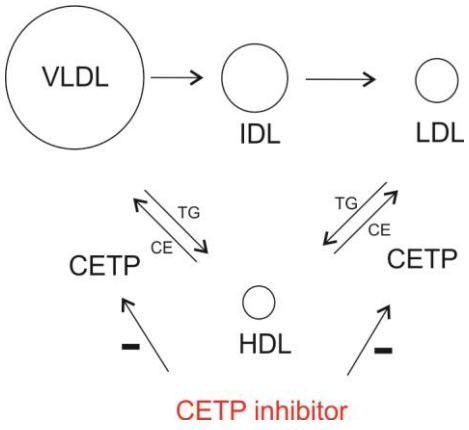

Figure 1. Mechanism of action of PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitors (adapted from reference 1)
LDL - low-density lipoproteins; LDLR – LDL receptor

Results of clinical trials have shown that PCSK9 inhibitors reduce LDL-C level by 50-60% independent from the presence of a background therapy with statins or other lipid-lowering agents (2). They are well tolerated, while the most common adverse effects include injection site reaction (erythema, pain, bruises, itching, swelling), as well as nasopharyngitis, upper respiratory tract infections, influenza, back

pain, arthralgia, myalgia, nausea. Hypersensitivity reactions (e.g., pruritus, rash, urticaria), including some serious events, like hypersensitivity vasculitis and hypersensitivity reactions requiring hospitalization, have been reported with alirocumab treatment. These allergic reactions, as well as elevated liver enzymes, were the most common causes of alirocumab treatment discontinuation. It should be noted that small but significant number of neurocognitive effects was described, but this finding requires further scrutiny. PCSK9 inhibitors are administered subcutaneously. The recommended doses for evolocumab are 140 mg once every 2 weeks or 420 mg monthly (both doses are equivalent), while alirocumab is administered from an initial 75 mg to a maximum of 150 mg every 2 week (or alternatively 300 mg monthly). After single-dose subcutaneous administration, the maximum serum concentrations of evolocumab are reached in 3-4 days, i.e. in 3-7 days after administration of alirocumab, while their bioavailability is 72% and 85%, respectively. They are primarily distributed in circulation with limited distribution in tissues. Metabolism is not investigated as proteins (human monoclonal antibodies of the class IgG2 (evolocumab) and IgG1 (alirocumab)) are expected to degrade to small peptides and individual amino acids. The median apparent half-life of evolocumab at steady state was 11 to 17 days, i.e. 17 to 20 days of alirocumab. Concomitant administration of PCSK9 inhibitors with a statin leads to an increase in clearance and a decrease in the median apparent half-life of PCSK9 inhibitors, but without affecting their pharmacodynamic effect and the need for dosage adjustment. Also, no dosage adjustments are required depending on age, sex, race, body weight, nor in patients with mild and moderate liver and renal impairment (they were not studied in patients with severe impairment) (9, 10).

Until now, PCSK9 inhibitors have been investigated in healthy subjects, patients with statin intolerance, patients at high CV risk, as well as in patients with FH receiving maximally tolerated statin doses (± other lipid-lowering agents) (11).

Evolocumab has been investigated within 5 phase II and 7 phase III trials. However, the problem is that all of them were short-term studies; therefore it was necessary to conduct long-term investigation of evolocumab. Hence, OSLER program, that included 4,465 patients, was designed with primary goal of obtaining the longer-term data on safety, side-effect profile and LDL-C reduction and also included an exploratory analysis on CV outcomes.

OSLER program consists of two clinical trials OSLER 1 and OSLER 2 that included patients who had already been involved in a variety of phase II and phase III trials that examined evolocumab.

One group of patients received the evolocumab (140 mg every 2 weeks or 420 mg once a month) in combination with statins and other lipid-lowering agents, while the other group received standard therapy (statin or other lipid-lowering agents). The LDL-C level, safety and the incidence of CV events (myocardial infarction, stroke, need for revascularization and CV death) were followed during the average period of 11.1 months (12).

The OSLER program results showed that evolocumab, when compared with standard therapy, reduced the LDL-C level by 61% ($p < 0.001$), from the median baseline of 120 mg/dL to 48.3 mg/dL after 12 weeks. This reduction in LDL-C level was consistent over 48 weeks. Changes in other atherogenic lipid fractions were similar, non-HDL-C was reduced by 52.0%, apoB by 47.3%, total cholesterol by 36.1%, triglycerides by 12.6% and lipoprotein(a) (Lp(a)) by 25.5% ($p < 0.001$ for all comparisons). Evolocumab raised levels of HDL cholesterol by 7.0% and apolipoprotein A1 (apo A1) by 4.2%, as compared with standard therapy ($p < 0.001$ for both comparisons). There were no differences in the rates of overall adverse events, serious adverse events, elevations in AST and ALT (> 3x the upper limit of normal) or creatine kinase (> 5x the upper limit of normal) levels. However, injection-site reactions, neurocognitive adverse events, as well as some non-specific adverse events, such as arthralgia, headache, fatigue, were reported more frequently in the evolocumab group (12).

The results of particular interest, which should be confirmed by additional studies, are those showing that patients in evolocumab group had significantly lower rate of all CV events (0.95%) than did patients in the standard-therapy group (2.18%) ($p = 0.003$) during one year (12). In May 2017, results from randomized, double-blind, placebo-controlled FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) trial involving more than 27,500 patients with atherosclerotic cardiovascular disease who were receiving statin therapy, were published. The primary efficacy end point was the composite of myocardial infarction, stroke, hospitalization for unstable angina, coronary revascularization or cardiovascular death, and the median duration of follow-up was 2.2 years. Patients were randomly assigned to receive evolocumab or matching placebo as an addition to standard statin therapy. Results showed that reduction in LDL cholesterol levels with evolocumab was from a median baseline value of 2.4 mmol/l to 0.8 mmol/l and, relative to placebo, evolocumab treatment significantly reduced the risk (15%) of the primary end point (9.8% in evlocumab group vs. 11.3% in placebo group, $p < 0.001$). There was no significant difference between the study groups with regard to adverse events (including new-onset diabetes and neurocognitive events), with the exception of injection-site reactions, which were more common with evolocumab (2.1% vs. 1.6%) (13). Secondary analysis of this study showed that patients who achieved progressively lower LDL-C concentrations in the FOURIER trial had progressively fewer cardiovascular events with no evidence of a plateau and with no increase in adverse events. Although most evaluated safety endpoints would be expected to manifest within the 2.2 years of follow-up, the adverse events such as cancer can take longer to manifest. Consequently, two long-term extension studies of FOURIER following approximately 6,600 patients (NCT03080935 and NCT02867813) that are planned to last 5 years should provide longer-term insights (14).

Alirocumab was investigated during 14 phase III trials within ODYSSEY program, which included

more than 23,500 patients with uncontrolled hypercholesterolemia (those with FH, those at high CV risk and those with statin intolerance) (11).

Most attention was attracted by ODYSSEY LONG TERM trial which was aimed to obtain longer-term data on safety and reduction in LDL-C levels by alirocumab, but the data on the incidence of CV events was also collected for a post hoc analysis. The study included 2,341 patients at high CV risk (patients with heterozygous FH with or without documented CV disease or patients with hypercholesterolemia and documented CV disease) who had LDL-C levels ≥70 mg/dL (1.8 mmol/L) despite therapy with statins at maximally tolerated dose, with or without other lipid-lowering drugs. Patients received alirocumab 150 mg or placebo every 2 weeks for 78 weeks. After 24 weeks, there was a significant difference in the LDL-C levels reduction between alirocumab and placebo (62%, $p < 0.001$), with the mean absolute reduction of 74 mg/dL (1.9 mmol/L) and 4 mg/dL (0.1 mmol/L), respectively. This effect remained consistent over a period of 78 weeks. The percentage of patients with any adverse events was similar in the two groups (81% with alirocumab and 82.5% with placebo). Specific adverse events that were reported with higher rates in the alirocumab group involve injection-site reaction, myalgia, neurocognitive events (amnesia, memory impairment, confusional state) and ophthalmologic events (15).

It should be noted that in a post hoc analysis there was a reduction in the rate of CV events (CV death, nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, unstable angina requiring hospitalization) in patients who received alirocumab (1.7%) compared to patients who received placebo (3.3%) ($p = 0.02$) (15). Given that the number of CV events was small, a follow-up period relatively short, and that post hoc analysis was applied, the obtained data must be confirmed additionally.

ODYSSEY OUTCOMES trial will assess whether alirocumab significantly reduces CV morbidity and mortality in more than 18,000 patients with recent acute coronary syndrome. Results of the study are expected in 2018 (11).

ApoB synthesis inhibitor – mipomersen

Mipomersen was approved in the United States of America (USA) for treating patients with homozygous FH as an addition to diet and maximally tolerated doses of statins and other lipid-lowering medications (16). However, in 2013, The Committee for Medicinal Products for Human Use (CHMP) of European Medicines Agency (EMA) recommended, for the second time, the refusal of the marketing authorisation for the mipomersen in European Union (EU). Reasons are as follows: a high proportion of patients stopped taking the medicine within two years (even in the group of patients with homozygous FH) mainly due to side effects, then, long-term consequences of a built-up of fat in the liver and increased enzyme levels and potential irreversible liver damage, as well as more CV events reported in patients taking this medicine (17).

Mipomersen is a drug which can reduce LDL-C level by an additional 25% in homozygous FH patients when given in combination with maximum tolerated doses of lipid-lowering agents. It is an „anti sense" oligonucleotide, 20 nucleotides in length, designed to block the production of apoB by attaching to the genetic material of cells responsible for producing it (Figure 2) (18).

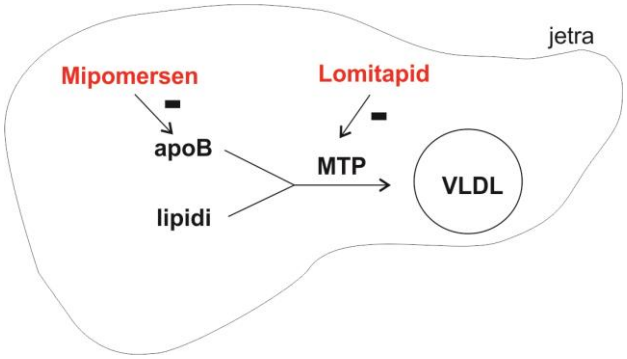

Figure 2. Mechanism of action of mipomersen and lomitapide
apoB – apolipoprotein B;
MTP - microsomal triglyceride transfer protein;
VLDL - very low-density lipoproteins.

ApoB is a crucial structural and functional component of all atherogenic lipoproteins. The human APOB gene encodes a single RNA transcript from which 2 isoforms, apoB-100 and apoB-48, are translated as a result of messenger RNA editing. ApoB-100, which is an essential structural component of very low-density lipoproteins (VLDL) and LDL-C, is produced predominantly by hepatocytes, while apoB-48, which is an essential structural component of chylomicrons, is produced predominantly by enterocytes. Mipomersen is complementary to the coding region of the messenger RNA for apo B-100. The hybridization of mipomersen to the target messen-ger RNA results in ribonuclease H1-mediated degradation of messenger RNK, leading to the reduction of apoB-100 production and hence, synthesis of VLDL and LDL-C is reduced (18, 19).

It should be noted that apoB has been identified as a new risk factor for atherosclerosis development. It is shown that apoB level is correlated to CV risk (19). The AMORIS study (Apolipoprotein-related MOrtality RISk study) indicated that elevated apoB is a more significant predictor of fatal myocardial infarction than LDL-C alone (20).

Mipomersen is administrated subcutaneously, usually in the dose of 200 mg once weekly. Following subcutaneous injection, maximal plasma concentrations are reached in 3 to 4 hours, while bioavailability of mipomersen ranged from 54% to 78%. Mipomersen is highly bound to human plasma proteins (≥ 90%) and, with once weekly dosing, plasma levels approach steady state typically within 6 months. Elimination half-life for mipomersen is approximately 1 to 2 months. This drug is not a substrate for the CYP450 metabolism, so, there are no clinically rele-

vant pharmacokinetic interactions between mipomersen and warfarin or between mipomersen and simvastatin or ezetimibe (18).

In clinical trials, mipomersen has been studied in healthy volunteers (21), in patients on statin therapy (22, 23) and in patients with FH (24-26).

In a study which involved 36 healthy volunteers with mild hypercholesterolemia, administration of mipomersen in doses ranging from 50 to 200 mg for 4 weeks, showed a dose-dependent and prolonged reduction in apoB and LDL-C with the maximum reduction from baseline of 50% and 35%, respectively, in the 200-mg dose group (21). In addition, a study (Dose-Escalating Safety Study in Subjects on Stable Statin Therapy) which involved 74 patients on stable statin therapy, showed that application of mipomersen during 5 weeks reduced LDL-C levels ranged from 18% to 49% relative to the placebo-control group at doses of 100 mg and higher. Within this study, the reduction in apoB and LDL-C of 36% from baseline was demonstrated in a group of 12 patients treated with 200 mg mipomersen over 13 weeks, what is more significant than the effect observed in the same-dose 5-week treatment group (22).

The efficacy of mipomersen therapy in patients with FH was confirmed in several phase II and III trials on the basis of which it is approved for this indication by the US Food and Drug Administration (FDA). For example, mipomersen was studied in phase III trial which involved 51 patients with homozygous FH already receiving maximum tolerated dose of lipid-lowering medications. The primary endpoint was percentage change in LDL-C concentration from baseline. After 26 weeks, patients receiving 200 mg of mipomersen subcutaneously once weekly had more significant LDL-C reduction (-24.7%) compared with placebo (-3.3%, $p = 0.0003$). The most common adverse events were injection site reactions (76% mipomersen vs. 24% placebo), and 4 patients (12%) receiving mipomersen had elevation in ALT ≥ 3x the upper limit of normal (21).

The adverse reactions of mipomersen for pooled Phase III clinical trials are: injection site reactions (84%), flu-like symptoms (~ 30%, which typically occur within 2 days after an injection), elevations in serum transaminases (specifically ALT, ~ 10%), hepatic steatosis (with or without concomitants increases in transaminases), gastrointestinal (30%), neurological (25%) and psychiatric (10%) disorders. Due to the risk of hepatotoxicity, mipomersen is available only trough a limited REMS (Risk Evaluation and Mitigation Strategy) program in which it could be prescribed and distributed only by certified physicians and pharmacists aiming to monitor patients because of the risk of side effects (18).

MTP inhibitor – lomitapide

Lomitapide was approved in 2012 by FDA, in 2013 by EMA, followed by marketing authorisation in Canada, Mexico, Taiwan and Japan for treating patients with homozygous FH as an addition to diet and maximum tolerated doses of statins and other lipid-lowering medications (11, 27-29). In homozygous FH patients, lomitapide can additionally reduce LDL-C levels by up to by 50%.

Lomitapide is an orally active, small molecule that directly binds to MTP, a protein localized in an endoplasmic reticulum, the role of which is to transport triglycerides, phospholipids and cholesterol esters to the newly synthesized apoB. This is a key step in the synthesis of VLDL in hepatocytes and chylimicrons in enterocytes (Figure 2) (30, 31). Inhibition of this protein in hepatocytes leads to a decrease in the VLDL concentration in the circulation and, consequently, to a decrease in LDL-C (32). In addition, inhibition of MTP in enterocytes can reduce plasma triglyceride levels by reducing fat absorption from foods through chylomicron (1).

After oral administration, the bioavailability of lomitapide is 7%, which is largely due to the extensive first pass effect. Maximum plasma concentrations were reached after 4-8 hours, and despite a high binding rate for plasma proteins (> 99.8%), it has a high volume of distribution. Lomitapide has an extensive liver metabolism, predominantly via CYP450 3A4, while other isoforms (2E1, 1A2, 2B6, 2C8 and 2C19) are involved to a lesser extent. The elimination half-life of lomitapide was approximately 29 hours. Because lomitapide is metabolized by CYP3A4, concomitant use with strong or moderate CYP3A4 inhibitors (e.g., antifungal azoles such as itraconazole, fluconazole, ketoconazole, voriconazole, posaconazole; macrolide antibiotics such as erythromycin or clarithromycin; ketolide antibiotics such as telithromycin; HIV protease inhibitors; the calcium channel blockers diltiazem and verapamil, and the anti-arrhythmic dronedarone) is contraindicated.

If treatment with these drugs is unavoidable, lomitapide should be stopped during the course of treatment. When administered with atorvastatin, a weak CYP3A4 inhibitor, the dose of lomitapide should either be taken 12 hours apart or be decreased by half. Lomitapide increases plasma concentrations of statins, which increases the risk of myopathy, requiring additional caution and monitoring. Concomitant administration of > 40 mg simvastatin is contraindicated. There is no need for dose adjustment in concomitant administration with fenofibrate, niacin or ezetimibe. Also, lomitapide increases the plasma concentration of warfarin, so regular monitoring of the INR (international normalized ratio) is recommended. Simultaneous use of St. John's Wort, grapefruit and alcohol with this drug should be avoided, and it may affect the absorption of fat-soluble nutrients (e.g. vitamin E) (33).

Lomitapide was studied in several phase II and III trials. For example, in the phase III HoFH Lomitapide Study, efficacy and safety of lomitapide was investigated in 29 patients with homozygous FH receiving appropriate lipid-lowering therapy. The primary endpoint was mean percent change in levels of LDL-C from baseline to week 26, after which patients remained on lomitapide through to week 78 for safety assessment. Lomitapide, at median dose of 40 mg (a starting dose was 5 mg and then escalated to the maximum of 60 mg), reduced LDL-C by 50%, from 8.7 mmol/L at baseline to 4.3 mmol/L after 26 weeks ($p < 0.0001$). Concentrations of LDL-C remained reduced by 44% ($p < 0.0001$) at week 56 and 38% ($p < 0.0001$) at week 78. Changes in levels of total cholesterol, apoB and triglycerides were similar with

change in LDL-C. During this study, side effects of lomitapide were mainly related to the gastrointestinal system. Four patients had AST/ALT > 5x the upper limit of normal, which resolved after dose reduction or temporary interruption of lomitapide, but there were no permanent discontinuation of treatment because of liver abnormalities (34).

The most serious adverse reactions during treatment with lomitapide were just liver aminotransferase abnormalities. Hepatic steatosis (with or without concomitants increases in transaminases) is also reported, and because of the risk of hepatotoxicity, like mipomersen, it is available trough the REMS program (33).

The most common adverse events of lomitapide, reported by 93% of patients, were gastrointestinal. Among them, diarrhoea occurred in 79% of patients, nausea in 65%, dyspepsia in 38%, vomiting in 34%, while flatulence, constipation and abdominal pain were reported by at least 20% of patients. These side effects are the result of its mechanism of action, and may affect the absorption of the concomitantly administered oral drugs (33).

Due to the fetal toxicity, based on findings of teratogenicity animals, females of reproductive potential should have a negative pregnancy test before starting lomitapide and use contraception during treatment (33).

Bempedoic acid

Bempedoic acid is a novel, oral LDL-C lowering drug that significantly reduces elevated LDL-C levels in patients with hypercholesterolemia, including patients inadequately treated with current lipid-modifying therapies. It works in the liver to block cholesterol biosynthesis by direct ATP citrate lyase (ACL) inhibition, a key enzyme that supplies substrate for cholesterol and fatty acid synthesis in the liver, resulting in up-regulation of LDL receptor (35).

To date, bempedoic acid has been studied in eighteen completed Phase 1 and Phase 2 clinical studies which showed clinically relevant LDL-C lowering results of up to 30% as monotherapy, approximately 50% in combination with ezetimibe, and an incremental 20% + when added to stable statin therapy (any statin at any dose). Currently, bempedoic acid is evaluated in four global Phase 3 LDL-C lowering efficacy and safety studies on approximately 3,200 patients at high CV risk with hypercholesterolemia on optimized background lipid-modifying therapy, and one cardiovascular outcomes trial (Cholesterol Lowering via BEmpedoic Acid, an ACL-inhibiting Regimen (CLEAR) Outcomes) which is expected to enrol approximately 12,600 patients in approximately 30 countries (35, 36). In addition, initiation of a single global Phase 3 bridging study (1002FDC-053), to evaluate the efficacy and safety of the bempedoic acid / ezetimibe combination, is planned and it is expected to enroll up to 350 patients (37). Results of Phase III program are expected during 2018 (36, 37).

CETP inhibitors

In recent years, extensive research is being carried out to identify new HDL-raising drugs hoping that this would lead to further reduction of cardiovascular risk. Currently, CETP inhibitors are being actively studied for that purpose (38).

The role of CETP is to promote the transfer of cholesteryl esters between plasma lipoproteins, therefore, CETP inhibition raises HDL-C levels and decreases LDL-C levels (Figure 3) (39). Epidemiological data have clearly demonstrated a strong inverse relationship between HDL-C levels and the risk of CV events (40-42).

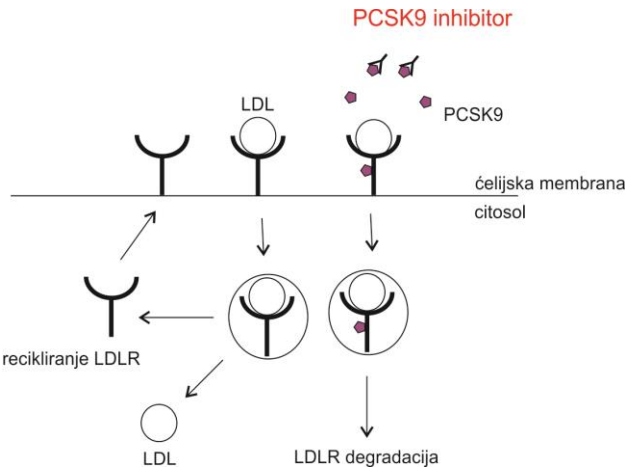

Figure 3. Mechanism of action of CETP (cholesteryl ester transfer protein) inhibitors
VLDL – very low-density lipoproteins;
IDL – intermediate density lipoprotein;
LDL - low-density lipoproteins;
HDL – high-density lipoproteins;
CE - cholesteryl esters; TG – triglyceride.

Torcetrapib, dalcetrapib, evacetrapib and anacetrapib are CETP inhibitors reaching phase III clinical trials. However, the torcetrapib trial was terminated prematurely because of mortality increase (CV and overall). Since the increase in mortality and increase in blood pressure (5.4 mmHg) was probably not caused by mechanism of action of torcetrapib, but, at least in part, by increased level of aldosterone as well as plasma electrolyte composition changes - lower potassium levels and increased sodium and bicarbonate levels (43), the testing of new CETP inhibitors is continued. Trials with dalcetrapib and evacetrapib were terminated due to insufficient efficacy. Although these drugs significantly raised HDL-C level, they failed to show a significant reduction in cardiovascular events (44, 45). Effects of anacetrapib on cardiovascular outcomes are currently being evaluated in the ongoing REVEAL (Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification) trial, a phase III trial on over 30,000 patients with atherosclerotic vascular disease who were receiving intensive atorvastatin therapy. Although the study completion is planned for 2019, the first results, after the median follow-up period of 4.1 years, showed that the addition of anacetrapib to intensive statin therapy resulted in a lower incidence

of major coronary events (coronary death, myocardial infarction, coronary revascularization) than the addition of placebo, despite very well-controlled baseline LDL cholesterol levels. The proportional risk reductions appeared to be larger with more prolonged follow-up. Anacetrapib did not affect the mortality rate (from cardiovascular or non-cardiovascular causes), the incidence of cancer, or any significant excesses in any major category of the adverse events. Patients who received anacetrapib had slightly higher blood pressure levels (0.7 mmHg systolic and 0.3 mmHg diastolic) compared to the placebo group, but there was no difference in the rate of adverse effects associated with hypertension. Also, there were slightly higher rates of moderate elevations in creatine kinase in the anacetrapib group than in the placebo group, but slightly lower rates of more severe elevations (> 40 times the upper limit of the normal range) (46). Results of this trial are expected to provide a final answer to the question whether CETP inhibitors might actually be effective for the reduction of cardiovascular risk or further investigation of this group of drugs should be discontinued.

Conclusion

Results of numerous clinical studies conducted during the past three decades clearly show that statin therapy significantly reduces the level of LDL-C as well as the occurrence of CV events and mortality. However, a significant number of patients using statins do not achieve the desired levels of LDL-C, especially those with high or very high CV risk. In addition, patients intolerant of statins should also be mentioned, as well as those with contraindications to these drugs. For these reasons, there is a need for some new lipid-lowering agents in therapy, which will further reduce not only the level of LDL-C, but also the occurrence of CV events.

Currently, the most promising agents are PCSK9 inhibitors. It has been shown that they produce significant LDL-C level reduction, and that they are well tolerated by patients. They are approved for use in combination with statins for the treatment of heterozygous FH, and in patients with clinical atherosclerotic CV diseases. In addition, evolocumab is approved for use in patients with homozygous FH.

Mipomersen, apoB synthesis inhibitor, and lomitapide, an oral MTP inhibitor, are currently approved, for treating patients with homozygous FH as an addition to statins and other lipid-lowering medications. Their usage is significantly limited by the high risk of hepatotoxicity.

Although the new lipid-lowering agents produce significant LDL-C level reduction, it is necessary to conduct longer trials with more patients, confirming their efficacy and safety, thus enabling their wider use for the treatment of dyslipidemia.

Conflict of interest

Authors declare no conflict of interest.

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NOVI LEKOVI U TERAPIJI DISLIPIDEMIJA

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Dislipidemije su vodeći faktor rizika za razvoj ateroskleroze i njenih posledica, kao što su koronarna bolest srca, ishemična cerebrovaskularna i periferna vaskularna bolest. Ove bolesti su glavni uzrok mortaliteta, kako u svetu tako i u Evropi, gde su odgovorne za 45% ukupne smrtnosti. Terapija dislipidemija uključuje primenu: statina, ezetimiba, fibrata, niacina, smola koje vezuju žučne kiseline i omega-3 masnih kiselina. Od pomenutih lekova, vodeću ulogu u terapiji dislipidemija imaju statini. Treba istaći da uprkos primeni statina, koji redukuju rizik od pojave kardiovaskularnih (KV) događaja za oko 30%, još uvek ostaje tzv. rezidualni rizik za nastanak KV događaja, što ukazuje da su potrebni novi lekovi koji će dalje redukovati rezidualni rizik. Novi lekovi u terapiji dislipidemija uključuju PCSK9 inhibitore, inhibitore sinteze apolipoproteina B (apoB), MTP inhibitore i CETP inhibitore, koji su ili već odobreni za primenu u određenim indikacijama, ili se nalaze u odmaklim fazama kliničkog ispitivanja. Alirokumab i evolokumab, dva PCSK9 inhibitora, odobrena su za primenu, u kombinaciji sa statinima, u terapiji heterozigotne familijarne hiperholesterolemije (FH), kao i kod bolesnika sa kliničkim aterosklerotičnim KV bolestima koji zahtevaju dodatnu redukciju nivoa lipoproteina male gustine (low-density lipoprotein cholesterol, LDL-C). Pored toga, evolokumab je odobren za primenu kod bolesnika sa homozigotnom FH. Mipomersen, inhibitor sinteze apoB, i lomitapid, oralni MTP inhibitor, su, odobreni za primenu samo kod bolesnika sa homozigotnom FH kao dodatak maksimalnoj tolerišućoj dozi statina i drugih hipolipemika. Iako novi hipolipemici značajno redukuju nivo LDL-C, neophodno je sprovesti studije, duže i sa većim brojem ispitanika, koje će potvrditi njihovu efikasnost i bezbednost i time omogućiti njihovu širu primenu u terapiji dislipidemija.

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Ključne reči: *dislipidemije, PCSK9 inhibitori, mipomersen, lomitapid, CETP inhibitori*

Review article

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MEDICAL SIMULATION: MORAL AND ETHICAL ISSUES

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Medical simulation is now widespread as an integral part of medical education. Simulation begins with an important moral claim: we must do the best we can to keep patients safe while training the next generation of clinicians and retraining current clinicians so that they are kept up-to-date. As a powerful teaching tool, simulation allows practicing communication, decision-making, practical skills and leadership as well as evaluation which can be standardized and poses no risk to patients associated with experiential learning conducted in the actual clinical setting. It is also the fact that simulation raises ethical questions of its own. That training is not simply technical. It is also a way to learn and practice dealing with the emotional challenges of real-life ethical situations. Simulation also provides a safe zone for students to make practical skills and communication mistakes and to develop moral imagination. Despite the growing acceptance of clinical simulation to enhance quality and safety in medical education, the question of whether students actually acquire and transfer the ethical principles that takes place in a simulation setting is unknown.

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Key words: *medical simulation, education, ethics*

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Background

Medical simulation is a widely used method for practicing communication, decision-making, practical skills and leadership (1) in an effort to increase patient safety (2). The three most important imperatives in simulation are: the safety of patients, students and faculty members; preventing errors, and enabling engaged learning. Many ethical issues encountered in everyday practice can be seen in simulation as well. Clinicians who have already challenged many problems and have more "experience" with mannequins make fewer mistakes while treating real patients. Ethics is not an add-on to simulation; it is an integral part of simulation and it is learned during simulation. Simulation begins with an important moral claim: we must do the best we can to keep patients safe while training the next generation of clinicians and retraining current clinicians so that they are kept up-to-date (3) and able to implement current medical guidelines.

In practice, guidelines are based on propositional knowledge (fictive/declarative) derived from the best available domain-specific evidence and on the domain expert consensus on the current best practices. Whether designed for individual professionals or for health delivery systems, the guidelines regularly lack information regarding how they will be adequately implemented so they may be indeed necessary but not sufficient for providing good outcomes, given their strategic incompleteness. Their effectiveness on the ground depends first on the quality of their actual propositional content knowledge and second, on how it is implemented ("know-how") (4-7). However, the regular absence of implementation knowledge in the formation of guidelines also lacks moral justification, in addition to epistemic justification (8). Every implementation process involves learning curve period, as the implementers and end users figure out how to translate guidelines into practice and apply the new system in everyday clinical practice. It is the medical simulations that provide gaining critical knowledge for supporting ethical controls to guideline implementation, adding a much needed epistemic and ethical grounding for minimizing harms to every learning cycle.

Simulation-based medical education (SBME) is increasingly recommended, as an educational strategy and for improving patient safety. SBME has largely been conducted in an off-site simulation (OSS) setting in simulation centers, which range widely from publically financed simulation centers at hospitals and universities to simulation centers that are detached facilities funded by sponsors and user payment. Some hospital departments also provide OSS as in-house training room(s) specifically set up for simulation training away from the clinical setting but within the hospital department (9).

Why is medical simulation so important for teaching?

Overall, SBME is a complex educational intervention. SBME was defined by Issenberg et al. (10) as: "In broad, simple terms, a simulation is a person, device, or set of conditions which attempts to present education and evaluation problems authentically. The student or trainee is required to respond to the problems as he or she would under natural circumstances". Simulation techniques and devices can comprise, for example high-tech virtual reality simulators, full-scale mannequins, plastic models, instructed or standardized patients, animal or animal products, human cadavers, or screen-based simulators.

In a typical setting, a teacher designs a simulation-based clinical scenario using various teaching tools such as manikins, task trainers, standardized patients (SPs), or role-play by staff or students. Students then participate, with facilitator guidance, by demonstrating the key integrated skills, tasks, and decision-making according to predetermined objectives. Following the simulation experience, a structured faculty-facilitated debriefing and discussion are conducted in order to highlight the most important part of the lesson and to allow maximal long-lasting learning through reflection. Debriefing is important because it emphasizes the educational principles and objectives, and identifies gaps in a learner's knowledge or skill. Simulation is a powerful teaching tool (11).

What makes simulation so unique is that no patient is at any risk associated with experimental learning conducted in the actual clinical setting (12). Also, simulation makes a bridge between didactic and observational learning to clinical practice. It allows deliberate practice and learning experience by repetition prior to or in parallel with traditional one besides training (13). Also, it enables learners to develop skills such as communication competence and other non-technical skills (14).

Teamwork has become a major focus in healthcare. Teamwork can be defined as the ability of team members to work together, communicate effectively, anticipate and meet one another's demands, and inspire confidence resulting in a coordinated collective action (15). The teamwork is required for effective patient management because of the increased specialization of tasks, the increased complexity and risks associated with treatment options, and the need to ensure appropriate healthcare outcomes and patient safety (16).

Based on the classic Bloom's taxonomy of learning objectives (17), simulation-based inductive learning promotes learning at the highest levels of all three objectives including cognitive (analyze + synthesis + evaluation), affective (organizing + characterizing) and psychomotor (adaptation + origination) abilities.

Kwanet al. (18) determined the effectiveness of simulation in improving student confidence in acute coronary syndrome (ACS) and the Advanced Cardiac Life Support (ACLS) curriculum. Secondary goal of their study included examining student perceptions of the role of simulation in medical education. Third-year medical students enrolled in the internal medicine clerkship between January and June 2014 attended a simulation course during their ambulatory block. Following a 2-hour session, participants completed a 17-item questionnaire. Students reported improvements in their ability to identify and manage ACS and ACLS before and after the simulation course: 93% felt that simulation boosted their self-confidence in performing these tasks on a real patient; 86% reported receiving useful feedback during the training sessions; 98% agreed that their experience was enjoyable; and 95% would recommend this course to other students.

Wayne et al. (19) assessed baseline proficiency in ACLS among internal medicine residents and determined that performance improved significantly after simulator training.

Ojha et al. (7) conducted an online search of original and review articles related to simulation and pediatric medical education and provided an overview of the role and utility of simulation in pediatrics that included 147 studies, and concluded that simulation had tremendous potential as a teaching and assessment tool for pediatric acute care providers.

The research evidence is clear that high-fidelity medical simulations facilitate learning among trainees when used under the right conditions (10). Several important features and aspects of simulation-based learning that will lead to effective learning are summarized in Table 1. McGaghie et al. (20) identified and discussed 12 features and best practices of SBME that medical educators should know and use. The first five are named in Issenberg's review (10) followed by the seven that are evident from later researches and practice (Table 2).

Simulation-based medical training is an "ethical imperative"

An ethical imperative has been presented for using simulation to train health care professionals. Simulation leads to better-educated students who developed a more humanistic care towards patients. Every year, millions of people die or get seriously injured due to errors in health practices. Therefore, it is crucial that safe care is enabled and medical errors

Table 1. Features and uses of high-fidelity simulators that lead to effective learning (10)

Important features and aspects of simulators
Feedback is provided during the learning experience
Learners engage in repetitive practice
Simulation is integrated into the overall curriculum
Learners practice with increasing levels of difficulty
Adaptable to multiple learning strategies
Clinical variation
Controlled environment
Individualized learning (in addition to team)
Outcomes / benchmarks clearly defined
Validity of simulator

Table 2. Medical simulation and gaps in understanding (adapted from McGaghie et al. (20)

Simulation features	Gaps in understanding
Feedback	What model of feedback?
Deliberate practice	Verify value of distributed practice versus massed practice
Curriculum integration	How and when to best integrate with other modalities?
Outcome measurement	Narrow bandwidth versus complex professional practise
Simulation fidelity	How does trainee readiness shape simulation use?
Skill acquisition and maintenance	What are the mechanisms of skill maintenance?
Mastery learning	What are the sources of variation in time to mastery standard: cognitive aptitude, motor skill, professional experience?
Transfer to practice	Pathway: simulation laboratory - health care
Team training	Team skill maintenance
High-stakes testing	Creation of test mechanisms (for crisis management e.g. resuscitation)
Instructor training	Should simulation instructors be certified for various devices?
Educational and professional context	How to break down barriers and overcome inertia?

are minimized in clinical practices and rapid response is given to changing health conditions. Ziv et al. (12) discuss that training in simulated contexts before practitioners interecting directly with patients should be mandatory for ethical clinical care. Simulation activities need to ensure patient safety without danger of harming the patient during the learning process. Hence, students would be allowed to perform medical procedures on actual patients after acquiring knowledge and skills in various simulated clinical scenarios. Simulation scenario is critical in the preparation of students and clinicians prior to the first actual patient experience including collaboration through team training, interprofessional, critical thinking, and independent decision-making skills (21, 22). Simulation scenarios also help young doctors and specialists in refining advanced techniques. The vital component in these scenarios is the prebriefing and debriefing. Especially, effective feedback and debriefing after the simulation can provide the opportunity to learn about ethical knowledge gaps (23). In other words, simulation training also assists in the determination of ethical problems that relate to principles of autonomy, beneficence, justice, informed consent, non-maleficence, fairness, truthfulness, advocacy, and dedication.

For sure, simulations can be stressful to students. They may experience both physiological and psychological stress while thinking how to perform a simulation and how to address a virtual patient. Also, they do not feel comfortable being videotaped and they feel anticipatory anxiety by thinking about

the feedback from the faculty. A key part of learning is the debriefing session. It is also a skill that needs to be exercised during every simulation. It is not something that should be taken easily and happening only if there is time before the next scheduled task. Facilities where simulation is performed must be safe places, where students can both learn and make mistakes. Even some level of stress is good, highlighting the awareness of the situation. In that way, an impact on students is made i.e. in a real-life situation they will act more effectively and efficiently. At the same time students should not be placed in situations where the level of stress is too high, because it may affect their cognitive abilities (3).

Many hospitals and medical schools have begun using high-fidelity simulation medicine in their educational curriculum. Despite the growing popularity of clinical simulation to improve quality and safety in healthcare education, the question of whether students actually transfer the ethical values that takes place in a simulation setting is unknown.

How to teach ethics using simulation?

Teaching ethics and using simulation share a common obstacle which is lack of faculty resources (24). There is a clear distinction between teaching clinical content and medical ethics. Ethical principles are based on many factors like social science, medical science, religion, law, economy, culture, language, and more. Many different ethical considerations are more difficult to teach than any medical issue. This renders facilitation of simulation scenarios designed to teach medical ethics more complicated and difficult than topics with a limited focus. It may be a reason why many medical students may feel underprepared to deal with the emotional challenges of real-life ethical situations.

Simulation also provides a safe zone for students to make communication mistakes (14) and to develop a moral imagination. The moral imagination is necessary because it represents a capability to feel empathy with others, thus making right ethical decisions. Unfortunately, during medical education clinicians receive little or no training in dealing with death, dying and end of life issues. The biggest and only concern about allowing the mannequin to die is the psychological safety of the students. Prior to implementing a scenario where a simulating patient must die, discussion must be made among the members of the simulation team.

Conclusion

At the end of their education, medical students are expected to be able to transfer their experiences from this education process to real life and to find a solution more easily for problems encountered in the everyday clinical practice. Many various scenarios fictionalized in simulation education bring along useful ethical approaches.

Invaluable importance of simulation is that students get a personal experience by this method while having the opportunity to share it with others. Great asset of simulation as a supporting learning is the feedbacks of the teachers. It is up to educators who must provide evidence-based strategies to enrich the students' clinical judgment. It is indicated that high-fidelity manikin-based simulation contributes not only to the development of the students' skills and medical knowledge, but also supports achieving communication, leadership and team collaboration.

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MEDICINSKA SIMULACIJA: MORALNA I ETIČKA PITANJA

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Medicinska simulacija je danas široko rasprostranjena kao deo medicinske edukacije. Osnove simulacije temelje se na važnom moralnom principu: moramo da učinimo sve što možemo kako bi sačuvali bezbednost bolesnika, kako tokom obuke budućih generacija lekara tako i tokom sticanja novih znanja i veština lekara brojnih specijalnosti. Kao izuzetno korisno sredstvo učenja, simulacija omogućava vežbanje komunikacije, donošenje odluka, praktičnih veština i rukovođenja, uz mogućnost analize i procene uspešnosti u standardizovanim uslovima bez rizika po zdravlje bolesnika - što nije odlika učenja zasnovanog na iskustvu koje se dobija u kliničkom okruženju. Činjenica je, takođe, da simulacija sama po sebi nosi brojna etička pitanja proistekla iz toga što ova vrsta vežbi nije samo tehničke prirode, već istovremeno predstavlja i učenje prihvatanja emocionalnih izazova sa kojima se susreće u realnim okolnostima. Simulacija istovremeno obezbeđuje zonu sigurnosti u okviru koje je studentima omogućeno da greše u izvođenju praktičnih veština i komunikaciji, kao i da razviju moralnu imaginaciju. Uprkos rastućem prihvatanju kliničke simulacije, kako bi se poboljšao kvalitet i bezbednost medicinske edukacije, ostaje otvoreno pitanje u kojoj meri studenti zaista primenjuju etičke principe koje su usvojili tokom vežbanja simulacionih scenarija.

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Ključne reči: *medicinska simulacija, edukacija, etika*

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THE ROLE OF ULTRASONOGRAPHY IN DIFFERENTIATING BETWEEN BENIGN AND MALIGNANT OVARIAN TUMORS IN POSTMENOPAUSAL WOMEN

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Malignant ovarian tumors occur at all ages, with the total incidence dramatically increasing with age. Ovarian cancer survival rate depends on the stage at which the disease is detected.

The aim of this study was to determine sensitivity, specificity and predictive values of the tests for pre-operative monitoring of the state of ovarian tumors in postmenopausal women and to assess possible malignant potential and examine the correlation of clinical finding and significance of ultrasonography in differentiating benign from malignant ovarian tumors.

The research is defined by the models of prospective-retrospective study, involving 60 postmenopausal women diagnosed with ovarian tumors.

The following medical tests and examinations were performed for all patients: anamnestic analysis of the medical records (history of the disease with the data on age, parity, duration of menopause, use of oral contraceptives, symptoms, and small pelvis sonography).

There is an age difference between the women with benign ovarian tumors and those with malignant ones. Women with benign tumors had 1.96 children on the average, compared to the average of 1.40 children of women with malignant ovarian tumors. Duration of oral contraceptive use in women with benign changes was 2.84 years on the average and 1.27 years was the average of women with a malignant process, showing a high statistical significance of 5%. Among the subjects with benign tumors, the dominant tumor structure was cystic, as opposed to mixed-type tumors in those with malignancies. Tumor location is, with a high statistical significance, more often bilateral in subjects with histopathologically confirmed malignant tumors, while it is predominantly unilateral in benign tumors. Tumor size is a reliable factor in differentiating benign from malignant ovarian tumors. The wall thickness of benign tumors is higher in relation to that in malignant ones. The presence of free fluid in the pouch of Douglas is rare in benign ovarian tumors, while it is quite frequent in malignant ones.

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Key words: *ultrasonography, ovarian tumors, postmenopausal*

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Introduction

In the last few decades, the incidence of morbidity and mortality from malignant ovarian tumors is constantly on the rise. Therefore, they represent a constant challenge for gynecologists-clinicians. To date, the true reason for this phenomenon has not been clarified. Ovarian carcinoma makes 5% of all malignant tumors in women. Although ovarian cancer accounts for 23% of all gynecologic cancers, 47% of all deaths due to malignancy of genital organs were caused by ovarian cancer (1). The data from Western Europe, the U.S. and Canada, indicate a high inci dence, with 10-15 out of 100.000 women being affected (2). The total incidence rate for Europe is 13,4 women out of 100.000. The incidence of this malignancy in our country is between 9,1 and 11,3/100.000 women and there has been an unbroken increasing trend (3).

Malignant ovarian cancers occur at all ages, including early childhood, but also in advanced old age, with the total incidence dramatically increasing with age. The risk of developing ovarian cancer increases after the age of 40, with the incidence peak between 50-55 years. Ovarian cancer is in the fifth place, immediately after breast, lung, rectosigmoid colon and lymphoma cancers, with the highest mortality rate among all gynecological cancers (4,5).

The survival rate for ovarian cancer depends on the stage of the disease at detection. In this respect, the survival rate is 93% in stage I, 70% in stage II, 37% in stage III and 20% in stage IV. Three-quarters of newly discovered ovarian cancers are in stage III and IV, where the five-year survival rate is below 50%. This suggests the importance of early detection and timely treatment of ovarian cancer.

The development of ovarian cancers is influenced by many factors. Hereditary, environmental factors, prior pregnancies, breast feeding, oral contraceptives, infertility, substitution hormone therapy, oncogenic viruses, etc. should be mentioned in this regard.

Hereditary factors have a major impact on the development of ovarian cancer. Although in recent years much has been written about prophylactic oophorectomy, Chen et al. reported that in women with familial history of ovarian cancer, three years after prophylactic oophorectomy, intraabdominal carcinomatosis has occurred (6). Regardless of this phenomenon, for women with a family history of ovarian cancer who have completed their reproductive function prophylactic oophorectomy represents the recommended therapeutic approach (7, 8).

Pregnancy has been proven to have a protective effect, i.e. it prevents the development of malignant ovarian tumors. The study of the patients with these tumors confirmed that they had fewer pregnancies and childbirths, they were older at the time of first gravidity, and they had a greater incidence of spontaneous abortions than the healthy women used as controls. This claim was confirmed by joint studies of Whittemore and associates in America and in Europe, as well as by the studies in Canada and Sweden (9).

Many studies indicate the protective effect of breastfeeding. It is believed that this protective effect works by way of ovulation suppression. Whittemore and associates estimate that with each month of breastfeeding, the risk is reduced by 1% (9).

Infertility can be one of the significant risk factors for the development of ovarian cancer. This is explained by an increased exposure to drugs which stimulate ovulation.

Combined oral contraceptives reduce the risk of ovarian cancer. This protective effect is the strongest finding in all epidemiological studies of epithelial ovarian cancer. The risk is reduced proportionally to the duration of oral contraceptive use. A reduction in the risk of ovarian cancer is estimated to be 11% for each year of use, and 46% after 5 years of oral contraceptive use. The protective effect lasts for 10 years after taking oral contraceptives (1, 10).

There are many opinions about the effect of hormonal substitution therapy. Some studies show a reduction in risk; other studies do not show that the use of supstitution therapy affects the occurrence and activity of ovarian tumors in postmenopausal women [23].

Recently conducted epidemiological studies indicate that environmental factors have a major impact on the appearance of ovarian tumors. Ovarian cancers more often occur in women living in highly industrialized countries (for example, the incidence in Jewish women who live in Israel and the U.S.A. is 14.3/100.000, while the incidence in Jewish women living in underdeveloped countries in Africa is significantly lower) (9) .

Based on this, it can be concluded that many factors affect the occurrence of ovarian cancer, so etiopathogenesis is very complex and there is a number of theories concerning the issue.

The information obtained by ultrasound examination should be used to decide on the need and type of surgical procedure. In general, the presence of a mass greater than 50 mm containing an irregular solid component or the presence of free fluid at a significant amount (over 20 ml) requires surgical treatment. On the other hand, masses that are cystic and less than 40-50 mm can be sonographically monitored for several months in order to document changes in the size of the cyst. Ultrasound can diagnose three large groups of tumors of the ovary: cystic, solid, and mixed-type with the predominance of one or another type of tissue.

In order to improve the prognosis of ovarian cancer, an emphasis is placed on early detection. In the past 20 years, various methods for the detection of ovarian cancer (Papanicolaou smear, peritoneal cytology examination, etc.) have been applied, but have not proved to be good enough. The latest methods of immunoscintigraphy, nuclear magnetic resonance and computed tomography can reveal small cancers, but their invasiveness and price prevent them from becoming the mainstay of screening for ovarian cancer. The methods studied today as screening methods are bimanual gynecological examination, ultrasound, and tumor markers.

Bimanual examination has the following advantages: it is relatively easy, it can be incorportated into the already existing cervical screening programs, and it does not require special equipment, so it is not costly. However, neither the sensitivity nor the specificity of this test has been known so far.

The majority of studies studying the value of ultrasound in the diagnosis of ovarian tumors used women with symptoms who were about to undergo laparotomy for suspicious 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 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, Sasona and associates published a study in 1991 presenting a scoring system for an objective description of pelvic disease based on transvaginal ultranosographic characterization of the change (11). The proposed scoring system used both for ovaries and extrauterine pelvic masses of unknown origin is based on the following four criteria: structure of the interior tumor wall (1-4 points), thickness of the tumor wall (1-3 points), presence and thickness of septa (1-3 points), 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 a curve is formed, the shape of which shows that the total score of 9 points best distinguishes between benign and malignant lesions.

Tumor markers, proteins produced by certain ovarian tumors, are used in the examination of ovarian tumors. Primarily, serum levels of alpha-fetoprotein markers (AFP), CA 125, lactate dehydrogenase (LDH), CEA, and inhibin B are monitored. If there is a suspicion that the tumor is hormonally active and produces certain hormones, appropriate 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 therapy, as well as for early detection of relapse of certain ovarian tumors (12).

CA 125 is an antigenic determinant of high molecular weight glycoprotein recognized by the monoclonal antibody OC125. CA 125 is a highly sensitive, but not 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 (12). Bast and associates (6) also showed that only 1% of healthy women had serum Ca 125 levels higher than 35 ml/U (13). 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 for developing ovarian cancer, is low. However, a more detailed analysis shows a dependence on the stage of the disease. The disease spread outside the ovary is associated with elevated levels of CA 125 in the serum in over 90% of cases. If the tumor is restricted to ovarian tissue, CA 125 levels in the serum are increased in only 50% of cases (13).

Given that a high degree of specificity is required for a prospective screening program for ovarian cancer and given the association between CA 125 and non-malignant pathology, the positive predictive value of elevated serum CA 125 for this disease is considered to be too small for CA 125 to be used as a sole screening test. The specificity could be sufficient if CA 125 is combined with ultrasonography. Such a screening program has been used in large centers worldwide, including the Royal London Hospital since 1985 (13).

The Aim of the studies

The aim of this study was to determine sensitivity, specificity and predictive values of the tests for pre-operative monitoring of the state of ovarian tumors in postmenopausal women and to assess possible malignant potential and examine the correlation of clinical findings and the significance of ultrasonography in differentiating between benign and malignant ovarian tumors.

Material and methods

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

The study was carried out in the following institutions: Clinic of Gynecology and Obstetrics, Clinical Center Niš, Women's Health Service of the Health Center Niš, Clinic of Gynecology and Obstetrics of the Clinical Center Kragujevac and Clinic of Gynecology and Obstetrics „Narodni front" from Belgrade.

Results

The study was conducted on 60 subjects, with the youngest being 45 and the oldest 66 years old. The average age was 52.17 years in the group of women with benign ovarian tumors, and 54.13 years in the category of women with malignant tumors (Tables 1 and 2).

Table 1. Total number of subject by age

Age	Benign tumors	Malignant tumors
< 45	0	0
46 - 50	16	3
51 - 55	18	6
56 - 60	7	3
60 >	4	3
∑	45	15

Table 2. Average age of subjects in the categories of benign and malignant tumors with standard deviations in both categories

	$\overline{X}$	**SD**	**Σ**
Benign	52.17	± 7.4	45
Malignant	54.13	± 6.39	15

T test = 0.91, P = 0.366 (no statistical significance), df = 58

The average duration of postmenopausal amenorrhea in the category of women with benign ovarian tumors was 4.4 years, compared to 5.87 years in the group of subjects with malignant tumors, which did not show a statistical significance at the level of 3%, as shown in Tables 3 and 4.

Table 3. Duration of amenorrhea in examined women.

Duration of amenorrhea	**Benign tumors**	**Malignant tumors**
< 1	1	0
1-2	15	4
3-4	9	3
5-6	6	2
6 >	4	6
Σ	45	15

Table 4. Average duration of amenorrhea in the categories of benign and malignant tumors with standard deviations in both categories

	$\overline{X}$	**SD**	**Σ**
Benign	4.4	± 3.9	45
Malignant	5.87	± 4.94	15

T test = -1.2; P = 0.234 – no statistical significance, df = 58

In the group of examined women with benign tumors of the ovaries, the average number of births was 1.96, while women in the group of subjects with malignant tumors averaged 1.4 children. Although the difference in the number of births is evident, it does not have a statistical significance at the level of 3%, as shown in Tables 5 and 6.

Table 5. Number of childbirths of the examined women

Number of childbirths	**Benign tumors**	**Malignant tumors**
0	3	4
1	12	4
2	18	4
3	7	3
3 >	5	0
Σ	45	15

Table 6. Average number of childbirths in the categories of benign and malignant tumors with standard deviations in both categories.

	$\overline{X}$	SD	Σ
Benign	1.96	± 1.11	45
Malignant	1.4	± 1.12	15

T test = 1.66; p = 0.102, df = 24

The use of oral contraceptives is more common in the category of women with benign ovarian tumors compared to women with malignant tumors of the ovary. The length of oral contraceptive use in the category of women with benign tumors of the ovary was 2.84 years, compared to 1.27 years in the group of women with malignant tumors, as shown in Tables 7 and 8. A high statistical significance (p = 0.046) was determined based on the duration of OC use.

Table 7. Duration of use of oral contraceptives (in years)

Duration of OC use	Benign tumors	Malignant tumors
0	18	8
< 1	2	1
1 - 2	2	4
3 – 4	4	2
5	11	0
6 >	8	0
Σ	45	15

T test = 2.03; p = 0.046, statistical significance 5%, df = 58

Table 8. Average duration of use of oral contraceptives in the categories of benign and malignant tumors with standard deviations in both categories

	$\overline{X}$	SD	Σ
Benign	2.84	± 2.80	45
Malignant	1.27	± 1.79	15

T test = 2.03; p = 0.046, statistical significance 5%, df = 58

Benign tumors were predominantly of cystic structure in the examined groups of women, in contrast to the mixed-content structure of malignant tumors, which showed the highest degree of statistical significance, as shown in Tables 9 and 10.

Table 9. Number of benign and malignant tumors differentiated by structure

Tumor structure	Benign tumors	Malignant tumors
Cystic	38	5
Solid	2	2
Mixed-type	5	8
Σ	45	15

Table 10. Correlation of cystic tumor structure to the mixed-type structure

	$\overline{X}$	SD	Σ
Benign	1.96	± 1.11	45
Malignant	1.4	± 1.12	15

$x^2 = 16.1$, $p > 0.001$ – high statistical significance, df = 1

Tumor location was predominantly bilateral in cases with malignant tumors, while in cases with malignant tumors it was unilateral resulting in high statistical significance, as shown in Table 11.

Table 11. Correlation of the location of benign to 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

The size of benign tumors was 7 cm on the average, while malignant tumors in the examined group of patients were over 9 cm, which was highly statistically significant, as shown in Tables 12 and 13.

Table 12. Size of the tumor process 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 13. Correlation of the size of benign to 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 su-

bjects was 2 and below 2 mm, which was highly statistically significant. These parameters are given in Table 14.

Table 14. Correlation of the thickness of the wall of benign to 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 were significant differences in the appearance of the tumor wall. While in benign ovarian tumors the interior of the wall was smooth, in malignant tumors the interior of the walls was uneven and with numerous excrescences, as shown in Table 15.

Table 15. Correlations of the structure of the wall of benign to 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

In benign ovarian tumors, a sporadic presence of free fluid was found in the pouch of Douglas, whereby the quantity was small, regularly below 50 ml. In malignant tumors, the presence of fluid in the pouch of Douglas is twice as frequent as its absence. In this sense, the finding of a larger quantity of 50 ml of free fluid is statistically highly significant and indicates the presence of a malignant tumor of the ovary. These data are shown in Table 16.

Table 16. Correlation of the presence of free fluid in the pouch of Douglas of the benign to malignant tumors with standard deviations in both categories

	No	Yes	Σ
Benign	40	5	45
Malignant	5	10	15

Discussion

The largest number of examined women was in the age group between 51 and 55 years. The average age of those with benign changes in the ovaries was 52.17 years, while the value for those with malignancies was 54.13. In this respect, this study did not confirm the significant role of age in distinguishing between benign from malignant tumors, nor did it confirm the results of studies of certain authors about malignant ovarian tumors occurring in younger women.

The duration of postmenopausal amenorrhea and the development of ovarian cancer is one of the most studied associations. Numerous studies have not produced any concrete and clear conclusions. The analyses conducted in Europe (14) indicate an increase in malignant potential with the duration of amenorrhea, while in Asian case-control studies (15, 16), as well as in America (17), this type of connection was not established. In addition, the results speaking in favor of the substitution therapy and the length of menopause not being the factors that affect the development and activity of ovarian tu-

mors in postmenopausal women (2,3) were presented. In the examined groups of women, the average length of amenorrhea was 4.4 years in those with benign alterations, compared to 5.87 years in women with malignant ovarian tumors. Based on this, the examinations did not establish close connection between the length of postmenopausal amenorrhea in distinguishing benign from malignant tumors of the ovary.

The examined group of patients differed by the number of childbirths. This ratio was 2:1.4 in favor of those with benign ovarian changes, but statistical significance was not established. However, the results of meta-analyses and case-control studies conducted both in Europe and in America point to the protective effect of childbirth. Thus, a linear increase in a protective effect of childbirth was demonstrated to be proportional to the number of childbirths (17, 18).

Back in 1992, the researchers from Harvard University analyzed the relationship between the use of oral contraceptive and ovarian tumors in 20 studies. The conclusions were that the risk of ovarian cancer was reduced with the length of oral contraceptive use. The results show 10-12% reduction of risk after only one year of use and about 50% reduction after 5 years of continuous use of oral contraceptives (19). Burkman and associates showed similar results: the use of oral contraceptives for 15 years and more reduces the risk of developing ovarian cancer by 58%; 10-14 years by 44%; 5-9 years by 36%; and for 1-4 years the risk was reduced by 22% (19). One of the studies in the Harvard Analysis of „Cancer and Steroid Hormones" (CASH) showed that the reduction of cancer prevalence was the same regardless of the type or concentration of estrogen, i.e. progestin in the tablet (20). In the examined population of women with benign changes, the length of oral contraceptive use was 3 years, while that length in the group with malignant changes was 1.2 years. The statistical significance was present at the level of 5% and corresponded to the results of most of the worldwide studies.

In ultrasound diagnosis and the estimation of malignant potential of ovarian tumors, many scoring systems were made that have not been broadly used in clinical practice (20, 21). However, all the data suggest that the tumor location in malignant processes is most often bilateral, in contrast to the predominant unilaterality in benign conditions. That assumption was proven in the presented study. In addition, the size of benign tumors reaches a little less than 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 of 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 tumor changes. While the interior of the wall is smooth in benign tumors, it is uneven and with numerous excrescences in malignant tumors. Papillary projections constitute a significant ultrasound sign of a malignancy. The degree of malignancy is proportional to the number of these papillary formations (22). Granberg et 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 (22).

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

Differentiation between benign and malignant ovarian tumors is most important both for the patient and the physician. In most institutions, surgical procedure (laparoscopy or laparotomy) depends on the assessment of the malignant change, but the malignancy can only be excluded with certainty by histopathological confirmation (23, 24). Therefore, many prognostic models for the differentiation of malignant and non-malignant ovarian tumors, including Doppler criteria, have been published so far and show significant validity.

Conclusion

There is an age difference between women with benign ovarian tumors and those with malignancies. Malignant tumors occurred 1.96 years later compared to benign ones. This age difference had no statistical significance. In women with benign tumors of the ovary, a shorter duration of postmenopausal amenorrhea was observed, with a difference of 1.47 years compared to women with malignant tumors of the ovary. However, such a difference in age did not have statistical significance in distinguishing benign from malignant tumors.

Women with benign tumors averaged 1.96 children, compared to 1.40 children averaged by women with malignant ovarian tumors (0.56 more), but the parameter had no diagnostic statistical significance. The length of use of oral contraceptives in women with benign changes was 2.84 years on the average, and 1.27 years was the average length of contraceptive use in women with malignant process, indicating a high statistical significance of 5%.

The examined women diagnosed with malignant ovarian tumors had pain in the small pelvis as a dominant disease symptom, while in the group with benign tumors, the most frequent reason for a visit to the doctor was regular control of gynecological health.

Among those with benign tumors, the dominant tumor structure was cystic, in contrast to the mixed-type structure in malignant tumors. In this sense, the parameter of tumor structure is a serious factor in distinguishing benign from malignant tumors of the ovary.

In subjects with histopathologically proven malignant tumors the disease was more often bilateral, while it was predominantly unilateral for benign tumors (the difference being highly statistically significant).

Benign tumors measured approximately 70 mm in size, while malignant tumors in the examined group of patients were over 90 mm. Based on this, the size of the tumor is a reliable factor in distinguishing benign from malignant ovarian tumors. The thickness of the wall of benign tumors was higher than the thickness of the malignant tumor wall, and this was 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, when seen, 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.

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ULOGA ULTRASONOGRAFSKOG NALAZA U RAZLIKOVANJU BENIGNIH OD MALIGNIH TUMORA JAJNIKA ŽENA U POSTMENOPAUZI

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Maligni tumori jajnika javljaju se u svim životnim dobima, pri čemu ukupna incidencija dramatično sa godinama raste. Preživljavanje od karcinoma jajnika zavisi od stadijuma u kome se bolest otkrije.

Cilj ovog rada bio je utvrđivanje senzitivnosti, specifičnosti i prediktivne vrednosti testova za preoperativno praćenje stanja tumora jajnika kod žena u postmenopauzi, procena eventualnog malignog potencijala i ispitavanje korelacije kliničkog nalaza i značaja ultrasonografskih pregleda u razlikovanju benignih od malignih tumora jajnika.

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 i ultrasonografski pregled male karlice.

Postoji razlika u godinama kod žena sa benignim tumorima jajnika u odnosu na one sa malignim. Žena sa benignim tumorima prosečno je rađala 1,96 dece, prema 1,4o dece koliko je prosečno rađala žena sa malignim tumorima jajnika. Dužina upotrebe oralne kontracepcije kod žena sa benignim promenama iznosila je prosečno 2,84 godine. Prosečna dužina korišćenja kontraceptiva je 1,27 godina kod žena sa malignim procesom, što pokazuje visoku statističku značajnost od 5%. Među ispitanicama sa benignim tumorima, dominantna građa tumora bila je cistična, nasuprot malignim tumorima mešovite građe. Lokalizacija tumora je sa visokom statističkom značajnošću češće bilateralna kod ispitanica sa histopatološki dokazanim malignim tumorima, dok je kod benignih tumora pretežno unilateralna. Veličina tumora je pouzdan faktor u razlikovanju benignih od malignih tumora jajnika. Debljina zida benignih tumora je veća u odnosu na debljinu zida malignih tumora. Prisustvo slobodne tečnosti u Duglasovom prostoru je retkost kod benignih tumora jajnika, dok je kod malignih tumora jajnika postojanje slobodne tečnosti česta pojava.

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ASSESSMENT OF HEALTH-RELATED QUALITY OF LIFE OF POST-STROKE SURVIVORS

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The objective of the paper was to assess the health-related quality of life (HRQOL) in post-stroke survivors. Method: A prospective cohort study was done. The study involved 136 post-stroke survivors who had inpatient rehabilitation. Their functional status was assessed by Barthel Index (BI) and modified Rankin scale (mRS). The Mini Mental State Examination (MMSE) was used for screening the cognitive function. HRQOL was assessed by the Medical Outcomes Study 36-item Short Form (SF-36). Functional status, cognitive function and HRQOL were assessed at the admission, one, and six months after the stroke. The repeated-measures analysis of ANOVA variance test was used as a correlation analysis. Results: There were marked declines in all eight SF-36 questionnaire domains at admission. After one month and after six monts of follow-up, BI and MMSE scores increased and mRS decreased. All eight domains of the SF-36 improved, but six showed a statistically significant increase. The domains of bodily pain and vitality showed non-significant improvements. Six months after the stroke, five domains continued to increase significantly, except for vitality, bodily pain and mental health. ANOVA showed that the values of mRs significantly decreased during the investigation ($p < 0.001$) while the BI and MMSE scores significantly increased ($p < 0.001$). Conclusions: A strong correlation was found between higher BI scores and physical and social domains, and emotional role, mental and general health. Improvements in motor ability and improvements of cognitive function were statistically significantly associated with HRQOL increase. All the domains of SF 36 improved during the six-month follow-up. Bodily pain, vitality and mental health improved non-significantly during the six months after the stroke.

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Key words: *stroke, inpatient rehabilitation, health-related quality of life*

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Introduction

Despite the advances in the diagnosis and treatment of cerebrovascular disease, stroke remains the third most common cause of death worldwide and leading cause of disability (1,2,3). The prevalence of stroke survivors who experience an incomplete recovery is 461 per 100,000, and one-third of these survivors require assistance with at least one activity of daily living (3).

Stroke remains one of the most devastating of all neurological diseases, often causing death or physical impairment or disability (4). According to the World Health Organization (WHO), 15 million people present with stroke annually, and of these five million die as a result of the event and a large part of the survivors present physical and/or mental seuqelae (5).

Post-stroke functional deficits and psychological problems disrupt the patient's ability to perform activities of daily living, which negatively impacts their health-related quality of life (HRQOL) (5). The most important consequence of stroke for stroke survivors is decreased HRQOL (6, 7).

In many studies, stroke patient HRQOL scores were evaluated and some were reported as disrupted (8-18). Patients often experience a loss of self-identity following a stroke (19). Speech loss or difficulties with speech (19) are significant factors that reduce social interactions (20).

Several studies suggest that HQOL decreases after stroke because of functional impairments, depression and insufficient social support (17, 21), home circumstances and standard of living (21), and also gender and age, with women and older adults, as well as more dependent stroke survivors, reporting lower QOL (22).

HRQOL measurements reflect the physical, functional, psychologic, and social aspects of health (19). HRQOL is usually a reflection of the patients subjective and personal evaluation of their own health status (23). Rehabilitation helps stroke survivors maximize their HRQOL, including physical, cognitive, emotional and social aspects (24).

The results of treatment are assessed applying the tests that evaluate physical limitations and/or functional impairments (25).

The objective of the paper was to assess the impact of physical and mental factors on HRQOL during and after inpatient rehabilitation in post-stroke survivors.

Methods

A prospective study was done. The study involved 216 post-stroke survivors aged 30-79 from the Nishava District, out of which 196 completed the study. Dropping-out was caused by the following: 11 patients declined to participate; 60 patients had outpatient rehabilitation; and 9 died. Only 136 post-stroke survivors had inpatient rehabilitation after stroke. The observed period was January 1, 2011 to August 15, 2013. The HRQOL was assessed by means of the Medical Outcomes Study 36-item Short Form (SF-36), which is a self-administered questionnaire. The Mini Mental State Examination (MMSE) was used for screening the cognitive function. Functional status was assessed by the Barthel Index (BI) and modified Rankin Scale (mRS). Functional status and questionnaires were assessed at the admission to the Clinic, after one month of follow-up, and after six months of follow-up.

The criteria for inclusion in the study were the first-ever stroke (cerebral infarction or hemorrhage), confirmed by either brain CT or MRI findings consistent with the clinical presentation, patient willingness to participate, and availability of a complete Mini-Mental State Examination (MMSE), mRS, BI score and SF-36 questionnaire.

The exclusion criteria were the second stroke or personal history of stroke, severe cognitive impairment, aphasia.

All the patients were informed in detail about the aims of the study. the Ethics Commitee of the Faculty of Medicine in Nis and the Ethics Commitee of the Clinical center Niš gave their approval for the study.

Questionnaires

The patients' functional status was assessed using the modified Barthel Index (BI) and modified Rankin Scale (mRS) (26-30).

The BI was developed in 1965 (27) and later modified by Granger and coworkers (28) as a scoring technique that measures the patient's perfor mance in 10 activities of daily life. The BI is considered a reliable disability scale for stroke patients (29). The items can be divided into two groups, one related to self-care (feeding, grooming, bathing, dressing, bowel and bladder care, and toilet use) and the other related to mobility (ambulation, transfers, and stair climbing). The maximal score is 100 if 5-point incre-ments are used, indicating that the patient is fully independent in physical functioning. The lowest score is 0, representing a totally dependent, bedridden state.

The mRS measures independence rather than performance of specific tasks. In this way, mental as well as physical adaptations to the neurological deficits are incorporated. The scale consists of 6 grades, from 0 to 5, with 0 corresponding to no symptoms and 5 corresponding to severe disability (29).

Cognitive function was assessed using the mini mental state examination (MMSE). The MMSE is a widely used, reliable and validated instrument used in screening for cognitive impairment. The exam assesses the aspects of cognition and is easily performed. The contents include orientation, attention, learning, calculation, abstraction, information, construction and delayed recall. The MMSE provides measures of orientation, registration (immediate memory), short-term (but not long-term) memory, as well as language functioning. The examination has been validated in a number of populations. The scores of 25-30 out of 30 are considered normal; the National Institute for Health and Care Excellence (NICE) clas-sifies 21-24 as mild, 10-20 as moderate, and < 10 as severe impairment. The MMSE may not be an appropriate assessment tool if the patient has lear-ning, linguistic/communication or other disabilities (sensory impairments) (31).

HRQOL was assessed by means of the Medical Outcomes Study 36-item Short Form (SF-36), which is a self-administered questionnaire containing 36 items that, when scored, yield 8 domains. The physical functioning domain assesses limitations in physical activities such as walking and climbing stairs. The role physical and emotional role domains measure the problems with work or other daily activities as a result of physical health or emotional problems. Bodily pain assesses the limitations resulting from pain; vitality measures energy and tiredness. Social function domain examines the effect of physical and emotional health on normal social activities, and mental health assesses the feelings of happiness, nervousness, and depression. General health perception domain evaluates the personal opinion of one's health compared with that of one's peers, as well as the expectation of health changes. All the domains are scored on a scale from 0 to 100, with 100 representing the best possible health state (32).

Change scores were calculated in such a way that positive change scores indicated improvement and negative change scores indicated deterioration.

Statistical analysis

All the calculations were done using the SPSS version 10.0 and S-PLAS programme, version 2000. The analyses included descriptive statistics (mean, SD, frequencies), independent Student's t-test to compare the numerical differences of normal distri-

bution. The Mann-Whitney U test was used to compare two values when the dependent variable is either ordinal or continuous, but not normally distributed.

The repeated-measures analysis of variance ANOVA test was used to gain an understanding of how the changes in independent variables were associated with changes in quality of life one and six months after the stroke. A correlation analysis was used for the relationships among the continuous variables (sex, age, side of stroke, mRS, BI and MMSE score) and domains, and Spearman coefficient of rang correlation was calculated. The p-value below 0.05 ($p < 0.05$) was considered as statistically significant.

Results

A total number of 136 post-stroke survivors completed the questionnaires at admission, one month, and six months after the stroke. The average age of post-stroke survivors was 63,72 ± 8,73. There were 66 (48,5%) men and 70 (51,5%) women. The determined differences were not statistically significant in gender distribution Ischaemic brean damage was present in 105 patients (77,2%) and hemorraghia was present in 31(22,8%). The basic characteristics of the patients are shown in Table 1.

Table 1. Basic characteristics of the studied post-stroke survivors

Characteristics	Number	Percentage (%)
Sex		
Male	66	48.5
Female	70	51.5
Type of stroke		
Ischaemia	105	77.2
Hemorraghia	31	22.8
Side of stroke		
Right hemisphere	60	44.1
Left hemisphere	62	45.6
Other (brainstem)	10	7.6
Both hemispheres	4	2.9

of all post-stroke survivors, 62(46,9%) had stroke in the left hemisphere, 60 (45,5%) had stroke in the right hemisphere and 10 patients (7,6%) had stroke of the brainstem.

Table 2 describes the changes in SF 36 at admission, one month, and six months after the stroke.

Table 2. Changes in SF-36, from admission to one-month and to six-month follow-up

Measure	Scores	Significance p	
Changes in SF-36	**admission**	**to one month follow up**	
Physical Functioning	1.91 ± 6.72	18.53 ± 24.21	0.05
Role Physical	0.00 ± 0.00	2.02 ± 9.65	
Role Emotional	0.00 ± 0.00	4.17 ± 19.22	
Vitality	60.55 ± 22.29	68.68 ± 20.12	n.s.
Menthal health	60.55 ± 22.29	77.00 ± 19.20	0.05
Social Functioning	2.76 ± 12.33	13.42 ± 21.76	0.05
Bodily Pain	90.63 ± 25.65	93.64 ± 20.12	n.s.
General Health Perceptions	1.10 ± 5.15	6.62 ± 11.07	0.05
Changes in SF-36	**one month follow up**	**to six month follow up**	
Physical Functioning	18.53 ± 24.21	56.54 ± 34.790.05	
Role Physical	2.02 ± 9.65	6.84 ± 41.57	0.05
Role Emotional	4.17 ± 19.22	28.19 ± 40.75	0.05
Vitality	68.68 ± 20.12	74.60 ± 17.48	n.s.
Menthal health	77.00 ± 19.20	78.82 ± 20.46	n.s.
Social Functioning	13.42 ± 21.76	57.35 ± 36.97	0.05
Bodily Pain	93.64 ± 20.12	95.72 ± 10.14	n.s.
General Health Perceptions	6.62 ± 11.07	23.90 ± 18.48	0.05

At admission, RF and RE summary scores were both zero. All other domains were significantly lower compared with the average domain scores one month later. During rehabilitation, in the first month after the stroke there were improvements in all 8 domains, but in two domains, that of vitality and bodily pain, the improvements were non-significant.

Six months after hospital discharge, 5 domains continued to show a significant increase, but 3 domains, that of mental health, vitality and bodily pain, showed non-significant improvement.

Table 3. The values of mRS, BI and MMSE scores in stroke survivors at admission, one month and six months after the stroke onset

Time	Post stroke survivors Number 136		
	mRs	**BI**	**MMSE**
At admission	4,75±0,55	25,00±24,66	22,84±6,01
One month after	3,82±0,73	57,28±24,88	25,34±4,73
Six monts after	2,60±1,08	83,75±18,59	27,40±4,20

The value of mRS at admission was 4,75 ± 0,55 and six moths after the stroke it was 2,60 ± 1,08. The average value of mRS was 1,8 times lower after six months than at admission.

There was a continued decrease of mRS during the study.

BI score was lowest at admission (25,00 ± 24,66) and highest six months after the stroke - 83,75 ± 18,59. BI score was 3,35 times higher than at admission.

The average MMSE score in the period from admission to six months after admission increased from 22,84 ± 6,01 up to 27,40 ± 4,20. Mild impairments were seen in post-stroke survivors only at admission to the Neurological Unit MMSE (< 24), and there was no cognitive dysfunction one and six months after the stroke.

Analysis of variance for repeated measures showed that mRS values significantly decreased during the investigation ($p < 0,001$), while BI and MMSE scores significantly increased ($p < 0,001$).

Table 4. Correlation between changes of domain in SF 36 questionnaire from admission up to six months after stroke

Characteristics	**Domains**								
	PF	RF	RE	VT	MH	SF	BP	GH	CGH
Sex	0,039	0,048	0,081	0,174*	0,096	0,113	0,036	0,100	0,164
Age	-0,026	-0,041	-0,001	0,013	0,017	-0,055	0,042	-0,035	0,036
Type of stroke	0,030	0,015	0,081	0,061	-0,106	-0,024	-0,044	0,035	-0,016
Right hemisphere	-0,052	-0,086	-0,017	0,060	0,158	0,031	0,005	-0,167	-0,055
Left hemisphere	-0,007	0,037	0,034	0,003	-0,099	-0,104	-0,102	0,095	-0,050
Both hemisphere	-0,145	-0,116	-0,131	0,165	0,067	-0,115	0,100	-0,018	-0,098
mRS at admission	-0,207*	-0,397†	-0,287†	0,085	0,087	-0,221†	-0,031	-0,013	-0,212*
Change of mRS	0,346†	0,341†	0,315†	-0,008	0,037	0,262†	0,167	0,220*	0,443†
BI score at admission	0,296†	0,123	0,203*	-0,032	0,013	0,357†	0,013	0,070	0,203*
Changes of BI score	-0,457†	-0,415†	-0,423†	0,126	-0,219*	-0,440†	0,098	-0,256†	-0,446†
MMSE score at admission	-0,721†	-0,475†	-0,592†	-0,014	-0,352†	-0,631†	0,117	-0,590†	-0,590†
Change of MMSE score	0,255†	-0,037	0,052	0,265†	0,312†	0,281†	-0,151	0,208*	0,115

* - P < 0,05; † - P < 0,01; PF – Physical Function; RF –Role Physical; ER – Role Emotional; VT – Vitality; MH – Mental Health; SF – Social Functional; GH – General Health; Change of General Health

A significant positive correlation was found between the increase of PF and mRS and MMSE scores during the investigation ($r = 0,346$; $p < 0,01$) and BI score ($r = 0,296$; $p < 0,01$) at admis-

sion. A significant negative correlation was established between the increase of RF and increase of mRS ($r = 0,341$; $p < 0,01$) and BI scores during the study. A significant negative correlation was found between the increase of PF and decrease of BI score during the investigation ($r = -0,457$; $p < 0,01$). A significant negative correlation was found between the increase of PF and decreased values of mRS ($r = -0,207$; $p < 0,05$) at admission.

A statistically significant correlation between the role physical, lower mRS values ($r = 0,315$; $p < 0,01$) and higher BI scores ($r = 0,203$; $p < 0,05$) at admission was determined. A negative significant correlation was determined between the increase of RF and decreased BI score during the study ($r = -0,415$; $p < 0,01$), as well as with decreased mRS values ($r = -0,397$; $p < 0,01$) and MMSE score ($r = -0,475$; $p < 0,01$) at admission.

A positive correlation was found between the increase of domain RE and increase of mRS ($r = 0,315$; $p < 0,01$) during the study and higher BI score at admission ($r = 0,203$; $p < 0,05$). A significant negative correlation was determined between the increase of RE and decreased BI score during the study ($r = -0,423$; $p < 0,01$), and with lower mRS values ($r = -0,287$; $p < 0,01$) and MMSE ($r = -0,592$; $p < 0,01$) score at admission.

A significant positive correlation was found between the increase of vitality domain and increased MMSE score ($r = 0,265$; $p < 0,01$) during the study. The domain of vitality showed a statistically significant improvement in women than in men ($r = 0,174$; $p < 0,05$).

A statistically significant negative correlation was found between the domain of MH and decreased BI score during the study ($r = -0,219$; $p < 0,05$).

A statistically significant positive correlation was found between the domain SF and increased mRS during the investigation ($r = 0,262$; $p < 0,01$) and between SF and decreased BI score at admission ($r = 0,357$; $p < 0,01$). A statistically significant negative correlation was found between the domain of SF and decreased BI during the study ($r = -0,440$; $p < 0,01$), and between decreased mRS at admission ($r = -0,221$; $p < 0,01$).

A statistically significant positive correlation was found between the domain of GH and increased mRS ($r = 0,220$; $p < 0,05$) and with increased MMSE score ($r = 0,208$; $p < 0,05$) during the study.

There were statistically significant correlations between the domain of bodily pain and all examined factors.

A statistically significant negative correlation was found between the increase of GH domain and decrease of BI score during the study ($r = -0,256$; $p < 0,01$), and with the decreased MMSE score ($r = -0,590$; $p < 0,01$) at admission.

A statistically significant positive correlation was found between the domain of CGH and mRS increase ($r = 0,443$; $p < 0,01$) during the study and with a higher BI score ($r = 0,203$; $p < 0,05$) at admission. A statistically significant positive correlation was found between the domain of CGH and BI score decrease ($r = -0,446$; $p < 0,01$) during the study and with the lower values of mRS ($r = -0,212$; $p < 0,05$) at admission.

Discussion

In this investigation, HRQOL was assessed using the SF-36, as an adequate measure for assessing post-stroke function (8). The importance of post-stroke QOL measurement has been increasingly accepted (19). We investigated possible associations between demographic characteristics, functional status, cognitive function and HRQOL.

During the six months of follow-up HRQOL constantly increased. During the investigation, all eight domains of SF-36 increased, but the domains of bodily pain, vitality and mental health showed a non-significant improvement.

Hopman and Verner (2003) found statistically significant declines in 5 domains (emotional role, mental health, social functioning, bodily pain and genaral health perceptions) of the SF-36 in the six months after the discharge (except for the physical role, physical functioning) (34).

According to the presented results positive changes in physical function and role function were strongly positively associated with increased HRQOL. During the six-month follow-up, a HRQOL improvement was seen.

Physical function was higher at admision in those survivors with right hemisphere stroke but these differences were no longer present one month and six months after the stroke. Physical function was higher, but non-significantly, in survivors with ischaemic stroke.

At admission, men had higher scores of physical function domain than women, but this difference was not significant. Hopman and Verner (2003) found that after six-month follow-up, there were no significant differences between men and women, but the differences in vitality ($p < 0,001$) and mental health ($p < 0,001$) became more pronounced (34).

Granger, Deric, and Denham found higher BI scores in post-stroke men compared with women (35). Similar results in post-stroke survivors a year after the stroke were reported by Wilkinson-a (36), Bethoux-a (37), Santus-a (38), Johansson-a (39).

In this study, a strong correlation was found between higher BI scores and higher scores of physical function, emotional role, mental health and general health.

There were more of post-stroke survivors with stroke located in the left hemisphere (46,9%: 45,5%). The side of stroke at ad-mission had non-significant impact on BI score (32). The side of hemiplegia had little impact on HRQOL (39). At admission, physical function of SF 36 was higher in patients with right-side haemiplegia, but after one month and six months there were no statistically significant differences.

A study of 46 stroke survivors 4 years after their first stroke showed that despite a good outcome in terms of hospital discharge, ADL, and return

to work, the HRQOL of 83% of the patients had not been restored to the pre-stroke level (18).

In one study, mean QOL scores decreased in the domain of physical function between 4 to 16 months after the stroke and the important determinants of QOL after 16 months were functional status, age and gender (41). Another study showed that neither age, gender, comorbidities, nor baseline disability was an important determinant of change in HRQOL from 1 to 6 months following an acute stroke (42).

Hackett et al. (2006) reported a decreased HRQOL in patients 4 years after stroke and found that the important determinants were physical state and cognitive impairment (22). Poor physical health 1 year after the stroke was independently associated with being female and having diabetes mellitus, right hemispheric lesions and cognitive impairment. In another study, poor mental health 1 year after the stroke was independently associated with being under 65 years, the presence of ischemic heart disease and cognitive impairment (22).

According to the data from one study that assessed 63 stroke survivors during inpatient rehabilitation, one month after the stroke and again at home 6 months after the stroke, it was found that functional independence and HQOL improved over time, but this improvement was strongly correlated with self-care and self-efficacy (32).

Cognitive impairment (CI) is a frequent complication in stroke survivors and predicts post-stroke death, dependency, and institutionalization (33).

According to presented results, post-stroke survivors had only mild impairments at admission to the Neurological Unit MMSE (< 24), and there were no cognitive dysfunctions one and six months after the stroke onset.

There was not any statistically significant difference in MMSE score at admission by gender. De Paulo (43) i Folstein (31) stated that in relation to gender, a greater decrease of cognitive function was noticed in women.

The social dimension of quality of life was assessed through the domains of social support, social role and loneliness. In post-stroke survivors, the domain of social function was statistically significantly increased a month after the stroke onset compared to the status at admission ($p < 0,01$).

Domain social function didn`t show statistically significant differences related to gender, side of hemiplegia, etiology of stroke and localisation of stroke.

Longitudinal data about HRQOL in post-stroke survivors are limited (34).

Limitations of the study must be taken into account when these results are interpreted. One limitation was the sample size, which was relatively small. Another issue was the inpatient rehabilitation, which was too short. Inpatient rehabilitation have a strong, positive impact on HRQOL. However, not all of the patients could have inpatient rehabilitation. Another 60 post-stroke survivors had rehabilitation in their homes or did not have any. The lack of assessment of Depression in post-stroke survivors was not assessed, while cognitive status was assessed only by the MMSE. MMSE is known to be insensitive to mild CI. Although acceptable validity is found in some studies, other studies reported that MMSE is not an appropriate screening test for cognitive dysfunction in cerebrovascular diseases due to shortcomings regarding right-sided lesions (41).

Conclusion

Changes in physical function and role function were strongly associated with changes of BI scores. A strong correlation between higher BI scores and physical, social domains, emotional role, mental and general health was found. Improvements in motor disability and improvement of cognitive function were statistically significantly associated with increased HRQOL. All the domains of SF 36 improved during the six month follow-up. The domains of pain and mental health improved non-significantly during the six months after the stroke onset.

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PROCENA KVALITETA ŽIVOTA BOLESNIKA POSLE MOŽDANOG UDARA

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Cilj rada bio je da proceni uticaj fizičkih i mentalnih faktora na kvalitet života bolesnika posle moždanog udara (MI) i to za vreme i posle bolničke rehabilitacije. Primenjena je prospektivna kohortna studija. Studijom je obuhvaćeno 136 bolesnika koji su bili na bolničkoj rehabilitaciji u trajanju od 30 dana. Funkcionalno stanje bolesnika procenjivano je modifikovanim Bartelovim indeksom (BI) i modifikovanom Rankinovom skalom (mRs). Za skrining kognitivne funkcije primenjena je »Mini-mental state examination« (MMSE) skala. Za procenu kvaliteta života primenjen je generički upitnik SF-36. Kvalitet života procenjivan je pri prijemu na Kliniku za rehabilitaciju i fizikalnu medicinu, mesec dana i šest meseci posle MI. Primenjen je test ponovljenih analiza varijanse (ANOVA) i korelaciona analiza. Prosečan BI skor na prijemu iznosio je 25,00 ± 24,66, a posle šest 83,75 ± 18,59. Vrednosti mRs na prijemu bile su 4,75 ± 0,55, a šest meseci posle moždanog udara 2,60 ± 1,08. Prosečni skorovi na skali MMSE na prijemu iznosili su 22,84 ± 6,01, a šest meseci posle MI, 27,40 ± 4,20. Prema ANOVA analizi vrednosti mRs značajno su se smanjile tokom istraživanja, a vrednosti BI ($p < 0,001$) i MMSE značajno su porasle ($p < 0,001$). Utvrđen je značajan pad svih osam domena kvaliteta života bolesnika na prijemu. Šest meseci posle MI, svih osam domena imalo je veće vrednosti, ali je samo 6 imalo statistički značajno veće vrednosti. Domeni za bol i vitalnost nisu imali statistički značajni porast. Došlo je do statistički značajnog porasta 6 domena kvaliteta života šest meseci posle Mi. Domeni za bol i vitalnost nisu imali statistički značajan porast. Porast kvaliteta života bio je statistički značajno povezan sa oporavkom fizičke i kognitivne funkcije. Utvrđena je jaka korelacija između većih vrednosti BI i fizičkog i socijalnog domena, emotivne uloge, mentalnog i opšteg zdravlja.

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DIAGNOSTIC IMPORTANCE OF THE THICKNESS OF THE INTIMA-MEDIA COMPLEX OF CAROTID ARTERIES IN PATIENTS SUFFERING FROM HYPERTENSION AS A RISK FACTOR FOR THE DEVELOPMENT OF CEREBROVASCULAR DISEASES

Snežana Zlatanović

Carotid diseases are very important for the increase of total morbidity and mortality of the population affected by cerebrovascular diseases. The primary pathologic factor responsible for the diseases of the cerebrovascular system is atherosclerosis, and the measurement of the thickness of the intima-media complex (IMC) in the carotid artery extracranial segment represents a measurable indication of atherosclerosis. The thickness of the IMC of the carotid arteries is influenced by many factors such as local hemo-dynamics, wall stress, arterial hypertension, and other.

The aim of our research was to confirm the influence of hypertension as a risk factor for the development of cerebrovascular diseases which influence the thickness of the intima-media complex of carotid arteries in asymptomatic and symptomatic observed patients concerning gender and age.

The research was conducted in 100 patients of both sexes who were treated at out-patient department or while hospitalized at the Neurology Ward, with hypertension as the main risk for the development of the cerebrovascular diseases. The measurement of the IMC of the carotid arteries was conducted by the ultrasound examination, and the value of IMC>0,9 mm was taken as a border line between normal and pathologic findings.

The research included 100 patients, out of which 36 were male, and 64 female patients. All patients had elevated values of blood pressure. In the male group, just 33.3% of the patients were affected by a hypertension disease only, while the remaining 66.7% had also suffered a cerebrovascular insult, and they had arterial hypertension as a risk factor in their anamnesis, as well. In the female group of 64 patients, 31.3% had a hypertension disease, 68.75% also suffered a cerebrovascular insult, as well as arterial hypertension.

The measurement of the thickness of the IMC of the carotid arteries by the ultrasound method is important for the detection of the subclinical structural damage of the arterial walls and is a part of the algorithm for the assessment of the cerebrovascular risk in patients who suffer from the elevated blood pressure. There is a significant corre-lation among the thickness of the carotid IMC, hypertension disease, and cerebrovascular diseases.

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Key words: *Carotid arteries, intima-media complex, hypertension, atherosclerosis, ultrasound diagnostics*

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Introduction

Cerebrovascular insult (CVI) in most of the countries of the world presents the third main cause of morbidity and mortality, next to cardiovascular diseases, and malignity. In this entity over 80% of the cases deal with diseases connected with brain ischemia, and in one third of the cases the syndrome of transitory ischemia attack appears (TIA) which represents an initial symptom for the presence of the carotid disease. Carotid disease, depending on the extent of the changes, can lead to hemodynamic dis-orders and can be the source of embolus, depending on the changes in the artery wall (that is, on the characteristics of surface, material and size of the arterial plaque and thickness of the intima-media complex) (1).

In most patients, atherosclerosis of the brain arteries, especially carotid arteries, is responsible for the occurrence of the cerebrovascular diseases.

Numerous epidemiology studies have proved the existence of risk factors such as artery hypertension, hyperlipidemia, diabetes mellitus, smoking, obesity, positive anamnesis of the cardiovascular diseases, consumption of alcohol, and others, for the occurrence of cerebral atherosclerosis (2). Most of the observed patients had two or more risk factors (3).

Primary pathological contributor for the occurrence of cerebrovascular diseases is atherosclerosis of the carotid arteries, and the measurement of IMC thickness of the extracranial part of the carotid arteries shows great sensitivity in the detection of the presence, regression, stagnation or progression of atherosclerosis (4). The thickness of the intima-media complex of the carotid arteries is influenced by many factors such as local haemodynamics, wall stress, blood pressure and others. Further, the thickness of the IMC wall of the extracranial segment of the carotid arteries, presents a measurable indicator of the progression of cerebral atherosclerosis, and it makes the assessment of the morphological characteristics of the neck blood vessels' wall possible (5).

Arterial hypertension is most prevalent among the grown-up population. According to the data published by the World Health Organization, about a billion people suffer from elevated blood pressure, which makes about 20-25% of the total population. It is connected with elevated incidence of clinical manifestations of atherosclerosis, such as myocardial infarction, and especially with cerebrovascular diseases. About two thirds of those who experienced apoplexia cerebri, have the record of hypertension as the risk factor in their anamnesis, and, at the moment the elevated blood pressure values were established, more than 60% of the observed patients already had atherosclerotic changes in the walls of the carotid arteries.

As an effect of the arterial hypertension, blood vessels become less elastic, the arterial walls thicken, and the arterial plaque is formed. Arterial hypertension can significantly influence the thickness of the IMC carotid arteries through the remodeling of the blood vessel or by their hypertrophy. Variations in the IMC thickness on various parts of the arterial blood vessel show the differences in local hemodynamic forces inside the blood vessel (6).

By the consensus of the American Echocardiography Society reached in 2007, carotid arteries are defined as a place of choice for the measurement of IMC, and the thickness of 0.9 mm is defined as an indicator of atherosclerosis and a significant risk for cerebrovascular diseases (7).

Asymptomatic carotid disease means the presence of stenosis lesions and the changes in the walls of carotid arteries in patients who did not experience neurological symptoms of cerebral insufficiency. The screening of the asymptomatic carotid disease is mainly done by the ultrasound examination and is recommended for grown-up persons with a high level of risk factors. The other asymptomatic period of the elevated blood pressure and multi-year presence of other atherogenic factors in persons with a predisposition for cerebrovascular diseases significantly enlarges the risk of ischemic lesions in the brain tissue (8).

Aim

The aim of this research was to confirm the influence of hypertension, as a risk factor for the development of cerebrovascular diseases, on the thickness of the intima-media complex of carotid arteries in asymptomatic patients, regarding gender and age.

Material and methods

The research was conducted in 100 patients treated at the General Hospital in Prokuplje at out patient department and in patients hospitalized at the Neurology Ward, all with arterial hypertension in their anamnesis. A certain number of observed patients suffering from arterial hypertension (62) experienced a cerebrovascular insult, and a certain number of patients (38) showed the signs and symptoms of hypertension disease only, but the symptoms of carotid disease were found during the diagnostic procedure as well.

The examination of the observed patients was done by ultrasonography apparatus, type Vivid 3, Syne Master 796 MB, with the linear probe of 10 MHz in B-mode. In B-mode echo sonography, the artery was presented longitudinally as a double line. The double line is hyperechogenicity and one of them was bordered by the lumen of the blood vessel, and the other by the hypoechogenic layer of adventitia (9,10).

The thickness of the hyperechogenic layer, immediately bordered by the lumen of the blood vessel, was measured by the ultrasound. The measurement was conducted on the back wall of the common carotid artery (CCA), in the segment directly behind the bifurcation point of the common carotid artery, at about 1cm before the actual bifurcation. The measurements were performed mutually, on the back wall of both CCA, the value of the IMC thickness was taken for each side separately, obtained results were processed as independent samples and the average value was calculated and established.

The values below 0.9 mm were considered normal, while all the measured values above 0.9 mm were considered as wall thickening of the blood vessel. For each patient the data about the family anamnesis of cerebrovascular diseases and hypertension as a significant risk factor for the incidence of vascular brain diseases were gathered.

The blood pressure was measured during the examination of the patients (the average value of the both upper-arm parts). According to the National Guide arterial hypertension is defined as a disease characterized by the elevated values of systolic (SP) and diastolic (DP) blood pressure ≥ 140/90 mmHg (11), and these border values for hypertension are identically recommended in Europe and USA.

Statistical analyses of the obtained data were conducted by the use of the following statistical tests: Student's t-test, Hi square test, and Anova. Recording of data, tabular and graphical presentation of data were performed using the MS Office Excel program. Results of the statistical analyses were pressented as tabular and graphic presentations. The program SPSS, version 20, was used for the statistical calculations.

The basic descriptive statistical parameters used were, in fact, the standard statistical methods for qualitative and quantitative evaluation of the obtained results: absolute numbers, relative numbers, percentage (%), arithmetical mean value , standard deviation (SD), minimal and maximal values. The normal distribution was examined by the use of Kolomogorov-Smirnov test. The comparison of arithmetic mean values of the two samples was conducted by the use of Student's t-test, while, in the case of three results, the ANOVA was used.

For the testing of the statistical significances the absolute frequencies among samples, Hi square test was used. The causality among the variables was examined by the Pirson's coefficient of simple linear correlation.

Statistical hypothesis was tested at the level of significance for the risk of $\alpha = 0.05$, that is, the difference among the samples was significant if $p < 0.05$.

Results

The research was conducted in 100 patients, out of which 36 (36%) were males, and 64 (64%) were female patients. All the patients suffered from the elevated blood pressure. The average age of the whole group of patients was 64.28 ± 10.02 years, among which, the youngest patient was 38, and the oldest 78 years old.

Age structure, according to the sex of the patients, didn't show statistically significant difference ($p = 0.666$) (Figure 1).

In the tested group, 68 (68%) of patients suffered a cerebrovascular insult, while 36 (36%) showed signs of hypertension disease. In the tested male group of 36 patients, 12 (33.3%), showed the signs of hypertension disease and 24 (66.7%) suffered a cerebrovascular insult and had arterial hypertension as a risk factor in their anamnesis as well. Out of 64 women, 20 showed the signs of hypertension disease (31.3 %) and 44 (68.75%) suffered a cerebrovascular insult and had arterial hypertension as well.

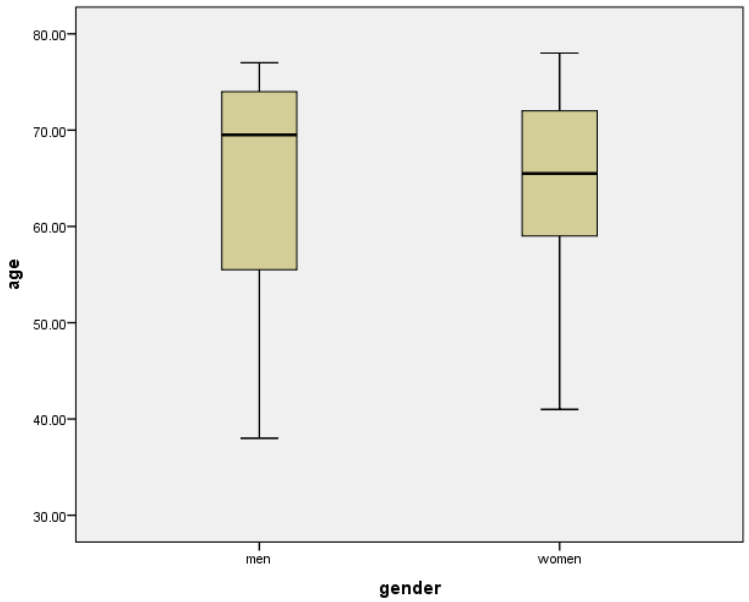


Figure 1. Age structure according to sex

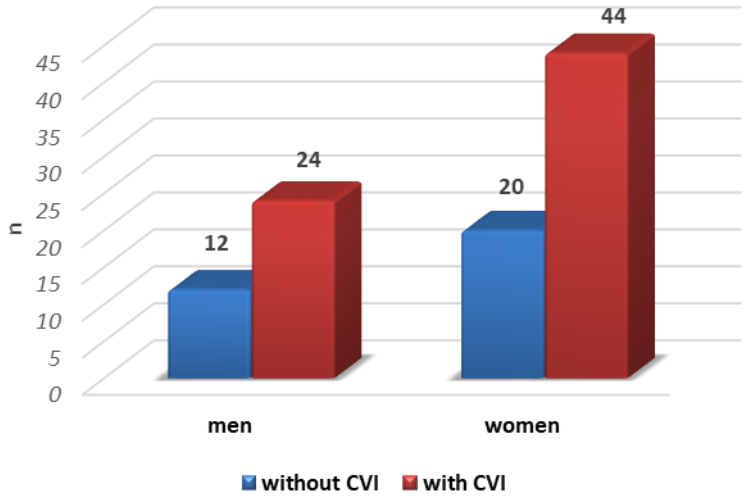


Figure 2. The distribution of CVI patients according to sex

There was no statistically significant difference in the presence of CVI with respect to the sex of the patients ($\chi 2$ =0.046; p= 0.830) (Table 1). The ageing of hypertensive patients contributes to the thickening of the intima-media complex of carotid arteries in both sexes (F=83.382; p<0.001) (Table 2). The presence of hypertension as a risk factor contributes to the thickness of carotid IMC. The ageing of hypertensive patients of both sexes who suffered cerebrovascular disease contributes significantly and shows statistically significant thickening of the intima-media complex of the extra cranial segment of carotid arteries (F=222.006; p<0.001), as showed in Table 3.

Table 1. The distribution of the cerebrovascular disease according to sex

	Elevated TA	CVI	χ^2	p
Men	12 (33.3%)	24 (66.7%)		
Women	20 (31.3%)	44 (68.8%)	0.046	0.830

Table 2. The distribution of patients with arterial hypertension without cerebrovascular diseases

age	men	women	Average thickness IMC/mm	F	p
30 – 49	1(8.3%)	0 (0.0%)	0.50 ± 0.00		
50 – 69	4(33.3%)	13(65.0%)	0.76 ± 0.05		
70 – 80	7(58.3%)	7(35.0%)	0.90 ± 0.00	**83.382**	**< 0.001**
total	**12(100.0%)**	**100(100.0%)**			

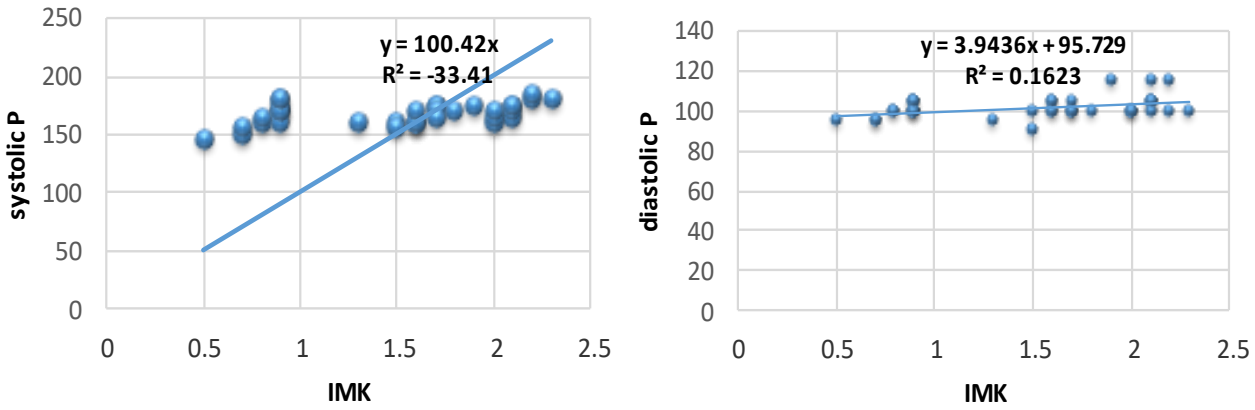
Table 3. The distribution of patients with both arterial hypertension and cerebrovascular diseases

age	men	women	Average thickness IMC/mm	F	p
30 – 49	3(12.5%)	8(18.2%)	1.40 ± 0.09		
50 – 69	10(41.7%)	20(45.5%)	1.70 ± 0.11		
70 – 80	11(45.8%)	16(36.40%)	2.12 ± 0.11	**222.006**	**< 0.001**
total	**24(100.0%)**	**44(100.0%)**			

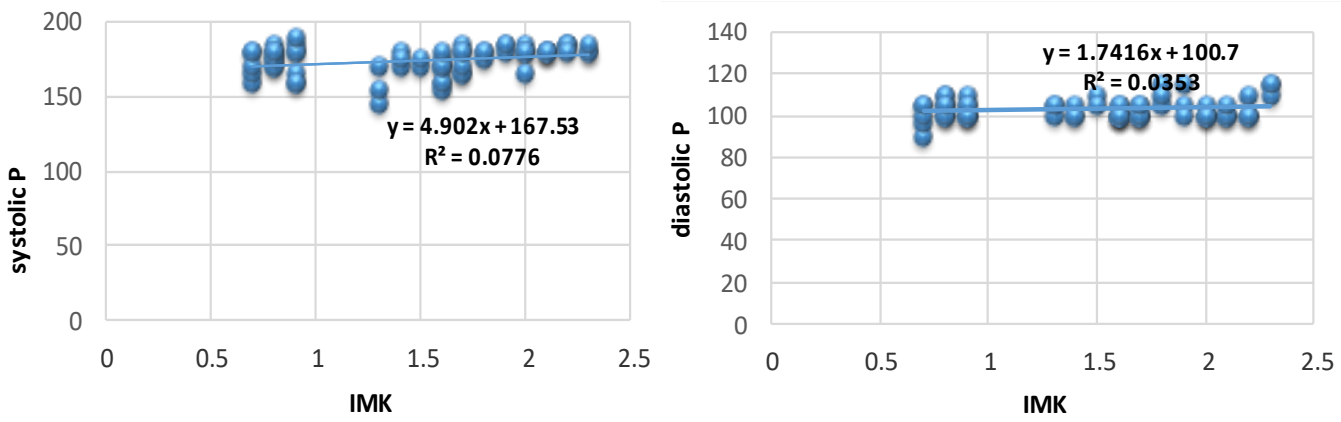
Table 4 shows the values of Pirson's coefficient of linear correlation of systolic and diastolic blood pressure with the values of the intima-media complex: in patients without CVI, a positive correlation was established with the systolic (r = 0.447; p = 0.010), as well as with the diastolic blood pressure (r = 0.413; p = 0.019); positive, statisticaly significant correlation was also noticed in patients suffering from CVI:
systolic P (r= 0.596; p = < 0.001),
diastolic P (r = 0.596; p = 0.008).

Table 4. Correlation between the systolic and diastolic blood pressure with IMC

	Patients without CVI		Patients with CVI	
	r	p	r	p
Systolic P	0.447	0.010	0.596	< 0.001
Diastolic P	0.413	0.019	0.318	0.008



Graph 1. The correlation between systolic and diastolic blood pressure and IMC in patients without CVI



Graph 2. The correlation between systolic and diastolic blood pressure and IMC in patients with CVI

A significant correlation was established between elevated values of systolic blood pressure and the IMC thickening in patients suffering from arterial hypertension but without the signs of neurological diseases ($\chi 2 = 35.034$; $p < 0.001$).

Table 5. The distribution of patients with elevated systolic blood pressure (SP) without cerebrovascular disease with respect to the thickness of the wall

	The values of the systolic blood pressure in mmHg						χ^2	p
The thickness IMC/mm	**140-149**	**150-159**	**160-169**	**170-179**	**180-189**	**190-200**		
0.5 – 0.6	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)		
0.7 – 0.8	0(0.0)	2(11.8)	4(23.5)	5(29.4)	6(35.3)	0(0.0)		
0.9	0(0.0)	0(0.0)	4(28.6)	4(28.6)	5(35.7)	1(7.1)	**35.034**	**< 0.001**

There was no statistically significant difference in the values of diastolic blood pressure and the IMC thickening in patients suffering from arterial hypertension, but without a sign of neurologic diseases ($\chi2=9.281$; $p=0.054$).

Table 6. The distribution of patients with elevated diastolic blood pressure (DP) who didn't suffer from cerebrovascular diseases with respect to the wall thickness

	The values of the diastolic blood pressure in mmHg			χ^2	p
The thickness IMC/ mm	**90 – 99**	**100 – 109**	**110 – 120**		
0.5 – 0.6	1(100.0)	0(0.0)	0(0.0)		
0.7 – 0.8	4(23.5)	11(64.7)	2(11.8)		
0.9	0(0.0)	13(92.9)	1(7.1)	**9.281**	**0.054**

The existence of a significant correlation was established between the elevated values of systolic blood pressure and the IMC thickening with the patients suffering from arterial hypertension with signs of neurological diseases ($\chi2 = 22.830$;$p = 0.004$). Statistically significant difference was establi-shed in the values of diastolic blood pressure and IMC thickening in patients suffering from arterial hypertension with signs of neurological diseases ($\chi2 = 11.712$; $p = 0.020$).

Table 7. The distribution of patients with elevated systolic blood pressure (SP) who suffered from cerebrovascular diseases with respect to the wall thickness

	The values of the systolic blood pressure in mmHg					χ^2	p
The thickness IMC/mm	**140-149**	**150-159**	**160-169**	**170-179**	**180-189**		
1.3 – 1.5	1(100.0)	2(18.2)	2(18.2)	5(45.5)	1(9.1)		
1.6 – 1.9	0(0.0)	1(3.3)	8(26.7)	12(40.0)	9(30.0)		
2.0 – 2.3	0(0.0)	0(0.0)	4(14.8)	5(18.5)	18(66.7)	**22.830**	**0.004**

Table 8. The distribution of patients with elevated diastolic blood pressure (DP) who didn't experience cerebrovascular diseases with respect to the wall thickness

	Values of the diastolic blood pressure in mmHg			χ^2	p
Thickness IMC/ mm	**90 – 99**	**100 – 109**	**110 – 120**		
0.5 – 0.6	2(18.2)	8(72.7)	1(9.1)		
0.7 – 0.8	0(0.0)	26(86.7)	4(13.3)		
0.9	0(0.0)	21(77.8)	6(22.2)	**11.712**	**0.020**

Discussion

Carotid disease is one of the main causes for the development of cerebrovascular diseases; atherosclerosis is the most common disease of the carotid arteries. Atherosclerosis of carotid arteries is the cause of almost one fifth of ischemic brain apoplexy incidences (12) and it starts most often in the distal part, more precisely at the bifurcation point of common carotid artery (CCA) and spreads in the first few centimeters of the proximal part of the internal carotid artery (ICA) and the external carotid artery (ECA), making the ICA much narrower. The initial examination for establishing the diagnosis of carotid arteries diseases is Color Doppler ultrasound examination (CDU) because of its noninvasiveness, portability and relatively low price (13).

The first morphological changes which signify the beginning of the atherosclerotic process are thickenings of the blood vessel walls. The arterial blood vessel wall consists of three layers. The interior layer, or intima is formed from a single layer of endothelial cells, and this layer is the most important in the structural and functional sense. The medial layer, or media, consists of muscular and elastic fibers. The outer layer, or adventitia, consists of connective tissues. The interior and medial levels form intima-media complex (IMC), the thickness of which is established by the ultrasound examination. IMC thickening normally begins with ageing, and these changes in the wall structure of carotid arteries are important for the blood flow in the brain, and serve for the assessment of already diagnosed atherosclerotic disease. In this way, the patients with elevated risk levels for the occurrence of cerebrovascular diseases can be detected early, and their status could be influenced by the application of certain preventive measures and therapy, with a much greater effect than the later treatment of cerebral apoplexy, accompanied with rehabilitation (14).

Structural alterations in the arterial wall, the intima-media thickenings, can be detected by the ultrasound examination of the carotid arteries in patients suffering from cerebrovascular diseases as well as in patients who developed risk factors. The evaluation of the risk for the development of cerebrovascular diseases in patients suffering from arterial hypertension depends on the severity of hypertension level (mild, moderate, severe), the present damage of the organs in question, the existence of previously combined clinical conditions and the presence of other risk factors (15).

The connection of risk factors for the development of cerebrovascular diseases and IMC thickness of carotid arteries are the subject of many conducted researches. The literature data show the existence of a correlation between the risk factors for CVI and the thickness of the carotid IMC (16).

The measurement of the IMC thickness is important for the establishing the subclinical damages to the carotid arteries and is a part of the algorithm for the assessment of cerebral risk in persons who suffer from the elevated blood pressure. Ultrasound measurement of the IMC thickness presents a noninvasive screening test for the identification of morphological changes in the structure and function of arteries in patients suffering from arterial hypertension (17). A great causality has been proved between the hypertension as a risk factor in the development of cerebrovascular diseases and the structural and functional changes in the walls of carotid arteries (IMC thickenings, the formation of atherosclerotic plaque).

Vascular aging, induced by atherosclerosis, can be tracked by the measurement of the IMC of the carotid arteries, thereby enabling the identification of patients with elevated risks, and application of preventive and therapeutic measures and procedures (18). The importance of the measurements of the IMC of the carotid arteries is confirmed by the fact that different national and international guidelines classify IMC as an additional factor in the assessment of the total risk for the occurrence of vascular diseases.

Our research showed that the older the age of patients of both sexes affected by arterial hypertension, the greater the thickness of the carotid IMC. There is a statistically significant correlation between the thickening of the carotid IMC and the elevated values of the blood pressure in patients who suffered a cerebrovascular insult (19).

Subclinical phase of atherosclerosis in the carotid arteries is the damage of the endothelium and gradual diffuse thickening of the IMC, further detailed researches of the altered IMC values of the carotid arteries will offer a better definition of this parameter as a predictable factor for cerebrovascular diseases (20).

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DIJAGNOSTIČKI ZNAČAJ DEBLJINE INTIMOMEDIJALNOG KOMPLEKSA KAROTIDNIH ARTERIJA KOD BOLESNIKA SA HIPERTENZIJOM KAO FAKTOROM RIZIKA ZA NASTANAK CEREBROVASKULARNIH BOLESTI

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Karotidna bolest doprinosi povećanju ukupnog morbiditeta i mortaliteta populacije obolele od cerebrovaskularnih bolesti. Primarni patološki činilac odgovoran za bolesti u ekstrakranijalnom delu cerebrovaskularnog sistema je ateroskleroza, a merenje debljine intimomedijalnog kompleksa (IMK) ekstrakranijalnog dela karotidnih arterija predstavlja merljiv pokazatelj ateroskleroze. Na debljinu IMK karotidnih arterija utiču brojni faktori, kao što su lokalna hemodinamika, zidni stres, hipertenzija arterialis i drugi.

Cilj našeg istraživanja bio je utvrđivanje uticaja hipertenzije kao faktora rizika za nastanak cerebrovaskularnih oboljenja na debljinu intimomedijalnog kompleksa karotidnih arterija kod asimptomatskih i simptomatskih ispitanika u odnosu na pol i starost.

Istraživanje je sprovedeno na 100 ispitanika oba pola koji su lečeni u ambulantnim uslovima ili hospitalizovani na Odeljenju neurologije, a u anamnezi bolesti su imali hipertenziju kao faktor rizika za nastanak cerebrovaskularnih oboljenja. Merenje IMK karotidnih arterija vršeno je ultrazvučnim pregledom, a kao granična vrednost normalnog i patološkog nalaza uzeta je vrednost debljine IMK > 0,9 mm.

Od ukupnog broja ispitanika bilo je 36 muškaraca i 64 žene. Svi ispitanici su imali povišene vrednosti krvnog pritiska. U ispitivanoj grupi bolesnika muškaraca sa znacima samo hipertenzivne bolesti bilo je 33,3%, a 66,7% je doživelo cerebrovaskularni insult i arterijsku hipertenziju kao faktor rizika. Od 64 žene sa znacima samo hipertenzivne bolesti bilo je 31,3%, a 68,75% je doživelo cerebrovaskularni insult, pri tom boluju od arterijske hiperenzije. Merenje debljine IMK karotidnih arterija ultrazvučnom metodom ima značaja u otkrivanju subkliničkog oštećenja strukture zida arterija i predstavlja deo algoritma za procenu cerebrovaskularnog rizika osoba sa povišenim krvnim pritiskom. Postoji značajna korelacija između debljine karotidnog IMK, hipertenzivne bolesti i cerebrovaskularnih oboljenja.

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Ključne reči: *karotidne arterije, intimomedijalni kompleks, hipertenzija, ateroskleroza, ultrazvučna dijagnostika*

Case report

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A SUCCESSFULLY TREATED IATROGENIC LEFT MAIN AND CIRCUMFLEX CORONARY ARTERY DISSECTION DURING A PRIMARY PERCUTANEOUS CORONARY INTERVENTION DUE TO A STENT THROMBOSIS: A CASE REPORT

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Stent thrombosis is one of the most dangerous complications after a percutaneous coronary intervention. Additionally, another worrisome complication is the iatrogenic left main coronary artery dissection. We present a case of an iatrogenic left main coronary artery dissection spreading toward the circumflex artery, successfully treated by a prompt bail-out stenting.

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Key words: *stent thrombosis, iatrogenic dissection, left main coronary artery*

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Introduction

Stent thrombosis, one of the most lethal complications after a coronary stent implantation, occurs in 0.5% to 1% of patients within the twelve months following the percutaneous coronary intervention and is usually presented as an acute myocardial infarction (MI) (1). Under these circumstances, the emergent PCI is the best approach to adopt and the treatment of stent thrombosis, with optimal reperfusion in two-thirds of patients (2, 3). The Iatrogenic left main coronary artery (LMCA) dissection remains, however, one of the most worrying complications in the catheterization laboratory, with a reported incidence of less than 0.1%.

An LMCA dissection often leads to the cessation of blood flow that supplies a large portion of the myocardium, resulting in a hemodynamic collapse. The first successful percutaneous bail-out type of an LMCA stenting was performed in 1993. Although a CABG is the treatment of choice, many patients died before entering the operating room. An immediate percutaneous coronary intervention (PCI) seems to be the appropriate and feasible alternative if performed by an experienced interventionalist (4).

We present a case of an iatrogenic left main dissection during a percutaneous coronary intervention of an acute coronary syndrome due to a stent thrombosis.

Aims

A 69-year old man had suffered an acute ST-elevation myocardial infarction (STEMI) two months ago and was treated with a primary PCI (PPCI). Two BMS were implanted in the mid portion of the LAD (Integrity 2.75 x 12 and Integrity 3.0 x 12). The patient accidentally stopped his clopidogrel intake for two days which resulted in a severe chest pain. He was admitted to the emergency department in the local hospital and the ECG revealed ST elevation in the anterior leads and was immediately referred to our center for coronary angiography and primary PCI. He was treated with a loading dose of Ticagrelor 180 mg, Aspirin 300 mg, and Heparin 8000 IU and transported to the catheterization laboratory. A right femoral approach was performed with an insertion of a 6 F introducer sheath and an Extra Back-Up 3.75 guiding catheter. The coronary angiography revealed an in-stent thrombosis of the mid-part of the left anterior descending coronary artery (LAD) and a stenosis of the proximal LAD segment of 70% (Figure 1).

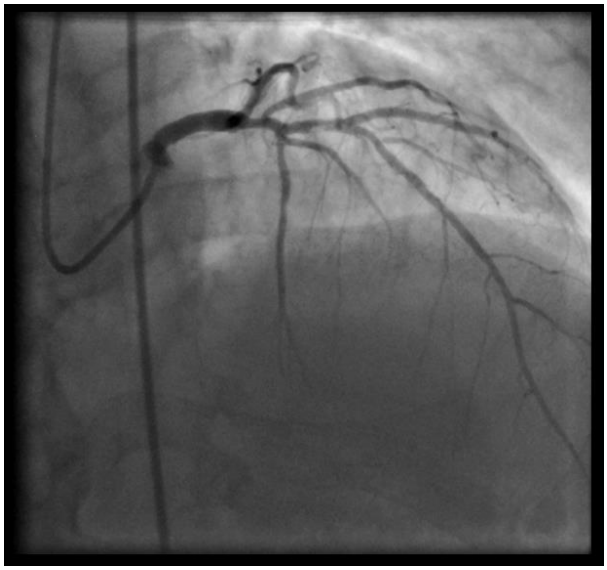

Figure 1. In-stent thrombosis in the mid-part LAD

After introducing the Sion Blue (Asahi) guidewire, a predilatation was performed using a noncompliant balloon (Maverick NC 3.0 × 20mm, Medtronic), after which a Xience V (Abbot) stent 3.5 x 28 was implanted at 13 atm. However, after the first injection of contrast dye after the stent implantation, a dissection extending from the proximal LAD to the distal LMCA was visualized (Figure 2.)

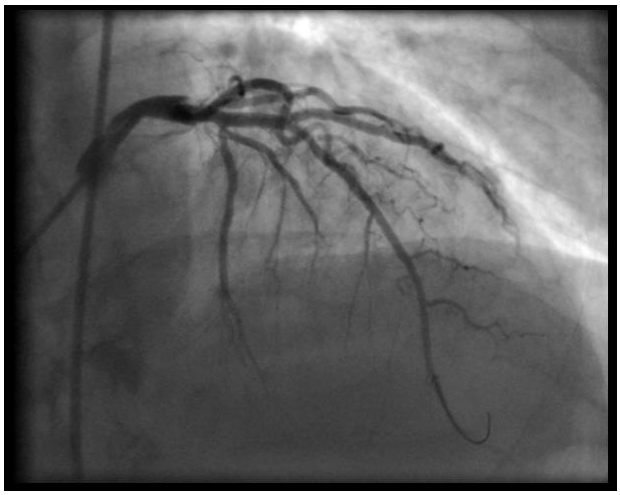

Figure 2. Left main dissection

The patient complained of a chest pain and the ST elevation reoccurred. We decided to perform a direct stenting of the LM using the Promus Premier (Boston) stent, 4.0 x 20 at 14 atm in order to prevent a LM thrombosis. After stenting, TIMI 3 flow was established, but with a compromised ostium and a dissection of the proximal part of the circumflex artery (Figure 3).

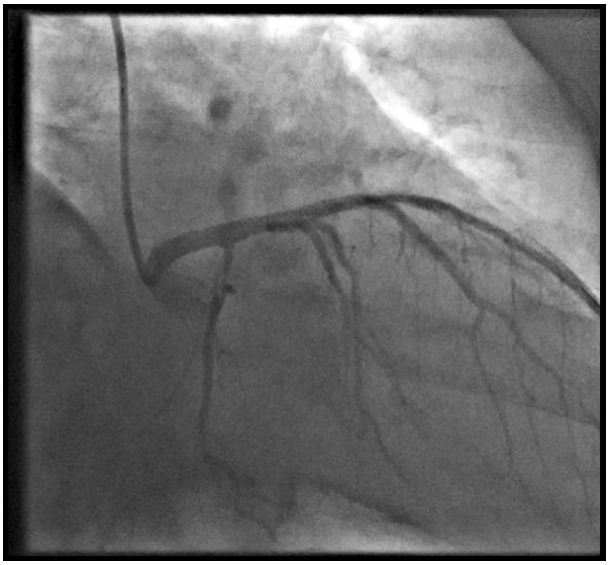

Figure 3. Dissection of the proximal part of the circumflex artery

The guidewire BMW was introduced in the circumflex artery and strut opening was performed using a balloon catheter Sprinter Legend (Medtronic) 2.0 x 10 at 13 atm. The dissection was solved using two stents Promus Element (Boston) 2.5 x 16 at 12 atm and the Promus Element (Boston) 2.75 x 16 at 12 atm (Figure 4).

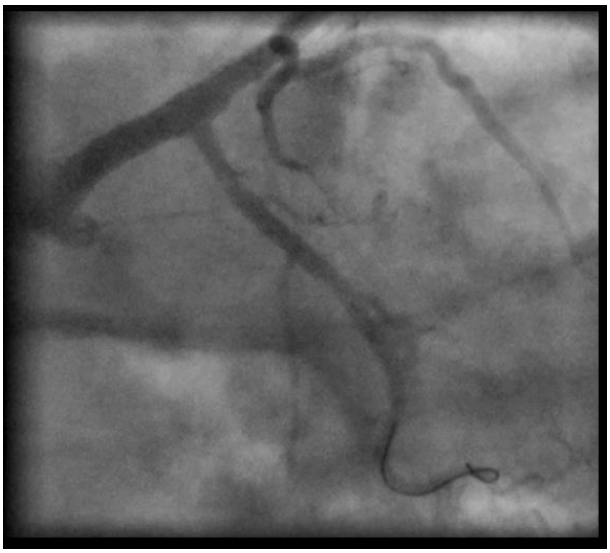

Figure 4. Stenting of the proximal and mid part of the circumflex artery

The final kissing was done using a balloon catheter Maverick (Medtronic) 3.75 x 14 in the left main at 10 atm and with a stent balloon 2.75 x 16 at 15 atm. TIMI 3 flow was established in the LM, LAD and the circumflex artery without any visual dissection or thrombosis (Figure 5).

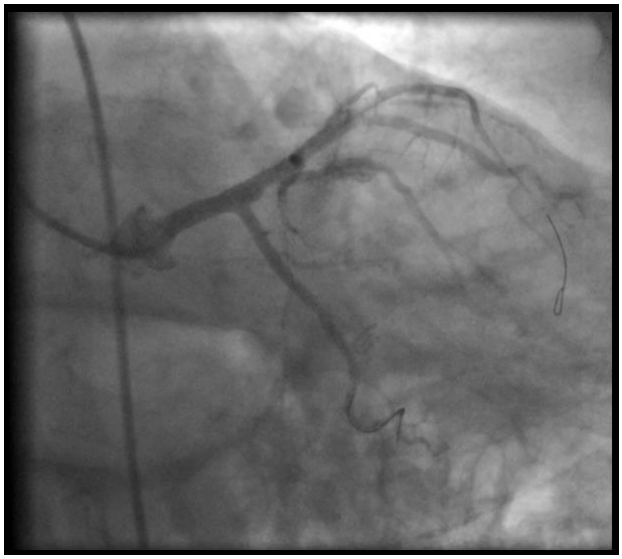

Figure 5. Final result after final kissing

Discussion

Stent thrombosis (ST) is potentially a life-threatening complication after a percutaneous coronary intervention (PCI), manifesting as an acute coronary syndrome (ACS) or a sudden cardiac death. The wide spectrum of risk factors related to a clinical presentation, the complexity of the lesion, stent type, and antiplatelet therapy may be responsible for the stent thrombosis (Table 1) (5).

Table 1. Potential risk factors for early stent thrombosis from the Dutch Stent Thrombosis Registry

Risk factors for early ST	Odds ratio
Premature clopidogrel discontinuation	36.5
Stent under-sizing	13.4
Coronary dissection	6.1
Postprocedural TIMI flow	5.2
≥ 50% lesion proximal to the culprit lesion	4.1
Malignancy	3.0
No aspirin at PCI	2.8
Impaired left ventricle ejection fraction (< 30%)	2.7

The procedural factors leading to the STEMI include a stent underexpansion and malposition (especially in the proximal part), a smaller stent diameter, and a coronary dissection (6, 7). A calcified lesion should be prepared before a stent deployment using a rotational atherectomy, cutting the balloon or the hugging balloon technique so as to avoid the stent underexpansion (8). In our case, the first PPCI was performed using direct stenting without a preparation of a calcified lesion. A proximal stenosis remained untreated, due to the operator's estimation that it was not a culprit lesion. A premature withdrawal of clopidogrel may be also an additional risk factor for an in-stent thrombosis.

Yet another complication related to the second PPCI was the LM dissection. The Iatrogenic LMCA dissection can be a result of a mechanical injury to the arterial wall during a catheter manipulation or administering of any kind of interventional devices. There is a whole range of possible causes: extensive catheter manipulation, special catheter type (e.g. Amplatz catheter or the small Judkins catheter with deep LMCA intubation), stiffer guide wires like the pressure wire, uncommon LMCA anatomy or take-off, a less experienced operator, and the a therosclerotic LMCA can be associated with an increased risk of dissection (9, 10).

In our case, the cause of the dissection spreading from the proximal LAD not only to LM but also to the unprotected circumflex artery was an expansion of the stent with a greater than usual diameter. LM stenting was a bail-out and after that, we had to rewire the circumflex artery with a great risk for entering the false lumen.

The wide spectrum of clinical presentations of the LM dissection is visible, depending on the remaining antegrade coronary flow: patients free of symptoms with a preserved TIMI 3 flow, or a patient in a refractory cardiogenic shock with a completely occluded LMCA. However, even in the case of an initial TIMI 3 flow and hemodynamic stability, rapid deterioration may suddenly occur because of an abrupt flow compromised due to a progressive dissection or superimposed thrombus formation, and a PCI or CABG should be performed immediately (4).

Different strategies have been proposed for the treatment of the LM dissection. Conservative management may be enough if its location does not compromise the coronary flow or a percutaneous intervention, if it is feasible. A coronary bypass surgery should be reserved for cases in which a percutaneous intervention cannot be successfully and safely performed promptly. 'Watchful waiting', as suggested by Alfonso et al. (2), is a reasonable option in the hemodynamically stable patient with a low-grade dissection. The hemodynamic instability is the main indication for a coronary intervention (11).

The literature review by Cheng et al. (12) of 36 patients stented due to an iatrogenic LMCA dissection showed a favorable immediate outcome with angiographic success in 32 patients (88.9%). Four patients needed an emergent CABG and two of them died. A percutaneous intervention with back-up surgery in those groups of patients resulted in an overall survival rate of 94.4%.

One-year follow-up of our patient was satisfactory. Unfortunately, we did not perform a coronary angiography, either IVUS or OCT, despite the obligation according to the guidelines of coronary

revascularization, because the patient did not accept a repeated invasive procedure. We had also suggested a prolonged dual antiplatelet therapy, lasting more than 12 months.

Conclusion

It is very important to prepare a lesion before stenting, especially if it is calcified even during the primary PCI. An iatrogenic dissection of the left main coronary artery may be a life-threatening complication and a bail-out PCI could be the only solution.

The left circumflex artery may be an innocent victim and should be treated in order to save the endangered myocardium.

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Prikaz slučaja

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USPEŠNO REŠAVANJE JATROGENE DISEKCIJE GLAVNOG STABLA I CIRKUMFLEKSNE GRANE LEVE KORONARNE ARTERIJE TOKOM PRIMARNE PERKUTANE INTERVENCIJE – PRIKAZ SLUČAJA

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Tromboza stenta je jedna od najtežih komplikacija perkutane koronarne intervencije. Ne manje važna je i jatrogena disekcija glavnog stable leve koronarne arterije. Opisali smo slučaj jatrogene disekcije glavnog stabla leve koronarne arterije koja se proširila na cirkumlekfsnu granu i koja je uspešno tretirana brzom perkutanom intervencijom.

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Ključne reči: *tromboza stenta, jatrogena disekcija, glavno stablo leve koronarne arterije*

Case report

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AGENESIS OF THE GALLBLADDER: A CASE REPORT

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Congenital agenesis of the gallbladder is a rare anatomical abnormality. A 75-year-old woman was admitted with a history of intermittent pain in the right upper abdominal quadrant in the past few weeks, suggestive of biliary colic. A physical examination showed some mild tenderness in the right upper abdominal quadrant. Abdominal ultrasonography was interpreted as "images consistent with a contracted gallbladder with multiple small stones". Multislice computerized tomography showed a common bile duct dilatation, and a mild intrahepatic dilatation of the left and right hepatic duct. Computerized tomography did not reveal any presence of gallbladder stones. Magnetic retrograde cholangiopancreatography did not show any anatomical variations and anomalies. Intraoperative ultrasonography failed to locate the gallbladder inside the liver. Intraoperative cholangiography confirmed the diagnosis of gallbladder absence, as well as absence of cystic duct and common bile duct stones. The patient recovered after surgery without any complications. A follow-up examination, one year after the surgery was without any complaints or complications.

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Key words: *gallbladder, gallbladder agenesis, biliary tract abnormality*

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Introduction

Congenital agenesis of the gallbladder (CAGB) is a rare anatomical abnormality. CAGB is usually asymptomatic; however, if symptomatic, it is accompanied with dyspepsia, nausea, vomiting or abdominal pain (1). Despite an absent gallbladder, half of the patients had symptoms resembling chronic cholecystitis or biliary colic (2). Isolated CAGB is extremely rare, with incidence ranging between 0.013 and 0.075% (3). CAGB may be associated with other congenital malformations (4, 5). Routine diagnostic methods frequently fail to diagnose gallbladder agenesis, and if not suspected, patients end up with a surgical intervention (5). However, nowadays, magnetic resonance cholangiopancreatography (MRCP) is a preoperative diagnostic method of choice for CAGB detection (1). The patient described in this case report was subjected to preoperative abdominal ultrasonography (US) which revealed gall stones. Other preoperative biliary imaging studies, such as multislice computerized tomography (MSCT) and MRCP, did not indicate any anatomic variations and anomalies. Both diagnostic imaging techniques suggested the need for a surgical intervention. The study presents a CAGB case detected by intraoperative exploration, as well as the shortcomings of the applied diagnostic and surgical methods.

Case report

A 75-year-old woman arrived to the surgical department with a history of intermittent pain for a few weeks in the right upper abdominal quadrant suggestive of biliary colic. The pain was exacerbated by eating, especially fatty foods. Recurrent episodes of pain occurred for six months. Clinically visible jaundice was found a week before admission. There was no relevant medical or family history of biliary disease.

Physical examination was in order, except for a mild tenderness in the right upper abdominal quadrant. Standard laboratory blood analyses showed atypical changes in total bilirubin - 50.1 μmol/L (normal range 1 to 20 μmol/L); serum glutamic-oxaloacetic transaminase - 276 U/L (normal range 5 to 48 U/L); alkaline phosphatase - 352 U/L (normal range 30 to 115 U/L); gamma-glutamyltransferase - 541 U/L (normal range 1 to 38 U/L); and lactate dehydrogenase - 491 U/L (normal range 120 to 246

U/L); leukocyte count - 9.1 x109/L (normal range 4 to 10 x109/L). Other laboratory parameters and urine analysis were within normal limits.

Abdominal US was interpreted as "images consistent with a contracted gallbladder with multiple small stones". MSCT showed common bile duct dilatation (up to 18mm) and initial intrahepatic dilatation of the left and right intrahepatic duct (9 mm and 12 mm, respectively). MSCT did not show the presence of gallstones. Furthermore, MSCT showed common bile duct stones and one impacted stone in the region of the papilla of Vater. MRCP confirmed bile stones and showed no anatomic variations and anomalies. After a review of both imaging studies, the radiologist indicated the need for surgical consultation. Since the symptoms did not resolve after conservative treatment, surgery was indicated.

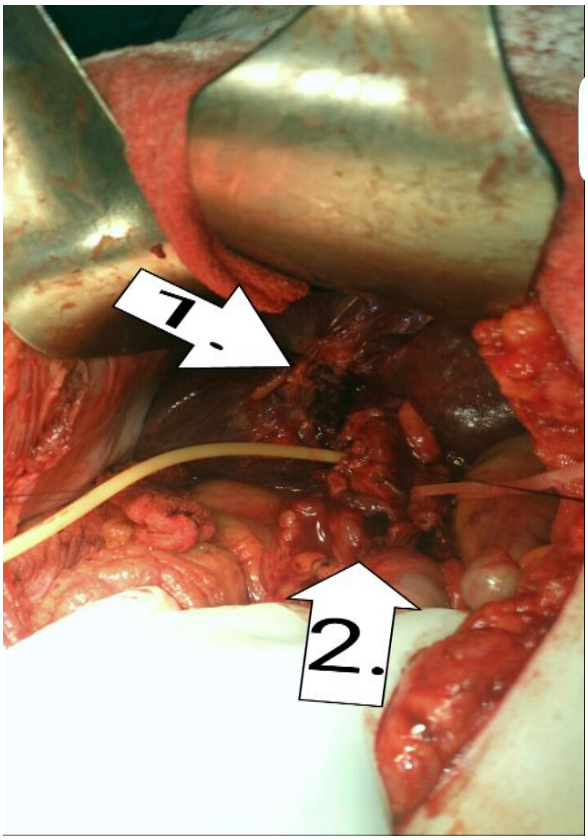

Figure 1. Position of the common bile duct before dissection (pointer 1), and position of the duodenal fistula after the separation (pointer 2) with T tube before cholangiography

The patient was classically operated because laparoscopy was not considered suitable for the case. Surprisingly, gallbladder was not found in the area of the gallbladder bed. An intraoperative dissection revealed that the common bile duct was deformed as protruding out of the liver (Figure 1, pointer 1). A very careful exploration of the falciform ligament, retrohepatic, retroduodenal, retropancreatic, retroperitoneal space, left side of the abdominal cavity and within the lesser omentum did not reveal the presence of gallbladder or cystic duct. Moreover, intraoperative US failed to locate the gallbladder inside the liver. The detected fistula between the common bile duct and duodenum (Figure 1, pointer 2) was disassembled during surgery in the further course of the operation.

After insertion of a T tube, an intraoperative tube cholangiography confirmed the diagnosis of absent gallbladder, absent cystic duct and gallstones in the common bile duct (Figure 2).

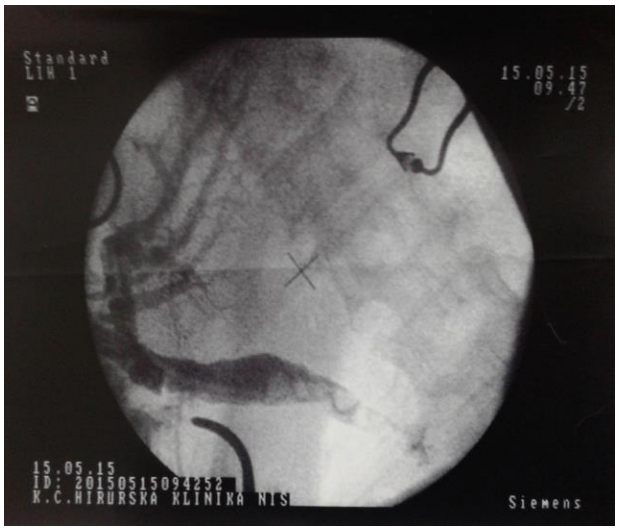

Figure 2. Intraoperative cholangiography confirming an absent gallbladder, absent cystic duct and common bile duct stones

The common bile duct was full of gallstones after the opening. The T tube was removed and the bile stones were washed out. After the removal of the stones from the common bile duct, the remaining digestive tract was operated with biledigestive Roux bypass. The procedure was chosen because of the suspicion of intrahepatic bile stones, bad quality of the common bile duct wall and duodenal wall, as well as bad duodenum contrast filling. The patient recovered well after surgery and was discharged on the 10th day after surgery. The follow-up visit 2 weeks after the discharge revealed no complications. The last follow-up visit one year after the surgery showed a healthy patient with no complaints and no signs of biliary system disease.

Discussion

CAGB is rare congenital anomaly characterized by the absence of the gallbladder with a normal bile duct system. CAGB is often associated with congenital abnormalities in other systems in approximately 30% (6). It can occur anytime during lifetime, most commonly at the median age of 46 years. The incidence in clinical series ranges from

0.007% to 0.027%, and in autopsy reports from 0.04% to 0.13% (6). The prevalence range is from 0.007% to 0.13% (3, 7). It is almost always an incidental finding at surgery or autopsy (1). Women to men ratio in clinical trials ranges three to one similar to other biliary diseases, but the autopsy reports suggest an equal (1:1) ratio (8).

Gallbladder agenesis is rare and occurs during embryonal development. In the fourth week of development, cranial and caudal part of the hepatic diverticulum develop from the hepatic diverticulum (9). From its larger caudal part, the liver parenchyma and intrahepatic biliary epithelium develop (4). The gallbladder and the cystic duct form from small vessels from the smaller caudal part (10). The anomalies that evolve during embryonal development may be in the form of gallbladder agenesis alone or with the absence of the cystic duct and many others congenital anomalies (1, 9, 11). The etiology of CAGB is unknown, but the reports of familial occurrence suggest a possible hereditary origin (4). Genetic factors may play an important role in the pathogenesis (12, 13).

Individuals with CAGB can be divided, according to Bennion (14), into 3 categories: 1) healthy subjects without symptoms (30% to 60%); 2) symptomatic patients (30% to 40%); and 3) patients with multiple congenital anomalies (15% to 30%). In his case report (12) in 2015, Li Ming Tang added 2 subcategories in the 3rd Bennion category: 3A) patients with lethal anomalies (15), and 3B) patients with nonlethal anomalies (5, 12).

Symptomatic patients have the symptoms suggestive of cholelithiasis (15). Most patients have right upper abdominal pain (90%), dyspepsia (30%), nausea and vomiting (66%), intolerance to fatty food and jaundice (12, 16, 17). With these patients it is difficult to determine what causes the symptoms. One of the explanations of the symptoms and clinical features is the combined biliary dyskinesia and constant pressure rise in the sphincter of Oddie. Some patients have a dilated common bile duct that takes up the function of bile storage. Finally, cholestasis arises from biliary dyskinesia and the resulting infection leads to future formation of common bile duct stones (5).

Around 40% to 60% of patients show the symptoms consistent with biliary disease: nausea, right upper abdominal pain, vomiting, bloating, and fatty food intolerance, as demonstrated in our case. In addition, 25% to 50% have choledocholithiasis with symptoms such as fever, chills, biliary colic and jaundice, as in our case presented above (18, 19).

Biliary tract diseases are diagnosed based on the usual imaging methods. Currently, these are abdominal US and MSCT. This led to a unique problem in diagnosing CABG, since cystic duct obstruction, chronic cholecystitis and gallbladder agenesis all lead to non-visualisation of the gallbladder and cystic duct with both modalities (8, 20, 21).

Preoperative diagnosis of CAGB is extremely difficult. Patient symptoms, ultrasonographic findings suggestive of gallbladder disease, lack of other reasonable clinical diagnoses, and rarity of this entity, weigh heavily in favor of the diagnosis of biliary tract disease. Our 75-year-old patient presented the symptoms of biliary tract disease that was later determined to be caused by gallbladder agenesis. Our patient was jaundiced, with suspected common bile duct stones. Ultrasonography of the right upper abdominal quadrant showed multiple hyperechogenic loci with significant shadowing in the gallbladder bed region. Ultrasonography of the same patient further demonstrated similar findings suggestive of multiple gallstones in a contracted gallbladder.

Ultrasonography, with its high sensitivity, is now the modality of choice for preoperative imaging of the gallbladder and acute biliary disease. In CAGB, intestinal loops occupy the expected location of the gallbladder causing significant shadowing, with an appearance similar to that of a contracted gallbladder filled with stones. The cystic duct, if present, may not be visualized as the result of intense shadowing from intestinal gas (18, 19, 22). These findings were present in our case, in which gallbladder agenesis could not be distinguished from chronic cholecystitis associated with choledocholithiasis, or simply a contracted gallbladder with stones (23, 24).

MSCT scanning or ERCP may raise the suspicion of CAGB in patients with questionable sonographic findings (16). MSCT may be useful in detecting a gallbladder in an intrahepatic or abnormal location, or suggesting the diagnosis of CAGB if the gallbladder cannot be visualized (16, 22). In our case, biliary duct dilatation was noted on MSCT. Both imaging methods are useful preoperative and postoperative modalities for diagnosis confirmation and for clinical follow-up. ERCP may demonstrate an enlarged common bile duct without evidence of a cystic duct or its remnant. This leads to a misinterpretation typical for cystic duct obstruction in many biliary tract diseases (22). CABG is rarely thought of in the differential diagnosis (25). MRCP revealed no anatomic variations and anomalies, but after a review, the radiologist indicated consultation with a hepatobiliary surgeon. MRCP is a noninvasive procedure but is readily available (8, 26). It is able to indicate the diagnosis of CABG, as well as of other biliary anomalies and diseases (26). Hepatobiliary scintigraphy with 99mTc – IDA can now potentially detect gallbladder anomalies (19). Selective arteriography of the hepatic artery has been proposed as a diagnostic tool for CABG (16, 19), but it is a very invasive procedure (20, 23).

During the open surgery, we discovered a winding common bile duct in the gallbladder bed (Figure 1, pointer 1). The confirmation of a truly absent gallbladder was made with T tube intraoperative cholangiography (Figure 2). Intraoperative cholangiography (16) should always be performed when gallbladder agenesis is considered, because 25% to 50% of these patients have coinciding common bile duct stones, like it was in our case (15). Intraoperative US and cholangiography can help with the diagnosis (12). In our case we performed both these procedures.

We can propose a diagnostic-therapeutic algorithm for gallbladder agenesis. If the diagnosis is

made preoperatively:
A) Patients without common bile duct stones, should undergo ERCP searching for missing coinciding bile stones and to confirm the diagnosis; the treatment is medicamentous, conservative.
B) Patients with common bile duct stones, should undergo ERCP stone extraction (if possible) with endoscopic sphincterotomy. Further treatment is medical and conservative for symptomatic patients, or no treatment for patients without symptoms. If the extraction is not possible, open surgery is recommended. Some even propose laparoscopy to confirm the diagnosis (7, 21, 27).

In the case when the diagnosis is made during laparoscopy, procedure should be aborted after searching for ectopic gallbladder (7, 27). Laparoscopic exploration depends solely on surgical skills. The confirmation is made postoperatively using the imaging methods (7, 28). Some advocate a conversion to open procedure and confirmation of the diagnosis with intraoperative US and cholangiography, if available (21).

If the diagnosis is made during open surgery, a surgeon should proceed searching for ectopic gallbladder in all known localizations with intraoperative US and cholangiography (21, 28). The special circumstances are common bile duct stones and fistulas discovered during surgery, which dictate further operative solutions (21, 27, 28, 29). Interestingly, with most symptomatic patients with pain, the pain resolves after exploratory surgical procedure (8, 13, 24).

In our case, after searching for an absent gallbladder, a bilio-digestive fistula was found and separated (Figure 1, pointer 2). T tube cholangiography confirmed an absent gallbladder and common bile duct stones. After the removal of common bile duct stones, the procedure was terminated with bilio-digestive anastomosis type side to side hepatico-jejunostomy. The reason for that was in the facts that common bile duct wall and duodenal wall were of bad quality and contrast duodenal filling was almost absent.

Conclusion

Agenesis of the gallbladder is a rare clinical entity most often diagnosed intraoperatively. Almost half of the patients have pain and symptoms of gallstones before the surgery. The other half are healthy subjects. The patients with CABG diagnosed preoperatively are referred for medical treatment, with or without potentially explorative laparoscopy. When CABG is incidentally diagnosed during laparoscopy, the procedure should be aborted and converted to laparotomy if the surgeon is not skilled enough to establish the diagnosis laparoscopicaly. When CABG is incidentally found during laparotomy, the procedure should continue and the diagnosis should be established. Although intraoperatively detected to have no gallbladder, most patients become asymptomatic postoperatively.

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Informed consent: We obtained a written and signed consent from the patient to publish her information in the form of this case report. The manuscript was translated into her native language and she approved of its contents. The copy of the signed informed consent can be obtained from the Journal Editor or corresponding author.

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AGENEZA ŽUČNE KESE: PRIKAZ SLUČAJA

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Kongenitalna ageneza žučne kese je retka anatomska abnormalnost. Žena stara 75 godina hospitalizovana je sa istorijom intermitentnog bola u trajanju od nekoliko nedelja, u desnom gornjem abdominalnom kvandrantu, sa suspektnom bilijarnom kolikom. Fizički pregled je ukazao na blagu bolnu osetljivost u desnom gornjem abdominalnom kvadrantu. Abdominalna ultrasonografija ukazala je na sliku "žučna kesa koegzistentna, kontrahovana, sa multiplim kamenjem manje veličine". Multislajsna kompjuterizovana tomografija ukazala je na dilataciju bilijarnog voda i blagu intrahepatičnu dilataciju levog i desnog hepatičnog voda. Kompjuterizovana tomografija nije ukazala na prisustvo kamenja u žuči. Holangiopankreatografska magnentna rezonanca nije pokazala nikakve anatomski abnormalne varijante, niti anomalije. Intraoperativna ultrasonografija nije uspela da otkrije žučnu kesu unutar tkiva jetre. Intraoperativna holangiografija potvrdila je dijagnozu odsustva žučne kese, kao i odsustvo cističnog voda i nalaza kalkuloze žučnog voda. Posle operacije, bolesnica se oporavila bez ikakvih komplikacija. Kontrolni pregled, godinu dana posle operacije, nije ukazao na bilo kakve tegobe, niti komplikacije.

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Ključne reči: *žučna kesa, ageneza žučne kese, abnormalnost bilijarnog trakta*

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BIOPTRON LIGHT THERAPY TO DRY EYE CAUSED BY LONG-TERM TOPICALLY APPLIED ANTIGLAUCOMA DRUG WITH BENZALKONIUM-CHLORIDE

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Benzalkonium-chloride is the most commonly used preservative in modern antiglaucoma topical therapy, with a broad range of advantages and cumulative side effects on the anterior eye surface. Benzalkonium-chloride is classified as a severe irritant of mucous membranes, resulting in dryness of the anterior eye surface.

The aim of the paper was a is scientific confirmation of the efficacy of Bioptron light therapy, its favorable response in healing and elimination of dry eye symptoms and signs caused by topical use of antiglaucoma therapy with Benzalkonium-chloride as a preservative.

The research included 36 patients who were divided into three groups: the first and second group with different approaches in the treatment of dryness of anterior eye surface, and the third control group. A complete ophthalmic examination of the patients was done before our examination and after Bioptron light therapy. The examination was done according to „Dry eye severity grading sheme", and as an objective parameter we did Shirmer's test 1. In the second group of patientis, cured by Hylo®gel solution and Bioptron light therapy, symptoms and signs decreased from 27.78% to 5.56% ($p < 0.001$). The values of Shirmer's test 1 showed an improvement from 30.56% to 13.89% deficit in tear secretion ($p < 0.001$). The symptoms and signs of anterior ocular surface dryness are statistically significantly reduced. Toleration of antiglaucoma drug therapy, comfort and patient compliance are significantly improved. It is necessary to include Bioptron light therapy in all patientes on antiglaucoma therapy with Benzalconium-chloride as a preservative.

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Key words: *Benzalkonum chloride, Keratoconjunctivitis sicca, Bioptron®light therapy*

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Introduction

Modern antiglaucoma therapy includes a large number of different topically applied drugs (1). The presence of one of many preservatives in antiglaucoma eye drops is necessary because of their effects on preventing contamination of the eye drops by pathogenic microbes as well as decompo-sition of active drug on which their shelf-life depends. Different eye solutions may have important and dangerous cytotoxic effects of preservatives on corneal, limbal and conjunctival cells, and if they are associated, especially with long-term use, may cause an additive cytotoxic effect (2). The most frequently used preservatives in ophtalmic preparations are Benzalkonium chloride (BAK), Chlorobutanol, Poliquad®, Stabilized oxichloro complex (Purite), sodium perborate (GenAqua™), SofZia™ ect. (3). Preservatives can have a negative effect on the structure of anterior segment of the eye. From toxicological standpoint, BAK is classified as a severe irritant of mucous membranes and skin (4). Its advantages lie in better penetration of active medication through tear film and cornea, limiting bacterial, viral, mycotic and amoeba infections, without biodegradation of the active medication at the same time. Side effects are cumulative cytotoxic effects on the front eye surface, tear layer disturbance, dry eye, and allergic reactions (5). Good penetration of the drug through the cornea is necessary because of the characteristics of the surfactant and dissolution of the lipid layer of the tear film (6). Its concentration in antiglaucoma drops is from 0.004% to 0.01%. Higher concentrations can cause

irreversible damage to the corneal endothelium (7). The most important collateral effect of BAK is apoptosis and necrosis of corneal epithelium cells, changes of corneal permeability for active drug substance, emergence of tear film instability, dry eye occurrence, discomfort caused by inflammatory changes of conjunctival and subconjunctival fibrosis (8). These changes on the eye front surface are observed 10-15 days after the beginning of use of ophthalmic drops with BAK as a preservative. If side effects of BAK occur in dry eye field, changes are much more undesirable, according to our evidence (9). Symptoms of dry eye are stinging, burning, pain, redeness and photophobia. "Dry eye severity grading scheme" distributes symptoms to four levels (Table 1). For conducting this clinical examination, it was necessary to test patient's psychometric abilities (10). In diagnostic tests for dry eye, the quantity and quality of tear secretion was measured (Shirmer's test) (Tear Break up Time-test). Shirmer's test is a first and simple method including Shirmer's test 1 without anesthesia, where pathological values are ≤ 10 mm in 5 min, and Shirmer's test 1 with anesthesia for basal tear secretion, with pathological values ≤ 8 mm in 5 min.

The symptoms of dry eye may decrease without therapy (11). They are more common in older patientis, menopausal women, during the therapeutic use of drugs for systemic diseases, and can be the result of constant exposure to air conditioning, wind, dust; they occur in people with long-lasting work on the computer, in case of perennial contact lens holders, in conditions when it is impossible to fully close eyelids, rare blinking, and in cases of various lacrimal gland diseases (12). Normal secretion of a triple-layer in tear film structure serves for the general protection, moistening and nutrition for cornea (13).

Table 1. Dry eye severity grading scheme

Dry eye severity level	**1**	**2**	**3**	**4**
Discomfort, frequency	Mild, episodic symptoms	Moderate, occasional or chronic symptoms	Severe frequent or constant symptoms	Severe, disabling, constant symptoms
Visual symptoms	None or episodic mild fatigue	Annoying, activity-limiting	Annoying chronic and constant, activity-limiting	Constant, disabling
Conjunctival injection	None to mild	None to mild	+/-	+/++
Schirmer score mm/5 min	Variable	≤ 10	≤ 5	≤ 2

Symptoms: scratching, fogging and redness

Discomfort upon the use of ophthalmic drops with BAK as a preservative influences the action of an active antiglaucoma drug substance and reduces the patient's compliance in regular application with ophhalmic therapy (14). The treatment of dry eye symptoms primarily consists of long-term use of different artificial tears available on the market, but this is not always enough to achieve an optimal effect (15).

Hylo®gel is lubricating eye drops for the special requirements of chronically dry eyes and particularly severe cases. They contain hyaluronic acid in the concentration of 0.2%, which forms an optimal viscosity of the tear layer on the corneal surface, without blurring vision, and supports the regeneration of the eye surface; the drops are free from preservatives and phosphates, and are packed in the patented multi-dose device that prevents the contamination of its contents. Starting with Bioptron light therapy, using the Bioptron lamp (Figure 1), Bioptron-AG, Swiss company, power of 100-240 V-T2A, we achieve a natural, medical method that boosts regenerative capacity and a favorable response to healing (16). Bioptron light therapy is efficient in the elimination of allergic reactions, signs and symptoms of dry eye, as a result of topicaly applied antiglaucoma therapy with Benzalkonium-chloride as a preservative.

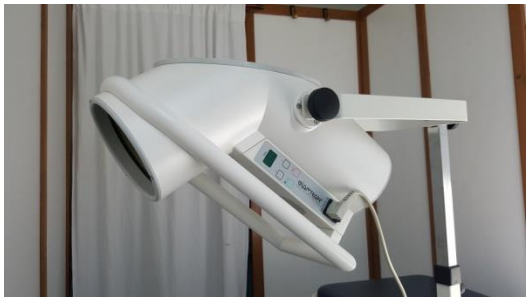

Figure 1. Bioptron®lamp

Bioptron light therapy makes balance within metabo-

lism, improving capillary circulation, immunity stimulation, relieving pain and establishing balance of tissue electromagnetic field (17). Through the eye, visible and infrared light come to the brain, influencng the function and balance of endocrine gland secretion (18). Bioptron biophysical effects depend on the polarized wave energy and polarized wave length. Polarized waves are of the same frequency but they are incoherent, the light intensity at the target tissue is constant, without variation in energy. Energy is very low, 1 - 2.4 J/cm without thermal effects, energy density is 40mW. Bioptron light is polychrome, wave frequency is from 400 nm (including blue, visible radiation) up to 2000 nm (representing infrared waves). Ultraviolet, chemically active radiation is completely eliminated with a special filter (19). Polarized beam penetrates into tissue up to 2.5 cm deep, depending on the exposure time. It stimulates fibroblast and collagen fiber production with no side effects. During and after the treatment, Bioptron light therapy activates immunity, both local and systemic. Being completely safe for eyes, even in pregnant women, the therapy contributes to the fight against viruses (20). The aim of this study was to determine the effectiveness of Bioptron light therapy in the elimination of symptoms and signs of discomfort on the anterior eye surface in patients with topical antiglaucoma therapy with preservative BAK, in whom therapy with Hylo®-gel solution is not enough to eliminate the symptoms and signs of dry eye.

Methods

From May 2014 up to January 2016, a sample of 36 middle-aged patients (72 eyes) was examined, 19 women and 17 men, from the age of 52 to 68. All of them had dry eye symptoms, ranging from mild/normal to moderate and severe. They were all treated longer than one year, with the same topical antiglaucoma drugs, Cosopt®2% (dorsolamide-hyd rochloride 20mg /ml and timolol-maleate 5mg/ml), twice a day, which contain the preservative BAK in a concentration ≤ 0.01%. Patients underwent complete internal examination for the purpose of exluding any other systemic disease which may cause dry eye syndrome and reduce regenerative capacity of mucous membranes and the skin (21). We did complete ophthalmic examination for the purpose of excluding any other disease, injuries and previous surgical treatment of eyes. All patients with diagnosis of chronic blepharitis, meibominitis or any other eye infection were excluded from the study as those diseases may also lead to dry eye due to a deficit in lipid outer layer, and consequently, to evaporative dry eye (22). We examined the symptoms like scratching, fogging and redness of the eyes. Patients were divided in the same three groups, with different topical treatment that followed for the next four weeks. During the four weeks, the first and second group were on different topical therapy, and the third, control group, was treated only with Cosopt® twice a day. The first group of 12 middle-aged patients (24 eyes), 6 women and 6 men, was treated with Cosopt and topical solution Hylo®gel three times a day. The second group of 12 middle-aged patients (24 eyes), 6 women and 6 men, was treated with Cosopt®, Hylo®gel three times a day, and Bioptron light therapy, three days a week. Patients were sitting in a comfortable chair, with their eyes closed, with previously cleaned eyelids, occasionally blinking. Bioptron lamp was lined at an angle of 90°, at a distance from 5 cm to 10 cm, exposure time was 5 min. The third, control group, involved 12 middle–aged patients (24 eyes), 7 women and 5 men, on Cosopt® therapy.

Parameters of better drug tolerability were criteria set by "Dry eye severity grading sheme" (23). As an objective parameter, Shirmer's test 1 was done in all patients, after putting in 0.5% tetracaine solution two times during five minutes for the purpose of measurement of the basal tear secretion, and elimination of the reflex tear secretion (Table 2). Measuement of basal tear secretion was done two minutes after the last use of 0.5% tetracaine drops (24). We used Alcon Laboratories Shirmer's tear test sterile strips, 35 mm in length, in the fornix of the lower eyelid at the junction of the middle and outer third, for 5 min with eyes closed. Humidity of strips was measured in mm, with a ruler. Finding the pieces of paper with humidity less than 10 mm was taken as a pathological value. During the treatment, patients were not on any other systemic therapy (25). The results processing was done using statistical Student's X^2 test and Student´s T-test.

Table 2. Previous measurement

	Mild/normal symptoms	Moderate symptoms	Severe symptoms
First group	4 eyes (5.56%)	6 eyes (8.33%)	14 eyes (19.44%)
Second group	4 eyes (5.56%)	6 eyes (8.33%)	14 eyes (19.44%)
Third control group	4 eyes (5.56%)	6 eyes (8.33%)	14 eyes (19.44%)

First group: 6 women and 6 men (24 eyes), treated with Cosopt®and Hylo®gel
Second group: 6 women and 6 men, (24 eyes), treated only with Cosopt®,Hylo®gel and Bioptron®light therapy
Third control group: 7 women and 5 men, (24 eyes), treated only with Cosopt®

Results

Symptoms: cratching, fogging and redness of the eyes

In previous measurement, before starting the therapy, mild/normal symptoms appeared in two patientis (4 eyes 5.56%) in each of the three groups, moderate symptoms appeared in 3 patients, (6 eyes, 8.33%) in each of the groups, and severe symptoms appeared in 7 patients (14 eyes, 19.44%) in each of the three groups (Table 2). They were divided into the same three groups according to symptoms. Age and gender were not statistically different.

Table 3. Previous measurement: Shirmer's test 1

Shirmer's test 1	> 10 mm mild/normal	≤ 10 mm moderate	≤ 5mm sever
First group	6 eyes (8.33%)	6 eyes (8.33 %)	12 eyes (16.67%)
Second group	2 eyes (2.78%)	10 eyes (13.89%)	12 eyes (16.67%)
Third control group	2 eyes (2.78%)	6 eyes (8.33%)	16 eyes (22.22%)

First group: 6 women and 6 men (24 eyes) treated with Cosopt®and Hylo®gel
Second group: 6 women and 6 men (24 eyes) treated only with Cosopt®, Hylo®gel and Bioptron®light therapy
Third control group: 7 women and 5 men (24 eyes) treated only with Cosopt®

Shirmer's test 1: basal tear secretion

In all three groups, 36 patients (72 eyes) in total, in the majority of patients severe decrease in the basal tear secretion (55.56%) was reported, moderate symptoms were found in 30.56% of participants, and mild/normal symptoms were reported in 13.89% of participants.

Symptoms: scratching, fogging and redness of the eyes

After 4 weeks of different treatment of the three groups with Cosopt®, Hylo®gel and Bioptron®light therapy, we repeated the measurements. In Group 1, severe symptoms in two patients were statistically significantly decreased: from 19.44% to 5.56% ($p < 0.001$). In Group 2, there were no patients with severe symptoms (0 eyes 0%), and the results in Group 3 (control group) were the same as in the previous measurement (Table 4).

Table 4. Results after treatment

	Mild/normal symptoms	Moderate symptoms	Severe symptoms
First group	14 eyes (19.44%)	6 eyes (8.33%)	4 eyes (5.56%)
Second group	20 eyes (27.78%)	4 eyes (5.56%)	0 eyes (0%)
Third, control group	4 eyes (5.56%)	6 eyes (8.33%)	14 eyes (19.44%)

First group: 6 women and 6 men (24 eyes) treated with Cosopt®and Sol.Hylo®gel
Second group: 6 women and 6 men (24 eyes) treated with Cosopt®,Sol.Hylo®gel and Bioptron®light therapy
Third control group: 7 women and 5 men (24 eyes) treated only with Cosopt®

Table 5. Results after treatment: Shirmer's I test

Shirmer's test 1	> 10 mm mild/normal	≤ 10 mm moderate	≤ 5mm sever
First group	8 eyes (11.11%)	6 eyes (8.33%)	10 eyes (13.89%)
Second group	14 eyes (19.44%)	8 eyes (11.11%)	2 eyes (2.78%)
Third control group	2 eyes (2.78%)	2 eyes (2.78%)	20 eyes (27.78%)

First group: 6 women and 6 men (24 eyes) treated with Cosopt®and Sol.Hylo®gel
Second group: 6 women and 6 men (24 eyes) treated with Cosopt®,Sol.Hylo®gel and Bioptron®light therapy
Third control group: 7 women and 5 men (24 eyes) treated only with Cosopt®

Shirmer's test 1: basal tear secretion

After the treatment, statistically significant improvement in severe deficit in tear secretion was found in 2 eyes (2.78%) in Group 1. Statistically significant improvement in severe deficit was found in 2 eyes (2.78%), whereas moderate deficit in tear secretion was observed in 2 eyes (2.78%) in group 2 patients. In third, control group, there was a decrease in tear secretion, and moderate deficit in 4 eyes turned into severe tear deficit. The role of Bioptron light therapy ($p < 0.001$) is evident especially in severe and moderate symptoms and signs.

Discussion

In Group 1 and Goup 2 patients, part of therapy was Hylo®gel solution, one of the best choises fot the treatment of dry eye in our country. This sterile solution without any preservatives, successfully relieved symtoms of dry eye in 2-4 weeks. It was our choice in therapy, because it is widely applied in our contry and well tolerated by patients. Topically applied Hylo®gel may help in the elimination of some symptoms, but it is not enough without Bioptron® light therapy. Because of dry eye, on a cellular level, discrete inflammatory changes appear, secretion of tears is decreased, corneal epithelium becomes thinner as a result of decreasing of corneal sub-basal nerve density. Treatment by Bioptron light therapy increases tissue anti-inflammatory cytokines and contributes to the regeneration of corneal nerves. Bioptron®light therapy reaches its full efficiency as addition to Hylo®gel therapy and by using both of them healing of severe cases of keratoconjunctivitis sicca can be achieved, as was confirmed in our research. With ageing, corneal epithelium becomes thinner and precorneal tear film becomes more and more unstable (26). In dry eye, which is a consequence of other systematic disorders and therapy, patient have healty corneal endothelium and basement-membrane, however, after therapy with BAK as a preservative, we found cytotoxic effects, higher endothelium damage, epithelium edema, bullous keratopathy, and higher incidence of eye inflammation (27).

Therapeutic potential of the Bioptron® light in the treatment and healing of wounds has been proven. There are no adverse reactions during and after this non-invasive therapy. Antimicrobial preventive effect, antidepressant effect and many other fields of application present additional benefits to each patient (28). It is necessary to attend a short course to apply Bioptron lamp. Low-power laser treatment has a stimulatory effect on wound healing. Bioptron light is also low-power light source, but differs from it as it is polychromatic rather than monochromatic (29).

Conclusion

We proved that Byoptron light terapy is successful and effective in disappearance of symptoms and signs of dry eye as a consequence of long lasting antiglaucoma therapy with BAK as a preservative. Bioptron light therapy is non-invasive, easily applicable, without any contraindications. Bioptron lamp is portable, suitable for home use and for immobile patients. Patients are very satisfied with the introduction of Bioptron light therapy in care of dry eye symptoms, so comfort and compliance of patient have been improved. Bioptron lamp is recommended for additional therapy for all antiglaucoma patients.

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BIOPTRON SVETLOSNA TERAPIJA SUVOG OKA USLED DUGOTRAJNE TOPIKALNE ANTIGLAUKOMNE TERAPIJE BENZALKONIUM-CHLORIDOM

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Benzalkonijum-chloride je najčešće korišćeni konzervans u savremenoj antiglaukomnoj topikalnoj terapiji, sa mnogobrojnim prednostima i kumulativnim neželjenim dejstvima na prednju površinu oka. Benzalkonium-chloride je klasifikovan kao težak iritans mukoznih membrana, što rezultira suvoćom prednjeg očnog segmenta.

Cilj našeg rada bio je naučna potvrda efikasnosti Bioptron svetlosne terapije u indukciji povoljnih reakcija na ozdravljenje, otklanjanju simptoma, znakova suvog oka kao posledicu dugotrajne topikalno primenjene antiglaukomatozne terapije Benzalkonijum-chloridom kao konzevansom.

Izvršeno je ispitivanje na 36 bolesnika (72 oka) koji su bili podeljeni u tri grupe: prva i druga grupa sa različitim pristupom u terapiji suve prednje površine oka i treća, kontrolna grupa. Kompletan oftalmološki pregled bolesnika urađen je pre početka ispitivanja i posle završene svetlosne terapije Bioptronom. Pregled je obavljen prema kriterijumima "Dry eye severity grading sheme", a kao objektivan parametar rađen je Shirmerov I-test. U drugoj grupi bolesnika, lečenih Hylo®gel kapima i Bioptron svetlosnom terapijom, simptomi i znaci su statistički značajno redukovani sa 27,78% na 5,56% ($p < 0,001$).

Vrednosti Širmerog I-testa pokazuju poboljšanje sa 30,56% na 13,89% deficita u sekreciji suza ($p < 0,001$). Simptomi i znaci suvoće prednje površine oka su statistički značajno redukovani. Tolerancija antiglaukomne terapije, komfor i komplijansa bolesnika su značajno poboljšani. Neophodno je uključiti Bioptron svetlosnu terapiju kod svih bolesnika na antiglaukomnoj terapiji Benzalkonijum-hloridom kao konzervansom.

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Ključne reči: *Benzalkonum-chloride, Keratoconjunctivitis sicca, terapija Bioptron® lampom*

Review article

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THE OPHTHALMIC INFORMATION SYSTEM

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The introduction of information systems to the healthcare industry has led to the automation and modernization of the system of work, increased the quality of diagnostic and therapeutic procedures, reduced consumables and increased the level of system utilization, simultaneously saving time and enabling the integration of heterogeneous systems into a single computer unit.

The ophthalmic information system (OIS) is a part of the hospital information system (HIS). The network, computer hardware and software, WEB technology, Digital Imaging and Communications in Medicine, Picture Archiving and Communication System, Health Level Seven protocol are essential to the functioning of the information system.

Teleophthalmology is a form of the medical information system that requires the use of telecommunication systems in order to provide ophthalmic services among remote locations.

The application of smart mobile phones for mobile ophthalmology has shown great efficacy in the diagnosis of macular degeneration, glaucoma, cataracts etc.

Web technologies in the ophthalmic information system have made health services available to everyone, enabled a fast and effective treatment, provided timely information and also enabled good communication between ophthalmologists.

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Key words: *information systems, ophthalmic information system, telemedicine, teleophthalmology, mobile ophthalmology*

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Introduction

The introduction of information systems to the healthcare industry has led to the automation and modernization of the system of work, increased quality of diagnostic and therapeutic procedures, reduced consumables and increased the level of system utilization, simultaneously saving time and enabling the integration of heterogeneous systems into a single computer unit (1–5).

The ophthalmic information system (OIS) is a part of the hospital information system (HIS). The network, computer hardware and software, WEB technology (telecommunication systems and technologies), Digital Imaging and Communications in Medicine (DICOM), Picture Archiving and Communication System (PACS), Health Level Seven protocol (HL7) are essential to the functioning of the information system (1–11). Modalities that make OIS (and present small OISs themselves) include: biomicroscope, fundus camera (FA, laser), OCT, ultrasound, computerized perimetry, ophthalmic surgical microscope, etc. The data collected in the form of electronic recording (e-recording) from the abovementioned modalities are stored and kept for a certain period of time in different formats. The ophthalmic information system must provide a quick and easy access to e-recordings, while the speed of access to the data may vary. The OIS should provide a greater and faster information flow of patient data from each part of the Eye clinic–examination rooms, depatments, operating rooms etc.

Integration of OIS into HIS

In order to be integrated into a single information system, the hospital information system and ophthalmic information system exchange the following information: patient registry (data on the new patients, updating the data on existing patients); patient examination results (who referred the patient and what type of examination is required, referral diagnosis, level of emergency, etc.); examination

status and various reports (radiological findings, specialist reports, laboratory findings, histopathological findings, etc.); delivery of findings; distribution of findings to the patient; data synchronization between HIS and OIS (examination methods, physicians, departments etc.) and other information (4).

It is possible to exchange information between OIC and other information systems integrated into a HIS, such as the radiology information system (RIS), surgical information system (HRIS) and others.

A radiology information system is one of the most advanced and most developed health information systems. It comprises a large number of different modalities such as: digital radiology, computed neurology, computed tomography (CT), magnetic resonance imaging (MRI), echocardiography, angiography, mammography, mobile radiology, teleradiology, dental radiology, orthopantomography, radiotherapy apparatus, radiosurgery appliances (gamma knife, cyber knife) and others (1, 2). OIS, HRIS and other medical information systems originated from the RIS.

The HL7 standard protocol is necessary for the integration of an ophthalmic information system into the health information system. The HL7 standard enables the exchange of medical information among different information systems of hospital organizational units, regardless of the programming language they are written in and regardless of the platform they are executed on. The HL7 is not a software application, but a standard encompassing thousands of pages with detailed explanations, which supply analysts and developers with a starting point on the standard, in order to implement it technically (1, 2, 6, 8).

PACS is based on the web technology (1, 2, 6–8). It is integrated into OSI, RIS, HRIS and other medical information systems. It represents a modern system for archiving images and communication. It is frequently used with the RIS. Designed as the computer systems for paper and filmed archives, PACSs handle medical images and information (1, 2). They store recordings from various medical devices-modalities (fundus cameras, biomicroscopes, computerized perimeters, fundus cameras, OCTs, ocular ultrasounds, standard ultrasounds, color Doppler ultrasounds and other appliances, then MRIs, CTs, angiography apparatuses, digital x-ray machines, mammography machines, PET scanners, nuclear medicine appliances, dental x-ray machines, and others). A PACS unites the functions of teleophthalmic, teleradiological and other services, and systems for archiving, searching, viewing medical images, patient data etc. The ophthalmic PACS configuration is given in Figure 1.

A PACS contains a device for medical diagnosis, servers, workstations to access the data, computer network that connects the system components, databases and interfaces to other systems. Computers of operating units are networked in a PACS and they represent the modalities that send the processed information (images) to the central computer

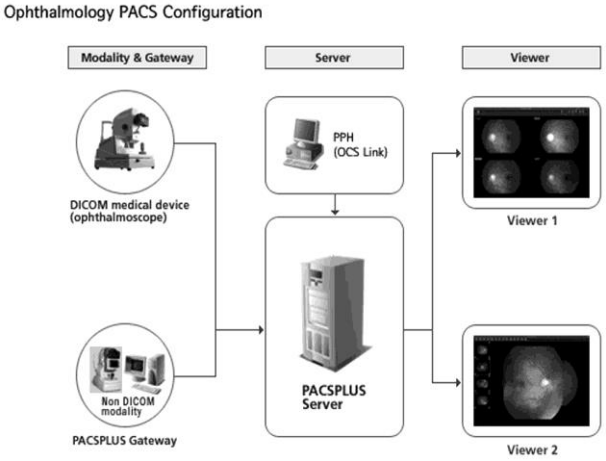

Figure 1. Ophthalmic PACS configuration

http://www.pacsplus.com/02_solution/sulotion_05.html

(server) and depositing it in PACS. Each computer in a PACS network is identified by its network address (1, 2, 4, 6, 8).

Benefits from the application of PACS are: saving space and time for filing the material (ophthalmic and/or radiological records), an easier search for the material with the purpose of staff training, cash savings (instead of buying ray films and photographic paper, only a CD is required), a higher quality of the deposited material, increased speed of establishing an ophthalmic diagnoses, possibility of visualizing records from remote locations. This leads to a better use of human resources as well as to an opportunity to connect within a network one or more health care providers, which results in the modernization of ophthalmic institutions in question, reducing the cost of servicing equipment etc.

DICOM is a standard for handling, storing, printing and transmitting information in medical practice. It is a set of rules that enable the exchange of medical images and information between computers of one or more health centers, by means of establishing a common information language, regardless of the manufacturer of the equipment and a digital system used (9–11).

DICOM and PACS are directly linked. DICOM consists of a file format definition and network communication protocol. Primary DICOM functions are the communication and exchange of digital medical images (fluorescent angiograms, echosonograms, CT orbits, etc.) (Figure 2) regardless of the manufacturer; the facilitation of the integration of PACS into the HIS, OIS, RIS and other information systems; the availability of a database of medical images regardless of the browser distance, enabling thus the functions of teleophthalmology, teleradiology, etc. (1, 2, 11–15).

The disadvantages of DICOM arise while searching databases and processing of images; with a simultaneous display of several images on the monitor, which results in the reduced quality of individual

images or even a cut-off of individual segments. Moreover, a false coloration at the adjacent gray level may occur, followed by a false coloring in contrasting colors, etc.

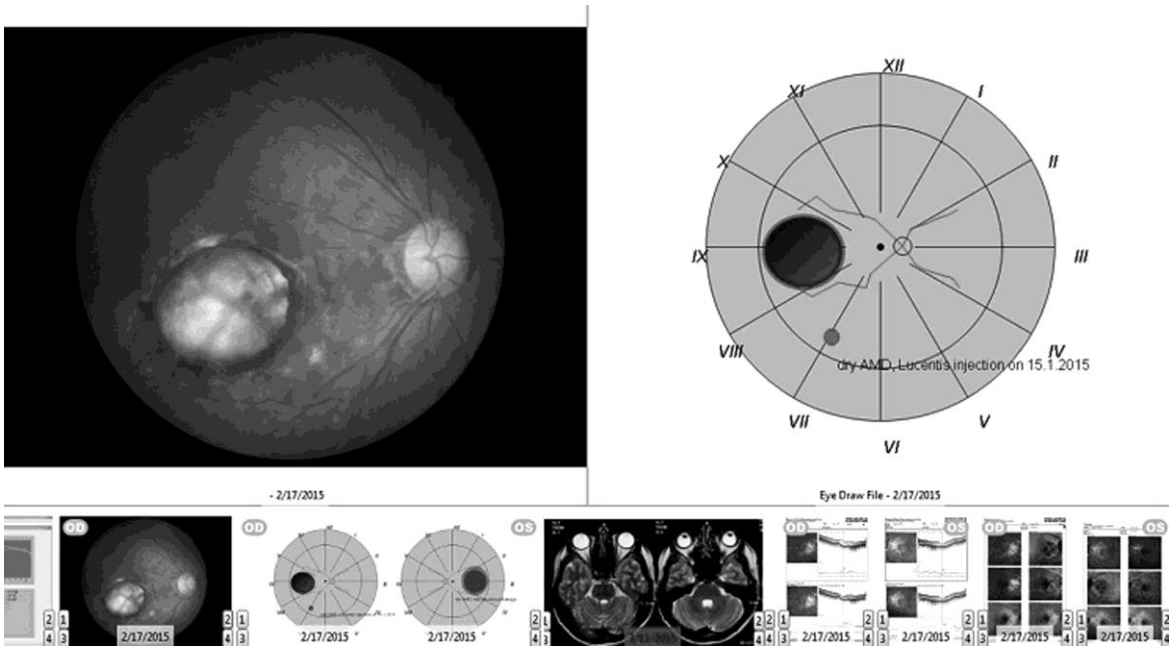

Figure 2. DICOM in an OIS

http://www.ifa4emr.com/index.php/News/Health-IT/VNA-for-ophthalmologists

Teleophthalmology

Teleophthalmology is a form of medical information system that requires the use of telecommunication systems in the form of satellites, the Inernet, mobile phones, computers and other devices for the exchange of data (images, video, audio or other ophthalmic and/or radiological, and similar information) with the aim of providing ophthalmic services between remote locations (11–15).

Teleophthalmology commonly utilises the Global System for Mobile Telecomunications (GSM), General Packet Radio Service (GPRS) and 3G systems for the transmission of information, because they allow the transfer of multimedia contents at high speed.

One of the requirements of telemedicine in general, and hence of teleophthalmology, is that information and communication technologies facilitate long-distance transfer of relevant medical data, by adhering to medical, technical and technological standards related to the collection, storage, transmission and retrieval of medical images, video, audio, and other similar ophthalmic and medical information, to a standardized and high-quality equipment and systems for telemedicine, quality devices, telecommunication equipment and connections.

Web technologies in teleophthalmology allow for the review and writing of ophthalmic findings on any computer, at any location, observing the relevant regulations and rights related to security and confidentiality of patient data, by means of the Internet Explorer (1, 2, 15).

The application of teleophthalmology is possible in ophthalmic institutions that have a digital ophthalmic camera, DICOM and PACS. The problem arises in areas where analogue ophthalmic devices are used. The question is how to digitize and archive ophthalmic findings, how to relate them to images, how to make information available to information systems of an ophthalmic clinic, hospital, clinic, other medical institutions in the country and abroad (1, 2, 4, 6, 9, 11).

Teleophthalmology is supposed to promote the development of telemedicine in Serbia, to modernize ophthalmic centers in the country, enable the timely availability of ophthalmic images (eye echosonograms, fluorescein angiography findings, radiographs, CT, MR, etc.) and their interpretation from rural and remote areas (mountains, forests, islands and other places); to provide a sub-specialist support to an ophthalmic diagnosis at a given time (e.g. the consulting body), to contribute to an easier establishement of the diagnosis by a quality analysis of identical examples or some rare diseases; to facilitate the distribution of ophthalmic images to other ophthalmic centers with the purpose of consulting;

to provide education and/or update of knowledge in ophthalmology, etc (1–4, 11–14).

The size of static images is the main technological challenge for telemedicine and teleophthalmology. Typically, uncompressed digital medical images vary from about 25 kB (nuclear medicine) to 50 MB (digitized mammography). Often, due to the need for repeated recordings (from different angles or in order to compare images from the same perspective), the total size of uncompressed examination data increases by 1 or 2 MB, up to almost 200 MB. The DICOM standard recommends the use of JPEG and JPEG 2000 compression formats, and the number of required shots depends on the device type. For most types of images only one shot is required, but FA, ocular echosonography, CT, MRI or Positron Emission Tomography (PET) scanning require a greater number of shots (e.g. 20 shots for a CT study of the orbit) (1, 2).

The flow of information necessary for the transmission of such images depends upon several factors. The time required for the transfer and the allowed compression level are particularly significant (image quality degradation is not allowed, since it would compromise data interpretation and diagnosis). Lossless compression techniques reduce the image size by 3 to 4 times, and while lossy compression reduces the image size by 10 to 20 times, yet still retaining a diagnostic image quality in some applications. An acceptable level of compression depends on the application domain (teleconferencing of ophthalmologist) and the image user (an ophthalmologist or a specialist in general practice). The security of data is also essential when using teleophthalmology, since the doctor-patient confidentiality and data integrity must be preserved during the transmission and storage of images. The confidentiality of personal data can be achieved by means of the techniques of user authentication, image access control and data encryption. Data integrity can be preserved (an important feature of a digital image is that it can easily be maliciously altered) by a digital signature technology. It is necessary to reconcile these requirements to the cost and ease of use.

Mobile ophthalmology

The development of mobile medicine is asso ciated with Maria Sklodowska Curie, who worked on the creation of a mobile X-ray room during the World War II (Figure3). The alliance of French women provided the funds for the first mobile „radiological car", later called "little Curie" in 1914 (15–17).

Nowadays her brilliant idea is used by well-known manufacturers of X-ray machines who install x-ray devices, CTs, MRIs, mammographs and other radiological devices in trucks with trailers, therefore making these devices mobile and accessible to all communities, under all weather conditions, in peacetime or wartime. Further, manufacturers of ophthalmic equipment install these devices on planes and helicopters, making them mobile as well (13, 15, 18).

Together with the development of telecommunications technology, the mobility of ophthalmic devices using mobile telephony has increased (15, 18).

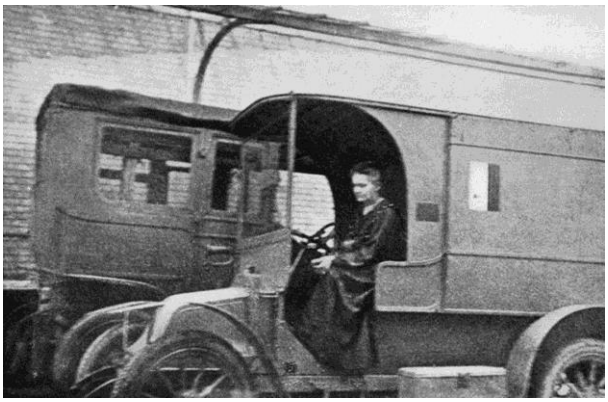

Figure 3. The first mobile "radiological car", later called "little Curie" from 1914.

This ingenious idea is just one of the links between telemedicine and teleophthalmology that made ophthalmic and other relevant medical information available everywhere, on any terrain in real time.

In radiology, the relevant characteristics of mobile phones are the screen diagonal of 3 inches, 250 CDL lighting, 256 MB of RAM memory, an 800 MHz processor and the mobile internet standard that supports HTML5 and Java script (2, 15). The use of the smart mobile phone is important in Mobile Ophthalmology (Figure4), since it would help teleophthalmology to become more relevant and far more realistic than the current reality (14, 18).

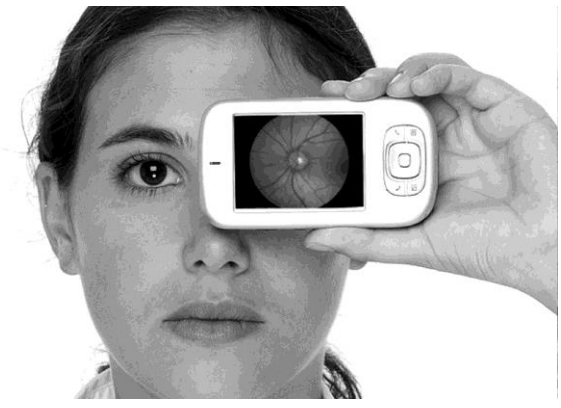

Figure 4. Mobile ophthalmology

http://www.kmendis.org/index.php/78-medical-informatics/124-teleophthalmology-an-annovation-that-could-change-medicine

The application of smart mobile phones in mobile ophthalmology has shown great efficacy in the diagnosis of macular degeneration, glaucoma, cataracts, anterior eye segment etc. It is believed that smart mobile phones in mobile ophthalmology will be initially applied in the treatment of diabetic retinopathy.

According to the World Health Organization (WHO), it is estimated that 135 million people suffer from diabetes, and that by 2025 this number will have increased to 300 million people. In order to prevent eye changes in patients with diabetic retinopathy, the use of smart mobile phones will be of great importance both for the needs of mobile teleophthalmology and static ophthalmology (18).

Contemporary teleophthalmology designs the future ophthalmology.

Conclusion

The ophthalmic information system has enabled the introduction of ophthalmology into the world of digital medicine. Web technologies within the ophthalmic information system have made health services available to everyone, enabled a fast and effective treatment, provided timely information, and facilitated the communication between ophthalmologists.

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OFTALMOLOŠKI INFORMACIONI SISTEM

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Uvođenjem informacionih sistema u zdravstvenu delatnost omogućena je automatizacija i modernizacija sistema rada, povećan kvalitet dijagnostičkih i terapijskih procedura, smanjen potrošni materijal, povećan stepen iskorišćenosti sistema, postignuta ušteda vremena i omogućena integracija raznorodnih sistema u jedinstvenu informatičku celinu.

Oftalmološki information sistem (OIS) je deo hospitalnog informacionog sistema (HIS). Mreža, hardver i softver kompjutera, WEB tehnologija, digitalne slike i komunikacija u medicini, sistem za arhiviranje slika i komunikaciju, kao i protokol "Health Level Seven", neophodni su za funkcionisanje informacionog sistema.

Teleoftalmologija je forma medicinskog informacionog sistema koja zahteva korišćenje telekomunikacionih sistema u cilju obezbeđivanja oftalmološke službe između udaljenih odredišta.

Primena smart mobilnih telefona za potrebe mobilne oftalmologije pokazala je veliku efikasnost u dijagostici makularne degeneracije, glaukoma, katarakte i dr.

Primena web tehnologije u oftalmološkom informacionom sistemu omogućila je da zdravstvene usluge postanu dostupne svima, brzo i efikasno lečenje, pravovremene informacije, kao i međusobne komunikacije oftalmologa.

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Ključne reči: *informacioni sistemi, oftalmološki informacioni sistem, telemedicina, teleoftalmologija, mobilna oftalmologija*

Original article

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LIGHT-MICROSCOPIC AND MORPHOMETRIC PROPERTIES OF ARGYROPHILIC NUCLEOLAR ORGANIZING REGIONS IN DEEP EPIDERMAL RIDGES OF HUMAN THICK SKIN

Aleksandar Petrović[1], Vladimir Petrović[1], Dragan Jovanović[2], Aleksandra Antović[3], Miroslav Milić[3], Hristina Kocić[2]

The epidermis of the thick skin on the flexor sides of hands and feet has a very complex superficial relief, known as dermatoglyphics, constantly maintained by precise, spatially coordinated regeneration and differentiation, whose potential lies within the deeper epidermal parts – rete ridges. One of the proliferative markers, AgNORs, represents neucleolar organizing regions, that after histochemical staining with silver ions can be observed as black dots in the nucleus. The aim of this study was to estimate morphometric properties of AgNORs in different micro-topographical compartments of thick skin epidermis, such as deep intermediate and limiting epidermal ridges. Necropsy samples of thick skin were taken from the tips of big toes of fifteen cadavers, and routinely processed to paraffinized microtome sections, which were stained with hematoxylin-eosin, and silver-based method for staining nucleolar organizing regions. Morphometric analysis was performed separately on basal keratinocytes of intermediate and limiting epidermal ridges. Suprabasal layer of tips, as well as basal layer of intermediate ridge sides, as a sign of higher proliferative status, showed a higher number of silver-stained nucleolar organizer regions with small average values of the area. According to AgNORs morphology, proliferation was sporadically and diffusely present in basal, as well as in suprabasal layer of tips, and sides of limiting ridges.

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Key words: *Silver Staining, Nucleolus Organizer Region, Epidermis, Computer-Assisted Image Analysis*

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Introduction

Glabrous skin of palms, soles, and flexor sides of fingers and toes, exposed to constant mechanical stimulation, has evolutionary considerably thicker epidermis than the skin on other anatomical integument regions (1,2). In these topo-anatomical regions, the epidermis is characterised by its prominent height and specific superficial relief composed of parallel linear ridges, and sulci between them, in the form of whirls, loops, and arches, making individually specific patterns, named dermatoglyphs. Their configuration is genetically determined and absolutely individual (3,4). The deep part of epidermis follows a diversified structure of the surface and forms deep epidermal rete ridges:

1) intermediate, which are positioned under superficial ridges,
2) limiting, located under superficial sulci, and
3) transverse, which intermittently bridge the previous two ridge types (5–9).

AgNORs (Argyrophilic Nucleolar Organizer Regions) have been defined as a set of proteins associated with ribosomal genes rDNA of nucleolar organizer regions, showing a selective affinity to bind silver ions (10). In cellular biology, the nucleolar organizer regions (NORs) are located on the secondary constrictions of acrocentric human chromosomes (13^{th}, 14^{th}, 15^{th}, 21^{st}, 22^{nd}) and contain rDNA. During interphase, these genes are located in nucleoli, with associated proteins of ribosomal subunits biogenesis. The major proteins involved in rRNA transcription and processing are RNA polymerase I, upstream binding factor (UBF), DNA topoisomerase I, nucleolin or C23 protein, fibrillarin and numatrin or B23 protein. In interphase, the NORs are located in the fibrillar centre, made of rDNA loops involved in rRNA transcription (except for 5S rRNA), and of NOR proteins (RNA pol-1, DNA topoisomerase-1, B23,

C23 and fibrillarin) (10). Among the argyrophilic nucleolar proteins, nucleolin and protein B23 were in normal proliferating cells estimated to account for 60–75% of the global AgNOR staining (11, 12). All of these proteins possess a great affinity toward silver ions, and argyrophila ultrastructurally extends from the fibrillar centre to dense fibrillar component (13–15). Upon staining with silver ions, NORs become apparent as black dots located inside the nucleolar region. The degree of proliferation in one cellular population could be evidenced by the detection of morphologic changes in nucleoli during cellular divisions. During interphase, the argyrophilic reaction toward AgNOR proteins is visible as black dots clustered in nucleoli. In the prophase of cell division, the AgNORs of nucleoli disassociate, and are shortly visible as distanced small black dots, which disap-pear during the rest of the division, reappearing at a reverse reassembly during the telophase, followed by formation of functional nucleoli (11, 16–19).

Epidermal regeneration is based on basal stratum stem cells, which by rare asymmetric divisions are self-maintaned and produce transit-amplifying cells, where the latter, in 3-5 further cell divisions, give rise to postmitotic keratinocytes that differentiate toward the corneal stratum (20–30). In the available literature only a few articles reported about the proliferative activity of deep epidermal ridges of the human thick skin by using different methods: [^{3}H] thymidine,(31) cell cycle S phase labeling with deoxyoligonucleotide probes to histone mRNAs, (30) and analyzing distribution of Ki67 positive nuclei in epidermal basal and suprabasal layers adjacent to acral and nonacral human nevi (32).

Although many papers, reporting about AgNOR expression in different epidermal lesions also presented the values for normal epidermis thin skin, the available literature lacks the data about proliferative activity of human thick skin epidermis by using the AgNOR staining methodology (33–36). The aim of this study was to quantify the number and area of AgNORs in nuclei of thick skin basal and suprabasal keratinocytes, in the regions of intermediate and limiting deep epidermal ridges.

Material and methods

This research was conducted at the Department of Histology and Embryology and Department of Forensic Medicine (Faculty of Medicine, University of Nis). The material consisted of skin samples taken from the tips of big toes from 15 cadavers of male gender, with age range from 35 to 52 years. The necropsies were excised perpendicularly to the skin surface, and transversally to the longer axis of superficial ridges, obtaining tissue samples of about 7x4x4 mm. The material was fixed in aqueous 4% formaldehyde solution, and routinely processed to 4 μm thick paraffinized tissue sections. The tissue slides were stained routinely with hematoxylin and eosin, and histochemically for AgNOR as recommended by the International Committee on AgNOR Quantitation – ICAQ (17). The research was carried out in compliance with the legal regulations and ethical standards for retrospective studies, which was approved by the local Ethics Committee.

A short description of AgNOR staining method by ICAQ

Tissue slides were deparaffined, rehydrated through decreasing concentrations of aqueous solution of ethanol and were brought to distilled water. Just before the staining, a developer for staining was made of 0,6% gelatin dissolved in deionised water, to which formic acid was added in order to obtain a 0,33% solution. The developer was warmed up to 37^{O}C before the addition of silver-nitrate, to make the final 33% staining solution, in which microscopic slides were immersed. The staining was performed in a dark chamber, incubation was carried out at 37^{O}C for 13 minutes. In continuation of the staining process, the stain was poured out, slides were washed several timed in deionised water, fixated in 5% sodium-thiosulphate, dehydrated, and mounted in glycerine.

Microscopic slides were analysed on a light microscope (Olympus BX50, Japan) equipped with Leica DMR digital camera (Leica Micro-Systems, DFC 295). From each of the examined tissue sample, for the purpose of morphometric AgNOR analysis, with the use of immersion oil, digital micro-photographs of minimally 5 pairs of deep epidermal ridges (intermediate and limiting) were taken under x2000 magnification. Morphometric analysis of the number and transectional surface area of AgNORs was done by interactive separation of AgNORs in Olympus Micro-Image Software, v. 4.0 for Windows (Media Cybernetics, Silver Spring, USA). Statistical analysis was done using the Jandel Sigma Stat 2.0 (SPSS Inc., Chicago, USA) software, using its functions for descriptive and comparative statistics of Mann-Whitney test. The differences between the values were considered statistically significantly different for $p < 0.05$.

Results

Microscopic slides of thick skin, stained with hematoxylin-eosin, displayed normal morphology characterized by well developed epidermis, which showed on its dermal side repeating and alternating, narrower intermediate and wider limiting ridges (Figure 1).

Those two types of deep epidermal ridges are different not only by their location and general morphology, but also by specific cellular composition, and their distribution. The basal layer of intermediate ridge is composed of small cubical cells with scarce basophilic cytoplasm, one centrally positioned rounded or slightly oval nucleus, and one variably apparent nucleolus. The basal cells of limiting ridge are elongated, almost prismatic and have more developed acidophilic cytoplasm. One larger oval euchromatic nucleus is located in the apical part of these cells, containing one or two well developed nucleoli.

On thick skin samples stained with ICAQ methodology, a lesser number of larger AgNORs could

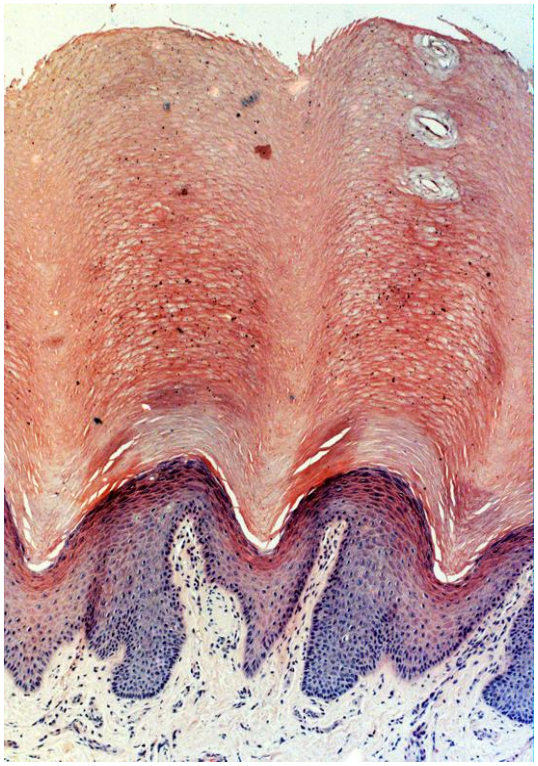

Figure 1. Histology of the thick skin epidermis. Deep intermediate ridges positioned under superficial ridges (on the right, spiral lumen of acrosyringia), and limiting under superficial sulci (in the middle) (hematoxylin-eosin, x100).

be observed in the nuclei of intermediate ridge tips basal layer. The intermediate ridge suprabasal layer had slightly larger nuclei with noticeably more numerous AgNOR structures and smaller average surface area per object (Figure 2a). The number of AgNORs and their areas showed a high statistical difference between the basal and suprabasal layer of intermediate ridge tips ($p < 0,001$) (Tables 1 and 2). On the sides of intermediate ridges, basal layer AgNORs were more numerous compared to suprabasal layer ($p < 0,05$), while smaller average values of their transectional area showed no statistically significant differences (Figure 2b, Table 1 and 2).

Within the tips of limiting ridges, a lower number of basal layer cells displayed "disassembling" nucleoli on separate, smaller components, observable as AgNOR single positive dots or partially grouped dots. However, single nucleoli could be observed in the majority of nuclei. In this region, measured AgNORs number and area values did not show statistically significant differences between the basal and suprabasal layer ($p > 0,05$) (Figure 2c, Table 1 and 2). The nuclei of basal layer in limiting ridge sides show lower values of AgNOR area compared to nuclei of suprabasal layer, with a statistically significant difference ($p < 0,05$). AgNOR structures were more numerous in basal than in suprabasal layer, however without a statistical significance ($p > 0,05$) (Figure 2d, Table 1 and 2). The higher parts of spinous stratum showed single nuclei clustered AgNOR pattern.

Discussion

Interpretation of AgNOR distribution pattern as a manifestation of cell cycle phase was mainly defined in the previous studies for the neoplastic cells, and mostly in the light of its prognostic or predictive significance. Combined MIB-1 and AgNOR staining and cytometry of cancer cells have been used to show that cell cycle time and the size of the ribogenesis machinery are co-regulated (10, 37). However, Ag-NOR distribution interpretations done by different authors for the same tumor types are rarely comparable. A definitive standardization of AgNOR staining and quantification has not yet been achieved, and this could be related to the use:

1) of different silver-staining methods for NOR labeling; and

2) variety of procedures for AgNOR proteins quantification (16).

Although many molecular studies reported the association of argyirophilic nucleolar proteins with cell cycle, it is interesting that the relation between morphology/pattern of AgNORs expression and life cycle of normal cells was almost a neglected question. A number of studies carried out in different tumour types have demonstrated that malignant cells frequently present a greater content of AgNOR proteins than the corresponding non-malignant cells (10). In cancer cells, AgNOR proteins expression was seen as strictly related to cell duplication rate, and there was a general consensus that AgNOR size or number was related to proliferative activity – the larger the AgNORs, the shorter the population doubling time (10, 37).

However, the interpretation of AgNOR expression pattern in normal cells should be considered quite differently than in malignant cells. Namely, the expression of AgNOR number and quantity of argyrophilic proteins are different for various normal diploid cellular phenotypes, and it seems that further standardized quantification should be implemented. The most important event to be taken in consideration when aiming at AgNOR distribution interpretation in normal cellular phenotypes is their nucleolar disassembly during prophase and their reassembly during telophase. During that process, NORs of nucleoli physically separate and reduce their volume, become invisible during the rest of cell division, and reappear and reassemble at telophase in the process of nucleologenesis, continuing with the onset of rRNA synthesis at the NORs (38–41). The main AgNOR proteins detected during active transcription and proliferation are nucleolin and protein B23. The amount of these proteins rises during S phase, and accumulates maximally at G2 phase, being 1.5 times more abundant compared to G1. During interphase, RNA polymerase I subunits, proportionally to their lower presence compared to nucleolin and B23, take a lesser part in general AgNOR staining. On the contrary, during mitosis, AgNOR staining of mitotic NORs reveals the presence of RNA polymerase I

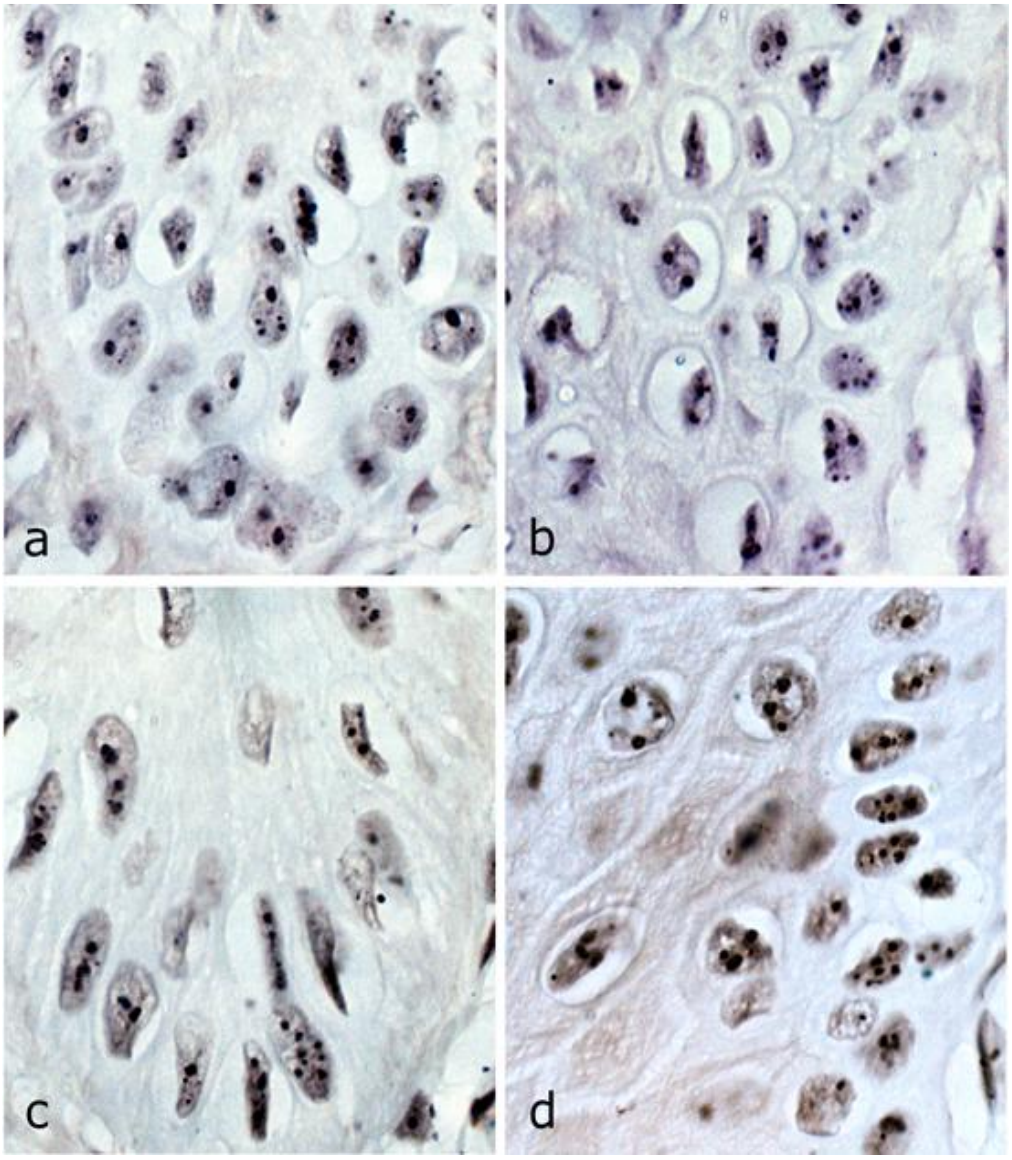

Figure 2. Morphology of AgNORs in the nuclei of thick skin epidermal keratinocytes by different microtopographical regions/compartments: a) tip of intermediate ridge, b) side of intermediate ridge, c) tip of limiting ridge, d) side of limiting ridge (ICAQ – AgNOR, x2000, immersion).

Table 1. Average values of surface area (μm^2) and their standard deviations (X ± SD) of AgNORs in tips and side of intermediate and limiting ridges. (n = 15)

Part of ridge	IB	IS	LB	LS
	(P value)		(P value)	
Tip of ridges	1.72 ± 0.46	0.38 ± 0.17	1.17 ±1.04	1.98 ± 0.75
	(< 0.001)		(>0.05)	
Ridge sides	0.27 ± 0.07	0.50 ± 0.26	0.80 ± 0.34	1.55 ± 0.25
	(> 0.05)		(< 0.05)	

IB – basal layer of intermediate ridge, IS – suprabasal layer of intermediate ridge, LB – basal layer of limiting ridge, LS – suprabasal layer of intermediate ridge; * statistical differences were tested by Mann-Whitney test

Table 2. Average number of AgNORs and their standard deviations (X ± SD) per nuclei of keratinocytes in tips and sides of intermediate and limiting ridges. (n = 15)

Part of ridge	IB	IS	LB	LS
	(P value)		(P value)	
Tip of ridge	1.15 ± 0.37	4.6 ± 1.31	2.07 ±1.06	1.88 ± 1.25
	(< 0.001)		(>0.05)	
Ridge sides	5.50 ± 1.30	3.67 ± 0.82	2.17 ± 2.40	1.50 ± 0.58
	(< 0.05)		(> 0.05)	

IB – basal layer of intermediate ridge, IS – suprabasal layer of intermediate ridge, LB – basal layer of limiting ridge, LS – suprabasal layer of intermediate ridge; * statistical differences were tested by Mann-Whitney test

subunits and upstream binding factor (UBF) which are the proteins of the transcription machinery. Therefore, the test for human cancer cell proliferation is mostly based on the level of nucleolar proteins that are not directly involved in rDNA transcription (11).

Examining epidermal differentiation in cynomoglus monkies and humans, Lavker and Sun noticed that palmar epidermis has two morphologically different, spatially segregated populations of basal keratinocytes (31). According to ultrastructural characteristics and kinetics of the cell cycle, the mentioned authors hypothesized that low proliferative basal layer keratinocytes of intermediate ridge tips were primitive differentiated cells, closer to stem cells, while serrated keratinocytes of limiting ridges, because of specifically differentiated cellular protrusions on basal pole and cytoplasmatic presence of tonofilaments, were considered as cellular population involved in augmentation of epidermal-dermal contact surface.

The differences noticed in AgNOR distribution, between intermediate and limiting ridges could be influenced by ridge morphology, cellular differentiation (31, 42, 43) and by specific local demands toward germinative compartment (31, 44–46). More numerous and smaller AgNORs per nucleus, evidenced in our research, primarily in the suprabasal layer of intermediate ridge tips and basal layer of its flanks, may be interpreted as the presence of expected and more intense proliferative activity. Larger and single nucleoli in the nuclei of intermediate tips basal layer cells, indicate the population of cells detoured form proliferation activities, and their statistically different number of AgNORs and average area, compared to the nuclei of suprabasal layer, suggest a model of quick and rare divisions within basal layer and switching proliferation and regenerative activity in the population of suprabasal layer. Non-proliferative nature of the intermediate ridge tips basal layer may be partially explained by the "dilution" of keratinocyte population by the presence of Merkel cell complexes, melanocytes, and acrosyringia. The basal layer of intermediate ridge sides / flanks, which shows a statistically significant difference of AgNOR structure area, does not show a statistical difference in their number when compared to suprabasal region, although it contains more AgNORs. Such a finding implies intensive proliferation, which continues through suprabasal compartment, giving "power" to "stream of keratinocytes" in the region of intermediate ridge, supposedly for the purpose of protruded surface ridge formation, by generation of larger number of newly produced, and latter through the rest of commited compartment, terminally differentiated keratinocytes. A higher distribution of proliferating cells, as seen by AgNOR interpretation, located in the germinative compartment of the intermediate ridge sides could represent a larger transit-amplifying compartment, based on a steeper basement membrane orientation of the ridge side (25, 47).

The contrast between the presence of epidermal suprabasal proliferation (32, 45, 46), and its insignificant appearance in higher layers (24, 48) was also reflected in our material as a pattern of AgNOR expression in the form of single nucleoli cluster in the cells of the higher parts of spinous stratum. The regulation of transit-amplifying compartment above the basal layer (49–51), and its specificity compared to upward terminally differentiating compartment, (24, 48) has a significance in the pathogenesis of epidermal hyperproliferative diseases, i.e. in psoriasis (52–57).

In the limiting ridge, basal and suprabasal layer AgNOR distribution pattern differed from the one evidenced in the intermediate ridge. The cells of the limiting ridge basal and suprabasal layer did not differ significantly in the number of AgNORs per nucleus, and area of AgNORs in tips of limiting ridge basal layer had smaller values compared to suprabasal layer, however without a statistically significant difference, leaving the impression of diffuse proliferation equally distributed within both of these layers. If we take into consideration the higher value of standard deviation in basal layer, it could be assumed that statistically significant difference was absent due to mixed population of cells with proliferative and cells with resting (interphase) morphology. Generally, the limiting ridges, as in the results of Lavker and Sun (31) that had more of an anchoring role toward papillary dermis and formation of superficial sulci, expectedly should have had a more tardive proliferative activity, the property which was almost equally distributed throughout the whole length of basal and suprabasal layer.

The number and area of transsectioned AgNORs, given our results, are generally in the va-

lue range reported in the available literature for human thin skin epidermis (33, 34, 36). However, these data are not directly comparable, due to specificities of thick skin epidermis, as well as because we focused on the germinative compartment and its components, the basal layer and suprabasal layer of spinous stratum. Heinisch et al. have reported the values for normal basal layer of human epidermis, where the AgNOR number was 3.3 ± 0.5, and AgNOR area 1.76 ± 0.21 μm^2. The mentioned findings are similar to our results, representing average values for whole length of examined basal layer, with the difference that our results are divided in four different topographic locations, within epidermal intermediate and limiting ridges.

It is much more likely that obvious morphological difference between intermediate and limiting ridges, followed by a specific expression pattern of various molecular indicators of cell cycle and differentiation, strengthen the impression of two separate tissue differentiation systems which again function in equilibrium to maintain epidermal homeostasis. In addition, the spinous strata cell morphology and different keratin types expression, separately specific for intermediate and limiting ridges (43,58), could be the evidence of different keratinocyte lines, originating from two stem cell precursors (59). Moreover, genetical labeling has proven that normal epidermal proliferating units are spatially organized without regularity of segregation or lateral migration of labeled cells (47).

Conclusion

Intermediate and limiting deep epidermal ridges express two different patterns of proliferation, recorded by silver labeled nucleolar organizer regions. According to AgNORs, higher proliferative activity in deep intermediate ridges is present in the suprabasal layer of tips and basal layer of the ridge flanks, while the proliferation is sporadically and diffusely distributed in tips and flanks of limiting ridges basal and suprabasal layers.

The thick skin represents a suitable research model of epidermal organization, necessary for understanding not only the complexities of regional histo-architectonics but also general mechanisms of tissue homeostasis and cell cycle morphology.

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SVETLOSNO-MIKROSKOPSKE I MORFOMETRIJSKE KARAKTERISTIKE ARGIROFILNIH NUKLEOLARNIH ORGANIZACIONIH REGIONA U DUBOKIM GREBENIMA EPIDERMA DEBELE KOŽE ČOVEKA

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Epiderm debele kože fleksornih strana šaka i stopala svoj složeni površinski reljef, dermatoglife, održava konstantnom i prostorno-koordinisanom regeneracijom, čije rezerve su smeštene unutar njegovih dubokih grebena. Jedan od proliferativnih markera, AgNOR, predstavlja nukleolarne organizacione regione (NOR), koji se po histohemijskom obeležavanju jonima srebra (Ag) vide kao crne tačke unutar nuklearnog područja. Cilj ove studije bio je utvrđivanje morfometrijskih osobina AgNOR u dubokim intermedijarnim i limitantnim grebenima epiderma debele kože. Uzorci debele kože jagodica palaca stopala petnaest leševa rutinski su obrađeni do parafinizovanih mikrotomskih tkivnih isečaka, a zatim obojeni hematoksilin-eozinom i histohemijskom metodom za obeležavanje nukleolarnih organizacionih regiona jonima srebra. Morfometrijska analiza je izvedena odvojeno na bazalnim i suprabazalnim keratinocitima intermedijarnih i limitantnih grebena. Suprabazalni sloj vrhova, kao i bazalni sloj strana intermedijarnih grebena, kao znak višeg deobnog stanja, pokazali su veći prosečni broj AgNOR, male površine preseka. Prema morfologiji AgNOR, proliferacija je sporadično i difuzno zastupljena, kako u bazalnom tako i u suprabazalnom sloju vrhova i strana limitantnih grebena.

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Ključne reči: *bojenje srebrovim solima, organizacioni regioni jedaraca, epiderm, kompjuterizovana analiza slike*

Review article

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PRIMARY HEALTH CARE: MEANING AND OPPORTUNITIES

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In the world today, there is a growing number of chronic conditions, and assurance of good primary health care for all persons requires constant improvement in organization, as well as harmonization with the fast development of medical technologies. The growth of welfare states has brought about new organization in health care systems in order to re-evaluate the costs. There are many benefits of a well managed primary health care system, because it results in fewer visits to emergency departments and less hospitalizations. Scientific evidence is making a great contribution to the measurement and accountability of Primary Health Care, and this evidence could inform and sustain choices that can be made at different levels of health care decision-making (the macro-policy level, the meso level, meaning the organizational model of providers, the micro level of professional interactions and the nano level, which refers to patient and care-giver collaboration with the providers).

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Key words: *primary health care, health system, health service research, continuity of patient care*

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Introduction

Chronic conditions are the most prevalent health problems worldwide, causing almost 70% of all deaths, which is an ever increasing trend (1). The economic burden of chronic diseases was estimated at 22.8 US$ trillion in 2010, and it is expected to rise to 43.8 US$ trillion by 2030 (2). Chronicity is characterized by multimorbidity. In fact, 65% of people aged 65 years and over and 82% of people aged 85 years and over suffer from two or more chronic conditions (3). Additionally, the population is aging, and average life expectancy increases by three months of life per year (4). The combination of chronicity and aging leads to an increase in disability, especially in the elderly (5).

This scenario is taking place in the context of limited financial resources, rising patient expectations and rapid development of medical technologies. For this reasons the shift from the paradigm of acute care to that of chronic care is needed. In acute care, the patient is a passive disease carrier treated in a hospital or clinical settings, "here and now", on the basis of the specific signs and symptoms of a disease. In chronic care, the patient is an equal partner with health professionals, and on the bases of his or her own resources it is possible to accept being able to live well with chronic conditions on a daily basis and navigate properly through the health care environment (6).

A network of social and health care providers can produce a personalized individual plan, whereby preventive actions are taken in advance, followed by early diagnosis, adherence to the therapy and timely follow-up. This can be carried out with the development of primary care (PC). According to the European Commission Expert Panel on the effective ways of investing in Health, primary care is defined as the provision of universally accessible, person-focused ongoing integrated health and community services provided by a multidisciplinary team of professionals able to address the majority of personal health needs. These services play a central role in the continuity of people's care, and they are delivered in continuous collaboration with informal caregivers and patients (7,8).

Primary care delivery is part of a broader vision for health development. In fact, primary health care (PHC) is an approach to the health of individuals and the community upon which an entire health system is based. It goes beyond the delivery of procedures and services, paying attention to all of the determinants of health, involving policies, practices, patients and communities (7, 9).

There are five basic primary health care functions. Accessibility as a concept of care is first sought from the primary care provider when a new health or medical need arises. Comprehensiveness implies the provision of intergrated health promo-

tion, disease prevention, curative care, rehabilitation, and physical, psychological, and social support. Coordination as a function indicates that whenever necessary an appropriate and timely referral of the person to specialist services or to another health professional should take place. "Patient-focused care" is a type of care that is respectful of and open to individual preferences, needs, and values. It relates to a model of whole-person care, including biological, emotional, social and cultural aspects. The responsibility of primary health care is to address the issue of unselected health problems of the whole population.

Today there is evidence of greater effectiveness, cost-effectiveness and equity of systems based on "Primary Health Care" (10). The main characteristics required for the improvement of PHC can be referred to at different levels of decision making. One of the strategies for organizing complicated networks in a health care system is to divide the system into strata or levels. Macro, meso, micro and nano levels provide a reasonable framework that refers to the policy level, the health care organization and community level, and to the patient interaction level. Each of those levels interacts with and influences the others.

At the macro or a policy level, a clear governance of the system (stewardship) is needed. This implies an overall system design related to policy formulation at the broadest level. It involves the way in which all the health system functions are put together. The assessment of performance is an essential ingredient for providing strategic direction and assuring a level playing field, carried out by assessing the performance of institutions involved in revenue collection, purchasing, provision and resource development. Priority setting has both a technical and a political aspect. Moreover, choosing the criteria for setting priorities and building consensus around them are the important elements for defining strategic direction for primary health care. Promotion of policies in other social systems that are not themselves part of the health system results in intersectoral advocacy. Social and economic determinants of health status, such as female education, do not present themselves as part of the health system. However, investing in them is a part of health action and therefore is part of the health system. Stewardship plays an important role in consumer protection. Health care markets and insurance are organized by information and power asymmetries between consumers and producers, and providing citizen safety in the use of health services. Part of stewardship function is achieved via a level playing field of the health system. Setting regulations is an important function of the health system, the two main types of which are health care regulation and sanitary regulation of goods and services. Determination of the rules through instruments such as accreditation, certification and rate settings helps to clarify the performance appraisal system and responsibilities in a health system (11).

The meso level concerns the organization of how care is delivered and where the reconfiguration of current practice is needed. In particular, the organizational framework of a Chronic Care Model has been proposed and studied. The model is based on six key elements that focus on quality health care promotion, patient self-management support, implementing care based on scientific evidence and patient preferences, health promotion, care coordination, cultural competences, effectively using patient/population data and mobilizing community resources (12).

There is a lot of evidence concerning the effectiveness of the Chronic Care Model, and some evidence of its cost effectiveness but no evidence of cost saving, at least in the short run (13). The evolution of the Chronic Care Model is represented by the Patient Centered Medical Home model of care. This model highlights the role of interdisciplinary team based care, the need for a strong relationship between health professionals and patients and their families, caregivers and the community, and the relevance of public reporting (14). This model is sustained by promising positive evidence, both for its effectiveness and its sustainability (15).

At the micro level, interdisciplinary and interprofessional teams can contribute to the achievements of patients' goals. Therefore, interprofessional education and collaboration, has to be fruitfully pursued. In fact, professionals learning from each other in different fields and applying this knowledge in the workplace with patients and care givers can produce successful outcomes (16,17).

Another relevant opportunity for improving PHC at the micro level is represented by different combinations of professionals in the workplace (skill mix). The need for a skill mix change is based on economic restrictions, technical development of professions and procedures, and political opportunities (18). Scientific evidence for changing the skill mix in primary health care has been reported to show positive clinical effects, as well as an increase in patient-perceived quality and process of care, although without cost saving (19).

The nano level refers to direct interaction between the provider and patient, which shows a promising influence on health benefits at the population level according to previous studies. Boivin et al. Suggested in their study that when patients and professionals join together to make choices regarding community health, they prefer to give priorities to person-centered care, as opposed to priorities being set and carried out exclusively by professionals who prefer to focus on technical disease management (20). Proactive involvement of patients in health choices and health care decision-making is supported by robust scientific evidence (21-24). On this basis patient and caregiver engagement can put new resources and accountabilities for the primary care networks actions, whereby the patient or care giver is a partner equal to health professionals.

For this reason, a shift should be made from the "working to patients" approach in PC to the approach of "working with patients". A process supporting patient motivations, knowledge and skills (25) is required.

A framework for moving from experiments to routine practice in primary health care has to take into account the following five pillars. A strong commitment has to be pursued at the policy level, not only in the regulatory approach, but also in the investment and training to enhance primary health care approach. Shifting from fees for service to value based payments is another relevant issue to promote care coordination. The organization of primary health care with central regulation where local autonomies have the power of decision can be a perfect balance that allows the continuity of effective and sustainable innovations. Not only policy makers, but all the key stakeholders, such as professionals, patients and the public have to become protagonists in the improvement of the system. Finally, scientific evidence must support policy and practise in PC (6).

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PRIMARNA ZDRAVSTVENA ZAŠTITA: ZNAČENJE I MOGUĆNOSTI

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U današnje vreme postoji stalan rast u broju hroničnih bolesti, zbog čega obezbeđivanje dobre primarne zdravstvene zaštite podrazumeva konstantno poboljšanje u organizaciji, kao i usaglašavanje sa brzim razvojem medicinske tehnologije. Razvoj država kroz povećanje standarda uslovio je novu organizaciju zdravstvenih sistema radi ponovne procene troškova. Velike su prednosti dobro organizovane primarne zdravstvene zaštite koja rezultira smanjenom broju poseta hitne pomoći i smanjenoj hospitalizacije. Naučni dokaz daje veliki doprinos merama i odgovornostima primarne zdravstvene zaštite, i samim tim može informisati i podržati izbore koji mogu biti doneseni na različitim nivoima odlučivanja o zdravstvenom uređenju (makro nivo, nivo uređivačke politike; meso nivo, nivo koji organizuje model ponuđača usluga; mikro nivo, gde profesionalci dolaze u interakciju i nano nivo, koji se odnosi na saradnju pacijenata i staratelja sa ponuđačima usluga).

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Ključne reči: *primarna zdravstvena zaštita, istraživanje zdravstvene službe, kontinuitet nege bolesnika*

Original article

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ACTIVATED PARTIAL THROMBOPLASTIN TIME AS INDICATOR OF DABIGATRAN EFFICIENCY IN PATIENTS WITH NON-VALVULAR ATRIAL FIBRILATION

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Dabigatran, a new generation anticoagulant, is a direct thrombin inhibitor, which has a rapid onset of action and relatively wide therapeutic scope, with no need for monitoring of efficacy, as is the case with vitamin K antagonists. However, there are certain emergency situations that require immediate assessment of the effectiveness of dabigatran. The aim of this study was to determine whether the aPTT as a screening coagulation test, can be reliably used to assess the anticoagulant effect of dabigatran.

The study included 32 patients with non-valvular atrial fibrillation who received dabigatran (Pradaxa, Boehringer Inhelheim) in a single dose of 110 mg or 150 mg twice a day. In all patients screening coagulation (PT, aPTT, INR) was done before the treatment. aPTT was performed 4 hours, 8 hours and 12 hours after taking the drug.

There was a statistically significant prolongation of aPTT after 4 hours and 8 hours of taking the drug in patients who were treated with 150 mg of dabigatran compared to 110 mg, while after 12 hours there was no statistically significant difference in aPTT between these two groups. There was a strong correlation between the control values of aPTT and the total increase in aPTT after dabigatran administration (r = 0.96 for a dose of 150 mg IR = 0.83 for a dose of 110 mg).

aPTT is a useful test for assessing the effect of dabigatran and can be used as a screening test in patients who urgently need to determine the efficacy of the drug.

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Key words: *dabigatran, aPTT, anticoagulant, bleeding*

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Introduction

For more than 50 years, anticoagulant therapy has been effectively used in the treatment of venous thromboembolism and prevention of stroke and systemic embolism (1). In addition, anticoagulants are used in the prevention of ischemic stroke in patients with atrial fibrillation, in the prevention of early and late recurrence of thromboembolism, in patients with acute coronary syndrome (ACS), deep venous thrombosis (DVT) or pulmonary embolism (PE) (2). In practice, anticoagulant therapy involves the use of warfarin or acenocoumarol taken per os, non-fractionated heparin (NH), low molecular weight heparins (LMWH) and parenteral inhibitors of the activated factor Xa (e.g., fondaparinux). Despite the proven clinical efficacy of these drugs, there are some limitations to their use, such as warfarin interactions with other drugs and some foods, prolonged onset of action, narrow therapeutic range and mandatory laboratory control, while the use of NH or LMWH carries the risk of heparin-induced thrombocytopenia (HIT), it requires laboratory control and dose adjustments. That is the reason why clinicians are introducing new oral anticoagulant drugs in routine practice, which are structurally direct inhibitors of thrombin (dabigatran) and activated factor Xa (rivoroxaban, apixaban, edoxaban), they are applied in a fixed dose and do not require laboratory control, have rapid onset of action and a relatively wide therapeutic range (1, 3). These drugs are more comfortable both for patients and for doctors, they have shown good clinical results so far and high profitability in the "cost-benefit" analysis (4). It is certain that the new oral anticoagulants bring significant changes in the management of anticoagulant therapy, both in prevention and treatment of thrombosis.

Dabigatran etexilate (Pradaxa, Boehringer In-

gelheim) is an oral anticoagulant that is hydrolyzed in the liver to dabigatran, which is a direct thrombin inhibitor. Recommended doses for clinical use are 110 mg and 150 mg twice a day, whereby the maximum drug concentration in plasma (100-400 ng/ml) is reached after 2-3 hours of ingestion. Dabigatran is mostly eliminated in the kidney (80%), in the glomerular filtration rate (GFR) > 80 ml/min half-life is about 13 hours, while at the GF of 30-50 ml/min half-life of dabigatran is about 18 hours (1,5). Dabigatran affects coagulation screening tests, in terms of their prolongation, which depends on the dose and the time that has elapsed since the last dose of the drug is taken (6).

Although it is not necessary to test the efficacy of dabigatran routinely, there are some emergencies that require measurement and assessment of the anticoagulant effect of this drug. These are, primarily, the state before surgical or invasive procedure when there is an indication that the patient took the drug in the last 24 hours (or longer if the creatinine clearance is less than 50 ml/min), if the patient is bleeding, if the patient had taken a dose greater than prescribed, in the developing renal failure or if there is a development of thrombosis while the patient is on therapy (3, 7). The literature describes the cases of serious gastrointestinal and intracranial bleeding associated with taking dabigatran, so in these cases it is very important to assess whether dabigatran is achieving supratherapeutic, therapeutic or subtherapeutic anticoagulant effect (5, 8). For this purpose, it is necessary to employ rapid laboratory testings, sensitive to dabigatran, which will give results within 30-60 minutes. These are: activated partial thromboplastin time (aPTT), thrombin time (TT) and ecarin clotting time (ECT)

Aim

The aim of this study was to determine:

- the effect of dabigatran on aPTT as a screening coagulation test,
- whether this test can be reliably used to assess the effect of dabigatran, especially in patients who are preparing for surgical intervention or those with bleeding.

Patients and methods

The study included 32 patients with non-valvular atrial fibrillation taking dabigatran (Pradaxa, Boehringer Inhelheim) in a single dose of 110 mg or 150 mg twice a day. A total of 14 patients were previously treated with anticoagulant therapy, vitamin K antagonists (warfarin (Farin), acenocoumarol (Sinkum, Sintrom), but because of the poor therapeutic efficacy of these drugs dabigatran was used instead, while 18 patients were on oral anticoagulant therapy for the first time.

Before the introduction of dabigatran, in all patients coagulation screening tests were done in the Department for monitoring of coagulation disorders in the Blood Transfusion Institute (BTI) of Niš, by determining the prothrombin time (PT/INR) and aPTT-on ACL Elite Pro (Instrumentation Laboratoty, USA). After two weeks of starting the therapy, patients came again to the BTI Niš when we took three blood samples in order to determine their, aPTT on the same day but in different time interval from taking the drug: 4 hours, 8 and 12 hours after the last taken dose of dabigatran. Blood samples for determining the aPTT were taken into 3.8 mL tubes with sodium citrate, aPTT was determined using the aPTT reagent HemosIL SP within 30 min of sampling.

Statistical analysis was performed using the Statistical Package for Social Science (SPSS Software GmbH, Germany), version 18.0. The results were presented in tables and graphs, using the mean values and standard deviations (SD). The relationship between aPTT before dabigatran therapy and aPTT after dabigatran administration was determined using the Pearson's correlation analysis.

Results

From the total of 32 patients in this study, there were 20 men (20/32 or 62.50%) and 12 women (12/32 or 37.50%), which represented a statistically significant difference ($p > 0.05$). The average age of patients in the study was 52.36 ± 10.14 years (the youngest patient was 42 and the oldest one was 70 years old). A total of 20 patients were treated with dabigatran in the dose of 150 mg twice a day, while 12 patients received 110 mg of dabigatran twice a day.

The average values of aPTT in the examined patients are presented in Table 1.

Table 1. aPTT (sec) in patients who are treated with dabigatran ($\overline{X}$ ± SD)

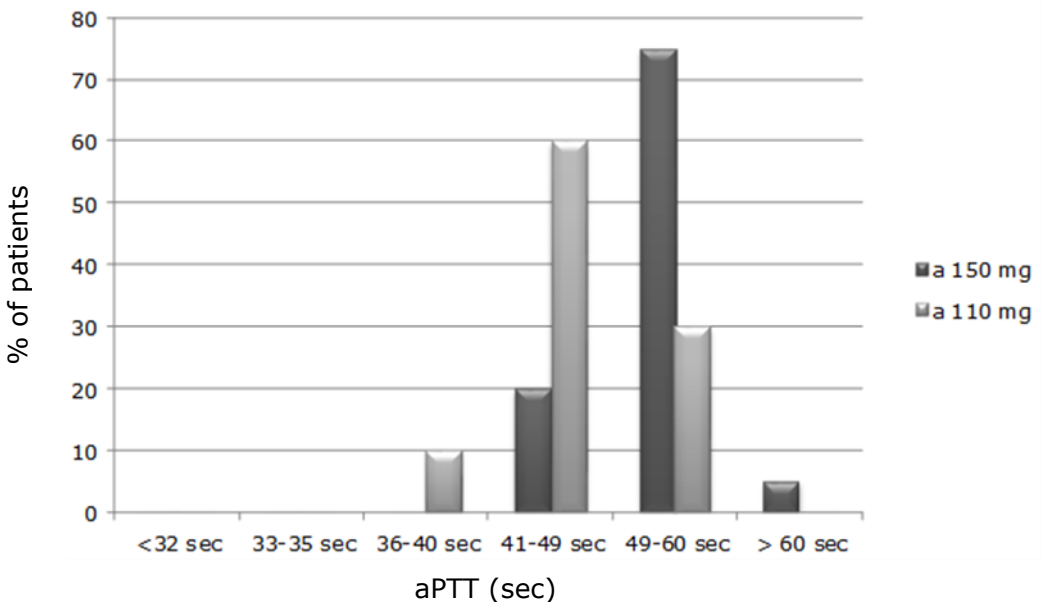
	a 150 mg	a 110 mg	p
Control*	31,23 ± 3,17	30,46 ± 2,79	> 0,05
After 4 hrs	52,95 ± 7,27	45,00 ± 2,64	< 0,001
After 8 hrs	44,15 ± 7,51	38,10 ± 2,05	< 0,001
After 12 hrs	36,70 ± 6,48	34,12 ± 1,12	> 0,05

* aPTT before dabigatran therapy

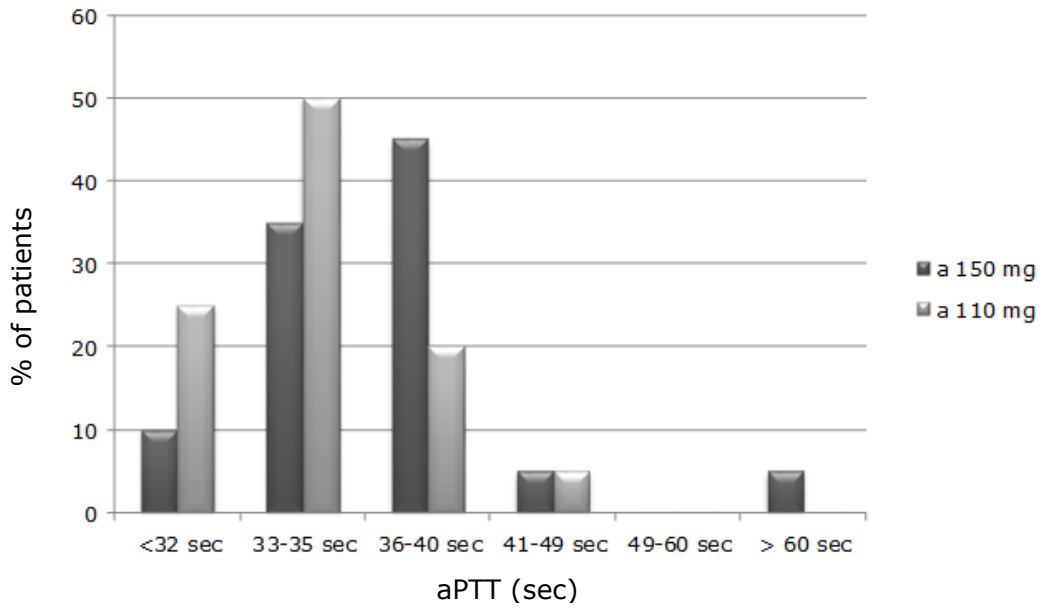
All patients had aPTT within normal ranges (25-35 sec) before the introduction of dabigatran. There was a statistically greater prolongation of aPTT values after 4 hours and 8 hours of taking the drug in patients who were treated with 150 mg of dabigatran as compared to patients taking 110 mg of dabigatran ($p < 0,001$), while after 12 hours of taking dabigatran there was no statistically significant difference in aPTT values between these two groups.

In patients who were taking 150 mg of dabigatran after 4 hours, aPTT was prolonged 1.5 to 1.8 times compared to controls; after 8 hours, 1.3 to 1.6 times; whereas after 12 hours it was 1.2 to 1.4 times greater then the initially measured aPTT values. In patients who received 110 mg of dabigatran after 4 hours, aPTT was prolonged 1.4 to 1.7 times, while after 12 hours of taking the drug aPTT was almost normalized (1.05 to 1.2 times greater). Patient distribution according to aPTT values after 4 hours and 12 hours of taking dabigatran are shown in Graph 1 and Graph 2.

Graph 1. Distribution of patients (%) according to aPTT values 4 hours after taking dabigatran



Graph 2. Distribution of patients (%) according to aPTT values 12 hours after taking dabigatran



Only one patient who was taking 150 mg of dabigatran had aPTT after 4 hours 2.8 times higher then normal, while after 12 h of dabigatran ingestion aPTT was still significantly prolonged (2.1 times). At the time of testing, the patient had bruises on the left arm and abdomen, a hematoma on the left forearm and complained of occasional epistaxis; the laboratory testings on the same day showed disturbed renal function (creatinine clearance about 35 ml/min).

Pearson's correlation analysis showed a strong positive correlation between initial aPTT (control) and total aPTT increase after taking dabigatran at a dose of 150 mg (r = 0,96, R2 = 0,93), as well as after 110 mg of dabigatran (r = 0,83, R2 = 0,89).

Discussion

When dabigatran was approved in October 2010 by the U.S. Food and Drug Administration (FDA) as an oral anticoagulant that can be used in the prevention of cerebrovascular events in patients with non-valvular atrial fibrillation, a major strength of this drug over vitamin-K antagonists, as indicated, was the drug pharmacokinetics that can be predicted, which eliminated the need for frequent laboratory testing to monitor its anticoagulant effect (9). The RE-LY study (Randomized Evaluation of Long-term anticoagulant therapy with dabigatran etexilate), conducted in order to investigate the efficacy and safety of dabigatran compared with warfarin in patients with non-valvular atrial fibrillation, showed that dabigatran at a dose of 150 mg twice a day had an efficacy equal to warfarin in achieving the anticoagulant effect, without any significant difference in the incidence of bleeding (10). The results in this study also showed that in patients older than 80 years and those with impaired renal function, bleeding occurred more frequently, but in these patients dabigatran is recommended in a single dose of 110 mg twice daily, while in those with significantly reduced renal function (creatinine clearance of 15-30 ml/min) a total daily dose of 150 mg (75 mg twice a day) is recommended. The factors that increase the risk of stroke, according to CHADS2 score (congestive heart failure, hypertension, age over 75 years, diabetes mellitus, previous stroke or transient ischemic attack), increase the risk of bleeding in patients with atrial fibrillation who are taking anticoagulant therapy (10). The later RELY-ABLE study (Long-term Multicenter Extension of Dabigatran Treatment in Patients with Atrial Fibrillation) showed no statistically significant difference in the incidence of stroke or mortality rate both in patients who received 150 mg of dabigatran and those taking 110 mg of the drug, but the higher dose of dabigatran was associated with a higher rate of bleeding (11). The literature describes 280 cases of fatal bleeding associated with dabigatran use in the last six years, especially in Japan and in the area of New Zealand (12), and the Australian Ministry of Health has confirmed that there are certain drugs that potentiate the effect of dabigatran and therefore increase the risk of bleeding, and those are primarily P-glycoprotein inhibitors such as ketoconazole, dronedarone and panrazol (13). Because of the risk of bleeding (primarily gastrointestinal and intracranial), as well as of overdosing dabigatran, it is necessary in certain situations to measure the anticoagulant effect of dabigatran and possibly introduce a reverse therapy.

Numerous studies have shown that aPTT as a screening coagulation test routinely used in almost all laboratories for testing hemostatic disorders is sensitive to the presence of dabigatran, and that there is a linear prolongation of aPTT with increasing concentrations of dabigatran up to 200 ng/mL (1, 14-16). In the presence of higher dabigatran concentrations there is a curvilinear prolongation of aPTT, which indicates that aPTT can not be used as a reliable test in the presence of supratherapeutic dabigatran concentration in the blood. On the other hand, TL Lindahl et al. in their study showed a linear response of aPTT even in the concentrations of dabigatran greater than 200 ng/mL (17). Our investigation confirmed that aPTT was a sensitive test for the presence of dabigatran, and it was most prolonged 4 hours after taking the drug (1.5-1.8 times when taking dabigatran in a single dose of 150 mg, and 1.4 to 1.7 times in the presence of 110 mg of dabigatran), but after 12 hours (immediately before taking the second dose of dabigatran) aPTT returned to the reference value or was barely prolonged. aPTT prolongation for more than twice, even 12 hours after taking the drug, was associated with bleeding and decreased renal function.

If bleeding occurs during the treatment with dabigatran, it should be immediately discontinued, and in case of bleedings that are life-threatening (especially intraocular, intracranial, gastrointestinal, retroperitoneal) it is recommended to apply prothrombin complex concentrate (PCC, activated and inactivated), activated factor VIIa or access hemodialysis (18). Today, a specific dabigatran antidote is available, idarucizumab (Praxbind), approved by the FDA and the European Medicines Agency (EMA), which reverses the effect of dabigatran in a few minutes, while the complete reversal of dabigatran is achieved in about 12 hours (19, 20).

Dabigatran therapy discontinuation because of surgery or other invasive procedures depends on the type and severity of the procedure and patient comorbidities, especially renal diseases. It is recommended that in the case of a minor intervention dabigatran has to be discontinued 2-3 days preoperatively and started again after 24 hours, while in the case of major surgical procedures or in those with impaired renal function (creatinine clearance less than 50 ml/min) dabigatran has to be discontinued 3-5 days before surgery and continued again after 48 hours (1). However, individual assessment of the anticoagulant effect of dabigatran in these cases is very important, and exactly here aPTT can be used as a semi-quantitative screening test that shows whether there is a pharmacologically significant anticoagulant effect of dabigatran at the time of testing. We must be particularly careful in the post-

operative period, because within 2-3 days after surgery false positive prolonged aPTT can occur due to heparin therapy in the perioperative period.

Conclusion

aPTT is a useful test for assessing the effect of dabigatran and can be used as a screening test in patients in whom the efficacy of the drug has to be urgently determined. Further studies should be directed towards determining the efficacy of aPTT in the assessment of the reverse effect of dabigatran in patients who are bleeding.

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AKTIVISANO PARCIJALNO TROMBOPLASTINSKO VREME KAO INDIKATOR PROCENE EFEKTA DABIGATRANA KOD BOLESNIKA SA NEVALVULARNOM ATRIJALNOM FIBRILACIJOM

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Dabigatran, antikoagulans nove generacije, po svojoj strukturi je direktni inhibitor trombina, ima brzi početak delovanja i relativno širok terapijski opseg, a njegova primena ne zahteva praćenje efikasnosti, kao što je to slučaj sa antagonistima vitamina K. Međutim, postoje određena hitna stanja koja zahtevaju procenu efikasnosti ovog leka. Cilj ovog rada bio je utvrditi da li se aPTT, kao skrining koagulacijski test, može pouzdano koristiti za procenu antikoagulantnog efekta dabigatrana.

Ispitivanje je obuhvatilo 32 bolesnika sa nevalvularnom atrijalnom fibrilacijom koji su uzimali dabigatran (Pradaxa, Boehringer Inhelheim) u pojedinačnoj dozi od 110 mg ili 150 mg dva puta dnevno. Svim bolesnicima je pre početka terapije urađen skrining koagualcije (PT, aPTT, INR). Merenje aPTT-a je vršeno 4 sata, 8 sati i 12 sati posle uzimanja leka.

Postoji statistički znajno veće produženje vrednosti aPTT-a posle 4 sata i 8 sati od uzimanja leka kod bolesnika koji su na terapiji dabigatranom od 150 mg u odnosu na bolesnike koji uzimaju 110 mg dabigatrana ($p < 0,001$), dok se posle 12 sati ne uočava statistički značajna razlika u vrednostima aPTT-a između bolesnika ove dve grupe. Postoji jaka korelacija između kontrolnih vrednosti aPTT-a i ukupnog porasta aPTT-a nakon uzimanja dabigatrana ($r = 0,96$ za dozu od 150 mg i $r = 0,83$ za dozu od 110 mg).

Za procenu efekta dabigatrana aPTT je koristan test i može se koristiti kao skrining test kod bolesnika kod kojih je potrebno hitno odrediti efikasnost leka.

Acta Medica Medianae 2018;57(1):135-140.

Ključne reči: *dabigatran, aPTT, antikoagulans, krvarenje*

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.

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