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The laureates of the Nobel Prize in Physiology or Medicine in 2014 are: an American-British neuroscientis John O'Keefe (born 18 Novemebr 1939), and Norwegian physiologists and neuroscientists May-Britt Moser (born 4 January 1963) and Edvard Ingjald Moser (bron 27 April 1962). They are awarded for discoveries of cells that constitute a positioning system in the brain (see Editorial, p. 995−6).

Dobitnici Nobelove nagrade za fiziologiju ili medicinu u 2014. godini: američko-britanski neuronaučnik John O'Keefe (rođen 18. novembra 1939) i norveški fiziolozi i neuronačnici May-Britt Moser (rođena 4. januara 1963) i Edvard Ingjald Moser (rođen 27. aprila 1962). Oni su nagrađeni za otkriće ćelija koje čine sistem za prostorno pozicioniranje u mozgu (vidi uvodnik, str. 995−6).

E D I T O R I A L

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Cognitive maps discovery – Far-reaching implications for contemporary neuroscience

Otkriće kognitivnih mapa – dalekosežni značaj za savremenu neuronauku

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The Nobel Prize in Medicine this year is shared by the three scientists whose research shed light on the concept of spatial orientation in the brain.

The movement started in the early development of life on Earth, from the most primitive to highly complex forms equipped with systems for multimodal navigation in space and time.

You guess, it is about the ability of man to recognize the coordinates of the current position and future projection of the trajectory in the world that surrounds it.

And even though this ability has been perfected from generation to generation for millions of years, marking the development of primates, understanding this infallible mechanism in neuroscience have been indicated only in recent decades.

More than 40 years ago, a young researcher, John O'Keefe, at the University College London published the first results of several years of research related to the recordings of neuronal firing in the dorsal hippocampus of freely moving experimental animals capacities [1, 2]. The results of these studies suggest the striking spatial correlates of neuronal firing that come from the so-called "place-cells" whenever the animal is at a particular location in a maze. This finding later led John O'Keefe and Nadel [3] to propose that the hippocampus could be the seat of a cognitive map.

Such cognitive maps, to further speculate, could be used not only for spatial navigation throughout the environment, but also as a memory framework upon which the significant items and episodes of experience could be superimposed. So, declarative memory in mammals could be lay down over the cognitive maps of spatial orientation.

Let me be provocative for a moment.

Was not this already known in the ancient times, as the Art of Memory, attributed to Pythagoreans ?

Actually, it was.

The Art of Memory is a mnemonic technique that was used to organize memory, especially during the epoch of the Renaissance as well as in the Western esoteric tradition, as for very long speeches of public orators.

To use this method one should walk through a building several times, viewing distinct places within it, in the same order each time, using it as the framework where one would place images or signs that would be used in the chains of associations later to connect one memory with another. One goal of the technique was without doubt, to maximize human mental capacities [4].

Does this resemble to the experimental animals or subjects who store spatial maps and declarative memory in the hippocampus and the entorhinal cortex as O'Keefe and Nadel were speculated?

Research that followed the O'Keefe's pioneering work, in the next decades showed that the neural cells of the adjacent brain region, the medial entorhinal cortex show hexagonal patterns of activity stretching over the space traversed, similar to the lines that indicate the geographic coordinates on the earth's surface [5–7]. These findings of the so-called "grid-cells" come out from research of a Norwegian couple May-Britt and Edvard Moser, from Kavli Institute for Systems Neuroscience in Trondheim, Norway, making a "whole- frame image" complete.

But why were these discoveries choosen as ground breaking in the field of medicine for this year?

At the elementary level it is about knowing how mental functions are represented in the brain, but far-reaching implications are more complex.

Decoding the patterns of electrical activity in the neural networks was limited until recently primarily to the earliest stages of cortical processing, e.g. allowing the brain to repre-

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sent sensory experiences from the external world – such as light and sound.

On the contrary, many were skeptical that it is possible to connect the complex brain functions such as memory, reasoning or imagination with neural firing at most high-end association cortices, primarily due to the increasing decoupling of neural activity as the more synaptic relays are added.

It is about getting to know the programming language which is used by the human nervous system to provide ways that information is integrated across hierarchical levels.

O'Keefe and the Moser couple discoveries of "place" and "grid" cells, led for the first time to breaking the "programer code" deep in the brain, at the high end of the cortical hierarchy. Those findings could be the first steps at the long journey of learning the operating language of the brain.

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ORIGINAL ARTICLES

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Cyclooxygenase-2 expression in cervical cancer

Ekspresija ciklooksigenaze-2 kod karcinoma grlića materice

Aljoša Mandić, Slavica Ušaj-Knežević, Tatjana Ivković Kapicl, Dejan Ninčić, Goran Malenković

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Abstract

Background/Aim. Cyclooxygenase (COX) or prostaglandin H2 synthase is the first enzyme that catalyzes the first two steps in the biosynthesis of prostaglandins from arachidonic acid. The aim of the study was to determine the expression level of COX-2 in patients with cervical cancer and compare it with that in the control group with no cervical pathology. **Methods.** The study included 76 patients divided into two groups: the control group – 30 patients without histopathological changes and the group A – 46 patients with cervical cancer, FIGO stage IB-IIA. Histopathological and immunohistochemical analyses were performed in these two groups of patients. **Results.** In the control group, the expression of COX-2 was not confirmed compared to the group A of 26 (56.52%) patients. The expression of COX-2 showed a statistically significant difference in the presence of lymphocytic stromal infiltration (p = 0.0053). The expression of COX-2 was more pronounced in the stromal tissue without lymphocytic infiltration (80% *vs* 20%). **Conclusion.** A higher expression of COX-2 in cervical carcinoma without stromal lymphocytic infiltration suggests a possible paradoxical effect of COX-2 in immunosuppression. Frequent COX- 2 expression in the subgroup with poor prognostic histological parameters in the group A indicates the importance of COX-2 expression in the carcinogenesis of cervical cancer.

Key words:
uterine cervical neoplasms; prostaglandin-endoperoxide synthases; immunohistochemistry; gene expression; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Ciklooksigenaza (COX) ili prostaglandin H2 sintaza (PGHS) prvi je enzim koji katalizuje prva dva koraka biosinteze prostaglandina iz arahidonske kiseline. Cilj rada bio je da se ispita prisustvo i stepen ekspresije COX-2 u tkivu grlića materice ispitanica sa cervikalnim karcinomom i uporedi sa kontrolnom grupom bez cervikalne patologije. **Metode.** Istraživanjem je obuhvaćen patohistološki materijal uzet od 76 bolesnica podeljenih u dve grupe: kontrolna grupa – 30 bolesnica bez patohistoloških promena na grliću materice i grupa A – 46 bolesnica sa verifikovanim karcinomom grlića materice, FIGO stadijum IB-IIA. U obe grupe na patohistološkom materijalu izvršene su histološke i imunohistohemijske analize. **Rezultati.** U kontrolnoj grupi nije potvrđena ekspresija COX-2, a u grupi A jeste kod 26 (56,52%) bolesnica što je statistički značajna razlika u odnosu na kontrolnu grupu. Ispitivanjem ekspresije COX-2 i patohistoloških parametara, uočena je statistički značajna razlika u odnosu na postojanje limfocitne infiltracije (p = 0,0053). Ekspresija COX-2 bila je izraženija u tkivu bez limfocitne stromalne infiltracije (80% *vs* 20%). **Zaključak.** Ekspresija COX-2 bila je izraženija kod karcinoma grlića materice bez limfocitne stromalne infiltracije što navodi na zaključak o mogućem paradoksalnom efektu COX-2 na imunosupresiju u tumorskom tkivu. Nalaz češće ekspresije COX-2 u podgrupi sa lošijim prognostičkim patohistološkim parametrima u grupi A upućuju na značaj aktivnosti ekspresije COX-2 u procesu karcinogeneze karcinoma grlića materice i uticaja na njegovu progresiju.

Ključne reči:
grlić materice, neoplazme; prostaglandin sintetaza; imunohistohemija; geni, ekspresija; osetljivost i specifičnost.

Introduction

According to the global scale, cervix uteri carcinoma remains in the second place among female population of diseased from the malignant neoplasia, with about 400,000 newly diagnosed cases *per* year with annual mortality of about 250,000 women. Approximately 83% of cervical carcinoma is diagnosed in underdeveloped and developing countries, which do not have any adequate screening programs [1]. According to the data obtained from the Cancer Registry of the Central Serbia, cervical carcinoma is the most frequent malignant tumor of the female reproductive organs with the incidence rate of 26.9/100,000. The same data from 2001 show the frequency of cervical carcinoma of 9.4% in

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the total number of the diseased and 5.8% in total mortality from malignant tumors of the female population [2]. According to the Malignant Diseases Registry of the Oncology Institute of Vojvodina, the incidence of 26.6/100,000 was registered in Vojvodina, for the period from 1993 to 2002 [3]. Former research in oncology contributed to the definition of the cyclooxygenase-2 (COX-2) expression significance in tumor cell oncogenesis. In oncogenetic expression, a more significant role is played by COX-2, whose expression is related to various pathophysiological conditions of inflammation or oncogenesis [4]. Furthermore, studies show the significance of COX-2 expression in regulation of apoptosis, disease progression, neoangiogenesis and the therapeutical response [4–7]. Liu et al. [8] presented COX-2 expression growth in more severe cases of esophagus squamous neoplasia. Lim et al. [9] also confirmed the increase in COX-2 expression in adenomatous and metaplastic stomach lesions. Saukonen et al. [10] presented COX-2 expression in 44% of patients with dysplastic changes in stomach. Also, COX-2 expression was confirmed in other neoplastic lesions of the breast, urinary bladder and pancreas [7, 11, 12]. Ferrandina et al. [13] also published the results of COX-2 expression study in precancerous and cancerous changes of vulva. They confirmed a higher level of COX-2 expression in more advanced stages of vulva carcinoma (FIGO stages III/IV), with metastatic lymph nodes and deeper stromal infiltration.

COX-2 expression was a significant factor in the increase of tumor angiogenesis and the reduction of apoptosis, which appeared as a possible, important connection within the development of carcinogenesis and tumor growth [14, 15].

The aim of this paper was to: test the presence and the level of COX-2 expression in cervical tissue of the female patients divided into two groups – the control group and the group A (cervical carcinoma, FIGO stage I-IIA), and to compare the COX-2 expression level in the patients diagnosed with the cervical carcinoma in relation to the level of tumor differentiation, stromal invasion, tumor size, the presence of lymphovascular invasion and the existence of metastases in the lymph nodes.

Methods

The study included histopathological material from 76 patients who underwent surgery with performed hysterectomy with or without adnexectomy due to benign changes in the uterus (myomas) or, with performed radical hysterectomy on the basis of biopsy verified cervical carcinoma, FIGO stage IB-IIA. The trial was conducted at the Clinic for Operative Oncology and the Center for Pathology and Diagnostic Cytology of the Oncology Institute of Vojvodina in Sremska Kamenica.

Based on the definite histopathological findings, the patients were divided into two groups: the control group (without changes at the cervix uteri) and the group A (cervical carcinoma, FIGO IB-IIA).

The control group included histopathological material of 30 patients who underwent total hysterectomy due to benign changes in the uterus and/or ovaries.

Exclusion criteria for this group of patients were the patients with: previous excision or ablation of the cervix uteri: precancerous or cancerous lesions diagnosed in the cervix uteri; verified chronic inflammation of the cervix uteri; verified malignant disease of the genital tract; verified malignant disease of any other localization.

The group A included histopathological material of 46 patients with verified cervical carcinoma FIGO stage IB-IIA, who underwent radical Piver, class III surgery, with lymphadenectomy.

Exclusion criteria for this group were the patients: with verified cervical carcinoma who were previously treated by irradiation therapy or neoadjuvant cytostatic therapy; who previously underwent an excision or ablative type of cervical treatment; with verified malignant disease of the genital tract of other localization; with verified malignant disease of any other localization.

The obtained surgical material was sent to histopathological (HP) examination.

Histopathological examination enabled the definition of the final histopathological diagnosis, determination of the stage of the tumor disease and the analysis of the standard histopathological prognostic parameters: histological type of the tumor; tumor size and the depth of the stromal invasion; grade of histological differentiation; the presence of lymphovascular invasion; the total number of removed lymphatic nodes; the presence of metastases and the number of metastatically changed lymphatic nodes.

Examination included all resection edges of parametrium and vagina, for determination of the presence or absence of the tumor.

Based upon the HE stained preparations, a representative sample from the examined material was selected for immunohistochemical testing.

Immunohistochemical analysis of COX-2

For immunohistochemical analyse, the selected tissue samples from the control group (hysterectomy due to myomatous uterus) and the group A (radical hysterectomy) were used. The samples were fixed in formalin and blocked in paraffin, sliced into sections of 4 micron thickness, and then "glued" to Superfrost (Menzel-Glaser) positively electrified glass slides, previously prepared for immunohistochemical reactions. After deparaffinization, we started blockage of endogenous peroxidase with 3% H2O2 for 5 minutes. Immunohistochemical identification of the tested antigens was performed by application of Streptavidin-biotin-peroxidase technique (B-0SA), according to the standard LSAB procedure (Dakocytomation-DAKO). The fragments were incubated for 30 minutes at room temperature with biotinylated anti-mouse antibody, and then incubated with streptovidin-peroxidase complex system, in duration of 30 minutes. As a hromogenous substrate, a 3-amino-9-ethylcarbazole (AEK, DAKO) was applied. After each incubation the samples were rinsed in Tris buffer solution (TBS: 0.05 M, pH 7.6). Contrasting was performed by hematoxylin. The tissue samples, which, during the treatment missed the primary antibody, were used as the negative control for antibody, while the

other phases of the immunohistochemical procedure were applied. The analysis of immunohistochemically processed tumor tissue samples was performed by light-microscopy, by qualitative and semi-qualitative method, expressed as the percentage of positive cells in relation to the total number of cells in the representative fields.

The value of COX-2 expression was analyzed semi-qualitatively, by determination of the percentage of the stained tumor cells [16]: absence of expression – negative findings; mild level of expression - < 25% of the changed cells were positive; medium level of expression – > 25.1–50% of the changed cells were positive; high level of expression – ≥ 50.1% of the changed cells were positive.

As internal negative control, tumor unchanged epithelial cells of the cervix were used. As positive external tissue control for COX-2 expression, a high-grade transitional cellular urinary bladder carcinoma was used [17].

During statistical analysis of data, descriptive statistics were calculated – frequencies, percentages, mean values, and a standard deviation.

We used graphical presentation of data and the results with the aid of column diagrams and box-whiskers diagrams.

Comparisons were performed by the *t*-test, numerical-feature-variance analysis.

For attributive features, the non-parametric, Pearson's χ^2-test and Fisher's exact test were used.

Statistically significant differences ($p < 0.05$) were marked by the asterisk (*), and highly significant difference ($p < 0.01$) by two asterisks (**).

Statistical data analysis was performed with the aid of the software package STATISTICA 9.0 for which, there is a University license at the Novi Sad University. Data analysis and presentations (tables and graphs) were prepared by the computer technique in programs Microsoft Word, Excel and Power Point.

Results

The study results were obtained by the analysis of histopathological material from 76 patients and their statistical processing. The material was collected at the Gynecological Oncology Department and analyzed at the Pathology and Cytodiagnostics Department of the Oncology Institute of Vojvodina in Sremska Kamenica.

The mean age of the patients in the control group was 46.19 years. The youngest patient was 30 and the oldest one 67 years of age ($\bar{x} \pm SD = 46.19 \pm 8.284$).

The mean age of the patients in the group A was 50.13 years. The youngest patient was 31 and the oldest 66 years of age ($\bar{x} \pm SD – 50.13 \pm 10.417$).

In the control group, histopathological findings showed the normal cervix uteri without any histopathological changes.

In the group A, in all 46 patients, a planocellular type of carcinoma was verified in the final histopathological findings. According to the FIGO classification in 34 (74%) of the patients, the disease was staged as IB1, i.e. stage IB2 in 12 (26%) of the cases.

Beside histological type and the stage of the disease, histopathological examination also analyzed standard prognostic parameters: size of the tumor, depth of stromal invasion, total number of removed lymphatic nodes, the number of metastatically changed lymphatic nodes, degree of histological differentiation, the presence of lymphovascular invasion, involvement of parametrial and vaginal resection edges, involvement of "isthmus" of uterus and the presence of lymphocyte infiltrate. The tumor size was up to 2 cm in 47.83% of the patients. In 69.57% of the patients, the depth of stromal invasion was greater than 10 mm, and more than 10 lymph nodes were removed in 71.74% of the patients. In 17 patients, out of 46, the presence of metastases in the lymph nodes was diagnosed. In 11 patients, two or more lymph nodes were positive. The degree of histological differentiation was distributed in the following manner: G1 (17.39%), G2 (58.69%) and G3 (23.92%). Lymphovascular infiltration was not registered in 60.87% of the patients. "Isthmus" of uterus infiltration was verified in 23.91% of the patients. In 56.52% of the patients, there was lymphocyte stromal infiltration. Parametrial and vaginal infiltration of the cuff was not confirmed in 93.48%, i.e. 91.30% of the patients (Table 1).

After histopathological analysis, the preparations were stained immunohistocemically for testing of the COX-2 expression characteristics. Fisher's exact test and Pearson's χ^2-test were used for testing of the difference between the examined groups.

In the control group, there was no COX-2 expression confirmed, while in the group A, it was verified in 26 (56.52%) patients (Figure 1).

By comparison of COX-2 expression between the examined groups, it was determined that there was a highly statistically significant difference between the control group and the group A ($p = 0.0001$) (Figure 2).

The control group did not show COX-2 expression in the entire examined histopathological material. Further comparison of expression was tested within the group A.

The level of COX-2 expression was divided into: the absence of expression or negative COX-2; mild level of expression – < 25% of the changed cells were positive; medium level of expression – 25.1–50% of the changed cells were positive; high level expression – ≥ 50.1% of the changed cells were positive.

In the group A, mild, medium and high expression of COX-2 was in 17.39%, 15.22% and 23.91% of cases of the examined histopathological material respectively (Figure 3).

Testing of COX-2 expression and histopathological parameters showed a statistically significant difference in relation to the existence of lymphocyte infiltration ($p = 0.0053$). Positive COX-2 expression was greater in the tissue of the patients without lymphocyte stromal infiltration (16/20, 80%). In the patients with lymphocyte stromal infiltration, positive expression was verified in 10/26 (38.46%) patients. There was no statistically significant difference confirmed regarding other histopathological parameters ($p > 0.05$) (Table 2).

Table 1

Frequency of histopathological parameters in the group A (with cervical cancer)

Histopathological variables	Frequency	Percentage
Size of the tumor		
up to 2 cm	22	47.83
over 2 cm	24	52.17
Total	46	100
Depth of stromal invasion		
up to 10 mm	14	30.43
over 10 mm	32	69.57
Total	46	100
Number of lymph nodes		
up to 10	13	28.26
more than 10	33	71.74
Total	46	100
Number of lymph nodes with metastases		
1 positive lymph node	6	35.29
2 or more positive lymph nodes	11	64.71
Total	17	100
Degree of histological differentiation		
G1	8	17.39
G2	27	58.69
G3	11	23.92
Total	46	100
Lymphovascular infiltration		
Yes	18	39/13
No	28	60.87
Total	46	100
Lymphocyte stromal infiltration		
Yes	26	56.52
No	20	43.48
Total	46	100
"Isthmus" infiltration		
Yes	11	23.91
No	35	76.09
Total	46	100
Parametrial infiltration		
Yes	3	6.52
No	43	93.48
Total	46	100
Vaginal cuff infiltration		
Yes	4	8.70
No	42	91.30
Total	46	100

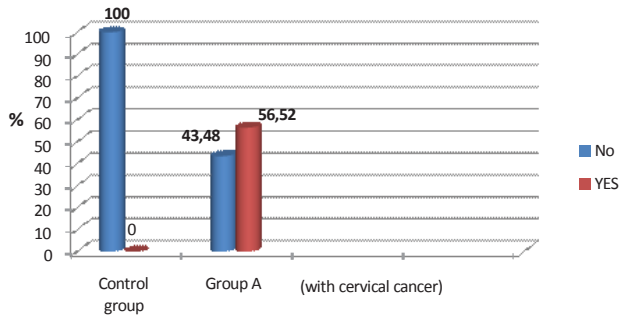

Fig. 1 – Frequency of COX-2 expression *per* groups.

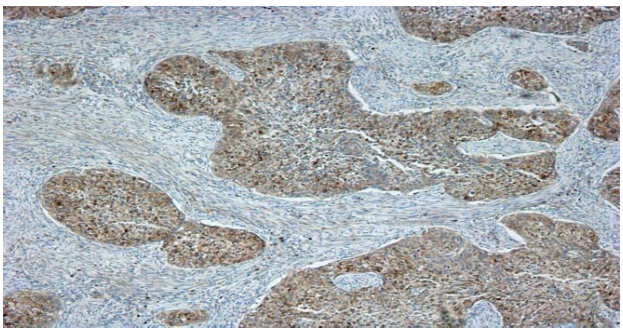

Fig. 2 – Immunohistochemical identification of COX-2 in the group A (with cervical cancer) (LSBA, ×200).

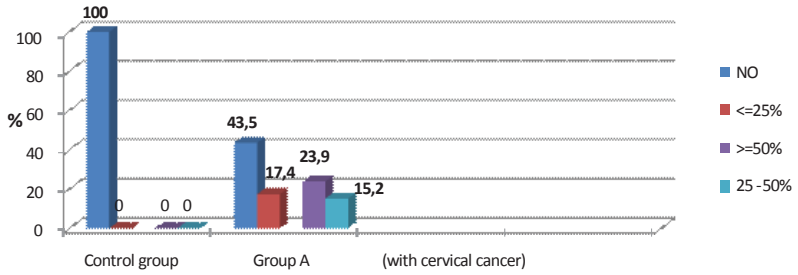

Fig. 3 – Frequency of COX-2 expression level.

Table 2

Comparison of COX-2 expression of lymphocyte stromal infiltration in the group A (with cervical cancer)

COX-2 expression	Positive, n (%)	Negative, n (%)	Total, n (%)	Fisher exact test p – values
Lymphocyte stromal infiltration				
Yes	10 (38.46)	16 (71.54)	26 (100)	0.0053*
No	16 (80)	4 (20)	20 (100)	
Total	26 (56.52)	20 (43.48)	46 (100)	

In the subgroup of patients with worse histopathological prognostic parameters, positive lymph nodes (17/46), lymphovascular infiltration (18/46), "isthmus" of uterus infiltration (11/46), there was a statistically significant difference in relation to the existence of COX-2 expression (positive COX-2 – 70.59%, 66.66%, 63.54%, and negative COX-2 – 29.41%, 33.34%, 36.36%, respectively; $p < 0.05$) (Figure 4).

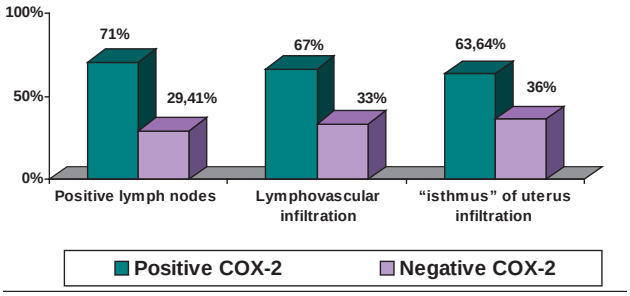

Fig. 4 – Frequency of COX-2 expression in the subgroup of patients with worse histopathological prognostic parameters within the group A (with cervical cancer).

In the other subgroups in the patients with the tumor greater than 2 cm, the level of histological differentiation and the depth of stromal invasion greater than 10 mm, there was no statistically significant difference in relation to COX-2 expression ($p > 0.05$). In a subgroup of patients with parametrial and vaginal infiltration of the cuff, due to a small sample (3, i.e. 4), statistical analysis was not performed.

The existence of a highly statistically significant difference in relation to the presence of lymphocyte stromal infiltration ($p = 0.003$) and the degree of COX-2 expression was determined. In patients with a medium degree of expression, 100% of the tested samples did not have lymphocyte stromal infiltration. Lymphocyte stromal infiltration was not present in 54.55% of the patients with the high COX-2 expression (over 50%) (Figure 5).

The degree of COX-2 expression in relation to the other histopathological parameters did not show a statistically significant difference ($p > 0.05$).

Discussion

Over 85% of deaths caused by cervical carcinoma are registered in the undeveloped countries. Observing the entire epidemiological picture, at the moment, Serbia is in the fifth place (19.6/100,000) in Europe for cervical carcinoma incidence, and in the third place for mortality (8.6/100,000) [18].

Researching COX-2 expression as a pro-oncogene activator contributed to wider knowledge of oncogenesis, but also imposed some new questions and goals to researchers. COX expression was proved in many premalignant, malignant and metastatic diseases regardless the tumor type and localization [4, 19]. Studies confirmed COX-2 expression in premalignant breast changes, adenomatous colon polyp, leukoplakia of buccal mucosa [20–22]. Expression was also determined in urinary bladder carcinoma, breast, colon, lung, pancreas, stomach, kidneys, skin carcinoma, lymphoma, sarcoma, leukemia, brain tumor [19, 23–30]. Researching COX-2 expression, its role in carcinogenesis and as a prognostic factor, was also presented in studies on malignant diseases of the lower genital systems in women.

Li et al. [31] presented expression of COX-2 in ovarian low malignant potential tumor (borderline) (57.9%) and carcinoma (81.5%) in relation to benign ovarian tumors (38.9%). They also determined a statistically significant difference between COX-2 expression and the clinical stage of ovarian low malignant potential tumor (borderline) (FIGO stage I/II –51.7% and stage III/IV – 90.9%). Denkert et al. [32] analyzed COX-1 and COX-2 expression in 117 ovarian tumor samples and 2 ovarian tissue samples without any changes. COX-2 was only detectible in malignant, changed tissue (42%). In univariate analysis, COX-2 expression was a bad prognostic factor. The mean survival in the patients with

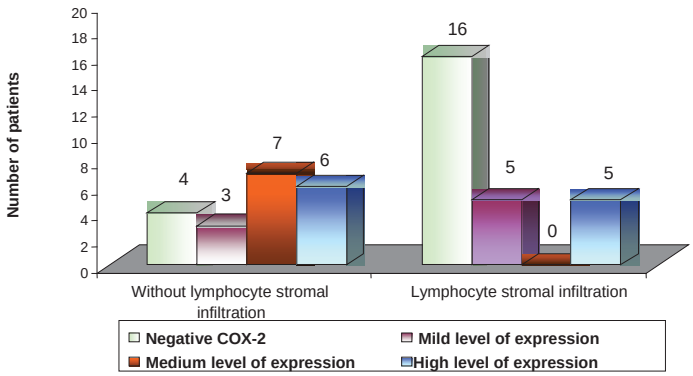

Fig. 5 – Frequency of the degree of COX-2 expression in relation to lymphocyte stromal infiltration.

negative COX-2 was 52 months in comparison to 30 months in COX-2 positive. In this study, the multivariate analysis showed that COX-2 expression is an independent bad prognostic factor (relative risk, RR = 2.74, 95% CI : 1.38–5.47). Chou et al. [33] showed more expressed COX-2 expression in ovarian carcinoma (which was related to endometriosis) than in the isolated carcinoma (27.8% *vs* 5.6%). In endometrial type of ovarian adenocarcinoma, a statistically significantly greater COX-2 expression was confirmed when compared to the isolated ovarian carcinoma (50% *vs* 0%; $p = 0.023$). Nasir et al. [34] tested COX-2 expression in endometrial adenocarcinoma (EAK), atypical complex hyperplasia (AKH) and endometrial hyperplasia (EH). COX-2 expression was proved in 88% of EAK, 80% of AKH and 44% of EH. The mean value of COX-2 immunohistochemical score for EH i.e. EAK was 33 (SD ± 24.11) and 76 (SD ± 54.57), respectively; $p = 0.022$. Ferrandina et al. [35] tested 69 samples of primary endometrial carcinoma. The expression of COX-2 was confirmed in 39.1% of cases. The COX-2 positivity was more pronounced (60.8%) in case of endometrial expansion to the cervix and outside the uterus, in contrast to carcinoma limited to the body of uterus (28.3%; $p = 0.017$). The authors showed the increase of COX-2 expression with the increase of the histological grade level (G1 – 13.6%, G2 – 41.7%, G3 – 60.9%; $p = 0.0049$). A statistically significant difference in expression was determined in relation to the depth of the myometrial invasion (< 50–15.6%, > 50–66.7%). Nofech-Mozes et al. [36] tested the immunohistochemical score of COX-2 expression in a group with inflammatory changes in the vulva (1.6), VIN I and II (1.4), VIN III and carcinoma *in situ* (0.7) and invasive vulvar carcinoma (1.2) with the existence of a statistically significant difference, but not the relation with the level of dysplasia or age.

In our study, we tested COX-2 expression in cervix uteri tissue without pathological changes and with carcinomatous changes. The expression of COX-2 was not confirmed in the control group, in opposition to the group A (56.52%). A statistically significant difference was determined in relation to positive expression between the control group and the group A. The control group did not show COX-2 expression in the entire examined material and thus, it was confirmed as an internal negative control for normal cervix uteri tissue. The specificity of COX-2 protein expression is in its significant role in an inflammatory process, and an important precursor of premalignant and malignant changes in the cervix uteri is a long-term inflammatory process [37]. A significant role in this mechanism has a persistent viral infection with human papillomaviruses of high oncogenic potential. Subbaramaiah et al. [38] presented a complex mechanism of COX-2 expression activation, activated by oncoproteins HPV 16 E6 and E7 at line cells of normal cervix and carcinoma. The activation of COX-2, induced by HPV 16 E6 was done through a complex mechanism by activation of epidermal growth factor receptors (*EGFR*), Ras, mitogen-activated protein kinase (*MAPK*) and activator protein-1 (*AP-1*). The expression of E6 and E7 leads to corepressor inhibition (*NCoR*). A potential mutual activity also coactivates the corepressor with HPV16 E6 and E7 oncoproteins in a complex mechanism of mutual activation and degradation, inducing expression of COX-2. Farley et al. [39] published a paper on the expression of COX-2 in precancerous cervical changes. The study included 62 cervical samples obtained by the LEEP technique (loop electrosurgical excision procedure), which included 18 CIN 1, 19 CIN 2 and 25 CIN 3 changes. The positive expression of COX-2 was marked if there was positivity in more than 50% of cells in the examined sample. In CIN 1 changes, the expression of COX-2 was observed in 50%, CIN 2 in 42% and CIN 3 in 68% of the patients. The average intensity of expression was growing with the level of dysplasia (CIN 1 – 1.6; CIN 2 – 1.8; CIN 3 – 2.1). The authors pointed out COX-2 expression, which might play a role in carcinogenesis of cervical carcinoma. Dai et al. [16] presented 45% of patients with CIN changes, who were positive to COX-2 expression. Similar results were confirmed by Kim et al. [40] with the expression of COX-2 in 24% of the patients with CIN 3 changes in the cervix uteri, in 37.9% in microinvasive cervical carcinoma and in 51.6 % of patients with invasive carcinoma. In their study, Dursun et al. [41] tested the expression of COX-2 in CIN changes of the cervix uteri and planocellular carcinoma and made comparisons with clinicopathological factors. The study included 25 patients with CIN 3 changes and 67 patients with cervical carcinoma. Positive expression of COX-2 was confirmed in 24% of patients in the group with CIN 3 changes, while it was confirmed in 55.2% of patients with carcinoma. A correlation of COX-2 expression and the presence of lymphovascular invasion (LVI) showed a statistically significant difference (positive LVI – 61.9% of the patients with positive COX-2; negative LVI – 33.3%, $p = 0.02$). Furthermore, statistically significant difference was shown in relation to the size of the tumor (up to 4 cm – 39% of patients with positive COX-2 and over 4 cm – 65.9%; $p = 0.028$). A statistically significant difference related to parametrial infiltration, lymph node status or recurrence disease and survival was not confirmed. In multivariate analysis, lymphovascular invasion was the only factor connected to the expression of COX-2, unlike the size of the tumor [41]. In Khunamornpong et al. [42] study COX-2 expression was significantly associated with lymph node metastasis but lacked a significant correlation with tumour stage, size, histologic grade, deep stromal invasion, lymphovascular space invasion (LVSI), and parametrial involvement. COX-2 expression was not associated with lymph node metastasis in the absence of parametrial involvement or LVSI. In the cases with LVSI, COX-2 expression was significantly associated with lymph node metastasis.

Comparison of risk factors and the stage of the disease in relation to COX-2 expression and its level within the group A did not prove a statistically significant difference. Luo et al. [43] tested COX-2 expression in cervical carcinoma and its clinical significance. Seventy-two cervical samples with invasive carcinoma and 16 cervical samples without tumor were examined. Within the group with the invasive carcinoma, COX-2 expression was present in 88.9% of cases and in the group without the tumor in 12.5 %. COX-2 expression was positively related to metastases in lymph nodes

and stromal invasion. A similar study was conducted by Manchana et al. [44] where they tested the prevalence of COX-2 and compared it with the clinicopathological factors. The study included 89 samples of cervical carcinoma, which were obtained after radical hysterectomy. COX-2 expression was confirmed in 49.4% of samples, while the greatest number was related to adenocarcinoma (86.7%) when compared to planocellular type (40.6%). A statistically significant difference was confirmed by comparison of COX-2 expression, the presence of lymph nodes metastases (100% *vs* 46.4%) and the presence of parametrial infiltration (80% *vs* 47.6%). A significant difference was not confirmed in relation to age, size of the tumor, depth of stromal invasion and lymphovascular invasion. The correlation between COX-2 expression and a five-year-long survival was not confirmed (positive COX-2 – 81%, negative COX-2 – 98%). Although the correlation of COX-2 expression, histological type and lymph node status was not confirmed, the authors concluded that COX-2 expression cannot be pronounced as a significant prognostic factor with absolute certainty.

A meta-analysis of Huang et al. [45] indicated that COX-2 overexpression might be an unfavorable prognostic factor and chemoradiation resistance predictive factor for cervical cancer.

An association between COX-2 expression and paraaortic lymph node recurrence has been reported in advanced stage patients treated with radiation therapy [46]. Also, some reports documented a better pathological response to chemotherapy in patients with negative COX-2 protein [47]. Still further studies need to correlate the expression of COX-2 with reccurence cervical cancer and the correlation of survival rate with COX-2 expression.

In the group A, with planocellular histologic type of the tumor and FIGO stage of the disease IB, COX-2 expression was confirmed in 56.5% of cases, similar to previously published results. A correlation of histopathological parameters and COX-2 expression, unlike the presented results of the other authors, did not show a statistically significant difference, but it should be noted that their data also differed.

This difference in the results is related to clinicopathological factors and it is possible that it lies in heterogeneity of the samples (number, stage, histological type), methods of testing of COX-2 expression (immunohistocemically, titer in blood, COX-2mRNA). The results of more frequent positive expression of COX-2 in the subgroup with worse prognostic histopathological parameters refer to the significance of the activity of these factors in carcinogenesis of cervix uteri carcinoma.

Observing COX-2 expression in our study, a statistically significant difference was determined in relation to lymphocyte stromal infiltration ($p = 0.0053$). In the available published papers, the presence of lymphocyte infiltration was not observed as a clinicopathological factor. Immunosuppression is an important factor in regulation of the activity and aggressiveness of a malignant disease. This represents a controversy in positive expression of COX-2 and its role in prostaglandin stimulation in tumor tissue, which leads to suppressing of immune system cells, thus creating an immunosuppressive area with the reduced immunological defense mechanism against the tumor tissue. Fourteen years ago, Staveley-O'Carroll et al. [48] described the induction anergy of T-lymphocytes in early oncogenesis. T-cell and dendritic cell defect, caused by the production of prostaglandin, which are stimulated by COX-2 expression, can play a significant role in tumor evasion of the immune system [4]. This can be indirectly observed in relation to the presence or the absence of lymphocyte infiltration in and around tumor tissue in relation to COX-2 expression.

Conclusion

There was the outstandingly significant COX-2 expression in the group with cervical carcinoma when compared to the control group. The positive correlation of COX-2 expression in the group with carcinoma shows a possible correlation with carcinogenesis of cervix uteri carcinoma. A statistically significant positive correlation between COX-2 expression and certain individual histopathological parameters in the group A was not proved, which was the basis for discarding the possibility of implementation of COX-2 as a marker for prognostic purposes in patients with neoplastic cervical lesions. COX-2 expression was more pronounced in cervical carcinoma without lymphocyte stromal infiltration, which led to the conclusion of a possible paradoxical effect of COX-2 to immunosuppression in tumor tissue. This conclusion requires some additional research. More frequent COX-2 expression in the subgroup with worse prognostic histopathological parameters in the group A, points to the significance of COX-2 expression activity in the process of cervix uteri carcinoma carcinogenesis and the impact to its progression.

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ORIGINAL ARTICLE

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Evaluation of conventional and digital radiography capacities for distinguishing dental materials on radiograms depending on the present radiopacifying agent

Ispitivanje kapaciteta konvencionalne i digitalne radiografije za utvrđivanje razlika kod materijala na radiogramu zavisno od prisutnog kontrastnog sredstva

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Abstract

Bacgroun/Aim. The radiopacity of an endodontic material can considerably vary as measured on film and a digital sensor. Digital radiography offers numerous advantages over convential film-based radiography in dental clinical practice regarding both diagnostic capabilities and postintervention procedures. The aim of this study was to investigate the capacity of conventional and charge-conpled device (CCD) based digital radiography to detect material on radiograph depending on the radio-pacifying agent present in the material. **Methods.** Experimental cements were formulated by mixing Portland cement with the following radiopacifying agents: zinc oxide (ZnO), zirconium oxide (ZrO_2), titanium dioxide (TiO_2), barium sulphate ($BaSO_4$), iodoform (CHI_3), bismuth oxide (Bi_2O_3) and ytterbium trifluoride (YbF_3). In addition, 5 endodontic materials comprising Endomethasone®, Diaket®, N_2®, Roth 801® and Acroseal® were investigated to serve as control. Per three specimens of each material were radiographed alongside an aluminum step wedge on film (Eastman Kodak Company®, Rochester, NY) and a CCD-based digital sensor (Trophy Radiologie®, Cedex, France). Radiopacity values were calculated by converting the radiographic densities of the specimens expressed as a mean optical densities or mean grey scale values into equivalent thickness of aluminum. **Results.** Two-way ANOVA detected no significant differences with respect to the imaging system ($p > 0.05$), but the differences were significant with respect to radiopacifier ($p < 0.001$) and the interaction of the two factors ($p < 0.05$). Paired *t*-test revealed significant differences between the methods used for pure Portland cement, all concentrations of $BaSO_4$ and CHI_3, 10% and 20% additions of ZrO_2 and Bi_2O_3 and 10% and 30% addition of YbF_3 ($p < 0.05$). **Conclusion.** The materials which incorporate CHI_3 or $BaSO_4$ as radiopacifying agents are expected to be significantly more radiopaque on a digital sensor than on film. During clinical practice one should concern to the quality of contrast assessement obtained by digital according to conventional radiography.

Key words:
radiography, dental; radiography, dental, digital; contrast media; dental cements.

Apstrakt

Uvod/Cilj. Radiokontrastnost jednog endodontskog materijala može znatno varirati u zavisnosti od toga da li je određivano na filmu ili digitalnim senzorom. Digitalna radiografija pruža mnogobrojne prednosti u odnosu na konvencionalnu radiografiju u svakodnevnoj stomatološkoj kliničkoj praksi, kako u pogledu dijagnostičkih mogućnosti, tako i u praćenju rezultata lečenja. Cilj ove studije bio je da se ispitaju mogućnost i konvencionalne i *charge-conpled device* (CCD) digitalne radiografije za vizualizaciju materijala na radiogramu u zavisnosti od kontrastnog sredstva prisutnog u materijalu. **Metode.** Eksperimentalni cementi su pripremljeni dodavanjem sledećih kontrastnih sredstava u Portland cement: cink-oksid (ZnO), cirkonijum-oksid (ZrO_2), titanijum-dioksid (TiO_2), barijum-sulfat ($BaSO_4$), jodoform (CHI_3), bizmut-oksid (Bi_2O_3) i iterbijum-trifluorid (YbF_3). Takođe, ispitivano je pet kontrolnih endodontskih cementa: Endomethasone®, Diaket®, N_2®, Roth 801® i Acroseal®. Po tri uzorka svakog materijala su radiografisana pored aluminijumskog stepeničastog etalona na filmu (Eastman Kodak Company, Rochester, NY) i CCD digitalnom senzoru (Trophy Radiologie, Cedex, France). Vrednosti rendgenkontrastnosti izračunavane su konverzijom radiografskih

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gustina uzoraka izraženih optičkim gustinama ili stepenom tona sivo-bele skale u odgovarajuću debljinu aluminijuma. **Rezultati.** Dvostrukom analizom varijanse nije ustanovljena statistički značajna razlika između primenjenih metoda radiografisanja ($p > 0.05$), ali su vrsta kontrastnog sredstva ($p < 0.001$) i interakcija ova dva faktora ($p < 0.05$) bili značajno različiti. Upareni *t*-test pokazao je statistički značajnu razliku između korišćenih metoda za čisti Portland cement, sve koncentracije $BaSO_4$ i CHI_3, 10% i 20% dodatka ZrO_2 i Bi_2O_3 i 10% i 30% dodatka YbF_3 ($p < 0.05$). **Zaključak.** Očekuje se da materijali koji sadrže CHI_3 ili $BaSO_4$ kao kontrastna sredstva budu lakše uočljivi na digitalnom senzoru nego na konvencionalnom dentalnom filmu. U kliničkom radu mora se imati u vidu kvalitet procene kontrasta koju pokazuje digitalna slika u odnosu na sliku dobijenu konvencionalnom radiografijom.

Ključne reči:
radiografija, stomatološka; radiografija, stomatološka, digitalna; kontrastna sredstva; zub, cement.

Introduction

An ideal root canal sealer should present, among other physical properties, sufficient radiopacity to allow distinction from bone and dentine on radiographs [1] and to facilitate the evaluation of the quality of endodontic treatment [2]. In addition, assessments of the voids within the restoration are facilitated when material experiences adequate radiopacity [3]. Radiopacity of an endodontic material should be compared to dental hard tissues; however, the radiopacity of human dentin varies considerably depending on the individual, age and storage conditions [4]. Therefore, aluminum alloy 1100 is chosen as the reference standard for measuring radiopacity because literature data show that its radiopacity is similar to dentine [5]. According to the American National Standard Institute/American Dental Association (ANSI/ADA) no. 57 endodontic filling material should present a difference in radiopacity equivalent to at least 2 mm Al in comparison to the bone or dentine [6] while the International Organization for Standardization (ISO) 6876 requires a minimal radiopacity equivalent to 3 mm Al [7].

The ISO protocol stipulates that the radiopacity of root canal sealer should be calculated by converting the optical density of the specimen measured on film by densitometer into a equivalent thickness of aluminum. Tagger and Katz [8] modified the method by performing digitization of radiographic films and correlating the radiodensity of the specimens expressed as a grey scale value (0–255) with the thickness of aluminum. Gu et al. [9] introduced direct digital radiography for radiopacity assessments that requires the use of digital sensors and computer radiographic image analysis. It is noteworthy that the ISO 4049 for polymer-based filling, restorative and luting materials [10], in contrast to the ISO 6876, recently adopted the method to allow for digital sensors.

The radiopacity of an endodontic material can considerably vary as measured on film and by a digital sensor. For example, Epiphany® sealer (Pentron Clinical Technologies, Wallingford, CT) has been reported to be equivalent to 7.34 mm Al as determined by Gendex® digital radiography (Gendex Dental Systems, Milano, Italy) [11], 8.0 mm Al as measured by Digora® digital radiography (Soredex Orion Corporation, Helsinki, Finland) [12], 8.2 mm Al as measured by Kodak® digital sensor (Eastman Kodak Company, Rochester, NY) [13], 8.8 mm Al as measured by digitized film [14] and 10.35 mm Al as measured by densitometry [15]. Yet, Roekoseal® (Coltene/Whaledent, Langenau, Germany) experiences the radiopacity that complies with the ISO 6876 according to film radiography (3.17 mm Al), but is not acceptable as obtained from digital assessments (2.83 mm Al) [14]. However, the physical cause of this discrepancy is still the matter of debate. It has been found that resin-based restorative materials that incorporate barium as a radiopacifier appear averagely 13% more radiopaque on a storage phosphor plate digital sensor (PSP) than on film [16]. Similar approximation was noted for barium containing endodontic sealers as analyzed by a charge-conpled device (CCD) based digital and film radiography [11]. It is surprising that the impact of elemental composition upon the differences in radiopacity, as obtained by densitometric and digital measurements, has not yet been investigated.

Therefore, the aim of this study was to evaluate the capacity of radiographic methods, conventional and digital CCD-based radiography, in differentiation of dental material's radiopacity.

Methods

Experimental endodontic cements were prepared by mixing one of seven radiopacifiers with Portland cement (PC) (Italcementi SPA®, Bergamo, Italy). The following radiopacifying agents were used: zinc oxide (ZnO) (Alkaloid, Skoplje, Macedonia), zirconium oxide (ZrO_2) (Kemika, Zagreb, Croatia), titanium dioxide (TiO_2) (Moss Hemos, Belgrade, Serbia), barium sulfate ($BaSO_4$) (Kemika), iodoform (CHI_3) (Galenika, Belgrade, Serbia), bismuth oxide (Bi_2O_3) (Alfa Aesar, Karlsruhe, Germany) and ytterbium trifluoride (YbF_3) (Alfa Aesar Word Hill, USA). Radiopacifiers were added to PC replacing 10%, 20% and 30% of the cement powder by weight. In addition, 5 commonly used root canal sealers were evaluated in this study. The commercial names, manufacturers, compositions and recommended power to liquid (P/L) or base to catalyst (B/C) paste ratios are listed in Table 1.

Each experimental cement was mixed in the ratio of 1 g powder per 0.37 mL distilled water and poured into metal ring mold (8 mm in diameter and 1 mm in thickness) placed on the glass slab. Another glass slab was used to press the cement onto the mold to obtain specimen of standardized thickness. After removal from the mold specimens cements were kept in an incubator at 37°C and 95% humidity for 24

hours for complete setting. The thickness of the specimens was controlled with a digital caliper (Mitutoyo, Tokyo, Japan). If necessary, specimens were ground wet with 600-grit silicon carbide paper to standard thickness. Commercial endodontic sealers were prepared with the accordance to manufacturer's instructions (Table 1). An electronic analytic balance (Mettler, Zurich, Switzerland) was useed to weight cement powder while syringe to measure liquid and two pastes of Acroseal®.

Results

Figure 1 depicts digitized conventional and digital radiographs of Acroseal® alongside with aluminum step wedge.

Radiographs of experimental cement with 30% addition of barium sulfate taken by using two radiographic systems are shown in Figure 2.

Table 1

Commercial names, manufacturers, composition and power to liquid/base to catalyst ratio of investigated endodontic materials

Commercial name	Manufacturer	Composition	Powder to liquid/base to catalyst paste ratio
Endomethasone®	Septodont Specialities, Saint Maur, France	Powder: zinc oxide, diodthymol, barium sulphate and hydrocortisone acetate. Liquid: eugenol and peppermint oil.	0.46 g : 0.15 mL
N_2®	Ghimas, Bologna, Italy	Powder: zinc oxide (69%), bismuth nitrate (2%), bismuth subcarbonate (5%), titanium dioxide (2%), lewad tetraoxide (12%), barium sulphate (2%) and iron oxide. Liquid: eugenol, peanut oil, rose oil and lavender oil.	0.84 g : 0.25 mL
Roth 801®	Roth International Limited, Chicago, IL, USA	Powder:zinc oxide (40%), staybelite resin (30%), bismuth subcarbonate (15%) and barium sulphate (15%). Liquid: eugenol and oil of sweet almond.	0.13 g : 0.03 mL
Diaket®	ESPE, Seefeld, Germany	Powder: zinc oxide (97%) and bismuth phosphate (3%). Liquid: prophionyl acetophenone nd vinyl isobutyl ether.	0.2 g : 0.05 mL
Acroseal®	Septodont Specialities, Saint Maur, France	Base: calcium hydroxide, diglycidyl ether of bisphenol A (DGEBA), and bismuth carbonate. Catalyst: glycyrrhetic acid (enoxolone) methenamine and bismuth carbonate.	1 mL base paste : 1 mL catalyst paste

Per three specimens of each cement were radiographed alongside an aluminum step wedge made of 99.6% pure aluminum alloy 1100 with the thickness varying from 1 mm to 10 mm in uniform steps of 1 mm each. The images were taken using an x-ray generator (Gendex GX, Lake Zurich, IL) operating at 70 kVp, 7 mA and a focus to target distance of 35 cm. The specimens were radiographed on Extraspeed occlusal film (Eastman Kodak Company) with a 0.32 s exposure. The films were processed in an automatic developing machine (Dent-X 9000, AFP Imaging Co., Elmsford, NY, USA) using the same developer at 28°C and standard processing time of 6 min. Each specimen was also radiographed using a radiovisiography (RGV-4) sensor (Trophy Radiologie, Cedex, France) with the exposure time of 0.074 s. Radiographic densities on film were expressed as mean optical densities measured by a transmission densitometer (X Rite 341, Grand Rapids, MI) while radiographic densities on digital images were expressed as mean grey scale values using the Adobe Photoshop CS4 software (Adobe Systems, San Hose, CA). Three readings were made for each specimen and each step of the step wedge. To determine the radiopacity of the cements, a graph was plotted for the logarithm of the thickness of the aluminum step wedge versus the corresponding radiographic density of the step wedge. The radiographic densities of the materials were then used to calculate radiopacity from this graph. The data were subjected to two-way ANOVA and paired *t*-test ($p < 0.05$).

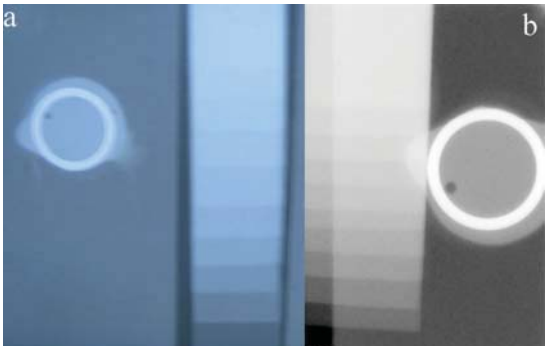

Fig. 1 – Film (a) and digital (b) images of Acroseal®.

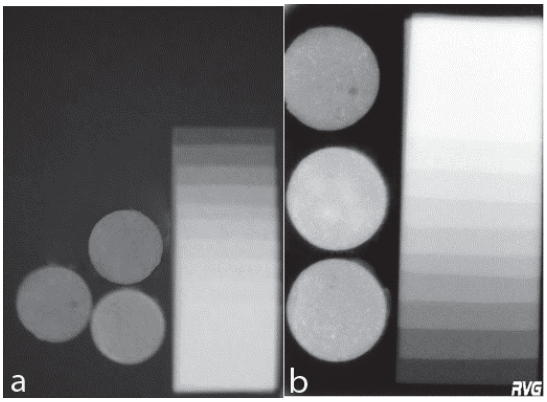

Fig. 2 – Film (a) and digital (b) images of Portland cement with 30% addition of barium sulfate alongside aluminum stepwedge.

The measured mean radiopacity values of commercial experimental cements are shown in Figures 3 and 4. Addition of Bi_2O_3 at 30% resulted in the highest radiopacities on both film and digital sensor (8.68 and 8.03 mm Al, respectively) whilst 30% addition of CHI_3 induced the highest differences between two imaging systems (50.2%). Two-way ANOVA testing indicated no significant differences with regards to imaging system ($p > 0.05$), but it was significant with respect to radiopacifier type ($p < 0.001$) and the interaction of two factors ($p < 0.05$).

Figure 5 presents the percentage difference between the results obtained by each radiographic methods for the investigated cements.

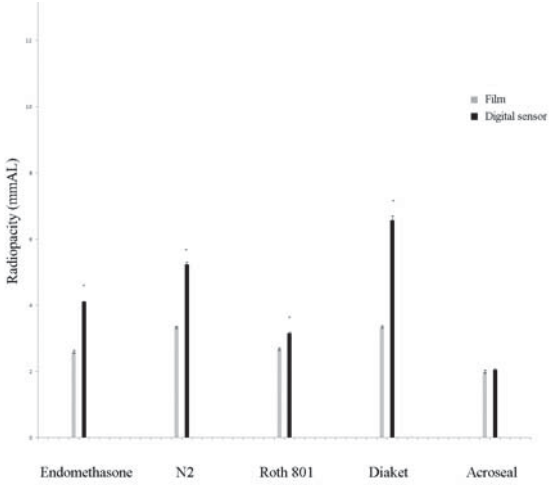

Fig. 3 – Radiopacity of the tested endodontic materials expressed as an equivalent thickness of aluminum. * – statistically significant difference on the digital sensor related to that on film (paired *t*-test; $p < 0.05$).

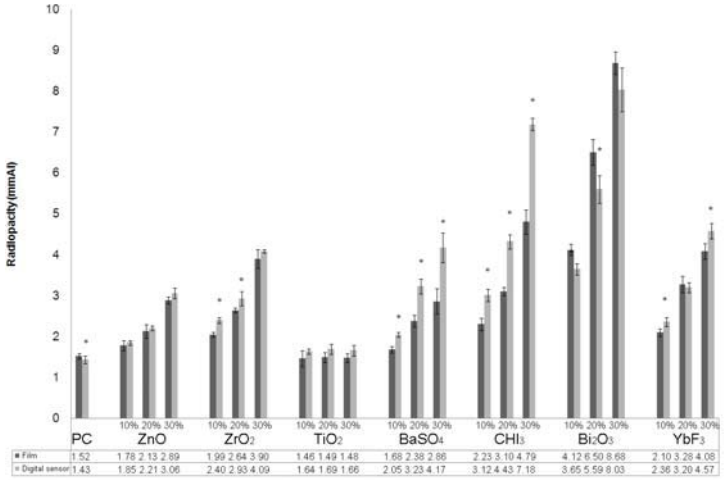

Fig. 4 – The mean radiopacity values and standard deviations expressed in thickness of aluminum (Al) for Portland mixtures. The columns marked with * represent groups with significantly different radiopacity on digital sensor when compared with film (paired *t*-test; $p < 0.05$).

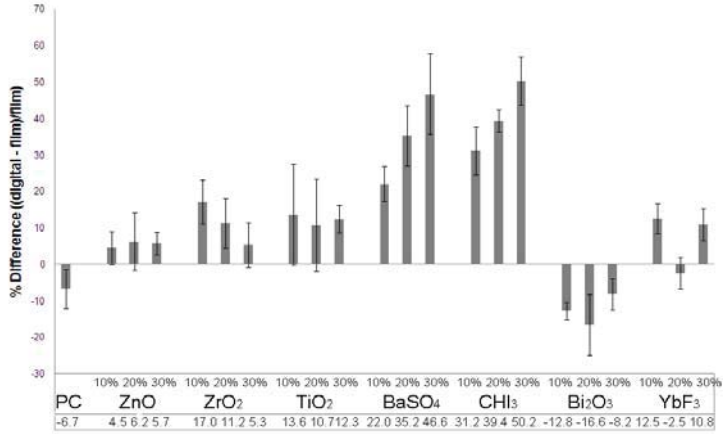

Fig. 5 – The percentage difference between the radiopacity measured by conventional and digital charge-conpled device (CCD)-based radiography. The positive values indicate increased radiopacity on the RVG® sensor compared with the film.

Discussion

A number of investigations have been carried out to compare digital to film radiography with respect to the diagnostic accuracy of secondary decay [17], root fractures [18] and root canal length [19]. This study focused on examining the impact of various radiopacifiers on the differences in radiopacity of dental materials in 2 different radiographic image modalities: film and CCD-based digital radiographs. Addition of CHI_3 and $BaSO_4$ induced the most significant differences; addition of 30% CHI_3 and $BaSO_4$ caused the experimental cements to present 50.2% and 46.6% more radiopaque on digital sensor, respectively.

Several factors affect the radiopacity of dental materials: a technique used for evaluation, specimen's thickness, particle size related to the water absorption of the material, film development, atomic numbers of materials' constituents and P/L respectively B/C paste ratio [20]. Among those factors the atomic number of materials' constituents is the most important one since it influences the radiopacity raised even to the exponent of four.

With a few exceptions, dental materials tended to appear more radiopaque on the digital sensor than on film. This is in agreement with the results of the previous study in which the CCD-based digital sensor was compared to film [21], but in contrast with an investigation that compared the radiopacity of endodontic sealers on film and PSP digital sensor [14]. The addition of 20% YbF_3 and all mixtures of Bi_2O_3 induced higher radiopacity values obtained by densitometric analysis. The results found for 20% additions of ZnO, ZrO_2, $BaSO_4$ and Bi_2O_3 as imaged on film are consistent with the outcome of Hungaro Duarte et al. [22] observed by digitization of radiographic films, but the 20% addition of CHI_3 led to the divergent results. Bortoluzzi et al. [23] used indirect digital technique and found similar results as it was found in the present study using conventional film radiography for 20% addition of Bi_2O_3, $BaSO_4$ and CHI_3, but significantly higher radiopacity for 20% addition of ZrO_2. In the previous reports densitometric analysis of radiographic films exhibited the results for TiO_2, ZnO, $BaSO_4$ and Bi_2O_3 additions that corroborate the results of the current study [23, 24]. The barium containing materials such as InnoEndo® (Haraeus-Kulzer, Armonk, NY), Epiphany® sealer (Pentron clinical technologies), Pulpdent RCS® (Pulpdent Corporation, Watertown, MA) and Nogenol® (GC America Inc., Alsip, IL) are reported to appear 44.1%, 9.4%, 17% and 12.5%, respectively, more radiopaque on PSP digital system than on conventional film radiography. Conversely, Bi_2O_3 containing materials Ez-Fill® (Essential Dental Systems, South Hackensack, NJ), Ez-Fill Express® (Essential Dental Systems) and Resilon® (Pentron Clinical Technologies) were averagely 3% more radiopaque on film-based radiography [11]. Although these results are consistent with the findings of the present investigation, manufacturers do not claim the percentage of the radiopacifiers incorporated within materials; thereby, exact correlation could not be observed.

Diaket® and N_2® were the most radiopaque materials in the present study; ZnO and bismuth in the form of bismuth subnitrate, bismuth phosphate and bismuth subcarbonate conferred radiopacity to the sealers. The difference in radiopacity on film versus a digital sensor raised as the percentage of ZnO in the material raised; the greatest difference was found for Diaket® with 97% of ZnO, lower difference was found for N_2® with 69% of ZnO and the lowest difference was found for Roth 801® with 40% of ZnO. In this study, Diaket® experienced radiopacity equivalent to 3.35 mm Al on the film and 6.57 mm Al on a digital sensor; in previous studies it was as radiopaque as 1.29 mm Al and 2.19 mm Al by conventional radiography measurements [13] and 6.5 mm Al [14] and 2 mm Al [13] on a digital sensor. However, it should be highlighted that the results found by Baksi et al. [14] (2.19 mm Al on film and 2 mm Al on a digital sensor) differ from the results found by other authors for all sealers investigated in that study. Endomethasone® exhibited radiopacity of 2.6 mm Al and 4.1 mm Al on film and a digital sensor, respectively, which is contaminant with the results of Gaur [25] who found it to be 3 mm Al and 4 mm Al, respectively. The results found for the radiopacity of Acroseal® (2 mm Al and 2.05 mm Al on film and a digital sensor, respectively) are in agreement with the results found by Baksi et al. [14] (2.04 mm Al on film and 1.9 mm Al on a digital sensor), but significantly lower than of Tanomaru-Filho et al. [26] (4.03 mm Al) who used indirect digital technique. Although Acroseal® contains bismuth carbonate as a radiopacifying agent and it is expected to present high level of radiopacity due to a high atomic number of bismuth, it exhibited lower radiopacity, which means that this component is presented in relatively low quantity.

The differences in the radiopacity of experimental cements on film and CCD-based digital sensor presumably arise from the different sensitivity of the detector used. Since a typical x-ray beam contains a rather broad spectrum of photon energies, all of the energies do not produce the same level of contrast. Silver on x-ray film is most sensitive to 26 keV, while iodine in a CCD-based digital x-ray sensor is most sensitive to 37 keV photons. Thus, elements that selectively filter out high energy photons (when compared to aluminum alloy 1100) should appear more radiopaque on CCD sensor; elements that preferentially filter out photons with energies less than 35 keV (compared to aluminum alloy 1100) should appear more radiopaque on film. Furthermore, if a dental material and an x-ray detector have similar compositions, the material will absorb most of the energies which the x-ray detector is most sensitive to. Hence, the material will appear more radiopaque on that detector versus a detector with different composition. Among investigated radiopacifiers, chemical elements incorporated in CHI_3 and $BaSO_4$ (iodine and barium), have ideal K-absorption edge (33 keV for iodine and 37 keV for barium) to absorb most of the energies that a CCD-based detector is most sensitive to. Therefore, in the present study, CHI_3 and $BaSO_4$ induced significantly higher radiopacity values on digital sensor than on film.

PC used in the current study has very similar composition as mineral trioxide aggregate (MTA) [27, 28], except for the presence of Bi_2O_3, which is contained in MTA at 20% ratio to improve its radiopacity. However, Bi_2O_3 adversely affects some of the material's properties by retarding its setting time [29], increasing the porosity [30], decreasing the compressive strength [29] and interfacing its hydration mechanism [31]. Thus, alternative radiopacifiers have been proposed to be associated with PC. According to the results of this study, the minimum radiopacifiers' amounts that allow the PC to reach 3 mm Al were 30% ZnO, 30% ZrO_2, 20% $BaSO_4$, 20% YbF_3, 10% CHI_3 and 10% Bi_2O_3 as measured by digital radiography and 30% ZrO_2, 20% CHI_3, 20% YbF_3 and 10% Bi_2O_3 as determined by conventional radiography. Neither concentration of ZnO and $BaSO_4$ ensured satisfactory radiopacity of the PC on conventional radiography while TiO_2 was not able to enhance the radiopacity neither on film nor the digital sensor. The interference of potential radiopacifiers with other physical and biological properties of PC should be further examined. The results of the present and previous studies [24, 27] imply, however, that Bi_2O_3 content may be reduced in MTA from 20% to 10% in order to minimize its adverse effects on material's properties.

To remind, the ISO 6876 stipulates that an endodontic material must present the radiopacity equivalent to at least 3 mm Al. In contrast to the ISO 4049 for polymer based restorative, filling and luting materials [10] which adopted the method to allow for digital sensor measurements, the ISO 6876 does not address this issue. Several researchers suggested that ISO 6876 needs modifications for electronic imaging [14, 26].

Conclusion

The obtained results reveal that characterization of cement type cannot be established using radiography. However, materials that incorporate CHI_3 or Bi_2O_3 are expected to be highly radiopaque. Conversely, materials that incorporate TiO_2 as a radiopacifying agent should be less radiopaque. These results are of practical importance concerning that the radiopacity of CHI_3 or $BaSO_4$ containing materials as recorded on film is not indicative of radiopacity as recorded on charge-conpled device-based digital sensor. Because material's composition influences the differences in radiopacity it is imperative to be taken into consideration when trying to establish any correlation between radiopacity values obtained by film and a digital radiography. Collectively, these lines of evidence suggest that the clinician must be cautious when comparing radiographs made by different methods because sudden decrease in radiopacity due to the choice of radiographic method may lead to suspicion of resorption or loss of restoration integrity. Barium in PSP digital system is sensitive to similar photon energies (38 keV) as iodine in chageconpled device-based digital sensor (37 keV); thus, the results of this investigation are expected to be applicable to most intraoral digital systems. Some further work would be useful to ascertain the source of the remaining variations between the two imaging systems.

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ORIGINAL ARTICLE

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Highly selective vagotomy and gastrojejunostomy in the treatment of peptic ulcer induced gastric outlet obstruction

Supraselektivna vagotomija i gastrojejunostomija u lečenju stenoze pilorusnog kanala uzrokovane peptičkim ulkusom

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Abstract

Background/Aim. The incidence of peptic ulcer-induced gastric outlet obstruction is constantly declining. The aim of this study was to present our results in the treatment of gastric outlet obstruction with highly selective vagotomy and gastrojejunostomy. **Methods.** This retrospective clinical study included 13 patients with peptic ulcer – induced gastric outlet obstruction operated with higly selective vagotomy and gastrojejunostomy. A 3-year follow-up was conducted including clinical interview and upper gastrointestinal endoscopy on 1 and 3 years after the surgery. **Results.** The most common preoperative symptom was vomiting (in 92.3% of patients). The mean preoperative body mass index was 16.3 ± 3.1 kg/m^2, with 9 patients classified preoperatively as underweight. There were no intraoperative complications, nor mortality. At a 3-year follow-up there was no ulcer recurrence. Delayed gastric emptying was present in 1, bile reflux in 2, and erosive gastritis in 1 patient. Two patients suffered from mild "dumping" syndrome. **Conclusion.** Higly selective vagotomy combined with gastrojejunostomy is a safe and easily feasible surgical solution of gastric outlet obstruction induced by peptic ulcer. Good functional results and low rate of complications can be expected at a long-term follow-up.

Key words:
peptic ulcer; pyloric stenosis; vagotomy, proximal gastric; digestive system surgical procedures; treatment outcome.

Apstrakt

Uvod/Cilj. Incidencija stenoze pilorusnog kanala uzrokovane petičkim ulkusom u stalnom je padu. Cilj ove studije bio je da prikaže naše rezultate u hirurškom lečenju stenoze pilorusnog kanala supraselektivnom vagotomijom i gastrojejunostomijom. **Metode.** Retrospektivnom studijom bilo je obuhvaćeno 13 bolesnika sa stenozom pilorusnog kanala uzrokovanom peptičkim ulkusom kod kojih je učinjena supraselektivna vagotomija i gastrojejunostomija. Sprovedeno je trogodišnje praćenje, koje se sastojalo od kliničkog ispitivanja i endoskopije gornjeg dela digestivnog trakta, jednu i tri godine nakon operacije. **Rezultati.** Najčešći preoperativni simptom bilo je povraćanje, kod 92,3% bolesnika. Srednja vrednost preoperativnog indeksa telesne mase bila je 16,3 ± 3,1 kg/m^2, pri čemu je 9 bolesnika preoperativno klasifikovano kao pothranjeno. Nije bilo intraoperativnih komplikacija ili mortaliteta. Nakon trogodišnjeg praćenja nije bilo rekurentnih ulkusa. Odloženo gastričko pražnjenje uočeno je kod jednog, bilijarni refluks kod dva, a erozivni gastritis kod jednog bolesnika. Kod dva bolesnika bili su prisutni simptomi "*dumping*" sindroma. **Zaključak.** Supraselektivna vagotomija kombinovana sa gastrojejuno anastomozom je bezbedna hirurška procedura i dobro rešenje za bolesnike sa stenozom pilorusnog kanala uzrokovanom peptičkim ulkusom. U dugoročnom praćenju mogu se očekivati dobri funkcionalni rezultati i niska incidencija kasnih komplikacija.

Ključne reči:
želudac, ulkus; pilorus, stenoza; vagotomija, proksimalna, gastrička; hirurgija digestivnog sistema, procedure; lečenje, ishod.

Introduction

From the historical point of view, surgery for peptic ulcer disease (PUD) used to be a cornerstone of general surgery. Much that once was, now has changed. The role of surgery, and especially elective one, in the treatment of PUD is declining, and currently is reserved for the complications of PUD, such as bleeding, perforation and gastric outlet obstruction (GOO) [1]. This shift of treatment paradigm has been influenced by improvement in antisecretory medications,

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knowledge of *Helicobacter pylori* (HP) role in pathophisiology and treatment of PUD, and introduction of endoscopic therapeutic approach [2]. HP eradication combined with proton pump inhibitors is now mainstay therapy for PUD.

Nowadays, GOO will occur in approximately 6–8% of PUD patients [3, 4]. GOO is usually accompanied with severe gastric distension and stasis which raises gastric pH, and will in return lead to increased gastrin and acid secretion, the example of "vicious cycle" [4]. Therefore, the goals of treatment should be to ensure gastric emptying and to diminish gastric acid secretion. Gastric emptying could be accomplished by endoscopic dilatations of pyloric stricture or by several surgical procedures, such as pyloroplasty, gastrojejunostomy or antrectomy [5]. Gastric acid secretion regulation on the other hand, can be achieved by employing acid suppressing medications, or surgically, by some form of vagotomy procedure. Highly selective vagotomy (HSV) had a major part in the treatment of PUD historically, although there are less and less recent reports which would enlighten the role of HSV nowadays. The rationale for HSV is in fact that this procedure should minimize gastric acid secretion, and preserve normal gastric emptying.

The aim of this study was to present our experience with HSV and concomitant gastrojejunostomy in the treatment of PUD – induced GOO.

Methods

The study was conducted in the Department of Esophagogastric Surgery, the First Surgical University Clinic, Clinical Center of Serbia, Faculty of Medicine, University, of Belgrade. The study was approved by Hospitals Board and Ethics Committee. It was designed as retrospective clinical study. We included 13 consecutive patients in whom HSV and gastrojejunostomy were performed due to GOO caused by PUD in the period from January 2004 till December 2010.

Microsoft Excel data base was created and it included the following: demographic data, upper gastrointestinal endoscopy data preoperatively and postoperatively, results of preoperative upper gastrointestinal endoscopic biopsy, and the data related to surgical procedure and postoperative course.

Two consecutive upper gastrointestinal endoscopies with biopsies were obligatory before surgery. This was performed in order to exclude malignancy, and HP infection evaluation.

Nasogastric tube was placed in all patients preoperatively, in order to prevent vomiting and aspiration of gastric contents.

Exclusion criteria were prior gastric surgery and GOO caused by malignancy. We excluded also the patients in whom postoperative upper gastrointestinal endoscopy was not performed.

A patient was placed in supine position, in a reverse Trendelenburg. Operation was performed using proximal median laparotomy. Sternal retractor usage was obligatory. After retracting the left lobe of the liver, the lesser sac of omentum was exposed. We used to begin dissection after identifying the anterior aspect of Latarjet's nerve, after which careful dissection was carried out from the level of angular incisure proximally towards phrenoesophageal ligament. Harmonic scalpel proved to be a useful tool in performing fine and precise dissection. The anterior trunk of the vagus nerve next to the lesser curve of the stomach was pulled laterally with a tape. After reaching the phrenoesophageal ligament complete encroaching of the esophagus was performed in order to divide all the anterior and posterior vagal braches. Then dissection was carried out towards the lesser curve, this time ligating the branches of the posterior vagal trunk, and the vagotomy procedure was finished with ligation of the most proximal branch of the posterior Latarjet's nerve. Serosal layers on the posterior and anterior aspects of the lesser curve were approximated with interrupted sutures. Gastrojejunostomy was performed on the posterior wall of the stomach trough the mesocolon of the transversal colon. It is performed in two layers with continuous sutures, approximately 20–30 cm from the ligament of Treitz. In all the patients we performed reconstruction of the angle of His by two or three interrupted sutures.

All patients underwent regular yearly check-ups including clinical interview, upper gastrointestinal endoscopy one and three years after the surgery. Data regarding medication usage after the surgery were also collected. Follow-up using upper gastrointestinal endoscopy data included the following features: erosions or ulcer formation, the presence of corporal liquid "pool" as a marker for delayed emptying, inspection of the strictured pyloric chanel, the presence of bile and obligatory biopsies of gastric mucosa.

All the data are expressed as means with standard deviations. Descriptive statistics is presented. Due to the limited number of patients, we did not perform comparative statistics.

Results

Overall 13 patients met the inclusion criteria. There were 10 men and 3 women. The mean age of patients was 67 ± 17 years. The mean duration of preoperative symptoms was 26 ± 9 months. Vomiting was the leading symptom in the great majority of the patients; 6 of them had pain in the upper abdomen (Table 1), and 3 were presented with aspira-

Table 1

Preoperative symptoms of the study participants

Symptom	Patients n (%)
Vomiting	12 (92.3)
Pain in the upper abdomen	6 (46.2)
Heartburn	7 (53.8)
Dyspepsia	8 (66.6)
Cough	3 (23.1)

tion pneumonia. The mean body mass index (BMI) was 16.3 ± 3.1 kg/m^2, and 9 (69.2%) patients were classified as underweight with BMI less than 18.5 kg/m^2.

Preoperative endoscopy revealed the presence of peptic pyloric stricture in all of the patients. Endoscope passage trough the pyloric channel was not possible in all the patients. Preoperative histology based on endoscopic biopsy specimens showed the presence of HP in 6 (46.2%) patients.

There was no intrahospital mortality. In one patient postoperative pneumonia was developed, and the patient was treated successfully. There were no intraoperative complications. The mean hospital stay was 9.4 ± 3.1 days. There were no readmissions in early postoperative course.

At a 6-month follow-up, one patient reported occasional nausea and vomiting. He was treated with proton pump inhibitors and prokinetics with a good symptomatic outcome. A total of 12 patients reported satisfactory results after the surgery, 9 of them reported weight gain.

Upper gastrointestinal endoscopy showed the presence of delayed gastric emptying in 2 patients. In one patient presence of erosive gastritis was notified. The presence of bile reflux was documented in 4 of the patients. Both patients with the presence of erosive gastritis and peptic ulcer were HP positive based on endoscopic biopsy and patohistology. Also, both patients were asymptomatic, and were submitted to HP eradication therapy. None of the patients was presented with recurrent ulcer.

Three years after the surgery we completed upper gastrointestinal endoscopy in 12 patients. In one patient there were endoscopic signs of delayed gastric emptying (Table 2). None of the patients were presented with peptic ulcer. In one patient erosive gastritis was found (Table 2). Pylorus recanalization was present in 3 of the patients. In 2 of the patients there was a significant amount of bile present in the stomach (Table 2). Eight patients had normal appearance of gastric mucosa on a 3-year follow-up endoscopy and were completely asymptomatic. Ten of the patients gain more than 5 kg of weight 3 years after surgery.

Two of the patients reported symptoms that could point out to mild "dumping" syndrome.

Table 2

Symptomatic status and endoscopic data obtained by 3-year follow-up

Endoscopic findings	Symptoms	Patients (n)	Positive for *Helicobacter pylori* infection
Delayed gastric emptying	Asymptomatic	1	-
Bile reflux	Dyspepsia, asymptomatic	2	-
Erosive gastritis	Pain in upper abdomen	1	+

Discussion

The focus of surgery for PUD has switched to disease complications, as a growing knowledge of patophisiology brought to light successful medicamentous treatment. GOO remains one of PUD complications in which surgery still has its role. It has been estimated that 1–2% of patients with PUD will eventually develop GOO and require surgery [6]. Patients with GOO are usually malnourished, presented with severe vomiting and early satiety. Initial treatment should be placement of nasogastric tube for gastric decompression, and fluid and electrolyte resuscitation, as the majority of patients will suffer from hypokalemia, hypochloremia and metabolic alkalosis due to persistent vomiting [5]. This was also the case in our study. The majority of the patients had BMI less than 20 kg/m^2 at the time of disease presentation, and 85% of them had electrolyte imbalance. After the initial phase in treatment, diagnostic workout should be concentrated on etiology of PUD. PUD is now the second most common cause of GOO, and malignancy is the main etiological factor [7]. Therefore the diagnosis should be concentrated on ruling out malignancy, which can be best accomplished by upper gastrointestinal endoscopy with biopsies. Due to intense scarification, edema and gastric stasis, endoscopic biopsy can sometimes fail to deliver the proper diagnosis, so computed tomography can be applied in order to get more precise view of the disease [8]. Even so, in some cases surgical exploration followed with *ex tempore* biopsies will be necessary to solve the disease etiology.

Endoscopic balloon dilatation is recommended as the primary treatment modality in patients with PUD induced GOO [9]. The procedure is not burdened with a high complication rate, but single dilatation is usually not enough to achieve normal gastric emptying. The success rate of endoscopic balloon dilatation is up to 50%, and these are the data from a relatively short follow-up [10, 11]. There are no comparative studies between endoscopic dilatation and surgical treatment of GOO, which could give some better perspective upon this issue. None of the patients included in our study went through the endoscopic dilatation sessions. Explanation for this is in the fact that all the patients were presented with long-lasting strictures, intense scarifications and severe gastric stasis.

The goal of surgical treatment of PUD-induced GOO is to provide the gastric emptying, and additionly to this, to decrease gastric acid secretion. This should be ideally performed in a single procedure, with lowest morbidity and postoperative complications. Early surgical attempts were oriented towards subtotal or partial gastric resection. These procedures resulted in a very low ulcer recurrence rate, but were burdened with severe postoperative complications, such as dumping, diarrhea and weight loss. One must not forget the fact that severe scarification can lead to intraoperative problems with closure of the duodenal stump and subsequent leakage. With the introduction of vagotomy procedures, surgeons tended to less radical solutions and a place was made for pyoloroplasty or gastrojejunostomy. Good functional results were obtained with a lower risk.

Vagotomy was introduced in the late 1940 by Lester Dragstedt [12]. This rather revolutionary procedure brought excellent results in terms of ulcer recurrence rates, but also was followed with large percentage of undesirable side effects in up to 50% of patients [13]. After experimental works of Griffith and Harkins [14] a HSV was introduced in 1969 also known as parietal cell vagotomy [15]. HSV divides only the nerve branches supplying corporal and fundic parts of the stomach, with the tendency to preserve normal innervation of the antral part of the stomach. Basal acid gastric secretion is reduced approximately for 85%, and stimulated secretion up to 50% [16]. Although effective in reducing acid secretion, HSV has been reported to have ulcer recurrence rate up to 25%. It was postulated that this was due to technical faults, and there were reports indicating that lower ulcer recurrence rates have been obtained in experienced surgical hands. Special emphasis is brought upon careful dissection of posterior vagal branches, and criminal nerve of Grasi. Donahue et al. [17] reported modification of HSV, extending the dissection upon recurrent vagal innervation along the right gastroepiploic vessels. Donahue [18] also reported the ulcer recurrence rate with this technique to be 1%. Similar results were obtained in our study. No ulcer recurrence and low rate of postprocedural side effects were noted. This could emphasize the importance of precise dissection, and the roll of experienced and specialized surgical team.

HSV was a mainstay vagotomy option in our study. Although we reported experience with limited number of patients, we have not encountered intraoperative complications with this procedure. This procedure was not time consuming, and was easily feasible in all the patients included in this report. In a 3-year follow-up we did not meet ulcer recurrence. Two patients suffered from mild dumping. Although present endoscopically in one patient delayed gastric emptying was completely asymptomatic. One patient was present with severe dyspeptic symptoms due to extensive alkaline reflux which was noted endoscopically. In three of the patients complete recanalization of the pyloric channel occurred indicating the importance of antral innervation preservation. HP infection was verified in 46% of the patients. This is in conjunction with other study which reported similar incidence of HP infection in patients with PUD-induced GOO [19].

When dissecting vagal branches for the corporal part of the stomach receptive relaxation phenomenon is interrupted and this can result in rapid liquid emptying, then in dumping syndrome. According our experience, this can be overcome by dietary measures.

One prospective study on 90 patients operated on due to PUD induced GOO, compared three groups of patients with the following randomization according to the type of treatment: HSV with lateral Jaboulay gastroduodenostomy, HSV with gastrojejunostomy or selective vagotomy with antrectomy [20]. Clinical outcomes were graded using the Visick classification. Patients operated with HSV and gastrojejunostomy had a statistically significantly better functional outcome than those operated with HSV and Jaboulay gastroduodenostomy, and a slightly better score than patients operated with selective vagotomy and antrectomy. The authors [20] concluded that HSV with gastrojejunostomy is the treatment of choice for patients with PUD-induced GOO.

Conclusion

Gastric outlet obstruction caused by peptic ulcer disease is nowadays a rare disease. It is hard to accomplish large surgical series and obtain more data. According to our experience, highly selective vagotomy in combination with gastrojejunostomy represent a safe and easily feasible surgical solution. Follow-up data point out that good functional results were obtained with this type of procedure, especially in terms of ulcer recurrence rate, and low incidence of postoperative side effects. Highly selective vagotomy is a well-established and proven procedure, with constant decrease in the number of indications, so surgeons should guard its place in the years that come.

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Reliability of fine needle aspiration and *ex tempore* biopsy in the diagnosis of salivary glands lesions

Pouzdanost aspiracije tankom iglom i biopsije *ex tempore* u dijagnostici lezija pljuvačnih žlijezda

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Abstract

Background/Aim. Interpretation of cytological material obtained by fine needle aspiration (FNA) of salivary glands is one of the most challenging areas in cytopathology. FNA is performed easily, it is minimally invasive, inexpensive, fast, reliable and provides valuable information to clinicians about the nature of the lesion and therapeutic modalities. *Ex tempore* diagnosis, frozen section (FS) is a diagnostic tool that is essential in determining the modalities of surgical treatment of lesions of the salivary glands. Today this method is used in determining the status of resection margins and infiltration of adjacent anatomical structures. The aim of this study was to present our experiences in the application of FNA and FS in the diagnosis of salivary gland lesions and to determine the sensitivity, specificity, predictive value, and diagnostic reliability of these methods. **Methods.** The study included 36 patients. In all the patients, cytological analysis was done before surgery and histological analysis of the surgical material. In 23 of the patients the FS diagnostics was done. Then we compared FNA and FS findings with histopathological findings. **Results.** Correlation of cytological and histological diagnosis showed sensitivity of 83.3%, specificity 96.67%, positive predictive value 83.3%, negative predictive value of 96.77% and diagnostic accuracy of 97.2%. Based on the relationship between FS diagnosis and histopathological diagnosis, the sensitivity was 100%, specificity 96.67%, while positive predictive value and diagnostic accuracy were 100% each. **Conclusion.** The study confirmed that FNA is a sensitive, reliable diagnostic method for differentiation of lesions of the salivary glands. In cases with no posibility to definite differentiation in FNA samples, and with the need to assess the resection margins and invasion of anatomical structures, it is recommended to use FS diagnostics.

Key words:
biopsy, fine needle; frozen sections; salivary glands; histological techniques; sensitivity and specificity.

Apstrakt

Uvod/Cilj. Citološka interpretacija materijala dobijenih aspiracijom tankom iglom (*fine-needle aspiration* – FNA) pljuvačnih žlijezda predstavlja jedno od najzahtjevnijih područja u citopatologiji. FNA se izvodi lako, brzo, pouzdano, minimalno invazivno, jeftino, pružajući značajne informacije kliničarima o prirodi lezije i terapijskim modalitetima. *Ex tempore* dijagnostika (*frozen section* – FS) je dijagnostička metoda koja je bitna pri određivanju modaliteta hirurškog liječenja lezija u pljuvačnim žlijezdama. Danas se ova metoda koristi za određivanje statusa resekcionih rubova i infiltracije susjednih anatomskih struktura. Cilj istraživanja bio je da se prikažu sopstvena iskustva u primjeni FNA i FS u dijagnostici lezija pljuvačnih žlijezda, te da se utvrdi osetljivost, specifičnost, vrijednost predviđanja i dijagnostička pouzdanost ovih metoda. **Metode.** Ispitivanjem je obuhvaćeno 36 bolesnika. Kod svih je urađena citološka analiza prije operativnog zahvata i histološka analiza operativnog materijala. Kod 23 bolesnika urađena je dijagnostika FS. Izvršeno je poređenje FNA i FS nalaza sa patohistološkim nalazima. **Rezultati.** Korelacijom citoloških i patohistoloških dijagnoza osetljivost je iznosila 83,3%, specifičnost 96,67%, pozitivna vrijednost predvi-

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đanja 83,3%, negativna vrijednost predviđanja 96,77% i dijagnostička pouzdanost 97,2%. Na osnovu odnosa FS dijagnoza i patohistoloških dijagnoza osjetljivosti je iznosila 100%, specifičnost 96,67%, a pozitivna vrijednost predviđanja i dijagnostička pouzdanost iznosili su 100%. **Zaključak.** Istraživanje je potvrdilo da je FNA osjetljiva i dijagnostički pouzdana metoda za diferencijaciju lezija u pljuvačnim žlijezdama. U slučaju nemogućnosti definitivne diferencijacije u FNA uzorcima, te potrebe da se izvrši procjena resekcionih rubova i invazija anatomskih struktura, preporučuje se FS dijagnostika.

Ključne reči:
biopsija tankom iglom; zamrznuti isečci tkiva; pljuvačne žlezde; dijagnoza; histološke tehnike; senzitivnost i specifičnost.

Introduction

Cytological interpretation of material obtained by fine needle aspiration (FNA) of salivary glands is one of the most challenging areas in cytopathology. Specifically, it describes a wide range of reactive and neoplastic lesions which can be diagnosed in more than 500 salivary glands present in the human body. Histological and cytological analyses confirmed that tumors of salivary glands were the most heterogeneous group of human tumors. An additional difficulty in cytodiagnostics of salivary glands is cytomorphological overlap of many benign and malignant tumors [1–3].

FNA is currently used worldwide to evaluate palpable and deeply located lesions in most anatomic regions [1, 4].

Salivary gland tumors are uncommon and account for 2% to 6.5% of head and neck tumors, of which 21% to 46 % are malignant tumors [5]. Preoperative diagnosis of lesions of a salivary gland requires clinical examination, analysis of lesion by radiographic techniques and more recently the use of FNA [6, 7].

Patients who are candidates for FNA often have enlargement and/or pain on the face, and upper part of the neck and mouth. Sometimes, they have a clinical presentation as partial paralysis or paresthesia of the face. FNA is performed easily, it is minimally invasive, inexpensive, fast, reliable and provides a valuable information to clinicians about the nature of the lesion and therapeutic modalities for each patient. It can be used to obtain material for further analyses (microbiological, immunocytochemical, molecular). Complications are rare [1, 8].

Ex tempore diagnostic (frozen section – FS) is a diagnostic tool that is essential in determining the modalities of surgical treatment of lesions of the salivary glands. FS is often the first procedure in a definitive histopathological diagnostics. Also, this method is used in determining the status of resection margins, and involvement of the tumor's process of some anatomical structures (blood and lymph vessels, nerves, etc.). In recent years, the importance of FS in the diagnosis of lesions was significantly lower and the share in the evaluation of the margins of the pathological process is much higher. In most cases, FS is used as a diagnostic confirmation of FNA results that have been interpreted as nondiagnostic, benign but clinically suspicious, atypical or cytologically suspicious for malignancy [6, 9, 10].

The goal of this research was to present our experiences in the application of FNA and FS in the diagnosis of salivary gland lesions, and to correlate these methods with definitive histopathological analysis of the operation material and determine their sensitivity, specificity, predictive values and diagnostic reliability.

Methods

The study included 36 patients selected at random with puncture of the salivary glands in the period between October 2010 and October 2011. Data on the patients were obtained from the database of the Department of Pathology, Clinical Center Banja Luka, Bosnia and Herzegovina. Criteria in the selection of patients were: clinically confirmed lesion in salivary gland; lesion verified by imaging techniques [ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI)]; completed preoperative puncture of lesion in salivary gland; cytology report of preoperative FNA performed; definitive histopathological findings.

In most of the patients, FS diagnostics, histological and cytological diagnostics were also done. Punctures were made with a thin needle. In most of the cases, a palpable node was present. In a few cases, a change was observed and puncture was guided by US. After puncture, 2–8 direct smears (conventional preparation) were made. Smears were treated in two ways: air-dried and stained with May-Grunwald-Giemsa (MGG) method, and fixed in 95% alcohol and stained with Papanicolaou method. Interpretation of cytological findings was performed as recommended by the guide on how to categorize the changes in aspiration cytology (The United Kingdom National Health Service Breast Screening Programmes, UK NHSBSP) [1, 11]. FNA reports included confirmation of adequacy of a sample and were placed into the following categories: unsatisfactory, nondiagnostic, negative for malignant cells, atypical, suspicious for malignant cells and positive for malignant cells. In most reports, a specific diagnosis or description were indicated.

Operative treatment was carried out in all 36 patients. In 23 of the patients FS diagnostic was done. FS interpretation was performed on the samples and the clinical behavior of the lesion (benign or malignant) was determined. Out of operating material the representative amount of tissue samples were taken, then fixed in buffered formalin, embedded in paraffin, and analyzed using a light microscope. After the diagnostics was completed, the patient was treated with the appropriate type of therapy according to the results of diagnostic procedures. If cytology and FS diagnostics confirm benign lesion, surgical treatment is finished by resection of the lesion with clear resection margins. In cases in whom cytology and FS diagnostic confirm malignant lesion two approaches are used (depending on the degree of malig-

nancy): resection with negative resection margins or radical extirpation of the salivary gland with regional lymph node dissection.

The determined parameters are recommended by the UK NHSBSP, which are used for assessing the performance of FNA/FS diagnostic methods in lesions of salivary glands (analogous to lesions in other organs): absolute sensitivity, complete sensitivity, specificity, positive predictive value, false negative relationship, false positive ratio, an inadequate ratio and inadequate ratio of cancers [12, 13].

For statistical data processing the statistical program SSPS (version 15.0) was used. The processed data and the results are presented in tabular and graphical forms. In the processing and analysis of data, the following statistical methods were used: descriptive statistics, correlation, χ^2-test and Cramer's V test.

Results

Our study included materials obtained from 36 patients, taken during the surgery at the Clinical Center of Banja Luka during the period from October 1, 2010 to October 1, 2011. In all the patients, a palpable or ultrasonically detected lesion in the salivary gland was punctured, followed by processing and analyzing the material, and interpretation of cytological findings. Subsequently, all the patients were treated surgically, depending on the results of cytological analysis. In 23 of the patients the diagnostics was done during the surgery (FS). After the surgical treatment, processing and histological interpretation of the results was performed. Interpretation of tumor processes was carried out according to the World Health Organization (WHO) classification [14].

The youngest patient was one year old and the oldest 83 years. The average age of the examinees was 52.08 years. Most of the patients were aged 51 to 60 years (30.56%). Most of the patients were male – 20 (56%), while the number of female patients was 16 (44%) (Table 1).

In 33 (91.7%) patients changes were localized in the parotid gland, followed by changes in submandibular gland in 2 (5.5%) patients, and the rest of the changes were localized in the minor salivary glands of the oral cavity, in 1 (2.8%) patient (Table 1).

The obtained cytological material was evaluated with respect to the adequacy of a sample. Of 36 analyzed samples, in three cases (8.3%) material was not optimal for analysis and was classified as nondiagnostic (sufficient for analysis, but with limitations), with the stated reasons for this limitation. In the remaining 33 (91.7%) cases the material was sufficient for analysis.

In three cases there were some restrictions: in the first case, it was stated that there was only blood, and the definitive diagnosis was hemangioma; in the second case, serous content and rare ductal epithelial cells were present, and the final diagnosis was salivary duct cyst; in the third case, the cytological details of normal salivary glands and fatty tissue were described, and the final histological diagnosis was lipomatosis of the salivary gland (Table 1).

Table 1
Characteristics of the patients and general interpretation of changes in salivary glands

Characteristics	Number of patients (%)
Sex	
male	20 (56)
female	16 (44)
Localization	
parotid gland	33 (91.7)
submandibular gland	2 (5.5)
minor salivary glands in oral cavity	1 (2.8)
Adequacy of FNA sample	
satisfactory (no limitations)	33 (91.7)
satisfactory (with limitations)	3 (8.3)
unsatisfactory	0 (0)
General FNA interpretation	
unsatisfactory	/ (/)
nondiagnostic	3 (8.3)
negative for malignant cells	28 (77.8)
positive for malignant cells	5 (13.9)
Specific diagnose in FNA samples	
confirmed	26 (72)
not confirmed (descriptive report)	10 (28)
Interpretation in FS samples	
benign	21 (91.3)
malignant	2 (8.7)
Definitive HP diagnosis	
non tumor lesion	7 (19.4)
tumor lesion (benign)	23 (64)
tumor lesion (malignant)	6 (16.6)

FNA – fine-needle aspiration; FS – frozen section; HP – histopathological.

Interpretation of cytological findings was performed according to the guide UK NHSBSP adapted for aspiration cytology of salivary glands. The general interpretation contains six categories: unsatisfactory (0/0), nondiagnostic [3 (8.3%) patients], negative for malignant cells (a benign lesion) (28/77, 8%), atypical (0/0), suspected for malignant cells (0/0), positive for malignant cells (5/13, 9%) (Table 1).

A specific diagnosis was identified in cytological material in 26 (72%) cases. The following specific diagnoses were confirmed cytologically: tumor mixtus (pleomorphic adenoma) in 20 cases (Figure 1), Warthin's tumor in 3 cases and squamous carcinoma in 3 cases.

Descriptive interpretation was seen in 10 (28%) cases (Table 1). The following conclusions were stated in these interpretations: 5 cases of benign findings-inflammation, 2 cases of high grade malignancy, and 3 cases were described without statement (nondiagnostic).

Rapid diagnosis during surgery was performed in 23 (64%) of the patients. The interpretations were: benign lesions in 21 cases, malignant lesions in two cases (Table 1).

In our samples, benign tumors were diagnosed in 23 (64%) of the cases of histological material. Non-tumor processes were diagnosed less frequently (7 cases or 19.4%), and most rarely malignant tumors, in 6 (16.6%) cases (Table 1).

Analysis showed that benign tumors were detected more frequently in men, 13 (56%) cases. Malignant tumors were twice as common in men, 4 (66.7%) cases, while non-tumor lesions were more common in women, 4 (57.1%) of the cases.

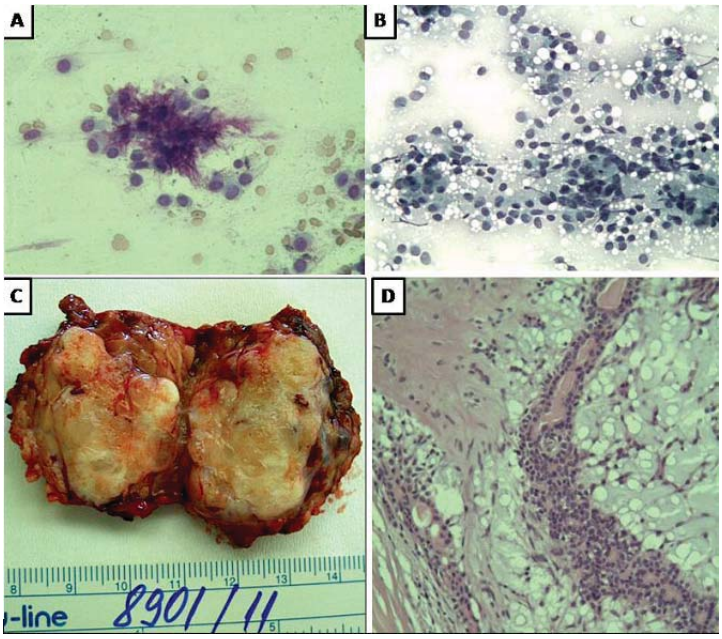

Fig. 1 – Pleomorphic adenoma (tumor mixtus) of the salivary gland.

A) – Myoepithelial cells detected, arranged individually within a gentle fibrillar matrix (MGG ×400); B) – Cellular pleomorphic adenoma. Myoepithelial cells arranged in groups (Papanicolaou ×400); C) – Macroscopic appearance, well-circumscribed, lobular nodule, whitish, glassy surface; D) – Histological appearance: epithelial, myoepithelial component of the tumor and myxoid matrix (HE ×200).

Definitive histological diagnoses of the operative material were established in all the 36 patients. Most frequently diagnosed was tumor mixtus (pleomorphic adenoma) in 16 (44%) of the cases (Figure 1), followed by Warthin's tumor (*cystadenoma lymphomatosum*) in 4 (11.1%) of the cases, squamous cell carcinoma and lymphoepithelial cyst in the 3 (8,3%) of the cases, high grade mucoepidermoid carcinoma (Figure 2), in 2 (5.5%) of the cases. The following histological entities had one case (2.8 %) each: lymphoma, schwannoma, hemangioma, lipomatosis, salivary duct cyst, rupture of epidermal cyst, lymphadenitis and abscessus.

Analysis of reliability of cytological diagnosis and FS diagnosis in terms of general interpretation of benign/malignant in relation to a definitive histopathological diagnosis was determined using the χ^2 test or Cramer's V-test. Based on statistical data, we can safely conclude that FNA and FS diagnostics are absolutely reliable in general interpretation of the lesion (benign/malignant) in salivary glands. [The value of Cramer's V = 0.898, c = 0.710, c = 1,000 ($p < 0.01$)].

Cytological and histological reports regarding specific diagnoses were the same in 22 (61.1%) of the patients. In 8

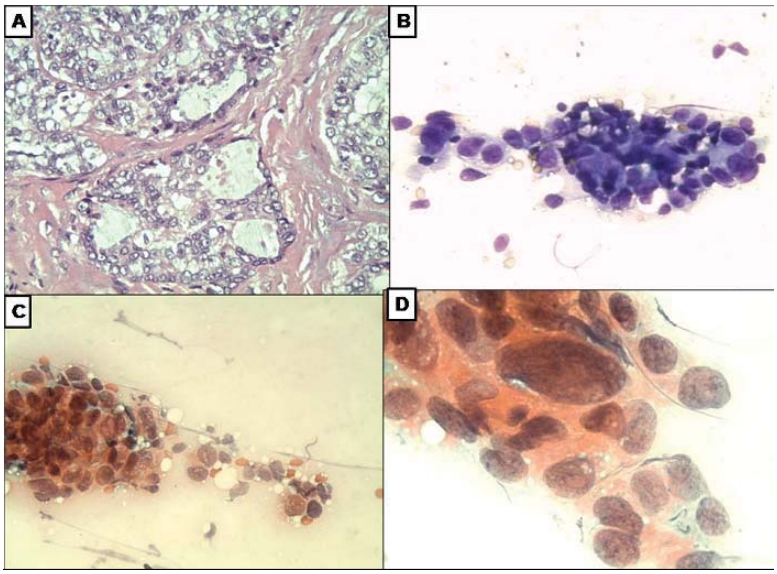

Fig. 2 – Mucoepidermoid carcinoma, high grade.

A) Histologically observed predominantly properly arranged tumor cells with characteristics of epidermoid and intermediate cells. Focally present glandular formations lined with mucinous cells (HE ×400); B) Cytologically, cells with squamoid appearance, sporadically vacuolated cytoplasm, large, polymorphic nuclei were found (MGG ×400); C) Cytologically observed cells with squamoid appearance, large polymorphic nuclei (Papanicolaou ×400); D) Cytologically, cells with squamoid appearance, large, polymorphic nuclei, prominent nucleoli were found (Papanicolaou ×1,000).

(22.25%) of the patients lesions were cytologically and histologically interpreted as benign, but specific diagnoses were different. In 2 (5.55%) of the patients lesions were interpreted cytologically and histologically as malignant, but specific diagnoses were not the same. Malignant cytological reports, which were not confirmed by histological diagnosis, were not present (false positives were not present). In one (2.8%) case, the lesion was interpreted as benign cytologically and as malignant histologically. Three (8.3%) of the cases were nondiagnostic in cytology (Table 2, Figure 3).

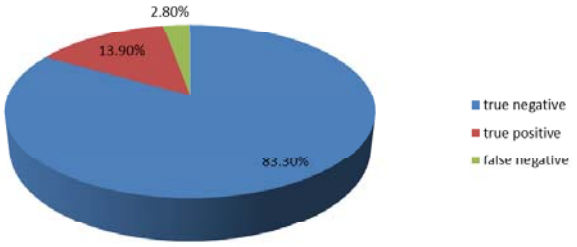

Fig. 4 – Presentation of examinees according to true positive (TP); true negative (TN); false positive (FP); false negative (FN) findings.

Table 2

Representation of matching of specific diagnoses in cytological and histological material

FNA diagnosis	Number of patients (n)	Histopathological diagnosis (n)	
		Congruous	Incongruous
Tumor mixtus	20	16	4 (1 lymphoepithelial cysts [1],1 schwannoma [1], 1 abscess [2], 1 Warthin's tumor [1])
Warthin's tumor	3	3	
Squamous carcinoma	3	3	
Description: benign – inflammation	5		5 (3 lymphoepithelial cysts [1], 1 reactive lymphadenitis, 1 mucoepidermoid carcinoma [3])
Description: malignant high grade	2		2 (1 mucoepidermoid carcinoma [1], 1 lymphoma DLBCL [1])
Description: nondiagnostic	3		3 (1 hemangioma [1], 1 lipomatosis [1], 1 salivary duct cyst [1])
Total	36	22	14

[1] – Minimal (insignificant) difference (n = 12); [2] – Difference of medium significance (n = 1); [3] – Huge (significant) difference (n = 1); DLBCL – Diffuse large B cell lymphoma.

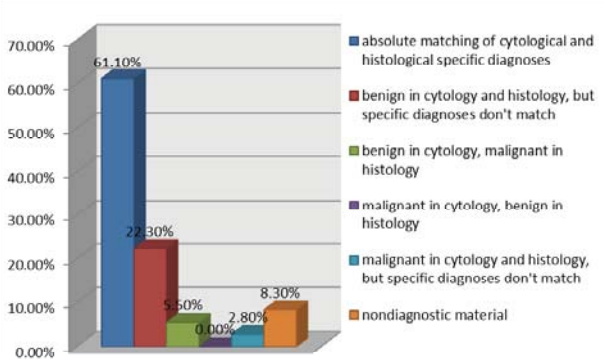

Fig. 3 – Representation of matching of cytological and histological specific diagnoses.

Findings of FS diagnostics and definitive histopathological diagnosis were absolutely matched, given that the interpretation was carried out in terms of clinical behavior of the lesion: benign or malignant. Specific diagnosis was not determined by FS diagnostics.

In the examined material, there were 30 (83.3%) true negative cytological findings, five (13.9%) true positive cases and one case (2.8%) was false negative. False positive cases were not present in our material (Figure 4).

Based on the ratio of cytological and histopathological diagnoses, calculated sensitivity was 83.3%, specificity 96.67%, positive predictive value of 83.3%, negative predictive value of 96.77% and diagnostic accuracy of 97.2%.

Based on the relationship of FS diagnosis and histopathological diagnosis, the calculated specificity was 96.67%, while the positive predictive value and diagnostic accuracy were 100% each.

Discussion

FNA is an effective and important diagnostic method for the diagnosis of diseases of various organs. If the method is correctly performed, cytological material adequately processed and the results interpreted by an experienced cytopathologist, FNA provides reliable information about the disease before surgical treatment [12, 13]. FNA of salivary glands is technically a simple procedure that is usually well-tolerated by the patient, and increasingly popular among clinicians and cytopathologists. Additional advantages of this method are rapid diagnosis and minimal opportunity for spreading of the tumor. To ensure more reliable and specific diagnosis in FNA, ongoing collaboration between clinicians, radiologists and cytopathologists is necessary [1].

One of the earliest analysis of the effectiveness of FNA in the diagnosis of salivary gland disease was published by Frable and Frable [15] in 1991. These authors analyzed 552 aspirates of salivary glands in a 15-year period and showed high sensitivity and specificity of FNA in the diagnosis of tumors of the salivary gland [15]. FNA in salivary glands may provide a saving of 8,000–24,000 dollar per 1,000 FNA of salivary gland tumors [16].

In our study, the average age of the patients was 52 years. Salivary gland tumors occur at all ages. Some of the most common tumors (pleomorphic adenoma, mucoepidermoid carcinoma) occur most frequently in the third and fourth decade of life. Most benign and malignant tumors occur between the fifth and seventh decade of life, and the average age is 46–47 years [1, 14, 17].

In our material, men had more frequently changes localized in the salivary glands and the ratio men : women was 1.25 : 1. Similar results were obtained by Das et al. [18], who analyzed the reasons for enlargement of salivary glands in 712 cases. In salivary glands, most commonly diagnosed are benign tumors, which were, in our samples, diagnosed in 23 (64%) of the cases. Non-tumor processes were seen in 7 (19.4%) of the cases, and malignant tumors in 6 (16.6%). Both benign and malignant tumors were more often seen in men – benign in 56% and malignant in 66.7% of the cases. Non-tumor processes were more common in women (57.1% of the cases). A similar distribution of benign and malignant tumors and non-tumorous processes was shown by other authors who were doing research on a larger number of patients [6, 18–21]. Salivary gland tumors are more common in women, but the differences are present with respect to the histological type (eg. Warthin's tumor and high grade cancers are more common in men) [1, 14, 17, 20].

In most of our patients (91.7%), the changes were seen in the parotid gland. Madani et al. [19] analyzed 169 patients who underwent FNA and subsequent histological analysis. In their samples, most of the changes are localized in the parotid gland – 152 (89.94%) of the cases.

Interpretation of cytological analyses was performed as recommended by the guide on how to categorize changes in aspiration cytology UK NHSBSP. Analysis showed that 77.8% of cases in the group were negative for malignant cells (benign lesion), followed by positive for malignant cells in 13.9% of the cases and nondiagnostic in 8.3% of the cases. In the examined material, three samples were named as nondiagnostic (8.3%). In these samples the final histopathologic diagnosis was lipomatosis of the salivary glands in one case, in the second hemangioma, and in the third case was a cyst of the salivary duct. The number of inadequate samples in FNA salivary glands differs and ranges from 2 to 8.5% [6, 18–20].

A precise criterion for assessing the adequacy of aspiration cytology of salivary gland does not exist. Evaluation of the adequacy is a job of a cytopathologist, who based on the present material determines whether the definitive diagnosis and interpretation can be made. Unsatisfactory FNA samples of salivary glands are usually acellular or hypocellular samples. Such samples are usually the result of various factors such as: abundant blood, inflammation, necrotic debris, artifacts of fixation, staining, and preparation of smears ("crush artifacts"). The exact number of cells required for analysis of salivary gland FNA has not been established, so the estimates based on this parameter are still subjective. Making statement that the sample is "satisfactory" instead of "unsatisfactory" or "nondiagnostic" may be the reason for false negative results in clinically verified changes in the glands. The presence of atypical cells should be stated in the report and provide guidance for further processing (testing). Hypocellular samples that the cytopathologist declared as "unsatisfactory" may be seen in cystic lesions, benign tumors, and so on. In such cases, the correlation of clinical and radiological images and the content of the sample can help to reduce the number of "unsatisfactory" samples [1, 13, 22].

FNA was positive in 5 (13.9%) cases. Postoperative histopathological analysis in all five cases confirmed malignancy of the lesions. Correspondence of specific diagnosis is absolute in the diagnosis of squamous cell carcinoma in cytology and histology. There were three cases with the same specific diagnosis of squamous cell carcinoma. In two cases the cytological findings were interpreted as positive for malignant cells and matched the high grade tumor. In histology, in one case, high grade mucoepidermoid carcinoma was diagnosed, and in the second case non Hodgkin's lymphoma (diffuse large B cell lymphoma). In the literature, the percentage of FNA positive for malignant cells varies and ranges from 10 to 25% [8, 19, 21, 23, 24].

In our study one (2.8%) case was false negative, while we did not find false positive cases. Numbers of false negative and false positive cytology findings are different. Madani et al. [19] had 10.65% of false negative findings and 5.91% of false positive. Rajwanshi et al. [21] in a study on 172 FNA cases had 4 false negative cases and 5 cases of false positive results of cytological analyses. Hughes et al. [8] presented 8 cases of false positive and 32 cases of false negative results.

The range of false negative results is from 1% to 15%, and in most cases negative interpretation is present in low grade tumors, hypocellular cysts. Most common false negative cytological interpretations were observed in mucoepidermoid cancer, low grade of malignancy, lymphomas, acinocellular carcinoma, and adenoid cystic carcinoma. The reason for this is hypocellularity of smears, containing only the content of a cyst, and besides that, epithelial cells appear cytologically without atypia or with minimal atypia, and mucinous cells can be interpreted as histiocytes (muciphages) [1, 3, 8].

False positive results were present in 5% to 8% of cases, mostly due to the presence of benign lesions with reactive and metaplastic changes especially present in inflammation. In the report of the College of American Pathologist Interlaboratory Comparison Program in Nongynecologic Cytology, most common false positive FNA findings are monomorphic adenoma, intraparotid lymph node, oncocytoma and granulomatous lymphadenitis [1, 8, 19, 21, 25].

Specific diagnosis on cytological material in our samples was determined in 26 (72%) of the cases. Matching between specific cytological and histological diagnosis was present in 22 (84.6%) of the cases. The difference was present in the specific diagnosis in four (15.4%) of the cases. In all four cases the interpretation was tumor mixtus and histology found the following entities: Warthin's tumor, lymphoepithelial cyst, chronic abscess, and schwannoma. So, in terms of general interpretation of benign changes there was no difference. Descriptive interpretation was seen in ten cases and the conclusions were: 5 cases of benign findings-inflammation, 2 cases of malignant lesinous-high grade, and in 3 of the cases de-

scription with no statement (nondiagnostic). For cytological statements benign – inflammation, in definitive histological report there were the following diagnoses: three samples were branchial lymphoepithelial cyst, reactive lymphadenitis in one case and one case interpreted as mucoepidermoid carcinoma, high grade (the only false negative cytological finding). The results of various studies indicate a low correlation of specific cytological and histological diagnosis of salivary gland material. Mihashi et al. [26] found that the sensitivity of FNA in the diagnosis of malignant tumors of the salivary glands was 88.2% and an extremely low (30%) matching of cytological and histological specific diagnoses. Jain et al. [27] had a correlation of histological and cytological diagnosis of malignant tumors of salivary glands in 64.2% cases.

Based on the results, the parameters of efficacy of FNA and FS in the diagnosis of salivary gland lesions were derived. Absolute and total sensitivity amounted to 83.3%, specificity of 96.67%, false positive ratio of 0%, false negative ratio of 16.6%, diagnostic accuracy of 97.2%, positive predictive value 100%, and negative predictive value of 96.77%.

Hughes et al. [8] analyzed 6,249 cases of salivary gland tumor and showed the sensitivity of the method of 73% and specificity of 91%. Our results are similar, but minimal differences are due to the sample size. Other studies show the reliability of the method in the range between 81% and 98%, accuracy benign/malignant between 81% and 100%, accuracy in specific diagnosis from 48% to 94%, false negative index from 1% to 15%, false positive index of 5% to 8%, sensitivity 86% to 100% and specificity of 90% to 100% [8, 18, 25–30].

histopathological findings is absolute. Specific diagnosis was not determined at FS samples. Seethala et al. [6] found that of 57 cases analyzed by this method 12% were false negative and no false positive findings. FS is a method that can provide a definitive diagnosis in cases where previous diagnostic methods are not sufficient (FNA). The authors noted that the sensitivity of FS was 77%, specificity 100%, and diagnostic accuracy 88%. The combination of FNA and FS increases sensitivity up to 90%, specificity to 100% and diagnostic accuracy to 95% [10, 31, 32].

FNA was compared with FS diagnosis. FNA had a similar accuracy as the FS diagnosis and the advantage of FNA is in providing preoperative diagnosis, in contrast to FS where the diagnosis is reached during the operation. FNA tends to be a more sensitive diagnostic method than FS and FS has a higher specificity and the advantage in possible evaluation of resection margins [1]. In the study of Seethala et al. [6], FNA and FS are complementary methods, and FS is most useful for assessing nondiagnosticly interpreted cases of FNA, as well as to confirm malignancy in some cases and evaluation of resection margins. According to our results, both diagnostic methods are important in the diagnosis of lesions of the salivary glands. Based on the literature data and experiences in our sample, the diagnostic algorithm for lesions in salivary glands requires clinical examination, radiological examination and FNA [1, 8]. In cases where FNA was interpreted as atypical, suspicious, or in cases of discrepancy between the findings of FNA and clinical and/or radiological findings the FS is indicated. A proposal for the treatment of patients with verified lesions in salivary glands is shown in Figure 5.

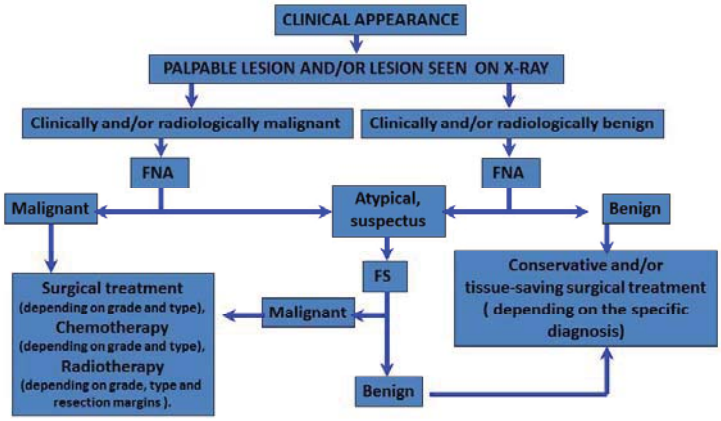

Fig. 5 – The diagnostic algorithm of the salivary glands lesions – treatment of palpable or radiologically detected lesions in the salivary glands [22].

The differences in the presented overall accuracy of FNA of salivary glands are consequences of classification of lesions in the salivary glands, the quality of the sample and the experience of the cytopathologist. FNA is very accurate in determining the diagnosis of certain neoplasms (e.g, tumor mixtus, Warthin's tumor, etc.), but is less effective in the definitive determination of other neoplasms (e.g, basal cell carcinoma, epithelial-myoepithelial carcinoma) [8, 27].

Rapid diagnosis during surgery (FS) was performed in 23 (64%) of the patients. The interpretations were: benign lesions in 21 of the cases, and malignant lesions in two cases. Matching of FS reports in terms of the nature of the lesion and definitive

Conclusion

The obtained results confirm that fine needle aspiration is a sensitive, reliable diagnostic method for differentiation of lesions in the salivary glands. Fine needle aspiration is highly accurate in distinguishing benign from malignant lesions in the salivary glands, and less accurate in determining the specific diagnosis. It be applied in the process of diagnosing of palpable or otherwise visualized lesions in the salivary glands. Fine needle aspiration in combination with clinical and radiological data will enable the establishment of a specific diagnosis in most of salivary gland lesions.

In cases where a definite differentiation in FNA samples is not possible, where there is a need to assess the resection margins and invasion of anatomical structures, frozen section diagnosis is recommended.

Both methods, fine needle aspiration and frozen section diagnostics, are important in evaluating lesions in the salivary glands.

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Cephalometric analysis of the middle part of the face in patients with mandibular prognathism

Cefalometrijska analiza srednjeg dela lica kod osoba sa mandibularnim prognatizmom

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Abstract

Background/Aim. The middle part of the face, that is the maxilla, has always been mentioned as a possible etiologic factor of skeletal Class III. However, the importance of the relationship of maxillary retroposition towards the cranial base is still unclear, although it has been examined many times. The aim of this study was to conduct cephalometric analysis of the morphology of maxilla, including the whole middle part of the face in patients with divergent and convergent facial types of mandibular prognathism, as well as to determine differences betweeen them. **Methods.** Lateral cephalometric teleradiograph images of 90 patients were analyzed at the Dental Clinic of the Military Medical Academy, Belgrade, Serbia. All the patients were male, aged 18–35 years, not previously treated orthodontically. On the basis of dentalskeletal relations of jaws and teeth, the patients were divided into three groups: the group P1 (patients with divergent facial type of mandibular prognathism), P2 (patients with convergent facial type of mandibular pragmathism) and the group E (control group or eugnathic patients). A total of 9 cephalometric parameters related to the middle face were measured and analyzed: the length of the hard palate – SnaSnp, the length of the maxillary corpus – AptmPP, the length of the soft palate, the angle between the hard and soft palate – SnaSnpUt, the angle of inclination of the maxillary alveolar process, the angle of inclination of the upper front teeth, the effective maxillary length – CoA, the posterior maxillary alveolar hyperplasia – U6PP and the angle of maxillary prognathism. **Results.** The obtained results showed that the CoA, AptmPP and SnaSnp were significally shorter in patients with divergent facial type of mandibular prognathism compared to patients with convergent facial type of the mandibular prognathism and also in both experimental groups of patients compared to the control group. SnaSnp was significantly shorter in patients with divergent facial type of mandibular prognathism compared to the control group, whereas SnaSnp was significantly smaller in patients with convergent facial type of mandibular prognathism compared to the control group. Additionally, there was a pronounced incisor dentoalveolar compensation of skeletal discrepancy in both groups of patients with mandibular prognathism manifested in the form of a significant upper front teeth protrusion, but without significant differences among the groups, while the maxillary retrognathism was present in most patients of both experimental groups. A pronounced UGPP was found only in the patients with divergent type of mandibular prognathism. **Conclusion.** The maxilla is certainly one of the key factors which contributes to making the diagnosis, but primarily to making a plan for mandibular prognathism treatment. Accurate assessment of the manifestation of abnormality, localization of skeletal problems and understanding of the biological potential are key factors of the stability of the results of surgical-orthodontic treatment of this abnormality.

Key words:
prognathism; mandible; malocclusion; angle class III; cephalometry; maxilla; face; orthodontics.

Apstrakt

Uvod/Cilj. Srednji masiv lica, odnosno maksila, skoro uvek se pominje kao mogući etiološki faktor skeletne klase III. Međutim, značaj odnosa retropozicije maksile u odnosu na kranijalnu bazu, mada dosta proučavan, još uvek je nejasan. Cilj ovog istraživanja bio je da se kefalometrijski analiziraju morfološke karakteristike maksile kao i celog srednjeg masiva lica kod pacijenata sa divergentnim i konvergentnim oblikom mandibularnog prognatizma, kao i da se ustanove ra-

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zlike između njih. **Metode.** Analizirani su rendgenkefalometrijski snimci ukupno 90 pacijenata Klinike za stomatologiju Vojnomedicinske akademije, Beograd, Srbija. Svi pacijenti bili su muškog pola, starosti od 18 do 30 godina i nisu ranije bili ortodontski lečeni. Pacijenti su prema dentoskeletnim odnosima vilica i zuba svrstani u tri grupe: grupu P1 (pacijenti sa divergentnim mandibularnim prognatizmom), P2 (pacijenti sa konvergentnim mandibularnim prognatizmom) i grupu E (kontrolna grupa ili grupa pacijenata sa normalnom okluzijom). Izmereno je i analizirano 9 kefalometrijskih parametara koji su se odnosili na srednji masiv lica: dužina tvrdog nepca (SnaSnp), dužina korpusa maksile (AptmPP), dužina mekog nepca, ugao između mekog i tvrdog nepca (SnaSnpUt), ugao inklinacije maksilarnog alveolarnog procesusa, inklinacija gornjih frontalnih zuba, efektivna dužina maksile (CoA), posteriorna maksilarna hiperplazija (U6PP) i ugao maksilarnog prognatizma. **Rezultati.** Dobijeni rezultati su pokazali da su CoA, AptmPP, kao i SnaSnp, značajno kraći kod pacijenata sa divergentnim oblikom mandibularnog prognatizma u odnosu na pacijente sa konvergentnim, a takođe i kod obe eksperimentalne grupe pacijenata u odnosu na kontrolnu. SnaSnp značajno je kraća kod pacijenata sa divergentnim oblikom mandibularnog prognatizma nego kod pacijenata kontrolne grupe, dok je SnaSnpUt značajno manji kod pacijenata sa konvergentnim oblikom mandibularnog prognatizma u nego kod pacijenata kontrolne grupe. Takođe, postoji izražena dentoalveolarna incizalna kompenzacija skeletne disharmonije kod obe grupe pacijenata sa mandibularnim prognatizmom u vidu značajne protruzije gornjih frontalnih zuba, ali bez značajne razlike među grupama, dok je retrognatizam maksile prisutan kod većine pacijenata obe eksperimentalne grupe. Izražena UGPP ustanovljena je samo kod pacijenata sa divergentnim tipom mandibularnog prognatizma. **Zaključak.** Gornja vilica svakako je jedan od bitnih faktora koji doprinose dijagnozi ali pre svega donošenju plana terapije kod mandibularnog prognatizma. Tačna procena ispoljenosti anomalije, lokalizacija skeletnog problema i razumevanje biološkog potencijala glavni su faktori postojanosti rezultata ortodontskohirurške terapije tog deformiteta.

Ključne reči:
prognatizam; mandibula; malokluzija; klase III; kefalometrija; maksila; lice; ortodoncija.

Introduction

Mandibular prognathism and skeletal Class III malocclusion are often used as synonyms, although they are not, because the importance of occlusal relationships is emphasized by the use of occlusal dental terms in describing skeletal intermaxillary relationships. Generally, mandibular prognathism is usually a part of skeletal Class III and includes morphological, dimensional and positionally modified mandible which gives a characteristic picture of skeletal Class III malocclusion together with modifications primarily found in the cranial base and probably in the middle part of the face. Accordingly, Nakasima et al. [1] and Thompson and Winter [2] emphasized a familiar tendency to mandibular prognathism. Mandibular prognathism also occurs as a part of numerous congenital anomalies: craniosynestosis (Apert and Crouzon's syndrome), cleidocranial dysostosis (*dysostosis cleidocranialis*), ectodermal dysplasia, achondroplasia, trisomy 21 chromosomes, Binder's syndrome, congenital cleft of the primary and secondary palate, which additionally proves its genetic etymology. Besides, Mackay et al. [3] identified 5 subgroups of Class III malocclusion manifested in mandibular prognathism. On the other hand, Class III malocclusion is not such unique and clear diagnostic clinical entity, because it is a combination of numerous skeletal and dentoalveolar components. Additionally, its etyology is still not clear enough. The results of various authors on the presence of mandibular prognathism in Class III are usually similar. They show that the frequency of mandibular prognathism is over 50% [4, 5] in the aforementioned malocclusion and different combination of intermaxillary relationships and relationships with other craniofacial structures.

There are 2 basic types of the real mandibular prognathism: divergent and convergent [6]. Divergent type is characterized by a divergent mandibular, occlusal and palatal plane [in other words, the nasion–sella line (NS) planes form larger angles compared to the reference ones], more obtuse gonial angle and open bite. Convergent type is characterized by the planes forming significally smaller angles with the NS plane, sharper gonial angle and vertical overlap of the front teeth. This classification is quite rough, so there is a necessity to classify these patients according to the other cephalometric criteria.

The maxilla is connected with the middle cranial fossa through numerous fissures and therefore their growth is interdependent. After the completion of cranial base growth, the maxilla continues to grow forward, laterally and downward in relation to the middle cranial fossa in numerous centers of its growth (sphenopalatine suture, sphenozigomatic suture and sphenoethmoidal suture). Hence, it can be concluded that the shape and position of the middle cranial fossa, (especially the large wings of the sphenoid bone) have an important role in the position of the back edge of the middle part of the face and its relationship with other parts of cranial base.

Diewert [7] found that the growth of the midfacial complex in a sagittal direction is closely connected to the cranial base, whose growth is almost completed in prenatal period, so that maxilla also takes its final sagittal position quite early, when all the changes related to the anterior cranial base are completed, enabling it to form Class I intermaxillary relationships. If any teratogenic factor influences the growth and development of the maxilla in this late embryonic period, it can cause irreversible changes on the morphology or position of the maxilla.

Using functional matrix hypothesis, many authors tried to explain a postnatal forward and downward growth of the middle part of the face. Moss suggested a passive role of the septal cartilage, Oyen assumed that masticatory function was the key factor of the growth of the midfacial complex,

Latham found the anteroposterior lagging in the development of the complex in dogs, if their vomer was not removed [8].

Bones of the middle part of the face, but primarily the maxilla, have the pronounced compensatory growth mechanisms on various sutures, so if their growth on one suture is blocked by any external factor (bad habit, bad position such as inclination, teeth, etc), they will grow more intensively on the sutures which are not blocked. Thus, if there is an oral inclination of the upper incisors, the maxilla will be "locked" and the mandible will generate a pseudo-progenia bite, so that the growth and development of the aforementioned bones could be modified. In that case, primary dental malocclusion can be developed into skeletal malocclusion. On the contrary, in the primary development of skeletal Class III malocclusion, a dentoalveolar compensatory mechanism (protrusion of the upper front teeth) can sometimes camouflage the anomaly. Therefore, usually after a dramatic growth during puberty, all compensatory mechanisms of the middle face are overcome due to deficient orthocephalization of the cranial base and increased anteroposterior growth of the mandibular corpus [8, 9] and skeletal Class III is manifested [8–10].

Guyer et al. [11] found that the maxilla measured by the angle of maxillary prognatism (SNA) in 15-year-old adolescents with mandibular prognathism was almost all the time in retroposition, as well as its length which was measured by the effective maxillary lenght (CoA) parameter. Mouakeh [4] also published similar results.

Analyzing the middle part of the face in patients with mandibular prognathism, Chang et al. [12] found that the palatal and maxillary lengths presented in cephalometric parameters [the length of the hard palate (SnaSnp) and the length of the maxillary corpus (APtmPP)] were significantly shorter compared to the control group. However, for vertical dimensions, they did not find a statictically important difference.

Assessing the craniofacial growth in patients with mandibular prognathism in their longitudinal study, Reyes et al. [13] among other things found that there were no significant differences in the patients from the age of 6 to 16 compared to the control group, regarding the position of the maxilla measured by angular and linear parameters. In addition, they recorded that the extrusion of the upper molars was almost a constant finding during the growth of children with mandibular prognathism, whereas Ellis and McNamara [14] found a larger extrusion of the upper first molars in patients with mandibular prognathism and more open bite compared to the patients who did not have an open bite.

Abu Allhaija [15] compared uvulo-glosso-pharyngeal dimensions of the midfacial complex in patients with different intermaxillary relationships and found among the other things that the soft palate in patients with mandibular prognathism was significantly thicker compared to the patients with skeletal Class I. Searching for differences in the airway and corresponding soft tissues of hyperdivergent and normodivergent facial patterns, Joseph at al. [16] found that the angle between the soft and hard palate was significantly larger in hyperdivergent facial patterns, probably due to the maxillary retroposition and more narrow nasopharynx. Dostalova et al. [17] found that the length of the soft palate (SnpUt) in patients with acromegaly was significally increased and the angle between the soft and palatal plate substantially reduced. These changes are not correlated with the concentration of growth hormone, but with the duration of the disease.

When all the aforementioned is taken into consideration, it seems that the variability of the maxilla and the whole midfacial complex in Class III malocclusion is the result of growth deficiency on sutures and especially on the transversal palatine suture, but it is often camouflaged by compensatory mechanisms (inclination of the upper front teeth, elongation of the anterior part of the midface, etc) [9, 10]. However, the final facial profile depends on the sagittal and vertical relationship of the aforementioned structures with a morphologically altered and antepositioned mandible, modified cranial base and soft tissues [8, 18].

Numerous studies have emphased the importance of selecting most appropriate treatment options for mandibular prognathism, which primarely depend on the localization and combination of skeletal relationships in adult patients with this deformity [19, 20].

The aim of this study was to conduct a cephalometric analysis of the morphological characteristics of the maxilla and the whole midfacial complex in patients with divergent and convergent facial type of mandibular prognathism and also to determine their differences.

Methods

For the purpose of this study, lateral teleradiograph images of 90 orthodontic patients were analyzed which were taken before their treatment at the Dental Clinic of the Military Medical Academy, Belgrade, Serbia.

According to the literature data on gender differences and dynamics of changes in growth [13, 21, 22], male subjects, aged 18–30 year were examined.

The control group, group E, consisted of 30 patients with normal intermaxillary relationships (skeletal Class I, eugnathic subjects): sella-nasion-B point (SNB) $\leq$ 80°; (ANB) = 0-5°; normal overlap of the front teeth and the relationship of the first permanent molars in Class I.

The group P consisted of the remaining 60 patients with mandibular prognathism diagnosed on the basis of the following criteria: SNB $\geq$ 80°; ANB $\leq$ 0°; B $\geq$ 30°; Björk $\geq$ 396°; anterior crossbite and relationship of the first permanent molars in Class III.

On the basis of the two cephalometric criteria, the group P was divided into two subgroups: the group P1 consisted of 30 patients with divergent type of mandibular prognathism who met the following criteria: B $\geq$ 300; Björk $\geq$ 396°; the group P2 consisted of 30 patients with convergent type of mandibular prognathism who met the following criteria: B $\leq$ 30°; Björk $\leq$ 396°.

All the patients from the group P were planned and later treated by orthodontic-surgical therapy including monomaxillary or bimaxillary surgical procedure, which was performed by the same team and lateral teleradiograph images in this study were taken before each therapy.

The obtained results were compared between the group E and the group P1, the group E and the group P2 and the group P1 and P2.

Cephalometric analysis

Lateral teleradiograph images of the skull were taken for each patient under standard conditions. The head was fixed in a cephalostat, and recording was conducted at the distance of 1.5 m. Analysis of the lateral teleradiograph images was preceded by drawing the corresponding structures on the tracing paper fixed on the film. Afterwards, the numerous points and surfaces were marked for analyzing certain angular and linear parametres taken from the analyses of Steiner, Jacobson, Rickettc, Downs and Björk. The measurements were performed twice by the same examiner, on different days, with accuracy of 0.5 mm or 0.50. Statistically significant differences did not appear between these 2 measurements.

The difference analysis of the 9 cephalometric parametres (Figure 1) was conducted between the patients with divergent type of mandibular prognathism and the patients with convergent type of mandibular prognathism and between these 2 groups and the control group of eugnathic patients.

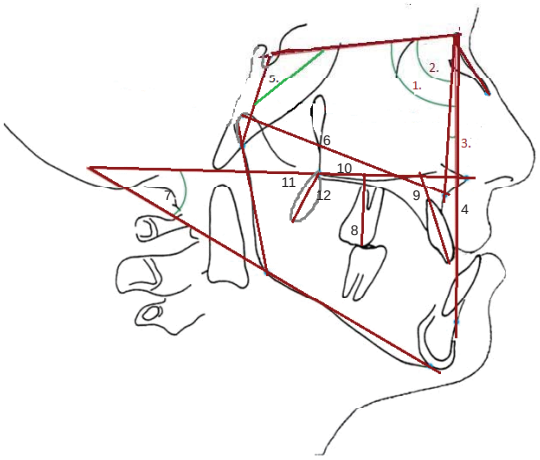

Fig. 1 – Cephalometric parameters

1 – SNA (the angle of maxillary prognathism); 2 – SNB (the angle of mandibular prognathism); 3 – ANB (the angle of sagittal intermaxillary relationships) 4 – SnaAPr (the angle of inclination of the maxillary alveolar process) 5 – NSAr (the angle of cranial base) 6 – CoA (the effective maxillary length) 7 – B (the angle of the vertical intermaxillary relationships) 8 – U6PP (the posterior upper dental height or posterior maxillary alveolar hyperplasia (perpendicular distance betweeen the mesial knob of the first permanent molar and the palatal plate) 9 – IPP (the angle of inclination of the upper front teeth) 10 – SnaSnp [the length of the hard palate (APtmPP – the length of the maxillary corpus is a projection of the points A and Ptm on the palatal plate)] 11 – SnpUt (the length of the soft palate); 12 – SnaSnpUt (the angle between the hard and soft palate).

Statistical analysis

On the basis of the data collected by cephalometric x-ray analysis, for each patient and each feature, the data base was formed in the SPSS12 windows program and the following statistical methods were used in statistical analysis: tables and graphical presentations, descriptive statistics methods and Bonferroni test to detect intergroup differences.

Results

Table 1 shows the statistical results of the following analyzed parametres of the maxilla: SnaSnp, AptmPP, SnpUt, SnaSnpUt, the angle of inclination of the maxillary alveolar process (SnaAPr), the angle of inclination of the upper front teeth (IPP), CoA, the posterior maxillary alveolar hyperplasia (U6PP) and SNA.

Table 1

Results of the analyzed parameters of the maxilla in the eugnathic examinees (E) and examinees with divergent (P1) and convergent type (P2) of mandibular prognathism

Parameters	N	$\bar{x} \pm SD$ (min – max)	
SnaSnp			
E	30	54.48 ± 3.48	(45.00 – 61.00)
P1	30	45.50 ± 2.93	(40.00 – 51.00)
P2	30	49.17 ± 3.96	(41.00 – 55.00)
Total	90	49.72 ± 5.06	(40.00 – 61.00)
AptmPP			
E	30	53.93 ± 3.74	(45.00 – 63.00)
P1	30	46.03 ± 3.14	(41.00 – 52.00)
P2	30	49.62 ± 4.17	(40.00 – 57.00)
Total	90	49.86 ± 4.90	(40.00 – 63.00)
SnpUt			
E	30	37.20 ± 4.12	(29.00 – 46.00)
P1	30	33.37 ± 4.33	(25.00 – 43.00)
P2	30	35.50 ± 4.34	(26.50 – 46.00)
Total	90	35.36 ± 4.50	(25.00 – 46.00)
SnaSnpUt			
E	30	129.00 ± 7.92	(114.00 –142.00)
P1	30	125.45 ± 8.32	(111.00 –141.00)
P2	30	121.92 ± 6.19	(111.00 –135.00)
Total	90	125.46 ± 7.99	(111.00 –142.00)
SnaAPr			
E	30	144.37 ± 8.73	(125.00 – 164.00)
P1	30	141.70 ± 6.15	(132.00 – 160.00)
P2	30	138.72 ± 9.66	(118.00 – 155.00)
Total	90	141.59 ± 8.54	(118.00 – 164.00)
IPP			
E	30	107.23 ± 8.31	(88.00 – 121.00)
P1	30	113.57 ± 6.69	(93.00 – 125.00)
P2	30	113.23 ± 7.56	(101.00 – 130.00)
Total	90	111.34 ± 8.02	(88.00 – 130.00)
CoA			
E	30	94.25 ± 5.80	(84.00 – 108.00)
P1	30	86.37 ± 4.15	(79.00 – 95.00)
P2	30	90.92 ± 3.96	(80.00 – 96.00)
Total	90	90.51 ± 5.68	(79.00 – 108.00)
U6PP			
E	30	24.95 ± 2.54	(19.00 – 29.00)
P1	30	29.57 ± 1.47	(27.00 – 32.00)
P2	30	25.57 ± 1.92	(22.00 – 28.00)
Total	90	26.69 ± 2.87	(19.00 – 32.00)
SNA			
E	30	82.38 ± 4.05	(73.00 – 89.00)
P1	30	77.67 ± 4.29	(71.00 – 86.50)
P2	30	79.77 ± 3.14	(74.00 – 86.00)
Total	90	79.94 ± 4.28	(71.00 – 89.00)

SnaSnp – the lenght of the hard palate; AptmPP – the lenght of the maxillary corpus; SnpUt – the lenght of the soft palate; SnaSnpUt – the angle between the hand and soft palate; SnaAPr – the angle of inclination of the maxillary alveolar process; IPP – the angle of inclination of the upper front teeth; CoA – the effective maxillary length; U6PP – posterior maxillary alveolar hyperplasia; SNA – the angle of maxillary prognathism.

A statistically significant difference was found in the values of all the measured parameters between the groups of examinees, including maxillary parameters and inter-

maxillary sagittal and vertical relationships parameters (Table 2).

Table 2
Results of one-factor analysis of the variance for all the measured parameters

Parameter	F	P
SnaSnp	50.525	0.000
AptmPP	34.142	0.000
SnpUt	6.080	0.003
SnaSnpUt	6.633	0.002
SnaAPr	3.466	0.036
IPP	6.686	0.002
CoA	21.195	0.000
U6PP	46.006	0.000
SNA	11.247	0.000

For key to abbreviations see under Table 1.

SnaSnp showed the highest values in the eugnathic subjects, statistically significantly lower values in the patients with convergent type of mandibular prognathism and the lowest values in the patients with divergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), we recorded a highly statistically significant difference in SnaSnp values among the groups of examinees (F = 50.525; $p = 0.000$). A highly statistically significant difference in SnaSnp values between the group E (54.48 ± 3.48) and the groups P1 (45.50 ± 2.93) and P2 (49.17 ± 3.96) was found. Additionally, there was a highly statistically significant difference between the groups P1 and P2 (Table 3).

AptmPP showed the highest values in the eugnathic subjects, significantly lower values in the patients with convergent type of mandibular prognathism and the lowest values in the patients with divergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), a highly statistically significant difference in AptmPP values was recorded among the groups of examinees (F = 34.142; $p = 0.000$). A highly statistically significant difference in AptmPP values between the group E (53.93 ± 3.74) and the groups P1 (46.03 ± 3.14) and P2 (49.62 ± 4.17) was found. Additionally, there was a highly statistically significant difference between the groups P1 and P2 (Table 3).

SnpUt showed the highest values in the eugnathic subjects, significantly lower values in the patients with convergent type of mandibular prognathism and the lowest values in the patients with divergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), we recorded a highly statistically significant difference in SnpUt values among the groups of examinees (F = 6.080; $p = 0.003$). A highly statistically significant difference in SnpUt values between the group E (37.20 ± 4.12) and the group P1 (33.37 ± 4.33) was found, while a statistically significant difference between the group E and the group P2 (35.50 ± 4.34) was not established. Additionally, a statistically significant difference between the group P1 and the group P2 was not found (Table 3).

SnaSnpUt showed the highest values in the eugnathic subjects, significantly lower values in the patients with divergent type of mandibular prognathism and the lowest values in the patients with convergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), a highly statistically significant difference in SnaSnpUt values was recorded among the groups of examinees (F = 6.633; $p = 0.002$). A statistically significant difference in SnaSnpUt values between the group E (129.00 ± 7.92) and the group P1 (125.45 ± 8.32) was not found, while a highly statistically significant difference between the group E and the group P2 (121.92 ± 6.19) was measured. A statistically significant difference between the group P1 and the group P2 was not found (Table 3).

Table 3
Results of intergroup differences of the characteristics measured on the maxilla by Bonferroni test

Parameter	(J) Group	Average value difference	*p*
SnaSnp			
E	P1	8.98	0.000
E	P2	5.32	0.000
P1	P2	-3.67	0.000
AptmPP			
E	P1	7.90	0.000
E	P2	4.32	0.000
P1	P2	-3.58	0.001
SnpUt			
E	P1	3.83	0.002
E	P2	1.70	0.379
P1	P2	-2.13	0.168
SnaSnpUt			
E	P1	3.55	0.214
E	P2	7.08	0.001
P1	P2	3.53	0.218
SnaAPr			
E	P1	2.67	0.653
E	P2	5.65	0.030
P1	P2	2.98	0.505
IPP			
E	P1	-6.33	0.005
E	P2	-6.00	0.008
P1	P2	0.33	1.000
CoA			
E	P1	7.88	0.000
E	P2	3.33	0.022
P1	P2	-4.55	0.001
U6PP			
E	P1	-4.62	0.000
E	P2	-0.62	0.724
P1	P2	4.00	0.000
SNA			
E	P1	4.72	0.000
E	P2	2.62	0.031
P1	P2	-2.10	0.114

E – eugnathic examinees; P1 – divergent and P2 – convergent type of mandibular prognathism examinees; For key to abbreviations see under Table 1.

SnaAPr showed the highest values in the eugnathic subjects, significantly lower values in the patients with divergent type of mandibular prognathism and the lowest values in the patients with convergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), we recorded a highly statistically significant difference in SnaAPr values among the groups of examinees (F = 3.466; $p = 0.036$). A statistically significant difference in SnaAPr values between the group E (144.37 ± 8.73) and the group P1 (141.70 ± 6.15) was not found, while a statistically significant difference between the group E and the group P2

(138.72 ± 9.66) was measured. A statistically significant difference between the group P1 and the group P2 was not found (Table 3).

IPP showed the highest values in the eugnathic subjects, while the average values between the experimental groups were slightly different. Using one-factor analysis of variance (Table 2), a highly statistically significant difference in IPP values was recorded among the groups of examinees (F = 6.686; $p = 0.002$). A highly statistically significant difference in IPP values between the group E (107.23 ± 8.31) and the group P1 (113.57 ± 6.69) and P2 (113.23 ± 7.56) was found. A statistically significant difference between the group P1 and the group P2 was not found (Table 3).

CoA showed the highest values in the eugnathic subjects, significantly lower values in the patients with convergent type of mandibular prognathism and the lowest values in the patients with divergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), a highly statistically significant difference in CoA values among the groups of examinees (F = 21.195; $p = 0{,}000$). A highly statistically significant difference in CoA values was recorded between the group E (94.25 ± 5.80) and the group P1 (86.37 ± 4.15) and P2 (90.92 ± 3.96) was measured. Additionally, a highly statistically significant difference between the group P1 and the group P2 was found (Table 3).

U6PP showed the highest values in the eugnathic subjects, higher values in the patients with convergent type of mandibular prognathism and the highest values in the patients with divergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), a highly statistically significant difference in U6PP values was found among the groups of examinees (F = 5.125; $p = 0{,}008$). A highly statistically significant difference in U6PP values between the group E (85.97 ± 7.79) and the group P1 (91.52 ± 7.29) was found, but not between the group E and the group P2 (87.17 ± 5.99). Additionally, a highly statistically significant difference between the group P1 and the group P2 was found (Table 3).

SNA showed the highest values in the eugnathic subjects, significantly lower values in the patients with convergent type of mandibular prognathism and the lowest values in the patients with divergent type of mandibular prognathism. Using one-factor analysis of variance (Table 2), a highly statistically significant difference in SNA values was found among the groups of examinees (F = 11.247; $p = 0{,}000$). A highly statistically significant difference in SNA values between the group E (82.38 ± 4.05) and the groups P1 (77.67 ± 4.29) and P2 (79.77 ± 3.14) was found. A statistically significant difference between the group P1 and the group P2 was not found (Table 3).

Discussion

Many studies have proved a significant reduction of the linear dimensions and retroposition [8, 11, 12, 23] of the maxilla in most patients with Class III malocclusion. Therefore, Singh [5] and Singh et al. [8] in their great study on the aforementioned malocclusion, emphasized its unclear entity and etiology, so that it is almost impossible to classify it. Hence, for the purpose of this study, we selected patients with mandibular prognathism and on the basis of vertical parameters, and classified them into the devergent and convergent types. These vertical parameters change mostly during the growth period, as it has been demonstrated in many studies dealing with Class III malocclusion growth and development, thus their estimation influences stability of the obtained treatment results [21–25].

Taking into consideration gender differences presented by a number of authors, primarely regarding the linear cephalometric parameters in adult patients [24, 25], and their dynamics of changes during the growth period [13 21, 22], only male patients were examined in this study in order to provide homogeneous samples.

The middle part of the face, that is the maxilla, has always been mentioned as a possible etiologic factor of skeletal Class III. However, the importance of the relationship of maxillary retroposition towards the cranial base is still unclear, although it has been examined many times. While one group of authors argues that there is a clear correlation between them the other group refutes it [5]. We estimated the maxillary anteroposterior position towards the cranial base, based on the SNA angle and obtained the results showing that the SNA angle was significantly reduced and similar in the both groups. In most patients with mandibular prognathism, the maxilla is in retroposition and there is no difference in the degree of retroposition between the divergent and convergent type of this anomaly.

AptmPP, SnaSnp and CoA are parameters which showed significant differences between the divergent and convergent type of mandibular prognathism in this study. All the three parameters were significantly lower in both groups compared to the control group, while they were the lowest in the patients with divergent type of mandibular prognathism. Similar results for skeletal Class III were found by Miyajima et al. [26], Guyer et al. [11], Reyes et al. [13] and Chang et al. [25].

Upper front teeth protrusion and the whole alveolar process, also recorded in this study, is one of many compensatory mechanisms of the midfacial complex growth formed to overcome skeletal discrepancy [10, 13, 27, 28].

Many authors used cephalometric analysis to examine differences in the pharyngeal area and corresponding structures between hyperdivergent and normodivergent facial patterns [15, 16]. The general view is that the pharyngeal area in the hyperdivergent facial pattern is significantly more narrow, which is explained by the maxillary and mandibular retrusion and increased vertical growth. Abu Allhaija et al. [15] noted that the soft palate was significantly thicker in patients with mandibular prognathism than in eugnathic patients. Dostalova et al. [17] found the increased SnpUt and smaller SnaSnpUt in patients with acromegaly who have a significant elongation of the mandible. In our study, we found a significantly reduced SnpUt in the patients with divergent type of mandibular prognathism which is in accordance with the aforementioned picture of the hyperdivergent facial pattern described by Joseph et al. [16] and Abu Allhaija et al. [15] In patients with convergent type of mandibular prognathism, we recorded a smaller SnaSnpUt. Given the fact that in the pre-

vious study on mandibular prognathism we found a significant anteposition of temporomandibular joint (reduced GoArNS angle) with the mandible moved forward and a significantly larger SNB angle in the covergent type compared to the divergent type, the currently obtained result can be in line with it [18].

Measuring a distance from the mesial cuspid of the first permanent upper molar to the palatal plate (U6PP), it was quite larger in the divergent type of mandibular prognathism compared to the control group and convergent type, whereas there were no differences in the convergent type compared to the control group, which speaks in favour of the posterior mandibular hyperplasia in the divergent type. Reyes et al. [13] also noted the increase in the U6PP parameter in patients with skeletal Class III in all developmental phases from the age of 6 to 16, which can cause elongation of the front lower facial height later in life and compromise the results of early treatment. Additionally, the posterior maxillary hyperplasia was in a pronounced correlation with the anterior and posterior facial height and the angle between the basic jaw planes (B), but only in patients with divergent type of mandibular prognathism. Thus, it is crucial to estimate vertical components, especially in younger age, when they can be camouflaged by various compensatory mechanisms.

Besides highly demanding orthodontic-surgical treatment of sagittal discrepancies in patients with mandibular prognathism, more attention has recently been paid to vertical discrepancies, which many authors consider as key factors for relapse [27–29].

In order to achieve esthetically satisfying and long term stability results in patients, a surgical correction must provide a bite closing, reduction of the mandibular plane angle and the angle between the basic jaw planes, but also the correction of the posterior maxillary hyperplasia as a main condition for the stability of results [28–31]. In our study, the posterior maxillary hyperplasia was found only in patients with divergent type of mandibular prognathism. In most patients, an adequate surgical treatment must include a surgical intrusion of the posterior maxilla, which will correct posterior maxillary hyperplasia and increased angle between the basic jaw plates. The posterior maxillary intrusion will allow the mandible to rotate around its axis without ramus elongation during bite closing and thus reduce the angle between the basic jaw planes and lower facial height with temporary deterioration of mandibular protrusion. However, since sagittal split osteotomy of the mandible is also performed along with maxilla intruded in this way, it will be retruded without stretching of masticatory muscles. In this manner, the stability of surgical results is significantly increased [32, 33].

In order to achieve satisfying therapy effects, it is necessary to accurately estimate a degree of the abnormality manifestation, problems of localization and understanding of the bilogical potential.

Conclusion

The effective maxillary length, the length of the maxillary corpus and the length of the hard palate are significantly shorter in the patients with divergent facial type of mandibular prognathism compared to the patients with convergent type and also in both experimental groups of patients compared to the control one. The length of the hard palate is significantly shorter in patients with divergent type of mandibular prognathism compared to the control group, whereas the angle between the soft and hard palate is significantly smaller in the patients with convergent type of mandibular prognathism compared to the control group. In addition, there is a pronounced incisor dentoalveolar compensation of skeletal discrepancy in both groups of patients with mandibular prognathism in form of a significant upper front teeth protrusion, but without a significant difference among the groups, while the maxillary retrognathism is present in most patients of both experimental groups.

A pronounced posterior maxillary hyperplasia was found only in the patients with divergent type of mandibular prognathism.

The maxilla is certainly one of the key factors which contribute to making the diagnosis, but primarily to making a plan for mandibular prognathism treatment. Nevertheless, it contributes to the apperance of the aforementioned deformity in more variable way than other craniofacial components (cranial base, mandible), primarely due to its morphology, way and time of growth completion as well as numerous compensatory mechanisms.

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Computer and experimental analyses of the stress state in the cement hip joint endoprosthesis body

Računarska i eksperimentalna analiza naponskog stanja tela cementne endoproteze zgloba kuka

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Abstract

Background/Aim. One of the possible complications after implantation of a cement hip-joint endoprosthesis is fracture in the endoprosthesis body. Fractures arise from overload or material fatigue of which an implant is made. The purpose of this research was to define the intensity of maximum stress and the positions of a critical cross-section in the endoprosthesis body. **Methods.** Unilaterally changing forces which act on the hip joint during walking as well as the loads result in flexible deformations of the endoprosthesis body. Biomechanical analysis of the forces acting on the hip joint determine their direction and intensity, whereas on the basis of Gruen's classification of the endoprosthesis body loosening the level of fixation is established. The bodies of cement hip joint endoprosthesis are made of cobalt-chromium-molybdenum (CoCrMo) alloy, suitable for vacuum casting, are submitted to the analysis. Analysis of the critical stress in the endoprosthesis body was performed on the endoprosthesis body by means of the finite element method. The experimental verification of the obtained results was carried out on the physical prototype under laboratory conditions. **Results.** Computer analysis, by means of the finite element method, determined the stress state by calculation of the maximum Von Mises stress and critical cross-sections for different angles of the resultant force action. The results obtained by the computer and experimental method correlate and are comparable to the results of similar analyses conducted on various endoprosthesis types. **Conclusion.** The analyses described in the paper make the basis for improving the process designing of hip joint endoprostheses and their customization to each individual patient (custom made).

Key words:
arthroplasty, replacement, hip; fractures, stress; computer simulation.

Apstrakt

Uvod/Cilj. Jednu od mogućih komplikacija posle ugradnje cementne endoproteze zgloba kuka predstavlja prelom tela endoproteze. Prelomi nastaju kao posledica preopterećenja ili zamora materijala od koga je napravljan implantat. Cilj ispitivanja bio je da se odredi intenzitet maksmalnih napona i kritičnog preseka u telu endoproteze. **Metode.** Jednosmerno promenjive sile koje deluju u zglobu kuka prilikom hoda i opterećenja imaju za rezultat elastične deformacije tela endoproteze. Biomehaničkom analizom sila koje deluju u zglobu kuka određuju se njihov pravac i intenzitet, a na osnovu Gruenove raspodele razlabavljenja tela endoproteze i nivo uklještenja. Ispitivana su tela cementne endoproteze zgloba kuka izrađene od legure kobalt-hrom-molibden (CoCrMo) pogodne za livenje u vakumu. Analiza kritičnih napona u telu endproteze izvedena je na telu endoproteze metodom konačnih elemenata. Eksperimentalna verifikacija dobijenih rezultata sprovedena je na fizičkom prototipu u laboratorijskim uslovima **Rezultati.** Računarskom analizom pomoću metode konačnih elemenata određeno je naponsko stanje kroz izračunavanje maksimalnih Von Misses-ovih napona i kritični preseci za različite uglove delovanja rezultantne sile. Rezultati dobijeni računarskom i eksperimentalnom metodom u korelaciji su i uporedivi su sa rezultatima sličnih analiza na različitim tipovima endoproteza. **Zaključak.** Istraživanja opisana u radu predstavljaju osnovu za usavršavanje procesa projektovanja endoproteza zgloba kuka i njihovo prilagođavanje svakom bolesniku posebno (*custom made*).

Ključne reči:
kuk, artroplastika; prelomi usled zamora; simulacije; kompjuterske.

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Introduction

The primary aim of contemporary orthopedics and bone and joint traumatology is to establish the function and eliminate pain in injured or degeneratively changed joints. As far as the hip joint is concerned, establishing its function usually implies installation of implants, i.e. an artificial joint.

Implantation of a hip joint endoprosthesis is the most widespread and most successful surgical procedure in orthopedic surgery, which is every year performed on 800,000 to 1,000,000 patients [1]. The success of this procedure relies on a great number of factors among which the most significant include: surgical technique, material, endoprosthesis properties and general mental and physical state of the patient [2].

Complications which may arise after a hip joint endoprosthesis implantation, and which require revision, may be classified into early complications including luxation and infection and the late ones comprising aseptic loosening, dislocation, bone tissue fractures in the endoprosthesis zone and endoprosthesis fractures.

The occurrence of fractures in the hip joint endoprosthesis body, as a complication, accounted for 1.3% of all complications due to which the revision had to be performed after the endoprosthesis implantation in the period which was analyzed from 1979 to 2010 [3]. It occurs due to material fatigue or overload and is the result of a number of factors. These include as follows: the occurrence of high stress exceeding the limits of the material strength, mechanical properties of the endoprosthesis' material, aseptic loosening and endoprosthesis geometry [4]. In order to decrease the occurrence of endoprosthesis fractures, frequent analyses are undertaken aimed at defining the load values leading to fractures as well as the zones in which this phenomenon occurs. Theoretical and experimental analyses of the intensity and distribution of load in the endoprosthesis body are demonstrated in the papers by Katouzian and Davy [4] and Bennet and Goswami [5], in the femur in the papers by Weinans et al. [6] and Schileo et al. [7], whereas in the assembly, which is the result of endoprosthesis implementation in the femur, is shown in the paper by Peters et al. [8].

In this paper the results of computer and experimental analyses of the BB2 type cement endoprosthesis are given. The principal aim of the analysis was to improve the processes of designing hip joint endoprosthesis and customize them to a specific patient.

Methods

Analysis of the stress state was conducted and the critical cross-section of the endoprosthesis body defined in the conditions of maximum load during monopedal leg support. Theoretical analyses were conducted by simulating the endoprosthesis' behaviour through the application of the finite element method in the product development software CATIA v5R21. Experimental analyses were implemented in the laboratory for material testing.

Hip joint endoprosthesis biomechanical loads

The task of a hip joint endoprosthesis is to shift the load occurring in the hip joint due to the mass of upper body part (torso) and additional loads to the lower extremity. The maximum loads in the state of quiescence occur when the body is supported by one leg (monopedal support) [9]. In that case, the external force, G_1 , (Figure 1) resulting from the weight of the upper body and unsupported lower extremity, as well as from the internal muscular force, ($\vec{M}$), acts on the hip joint. The value of $\vec{G}_1$ force is around 80% of the body weight - G_1 (equation 1).

$$G_1 = 0.8G \tag{1}$$

The direction and intensity of the resultant force (R) acting in the centre of the hip joint rotation was determined by adding up the vectors of external (G_1) and internal (M) forces, (equation 2).

$$\vec{R} = \vec{M} + \vec{G}_1 \tag{2}$$

On the basis of the steady state (3) and geometrical requirement (4), the intensity of the muscle force $\vec{M}$ (6) as well as of the resultant force $\vec{R}$ (7) was defined.

$$\vec{M} \cdot \vec{r} = \vec{G}_1 \cdot \vec{l} \tag{3}$$
$$l = 2 \cdot r \tag{4}$$
$$\vec{M} \cdot \vec{r} = \vec{G}_1 \cdot 2\vec{r} \tag{5}$$
$$\vec{M} \cdot \vec{r} = 2 \cdot \vec{G}_1 \tag{6}$$
$$\vec{R} = 3 \cdot \vec{G}_1 \tag{7}$$

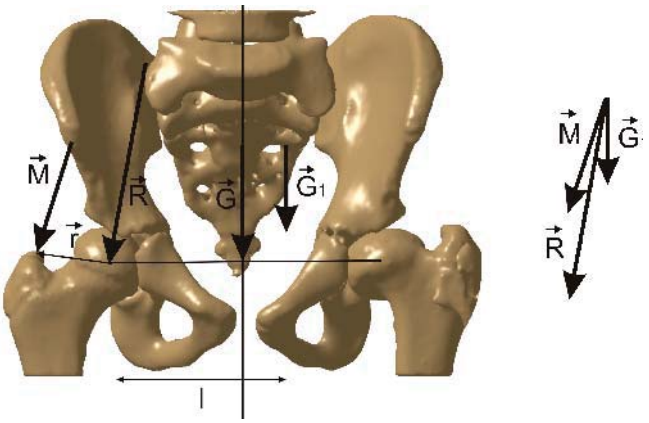


Fig. 1 – Distribution of load in monopedal support [9].

In motion, dynamic forces which occur in specific phases of the step are variable and significantly greater than the static ones. For the body mass of 100 kg the reduced maximum load of the hip joint in motion is around 4,000 N [9]. Figure 2 shows the load variations on the hip joint in specific phases of the step.

Hip joint endoprosthesis body modeling

Computer models obtained by discretisation of geometric models generated in the computer aided designing software's (CAD) [10] constitute the basis for analyses by means

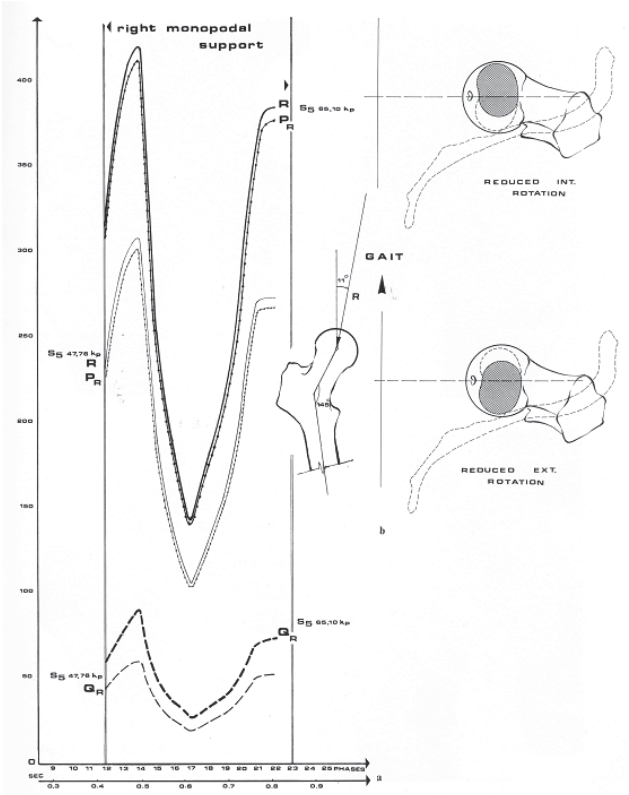


Fig. 2 – The hip load variations in different phases of the step [9].

of the finite element method, which results in creation of the mesh of three-dimensional finite elements. Figure 3 shows the geometric (3a) and discretized (3b) model of the hip joint endoprosthesis.

from the biomechanical analysis, whereas the experimental part of the analysis was conducted on the physical endoprosthesis model.

In computer simulation the properties of the material of which the physical model of the endoprosthesis body BB2 was made was adopted Cobalt-Chromium-Molybdenum (Co-Cr-Mo) alloy (ISO 5832/IV 1978) which is treated as a linear isotropic material in FEM analyses whose mechanical properties are shown in the Table 1.

Table 1
Mechanical properties of the Co-Cr-Mo material

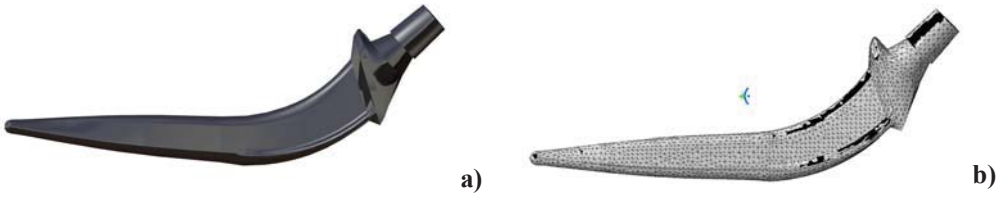
Parameters	Values
Flexibility module (MPa)	211
Paoson coefficient	0.33
Yield limit, $R_{0.2}$ (MPa)	680
Tensile strength, R_m (MPa)	720

Co-Cr-Mo – Cobalt-Chromium-Molybdenum

In the maximum stress analysis the most unfavourable case was adopted as a high fixation when, according to Gruen's zones of loosening, the endoprosthesis body was fixed in the zones 3, 4, and 5 (Figure 4a), that is, 1/3 of the height distally. Figure 4b shows constraints in the discretised model of the endoprosthesis body.

Computer analysis of the stress state (static behaviour) of the endoprosthesis body

In the phase of simulation of exploitation conditions the forces and constraints obtained by biomechanical analysis of the human locomotor system was defined. These included as follows: the force intensity of 4,000 N acting on the surface of the conical element of the endoprosthesis' proximal part



a) **b)**

Fig. 3 - Models of the hip joint endoprosthesis body: a) geometric, b) discretised

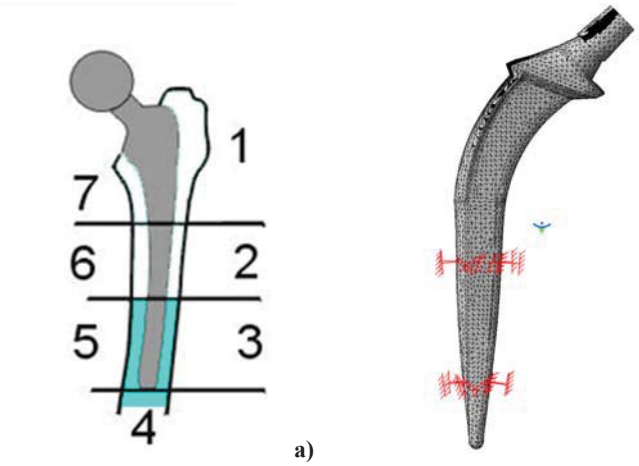
To define the geometric model of the endoprosthesis body the appropriate modules of the CAD software Dessault Systems Catia V5r21 were used for surface (Generative Shape Editor) and solid modeling (Part Modeling), whereas for discretisation and analysis of the static behaviour the Generative Structural Analysis module was employed. The discretised model consists of solid isoparametric finite elements of hexahedron of the 2 mm side length, thus having the endoprosthesis body circumscribed by the mesh of 26971 of finite elements.

Maximum stress analysis

Maximum stress analysis of endoprostheses was performed by means of computer simulation of the static behavior of endoprosthesis and experimental verification of the obtained results. Simulation has been carried out by employing the finite element method for the conditions resulting under the angle of α[7] (Figure 5). The femoral inclination angle (as well as of the anatomical endoprosthesis axis) is 6° in relation to the central axis. The degree of fixation equals 1/3 of the endoprosthesis body height distally. The variable value in the analysis is the resultant force action angle ranging from 15–20°. The analysis examined the position and intensity of maximum Von-Mises stress on the endoprosthesis body.

Analysis of the stress in the endoprosthesis body

The experimental definition of the stress in the endoprosthesis body was performed under laboratory conditions on the physical model cast in vacuum using the Cr-Co-Mo alloy. Stress measurements were carried out by means of strain gauges LY 1 × 3/120, manufactured by HBM, the one of which was placed on the lateral surface at the point maximum stress according to the computer analysis, and the two



a) b)

Fig. 4 – Fixation of the endoprosthesis body according to: a) Gruen zones of loosening; b) the computer model constraints.

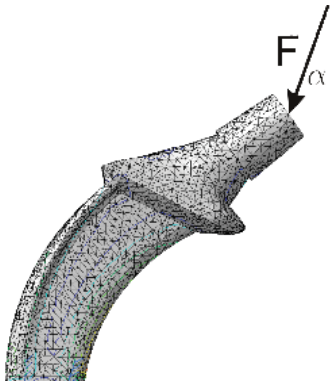


Fig. 5 – Direction of the load action in the analysis of the static behaviour.

of them on the lateral sides at the same level. The physical model was fixed by epoxy resin in the metal holder at 1/3 of the height distally. At the tensile strength testing machine (Figure 6) the resulting load was 4,000 N with the action direction corresponding to the direction angle of the resultant force action of 20°.

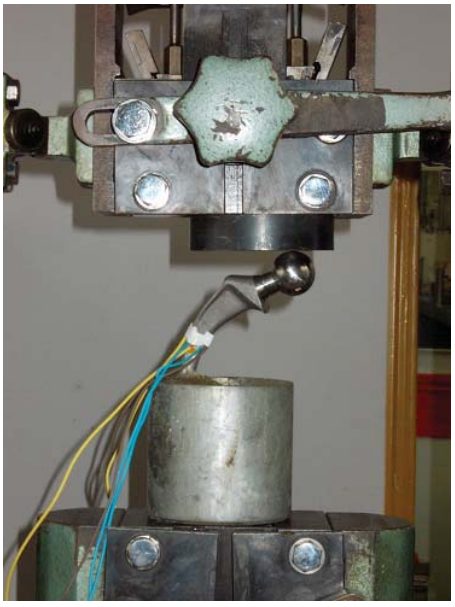


Fig. 6 – Experimental stress analysis.

Measurement of the stress variations resulting from the endoprosthesis body deformations was made by means of the corresponding DMS acquisition device, while the extent of deformations was measured by a displacement sensor of HBM W103 type.

To determine the real values of boundary stress at the critical cross-section of the hip joint endoprosthesis body, the samples were cut out of the physical model and a test specimen made (Figure 7) and subject to tension up to the point of tearing using the tensile strength testing machine.

Fig. 7 – A test specimen resulting from cutting the physical model out.

Results

Analysis of the stress state of the endoprosthesis body, under the set conditions, by applying the finite element method proved that the maximum Von Mises tension stress appears in the dorsal surface of the endoprosthesis body.

Figure 8 shows the position of the maximum Von Mises stress within the critical cross-section zone. The spectral diagram in the same figure demonstrates numerical stress values depending on the color.

On the basis of analyses the resulting maximum equivalent stress is 449 MPa (Table 2), which was significantly lower than the yield stress for Co-Cr-Mo alloy (720 MPa) according to the Table 1). Therefore, it was concluded that the reductions in the load action angle would not lead to exceeding the maximum acceptable stress values for the given material of the hip joint endoprosthesis body, but to significantly influence the stress increase at the critical cross-section. In all the conducted analyses the critical cross-sections were nearly in the same position (Figure 8, marked by a circle) coinciding with the common place for endoprosthesis' fractures (Figure 9). Decreasing the angle of the resultant force action direction results in increasing the stress at the critical cross-section (along with the linear dependency), which is evident in Figure 10. This means that if the endoprosthesis is placed in "Varus" position, implemented femoral head with longer neck or increased "offset", the angle of the resultant force action direction decreases while the stress at the critical cross-section increases.

Table 2

Maximum equivalent Von Mises stress

Load angle α (°)	Maximum equivalent Von-Mises stress, σ_{ekv} (MPa)
15	449.5
16	431.7
17	413.8
18	395.8
19	377.7
20	359.4

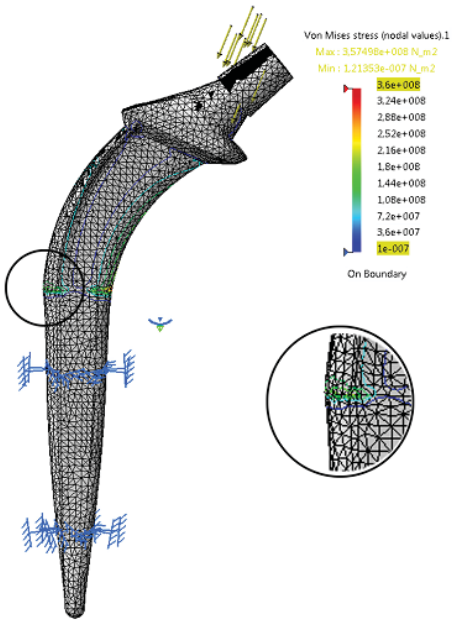


Fig. 8 – Equivalent Von Mises stress.

Fig. 9 – Endoprosthesis body fractures due to material fatigue.

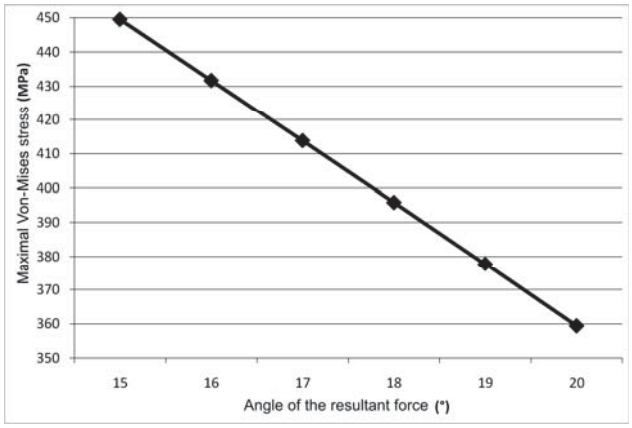


Fig. 10 – Dependency of the stress at the critical cross-section on the force action angle.

Based on experimental measurement of the strain gauges dilatation in critical zones, it may be established that the stress on the dorsal endoprosthesis surface at the critical cross-section was linearly increasing with the increased load, while it remained constant on the lateral sides, corresponding to the stress state of the computer model obtained by the FEA method application. By conversion of the obtained results on the basis of common relations for Hooke's law, the maximal stress on the endoprosthesis body was calculated (420 MPa).

Experimental analysis on the tensile strength testing machine determined the yield strength and tensile strength of the material. The resulting values were $R_{p0,\cdot 2}$ = 652 MPa for the yield strength and R_m = 695 MPa for the tensile strength.

Discussion

Evaluation of the achieved results may be performed through analysis and critical reflection on the results obtained by simulation of the behaviour of the endoprosthesis computer model by means of the finite element method and experimental verification.

In accordance with vector analysis of the composition of forces acting in the hip joint under the static conditions for the monopedal support, the resultant force intensity ($\vec{R}$) acting in the hip joint is $4 \cdot G_1$ while G_1 is the load resulting from the torso mass and the mass of the leg which is in the air. The total load in this case is $3 \cdot 0.8 \cdot G$, i.e. approximately 2,400 N. Under the dynamic conditions, the maximum value of the resultant force acting in specific

phases of the step is where G is the body mass weight. The higher value was used as a referential parameter in the computer model analysis and during the laboratory verification with the assumption that the patient body mass is 100 kg (as well as the resultant force intensity of 4,000 N).

Simulation of the endoprosthesis body's behaviour under exploitation conditions points to the fact that the maximal Von-Mises stress on the endoprosthesis body is significantly lower than the acceptable stress of Cr-Co-Mo alloy which is used for manufacturing of BB2 endoprostheses. The resulting values (360–450 MPa) match the values obtained by means of the finite element method, for the six endoprostheses types (150–600 MPa) [5]. The indicated differences in numerical values also result from the properties of the material used for the endoprosthesis manufacturing. In the computer model the material properties' values were used from the relevant literature and they differ according to the experimental measurements and in the specific example are lower by around 3.6%. In addition, the critical cross-section zones in BB2 endoprosthesis also coincide with the ones in the mentioned paper and corresponded to the point where the force acting on the endoprosthesis established the maximum bending moment [11].

A significant correlation between these factors was on the basis of impact analysis of the change of the resultant force action direction angle on the stress state at the critical cross-section.

The results of experimental measurements of the maximum stress intensity (420 MPa) are within the boundaries of the results obtained by the finite element method (deviations are within the range of 1.5–14.4 % depending on the load action angle). The measured values are significantly lower than the values of the material properties of which the physical model is made, which is also supported by experimental analysis of the material. The noted deviations in the results obtained by the theoretical and experimental analysis arise, on one hand, from the difference between the real data and those found in the literature on material properties and on the other, from the objective effects in experimental analyses including as follows: the dimensions of the used strain gauges (strain gauges are significantly larger than the maximum stress zone which leads to errors in defining the stress), bone cement [12] and the problem of precise positioning of the endoprosthesis during measurements (load angle error).

Future analyses should also encompass the effects of bone cement on the connection between the endoprosthesis and the femur, as well as the mechanical properties of the bone tissue itself [4, 6].

Conclusion

The results obtained in this study support the hypothesis that the application of theoretical analyses of endoprostheses by means of the finite element method is a reliable alternative to experimental analyses. The results of the finite element method application have a significant role in defining the shape and selection of the implant material, both from the medical and engineering point of view. From the orthopedic point of view, it is possible to analyse the effect of the surgical technique used for the endoprosthesis implantation. The computer model offers an opportunity to analyse the impact of various load actions on the endoprosthesis. In contrast, the application of these methods in the development of endoprostheses enables engineers to optimize the shape and dimensions of endoprostheses according to the requirements of surgical method, the type of disease and the patient himself/herself.

Acknowledgments

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ORIGINAL ARTICLE

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BCL10 aberations and NF-kappa B activation involving p65 are absent or rare in primary gastric MALT lymphoma

BCL10 aberacije i aktivacija p65 gena NF-kappa B puta su odsutne ili retke u primarnom MALT limfomu želuca

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Abstract

Bacground/Aim. Mucosa-associated lymphoid tissue (MALT) lymphoma accounts for 5–17% non-Hodgkin lymphomas (NHL). The molecular pathogenesis of MALT lymphomas is not well-established. The aim of this study was to evaluate immunohistochemically determined nuclear coexpression of BCL10 and NF-kappaB (NF-κB) in tumor cells of gastric MALT lymphoma and its impact on the patogenesis and outcome of the disease. **Methods.** Medical records of 35 patients with newly diagnosed gastric MALT lymphoma were analyzed and biopsy specimens were immunostained for BCL10 and NF-kB expression (p65 subunit). **Results.** The median age of 35 patients diagnosed with gastric MALT lymphoma was 63.5 years (male/female = 21/14). Symptoms were present in 23/35 (65.7%) patients with the weight loss as the most common symptom. Gastric MALT lymphomas were usually localized in the stomach corpus and corpus and antrum (45.7% and 31.2%, respectively). *H. pylori* infection was confirmed in 20 out of 30 (66.7%) patients. Treatment options were as follows: immunochemotherapy in 10 (28.5%) patients, surgery in 9 (25.8%) patients, combined surgery and chemotherapy in 14 (40%) patients and supportive measures in 2 (5.7%) patients. Complete remission was achieved in 13 (37.1%) patients and partial remission in two (5.7%) patients. Sixteen (45.7%) patients had disease progression ($p < 0.001$). Cytoplasmatic expression of BCL10 in tumor cells was detected in 19 (54.3%) specimens. Nuclear expression was detected in no specimen. Cytoplasmic expression of NF- κB was present in 22 (65.7%) specimens, but nuclear expression was not detected in any specimens. **Conclusion.** Nuclear expressions (activation) of NF-κB p65 subunit and BCL10 were not detected in specimens of gastric MALT lymphoma. The correlation of nuclear coexpression of BCL10 and NF-κB in gastric MALT lymphoma was not established. These results indicate that other mechanisms and signal pathways are active in lymphogenesis of gastric MALT lymphoma, as that apoptotic inhibition is not the main, nor the only mechanism in tumorogenesis.

Key words:
lymphoma, b-cell, marginal zone; stomach neoplasms; immunohistochemistry; gene expression; signal transduction.

Apstrakt

Uvod/Cilj. Limfomi limfnog tkiva pridruženog mukozi (MALT) čine 5–17% svih non-Hodgkin limfoma (NHL). Molekularna patogeneza MALT limfoma nije razjašnjena. Cilj ove studije bio je da se imunohistohemijski utvrdi prisustvo nuklearne koekspresije BCL10 i NF-kappaB (NF-κB) u tumorskim ćelijama želudačnog MALT limfoma kao i njihov uticaj na patogenezu i ishod bolesti. **Metode.** Analizirani su klinički podaci iz istorija bolesti 35 bolesnika sa novodijagnostikovanim želudačnim MALT limfomom i uzorci tkiva biopsije želuca bojeni su imunohistohemijskom metodom na ekspresiju BCL10 i NF-κB. **Rezultati.** Prosečna starost kod 35 bolesnika sa dijagnozom želudačnog MALT limfoma iznosila je 63,5 godina (muškarci/žene = 21/14). Simptomi su bili prisutni kod 23/35 (65,7%) bolesnika sa gubitkom težine kao najčešćim simptomom. Želudačni MALT limfom bio je najčešće lokalizovan u korpusu i korpusu i antrumu (45,7% i 31,2%). Infekcija *H. pylori* bila je potvrđena kod 20 od 30 (66,7%) bolesnika. Bolesnici su lečeni imunohemioterapijom (10 bolesnika, 28,5%), hirurški (9 bolesnika, 25,8%), kombinacijom hirurgije i hemioterapije (14 bolesnika, 40%) i suportivno (2 bolesnika, 5,7%). Kompletna remisija bila je postignuta kod 13 (37,1%) bolesnika, a parcijalna remisija kod dva (5,7%) bolesnika. Kod 16 (45,7%) bolesnika došlo je do progresije bolesti ($p < 0,001$).

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Citoplazmatska ekspresija BCL10 bila je nađena kod 19 (54,3%) tumorskih ćelija. Nuklearna ekspresija nije uočena ni u jednom uzorku. Citoplazmatska ekspresija NF-κB bila je prisutna u 22 (65,7%) uzorka, ali nuklearna ekspresija nije potvrđena ni u jednom uzorku. **Zaključak.** Nuklearna ekspresija (aktivacija) p65 subjedinice NF-κB puta i BCL10 nije otkrivena u uzorcima želudačnog MALT limfoma. Korelacija nuklearne koekspresije BCL10 i NF-κB u tumorskim ćelijama želudačnog MALT limfoma nije utvrđena. Ovi rezultati ukazuju da su neki drugi mehanizmi i signalni putevi aktivni u limfogenezi želudačnog MALT limfoma i da inhibicija apoptoze nije glavni i jedini mehanizam tumorogeneze.

Ključne reči:
limfom, malt; želudac, neoplazme; imunohistohemija; geni, ekspresija; signali, transdukcija.

Introduction

Mucosa-assciated lymphoid tissue (MALT) lymphomas are defined as extranodal B-cell lymphomas of marginal zone that originate from lymphatic tissue associated with mucosal and glandular epithelium [1]. MALT lymphomas account for 5–17% of non-Hodgkin lymphomas (NHL) [2]. They can occur in any organ, most commonly in the stomach, lungs, salivary glands and thyroid gland. MALT lymphoma of the stomach account for about 40% of all primary gastric NHL [3]. They predominantly occur between 50 and 60 years of age and there is no gender preponderance [4]. Morphologically, they are present as multifocal gastric lesions usually accompanied with gastrointestinal symptoms. At presentation, the disease is localized and usually have indolent course with histological transformation to large-cell lymphoma in 10% of cases later on [2]. The 5-year survival is between 95% and 85% [5]. Association with *H. pylori* infection in 90% of gastric MALT lymphomas was proven [6, 7]. It is assumed that malignant transformation of B lymphocytes is caused by chronic antigenic stimulation with *Helicobacter* infection. As a result of direct and indirect (T-cells specific for *H. pylori*) antigen stimulation B cells proliferate and can, at times, undergo a neoplastic transformation following the acquisition of genetic abnormalities [3]. In more than half of patients with MALT lymphomas structural cytogenetic abnormalities with translocation t (11; 18), t (1; 14), t (14; 18) are described, while the most common numeric cytogenetic abnormality is trisomy 3. These translocations result in the generation of the novel fusion proteins, aberrant nuclear BCL10 expression and activation of the nuclear factor kappaB (NF-κB) pathaway. NF-κB induction appears to drive antigen independent growth of lymphoma cells.

BCL10 activates NF-κB transcription factor and inhibits apoptosis. Binding beetween BCL10 and MALT1 is crucial for the oligomerisation and self-activation of MALT1, which leads to NF-κB activation [3]. Physiologicaly, BCL10 is found to be abundant in the cytoplasm of B lymphocytes in germinative center and moderately expressed in the cytoplasm of B lymphocytes of the marginal zone of follicules. An ectopic, nuclear localization of BCL10 was observed in some cells of gastric MALT lymphoma. The degree of BCL10 expression correlates to the type of cytogenetic abnormality. In gastric MALT lymphoma with t (11; 18) nuclear BCL10 expression is usually moderate; in t (1; 14) nuclear expression of BCL10 is prominent; while t (14; 18) is characterized with cytoplasmatic expression of BCL10 [8].

NF-κB is a dimeric transcription factor that belongs to the family of Rel (reticuloendotheliosis) proteins, composed of five proteins (p50, p52, p65, c-Rel, RelB). NF-κB is predominantly located in the cytoplasm of the cells as inactive cytoplasmic complex with the inhibitor κB (IκB) [9]. After phosphorylation of IκB, NF-κB is released from the complex NF-κB/IκB and goes into the nucleus, where it controls the transcription of various genes that play a key role in the regulation of many cellular processes such as inflammation, proliferation, immunity, angiogenesis and apoptosis [10]. NF-κB signaling pathway is activated after stimulation of cell proinflammatory cytokines, mitogens, growth factors, antigens, oxidative stress triggers and intercellular contact and plays an important role in the development of various tumors. Activity of NF-κB pathway in tumor cells is assesed on the basis of the nuclear localization of its subunits (commonly p50, p52 and p65).

The aim of this study was to determine nuclear coexpression of BCL10 and NF-κB in tumor cells of gastric MALT lymphoma and its impact on the patogenesis and outcome of the disease.

Methods

We analyzed medical records of 35 patients with newly diagnosed gastric MALT lymphoma between January 2001 and July 2007. The study was conducted in retrospective-prospective manner. The patients were followed until March 2010. The study included 35 patients: 21 male and 14 female. The median age was 63.5 years (range 35–77 years). There were 17 (48.6%) patients younger than 65 years and 18 (51.4%) patients older than 65 years. Clinical characteristics of patients were sumarized in Table 1. Histopathologic diagnosis was made according to the Revised European American Lymphoma/World Health Organization (REAL/WHO) classification. The presence of *H. pylori* in gastric mucosa was determined by standard Geimsa staining of biopsy sample.

The immunoexpression of BCL10 and NF-κB was determined in biopsy tissue samples of gastric MALT lymphomas by standard avidin-biotin-streptavidin immunohistochemical method. The expression of NF-κB in tumor cells of gastric MALT lymphoma was determined by using monoclonal antibody p65 NF-κB (Thermo Vision Corporation; dilution 1 : 50). The expression of BCL10 was determined by using monoclonal antibody BCL10 (DAKO; dilution 1 : 25). Paraffin embedded normal lymphoid tissue was used as a positive control.

Table 1

Clinical features of patients with gastric MALT lymphoma (χ^2-test)

Patients caracteristics	n	%	*p*
Age (years)			
< 65	17	48.6	0.866
> 65	18	51.4	
Gender			
male	21	60	0.237
female	14	40	
B symptoms			
yes	12	33.3	0.063
no	23	65.7	
Karnofsky index			
> 60%	30	85.7	0.0001
< 60%	5	14.3	
Disease symptoms			
epigastric pain	25	71.4	
epigastric pain + melena	8	22.9	0.001
peritoneal effusion	1	2.9	
without symtoms	1	2.9	
Disease dissemination			
yes	21	60	0.237
no	14	40	
Macroscopic tumor pattern			
ulceration	24	68.6	0.0001
polypus	3	8.6	
diffuse-infiltrative pattern	8	22.9	
H. pylori infection			
positive	20	57.4	0.007
negative	10	28.1	
not done	5	14.3	

MALT – Mucosa-associated lymphoid tissue.

BCL10 immunoreactivity was interpreted as positive or negative, as well as cytoplasmic, cytoplasmic and nuclear, or nuclear. Staining intensity was graded as weak, moderate or strong. Approximate cutoff of ≥ 20% was used to classify tumors as having weak and strong expression. NF-κB p65 immunoreactivity was categorized as cytoplasmic (inactive) or nuclear staining (active). Positive staining patterns were claimed if protein expression was detected in more than 10% of the cells.

A treatment response was evaluated a month after the therapy completition. Data were summarized by frequency and percentage for categorical variables. For continuous variables, the medians and ranges were computed. Statistical tests were 2-sided at the 5% level of significance. Univariate analysis using the nonparametric Wilcoxon rank-sum test or the Kruskall-Wallis rank-sum test when appropriate were performed to investigate the association between continuous variables and categorical variables. Statistical analyses were performed by using statistical package SPSS (version 11.5 for Windows).

Results

The most common symptom at presentation was epigastric pain registered in 25 (71.4%) patients ($p < 0.001$) (Table 1). Epigastric pain associated with gastrointestinal bleeding was registered in additional 8 (22.9%) patients. B symptoms were present in 23 (65.7%) patients with weight loss as the most common ($p < 0.063$). MALT lymphoma was usually localized in the gastric corpus (16 or 45.7% patients). The corpus and antrum were often simultaneously affected (11 or 31.2% patients; $p < 0.001$). Tumor lesions usually appeared as macroscopic ulcerations (24 or 68% patients), diffuse infiltrative growth was seen in 8 (22.9%) patients whilst polypoid growth pattern was seen in only 3 (8.6%; $p < 0.0001$) patients. Giemsa staining confirmed *H. pylori* infection in 20 out of 30 tested patients (histochemical analysis was not done in 5 specimens due to technical reasons) (Table 1). The most of the patients (14 or 40%) were in clinical stage IV according to the Lugano staging system while 8 (22.9%) were in clinical stage I and 13 (37.1%) in clinical stage II at the time of presentation. The most of the patients (21 or 60%) had disseminated disease at presentation. Disseminated disease was defined as the presence of multifocal lesions or as nonmucosal organs infiltration (distant lymph node, bone marrow, spleen, liver, pleura) together with the presence of the disease in one MALT tissue. The patients were treated as follows: immunochemotherapy in 10 (28.5%) patients, surgery in 9 (25.8%) patients, combined surgery and chemotherapy in 14 (40%) patients. *H. pylory* eradication in combination with chemotherapy was performed in 2 (5.7%) patients and supportive measures in 2 (5.7%) patients. Complete remission was achieved in 13 (37.1%) of the patients and partial remission in two (5.7%) patients. Sexteen patients had disease progression (45.7%; $p < 0.001$). There was no reliable data on treatment outcome in 4 patients (uncomplete medical records). The most of the patients (8 out of 13) achieved a complete remission when treated with a combined modality (surgery and chemotherapy). There was no significant difference in treatment modality among patients with progressive disease ($p > 0.05$).

The results of immunoexpression in tumor specimens of gastric MALT lymphoma are showed in Table 2.

BCL10 cytoplasmic expression was detected in 19 (54.3%) biopsy specimens: 15 had moderate, three low and one prominent cytoplasmic positivity (Table 2). In 16 (45.7%) specimens, BCL10 expression was not found in the nucleus, nor in the cytoplasm. BCL10 nuclear expression was found in no specimen.

Table 2

Immunoexpression in tumor specimens of gastric MALT lymphoma

Cytoplastatic staining	BCL10 (%)	NF-κB (%)
Positive	54.3	65.7
Negative	45.7	34.3

MALT – Mucosa-associated lymphoid tissue.

NF-κB cytoplasmatic expression was found in 22 (65.7%) patients (Tables 1 and 2). In four (11%) patients cytoplasmic and nuclear expressions of NF-κB were positive, but nuclear expression was present in less than 10% of cells, which is not considered significant in terms of activity of this transcription factor. In other words, cytoplasmic activity means the presence, but not the activity of NF-κB transcriptional factor.

Since nuclear expression of BCL10 or NF-κB was not found it could be concluded that some other mechanisms and signal pathways are active in lymphogenesis of gastric MALT lymphoma. So, the found cytoplasmic expression was not correlated to clinical patients features.

Discussion

The patients in this study diagnosed with gastric MALT lymphoma were mostly older than 50 years, with good performance score as alredy reported [5]. In contrast to recent tendencies (in developed health systems) to diagnose the disease at an early stage, gastric lymphoma in our patients was usually diagnosed at an advanced stage. Also, according to literature data, gastric MALT lymphoma is most frequently localized in the gastric antrum and presented as multiple ulcerations, but in our patients lymphoma was localized in the corpus and presented mostly as ulcerative lesions. Therapeutic approach in patients with gastric MALT lymphoma is controversial and not standardized. Our patients were first seen by the surgeon, since the main symptom at presentation was abdominal pain. Therefore the most common therapeutic approach was surgery and the reason while eradication therapy for *H. pylory* was not common.

The molecular pathogenesis of MALT lymphomas arising in the gaster is not well-established, but it is known that malignant transformation disrupt the cell signaling pathway and allows its autonomous behavior. As shown by others, at least three of the chromosomal translocations were identified in MALT lymphomas [t (11; 18), t (14; 18), and t (1; 14)] and result in deregulation of BCL10 and downstream activation NF-κB pathway [11, 12].

We used BCL10 immunostaining to indirectly assess for MALT lymphoma-associated translocations since BCL10 immunostaining has been reported to show characteristic expression patterns that correlate with the presence of specific translocations. In MALT lymphomas carrying the t (1; 14), BCL10 is predominantly and strongly expressed in the tumor cell nuclei. In MALT lymphomas with the t (11; 18) or t (14; 18), BCL10 is moderately expressed in the nucleus or strongly expressed in the cytoplasm of tumor cells, respectively [8, 12, 13]. However, nuclear expression of BCL10 can be found in up to 50% MALT lymphomasa without specific translocation [14, 15]. Therefore, the significance of nuclear expression of BCL10 in lymphogenesis stil remains unexplained.

In 16 (45.7%) samples of gastric MALT lymphoma in this study, immunohistochemical BCL10 staining was not detected either in cytoplasm or nucleus, suggesting that it is not the central mechanism responsible for lymphogenesis. Beside ectopic nuclear localization of BCL10 an altered function of mutant forms of BCL10 protein could be also responsible for lymphogenesis. However, mutated forms of BCL10 protein are difficult to detect using immunohistochemical metod [16]. Thus, although BCL10 immunostaining can be used as an initial screen for the t (11; 18) in MALT lymphomas, the presence of BCL10 nuclear expression should not be used as a surrogate for the presence of the t (11; 18). Nuclear expression of BCL10 has prognostic significance since it is a common feature of disseminated forms of gastric MALT lymphoma [15, 17]. Disseminated forms are associated with structural cytogenetic aberrations as t (1; 14) and t (11; 18) and usually without the effect of *H. pylori* eradication therapy) [8, 18]. However, 60% in our study group presented with disseminated form of the disease, but none of the patients had nuclear expression of BCL10.

Immunohistochemical staining was also performed to evaluate the expression of p65 subunits of NF-κB. In many different tumor types, and in some gastric and ocular adnexal MALT lymphomas, the evidence of NF-κB activation has been shown in a subset of cases [19]. As the p65 subunit is involved in many activated forms of NF-κB, immunohistochemical detection of nuclear p65 staining is used as evidence of NF-κB activation [20]. In all the studied cases, the staining pattern was only cytoplasmic, and therefore negative, suggesting that NF-κB is inactive. These results further suggest that MALT lymphoma-associated translocations that are known to activate NF-κB were absent or rare in our studied cases of gastric MALT lymphomas. However, we cannot exclude the possibility that NF-κB activation still exist in these MALT lymphomas cases, involving other members of Rel proteins family (p50, p 52).

In this study, in 35 MALT lymphoma specimens, nuclear coexpression of BCL10 and NF-κB in tumor cells was not found. It is interesting to note that Talwalker et al. [20] found

BCL10 positivity in some cases of breast MALT lymphomas despite NF-κB negativity in all of them, but none of the cases had MALT1 gene rearrangements confirmed by fluoreseence in situ hybridization (FISH). This suggests that BCL10 immunostaining overestimates the frequency of MALT1 gene rearrangements [21]. Moreover, Sagaert et al. [22, 23] studied 77 patients with MALT lymphoma and found translocations involving MALT1 and BCL10 gene in only 1% of patients, concluding that other structural or numerical chromosome disorders may be responsible for tumorigenesis and lymphogenesis. Similar conclusions can be widrawn from our study *ie* that lymphogenesis pathway in our cases should be explained other than by activation of BCL10 and NF-κB.

Conclusion

Nuclear expression of NF-κB p65 subunit and BCL10 were not detected in studied specimens of gastric MALT lymphoma. These results indicate that other mechanisms and signal pathways are active in lymphogenesis of gastric MALT lymphoma, and that apoptotic inhibition is not the main, nor the only mechanism in tumorogenesis.

Conflict of interest

The authors do not have any conflicts of interest to declare.

R E F E R E N C E S

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Surgical treatment and dilemmas in the treatment of basal cell carcinomas with intracranial propagation

Hirurško lečenje i dileme pri lečenju bazocelularnih karcinoma sa intrakranijalnom propagacijom

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Abstract

Background/Aim. Basal cell carcinoma (BCC) is one of the most common malignant skin tumors on the head in 90% of cases and is characterized by a high local infiltrating potential and destructive growth. The aim of this study was to show the characteristics of a correlation between pathohistological types of basal cell carcinoma and the size of this lesion, aggressiveness and infiltration of basal cell carcinoma, and its effect on the course of the therapy. **Methods.** We analyzed 27 patients operated on for BCC that affected the scalp and the bone. We described and considered the clinical characteristics (size, depth of invasion), duration and speed of intracranial propagation and then made comparison with the type of BCC. We described the extent of surgical treatment and the width of excision to determine the best course of the treatment. The patients went through examinations during the next three years. **Results.** According to the histopathological type the most common tumors were: infiltrative (60.2%), noduloinfiltrative (37.2%), and morpheaform (2.6%). Tumors were clinically manifested as ulcerative lesions, *ulcus rodens* and *ulcus terebrans*. Tumor diameters ranged from 2 to 25 cm. The depth of intracranial propagation depended on the histological type and tumor size. Most relapses (35%) occurred with morpheaform type of BCC. In 17 of the cases, BCC affected the bone without intracranial propagation. In 10 of the cases, basalioma infiltrated intracranial space – in 8 of the cases it infiltrated the dura and in 6 of the cases the brain parenchyma, of which in two of them, the superior sagittal sinus was affected and had to be surgically tied off. **Conclusion.** The aggressiveness and infiltration of basal cell carcinoma into the brain parenchyma is directly linked to the histological type and the size of the tumor. The larger the basalioma or if histopathological findings confirm morpheaform type of basalioma the larger surrounding healthy tissue, sometimes more than 3 cm in diameter, needs to be removed. In cases of these tumors postoperative radiotherapy is recommended.

Key words:
head and neck neoplasms; neoplasms, basal cell; neoplasm invasiveness; neurosurgical procedures.

Apstrakt

Uvod/Cilj. U predelu poglavine javlja se veliki broj tumoroznih promena. Tumorozne promene na glavi javljaju se kod 90% slučajeva i odlikuju se lokalnim infiltrativnim a ponekad i destruktivnim rastom. Cilj ovoga rada bio je analiza bazocelularnog karcinoma sa intrakranijalnom propagacijom, karakteristika i korelacija između patohistološkog tipa, veličine tumora, infiltracione agresivnosti i načina lečenja. **Metode.** Analizirali smo 27 bolesnika operisanih zbog bazocelularnog karcinoma sa zahvaćenim koštanim tkivom poglavine. Opisali smo i proučavali kliničke karakteristike (veličinu, dubinu invazije), vreme trajanja i brzinu intrakranijalne propagacije, a zatim ih upoređivali sa tipom bazocelularnog karcinoma. Razmatrali smo radikalnost operacije i širinu ekscizije i procenjivali najbolju hiruršku intervenciju. Bolesnike smo pratili tri godine nakon operacije. **Rezultati.** Prema patohistološkom tipu karcinoma, najzastupljeniji bili su: infiltrativni (60,2%), noduloinfiltrativni (37,2%) i morfoeiformni (2,6%) tip. Klinički su se manifestovali kao ulcerozne lezije: *ulcus rodens* i *ulcus terebrans*. Veličina tumora kretala se od 2 do 25 cm u prečniku. Dubina intrakranijalne propagacije zavisila je od histološkog tipa i veličine tumora. Najveći broj recidiva (35%) bio je prisutan kod morfoeiformnog tipa bazocelularnog karcinoma. Kod 17 bolesnika bazocelularni karcinom je bio zahvatio kost bez propagacije intrakranijalno, a vreme trajanja promene bilo je od jedne do dve godine. Kod 10 bolesnika bazeliom je bio prodro intrakranijalno i to u osam infiltrisao duru, a kod šest moždani parenhim, od toga kod dva bolesnika bio je zahvačen *sinus sagittalis* koji je morao biti podvezan. **Zaključak.** Agresivnost i infiltracija bazocelularnog karcinoma u moždani parehhim direktno su uslovljeni histološkim tipom i veličinom tumora. Što je bazeliom veći ili ako je prema patohistološkom nalazu morfoeiformni tip mora se izvršiti veće otklanjanje okolnog zdravog tkiva, nekada više od 3 cm u prečniku, uz postoperativnu radiološku terapiju.

Ključne reči:
glava i vrat, neoplazme; karcinom, bazocelularni; neoplazme, invazivnost; neurohirurške procedure.

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Introduction

Carcinoma basocellulare originates from pluripotent cells of the basal layer of the epidermis, the outer layer of hair follicles, sebaceous glands or other skin adnex [1–2]. It is characterized by local infiltrative and sometimes destructive growth. It very rarely gives metastases. In the scalp region a large number of tumorous lesions appear. Some types of cancer can be very aggressive and by location they can be intracranial with extracranial propagation or extracranial with intracranial propagation. One type of tumor that is by localization extracranial and has intracranial propagation are basal cell carcinomas.

There are several histological types of basocelular carcinoma [2] which may be manifested in over a dozen clinical forms. The most aggressive is infiltrating form which is usually clinically manifested as: *carcinoma basocellularae ulcerosum* (*exulcerans*) s. *ulcus rodens* – it appears as a sharply defined ulcerative lesion of various sizes and depths, with irregular edges [3–4]. The bottom appears as a crater covered with bloody discharge, hypergranulation and crust and easily bleeds. It grows very fast and infiltratively, devastating the surrounding tissue down to the cartilage and bone; *carcinoma basocellulare terebrans*, s. *ulcus terebrans* – it develops from *ulcus rodens* with a deeper and more extensive tissue destruction and decay. The tumor penetrates and infiltrates and destroys the subcutis, fascia, muscles and cartilage, bones [4]. Extensive mutilations and deformities appear with bleeding and secondary infections. They usually occur on the scalp and the middle of the face and are very difficult to treat [4]; *carcinoma basocellulare morpheaform* (*sclerodermiform*) – a less frequent clinical form, more common on the head as a small, raised yellow or white plate with a network of telangiectasia on the surface. This type of basocelular cacinoma is radioresistant.

We used multidisciplinary methodology, including the specialist in plastic surgery. Surgical treatment consisted of excision of basal cell changes, and closure of secondary defects with skin grafts and flap reconstruction depending on the location and size. We entered the following data in the protocol: age, sex, lesion diameter, duration and anatomic site. Preparations were placed in 1% formaldehyde and sent to histopathological (HP) verification. After receiving the HP findings we entered in the protocol the data on the presence of tumor tissue at the edges of the resection and the histopathologic type of basal cell carcinoma. On the basis of their clinical characteristics we compared the histopathologic type and the type of basal cell carcinoma and then described the radicality of surgery and the width of excision to determine the best surgical treatment. The patients were followed three years after the surgery. The diagnostic methods we used were computed tomography (CT), magnetic resonance (MR) and 64-multislice CT (MSCT) as a very accurate diagnostic procedure.

Results

Out of 27 analyzed patients with basal cell carcinoma 15 (56%) were male and 12 (44%) female. The age structure of the patients ranged from 82 years to 43 years (with the average age of $\bar{x}$ = 62 years). Tumor size ranged from 2 cm to 25 cm in diameter (average value of $\bar{x}$ = 3.8 cm). In relation to the anatomic site basal cell carcinomas were usually present in the frontal region (22%), parietal (31%), in the temporal (33%), and in the occipital region (14%).

By histopathologic type the most common were: infiltrative (60.2%), noduloinfiltrative (37.2%) and morpheaform (2.6%). They were clinically manifested as: ulcerative, *ulcus rodens* and *ulcus terebrans* (Figure 1). Non-infiltrative basal cell carcinoma (nodular, noduloadenoid and superficial) did not affect the bony part of the scalp and did not make intracranial penetration. The duration of the changes, i.e. the time between noticing and the first consultation with the doctor ranged from 6 months up to 3 years. Tumor sizes ranged from 2 to 25 cm in diameter. The depth of the intracranial propagation depended on the histological type and tumor size. Most relapses (42%) were present in the morpheaform type of basal cell carcinoma. In 17 cases the basaliom affected only the bone without intracranial propagation and the duration of changes ranged from one to two years. In 10 cases the basalioma penetrated intracranially (duration of changes over three years), of which in 8 cases the duru and in 6 cases it infiltrated the brain parenchyma, of which in two

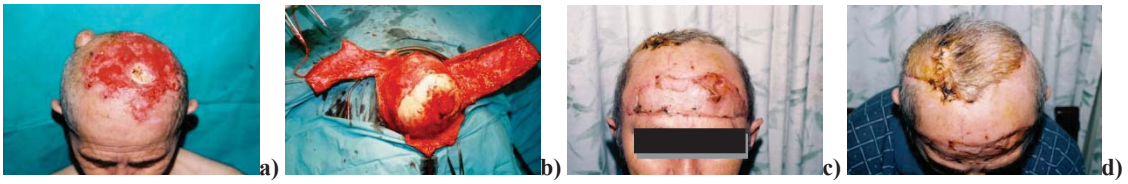

Fig. 1 – a) Clinical manifestation of infiltrative basal cell carcinoma; b) Intracranial propagation of the tumor and "Banana Peel" lifted flaps and reconstructed secondary defect by Orticochea; c) Front view; d) Top view.

Surgical treatment largely depends on the histological type, size, location and the depth of the intracranial propagation and its clinical manifestations.

Methods

We analyzed 27 patients operated on for basal cell carcinoma affecting the scalp and bone, of both sexes and different ages. We studied the clinical characteristics of basal cell carcinoma affecting the bone scalp. We described: the size, the depth of invasion, duration and speed of intracranial propagation and then made comparison with the histopathologic types i.e. the type of basal cell carcinoma.

cases the sagittal sinus was affected and had to be tied off (Figure 2). In all the cases with the brain parenchyma invaded we removed basalioma down to the macroscopically and microscopically healthy brain parenchyma and in all the cases we implemented radiotherapy.

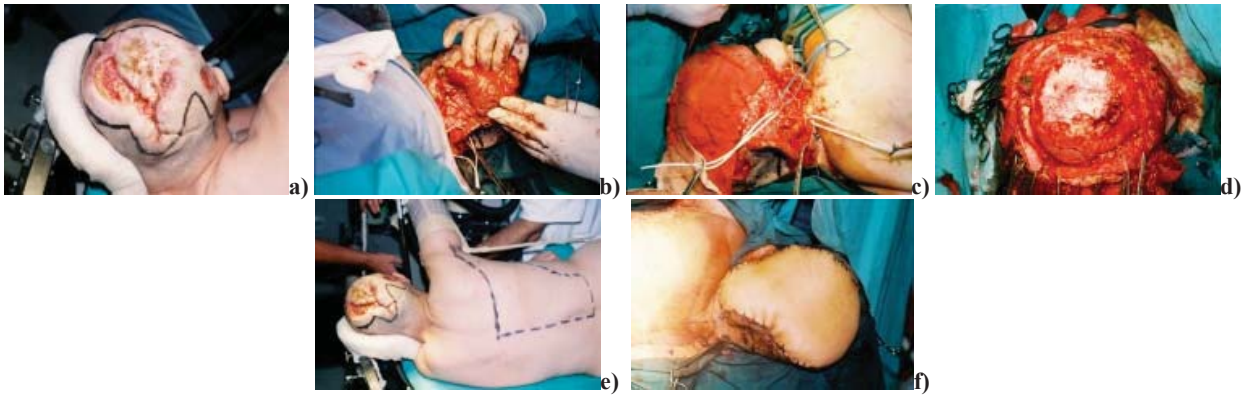

Fig. 2 – a) Clinical manifestation of infiltrated basal cell carcinoma (*ulcus terebrans*); b) Marked lines represent the site of delamination of free microvascular *latissimus dorsi* flap; c) Clear invasion of carcinoma into the tissue (which is much larger than the skin invasion) with the affected *sinus sagittalis*; d) Prepared *a.* and *v. occipitalis*; e) microvascular anastomosis *a.v. occipitalis* and *a.v. thoracodorsalis*; f) vital microvascular flap *latissimus dorsi*.

Discussion

Despite the fact that basal cell carcinomas are affected by radiation therapy (except for the morpheaform type), cryotherapy, curettage, local cytostatic therapy, retinoids or electrodisection, surgical treatment is the method of choice in the treatment of this type of tumor [5–10]. Surgically, the tumor can be entirely removed with safe histopathological checkup and confirmation [11, 12].

The rate of healing in surgical excisions ranges from 85% to 95% [5]. Epstein [9] finds that the visual assessment of basal cell carcinoma edges is within 1 mm from the right edge in about 94% of cases, which led him to conclude that the edges of 2 mm give 94% healing. Burg et al. [11] compared the clinical size of the tumor estimated by the naked eye with the right size determined by Mohsov's micrographic examination and received the distinction of 5.5 ± 3 mm in primary basocelular carcinomas and 8.9 ± 4.8 mm in recurrent basal cell carcinomas.

Based on the received results and clinical experience we believe that the width of excision and the presence of tumorous cells on the resection lines are in direct correlation with the histological type and size of basal cell carcinoma. So, the adequacy of surgical excision is directly dependent on the type and size of basal cell carcinoma.

We found that in all our cases with the affected scalp bone tissue and intracranial propagation, it was an infiltrative basal cell carcinoma and all its histological combinations with infiltrative component (no macroscopically visible edge compared to the healthy tissue), and that it required wider excision and depended on the clinical manifestation of the tumor and its size[4, 13–17]. If it is the infiltrative type (clinically most often with ulceration and covered with crust) and if it is larger than 1 cm (clinically most often manifested as *ulcus rodens* or *ulcus terebrans* excision should be made into the healthy tissue for about 1.5 cm to 3 cm, sometimes even more.

Noduloinfiltrative histological type is most often clinically manifested as nodus accompanied by tissue decay, ulceration. Because of the clinically present nodus it sometimes misleads in terms of the evaluation of the excision width (therefore a high percentage of the presence of tumor at the resection edge) [17–24]. But with this kind, there is no clear visual limits of tumor from healthy skin which requires, depending on the size, at least 5 mm excision into the healthy tissue.

In the morpheaform type of basal cell carcinoma (rate of the presence at resection lines 35%) excisions need to be made into the healthy tissue up to 3 cm from the tumor edge which is not macroscopicly clearly defined. In the diagnosis of the width of excision in morpheaform basal cell carcinomas we used 64-slice MSCT.

Conclusion

The aggressiveness and infiltration of basal cell carcinoma into the brain parenchyma is directly linked to the histological type and size of the tumor. The larger the basal cell carcinoma the wider excisions need to be made into the healthy tissue with obligatory removal of the affected bone down to the macroscopicly and microscopicly healthy brain parenchyma sometimes up to 3 cm in diameter with obligatory postoperative radiological therapy.

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The importance of sleep apnea index determination using 24 h ECG analysis in patients with heart rhythm disorders

Značaj određivanja indeksa apneje u snu analizom holter EKG-a kod bolesnika sa poremećajima srčanog ritma

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Abstract

Background/Aim. A possible cause of malignant heart rhythm disorders is the syndrome of sleep apnea (periodic cessation of breathing during sleep longer than 10 seconds). Recent 24h ECG software systems have the option of determination ECG apnea index (AI) based on the change in voltage of QRS complexes. The aim of the study was to determine the significance of AI evaluation in routine 24-hour Holter ECG on a group of 12 patients. **Methods.** We presented a total of 12 consecutive patients with previously documented arrhythmias and the history of breathing disorders during night. They were analyzed by 24 h ECG (Medilog AR 12 plus Darwin), that is able to determine AI. **Results.** We presented a case series of 12 patients, 8 men and 4 women, mean age 58.75 years and the average AI 5.78. In the whole group there was a trend of increasing prevalence of complex rhythm disorders with increasing of AI and increased frequency of arrhythmias in the night phase *vs* day phase. **Conclusion.** Determination of AI using routine long term (24 h) ECG analysis is important because sleep apnea can be successfully treated as an etiological or contributing factor of arrhythmias.

Key words:
sleep apnea syndromes; arrhythmias, cardiac; electrocardiography, ambulatory.

Apstrakt

Uvod/Cilj. Jedan od mogućih uzroka malignih poremećaja ritma srca na koji se ne obraća dovoljno pažnja je sindrom poremećaja disanja u toku spavanja poznat kao *sleep apnea* (periodični prekid disanja u toku spavanja duži od 10 sekundi). Noviji softverski sistemi holter EKG-a imaju mogućnost određivanja apneja indeksa (AI) na osnovu promene voltaže QRS kompleksa. Cilj ovog rada bio je utvrđivanje značaja određivanja AI u rutinskoj analizi holter EKG-a na uzorku od 12 bolesnika. **Metode.** Prikazana je grupa od 12 uzastopnih bolesnika sa prethodno dokazanim aritmijama i istorijom poremećaja disanja tokom noći. Svi su bili podvrgnuti 24 h holter EKG analizi (Medilog 12 plus Darwin) koja omogućava određivanje AI. **Rezultati.** Prikazali smo seriju od 12 bolesnika, 8 muškaraca i 4 žene, prosečne starosti 58,75 godina i prosečnog AI od 5,78. U celokupnoj grupi postojao je trend povećanja učestalosti kompleksnih poremećaja ritma sa povećanjem AI i učestalosti aritmije u noćnoj fazi u odnosu na dnevnu fazu. **Zaključak.** S obzirom na mogućnost lečenja apneje u snu kao etiološkog ili doprinosećeg faktora u nastanku i pogoršanju aritmija, određivanje AI rutinskom primenom holter EKG-a od velikog je značaja.

Ključne reči:
apneja, spavanje poremećaji, sindromi; aritmija; elektrokardiografija, ambulantna.

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Introduction

A possible cause of malignant arrhythmias that is often forgotten is a syndrome of breathing disorders during sleep, known as sleep apnea [1]. Besides, arrhythmias sleep apnea is a potential cause of many adverse cardiovascular events such as myocardial ischemia, and hypertension. Sleep apnea is defined as periodic interruption of breathing during sleep longer than 10 seconds. Hypopnea in adults is characterized by drop of nasal pressure at least 30% (compared to the basal state), of at least, 10 seconds associated with oxygen desaturation of ≥ 4%. Apnea index (AI) is the number of these episodes (apnea and hypopnea) *per* hour of sleep.

lation, although there is evidence that in the population of patients with atrial fibrillation the incidence of OSA is higher than in the general population [5]. In addition, a high incidence of recurrent atrial fibrillation was documented in the first year after cardioversion in patients with sleep apnea who were not treated with Continuous Positive Airway Pressure (CPAP) [6].

The newer software systems of long term ECG (12-channel ECG recording) have the option of determining the apnea index based on changes in QRS voltage that changes due to minimal changes in the axis of the heart that occur during respiration/breathing interruptions, known as ECG derived respiration signal (EDRF) (Figures 1 and 2).

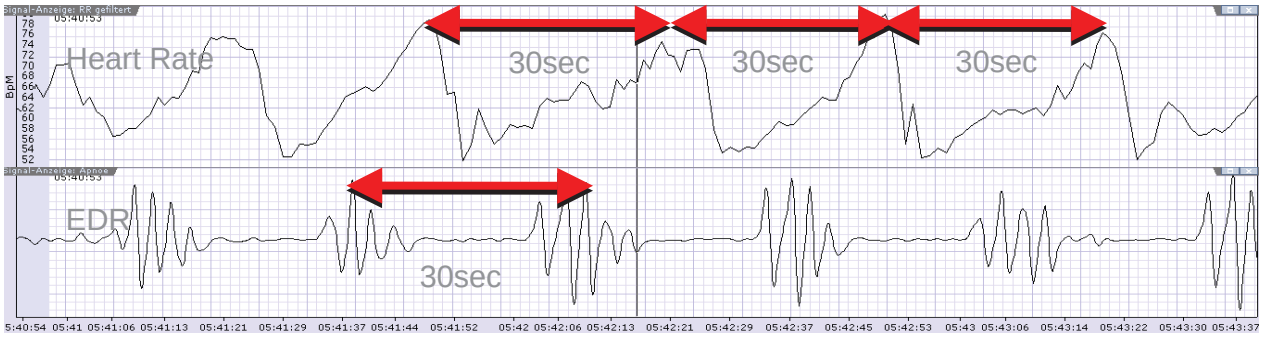

Fig. 1 – Episodes of apnea occur periodically leading to significant fluctuations of the heart rate. The length of these oscillations correlates with the period of time from one to the next apnea on the presented electrocardiogram (ECG) derived respiration (EDR) signal.

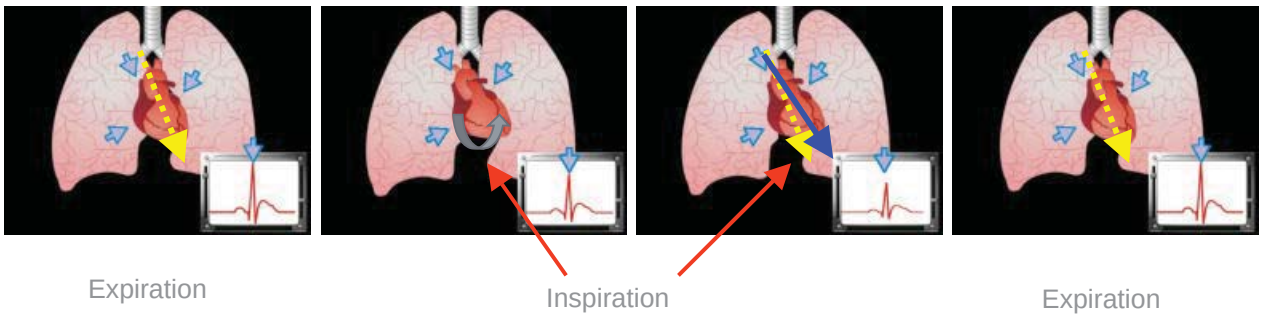

Fig. 2 – The heart is slightly raised and moves during inspiration and expiration flow related to a fixed position of the electrodes.

There are two main types of sleep apnea: obstructive sleep apnea (OSA), which occurs when the throat muscles collapse, due to different functional disorders of nose, use of sedatives, short neck, obesity; and central sleep apnea (CSA) the brain does not send proper signals to the muscles that control breathing.

Polysomnography is the gold standard in the diagnosis of sleep apnea in specialized laboratories. It is expensive and requires training staff and in our country is rarely used, only in scientific purposes.

The percentage of rhythm disorders caused by apnea is significant [2]. Guilleminault at al. [2] in the long term ECG analysis found that 3% of the 400 patients with OSA had atrial fibrillation [3, 4], which is significantly higher comparing the prevalence of atrial fibrillation in the general popu-

During episodes of interrupted breathing, heart rate compensatory increases and that period of heart rate oscillation correlates with the period of time from one episode to the next episode of apnea of EDRF signal (Figure 3).

Small changes of the vector result in very small changes in ECG amplitudes of a few μV which is possible to be recorded by the high resolute 24h ECG devices.

That is why a simple and inexpensive examination by 24 h ECG (comparing to the specialized respiratory laboratories) may be used as screening test for patients with breathing disorders during sleeping. This may improve etiologic treatment of arrhythmias, without unnecessary or improper use of antiarrhythmic drugs even implantation of pace-maker devices.

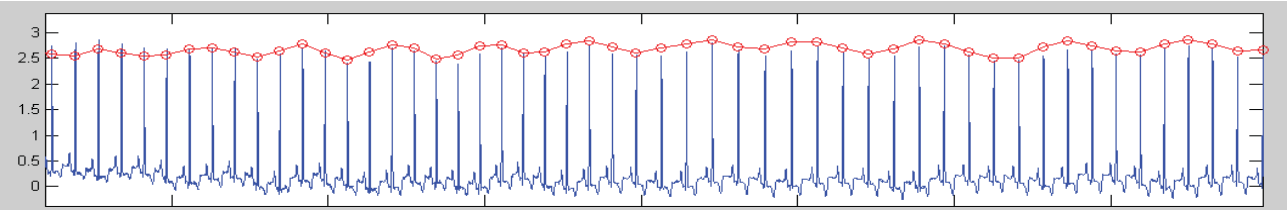

Fig. 3 – Heart rate compensatory increases during the episodes of interrupted breathing.

Methods

We presented a total of 12 consecutive patients with previously documented arrhythmias and history of breathing disorders during night. They were analyzed by 24 h ECG, (Medilog AR 12 plus Darwin), that is able to determine AI. The study group consisted of 8 men and 4 women aged from 42 to 65 years, average 58,75 years. The average AI was 5,78 (min AI = 0 , max AI = 18).

There was an increase in the incidence of complex rhythm disorders with an increase of AI (except for the patient ID 12, who had 78 episodes of pauses), and increase in the incidence of all kinds of arrhythmia during the night comparing with the the day phase. Concerning the previous data in the literature [6] that symptomatic patients with AI >5 are candidates for treatment with CPAP, and patients with AI > 20 have the absolute indication for administration of CPAP, we divided our patients into two groups, the group I = AI < 5 and the group II = AI > 5. In the group II (with breathing disorders during sleeping) all the patients (100%) were men, with hypertension and smoking habit, data that "they stop breath during the sleep", and 60% were obese according to body mass index (BMI). Also, 80% in this group met criteria for metabolic syndrome (Table 1).

Concerning complex rhythm disorders in the group I we found 42% of the patients with this arrhythmia (Lawn III, IV or V class) *vs* 80% patients in the group II. All the patients in the group II had more than 50% episodes of arrhythmia during the night phase. In the patients with AI > 15 we detected pauses of more than 2 s and their number was in correlation with AI (Table 2).

We selected and presented the patient ID 7 from the group I, with AI = 2.29, and arrhythmias by the Lawn classification IV, 2,700 premature ventricular complex (PVC). The

Table 1

Apnea index (AI), rhythm disorders and heart rate variability in the study population

ID	AI	Pause	Tachycardia	VES	Night arrh %	CI	SDNNi	SDANNi	Qtcdisp
1	0	0	0	L3	0	1.21	87.0	172.1	511
2	0.29	0	0	0	0	0.95	32.2	90.2	303
3	0.29	4	1	L1	0	1.30	62.7	136.7	284
4	0.71	3	3	L4	0	1.36	40.5	124.2	344
5	1,57	0	0	0	0	0.93	27.3	85.7	434
6	2.0	7	6	0	0	1.11	119.6	211.6	601
7	2.29	0	0	L4	80	1.35	67.5	137.2	458
8	5.29	0	0	L3	62	0.88	42.6	84.4	383
9	8.0	0	0	L4	52	1.23	106.9	75.2	717
10	15	5	0	L5	68	1.19	60.0	84.0	413
11	16	27	0	L4	73	1.09	56.1	98.6	416
12	18	78	2	0	89.7	1.24	88.6	47.1	787

ID – patient's identification number; CI – circardial index; VES – ventricular extrasystoles; Qtcdisp – Qtc disperzion; SDNNi – average value of all standard deviation PR interval; SDANNi – standard deviation of all the average values.

Table 2

Main characteristics of the study group

ID	Gender	Age (year)	BMI	Msy	Smoking	HTA	SAH	DN
1	F	64	1	0	0	0	0	0
2	F	51	0	1	0	1	0	0
3	M	42	0	0	0	0	0	0
4	F	53	1	1	1	1	0	0
5	F	55	0	1	1	1	1	1
6	M	69	1	0	1	1	1	1
7	M	42	0	1	0	1	1	0
8	M	54	1	0	1	1	1	0
9	M	66	0	1	1	1	1	1
10	M	73	0	1	1	1	1	1
11	M	58	1	1	1	1	1	1
12	M	78	1	1	1	1	1	1

ID – patient's identification number; BMI – body mass index, 1 > 0.25 kg/m^2;
Msy – metabolic syndrome; SAH – history of cessation of breathing at night;
DN – information on the deviation of the nose; F – female; M –male.

patient had 80% of complex arrhythmia episodes recorded in the night phase in the periods of breathing cessation (Table 2). The patient had the history of viral myocarditis a year ago when 24 h ECG was also performed using standard 24 h ECG (it was recorded PVC 5100, Lawn 4, but without the possibility of determining the AI). All the signs of myocarditis on this control check up were negative (clinical, laboratory and echocardiography findings), and VES might be induced not only by viral myocarditis. This patient also gave information about deviation nose which may be a predisposing factor for the development OSA. He was overweight, not a smoker, blood pressure values were slightly higher in the morning hours to a maximum of 150/90 mmHg without treatment. He was referred to additional throat examination and operative correction.

We also presented the patient ID 12 from the group II, with 78 episodes of OSA type block longer than 2.0 seconds and Mobitz type I and II [70 (89.7%) episodes in the night phase] (Figure 4). This patient was obese, excessive smoker with a large-scale neck, unregulated hypertension, morning headaches, disorientation and fatigue. Echocardiography showed slightly impaired global systolic function, diastolic dysfunction grade II. Hypertension was treated using angiotensin-converting enzyme (ACE) inhibitors and calcium antagonists.

The patients with AI > 5 were advised to perform polysomnography, but we have not been able to perform it.

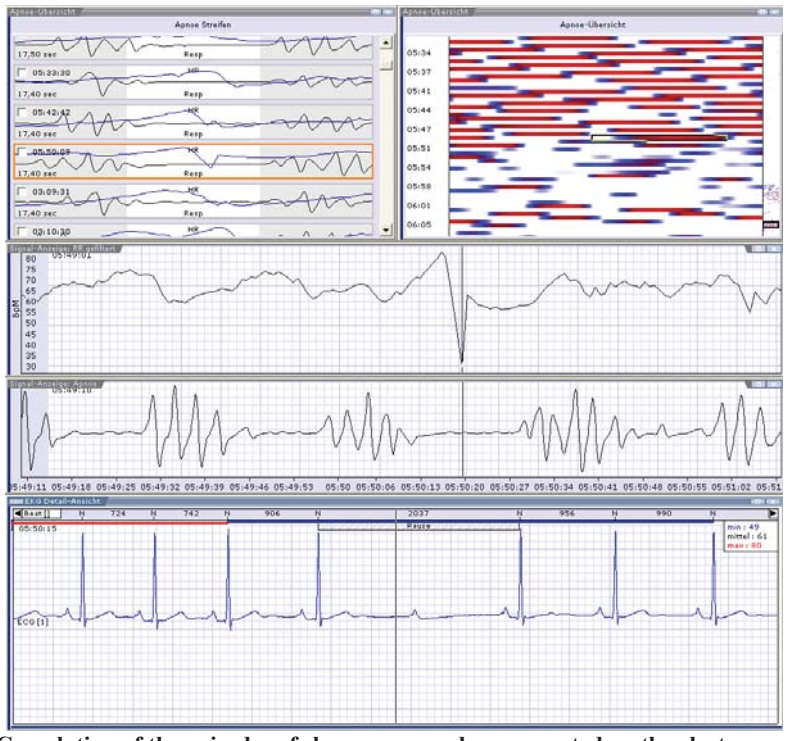

Fig. 4 – Correlation of the episodes of sleep apnea and pauses noted on the electrocardiogram.

Discussion

Sleep apnea is a disorder that in many ways diminishes the quality of life of patients and increases cardiovascular morbidity and mortality [7]. The literature rather analyzes the phenomena of cardiovascular events in patients with breathing disorders. The obstructive sleep apnea is associated with several heart diseases, most notably hypertension, ischemic heart disease, heart failure, stroke, rhythm disorders and pulmonary hypertension [2]. Our study included patients with both history of breathing disorders during night and rhythm disorders. We tried to determine if abnormal breathing during sleep was a contributing factor for arrhythmias.

Ventricular arrhythmias show circadian rhythm with the highest incidence of occurrence of midnight (0 h) to 6 h in the morning. This could be one of the reasons for the increased incidence of ventricular arrhythmias in patients with OSA, although increased oxygen desaturation during OSA is certainly a contributing factor [8].

The predisposing factors for sleep apnea are: obesity, hypertension, broad neck (short, thick neck), narrow airways, smoking [9]. People with this disorder often snore at night and have elevated blood pressure during the night and early morning, morning headaches, depression, memory problems. Data from the literature suggest that these noncardiac manifestations can lead to sudden cardiac death, because patients with sleep apnea are sleepy during the day phase, have loss of concentration and, therefore, have an increased incidence of traffic accidents [10]. All the presented patients in this study had all of the above predisposing factors (smoking, obesity, metabolic syndrome and hypertension). The consequences in terms of daily loss of concentration and sleepiness had only patient ID 11 and ID 12 in which the AI > 15, which is consistent with literature data.

Sleep apnea is associated with prolonged episodes of hypoxemia which triggers the reflex similar during drowning. This reflex has a duty to preserve oxygen to the vital or-

gans, the brain and the heart, reducing blood flow to the periphery which consists of sympathetic vasoconstriction (reflected in the periphery) and the vagal response that affects the heart. Effects on the heart are various conduction disorders that occur in patients with OSA [8, 10] such as sinoatrial block, sinus arrest, AV conduction disorders, even asystole. It has been shown that these patients have no structural and anatomical changes in the conduction system and rhythm disorders were the result of increased parasympathetic tone due to hypoxemia. These conduction abnormalities are reversible and disappear after the use of CPAP [8]. These bradyarrhythmias, SA and AV conduction abnormalities and ventricular premature beats and complex type of atrial fibrillation may predispose and lead to sudden cardiac death. This risk is increased in patients with associated risk factors for cardiovascular events, so detection of breathing disorders in these patients could be the obligatory diagnostic procedure, if technically possible. Since the presented group was a random sample of patients who came to perform 24 h ECG due to arrhythmias, complex ventricular arrhythmias were more common than pauses (higher incidence in the literature), but the patients ID 11 and ID 12 with AI > 15 had recorded a significant number of pauses which occurred during periods of apnea consistent with previous research [11–13].

In the previous studies the heart rate variability (HRV) showed that HRV was expressed in the severe cases of OSA. On the contrary, in patients with milder degrees of OSA, in patients who take amiodarone, sotalol, propafenon, nebivolol, bisoprolol and very elderly people with "rigid heart rhythm", HRV was not pronounced [14, 15]. In our study, there were no statistically significant difference in parameters of HRV, also.

This pilot study showed the significance of determining AI in patients with rhythm disorders in order to establish cause and determine further diagnosis and treatment. In fact people with AI > 5 are referred for further diagnostic of sleep apnea. Also, we advise them to correct habits of living, reduce and/or eliminate use of medications that predispose manifestation of respiratory distress (sedatives), and to visit the throat specialist to correct existing anomalies. If sleep apnea is confirmed in the specialized laboratory, than the application of CPAP will be the best form of treatment for arrhythmia and apnea.

Standard cardiorespiratory monitoring is complex including: nasal airflow, measurement of saturation (pulse oxygen meter), determination of HRV and determination of "respiratory movements". Laboratories for cardiorespiratory monitoring and polysomnography in our country are not available in routine clinical practice due to methods complexity, the need for special equipment and high costs of treatment.

Unfortunately, there are no studies on a large number of patients that compared the results of classical polysomnography 24 h ECG with respiratory monitoring. But the available literature data suggest that the sensitivity and specificity of this method in determining of sleep apnea are satisfactory [16, 17].

Conclusion

The conclusion of this pilot study is that respiratory analysis of apnea index by appropriate 24 h ECG is precious in patients with arrhythmias, as well as in the group of patients with suspected breathing disorders during sleep, as primary screening of patients with sleep apnea and dynamic screening of patients who are treated with CPAP. Further investigations by the cardiologists in our country in this field are necessary.

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CURRENT TOPIC

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Health care economics in Serbia: Current problems and changes

Ekonomija zdravstvenog sistema Srbije: tekući problemi i promene

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Key words:
health care; insurance, health; serbia; economics.

Ključne reči:
zdravstvena zaštita; zdravstveno osiguranje; srbija; ekonomski faktori.

Introduction

One of the fundamental rights of every human being is to enjoy "the highest attainable standard of health" [1]. Achieving better health requires no only adequate medical knowledge and technologies, laws and social measures in the field of health care, but also sufficient funding for fulfilling people's right to health. However, economic crisis has left every community with limited possibility of investing in health care and forced them to use the available resources more efficiently. This is the reason why health financing policy represents an important and integral part of the health system concerned with how financial resources are generated, allocated and used.

Development of new drugs and medical technologies, population aging, increased incidence of chronic diseases as well as the peoples' rising demands from health care providers lead to a constant increase of health system costs worldwide. In these circumstances, countries in transition, like Serbia, face difficult challenges in financing their health systems. Current economic crisis and budget constraints do not allow the Government to simply allocate more public revenues for health and solve the people's expectations by increasing the spending. Instead, Serbia is forced to start reforms to provide a more efficient health system. The reform processes are positioned within the wider context of European integration and public administration reforms. This paper provides a short description of the health care system in Serbia focusing on the healthcare economics and reforms and their influence on financial sustainability.

System of Obtaining Funds for Financing Health Care in Serbia

There are several main models of healthcare, depending on how the funds are collected: the Beveridge model, the Bismarck model and the Modified market or Consumer sovereignty model (Private Insurance model) [2].

Beveridge model originates from Britain's National Health Service. Health care is financed by the government trough tax payment and it covers the entire population. Doctors may be government employees or may work in privately owned hospitals and ordinations, but are always paid by the government. This system prevailed in Northwestern Europe, i.e. in the UK, Ireland and Scandinavia and in Southern Europe, i.e. in Spain, Portugal, Italy and in Canada. Similar health care funding was used in former USSR and Eastern block countries, but with much less independent providers, and without private practice. It is called the Semashko model.

Bismarck model is based on a premium financed social insurance system with a mixture of public and private providers. Funding is compulsory by employers and employees. Originally, it was not aimed at "universal coverage" because a right to health service was associated with labour status. Nowadays, it is based on the principles of solidarity and covers almost the whole population in many countries. Such type of health insurance represents an alternative form of taxation, because of its linkage to earnings and its detachment from benefit. However, it is more politically attractive than general tax because revenues are directed to health care. The Bismarck social insurance model was current in many Western European countries, e.g. Germany, France, Austria, Switzerland and Benelux.

In the Modified market or Consumer sovereignty model funding of the system is based on premiums paid into private insurance companies. In this model health is viewed as a commodity and ill health as an insurable risk. The great majority of the providers in this model belong to the private sector and the level of health care is directly connected with the cost of premium. This model in its pure form exists only in the USA.

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Rising costs of health care make a substantial burden to finance it at the desirable level, even for the high-income countries, so actual health care systems have more or less elements from different models. For instance, in Germany, the Bismarck system of compulsory insurance is prevalent, but it cannot cover health care expenses and some funds have to be transferred from the general budget, like in Beveridge model. In Norway, the system is financed from the budget, but because of the long wait for some free services, increased use of a parallel private market is planned. In USA there is mostly a private insurance system, but health care reforms allow free health care for some categories of people, like in the Beveridge model [3,4].

A former socialist Yugoslavia's health care system was unique among the European socialist countries in terms of financing, as it was financed through a compulsory social insurance (kind of Bismarck model), but the access to health care was a constitutional right of all citizens. However, the provision of service was more like in the Semashko model, with physicians as salaried state employees [5]. Private practice was prohibited soon after World War II. Political changes in former Yugoslavia allowed it again in 1989 within the Law of Individual Work [6]. After the breakdown of Yugoslavia, Serbia basically kept the same system, but also established some new laws on health care regulating conditions for activities of private enterprises in the field of medicine, dental care, laboratories and pharmacies. By the Current Low on Health Care [7] (2005, changed in 2012) private health care providers are not the integral part of the public health system, but may be included in by contracting. However, there is a short list of such services limited to those which are deficient.

Public health system is mainly financed by the Republic Health Insurance Fund (RHIF). It collects revenues from obligatory insurance, which represent the largest source of its incomes (about 70%) and distributes them to health providers. Though the health insurance system has many potential advantages, this model of financing may not be independently sustainable if the income from insurance does not cover all health care costs. In Serbia, as in many other countries, additional funds must be transferred from the general budget, and some services have to be paid by out of pocket money. It is usual that state or local authorities cover the costs of construction and maintenance of buildings, purchase of major equipment, epidemiological control, medical staff training and research. Funding of staff salaries, medical supplies, and medicines is under the jurisdiction of the RHIF and/or the Ministry of Health (MoH). Except for MoH, regional and local governments, a small part of the funds for health care is provided by the Ministry of Defence, Military Health Insurance Fund and the Ministry of Justice.

Serbian Health Insurance Fund is facing a difficult financial situation in recent years. One of the essential problems is the lack of adequate revenue collection. Economic downturn has led to a high rate of unemployment and low average salary, so the basis for taxation is small. Contributions are high, comparable to those in the European Union (EU), because there is an effective social tax on wages of about 36%, including health insurance, and pension insurance. Even continuous decrease of health care insurance rate, from 19.4% in 1991 to 12.3% since 2004 [8] was too much of a burden to employers, so many of them failed to pay compulsory contributions to the RHIF. Tax evasion has left a lot of employed people and their families' uninsured in the year 2012. In some cases, faced with the workers protests, the government decided to extend their insurance despite the fact that employers did not pay for it. Additionally, the number of insured persons, in accordance with the Law on Health Care Insurance, is increased by more than one million persons such as refugees, exiled persons, temporarily displaced persons from Kosovo and Metohia. They benefit the equal right to health care, and the government is obliged to pay for it from the state budget. According to the official data, the ratio between the insured non-employed and employed persons rate was almost 50 : 50 in 2010 (Table 1) [9]. As the employment rate in Serbia continued to decrease, this ratio is going to become more adverse.

Table 1

Number of health insured persons in Serbia in 2010

Type of health insurance	Number of persons	%
Employed persons*	2,875,243	42.01
Self-employed*	287,214	4.20
Farmers*	320.771	4.69
Unemployed persons	95.358	1.39
Retired persons	1.895.397	27.69
Other	1.370.015	20.02
Total	6.843.998	100.00

***Persons who actually earn funds for insurance**

Private funding through official copayment is practically irrelevant source of financing, because of very low prices and the wide range of persons excluded from this obligation (elderly over 65 years, children, pregnant women, persons with disabilities, unemployed and recipients of social welfare benefits).

Serbian health care has been severely under-funded for many years and consequently, equipment and facilities were not modernized. In the last twelve years, Serbia received multiple international support, mainly earmarked for capacity building (improvement of buildings, medical equipment and education) and to reform the way of health system functioning. For instance, European Union supported health care in Serbia since the year 2000 with more than 100 million Euros [10] and several soft loans were given from the World Bank (WB) and European Investment Bank (EIB) for a project with the similar purposes.

Expenditures for health care in Serbia

Data on expenditures on health care in Serbia differs, depending on the source. According to the World Bank, the total (public and private) health expenditure in Serbia accounts for about 10.4% of gross domestic product (GDP) in recent 4 years [11]. Serbia spends a larger share of GDP only for financing pension expenditures. It is a relatively high percentage, only few European countries spent more of GDP on health in 2010 (The Netherlands, France, Germany, Switzerland, Denmark, Austria, Portugal, Belgium, Bosnia and Moldova). However, Serbian GDP is significantly smaller than in the majority of European countries, so the actual amount *per capita* is low (only Russian Federation and some former members of USSR, Bosnia, Romania, Bulgaria, Macedonia and Albania spend less than Serbia). For comparison with other countries, when this amount is adjusted for purchasing power parity Serbian expenditures *per capita* are around half of the average of the new EU members, and about a sixth of that of the EU-15 [12]. An increasing trend of total health care spending *per capita* was clearly present during last decade world-wide. In Serbia its level reached a maximum of 673 US dollars (USD) in 2008. Since that time, mainly because of the global economic crisis, the GDP in Serbia declined, and so did the total health care spending *per capita* which amounted 546 USD in 2010 [11, 13]. Projections for the future do not predict increase of public funding for health care [14].

For many years there was no exact evidence or even reliable assessment on private expenditures on health care and current data are based on estimation from the household budget survey [13] because private funding is almost completely based on out-of-pocket money. There are two types of out-of-pocket patient payments: official copayments and informal (unofficial) patient payments. Official copayments for services in public health system in Serbia are very modest and do not represent the financial burden for patients. However, in Serbia, like in many low- and middle income countries, informal payment may create an access barrier to health care for the patients who cannot afford to pay. These expenditures mainly include buying of medicines which are not on the positive list and use of private health care services [15, 16]. Private Medical Chamber reported more than 20 million services *per* year estimating that more than 50% of the population use them, mainly for dental and specialist care and diagnostics [17]. Possibility for doctors employed in public health system to additionally work in private facilities made private expenditures more pronounced. As some measure for control of funds flow in private sector, providers are obliged to share fiscal invoices with patients.

In recent years, the flow of funds through the health system in Serbia is monitored by the National Health Accounts (NHA). Development of this institution was supported by MoH and financed by the World Bank, and the first national health accounts was produced at the beginning of 2006. Among the main challenges to deal with for NHA is weak transparency in public and private financial flows, particularly informal payments [15]. NHA assessed that private spending on health, including under-the-table payments to providers, was significantly larger than reported from official statistical data. It was estimated that health insurance covers approximately 61.9% of the total health care expenses, and 38.1% of payment is additional out-of-pocket money. Proportion of expenditure that can be attributed to private spending is much larger than in the EU making health care less accessible to the poor.

Public funds for health care are currently allocated on the basis of the number of staff and/or beds at health facilities. In 2010 the total number of public health institutions in Serbia was 375 which included primary health centers (158), general hospitals (24), specialized hospitals (24), clinical centers (4), clinical-hospital centers (4), public health institutes (23), pharmacies etc. with 122,695 employees (114,432 permanently employed). The total number of hospital beds in 2008 was 39,660 (540 *per* 100,000 people) but the reduction to 525 *per* 100,000 was planed. It is less than European average of 570 per 100,000. About 18% of employees are medical doctors, 35% nurses, 21.5% other health workers (pharmacists, dental doctors, lab staff, etc.), and 25.5% are workers in administration and logistics. There were 281 medical doctors employed in public health per 100,000 people (European average was 321 *per* 100,000). However, according the number of licensed medical doctor registered at Doctors Chamber, which included and doctors in private practice, this number was 387 per 100,000.

About half of medical doctors from the public health care facilities are employed in hospitals [18, 19]. The number of dental doctors in public health care system is relatively small because the financing of dental care is limited only to children, students, pregnant women and some special categories of patients. Facing inherited problems the MoH made the action plan for building of human resources to meet international standards. Human resources strategy was not appropriate for decades and education policy was not coordinated with the needs of health care, so the number of unemployed doctors was constantly increasing (about 2000 medical doctors and 1200 dental doctors were looking for the job in the health sector in 2012). At the same time, there was insufficient number of some specialists (radiologists, anaesthesiologists, cardiac surgeons, etc.) [20, 21]. Low salaries and high unemployed rate create an incentive for doctors to emigrate.

The largest proportion of RHIF's expenses is designed for the salaries of employees in the public system. In accordance with the effort to constrain public spending, the share of total expenditures for employee's salaries decreased from 61.20% in 2008 to 56.21% in 2010 [19]. Divergences between wages of different medical professions (e.g. specialists, general practitioners, pharmacists, nurses) are small and the doctors' salaries are among the lowest in Europe [12]. Salaries for medical staff represent the greatest part of the health services expenditures in many European countries, including EU. However, in these countries gross salaries of employees in health care are much higher than countries average, while, in Serbia, as in other Western Balkan countries, they are lower.

The structure of RHIF's expenditures in recent years has been almost the same: generally, more than 50% of funds are directed towards secondary and tertiary health care, almost 25% towards primary health care and more than 12%

are expenditures for prescribed drugs. The rest include all other expenditures (rehabilitation, dental care, sick leave benefits, etc.) [21].

Current efforts to achieve financial sustainability

Expenditures for healthcare in Serbia absorb a large share of GDP and there is no fiscal space to increase public expenditures, especially in current economic situation [22]. Despite the fact that the needs were recognized, from 2009 to 2012 less funds from the budget were always allocated than for the real needs of people without insurance. Some drug wholesalers also bear the responsibility for the financial problems in the health system.

Since 2010 some of them started the "inflating balloon", similarly to the other institutions on the planet in financial crisis – the money (they have been receiving from the pharmacies with a grace period of three months) were given to the producers very late, even up to a year, or more. This put the pharmaceutical industry in Serbia in a serious problem so producers stopped with the drug delivery to the health institutions.

As there is practically no possibility for the better financial support of the health care from the state budget, it has to be done by reforming the system. From the economical point of view, there are two essential ways to achieve financial sustainability: increasing revenue collection and allocating the available resources more efficiently.

Compulsory prepayment of health insurance with the controlled pooling mechanisms by RHIF is planned to continue to be the major source of funds for health care. Avoidance of insurance premium payment was identified as a serious problem. The practical solution for improvement of efficiency of revenue collection may be in increasing the financial discipline by imposing new laws on punishing tax evasion including evasion of compulsory insurance contributions.

Public health system had a chronic debt which continued to increase annually. For many years, RHIF revenue collection from obligatory insurance was less than expected, and the government has not always made adequate provisions for its contributions in respect to non-payers [8]. There is no available exact data on the level of the deficit and the only available information is from the statements published in press that contributors owe to RHIF more than 1,000 million Euros [23, 24]. This situation, low income from insurance payment and insufficient transfer of funds from the budget, on the one hand, and the extensive package of services covered by health insurance, on the other, commenced a cycle of debt in which RHIF failed to pay on refund to hospitals and other providers, who in turn delayed payment to suppliers such as drugs and utilities companies. It resulted in shortage of some drugs and patients were often forced to purchase them in private pharmacies. In recent years, the debt continued to increase, so with about 26 billion dinars (RSD) or almost 300 million USD, health sector accounts for the substantial part of Serbia's public debt in 2012. Of the total debt, hospitals owe more than 13 billion to the pharmaceutical industry, while the remaining 13 billion is to be paid by RHIF for the prescribed medicines distributed by pharmacies.[25] Recently, efforts to improve financial sustainability of the RHIF were made by the Government. Some agreements on debt reprogramming and 50% discount are made between the representatives of Government and pharmaceuticals suppliers.

After declaring the debt of health sector to the providers as public debt, the repaying of the rest (a half the amount of 26 billion RSD) was agreed in Parliament [26]. This means that in 2013 RHIF could start without burden of a huge chronic debt. As pharmaceutical procurement and pricing system had great impact on RHIF expenditures, to avoid further debts, a new system of centralized procurement will be established. It is expected that it could be the way of preventing the corruption and to save the funds by achieving the lower prices of pharmaceuticals negotiated with suppliers [27]. According the World Health Organization (WHO) estimates, appropriate use of medicines could save countries up to 5% of their health expenditure. It means prescribing equally effective but cheaper drugs if available, avoidance of drugs overusage (especially antibiotics and injections), better storage and wastage and appropriate procurement [28].

However, to achieve financial sustainability of the RHIF, additional funding may be needed. Financial planning in respect to contribution rates was not appropriate and though the rates for health insurance were high, they are not set according to an actuarial analysis of expected costs. They tend to be based on a combination of estimates of desired revenues (which may not reflect the actual revenue that can be feasibly collected) and the assessment of the political acceptability of adding to already high tax burden [12]. The WHO recommended some innovative financing of health care for countries world-wide. Taxes on products harmful to health may have dual positive effect by reducing consumption and increasing funds. Since 2006 one dinar of the tobacco excise tax is already allocated to the MoH. Additional funds from taxes on alcohol may be collected. Some countries are also considering taxes on other harmful products, such as sugary drinks and foods high in salt or transfats [29, 30]. Serbian Government may implement those that best suit the economy of the country and are likely to have political support. Serbia currently has a kind of paradox, since the Government subsides the price of the unhealthiest type of white bread, while the bakery products made of integral grain are much more expensive.

Health is one of the most important subjects that require global solidarity. High-income countries and the international community financially supported the Serbian health care. Donations currently come from various sources and there is no exact data on the purpose and total sum. Identifying the priorities and making action plan for using them could considerably improve their positive impact and even contribute to bring in more funds in future.

Efforts to improve efficiency and productivity through payment reform

Collecting sufficient funds is essential, but it could not guarantee sustainability and quality of health care if the resources are not used appropriately. From the economics

point of view, the intention of reforms is to allocate resources more efficiently, which means to distribute existing funds for health in a different way and achieve better outcomes. In the long-term, the increase of preventive services is expected to result in decreasing the need for more expensive diagnostic services and hospital treatment. Planned changes in treatment behaviour should lead to increased productivity and reduced costs, so some expenditure may be shifted from staff and utilities to medicines and supplies. Contracting and change of methods of payment may also be valuable tools to improve efficacy.

The introduction of the capitation system into the primary health care was the first major payment reform measure. Primary health care in Serbia is provided in out-patient centres, known as *Dom zdravlja* (DZ) by three types of doctors: general practitioners, paediatricians and gynaecologists. The introduction of the concept of selected doctors, or "chosen doctors" at the primary level is supposed to enable better coordination between different levels of care, but also to promote health and preventive services, as opposed to the current system which is dominated by a clinical (curative) approach [31]. Capitation as a method of payment was recommended by the World Bank which conducted a cost and efficiency study of 147 DZs [32]. The study showed a very little variation in the cost-efficiency of DZs, because expenditures were largely pre-determined by the prices of input factors, mainly personnel wages (70% of total cost) which were set according the line budgeting and not according the outcomes.

Payment *per capita* means that doctors should be basically paid by the number of patients who choose them. It started in 2012 and according the capitation formula, the main additional criteria for calculating salary include prescribing (the cost of drugs prescribed), the number of actually treated patients and the number of preventive and screening examinations.[33] Introduction of capitation should motivate doctors to provide more preventive exams and to reduce the need for more expensive diagnostic and therapeutic procedures, to prescribe less expensive medications, and to avoid unnecessary laboratory tests. So far, there have been no estimates of its functioning, except for the coverage of registered insured persons with chosen doctors.

The increase of salary according the proposed criteria is limited to 4% maximum, so there is a suspicion as to how effective it would be in changing the behaviour of medical staff.

Reforming hospital payment mechanisms is one of the areas where substantial efficiency gains could be made. Expenditure on hospital services is one of the largest shares of total health care spending in Serbia, as well in other European countries [34]. Both rich and poor countries face similar challenges with regards to ensuring efficiency and value for money through hospital payment mechanisms. These mechanisms mainly include global budgets, fee for service, daily rebates and case payments. Each of these modalities motivates providers' behaviour differently [35]. Many countries world-wide accept Diagnosis Related Groups (DRGs) as kind of case-based payment and it is the most common mechanism for reimbursing hospitals in Europe. DRGs are classification systems that group patients according to the consumption of resources required for their treatment and their clinical characteristics. Originally, DRGs are developed in the USA and its use as an instrument for cost containment for hospitals started in 1983. Many countries developed their most suitable variants (Australia, Germany, Switzerland, etc.) [36, 37]. Variables used to define DRGs are diagnosis code (principal and secondary), procedure code (i.e. surgical or non surgical), age, sex and discharge status (released home, transferred to other hospital, death, etc). These classifications have to be changed frequently due to the use of new diagnostic and treatment procedures.

Current funding on the basis of the number of staff and beds does not motivate providers to improve efficacy, quality of care and health outcomes, so the Serbian Ministry of Health plans to reform the payment system. The Serbia's Health Care Development Plan 2010–2015 [18] includes change of the funding of secondary and tertiary care. Implementation of payment by DRGs is planned for acute inpatient care. Expectations from this financing model are provision of equality of all hospitals and patients, increase of efficacy and transparency when contracting health care services and basing payments on the best available data. Reforms are supported by the MoH and financed by the World Bank soft lone. A system developed in Australia ("Australia refined" – AR-DRGs) has been chosen based on the experiences of neighbouring countries and positive results in an initial pilot study for reporting purposes in six hospitals. DRGs introduction would be incremental: in the beginning the system is planned to be applied as an analytical coding tool, then for reporting purposes and only after several years as a hospital reimbursement method. Trainers for coding skills have already been trained in order to disseminate knowledge about the new system [38].

Payment according to DRGs means that hospitals are basically paid the average cost for a case. It establishes a transparent link between funding and activity which is absent under retrospective global budgets. Formula may include indirect costs such as teaching, or be adjusted to some specific cost in the area or other economic conditions.

Introduction of DRGs based payment in hospitals is expected to minimize the cost of hospital stay. However, DRGs payment motivates hospitals to reduce the cost per episode of hospitalization irrespective of outcomes and may lead to lower quality of service as a way of cost saving. It is very difficult to monitor and control quality of care because it is almost impossible to distinguish whether a bad medical outcome is a result of low quality of care or the severity of illness. To optimize the payments they get, hospitals may implement organizational change and to introduce utilization of new technologies and procedures, but also may skip some medically indicated tests and therapies, or over-provide certain services to put the patient into a higher-paying category. They also may discharge patients earlier than clinically appropriate and–readmit them, or not admit patients whose treatment costs are likely to be higher than the average. Studies of the impact of DRGs on hospitals behaviour

showed the increased rate of admission, classification of illnesses as more severe and shortened length of hospital stay in many countries (Sweden, Norway, Denmark, Germany, England, etc.). In the majority of European countries, the introduction of DRGs payment increased total hospital costs, partly due to classification of diseases in higher DRGs and increased efficiency. This system of reimbursement may have contradictory effects for different patients groups depending on the price incentives provided by different DRGs [39–41].

It can be supposed that the implementation of the capitation formula and the diagnosis-related groups can make the system more efficient, but only with an adequate system of control. It includes the quality monitoring and more administrative costs. Some studies in the USA showed that the savings on clinical resources were almost fully invalidated by higher administrative cost [42, 43].

Conclusion

Each country has a system of health financing that it has developed over decades and there is no universal single effective strategy on how to finance health. When introducing reform measures, possible factors such as culture and tradition, the way of living and legislative aspects may have an important impact on the structure and quality of health care.

The Serbian public health system is founded on equity and solidarity and despite the political and economic changes the idea of universal coverage for the extensive level of services was kept. Some services, like dental care were cut, but there are no plans for radical market oriented reforms. The entire population has the right to use a large package of services (prevention, promotion, treatment and rehabilitation) and virtually everyone is protected from severe financial risks.

Countries, in which entire populations have access to a large package of public health services, including Serbia, usually have relatively high expenditures for health care – more than 5–6% of GDP. Nevertheless, it is not enough even for the high-income countries that are commonly said to have achieved universal coverage actually to cover the whole population for 100% of the services available and for 100% of the cost – and with no waiting lists. In the report on health financing published in 2010 by the WHO [28] it is stated: "All health systems, everywhere, could make better use of resources, whether through better procurement practices, broader use of generic products, better incentives for providers, or streamlined financing and administrative procedures". This report pointed to the changes of the Serbian health care system which could improve its financial sustainability and efficiency.

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PRACTICAL ADVICES FOR PHYSICIANS

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Understanding sensitivity, specificity and predictive values

Razumevanje osetljivosti, specifičnosti i prognostičkih vrednosti

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data interpretation, statistical; sensitivity and specificity; predictive value of tests.

Ključne reči:
statistička analiza podataka; osetljivost i specifičnost; testovi, prognostička vrednost.

Introduction

Modern medicine and medical science have experienced a change in knowledge and dramatic increase in technology. Nowadays "Hi-Tech" tests are widespread and doctors can hear a statement: "This new test is very reliable, it is the most sensitive test at the market, you should use it", practically every day. The basic idea for using diagnostic tests aims to easier making the diagnosis of a disease and so enable appropriate treatment. How to know if a test is useful? Will a test point out to a doctor whether a patient is ill or not?

Due to the fact that the doctors and medical research workers are not so common with the statistical methods, the aim of this paper was to explain the basics of sensitivity, specificity and predictive values in a very simple way, using simple logic formulas, and presenting the samples from medical practice.

Diagnostic test and the gold standard

Diagnostic tests are all the tests that physician can use in the process of making the diagnosis of a particular disease. It is a procedure performed to confirm or determine the presence of a disease in a person suspected of having the disease, usually following the report on signs and symptoms, or based on the results of other medical tests [1].

The most accurate test for determining a disease is a "gold standard". Since it represents the best of the existing tests, we may consider the "gold standard" as a currently preferred method for diagnosing a specific disease [2]. It is often invasive or expensive; therefore some other diagnostic test may be used instead. Hence, a newly designed test has to be initially validated by comparing its results with a gold standard due to establish the exact health status of a person. Some of the known test examples are: uriscreen for urinary tract infection, blood pressure for hypertension, pap smear for cervical carcinoma, mammography for breast cancer, prostate specific antigen (PSA) for prostate cancer, fecal occult blood for colon cancer, ocular pressure for glaucoma, colonoscopy to find early cancers and potentially cancerous polyps, ultrasound for thyroid cancers, nuclear medicine techniques to examine a lymphoma, measuring blood sugar for diabetes mellitus, taking a complete blood count for bacterial infection [3]. Gold standard may be arbitrary and may change. When a new test is under consideration for using in practice it should be good enough to replace the gold standard for some particular disease, otherwise should be discharged or used as a preliminary test. The doctor's goal is to realize which test and testing strategies are best for making the correct diagnosis.

Validity

Validity is the capability of a test to point out which people have a disease and which do not. It is the test accuracy, or the extent to which a test is able to measure what should be measured [4]. Validity is estimated by two objective measures: sensitivity and specificity [5].

The ideal test should correctly identify all tested people with or without disease with 100% of accuracy, which is practically impossible. Traditionally, to help understanding sensitivity, specificity and predictive values, the best method for explanation is based on 2 × 2 contingency table. Suppose a population of 1,000 people, 100 of them have a disease X, 900 do not have the disease according to the gold standard results (Table 1). Here, the rows represent the screening test results and the columns the true condition of a person according to the gold standard. Screening test is used to identify 180 people with the disease (a + b).

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Table 1

The table 2 × 2 (for diagnostic test results)

	Disease X		
Screening test results	present (n)	absent (n)	Total (n)
Positive	80 (a)	100 (b)	180 (a + b)
Negative	20 (c)	800 (d)	820 (c + d)
Total	100 (a + c)	900 (b + d)	1000 (a + b + c + d)

a – true positive (sick people correctly diagnosed as sick);b – false positive (healthy people wrongly diagnosed as sick); c – false negative (sick people wrongly diagnosed as healthy); d – true negative (healthy people correctly diagnosed as healthy).

Sensitivity

Sensitivity of a clinical test represents test ability to correctly identify people with illness (a) within all people with illness (a + c). It is a proportion of people with disease who positive, expressed in percentages. Sensitivity as a fixed test characteristic provides a true positive rate [5, 6].

$$\text{Sensitivity} = \frac{\text{True positives}}{\text{True positives} + \text{False negatives}}$$

If we apply screening test to our hypothetical population and receive that 80 of the 100 people with disease X test positive, than the sensitivity of this test is 80/100 or 80% (Table 1). A test with 80% of sensitivity detects 80% of true ill patients, while 20% (false negative) will not be detected.

Specificity

The specificity of a clinical test represents test ability to correctly identify people without illness (d) within all people free from illness (b + d). It is a proportion of people without disease who test negative. Specificity is also a fixed characteristic of the test and represents true negative rate [7, 8].

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}}$$

This hypothetical population (Table 1) demonstrates that 800 of the 900 people without disease X test negative, so specificity of this screening test is 800/900 or 89%. Practically, this test detects 89% of healthy people but 11% will be assumed as false positive.

For a test to be accurate, both sensitivity and specificity should be high. When measuring sensitivity, we only calculate those people with disease. High sensitive test detects a high percentage of positive cases while missing few. Also, a negative result would suggest the absence of disease according to test with high sensitivity. On the contrary, specificity highlights negative test results. A highly specific test is good for detection of a disease if a person tests positive, likewise it does not falsely diagnose disease when none is present.

It is worthy to mention that specificity and sensitivity of a quantitative test depend on a cut-off value. This is the value which determines the limit between positive and negative test results. In a situation when the cut-off is reduced, most people with the disease will be correctly identified, but at the same time the number of false positives will be increased. Raising the cut-off value will show more false negatives, but will reduce the number of false positives [9, 10]. Practically, sensitivity and specificity are inversely proportional, as one increases the other decreases and *vice versa*.

The lack of adequate education about interpreting test results (especially relevant to tests that may have minor and insignificant findings) may lead to misdiagnosing [11]. It is known that in two cases if test result doesn't correspond to the real situation an error has occurred. Therefore it is essential to pay attention to false positive and false negative test results. These errors are closely associated with the terms of type I and type II errors in hypothesis testing. In hypothesis testing "null hypothesis" matches to the natural state (in our situation people who are free from disease). As opposed to the null hypothesis there is an “alternative hypothesis” which corresponds to the ill people. Type I error, also known as “α” error appears when we reject null hypothesis which is actually correct [12, 13]. Type I error corresponds to the false positive results. Type II error or “β” error appears when accepting null hypothesis, when actually it is not really true [12, 13]. This error corresponds to false negative test results. It depends on a situation in which a false result is more undesirable. Minimizing false positives and false negatives at the same time maximizes sensitivity and specificity. Generally, it is not benign to tell someone after testing that he has a serious disease (false positive) when he does not really have the same (HIV for example). Moreover, it is inexcusable to overlook a disease when it really exists (false negative).

Sensitivity and specificity do not depend on the disease prevalence [14]. They are conditional on the patient either having or not having a disease and represent the power of a diagnostic test to discriminate between those with and without disease. When a patient has a positive test result, does it actually mean that he/she has disease or not? Sensitivity and specificity cannot answer such a question, thus it is worthy to know predictive values.

Predictive values

The real questions to be answered are the following: “What is the probability that a person with a positive test results will have the disease? Also if a person has a negative test, what is the likelihood that he is healthy?” These questions refer to what's called the “predictive values”. Therefore, the mission of the clinician is to determine the likelihood of a disease present given a positive test (positive predictive value – PPV), or the likelihood of a disease absent given a negative test (negative predictive value – NPV).

Positive predictive value

The positive predictive value or precision rate is defined as a proportion of people with a positive test result (a) who are actually ill (a + b) [5, 8]. It is calculated by the formula:

$$\text{Positive predictive value} = \frac{\text{True positives}}{\text{True positives} + \text{False positives}}$$

In our population from 180 (a + b) people who test positive, 80 (a) of them actually have a disease X. PPV in this case is 80/180 or 44%. This practically means that a person who tests positive has a 44% likelihood of having a disease X. Less false positives (higher specificity) signifies a higher PPV in the observed population.

examined (Table 2). Since this is the same test, sensitivity and specificity remain the same 90% and 80%, respectively. According to this new scenario, PPV is 450/550, or 82%, whereas the NPV is 400/450, or 89%. In the case when we repeat the same test to some other population with different disease prevalence, we will notice that the PPV increases with the increasing disease prevalence while the NPV decreases in the same situation (Table 3). Though the sensitivity and specificity remain the same, the PPV has changed remarkably.

Table 2

Calculation of predictive values at 50% disease prevalence

Test	Disease present (n)	Disease absent (n)	Total (n)
Positive	450	100	550
Negative	50	400	450
Total	500	500	1000

Prevalence = 500/1000 = 0.5; sensitivity = 450/ 500 = 0.9; specificity = 400/500 = 0.8; PPV = 450/550 = 0.82; NPV = 400/450 = 0.89; PPV – positive predictive value; NPV – negative predictive value.

Table 3

Relationship between disease prevalence and predictive values for a test of 90% sensitivity and 80% specificity

Prevalence (%)	Positive predictive value (%)	Negative predictive value (%)
5	0.19	0.99
10	0.44	0.97
50	0.82	0.89
70	0.90	0.77
90	0.96	0.47

Negative predictive value

The negative predictive value is defined as a proportion of people with a negative test result (d) who actually do not have disease (b + c) [5, 8]. The formula for this measure is:

$$\text{Negative predictive value} = \frac{\text{True negatives}}{\text{True negatives} + \text{False negatives}}$$

We can see from our example (Table 1) that out of 820 people disease free, 800 people test negative. That basically means, a person who tests negative has 800/820 = 0.97 or 97% likelihood of not having a disease. NPP of enormous 99% indicates that a negative screening test actually dismisses disease. On the other hand, a very low PPV (only 44%) indicates that every second ill patient is diagnosed wrongly according to this test. A highly sensitive test (small number of false negatives) will have a higher NPV in the observed population.

In clinical practice, the value of a test result for an individual patient depends on the prevalence of tested population (the proportion of the population that has the disease at a given time) [15, 16]. We should expect that for any given population, as the prevalence of a disease increases, the test PPV will also increase and *vice versa*, as disease prevalence in the population being tested decreases, the PPV of that test will also decrease, while the NPV will increase. To help you understand these relationships we will illustrate this through the following example. Imagine that we now apply the same screening test we used before (Table 1) to another population where the disease prevalence is 50%, respectively 500 sick patients out of 1,000

The utility of predictive values is limited because the statistics is determined by sensitivity and specificity of a test, as well as with the prevalence of disease which can vary [17]. In general, specificity has more impact on a positive predictive value in the case of low disease prevalence [18]. Constancy of sensitivity and specificity are an important feature of a test when using in similar patients and similar settings. Predictive values although associated with sensitivity and specificity will change with the prevalence of target disease (Figure 1).

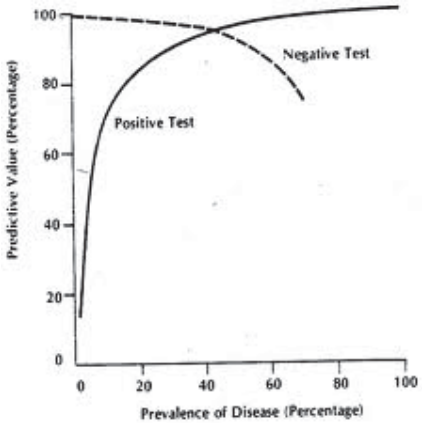

Fig. 1 – Disease prevalence and predictive values

illustrates the effect of the disease prevalence on the PPV and the NPV. Decreasing the disease prevalence increases the number of false-positive test results, while increasing the disease prevalence decreases the number of false-negative test results.

It is vital to note while comparing two populations that predictive values are only significant if the disease prevalence is the same in both populations. They are used along with the specificity and sensitivity when the prevalence in the target population is known. In the case of unknown prevalence, the sensitivity and specificity are the primary measurement used to evaluate the accuracy of a test [19].

Conclusion

To enable interpretation of diagnostic test accuracy it is necessary to understand the concepts of sensitivity, specificity and predictive values. These calculations require a design of the two-by-two table. Sensitivity and specificity of a test have limited clinical usefulness of the disease likelihood in an individual patient. Although sensitivity and specificity are not under the influence of the disease prevalence they can be affected by the differences in disease characteristics. In everyday clinical practice knowing the predictive values is more significant for measuring diagnostic accuracy. So, next time you hear about a new reliable test, ask yourselves what gold standard is performed, what is the disease prevalence, and most important of all, what are the sensitivity, specificity, positive and negative predictive values? If the answers are satisfactory, you can recommend this test for use.

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CASE REPORTS

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Syncope as initial symptom of ostial lesion of the left main coronary artery with cardiogenic shock

Sinkopa kao početni simptom ostijalne lezije glavnog stable leve koronarne arterije sa kardiogenim šokom

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Abstract

Introduction. Syncope represents a relatively atypical symptom of acute coronary syndrome. Syncope itself does not provide enough information to indicate an acute coronary event, especially a lesion of the left main coronary artery, without malignant rhythm and conduction disorders. **Case report.** A male patient, aged 63, was admitted to the intensive cardiac care unit because of a short loss of consciousness, in sinus tachycardia, with signs of acute heart failure and being hypotensive. Electrocardiogram showed a possible acute anterior myocardial infarction, followed by cardiogenic shock and emergency coronary angiography (subocclusive ostial lesion of the left main coronary artery) and primary percutaneous coronary intervention with intraaortic balloon pump therapy was performed. A direct drug eluting stent was implanted with the optimal primary result. **Conclusion.** The prompt diagnosis, especially in such relatively atypical clinical presentation, reperfusion therapy with primary percutaneous coronary intervention in acute myocardial infarction complicated by cardiogenic shock, contribute to the improvement in the survival rate and patient's quality of life. This case report is clinically educative due to relatively atypical presentation and performed interventions.

Key words:
myocardial infarction; syncope; shock, cardiogenic; coronary angiography; drug-eluting stents.

Apstrakt

Uvod. Sinkopa predstavlja relativno netipičan simptom akutnog koronarnog sindroma. Sama sinkopa ne pruža dovoljno informacija za dijagnozu akutnog koronarnog događaja, naročito lezije glavnog stabla leve koronarne arterije, bez malignih poremećaja ritma i provođenja. **Prikaz bolesnika.** Bolesnik, star 63 godine, primljen je u jedinicu intenzivne kardiološke nege zbog kratkotrajnog gubitka svesti, u sinus tahikardiji, sa znacima akutne srčane slabosti, hipotenzivan. Elektrokardiogram je pokazivao suspektne znake za razvoj akutnog infarkta miokarda prednjeg zida. Zbog toga, kao i zbog razvoja početnog kardiogenog šoka, indikovana je i urađena urgentna koronarografija (subokluzivna lezija ostijuma glavnog stabla leve koronarne arterije) i primarna perkutana koronarna intervencija sa implantiranom intraaortnom balon pumpom. Direktno je ugrađen stent obložen lekom sa optimalnim primarnim rezultatom. **Zaključak.** Brza dijagnoza, naročito sa ovakvom relativno atipičnom kliničkom slikom, brza primena reperfuzione terapije u vidu primarne perkutane koronarne intervencije u akutnom infarktu miokarda komplikovanim kardiogenim šokom, doprinosi poboljšanju preživljavanja i kvalitetu života bolesnika. Ovaj prikaz bolesnika klinički je poučan zbog relativno atipične prezentacije i izvršenih intervencija.

Ključne reči:
infarkt miokarda; sinkopa; šok, kardiogeni; angiografija koronarnih arterija; stentovi, lekom obloženi.

Introduction

Syncope is a transient loss of consciousness precipitated by cerebral hypoperfusion, which is associated with the absence of postural tone and usually followed by a complete recovery within a few minutes. This clinical condition is a common medical problem with an estimated incidence of *per* 1,000 persons *per* year and accounts for 1% of emergency department visits and 6% of all hospital admissions [1].

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Syncope represents a relatively atypical symptom of acute coronary syndrome. Only 3.1% patients with syncope were diagnosed with acute myocardial infarction [2]. Syncope itself does not provide enough information to indicate an acute coronary event, especially a lesion of the left main coronary artery, without malignant rhythm and conduction disorders. Many of the missed diagnoses included syncope that resulted in medico-legal action involved. Therefore, establishing the diagnosis of syncope is important so that specific treatment can be administered to prevent future recurrences and eliminate the underlying predisposing disease.

Treatment of unprotected stenosis of the left main coronary artery (LMCA) still remains a challenge for interventional cardiologists. According to the current recommendations of the American Heart Association (AHA) and American College of Cardiology (ACC), unprotected LMCA stenosis represents an indication for cardio-surgical treatment, except in special situations where interventional cardiology has its place [3]. Some of them are ostial lesion of the left main stem, proximal lesion of the left main stem, acute myocardial infarction, and initial and developed cardiogenic shock [3]. According to current recommendations, the patients with acute myocardial infarction (AMI), complex mechanical complications or cardiogenic shock are supported using intra-aortic balloon pump (IABP) [4]. Nowadays it is recommended that even percutaneous coronary intervention (PCI) on LMCA is performed with the support of IABP or other circulatory support [5].

For this reason, we presented a patient with syncope and acute myocardial infarction with incipient cardiogenic shock, diagnosed with unprotected ostial lesion of the left main coronary artery.

Case report

A male patient, aged 63, was admitted to the intensive cardiac care unit for a short loss of consciousness, without chest pain. There was no history of previous syncopal or presyncopal states or ischemic heart disease symptoms. Risk factors for ischemic heart disease were hypertension and smoking. At admission, the patient was conscious, hypotensive with blood pressure of 90/60 mmHg, with signs of acute heart failure and sinus tachycardia – heart rate of about 100/min, without malignant rhythm and conduction disorders. Electrocardiogram registered signs of possible acute anterior myocardial infarction, with incomplete left bundle branch block (LBBB) and first degree heart (AV) block (Figure 1). The initial laboratory tests showed increased values of myoglobin (261 ng/mL, reference values up to 107 ng/mL), whereas the levels of hs troponin and creatine kinase-MB (CK-MB) were within the reference range, due to patient's early presentation. Initial echocardiographic examination showed ischemic cardiomyopathy with ejection fraction (EF) of 50%, and inferolateral basal and medial hypokinesia, apical anterior, basal inferior and medial anterior akinesia, with hyperkinetic movements of other segments. Soon after admission, the state of the patient worsened and the first signs of cardiogenic shock were registered (the mean arterial pressure – MAP of 60 mmHg, diuresis 30 mL/h). For this reason, the patient was initially administered midazolam, and the sedation was later maintained using propofol, and was supported with invansive mechanical ventilation (IMV) in the intermittent positive pressure ventilation (IPPV) mode with PEEP (positive end-expiratory pressure) 5 cm H_2O. The patient was treated with dual antiplatelet therapy (aspirin 300 mg, clopidogrel 600 mg), statin 20 mg through nasogastric tube, heparin (1,000 IU/h), and inotropic stimulation with dopamine 8 mcg/kg/min. Clinical status, electrocardiographic changes, increased level of myoglobin, and echocardiographic findings gave indications for emergency coronary angiography, which was performed within two hours after syncope. Coronary angiography of the left coronary artery indicated subocclusive ostial lesion of the left main coronary artery with TIMI (thrombolysis in myocardial infarction) grade 2 flow (Figure 2). The right coronary artery was without significant lesions (Figure 3). After consultation with the cardiac surgeon, because of the possibility for emergency surgical revascularization, primary percutaneous coronary

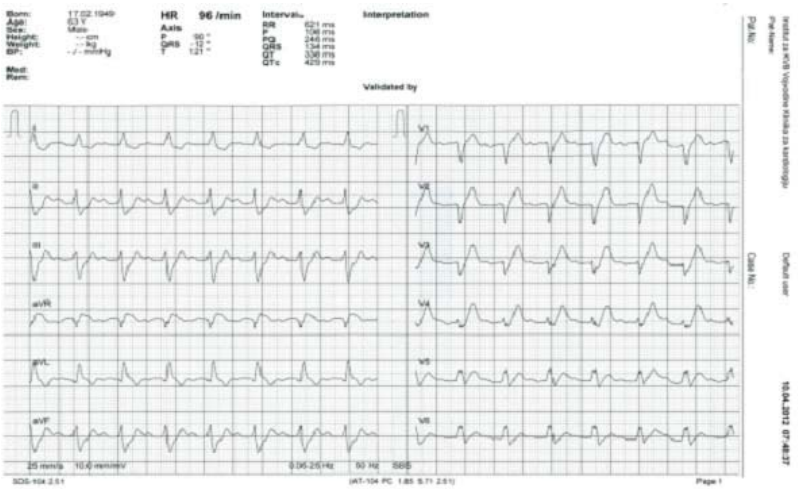

Fig. 1 – Electrocardiogram at admission – sinus rhythm, incomplete left bundle branch block, heart (AV) block 1st degree, heart rate 96/min, signs indicating possible acute anterior myocardial infarction.

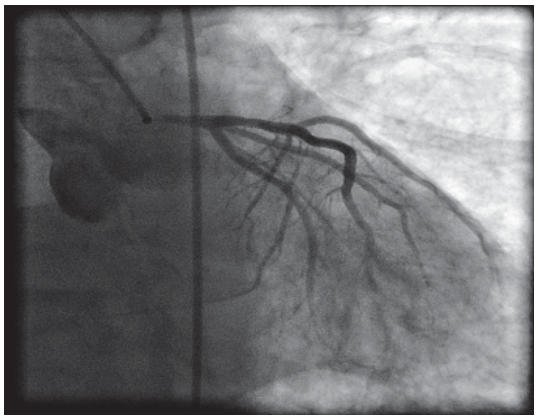

Fig. 2 – The right anterior oblique (RAO) projection of the left coronary artery – ostial lesion of the main stem.

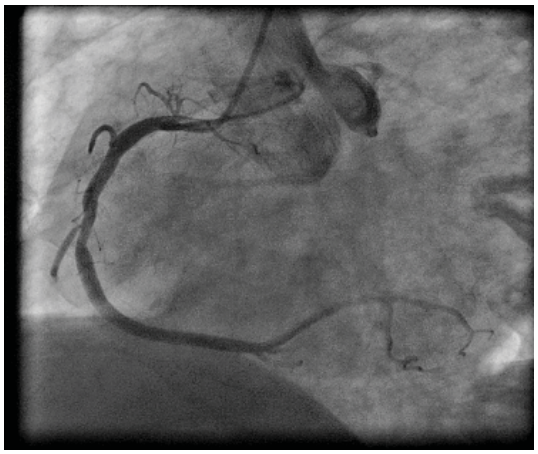

Fig. 3 – The left anterior oblique (LAO) projection of the right coronary artery.

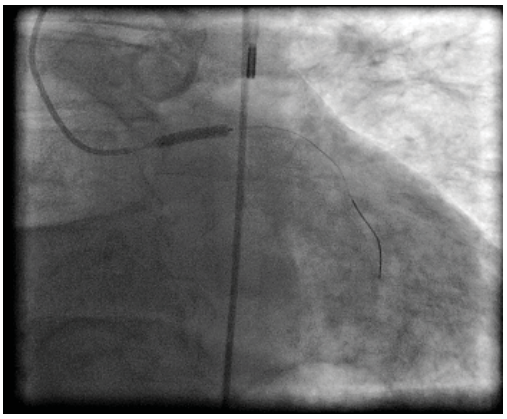

Fig. 4 – Direct implantation of the stent in the ostium of the left main coronary artery with the support of an intra-aortic baloon pump.

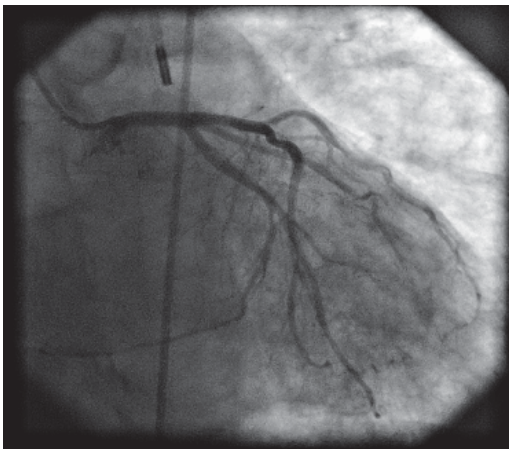

Fig. 5 – The right anterior oblique (RAO) projection of the left coronary artery after stent implantation – the optimal primary results of the intervention.

intervention (pPCI) was performed. Periprocedural heparin (10,000 IU) was administrated intracoronary. Initial heparin infusion was stopped. As a result of the developing cardiogenic shock and lesion of the left main coronary artery, an intra-aortic balloon pump (IABP) with the ratio 1:1 was implanted before the intervention. The following were used in pPCI: 5F left Judkins guideing catheter 4.0, guidewire (Runthrough, Terumo Europe) and a drug-eluting stent (Nobori 3.5 × 14 mm, Terumo Europe).

During cannulation of the left coronary artery, its occlusion occurred as a result of ostial lesion, so immediately before the intervention, the guidewire was placed with the stent in the catheter, together cannulating the left coronary artery. A direct stent was deployed at 14 atm and the primary result of the intervention was optimal (Figures 4, 5). In order to prevent acute stent thrombosis, after the stent deployment, glycoprotein IIb/IIIa inhibitor abciximab was administered intracoronary, and continued intravenouslly during following 12 hours. After the intervention the patient still had signs of cardiogenic shock and was supported with the intra-aortic balloon pump. Right cardiac microcatheterisation was performed through the right interior jugular vein with a pulmonary artery catheter (Swan-Ganz). The value of pulmonary capillary wedge pressure (PCW) was 8 mmHg. These hemodynamic parameters can be explained with the sedation of the patient on IMV with PEEP 5 cm H_2O, which caused systemic and pulmonary vasodilatation and lower PCW. Expansion of the volume was initiated using glucose and electrolyte solutions, along with inotropic stimulation with dopamine 8 mcg/kg/min. After this treatment, hemodynamic stabilisation was achieved (MAP 75 mmHg), PCW increased to 18 mmHg and diuresis was 150 mL/h. As a result of intermittent hemodynamic and respiratory instability, in the following four days the patient was on IMV in IPPV mode of ventilation, supported with IABP. After that, the patient was hemodynamically and respiratory sufficient, so he was extubated and circulatory support was stopped. The intra-aortic balloon pump removed without haemorrhagic complications. Carotid arteries ultrasound showed no significant lesions of carotid arteries. Follow-up echocardiographic examination showed EF of 45%, anteroseptal hypokinesia with medioapical septal akinesia. On the 15th day after admission, the patient was discharged with the diagnosis of acute anterior myocardial infarction (on the basis of electrocardiographic changes, and later increased levels of hs troponin and CK-MB, echocardiographic findings and the results of coronary angiography) (Figure 6), in cardiologically stable condition with double antiplatelet therapy (aspirin 300 mg, clopidogrel

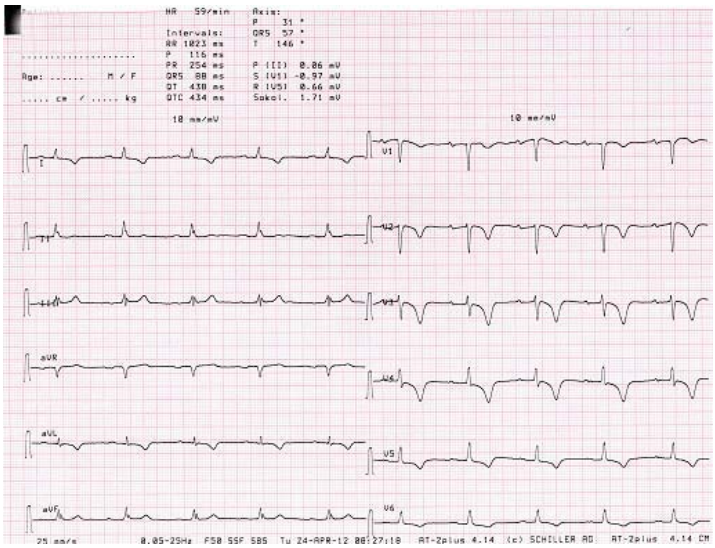

Fig. 6 – Electrocardiogram at discharge – sinus rhythm, heart rate 60/min, negative T waves in DI, AVL, V2-V6.

150 mg), beta-blocker (metoprolol 100 mg), ACE inhibitor (ramipril 2.5 mg), statin (atorvastatin 20 mg), diuretic (furosemid 40 mg) and aldosterone antagonist (spironolactone 25 mg). In the following 12 months the patient was without subjective complaints. Follow-up echocardiographic examination showed increased EF of 55%, hypokinesia apical-septal basal inferior. A stress exercise test using the Bruce protocol was performed and no signs of reduced coronary flow were registered. Ambulatory Holter-electroardiography monitoring showed sinus rhythm, with the average heart rate 69/min, rare ventricular and supraventricular premature beats. No conduction disorders were detected.

Discussion

Cardiovascular causes are the most common life-threatening conditions associated with syncope, and these can be divided into arryhthmogenic, structural, and ischemic [1]. Syncope from a sudden disruption in cardiac output is the deadliest form of syncope. Arrhythmogenic causes of syncope can include ventricular tachycardia, bradycardia [e.g. Mobitz type II or 3rd degree heart (AV) block], and significant sinus pauses (more than 3 seconds). Ischemia includes acute coronary syndromes, acute myocardial infarction. Among structural abnormalities are: valvular heart disease, such as aortic or mitral stenosis, cardiomyopathy (e.g., ischemic, dilated, hypertrophic), aortic dissection, atrial myxoma, and cardiac tamponade [1]. Cardiac syncope more often occurs in patients older than 45 years. Life-threatening causes of syncope beside cardiovascular causes, include hemorrhage, and subarachnoid hemorrhage. Approximately 15% of the following life-threatening conditions present with syncope: subarachnoid hemorrhage, acute coronary syndrome, aortic dissection, leaking aortic aneurysm, and ruptured ectopic pregnancy. Many of the missed diagnoses of these five conditions that resulted in medico-legal action involved presentations that included syncope [1].

Syncope without chest pain is not the most typical symptom of acute coronary syndrome. In acute coronary syndrome, syncope is mostly caused by malignant rhythm and conduction disorders, and in fewer cases is caused by large myocardial damage. There is no much literature data on syncope after acute coronary event as neuromediated reaction. Sympathetic withdrawal seems to be the most likely mechanism of syncope [6]. In the presented case, the most probable cause of syncope was cerebral hypoperfusion, which occurred due to incipient cardiogenic shock, since unstable atherosclerotic plaque in the ostium of the left main stem caused transient myocardial ischemia with lower cardiac output. Strategies in diagnostic syncope include the patients history, special tests like carotid sinus massage, tilt-table tests, echocardiography (ECHO), exercise stress test, cardiac monitoring, Holter monitor, external loop recorder, implantable loop recorder and electrophysiologic study. In emergency cases, like the presented one, for the diagnosis etiology of syncope were ECG, cardiac monitoring, ECHO and coronary angiography were important facts. Exercise stress tests, ambulatory ECG Holter monitor, loop recorders are important facts for the patient's follow up.

Emergency echocardiography is an important and useful tool for establishing the diagnosis. That provides diagnostic and prognostic information on heart diseases that predispose patients to syncope, including the assessment of cardiac size, left-ventricular function, wall motion, valvular heart disease, pulmonary pressure or right ventricular strain and pericardial effusions. It has also become an established tool for diagnosing coronary artery disease [7].

Following the algorithm for emergency treatment of cardiovascular patients (ECG, biomarkers, echocardiography, and monitoring hemodynamic parameters), the precise diagnosis of acute myocardial infarction was quickly made and adequate treatment was provided. Primary percutaneous coronary intervention is nowadays an option for treatment of acute myocardial infarction, and it should be performed by the center with a large number of patients, which is able to perform the interventions 24 hours, 7 days a week, 365 days a year. Concerning treatment of lesions of the left main coronary artery, surgical myocardial revascularization

(CABG) is considered a "golden rule" for the treatment of unprotected LMCA, but PCI is emerging as a possible alternative to surgery in certain groups of patients. Localization of the lesion is an important factor when considering PCI as the treatment for unprotected stenosis of the left main coronary artery. Stenting the ostium of the left main stem or medial segment is preferable to distal segment with bifurcation or trifurcation lesions, which requires a considerable experience of the operator [4]. Percutaneous coronary intervention on bifurcation lesions of the left main stem is accompanied by a higher incidence of restenosis in comparison with the ostium or the medial segment [8]. The importance of lesion localization for the choice of treatment was presented in the SYNTAX study [9]; for example, patients with left main stem lesion and SYNTAX score higher than 33, with more complex or extensive coronary disease, have higher mortality with PCI than with CABG. According to the recommendations of ACC/AHA from 2009 in treatment of lesions on the left main stem PCI does not require follow-up coronary angiography [10]. Emergency PCI is recommended for unprotected lesion of the left main stem in the treatment of acute myocardial infarction, with "culprit" lesions, when anterograde flow is compromised, and when the patient is hemodynamically unstable; it is believed that in these circumstances PCI may enable a faster outcome than CABG [11]. Taking all these facts into account, the treatment using PCI for the said patient was chosen, because the patient was in the state of initial cardiogenic shock, the ostial lesion of the left main coronary artery was suitable for PCI and it was quicker to treat it in this manner than by using surgical myocardial revascularization.

A drug-eluting stent (DES) was implanted, in the presented patient because the results of randomised studies showed that there was a significantly lower rate of major adverse cardiac events (MACE), mortality, and repeated revascularisation after 6 month, 12 months, 2 years and 3 years, in comparison with bare metal stents (BMS) [12].

Cardiogenic shock is a cause of a high mortality rate in patients with acute myocardial infarction. Treatment of cardiogenic shock as a complication of acute myocardial infarction includes hemodynamic stability achieved by medication therapy or circulatory support and emergency revascularization using PCI or CABG. Medication therapy includes antiplatelet and antithrombotic drugs, vasopressors and inotropic agents. Antiplatelet and antithrombotic therapy should be done automatically in acute myocardial infarction. Volume application is often used, although it has not been analyzed in randomised studies. Vasopressors and inotropic agents are used because of their positive hemodynamic effect, but neither of them leads to permanent symptomatic improvement, and many even reduce survival rate, which may be associated with cell dysfunction caused by these drugs [13]. A recent randomised study compared norepinephrine and dopamine in cardiogenic shock. Dopamine was associated with a higher mortality rate and more adverse effects, such as arrhythmia [14]. In hypotension with other signs of cardiogenic shock, norepinephrine is recommended as the first choice. It should be initially administered in low doses and gradually titrated until systolic pressure reaches values of over 80 mmHg. After that, dobutamine can be administered together with norepinephrine for better contractility [13]. In the presented case, hemodynamic stability was achieved using dopamine and volume application.

A SHOCK study [15] revealed that patients with cardiogenic shock treated with pPCI or emergency surgical revascularization had better prospects of survival in comparison with patients with initial medication treatment.

Intra-aortic balloon pump is widely used in the treatment of cardiogenic shock and it represents the first line of circulatory support. A TACTICS study [5] has shown that IABP does not contribute to the reduction in intra-hospital mortality, but it brings about the improvement in a six-month mortality rate. A IABP-SHOCK II study has shown that the use of intra-aortic balloon counterpulsation does not significantly reduce a 30-day mortality in patients with cardiogenic shock complicating acute myocardial infarction for whom an early revascularization strategy was planned [13, 16]. Recent meta-analysis evidenced it in relation to survival rate when using IABP in cardiogenic shock [17]. IABP therapy is considered to be a class IIb indication (European Society of Cardiology guidelines) for the management of cardiogenic shock [13].

Conclusion

This case report shows that the fast diagnosis, especially in patients with relatively atypical clinical presentation, and application of reperfusion therapy using percutaneous coronary intervention in patients with acute myocardial infarction complicated by cardiogenic shock, as well as application of adequate medication therapy and circulatory and respiratory support, contribute to the improvement in the survival rate and the quality of life of such patients.

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CASE REPORT

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Transluminal Nd:YAG laser embolysis – A reasonable method to reperfuse occluded branch retinal arteries

Transluminalno Nd:YAG lasersko razbijanje embolusa – razumna metoda za recirkulaciju okludiranih grana retinalnih arterija

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Abstract

Introduction. Central retinal artery occlusion (CRAO) and branch retinal artery occlusion (BRAO) result in partial or complete retinal ischemia and sudden loss of vision; at this moment, there is no effective therapy for CRAO and BRAO. Transluminal Nd:YAG laser embolysis (TYE) represents a therapeutic approach used for retinal vascular occlusive diseases. The main indication is branch retinal artery occlusion with visible embolus; for central retinal artery occlusion this tehnique is hardly applicable. The principle of this method consists of intravascular embolus breakage using the 1064 nm Nd:YAG laser, focused on the embolus surface. **Case report.** We presented 5 cases with BRAO, 3 with infero-temporal and 2 with supero-temporal BRAO, all of them treated with TYE, with variable results. All the patients had a visible embolus within the BRA, the laser applications being delivered directly to the embolus. **Conclusion.** Despite our short-term experience regarding this therapeutical approach, we can resume that the moment of emboli distruction, as close as possible to the onset of the occlusion, is decisive for regaining vision and that applying the procedure correctly is superior to observation in most cases. Worldwide experience with TYE is still limited, but the technique seems feasible also when treating CRAO caused by visible emboli on the optic disc surface. This most certainly calls for random trials for identifying precisely the role of TYE in treatment of retinal occlusion pathology, though the relatively small number of properly diagnosed cases affects this objective. In all cases, the risks of TYE must be weighed against the possibility of severe and permanent loss of vision secondary to retinal artery occlusions.

Key words:
laser therapy; lasers, solid-state; retinal artery occlusion; treatment outocme.

Apstrakt

Uvod. Okluzija centralne arterije retine kao i okluzija grana retinalnih arterija rezultiraju parcijalnom ili totalnom ishemijom retine, praćene iznenadnim gubitkom vida. U ovom trenutku, ne postoji uspešna terapija za ovu vrstu oboljenja. Transluminalno Nd:YAG lasersko razbijanje embolusa predstavlja terapeutski pristup okludiranim krvnim sudovima retine, najbolji rezultati se postižu kod okluzije grane arterije retine sa vidljivim embolusom unutar krvnog suda, dok je kod okluzije centralne arterije retine ova tehnika teško izvodljiva. Princip ove metode je u razbijanju intravaskularnog embolusa koristeći 1064 nm Nd:YAG laser, fokusiran na površinu embolusa. **Prikaz bolesnika**. U radu je prikazano pet bolesnika sa okluzijom grane arterije retine, tri sa okluzijom donje temporalne grane, dva sa okluzijom gornje temporalne grane. Svih pet je tretirano transluminarnim Nd:YAG laserom sa različitim rezultatima. Svi bolesnici imali su vidljiv embolus unutar arterije, tako da je laser usmeravan direktno na embolus. **Zaključak**. Uprkos našem kratkom i malobrojnom iskustvu sa ovim terapeutskim pristupom, može se zaključiti da je razbijanje embolusa, što je moguće tačnije i bliže mestu okluzije, neophodno za vraćanje vidne oštrine. Pravilno izvođenje ove laserske procedure superiornije je od praćenja stanja bolesnika. Svetska iskustva sa ovom metodom su još uvek skromna, pri čemu se ona može primeniti i kod okluzije centralne arterije retine sa vidljivim embolusom na površini papile očnog živca. Ova metoda sasvim sigurno zahteva jedno šire, veće ispitivanje da bi se odredila uloga razbijanja embolusa Nd:YAG laserom u lečenju okluzije krvnih sudova retine, mada relativno mali broj pravilno dijagnostikovanih slučajeva onemogućuje procenu uspešnosti. Kod svakog pojedinačnog slučaja okluzije, rizik od upotrebe ove metode mora biti procenjen u odnosu na mogućnost teškog i trajnog gubitka vidne oštrine.

Ključne reči:
lečenje laserom; laseri, kristalni; okluzija retinalne arterije; lečenje, ishod.

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Introduction

Branch retinal artery occlusion (BRAO) can be caused by fibrinoplatelet emboli, cholesterol emboli and calcific emboli, typically located on arteriolar bifurcations or areas of vascular stenosis. A rather frequent occurrence in retinal vascular pathology is temporal artery branch occlusion (superior or inferior) caused by emboli located at arterial branch's emergence from the optic nerve papilla. Blood flow blockage through the artery causes partial or complete retinal ischemia with sudden loss of visual acuity and visual field impairment. The ocular fundus exam reveals sectorial clouding of the retina and most of the times viewing the translumenal embolus is possible. The fluoresceine angiography (FAG) exam shows either a delay or complete absence of dye material filling the affected blood vessel. Histopathologically, retinal artery branch occlusion is characterized by intracellular oedema in the internal retinal layers with loss of cells (in several months) which extends from the nerve fiber layer to the inner nuclear layer. Medical literature still lacks a well-established treatment for such conditions, however there are a few published studies referring to dissolving or melting emboli with the aid of lasers. The first method which was used involved low power argon laser photocoagulation. Although the cholesterol emboli were successfully melted with the laser, no functional improvements have been made. Opremcak and Benner [1] introduced the idea of using photodisrupting Nd:YAG laser for selectively lysing an intravascular solid embolus without damaging vascular walls. The aforementioned authors presented in April 2002 two surprisingly efficient solved cases, both anatomically and functionally, through transluminal Nd:YAG laser embolysis (TYE). In both cases immediate clearing of the embolus was noted alongside full recovery of retinal blood flow and a relatively fast regain of visual function (1–2 weeks). There were also other authors reporting favorable results using TYE in some cases [2].

We have been applying this method since 2006 on around 20 cases until now. In this paper, we presented some of these cases, including technical features. We were among the first authors in Europe to publish results regarding TYE and some of our clinical cases were presented during the 2010 European VitreoRetinal Society (EVRS) Congress in Seville, Spain [3, 4]. We consider that TYE is a therapeutical approach which can be used in certain situations.

Worldwide experience with TYE is still reduced, but the technique seems feasible in treating branch retinal artery occlusion and also central retinal artery occlusion caused by visible emboli [5–7]. This most certainly calls for random trials for identifying precisely the role of TYE in treating retinal occlusions, though the relatively small number of properly diagnosed cases affects this objective.

Case 1

An-81-year old female patient was presented to another clinic with blurry vision and superior altitudinal visual field defect in her right eye. After thorough investigations including FAG and visual field (VF) testing, the diagnosis was inferior temporal artery branch occlusion with retinal oedema in the lower macular region, with a visible embolus at the point of emergence of the vessel from the papilla (Figure 1), and delayed filling of the affected inferior temporal artery by the fluorescein, with hypofluorescence in the surrounding inferior area (Figure 2). The visual acuity was good, 20/30 best corrected visual acuity (BCVA), due to a permeable optociliary vessel, and a superior arcuate scotoma was present (Figure 3).

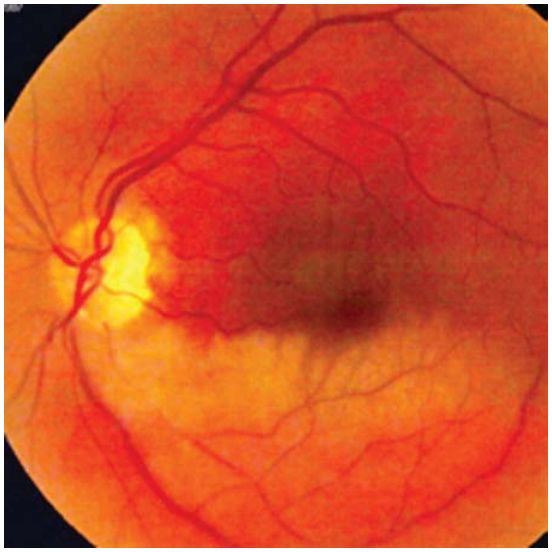

Fig. 1 – Colour retinography at presentation: inferior temporal artery branch occlusion, retinal oedema in the lower macular region, visible embolus at the point of emergence of the vessel from the papilla.

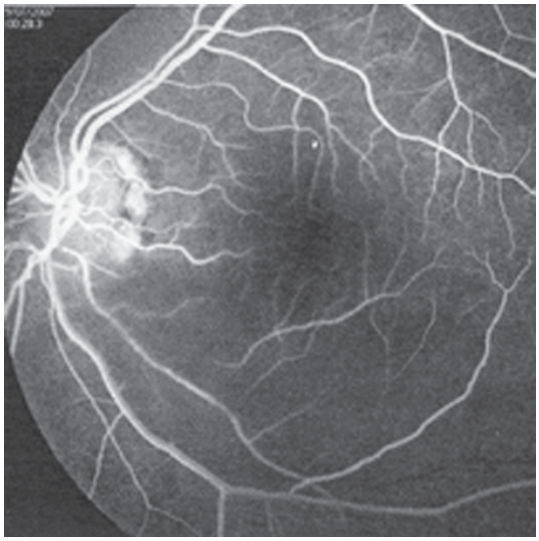

Fig. 2 – Fluoresceine angiography at presentation: delayed filling of the affected inferior temporal artery by the fluorescein, hypofluorescence in the surrounding inferior area.

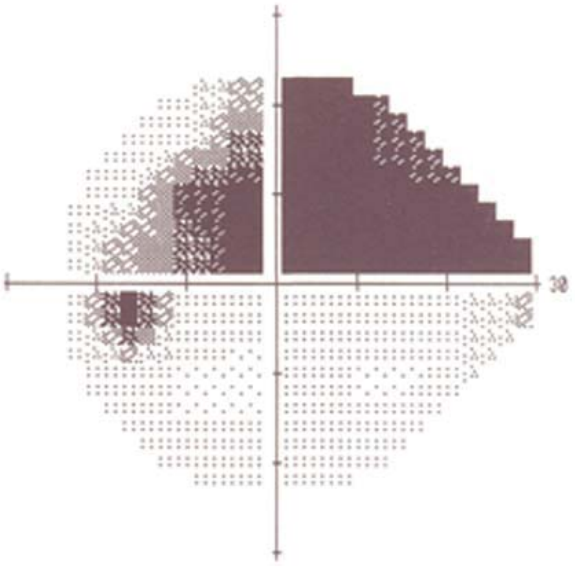

Fig. 3 – Visual field at presentation: superior arcuate scotoma.

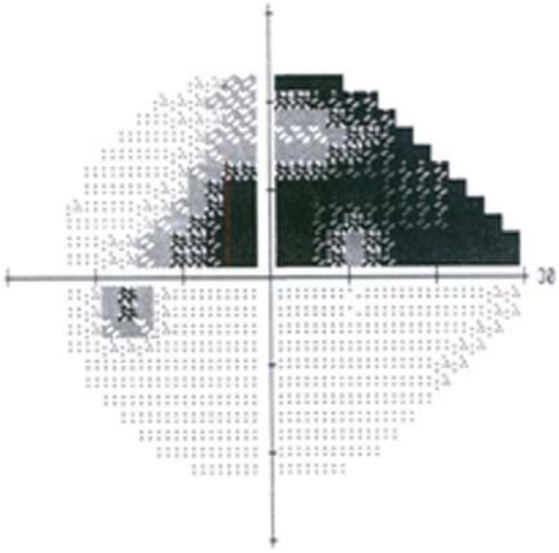

Fig. 5 – Visual field post-transluminal Nd:YAG laser embolysis (TYE): arcuate scotoma, slightly diminished in size.

The patient was referred to our clinic 10 days after the onset of symptoms, for the laser procedure, which was successfully completed, thus breaking the embolus and completely restoring blood flow (Figure 4). After resorbtion of

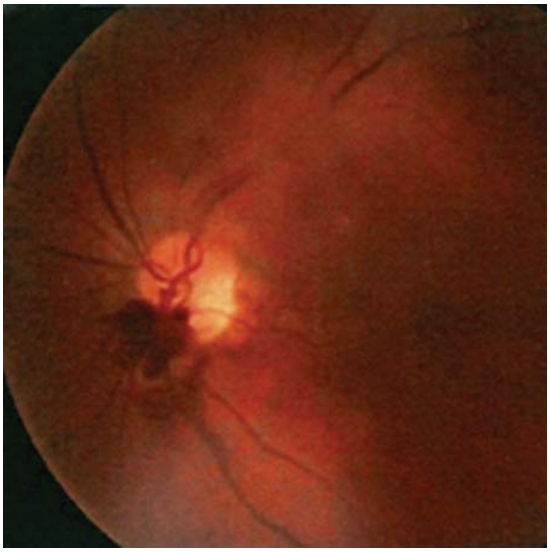

Fig. 4 – Colour retinography post-transluminal Nd:YAG laser embolysis (TYE): restored blood flow, juxtapapillary preretinal hemorrhage.

the small juxtapapillary preretinal hemorrhage induced by the TYE, another FAG exam was performed after 3 weeks, showing the absence of retinal oedema and restored blood flow. Subjectively, the patient's visual acuity was about the same and the arcuate scotoma slightly diminished in size without disappearing completely (Figure 5).

Obviously, because of the optocilliary artery and the long period of time between the start of the symptoms and the referral to laser, this case is not the best example about the utility of TYE, but this was the first case done using TYE and it gave us the courage to continue.

Case 2

A 66-year-old male patient, with a complex associated cardiovascular and hepatic pathology, was presented to another hospital's emergency room with sudden and dramatic loss of vision in his left eye (counting fingers) in the past 12 hours. He was diagnosed with inferior temporal artery branch occlusion with macular oedema affecting the foveola, with a visible white embolus at the vessel's emergence from the papilla and numerous other small agglutinated emboli scattered along the aforementioned arterial branch. The patient was referred to us for TYE, which was performed 2 days after the sudden loss of sight.

Three procedures (one every other day) were necessary in order to completely break the cholesterol emboli across the papillary area. Unfortunately our laser equipment does not have a video camera attached, because it is difficult to explain how the whole package of emboli was completely mobilized downstream the vessel after just one laser shot. We had to repeat the procedure three times because new emboli has arrised on the same place (on the optic disc surface). It is worth mentioning that despite the patient's systemic health problems, there was no hemorrhage during the procedures. After 4 weeks, the patient visual acuity was 20/20, with superior arcuate scotoma, normal aspect of the fundus and no visible oedema. Unfortunately, because of the emergency of the case, no VF or FAG exams were performed nor any other images were taken.

Case 3

A 73-year-old female patient was presented for an ophthalmological exam because of dramatic and sudden loss of sight in her right eye for approximately 3 days. Visual acuity of counting fingers was found and a superior temporal artery branch occlusion was diagnosed, with a visible embolus at the emergence of the blood vessel from the papilla. Fundus pictures were taken along with a 3D optical coherence tomography (OCT) revealing the macular oedema (Figure 6).

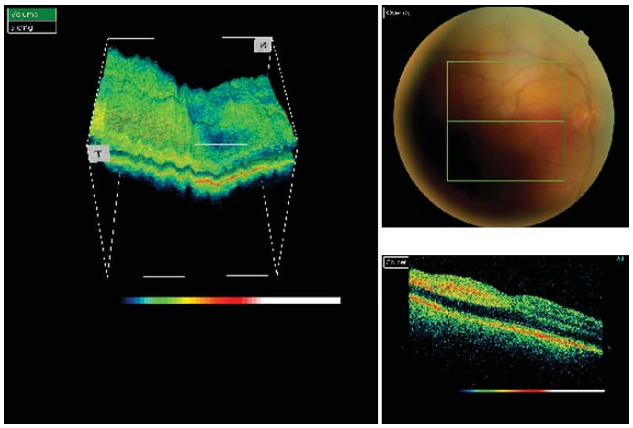

Fig. 6 – 3D optical coherence tomography (OCT) and colour retinography revealing the macular oedema.

The patient was referred to us for TYE procedure, seven days after the symptoms started. Two laser surgical attempts, a week apart, were needed for the complete embolysis. This was due mainly because of the patient's lack of cooperation, constantly moving her eyes and making it almost impossible for accurate targeting on the embolus surface; this also caused iatrogenic vessel perforation and subsequent hemorrhages.

Two weeks after the second procedure, normal blood flow was noticed, slight visual acuity improvements (20/200), retinal oedema remission and small hemorrhage in the posterior vitreous. In the images below, the presence of 4 small white emboli was presented, located at half disc diameter, inferior of the papilla, preretinal. Those emboli were pushed into the vitreous accompanied by a small blood jet, when we broke the initial embolus and the containing vessel (Figure 7).

Six weeks after the surgery, visual acuity improved up to 20/100, retinal oedema was noticeably reduced but not completely (Figure 8). The images below show an embolus fragment placed on the superior temporal artery at half disc diameter of the papilla, not completely obstructing the lumen. This is a risky location and we do not recommend laser in this situation because of the possibility of inducing retinal tears.

Case 4

A 62-year-old female patient was referred to us, presenting a superior altitudinal scotoma and reduced visual acuity (20/120) for about 48 hours. The diagnosis was inferior temporal branch arterial occlusion (left eye) with a visible emboli at the edge of the papilla (Figure 9).

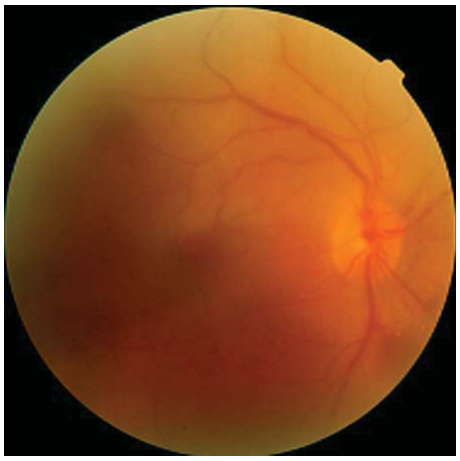

Fig. 7 – Colour retinography 2 weeks post transluminal Nd:Yag laser embolysis (TYE): small hemorrhage in the posterior vitreous; small white emboli, located at half disc diameter, inferior of the papilla, preretinal.

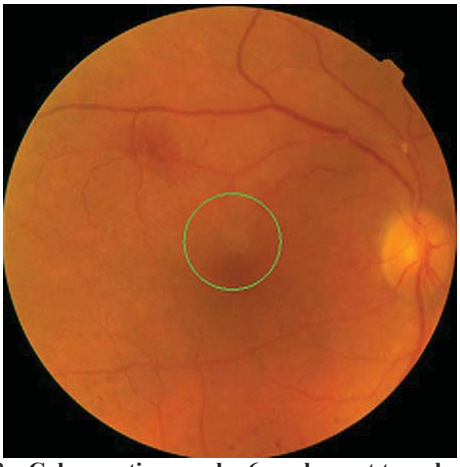

Fig. 8 – Colour retinography 6 weeks post transluminal Nd:YAG laser embolysis (TYE): reduced retinal oedema; embolus fragment placed on the superior temporal artery at half disc diameter of the papilla, not completely obstructing the lumen.

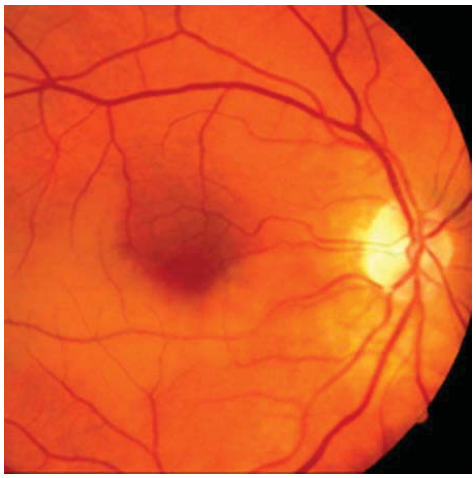

Fig. 9 – Colour retinography at presentation: inferior temporal branch arterial occlusion, visible emboli at the edge of the papilla.

TYE was performed thus destroying the embolus; blood flow and visual acuity up to 20/20 were restored while the visual field defect diminished considerably, being unnoticeable to the patient.

There was a cholesterol embolus and the laser procedure succeeded to break the embolus in smaller fragments inside the vessel, which went downstream.

The retinography performed 2 months after the laser procedure revealed: disappearance of the embolus from the inferior temporal quadrant of the optic disk and narrowing of the affected adjacent vessel (Figure 10).

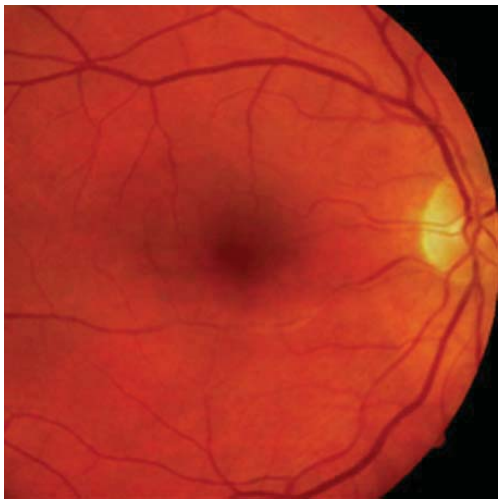

Fig. 10 – Colour retinography 2 months post transluminal Nd:YAG laser embolysis (TYE): disappearance of the embolus from the inferior temporal quadrant of the optic disk and narrowing of the affected adjacent vessel

Case 5

A 76-year-old male patient, presenting an inferior altitudinal scotoma for about 4 days, came to us for a laser procedure, being diagnosed with superior temporal branch arterial occlusion on the left eye (Figure 11). Vision (BCVA) on the left eye was 20/30.

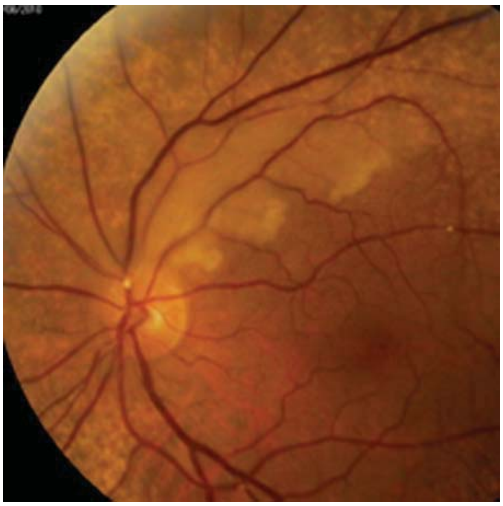

Fig. 11 – Colour retinography at presentation: superior temporal branch arterial occlusion, retinal oedema in the superior retinal sector.

TYE procedure was performed and blood flow was fully restored in the affected vessel (Figure 12) after 3 weeks; visual acuity recovered up to 20/20 and a substantial decrease in visual field defect was noticed. In this case the embolus was a little bit harder and a small haemorrhage was induced because of the laser impact inside the embolus against the vessel walls. There was not any extravasation of the embolus or fragments of it inside the vitreous cavity.

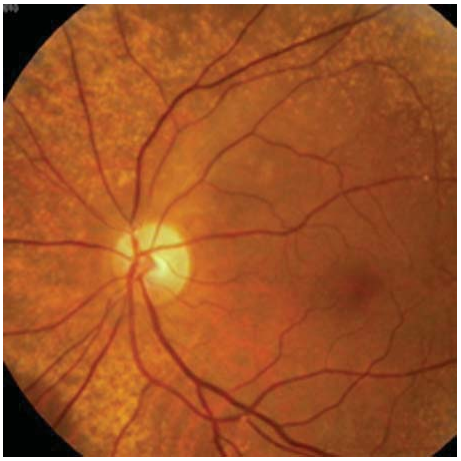

Fig. 12 – Colour retinography 3 weeks post transluminal Nd:YAG laser embolysis (TYE): fully restored blood flow in the affected vessel.

We summarized the cases in Table 1 below.

Discussion

Transluminal embolysis was performed with a photodisrupting Nd:YAG laser (1064 nm) manufactured by Nidek. The advantage of using this model is the possibility of focusing together the laser beam and the image of the slit-lamp perpendicularly on the center of the cornea, not just obliquely (there are also other laser equipment manufactured by different companies wich have this coaxial kind of focus); thus, using a specific laser contact lenses, it is possible to focus the target areas along the posterior pole. Throughout our cases we have used a Volk Centralis Direct contact lens, but also a three mirrors Goldman lens can be used. The focusing was performed perpendicularly on the embolus surface. The spot diameter was constant 50 μm in size and the power ranged from 0.8 mJ to 1.2 mJ. Firing was done "shot by shot". In each session there were fired between 2 and 4 shots. Potential complications regarding this technique are: retinal or vitreous hemorrhage, retinal breaks, choroidal neovascularization, epiretinal membranes. In most of our cases we faced just minor preretinal hemorrhages that were completely reabsorbed after 10–14 days and, in some cases, vitreous hemorrhages that were reabsorbed after 4 weeks. In all the cases improvement of visual function was observed and full restoration of blood flow.

The absolute therapeutic role of TYE in BRAO is debatable as it is known that over 70% of cases lean towards resolution, but the efficiency of the technique remains unquestionable speaking about the speed of emboli distruction and the recovery

Table 1

Centralized patient data

Parameters	Case				
	1	2	3	4	5
Occlusion type	Infero-temp BRAO	Infero-temp BRAO	Supero-temp BRAO	Infero-temp BRAO	Supero-temp BRAO
Embolus type	Calcific	Cholesterol and fibrinoplatelet	Calcific	Cholesterol	Cholesterol
Location	Optic disc	Optic disc	Optic disc	Optic disc	Optic disc
Macular edema	Yes-partial	Yes	Yes	Yes – partial	No visible
Arcuate retinal edema	Yes	Yes	Yes	Yes	Yes
Time of the TYE	10 days	2 days	7 days	2 days	4 days
Type of dislodging	Intra- and extravascular	Intravascular	Extravascular	Intravascular	Intravascular
Haemorrhages	Yes	No	Yes	No	Yes (very small)
VA preop.	20/30	CF	CF	20/120	20/30
VA postop.	20/30	20/20	20/100	20/20	20/20
Visual field defect	Diminished, but present	Very diminished, but present	Still present	Very diminished	Very diminished

TYE – transluminal Nd:YAG laser embolysis; BRAO – branch retinal artery occlusion; VA – visual acuity; CF – finger counting.

of blood flow. Despite a set of inherent riscs of any surgical intervention, speaking about TYE, the moment of emboli distruction as close as possible to the onset of occlusion is decisive for regaining visual function and, most probably, applying the procedure correctly is superior to observation.

We emphasize the importance of individualized analysis of any BRAO patient in deciding the treatment or not. For a successful TYE, the embolus have to be visible on the optic disc surface. We do not recommend laser treatment of emboli localized outside the optic disc, because of the retinal tears risks.

Any kind of arterial emboli can be treated in this way, but the TYE effect differs according with the embolus type. For hard emboli, like calcific ones, by using a YAG laser we can sometimes get a true extravasation of embolus, like in case 1. In such cases because of the high density of embolus the blood jet will pump up the full embolus or fragments of it in the vitreous cavity. For softer emboli, like fibrinoplatelet or cholesterol ones, the laser impacts usually dislodged them downstream the vessel in smaller fragments, if the power is set properly and the focus is done correctly. In this situation there is a true intravascular procedure by external approach. Obviously the sooner is the better, but even in prolonged occlusions (8–10 days) with persistent macular edema probably embolysis can help. We cannot prove yet the benefits of late TYE. It is very difficult to decide if the retina lesions are for good after one week of BRAO or there are still some viable retinal cells able to survive if the reflow will be set up again. Anyway "do no harm" is mandatory in judging each case, so when potential complications overwhelm the potential benefits of TYE, the surgeon has to adopt a conservatory approach.

Conclusion

Despite our short-term experience with this therapeutical approach, we can resume that the moment of emboli distruction, as close as possible to the onset of the occlusion is decisive for regaining vision and that applying the procedure correctly is superior to observation in most cases. Worldwide experience with TYE is still limited, but the technique seems feasible also when treating CRAO caused by visible emboli on the optic disc surface. This most certainly calls for random trials for identifying precisely the role of TYE in treatment of retinal occlusion pathology, though the relatively small number of properly diagnosed cases affects this objective. In all cases, the risks of TYE must be weighed against the possibility of severe and permanent loss of vision secondary to retinal artery occlusions.

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CASE REPORT

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The first case of papillary thyroid carcinoma in an adolescent with congenital dyshormonogenetic hypothyroidism in Serbia

Prvi slučaj papilarnog karcinoma štitaste žlezde kod devojčice sa kongenitalnim hipotiroidizmom u Srbiji

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Abstract

Introduction. Differentiated thyroid carcinoma (DTC) is a rare childhood malignancy, as it represents 0.3–0.4% of pediatric malignancies. Papillary carcinoma is the most common type of pediatric DTC and it represents about 90% of all DTC patients. Although rare, DTC arising from dyshormonogenetic goiter is the most serious complication of congenital hypothyroidism. **Case report.** We presented the development of thyroid papillary carcinoma in a 15-year-old girl diagnosed with congenital dyshormonogenetic hypothyroidism at neonatal age. Considering the early initiation and proper dosage of hormonal substitution, normal levels of thyreotropin and thyroid hormones were achieved quickly and maintained through a follow-up period. The girl remained euthyroid and asymptomatic until 13.8 years of age, when she presented with a large multinodular goiter. The patient underwent total thyroidectomy. Pathological examination revealed intrathyroid microcarcinoma in the right lobe. **Conclusion.** Although differentiated thyroid carcinoma is a rare pediatric malignancy, it is of great importance to have a certain degree of clinical caution and provide a multidisciplinary approach during the follow-up of patients with dyshormonogenetic hypothyroidism.

Key words:
congenital hypothyroidism; thyroid neoplasms; carcinoma, papillary; thiroidectomy; child; treatment outcome; serbia.

Apstrakt

Uvod. Diferentovani karcinom štitaste žlezde (DKŠŽ) je redak malignitet u detinjstvu i predstavlja 0,3–0,4% pedijatrijskih maligniteta. Najčešća forma DKŠŽ je papilarni karcinom pošto se javlja u približno 90% slučajeva. Iako redak, DKŠŽ koji se javlja kod dishormonogenetske strume predstavlja najozbiljniju komplikaciju kongenitalne hipotireoze. **Prikaz bolesnika.** U radu je prikazan papilarni karcinom štitaste žlezde kod petnaestogodišnje devojčice koja je lečena zbog kongenitalne hipotireoze od neonatalnog uzrasta. S obzirom na rano započinjanje i adekvatnu supstitucionu hormonsku terapiju, normalni nivoi tireotropina i tiroidnih hormona brzo su postignuti i devojčica je bila eutiroidna i bez simptoma do uzrasta od 13 godina i 8 meseci, kada je došlo do razvoja velike multinodularne strume. Konsultovan je onkološki hirurg i učinjena je totalna tiroidektomija. Patohistološkim pregledom otkriven je intratiroidni papilarni karcinom desnog režnja. **Zaključak.** Iako je diferentovani karcinom štitaste žlezde redak pedijatrijski malignitet, od izuzetnog je značaja imati određeni stepen kliničke sumnje i multidisciplinarni pristup tokom kliničkog praćenja bolesnika sa dishormonogenetskim hipotiroidizmom.

Ključne reči:
hipotireoidizam, kongenitalni; tireoidna žlezda, neoplazme; karcinom, papilarni; tireoidektomija; deca; lečenje, ishod; srbija.

Introduction

Differentiated carcinoma of the thyroid (DTC) is a rare childhood malignancy, as it represents 0.4–3% of all pediatric malignancies. More than 70% of these appear in patients aged between 11 and 17 and are usually related to radiation exposure. Papillary carcinoma is the most common type of pediatric DTC as it represents about 90% of all DTC cases [1–6]. Compared to adults, children tend to have more aggressive clinical course of this malignant disease, with up to 80% cases presenting with regional lymph node metastasis, and pulmonary metastasis in 10–20% [1, 2, 4, 7, 8]. Although rare, DTC araising from dyshormonogenetic goiter is the most serious complication of congenital hypothyroidism.

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In this case report, we present the developement of thyroid papillary carcinoma in a 15-year-old girl diagnosed with congenital dyshormonogenetic hypothyroidism at neonatal age.

Case report

This 15.2-year-old girl was diagnosed with congenital hypothyroidism as a newborn. The diagnosis of hypothyroidism was made by neonatal screening and the child underwent further examination which confirmed the diagnosis of primary dyshormonogenetic hypothyroidism. The patient started substitutional levothyroxine therapy, that she continued to take regularly. Considering the early initiation and proper dosage of hormonal substitution, normal levels of thyreotropin (TSH) and thyroid hormones were achieved quickly and were present all through the follow-up. Regular physical examinatitions showed no signs of goitre, and the girl was euthyroid and without any simptoms until 13.8 years of age.

At that time, the girl experienced loss of brother who was killed in a traffic accident. Almost immediately after the tragedy, her mother noticed the development of a large goitre and the girl came for an unscheduled appointment. Physical examination discovered a large multinodular goitre. Laboratory tests showed the following results: TSH 0.015 μIU/mL (normal range 0.27 to 4.2 μIU/mL), fT4 12.34 pmol/L (12.0 to 22.0 pmol/L), TPO antibodies over 600 IJ/mL (normal range less than 34 IJ/mL), Tg antibodies 140.4 IJ/mL (normal range ≤ 115 IJ/mL). Ultrasonography revealed a diffuse enlargement of the thyroid as well as the compression of the tracheal cartilage rings and hypoechodensity. Both of the lobi and the isthmus contained confluent nodular masses of 15–20 mm in diameter. These laboratory and ultrasonographic findings were consistent with autoimmune thyroiditis. There was no enlargement of the regional lymph nodes.

At the age of 10, the girl was also diagnosed with hypercholesterolemia, and the family history showed that the girl's mother and mather's father also suffered from this disorder. Also, the girl's grandfather had hyperthyroidism, and underwent thyroidectomy at the age of 63.

The patient was reffered to the surgeon, who recommended surgical treatment (thyroidectomy), with a complete preoperative work-up. Chest X-ray and abdominal ultrasound were normal. Laboratory tests, including parathyroid hormone and calcium levels were within allowed range. The patient was reffered to total thyroidectomy with *ex tempore* biopsy.

Pathological examination revealed the presence of intrathyroid papillary microcarcinoma 2.5 mm in diameter in the right lobe. The girl had two attacks of hypocalcemic tetany, so she was advised to take calcium and vitamine D, as well as substitutional levothyroxine therapy.

Currently, the girl is doing well and is euthyroid.

Data in this case report were presented with the written consent of the patient's parents.

Discussion

The developement of differentiated thyroid carcinoma in patients with dyshormonogenetic goiter is very unexpected, especially when it comes to pediatric patients. The case we presented was the first one the in western Serbia in the last 30 years, since the application of the neonatal screening program.

Most studies report a male predominance in children under the age of 10, that shifts to a female predominance in adolescents [3, 8–12]. As mentioned before, papillary thyroid carcinoma is the most common form of pediatric DTC.

Although the etiology of papillary carcinoma in patients with dishormonogenetic hypothyroidism remains unclear, certain risk factors have been recognised. Radiation exposure is known to be one of the major risk factors [5, 6, 3–15]. Another important element in the development of thyroid carcinoma is genetic factor [9, 16]. It has also been suggested that prolonged TSH stimulation, as well as inadequate substitutional therapy and intermitent TSH elevation can be associated with the development of DTC [17, 18].

Our patient's gender and age were consistent with the previous reports on DTC in children. On the other hand, in the presented patient there was no radiation exposure, pre- or postnatal, accidental or medical. Also, there were no data on thyroid carcinoma in the patient's family history, although her grandfather had suffered from hyperthyroidism and underwent total thyroidectomy. Levothyroxine treatment was initiated in neonatal age, the doses were adequate all the time and the consequent TSH levels were within normal range during the follow-up, so thyreotropin stimulation as a risk factor can be excluded.

In pediatric population, DTC has a great 20-year survival rate, that has been reported to vary between 90 and 95%. On the other hand, it is considered to be more agressive cancer in children, compared to adults [1, 2, 4, 7–9].

Conclusion

Differentiated thyroid carcinoma is a rare pediatric malignant tumor and the presented patient is the only reported case in Serbia. Although it has a more aggressive clinical course in children than in adults, the 20-year survival rate is excellent. Some risk factors are recognised, and should be kept in mind when assessing patients with congenital hypothyroidism. On the other hand, the presented patient did not have any of these risks in her history, so this case can serve as a remainder that it is of great importance to have a multidisciplinary approach and a certain degree of clinical suspicion even in cases with no apparent risks in patients medical history.

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CASE REPORT

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Dysplasia *epiphysealis hemimelica*: A case report

Hemimelična epifizealna displazija

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Abstract

Introduction. Dysplasia *epiphysealis hemimelica*, also known as Trevor's disease, is an extremely rare skeletal developmental disorder of unknown etiology, characterized by an osteocartilaginous outgrowth of one or more epiphyses or of a tarsal bone during childhood. **Case report.** We presented a sporadic case of dysplasia *epiphysealis hemimelica* developed in the talus. A 6-year-old boy complained of swelling of his right ankle joint, with painful and reduced movements. Radiographies suggested excessive overgrowth of the dorsomedial aspect of the talus. The tumor was surgically excised and the gross and histological findings were consistent with those of osteochondroma. **Conclusion.** Dysplasia *epiphysealis hemimelica* is diagnosed by clinical, radiographic and histopathological examination. Early diagnosis is important for the condition to be treated before the deformity becomes disabling.

Key words:
osteochondrodysplasias; talus; pain; diagnosis; orthopedic procedures; treatment outcome.

Apstrakt

Uvod. Hemimelična epifizealna displazija, takođe poznata i kao Trevorova bolest, ekstremno je redak poremećaj razvoja skeleta nepoznate etiologije, koji se karakteriše koštanohrskavičavom proliferacijom, lokalizovanom na jednoj ili više epifiza ili na talusu, u toku detinjstva. **Prikaz bolesnika.** U radu je prikazan bolesnik sa hemimeličnom epifizealnom displazijom lokalizovanom na talusu. Kod 6-godišnjeg dečaka bolest se prezentovala otokom desnog skočnog zgloba, bolom i otežanim pokretima. Radiografija je ukazala na postojanje izrasline na dorzomedijalnoj strani talusa. Tumor je ekscidiran, a makroskopski i histološki nalaz bio je identičan osteohondromu. **Zaključak.** Hemimelična epifizealna displazija se dijagnostikuje kliničkim, radiološkim i patohistološkim pregledom. Rana dijagnoza je važna, da bi se pravovremenom terapijom sprečio nastanak deformiteta.

Ključne reči:
osteohondrodisplazije; talus; bol; dijagnoza; ortopedske procedure; lečenje, ishod.

Introduction

Historically, dysplasia *epiphysealis hemimelica* (DEH) has been referred to by many names. It was originally described as "tarsomegalie" in 1926 by Mouchet and Belot [1]. In 1950 Trevor used the name tarso-epiphysial aclasis, and this abnormality is also commonly referred to as Trevor's disease [2]. Subsequently, in 1956 Fairbank [3] coined the current most frequently used term dysplasia *epiphysealis hemimelica* (*hemi* – half and *melos* – limb). According to Fairbank, DEH refers to a developmental disorder, which is confined to the medial or lateral half of an epiphysis of a single limb.

The etiology and pathogenesis of DEH are still not clear. DEH can be differentiated from osteochondroma of long bones using clinical, radiologic and pathologic parameters and this is an instructive example for the necessity of interdisciplinary collaboration in the assessment of bone dysplasias [4, 5].

We reported the clinical, pathological and radiological features of our case both for the extreme rarity of dysplasia *epiphysealis hemimelica.*

According to our knowledge, this is the first description of DEH in the Serbian literature.

Case report

A 6-years-old boy was admitted to the hospital for treatment because of motion pain and restriction of the right ankle. The patient had no history of trauma or excessive symptoms. The family history was negative for bone deformity or joint problems. At physical examination a painless

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and hard protrusion could be palpated on the dorsomedial aspect of the right ankle. The neurologic examination was intact. Muscle wasting was not noted in the lower extremity. Standard laboratory blood parameters (blood count, coagulation, electrolytes, kidney function tests, liver function tests, and thyroid function tests) were all within the normal range. Radiographs showed an abnormal ossified mass occupying the dorsomedial aspect of the talus (Figure 1a). No areas of pathological contrast enhancement of muscles and subcutaneous tissue or joint effusion were present. Surgery was proposed and performed to improve the range of ankle motion.

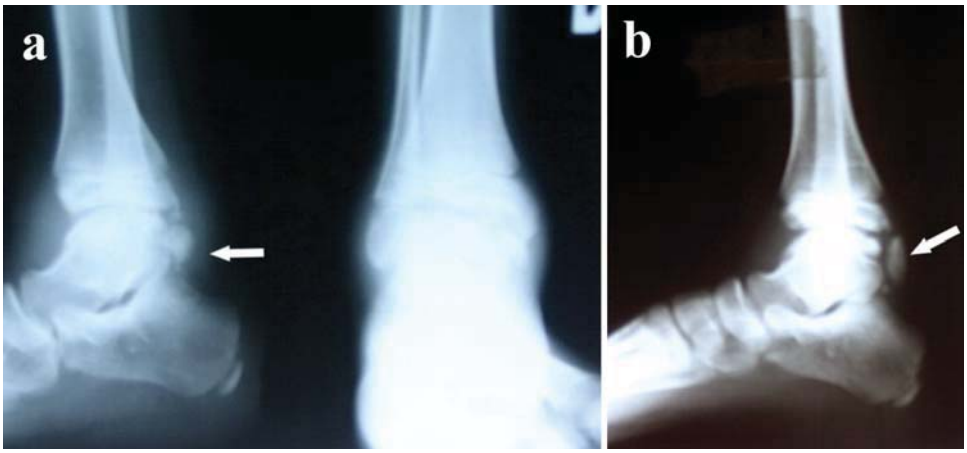

Fig. 1 – a) Preoperative radiographs (anteroposterior/lateral projection) and b) Radiograph of the recidive, showed an osteocortilaginous protuberance of the talus.

Histopathologically, resected specimen taken from the lesion, showed a typical appearance of osteochondroma (Figure 2). The periphery of the bone lesion was

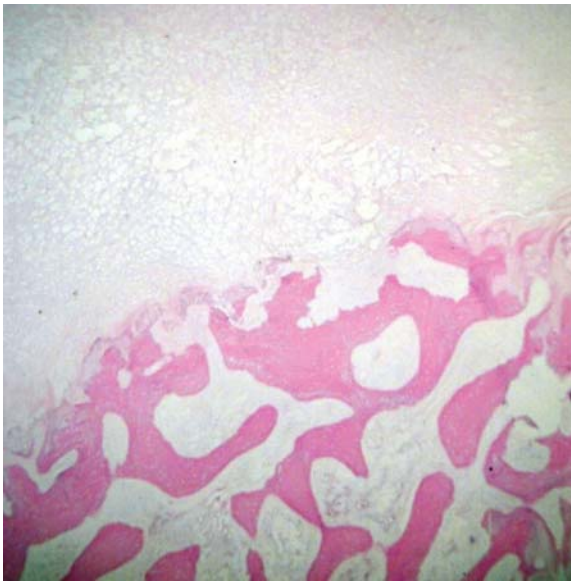

Fig. 2 – Photomicrograph of a specimen showing the appearance of the osteochondroma (hematoxylin-eosin staining, original magnification ×200).

covered by a cartilaginous cap. The cartilage cap was lobulated and lobules were arranged around blood vessels. At the base of the cap, areas of incomplete and irregular endochondral ossification were observed, i.e. the columns of chondrocytes were disorganized, mineralization was incomplete and bone was incompletely replaced by lamellar bone.

Eleven months later the patient again had pain and restriction in the range of right ankle motion, and visited our hospital. New radiography verified recidive (Figure 1b). Surgery was performed again and the specimen was sent for pathological examination. Again, the result showed that the trabecular bone was covered by a thick irregular cap of cartilage, suggestive of an osteochondroma.

Actually, two years after the surgery, the boy had a complete range of motion and walking without limping and did not complain of pain in the right ankle during daily activity. The radiographs demonstrated no progression of osteoarthritic changes in the ankle joint nor further expansion of this osteocartilaginous lesion.

Discussion

Trevor's disease appear in the latest International Nomenclature of Constitutional Disorders of Bone, the Group 31: "disorganized development of cartilaginous and fibrous components of the skeleton" [6]. In that list it is recorded as sporadic in inheritance and with no evidence of malignant transformation.

There are three forms of DEH based on its extent and distribution: a localised form involving only one *epiphysis*, a classic form involving more than one area in a single limb and a generalised or severe form involving the whole lower limb, from pelvis to foot [7]. The reported incidence is 1 in million and approximately 200 cases of DEH have been reported since 1957 [8].

The etiology and pathogenesis of DEH are not known. Its origin and evolution have, initially, similarities to the development and growth of the secondary ossification centers in the *epiphysis* and show centrifugal mode of growth, probably induced by invasion of capillary vessels followed by endochondral ossification [9]. A recent molecular study of DEH demonstrated normal expression levels of

EXT1 and EXT2 genes, and therefore a normal Indian Hedgehog pathway of cell growth, comparable to normal growth plate [10]. Osteochondroma, on the other hand, has low expression levels of EXT1 and EXT2 genes because of gene mutation. These findings strongly favor the distinction of DEH and osteochondroma as separate entities [10]. Perl et al. [11] found that the cellular phenotype of clustered chondrocytes in DEH exhibited characteristics of chondroprogenitor cells and terminally differentiated cells, suggesting dysregulation of the resident progenitor cells [11]. Limited data are available on the entity, mainly consisting of small case series.

Boys are affected approximately three times as often as girls [12]. The age of onset is usually between 2 and 14 years [13]. DEH commonly affects the lower extremity (tarsus, distal tibia, and distal femur) on one side of the body and often is restricted to either the medial or the lateral side of the limb (hemimelic) [12]. Although asymmetrical involvement has been reported, the lesions typically affect only one side of the joint, with the medial side affected twice as frequently as the lateral side [14]. In approximately 2/3 of the cases, more than one *epiphysis* is affected [15].

The most common symptom of DEH is painless swelling or deformity, and patients may complain of pain or decreased range of motion [14]. Radiographic findings are characteristic for DEH. Early lesions consisted of an irregular mass with multicentric ossification arising from either the lateral or the medial half of the affected *epiphysis* or tarsal bone; later this fused with the adjacent bone and resembled an exostosis [16].

Histopathologically, it was not possible to distinguish DEH from osteochondroma [17]. The lesion may be a pedunculated bone mass with a cartilaginous cap, or it may be seen only as an enlarged irregularity of the articular surface [14]. Histologic findings include clumping of chondrocytes in a fibrillary chondroid matrix and immunohistochemical positive expression of Indian hedgehog/parathyroid hormone-like hormone (IHH/PTHLH) [10].

DEH should be differentiated from other osteocartilaginous lesions such as synovial chondromatosis, capsular or para-articular chondroma, and particularly osteochondroma [11]. Differential diagnoses also include *myositis ossificans*, infection, chronic infantile neurologic, cutaneous, and articular (CINCA) syndrome, tumoral calcinosis, and vascular or parasitic calcification [18].

The treatment of DEH is still discussed in the literature. Because of the variety of location and the extent of involvement, patients must be treated on an individual basis [14]. Treatment ranges from simple observation to surgical excision. Asymptomatic lesions may be treated nonsurgically since there is no known risk of malignant transformation. Surgical intervention is more frequently required for these lesions than for solitary osteochondromas because the epiphyseal location is often associated with pain, deformity or loss of normal mechanical function [8, 19]. Surgery is often more directed at improving joint congruity to lessen subsequent development of secondary osteoarthritis; thus, treatment at an early stage of disease improves outcome [4, 19]. If the lesion is extraartcular, as in our case, simple mass excision yields favourable results.

The prognosis of DEH is variable, and depends on the location and size of the lesion. If the lesion is intraarticular, resection may increase the risk of degenerative joint disease even further. In all cases, there is a risk of recurrence of this disease until the epiphyses are closed. Continuous monitoring may be needed.

Conclusion

Dysplasia *epiphysealis hemimelica*, a rare deformity of unknown etiology, is diagnosed by clinical and radiographic examination. Early diagnosis is important for the condition to be treated before the deformity becomes disabling. Surgery is considered the treatment of choice, although surgical correction may lead to degenerative arthritis and instability of the ankle joint.

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B O O K R E W I E V

Health Economics with Pharmacoeconomics

Original title: Zdravstvena ekonomija sa farmakoekonomijom
Editor: Prof. Dr. Mihajlo Jakovljević
Publisher: University of Kragujevac, Faculty of Medicine Sciences, Kragujevac, Serbia
Published: 2014

The book written by a group of authors and edited by Prof. Dr. Mihajlo Jakovljević represents the first comprehensive reading material written in Serbian language that addresses areas of health economics, health care policy and financing, as well as pharmacoeconomics, i.e. evaluation of health and economic outcomes of drug therapies.

Health care systems throughout the world face increasing financial pressures and challenges to, on one hand, retain financial stability of the system and on the other hand allow patients access to new health technologies. Developed countries have implemented various mechanisms to control health expenditures and have established institutions specifically dedicated to assessment of new health technologies, both from cost as well as benefits perspective. Although there is no specific health technology assessment institute in Serbia, a number of interesting health economic analyses that relate to Serbian setting have been published by the editor and authors in national and international journals. Some of these are also presented in the current textbook. The textbook which was primarily written for students of medical and healthcare sciences is thus very useful and interesting reading for the whole professional community.

The textbook is segmented into three sections. The first section starts with introducing history of health economics; this is followed by chapters addressing specifics of health care markets, types of national health care systems and financing models, as well as health policy and organisational aspects of health care provision. The concluding chapters describe health technology assessment and evidence based medicine.

In the second section, authors present economic burden of illnesses and social trends that are most impactful on the health budgets. Among those are economic burden of population ageing, infectious, malignant, cardiovascular, neurological, mental and respiratory diseases, as well as physiotherapy and rehabilitation.

The third section of the textbook is dedicated to pharmacoeconomics. There is a particular scrutiny in the developed countries on evaluation of health benefits and costs related to new drug therapies. Namely, many new drugs, particularly biologics, are extremely expensive and in many cases their access to the market is strictly controlled or they are allowed to be used for specific patient populations only. In some cases, their financing is even rejected as they are not deemed to have sufficient added benefit to support their high price. In the textbook, authors describe various methodological approaches how to evaluate health and economic outcomes related to drug therapies. The reader is familiarized with different types of pharmacoeconomic analyses (cost-minimization, cost-effectiveness, cost-utility, cost-benefit analysis), modelling, statistical analysis of the results, as well as evaluation of health utilities and the quality of life. The section is written in a straightforward and comprehensive manner and provides the reader with essential knowledge and terminology used in this particular area of science.

Lastly, it is of particular value that so many authors from various backgrounds and institutes contributed to this textbook. The book thus covers all relevant and contemporary topics and it will be a very useful reading for several generations of students and professionals.

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E R R A T U M

The article „The first pharmacy in Vranje with the educated pharmacist and its development”. Vojnosanit Pregl 2014; 71(10): 978–984 (DOI: 10.2298/VSP130819048K).

Listed the authors as: Dušanka M. Krajnović, Jasmina B. Arsić, Andrijana M. Milošević Georgijev, Jelena M. Manojlović

The list of authors should read as: Dušanka M. Krajnović, Jasmina B. Arsić, Andrijana M. Milošević Georgiev, Jelena M. Manojlović

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DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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